Effects of Stocked Trout on Native Fauna of Productive Lakes

by

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Abstract

Salmonids are stocked around the world to create and enhance fishing opportunities. Most research into the effects of trout on lake ecosystems has occurred in alpine and other oligotrophic systems, often naturally fishless, where negative impacts are likely. In contrast, I investigated effects of stocked Rainbow Trout (*Oncorhynchus mykiss*) on native fishes, invertebrates and food webs in productive lakes in the boreal foothills of Alberta and examined mechanisms that may act to limit trout impacts. I used a variety of approaches, including whole-lake comparisons, Before-After-Control-Impact (BACI) analyses, and mesocosm experiments.

Unlike results from alpine lakes, I found few negative effects of trout on native species. In Chapter 2, I showed that stocked trout were dietary generalists and consumed a variety of prey taxa across multiple trophic levels, not dissimilar to the native fishes, and the structure of food webs in stocked lakes was generally unaffected by trout relative to unstocked lakes. Stable isotope analysis revealed that many native organisms primarily inhabit littoral areas and may therefore benefit—passively or actively—from the predation refuge provided by dense aquatic macrophytes. In whole-lake comparisons, trout did not affect the abundance or lengths of forage fishes, but both adult and young-of-year dace (*Chrosomus* spp.) were captured more frequently in vegetated habitats in stocked lakes relative to unstocked lakes (Chapter 3). BACI analyses revealed that invertebrate assemblages were likely affected by trout the first year after stocking; however, by the second year, assemblages in a focal stocked lake were similar to those in unstocked controls (Chapter 4). My mesocosm experiment demonstrated that native fishes will adjust their use of macrophyte beds in the presence of trout to avoid open-water habitat (Chapter 2) and that macrophyte stands and occasionally small-bodied fishes can shape the community composition and taxon-level abundance of invertebrates (Chapter 5). Indeed, the “pre-structuring” effects of forage fishes and macrophytes may limit the negative impacts of trout in my lakes compared to lakes that lack these features.

My results demonstrate that stocked trout do not always negatively affect native fauna and suggest that native forage fishes and/or dense beds of macrophytes buffer against impacts of trout. Although the precautionary principle should be applied before introducing a species into a novel ecosystem, some systems, such as these isolated productive lakes, appear to have the capacity to support introduced trout with few effects on native forage fishes and invertebrates.

Preface

Research for this thesis was conducted as part of the FIESTA (Fish, frog, and Invertebrate Effects of Stocked Trout and Aeration) Project, a collaborative partnership between the University of Alberta, Alberta Conservation Association (ACA), and Alberta Environment and Parks (AEP). The work in Chapters 2 – 5 and literature reviews in Chapters 1 and 6 are my original work, with contributions from co-authors in published manuscripts as detailed below.

Chapter 2 of this thesis will be submitted for publication as Hanisch, J.R., Tonn, W.M., Paszkowski, C.A., and Scrimgeour, G.J. I was responsible for data collection, analysis, and manuscript composition. William Tonn, Cynthia Paszkowski, and Garry Scrimgeour assisted with conceptual experimental design. William Tonn and Cynthia Paszkowski also provided editorial input to the manuscript and were supervisory authors.

Chapter 3 of this thesis was published as Hanisch, J.R., Tonn, W.M., Paszkowski, C.A., and Scrimgeour, G.J. "Complex littoral habitat influences the response of native minnows to stocked trout: evidence from whole-lake comparisons and experimental predator enclosures," *Canadian Journal of Fisheries and Aquatic Sciences* 69(2): 273-281. I was responsible for data collection with assistance from William Tonn, Cynthia Paszkowski and Garry Scrimgeour. I was also responsible for data analysis and manuscript composition with conceptual guidance and editorial input from all three coauthors. Additional data analysis and discussion of results from Before-After-Control-Impact (BACI) analysis of fish length and abundance were added to this Chapter after publication for inclusion in the thesis. Additional data used in these analyses were collected by Justin Hanisch and Leslie Nasmith, and data analysis and discussion were completed by Justin Hanisch.

Chapter 4 of this thesis was published as Hanisch, J.R., Tonn, W.M., Paszkowski, C.A., and Scrimgeour, G.J. “Stocked trout have minimal effects on littoral invertebrate assemblages of productive fish-bearing lakes: a whole-lake BACI study,” *Freshwater Biology* 58, 895–907. I was responsible for data collection, analysis, and manuscript composition. William Tonn, Cynthia Paszkowski, and Garry Scrimgeour assisted with conceptual experimental design and also provided editorial input to the manuscript. William Tonn and Cynthia Paszkowski were supervisory authors.

Chapter 5 of this thesis will be submitted for publication as Hanisch, J.R., Tonn, W.M., Paszkowski, C.A., and Scrimgeour, G.J. I was responsible for data collection, analysis, and manuscript composition. William Tonn, Cynthia Paszkowski, and Garry Scrimgeour assisted with conceptual experimental design. William Tonn and Cynthia Paszkowski also provided editorial input to the manuscript and were supervisory authors.

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Chapter 1. General Introduction

Human action, both intentional and inadvertent, has introduced many fish species outside of their native ranges, resulting in a large-scale homogenization of fish fauna (Rahel 2000, Rahel 2007). While effects of nonindigenous species have long been a topic of study (Elton 1958), anticipating the successful establishment and potential effects of introduced species remains difficult (Fausch 2008). Part of this difficulty lies in the complex interrelationships between biological and ecological characteristics of both the introduced species and ecosystem into which the species is introduced (Moyle and Light 1996, Mack et al. 2000). For example, the success of a single invading species can vary significantly across habitat types within the same geographic area (e.g., abundance of *Pomacea* gastropods in ponds, streams, and rice paddies; Burlakova et al. 2010). Because biotic homogenization caused by introduced species is expected to continue and because the ecological and evolutionary implications of introduced species are still poorly understood (Olden et al. 2004), continued study of introduced species across a variety of systems remains of principal importance in ecology and conservation.

Salmonids *(Oncorhynchus, Salmo, Salvelinus*) are among the most commonly introduced fishes throughout the world (Rahel 2007) and have been stocked extensively in North America to create or sustain recreational angling opportunities (Bahls 1992, Schindler 2000, Halverson 2008), frequently in areas devoid of native game fishes. Stocked trout, however, often have serious negative effects on native biota. For example, benthic macroinvertebrates, large bodied zooplankton, and amphibians are especially vulnerable to trout predation (Knapp et al. 2005; Tiberti et al. 2014), and in extreme cases, native zooplankton (Schindler 2000) and amphibians (Bradford et al. 1998) have been extirpated after trout stocking. Such negative results appear to occur most often in oligotrophic and alpine lakes characterized by low productivity, low species richness, and low complexity of native habitat—all factors thought to favor negative impacts of invading species (e.g., Vermeij 1991, Moyle and Light 1996). As well, alpine lakes are often devoid of native fishes, and the native populations of these lakes are thus naïve to predation by fish (Cox and Lima 2006).

Despite some understanding of how trout affect alpine and other oligotrophic systems, relatively little is known about how trout affect more productive, lower-elevation lakes. Recent research in productive lakes in Canada (Schank et al. 2011, Nasmith et al. 2010), New Zealand (Wissinger et al. 2006), Patagonia (Pascaul et al. 2007), and California (Hartman et al. 2013) has documented minimal effects of stocked trout on native organisms, including amphibians, macroinvertebrates and small-bodied fishes. These results contrast markedly with the effects of trout in alpine lakes and suggest that characteristics of small, productive lakes may ameliorate negative effects of stocked trout and promote the coexistence of these introduced predators and their prey. Accordingly, the overall objective of my Ph.D. research, using both comparative and experimental methods, was to determine how stocked trout affect the ecological communities of productive lakes. I compared fish and invertebrate communities of stocked lakes to unstocked lakes in the boreal foothills of Alberta, and because one lake was stocked with trout two years into my project, I also used a Before-After-Control-Impact (BACI) design (Downes et al. 2002) to determine effects of trout in this lake relative to three unstocked control lakes. Within a single, unstocked lake I also conducted a mesocosm experiment to investigate the importance of aquatic macrophytes in structuring the interactions between introduced trout, forage fishes, and macroinvertebrates. This thesis is organized into four data chapters, each with an objective of investigating the impacts of stocked trout on a specific aspect of the native ecological community.

Direct consumption of native species by trout is often the main cause of species decline or extirpation post-stocking (Bradford et al. 1998, Carlisle and Hawkins 1998), but the addition of trout can also elicit indirect effects, including changes in nutrient cycling (Schindler et al. 2001), changes in prey behavior (Bryan et al. 2002, Messler et al. 2007), and even reduced subsidies to the surrounding terrestrial ecosystem (Epanchin et al. 2010). Such effects often manifest as detectable changes in food web structure (Vander Zanden et al. 1999, Vander Zanden et al. 2003, Lepak et al. 2006). Accordingly, in Chapter 2 I used quantitative measures of trout diet in stocked lakes and of food web structure to investigate potential effects of trout on the structure of native invertebrate communities. I predicted that the abundance or size structure of some commonly consumed prey taxa may be affected by trout presence but that few, if any, changes in food web structure would occur.

Trout are commonly stocked in lakes without native populations of forage fish (Dunham et al. 2004, Halverson 2008), so knowledge of how forage fishes respond to trout introduction is generally lacking. Introductions of other fish species, such as Eurasian Perch (*Perca fluviatilis*; Tonn et al. 1992) and Smallmouth Bass (*Micropterus dolomieu*; Chapleau et al. 1997) into fish-bearing waters have impacted native fishes negatively. Research in Patagonia and New Zealand has found both negative and no effects of introduced salmonids on native fishes (Macchi et al. 1999, Habit et al. 2010), and it appears that in some cases, native fishes alter their habitat use to avoid predation by trout (Stuart-Smith et al. 2008; Nasmith et al. 2010, Elkins and Grossman 2014). To investigate potential effects of trout on native forage fishes, I used a variety of approaches in Chapter 3. These included comparing habitat use of forage fishes in stocked and unstocked lakes, Before-After Control-Impact (BACI) analysis to compare abundance and length of forage fish in a stocked lake relative to unstocked controls, and using experimental mesocosms, testing the interaction between stocked trout, forage fishes, and the refuge habitat provided by aquatic macrophytes. Because forage fish may use aquatic macrophytes as refuge from trout predation, I predicted that forage fish in stocked lakes would be found more often in littoral habitat than in unstocked lakes. The availability of this refuge habitat would result in few, if any, differences in abundance and length of forage fish populations in the stocked lake relative to unstocked controls.

Introduced trout are known to prey on large-bodied macroinvertebrates and zooplankton, often reducing their numbers (Knapp et al. 2005) and sometimes extirpating these taxa (Anderson 1980). Accordingly, predation by trout can change the composition and size structure of macroinvertebrate communities. As detailed above, however, macroinvertebrate communities of productive lakes appear to be less affected by trout introduction (Wissinger et al. 2006, Nasmith et al. 2012). Accordingly, I used BACI analysis to investigate potential changes in macroinvertebrate communities in a single lake after stocking compared to unstocked controls (Chapter 4). I predicted that the size structure of some commonly-consumed macroinvertebrate taxa may be affected by trout but that no large changes in community composition would occur after stocking or in stocked versus unstocked lakes.

Aquatic macrophytes play important roles in structuring aquatic communities (Carpenter and Lodge 1986). In lakes stocked with a predator, aquatic macrophytes can provide an important refuge from predation for native species (Schriver et al. 1995, Sass et al. 2006, Stuart-Smith et al. 2007), and such refugia are often cited as explanations when strong effects of introduced trout are not observed (Wissinger et al. 2006, Nasmith et al. 2010; Hartman et al. 2013). I conducted a two-factor mesocosm experiment, manipulating macrophyte density and native fish presence, to test the structuring role of macrophytes and native forage fishes in an unstocked boreal foothills lake (Chapter 5). Each replicate consisted of four mesocosms: two at "natural" macrophyte density and two at "reduced" macrophyte density. Each macrophyte density had one mesocosm that excluded fish and one that allowed forage fish unimpeded access. I predicted that abundance of invertebrates should be greater in natural macrophyte-density treatments relative to reduced macrophyte-density treatments. Similarly, if forage fish structure invertebrate populations, I predicted that invertebrate abundance would be greater in fish-absent treatments than in fish-present treatments.

Ecological effects of stocked salmonids have been the subject of numerous studies and reviews (e.g., Dunham et al. 2004, Eby et al. 2006, Crawford and Muir 2008, Fausch 2008, Leprieur et al. 2009), and salmonids continue to be stocked by the millions across North America and the world to sustain popular and economically important commercial and recreational fisheries (Rahel 2000, Halverson 2008). Effects of stocked trout can be negative; however, it appears that some systems, such as productive lakes characterized by complex habitat and diverse communities of native species, including native fish, may be more robust to the effects of these introduced predators (Wissinger et al. 2006, Stuart-

Smith et al. 2007, Nasmith et al. 2010). My research, which consists of comparisons of fish and macroinvertebrate communities between stocked and unstocked lakes, BACI analyses (considered one of the most robust methods to detect environmental impact; Downes et al. 2002), and experimental field manipulations, represents one of the most comprehensive investigations of the effects of stocked trout in productive lakes to date.

The results of my research have implications for both applied fisheries management and basic science. Because very little research has explicitly addressed the impacts of stocked trout in productive lakes, fisheries managers could use my results to inform their stocking decisions. Additionally, my research is more generally applicable to the ecology of nonindigenous species. Trout stocking is not a perfect analog to "natural" species invasions. However, promoting the introduction and spread of non-native species for ecological research is generally inadvisable, and most research into impacts of invasive species are post-hoc and preclude experimental manipulation. Trout stocking can be a controlled experiment, which can reveal important insights into the mechanisms governing the response of native communities to the addition of a top predator. Insights from my research should be applicable to other productive systems at risk or already invaded by similar top predators.

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Chapter 2. Trout diet and the effects of stocked trout on native food webs of small, productive lakes

Abstract

Stocking of non-native trout can have serious negative effects on native communities, including changes in species abundance and habitat use (Biro et al. 2003) or even extirpations. The most serious effects of stocked trout have been documented in frequently-studied alpine and oligotrophic lakes; however, it is unclear how trout affect other less-studied native communities—such as those typical of productive lakes—where stocking frequently occurs. To investigate the potential effects of stocked trout in productive, fish-bearing lakes, I first quantified the diets of stocked trout in a suite of lakes with various dates of first stocking. I then used several stable isotope techniques to determine whether stocked trout have impacted the food webs of several stocked lakes and within a single focal lake before and after stocking compared to unstocked control lakes. Top prey items of trout in stocked lakes included cladocerans and chironomids and generally did not differ between recently stocked lakes and lakes with a long history of trout stocking (e.g., > 60 years). However, trout from recently stocked lakes did consume a larger proportion of some taxa known to be vulnerable to trout (chaoborids and trichopterans) than did trout in long-stocked lakes.

Stable isotope analysis documented few differences in food-web structure between stocked and unstocked lakes and within the single focal lake before and after stocking. Forage fishes did appear to change their use of habitat in the presence of trout, with native fishes in some lakes increasing their use of littoral habitat; however, such shifts were not consistent across lakes, and forage fishes in one stocked lake were strongly pelagic. Stable isotopes revealed that native organisms in all lakes frequently inhabited littoral habitat and may therefore benefit—either passively or actively—from the refuge from predation by trout provided by aquatic macrophytes.

Introduction

Stocking of non-native trout can have serious negative effects on native communities, including changes in species abundance (Knapp et al. 2001) and habitat use (Biro et al. 2003) or even extirpations (Anderson 1980, Schindler 2001). The most serious effects have been documented in alpine and oligotrophic lakes (Dunham et al. 2004, Eby et al. 2006), which have characteristics thought to facilitate negative impacts of introduced fishes (e.g., Moyle and Light 1996). However, because these simple, vulnerable systems are also the most studied, it is unclear how trout affect other native communities—such as those typical of productive lakes—where stocking frequently occurs (e.g., Gozlan 2008).

Limited studies to date on stocked trout in productive lakes have documented a variety of effects, although few have been severe. For example, effects on native invertebrates have been limited to relatively minor changes in abundance or length of a few taxa, and few, if any, impacts have occurred at the community level (Wissinger et al. 2006 Nasmith et al. 2012, Hanisch et al. 2013). Effects of trout on forage fishes in these systems have also been weak, consisting of habitat shifts (increased use of refugia such as macrophyte beds; Biro et al. 2003, Hanisch et al. 2012) and minor changes in length distributions (Nasmith et al. 2010).

Previous research has offered a few potential reasons for the lack of strong negative effects of trout on native organisms in some types of stocked lakes. Trout in a recently stocked mesotrophic lake in central Alberta, Canada, exhibited a relatively diverse diet, consuming organisms across a variety of body sizes, functional ecological groups, and habitat types (Hanisch et al. 2013); thus, it is possible that direct effects of trout can be ameliorated by their "generalist" feeding habit (Fagan, 1997; Eriksson et al., 2011, Hanisch et al. 2013). A second potential explanation is the aforementioned macrophyte beds as refugia for prey fish in more productive systems, although little work has addressed the use of refugia by other organisms, including invertebrates, and few studies have examined potential changes in food webs in productive, fish-bearing stocked lakes, e.g., via stable isotope techniques.

Furthermore, few studies have quantified actual diets of trout from productive lakes. Decreases in the importance of particular invertebrate groups from trout stomach samples, especially groups known to be more susceptible to trout predation such as odonates or leeches, may be an "early warning" of subsequent decreases at the whole-lake level (e.g., Venturelli and Tonn 2005; Hanisch et al. 2013). As well, differences in the diets of trout among lakes with different histories of stocking (e.g., duration of stocking) may suggest which taxa are most vulnerable to effects of trout predation in productive lakes. In low-productivity Maine lakes, effects of stocked Brook Trout (*Salvelinus fontinalis*) were detectable within 3 years of first stocking and were most severe in lakes stocked for more than 40 years (Schilling et al. 2009). Because trout often select taxa that are slow-moving and/or easily-detected (Carlisle and Hawkins 1998, Knapp et al. 2001, Schilling et al. 2009), the proportion of such prey in trout diets should decrease through time.

Although stomach contents provide valuable direct information on trout diet, they offer only a "snapshot" of recently consumed food-items, and different digestion rates among taxa may bias results (e.g., Kawaguchi et al. 2006). Additionally, the habitat where trout capture prey is of interest because it can potentially reveal changes in habitat use by prey species. Such habitat capture information is difficult to obtain directly (but see Biro et a. 2003) or via stomach contents. However, stable isotopes of carbon and nitrogen are frequently and effectively used to document the diets of predators and the habitats of their prey (littoral vs. pelagic: France 1995, Vander Zanden and Rasmussen 1999), as well as changes in food webs and prey communities induced by introduced fishes (Vander Zanden et al. 1999, Carey and Wahl 2010, Correa et al. 2012).

To investigate how trout affect the structure of food webs in mesotrophic boreal foothills lakes, I first obtained stomach samples over four field season (2007 – 2010) from trout in five lakes with a range of dates of first stocking (1950 – 2007). Using a variety of stable isotope analyses—including mixing models (Phillips and Greg 2001), distance metrics (Layman et al 2007) and circular statics (Schmidt et al 2007)—I then compared food webs among i) a suite of stocked and unstocked lakes, and ii) within a single lake before and after stocking relative to three unstocked control lakes. The combination of stomach content analysis and stable isotope analysis should provide insight into what ways, if any, trout have altered the food web structure of stocked lakes relative to unstocked lakes (Beaudoin et al. 1999). Because previous research in my central Alberta study lakes suggested that a general “fish effect” results in stronger changes to resident communities than a more specific “trout effect” (Schank et al. 2011, Holmes 2012), I included one fishless lake in my analyses.

I predicted that trout diet in newly stocked lakes will be dominated by large, conspicuous prey items whereas smaller, less conspicuous prey items would predominate in lakes with longer history of trout stocking. In light of previously-documented habitat shifts within my study lakes (Hanisch et al. 2012), I also predicted that food webs of stocked lakes would be more strongly connected to littoral-based carbon (e.g., reflecting use of littoral macrophyte refugia) than webs of unstocked lakes. Similarly, the food web of my before/after lake (Fiesta Lake) should show a shift toward more littoral sources of carbon after stocking relative to unstocked control lakes. However, I did not expect quantitative stable isotope analysis to reveal differences in the larger-scale trophic structure of food webs between stocked and unstocked lakes (e.g., no differences in total niche area, species packing, etc.; Layman et al. 2007) because trout were expected to show a high level of trophic redundancy with native fishes and therefore have similar effects on food webs (e.g., Hanisch et al. 2012). Finally, I predicted that the trophic structure of food webs in fish-bearing lakes (unstocked and stocked) would be more similar to each other than to the web of the fishless lake.

Methods

Study Area

I sampled a suite of ten lakes in the boreal foothills of western Alberta, near the towns of Rocky Mountain House (52°22′39″N, 114°54′ 37″W) and Caroline (52°5’36’’ N and 114°44’22’’ W). All lakes were small, moderately deep, and mesotrophic, containing dense beds of littoral macrophytes, primarily *Potamogeton* and *Nuphar* (Table 2-1). One lake was fishless, and the remaining nine contained natural populations of small-bodied fishes. Five of the fish-bearing lakes were stocked with trout (Brook, Rainbow (*Oncorhynchus mykiss*), and/or Brown Trout (*Salmo trutta*); date of first stocking, 1950 - 2007), four were unstocked, and one lake, Fiesta Lake, was unstocked for 2 years (2006 – 2007) and stocked with Rainbow Trout for 2 years (2008 – 2009; Table 2-1). Ironside Pond and Fiesta Lake were first stocked in 2005 and 2007, respectively, and are considered “recently-stocked lakes;” Beaver Lake was first stocked in 1999, and Mitchel and Strubel lakes were first stocked in 1950. These three lakes are considered "long-stocked lakes."

Study Designs

For my investigations of trout diet, I sampled trout from five stocked lakes that varied in date of first stocking from 1950 to 2007 (Table 2-1). Stomach content samples were obtained from Rainbow Trout during summer months (May – September) in 2007 – 2010 from Ironside, Mitchell and Strubel lakes, and in 2008 – 2010 from Fiesta and Beaver lakes. Data from Yellowhead Lake were used in the CI analysis of food webs; however, all analyses of trout diet are restricted to the four lakes that contain Rainbow Trout (Table 2-1).

I used two study designs to investigate the effects of trout on native food webs of boreal foothills lakes. A "Control-Impact" (CI) design compared the food webs of four stocked lakes, four unstocked lakes, and one fishless lake over 4 years (2006 - 2009). Yellowhead Lake is stocked with Brook Trout. Stable isotope samples were not taken from Beaver Lake, and it is not included in analysis. Second, I used a "Multiple Before-After-Control-Impact" (MBACI) design (Downes et al. 2002) to assess effects of trout on food webs in Fiesta Lake relative to three unstocked control lakes (DL, GP, TE; Table 2-1), 2 years before (2006 – 2007) and 2 years after (2008 – 2009) Fiesta Lake was stocked with trout.

Field Sampling

Trout were captured by angling, anesthetized in a solution of clove oil (eugenol) and water, and stomach contents were obtained with gastric lavage (Light et al. 1983).

Stomach contents were stored on ice in the field and frozen later in the day for laboratory analysis. A tissue sample (either dorsal muscle or caudal fin clip; Hanisch et al. 2010) was also taken from each fish, stored on ice in the field, and frozen for future stable isotope analysis.

Littoral invertebrates were collected from all study lakes, with the exception of Beaver Lake. Four lakes included in BACI analysis (DL, GP, TE, and FI) were sampled 2006 – 2009, and the full suite of lakes included in CI design were sampled in 2006 – 2007 (Table 2-1); all sampling occurred in July and August. At least six random locations were visited in each lake and sampled with a 500 µm littoral sweep net. At each location, the net was placed approximately 5 cm into the lake sediments and swept quickly along the contour of the lake bottom and up through littoral macrophytes. Invertebrates— typically less abundant, large bodied taxa such as dytiscids and leeches—were also collected opportunistically in each lake. I collected both known prey items of trout as well as organisms not commonly consumed by trout, because the taxa directly orindirectly affected by trout are not always predictable, *a priori* (Hanisch et al. 2013). Ialso used a 250 µm vertical tow net to collect zooplankton from the deepest point of each study lake. Zooplankton and littoral invertebrates (sorted to family level) were held in filtered lake water for 24 hours to allow invertebrates to void their guts. Subsequently, all invertebrates were frozen and held at -20C until processed for stable isotope analysis.

To sample small-bodied fishes, three to six Gee-type minnow traps were set at 0.5 – 3 m for 2 – 24 hours, depending on typical catch rates in each lake. After capture, fishes were euthanized, frozen, and held at -20C until processed for stable isotope analysis.

Stomach Content Processing

Stomach contents were identified to the lowest feasible taxonomic level (LFTL), typically family or genus; for partial organisms, I used discrete body parts (legs, head capsules, etc.) to estimate the minimum number of organisms for each LFTL, and that number was added to the total for the LFTL in each trout stomach. Length, excluding cerci, tails or other appendages, was measured for the first 20 individuals of each LFTL from each trout stomach. For gastropods, shell length was taken. Only whole individuals were measured, and a lake*year average of each LFTL was calculated and assigned to a LFTL from stomachs lacking measureable individuals. I used length-to-mass relationships to generate biomass (dry weight) estimates for each trout stomach sample (Wrona, 1982; Leeper & Taylor, 1998; Benke et al., 1999; Baumgärtner & Rothhaupt, 2003, P. Venturelli, University of Minnesota, pers. comm).

For each LFTL in trout diets, I calculated Frequency of Occurrence (FO), Percentage by Abundance (PA) and Percentage by Biomass (PB) for each lake*year. I used these three metrics to calculate the relative importance (RI) of each LFTL to trout diet in each lake*year with the following formula, where AI_a= FO + PA + PB of each LFTL *a* (George and Hadley 1979).

$$RI_a = \frac{AI_a}{\sum_{a=1}^{n} AI_a}$$

Relative Importance was calculated for two categories of trout sizes: $\leq$300 mm and > 300 mm, as 300 mm was the median length of trout captured. Total number of trout stomach analyzed ranged from 16 – 138 across size classes in each lake*year. Only lake*years with more than five stomach content samples per size class were included in RI and stable isotope analysis.

Stable Isotope Processing

Invertebrate samples and trout fins or muscle were washed in distilled water. For small-bodied fishes, white muscle tissue was dissected from frozen fishes. Fish tissue and whole invertebrates were then transferred to scintillation vials and lyophilized for 24 hours, homogenized into a powder and weighed (2.0 ± 1.0 mg) into tin capsules. For smaller-bodied invertebrates, ca. 3-10 dried individuals were pooled for one sample. One bulk zooplankton sample was submitted for each lake to represent the isotope signatures of pelagic habitats; three were submitted from Ironside Pond to generate an estimate of variability. My goal was to submit two to five samples of each LFTL in each lake*year. This was achieved for approximately 62% of LFTL's across all lake*year; the remaining taxa were represented by 1 sample.

Stable Isotope Analysis

Samples were submitted to the University of Saskatchewan Department of Soil Sciences for stable carbon and nitrogen isotope analysis. Samples were processed with an ANCA G/S/L elemental analyzer coupled to a Tracer/20 mass spectrometer (Europa Scientific, Crewe, UK). Results are presented in δ notation, where $\delta^{15}N$ or $\delta^{13}C$= $[\{R_{sample}/R_{standard} - 1\}]$ X 1000 and R= $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The international reference standards are PeeDee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The internal reference was egg albumin, which had a SD (n= 335) of 0.080 for δ^{15}N and 0.14 for δ^{13}C.

When using stable isotopes to infer trophic relationships, it is necessary to correct the δ^{13}C signatures of samples with high lipid content. Lipids are often significantly depleted in ^{13}C (DeNiro and Epstein 1977); thus, samples with a high percentage of lipids can have biased δ^{13}C signatures when compared to organisms with low lipid content (Post et al. 2007). I used C:N ratios (which are correlated with lipid content) to calculate corrected δ^{13}C signatures for all samples (Post et al. 2007). Site-specific differences in ^{15}N/^{14}N ratios, often due to anthropogenic activity in watersheds, can also introduce biases into comparisons of food web metrics (Cabana and Rasmussen 1996). To avoid these biases, I subtracted the lake-specific, average δ^{15}N signatures of snails (primarily adult lymnaeid snails) from the δ^{15}N signature of all samples from each lake (Cabana and Rasmussen 1996). I only ran stable isotope analysis on trout captured in their second (or later) summer after stocking, to avoid stable isotope signatures reflective of the hatchery diet. This resulted in small (< 300 mm) trout being underrepresented or absent from some lake*years.

Stable Isotope Analysis of Trout Diet

I used linear regression to determine if δ^{15}N or δ^{13}C signatures changed with trout length in each year of sampling. δ^{15}N is representative of trophic position (higher values indicate higher trophic position) and δ^{13}C is representative of foraging location (more negative values indicate pelagic foraging and more positive values littoral foraging; France 1995). Additionally, Bayesian ellipses were generated in δ^{13}C:δ^{15}N space to visualize the niche area for trout populations in each lake year using the package SIBER (Jackson et al. 2011) in the statistical program R. Bayesian ellipses were used, as they are less sensitive to differences in sample sizes than are non-Bayesian methods. The area of a trout population's stable isotope niche is dependent, in part, on the diversity of prey consumed; thus, a larger niche area indicates that trout are consuming prey across a wider range of habitats and/or trophic levels (e.g., Layman et al. 2011). I then compared niche area as inferred by Bayesian ellipses between recently stocked lakes and lakes with a longer history of stocking with repeated measures ANOVA, with year repeated within lakes.

Characterization of Food Webs

To represent visually the food webs of my lakes, I created stable isotope biplots in $\delta^{13}C$:$\delta^{15}N$ space (Appendix 2-A). Food webs were inspected visually to detect patterns in food-web structure (e.g., Beaudoin et al. 2001) and to inspect habitat use (i.e., $\delta^{13}C$ signatures) and trophic position ($\delta^{15}N$) of food web members.

I used mixing models to determine the percentage of littoral-derived carbon in populations of dace (*Chrosomus*), the only forage fish taxon present in all fish-bearing study lakes. Two end-member mixing models were conducted in IsoError (Phillips and Gregg 2001), with lymnaeid snails as the littoral end member and zooplankton as the pelagic end member (e.g., Syväranta et al. 2011). No zooplankton samples were collected in 2006, so I used lake average zooplankton values from subsequent years in 2006 mixing models. Dace were sampled in 2006 and 2007 from IR, MI, ST and YE and 2006 – 2009 in all other lakes (see Table 2-1 for lake abbreviations). I ran similar mixing models on a composite category of mobile, predatory invertebrates that are consumed by trout, a group comprising Aranaea, Anisoptera, Belostomatidae, Dytiscidae, Gerridae, Gyrinidae, and Notonectidae. For three lake*years, (2006 and 2007 in Mitchell Lake and 2006 in Strubel Lake), the stable carbon isotope signature of mobile invertebrates fell outside of one of the two end members; these years were omitted from analysis. I used repeated measures ANOVA's as described above to determine if percentage of littoral carbon in forage fish or mobile invertebrates (reflecting use of inshore refugia) differed between treatments or after stocking in Fiesta Lake relative to control lakes. Percentage data were arcsine-square root transformed prior to analysis.

I also used circular statistics (Batschelet 1981; Schmidt et al. 2007) to investigate if shifts occurred in the prey community of Fiesta Lake after trout stocking compared to three unstocked control lakes. Stable isotope biplot data were converted to circular data, and I calculated the mean angle and magnitude of change in stable isotope signatures for each taxon in each lake for the 2 years after Fiesta was stocked relative to the 2 years before stocking. If shifts in carbon signatures occurred, indicating a change in habitat use, the angles of species' vectors would be oriented toward 90^{o} (enriched in ^{13}C; littoral) or 270^{o} (depleted in ^{13}C; pelagic). If shifts in nitrogen signatures (i.e., trophic position) occurred, the angle of species vectors would be oriented toward 0^{o} (higher trophic position) or 180^{o} (lower trophic position). I also plotted the mean angle of change for the entire community of each lake. All analyses with circular statistics were conducted in Oriana (version 4.0).

I used distance metrics proposed by Layman et al. (2007) to quantify food web structure in stocked, unstocked, and fishless lakes. These metrics use $\delta^{13}C$ and $\delta^{15}N$ of food web members to define a “trophic niche space” for each food web in $\delta^{13}C$ vs. $\delta^{15}N$ space. $\delta^{15}N$ Range (NR) is the distance between the two food web members with the highest and lowest $\delta^{15}N$ signatures, and $\delta^{13}C$ Range (CR) measures the distance between the two food web members with the highest and lowest $\delta^{13}C$ signatures. Total Area (TA) is the area of the convex hull that encloses all members of a food web and is a proxy for total trophic diversity in a food web. Mean Distance to Centroid (CD) is the average Euclidean distance of each member of a food web to the centroid of the food web, representing average level of trophic diversity. Mean Nearest Neighbor Distance (MNND) is the mean Euclidean distance of each food web member to its nearest neighbor, a measure of the packing of members within a food web. Finally, Standard Deviation of Nearest Neighbor Distance (SDNND) represents the “evenness” of the distribution of species in trophic niche space.

I compared these distance metrics among fishless, unstocked, and stocked lakes and also before and after trout were stocked in Fiesta Lake relative to my three control lakes. Comparing food webs using convex hulls has been criticized when different numbers of taxa are involved, as food webs with more taxa tend to have larger convex hulls (Jackson et al. 2011). I therefore used SPSS to bootstrap (n= 25) all lake-year food webs, which ranged from 11 to 20 members, to a common number (11 taxa). Distance metrics were generated in R (version 2.15.1) with the package SIAR. I used repeated measures ANOVA’s in SPSS (version 20.0 for Mac) to compare the metrics of stocked (n= 4), unstocked (n= 4), and fishless (n= 1) lakes. Year was repeated within lakes, with lakes being sampled for 2 - 4 years. Repeated measures ANOVA’s were also used to assess statistical interactions between time (before vs. after stocking) and treatment (stocked vs. unstocked).

Because of the small sample sizes inherent when conducting whole-lake studies, I considered results significant at $p< 0.05$ and marginally significant at $0.05 < p < 0.10$ for all statistical tests.

Results

Trout Diet

For all lakes and for both small (< 300 mm) and large (> 300 mm) fish, trout diets were dominated by six or seven prey categories, comprising ca. 50 – 80 % of the diet by RI (Figure 2-1a). Chironomidae and Cladocera were typically the top two prey items for large and small trout across all lakes, comprising collectively 20 – 50 % of diet by RI (Figure 2-1a). Other predominant prey items included Anisoptera, Corixidae, forage fish, and planorbid snails.

Over the duration of the study, the identity of the top taxa typically did not differ between recently stocked (FI, IR) and long-stocked lakes (BE, MI, ST) for either large or small trout. However, when comparing yearly averages (Figure 2-1 b-f), a few differences were apparent. Although chaoborids were not a top prey item in any long-stocked lake, they were a top prey item for large trout in four of four years in Ironside Pond and two of three years in Fiesta Lake, the two recently stocked lakes. Across all lake*years, trout in recently stocked lakes consumed an average of 6 chaoborids per trout, whereas trout in long-stocked lakes consumed only 0.6 chaoborids. Similarly, trichopterans were also a top prey item in all lake*years for the two recently-stocked lakes but not for long-stocked lakes, with trout from recently-stocked lakes consuming an average of 1.1 and 0.2 trichopterans per trout, respectively, in recently and long-stocked lakes.

There was no general relationship between trout length and stable isotope signatures across treatments or lakes. Only three of eight regressions between length and $\delta^{13}C$ signatures were significant, with $\delta^{13}C$ signatures increasing with length in two lake*years (Fiesta 2009; Mitchell 2007) and decreasing with length in another (Ironside 2008 Figure 2-2). Similarly, only three of eight lake*years showed significant relationships between $\delta^{15}N$ signatures and length, with $\delta^{15}N$ signatures increasing with length in two lakes*years (Ironside 2008, Strubel 2008) and decreasing in one (Mitchell 2008). There was also no difference in niche area between recently-stocked lakes and long-stocked lakes ($F_{1,2}$= 0.008, p= 0.94; Figure 2-3). For all but one lake (long-stocked Strubel), there was a large degree of overlap between ellipses for the two sampling years.

Food Webs

Food webs in all lakes showed a relatively consistent trophic structure. In unstocked lakes, forage fishes generally occupied the highest trophic position, although in a few lake*years, positions of leeches were similar (Appendix 2-A). In stocked lakes, trout typically had the highest $\delta^{15}N$, but often overlapped with forage fishes. Predatory or parasitic invertebrates, such as dytiscids, leeches, notonectids, odonates, mites, and aquatic spiders, typically exhibited intermediate $\delta^{15}N$ values, whereas primary consumers or detritivores, such as corixids, amphipods, and ephemeropterans, had the lowest $\delta^{15}N$ signatures. In my fishless lake, predatory invertebrates such as notonectids, leechs, spiders typically occupied the top of the food web (Appendix 2-A).

In unstocked lakes, $\delta^{13}C$ signatures were most negative for forage fishes and zooplankton and most positive for gastropods and amphipods (Appendix 2-A). Similar patterns were observed in stocked lakes, except that $\delta^{13}C$ signatures of forage fishes were generally more positive than in unstocked lakes. $\delta^{13}C$ signatures of trout and forage fishes generally overlapped in stocked lakes, with the exception of Pearl Dace and Fathead Minnow in the first year after stocking in Fiesta Lake. In fishless lakes, notonectids joined zooplankton in having the most negative $\delta^{13}C$ signatures.

There was no difference in the percentage of littoral carbon in forage fish tissue among treatments (Figure 2-4a; $F_{1,12.4}$= 0.005, p= 0.95). However, if Fiesta Lake is excluded from the analysis as a newly stocked lake and if Strubel Lake is excluded as the only stocked lake in which forage fishes were observed to be strongly pelagic (Hanisch unpublished data), the difference in percent littoral carbon in this subset of stocked and unstocked lakes was highly significant ($F_{1,\ 5.278}$= 27.95, p= 0.003), with the mean (± SE) values for stocked and unstocked lakes being 78 % ± 0.7 and 36.6% ± 0.3 littoral, respectively (a). The BACI results for Fiesta Lake vs. unstocked controls were marginally significant, with dace in Fiesta Lake showing a relatively stronger pelagic signal after stocking relative to control lakes (Figure 2-4b; $F_{3,5.2}$= 4.54, p= 0.065). There was no difference in the percentage of littoral carbon in mobile invertebrate taxa across the three treatments (Figure 2-4c; $F_{2,2.3}$= 0.068, p= 0.94) or after stocking in Fiesta Lake relative to the unstocked control lakes (Figure 2-4d; $F_{3,5.9}$= 0.474, p= 0.71).

Circular statistics did not reveal a food-web shift in Fiesta Lake toward more littoral sources of carbon. The mean angle of change (and 95% confidence interval) did not overlap 90º (i.e., a shift toward littoral habitat) for Fiesta or any of the control lakes after stocking relative to before stocking periods (Figure 2-5). The mean angle of change for all lakes generally occurred between or intersected 180º and 270º (i.e., slightly away from littoral habitat).

For food-web distance metrics, $\delta^{15}N$ range (NR) differed among lake types ($F_{2,9.32}$= 4.93, p= 0.035), with fishless lakes having a significantly smaller $\delta^{15}N$ range than either unstocked (p= 0.012) or stocked (p= 0.015) lakes (Figure 2-6). There was no difference between unstocked and stocked lakes (p= 0.86). $\delta^{13}C$ Range (CR) was marginally different among the three lake types ($F_{2,9.9}$= 3.80, p= 0.060; Figure 2-6), with unstocked lakes having a smaller CR than stocked lakes (p= 0.025). Although values for several of the other distance metrics were lower for fishless lakes, no differences were significant. For the BACI comparisons of distance metrics, most appeared to decrease in Fiesta Lake after stocking, but only MNND showed a marginally significant (p= 0.10) time* treatment interaction, decreasing in Fiesta Lake after stocking relative to the unstocked control lakes (Figure 2-7).

Discussion

Impacts of trout in stocked lakes were generally limited to a few marginally-significant effects on food web structure, and I documented few systematic differences in trout diet between recently stocked lakes and long-stocked lakes. Trout in all lakes consumed a variety of prey across all trophic levels, from gastropods to forage fishes. Some differences in trout diet were observed in recently-stocked lakes, including a higher levels of consumption of taxa susceptible to fish predation, e.g., chaoborids and trichopterans (Drouin et al. 2011, Schilling et al 2009). Trichopterans frequently dominate diet of both native (Angradi and Griffith 1990) and non-native (Whiting et al.

2005) trout and chaoborids often decrease dramatically in abundance after trout are stocked (Schilling et al. 2009); thus, their decreased abundance from trout stomach in long-stocked lakes is not necessarily surprising. However, other taxa typically susceptible to trout predation were not observed to decrease in abundance within trout stomachs in long-stocked lakes. For example, in low productivity lakes (Maine, USA), anisopterans and notonectids were significantly impacted by trout presence (Schilling et al. 2009). In my study system, these taxa both saw their highest RI in lakes that had been stocked since 1950 (Mitchell and Strubel lakes). Schilling et al. (2009) detected significant impacts of Brook Trout on abundance of taxa within 3 years of first stocking, and lakes with the longest history of trout presence (> 40 years) showed the strongest impacts. In contrast, such strong negative effects were not seen in my productive lakes, even in those with > 60 years of trout presence (see also Nasmith et al. 2012, Hanisch et al. 2013). Instead of the predicted decreases in RI of large, vulnerable prey taxa through time, trout diets appeared more lake-specific than treatment-specific (e.g., consistently high levels of forage fish consumption in Fiesta and Strubel lakes versus lower levels in Ironside and Mitchell lakes).

As in other systems where Rainbow Trout have been introduced (e.g., Clarke et al 2005, Whiting et al. 2014; Rowe 1984; Brandt 1986; Shelton et al. 2015), trout diet in my study lakes included both aquatic invertebrates and fish. Typically, Rainbow Trout show predictable increases in piscivory with size (e.g., Clarke et al. 2005), however, such a shift was not routinely observed in my lakes. Trout size also seemed to have little effect on overall trout diet or foraging location, as evidenced by the general lack of significant relationships between trout length and $\delta^{13}C$ and $\delta^{15}N$ signatures. It is likely that the primary growth-related diet shifts occur at lengths smaller than the sizes typically stocked in my lakes (ca. 250 mm) (Whiting et al. 2014), although, diets of both small and large size classes were typically dominated by cladocerans and chironomids. Because chironomids and cladocerans are among the most abundant taxa—both numerically and by biomass—within most of my study lakes (Nasmith et al 2012, Hanisch et al 2013), it appears that both large and small trout are responding to their abundance (e.g., Bres 1986), rather than keying in primarily on large-bodied or vulnerable taxa (e.g., Angradi and Griffith 1990), with the exceptions detailed above.

The overall food-web structure of study lakes was similar to other northern lakes, with native fishes and predatory invertebrates having the highest δ^{15}N signatures followed by herbivorous/detrivorous invertebrates (e.g., Beaudoin et al. 1999, Vander Zanden and Rasmussen 1999, Eloranta et al. 2015). There was a higher degree of overlap of δ^{15}N signatures across many taxa, and discrete trophic levels were not apparent in most isotope biplots, which is a signal of a relatively high degree of trophic omnivory in the food webs (Vander Zanden et al. 1999).

In most lake*years, trout appeared to occupy an isotope niche similar to forage fish, with slightly elevated δ^{15}N and overlapping δ^{13}C signatures. Food webs of stocked and unstocked lakes were generally similar in structure and both showed a strong linkage to littoral carbon. Because reliance on littoral habitat was documented behaviorally for small-bodied fish in my study lakes (Hanisch et al. 2012) and other lakes (e.g., Dupuch et al. 2009) after the introduction of trout, I had expected to see evidence of higher use of littoral habitat by forage fishes in stocked lakes. This was indeed the case for dace in three of five stocked lakes, however, there also were two clear examples where forage fishes relied on pelagic habit even in the presence of trout, and the percentage of littoral carbon in dace from Fiesta Lake actually decreased after trout stocking. Because both Common Loon (*Gavia immer*) and Osprey (*Pandion haliaetus*) were observed feeding frequently on Fiesta and Strubel lakes (Osprey only after stocking of Fiesta Lake), it is possible trout avoided surface pelagic areas and dace used this habitat as refuge in these two lakes (Post et al. 1998). However, it is unclear what combination of factors led to possible adoption of a littoral refuge in some lakes and a pelagic refuge in others, as piscivorous birds and littoral macrophyte beds were observed in all stocked lakes.

I uncovered even less evidence that trout affected habitat use of invertebrate taxa. Across the three treatments (fishless, unstocked and stocked lakes), mobile predatory invertebrate taxa exhibited comparably high (60% - 70%) levels of littoral-zone derived carbon. Although some taxa, such as notonectids, were caught offshore in tows for young-of-year fish in unstocked lakes (J. Hanisch, unpublished data), the inherent tendency of most conspicuous, mobile macroinvertebrates to inhabit the littoral zone likely provides refuge from trout predation (e.g., de Mendoza et al 2012) and negates the need for additional habitat shifts in the presence of trout. This pattern may also explain the absence of a community-wide shift in habitat use in Fiesta Lake towards additional sources of littoral carbon, as evidenced by circular statistics.

In many lake*years, forage fishes and stocked trout displayed overlapping stable isotope signatures, indicating that these fishes used similar resources despite large differences in body size. Indeed, stomach content analysis from dace in my study lakes showed considerable overlap with trout diet (Mee et al. 2013). Thus, if trout are using resources already exploited by high densities of forage fishes, trout may exert relatively small, additional predation pressure on the invertebrate prey base. Similar overlaps of fish species have been seen in boreal lakes between northern pike (*Esox lucius*) and native forage fishes (Beaudoin et al. 2001).

Distance metrics identified only a few differences in food web structure between fishless, unstocked, and stocked lakes. Not surprisingly, fish-bearing lakes had a larger range of nitrogen values (NR) than did the fishless lake, although this difference was not significant between stocked lakes and lakes with forage fishes only. Similar to other small productive lakes, fish in our lakes typically occupied a higher trophic position relative to most invertebrates, but large differences in trophic position were not documented among fish species, even among those of varying body sizes (e.g., small-bodied vs large-bodies fishes; Beaudoin et al. 2001). The Range of δ^{13}C values (CR) was also marginally higher in stocked lakes relative to other fish-bearing lakes. Trout had intermediate δ^{13}C signatures within most food webs, so their addition is not the direct cause of the increased CR. It is possible that indirect effects of trout have caused prey taxa to use a slightly larger range of basal energy sources in stocked lakes relative to unstocked lakes (e.g., Layman et al. 2007); indeed, forage fishes in some stocked lakes did show differences in littoral-derived carbon relative to unstocked lakes.

Because fish presence is known to affect invertebrate communities and food web structure in many systems (e.g., Zimmer et al. 2001, Schilling et al. 1999, Gonzalez-Bergonzoni et al. 2014), I was surprised at the overall lack of differences in food-web structure between fish-bearing and my fishless lake. Although a number of food-web metrics tended to differ in my fishless lake from those of fish-bearing lakes, these differences were generally not significant. Unfortunately, I only had two years of data for one fishless lake, which limited my statistical power. By expanding to three fishless lakes, Holmes (2012) found differences in planktonic microcrustacean and rotifer communities between fish-bearing and fishless lakes, and I suspect similar differences may exist in the littoral invertebrate communities between fishless and fish-bearing lakes in my study system.

Only one metric, Mean Nearest Neighbor Distance (MNND), was marginally different in Fiesta Lake after stocking relative to control lakes. MNND reflects the degree to which organisms are “packed” into a food web (Layman et al. 2007). Thus, a lower value for this metric after stocking could be evidence that trout constrained the foraging behaviour of food web members, “packing” them into a smaller trophic space. Visual examination of the food web of Fiesta Lake (Appendix 2-A m-p) does reveal a more densely-packed food web in the second year after stocking. However, there was no difference in MNND between my stocked and unstocked treatments, suggesting that this pattern may be a temporary one in Fiesta Lake (e.g., Hanisch et al. 2013).

The absence of either major long or short-term effects of trout presence on the food webs of stocked relative to unstocked lakes provides evidence that my productive lakes are able to absorb a new fish predator with few additional effects on food web structure. In some species poor, northern systems, fish presence can both increase food chain length (as seen in the present study) but also increase species packing and reduce the trophic diversity of a food web relative to fishless systems (Gonzalez-Bergonzoni et al. 2014). However, trout in my study lakes can be described as dietary generalists that can show relatively few differences in diet between recently-stocked and long-stocked lakes (see also Hanisch et al. 2013), and also appeared to show a relatively large degree of trophic overlap with native fishes. Trophic redundancy and omnivory can dampen potential effects of trophic cascades (Polis et al. 2000, Eriksson et al., 2011) and add stability to a food web (Fagan 1997). Thus, the relatively complexity of food webs of my productive lakes, combined with the omnivory of trout and some degree of trophic redundancy with native fishes have likely moderated the potential effects of this introduced predators (e.g., Polis et al. 2000).

These results are also consistent with limited effects of stocked trout documented on macroinvertebrates (Wissinger et al. 2006, Nasmith et al. 2011, Hanisch et al. 2013), fish (Nasmith et al. 2010), and amphibian communities (Schank et al. 2011) in other productive lakes. Complex habitat, such as macrophyte beds, can often dampen negative effects of introduced species (e.g., Moyle and Light 1996) and can promote the coexistence of antagonistic species (Langellotto and Denno 2004). Furthermore, food web structure and relative population sizes of invertebrates and forage fishes were quite variable among lakes and among years within a lake (Nasmith et al. 2010, Schank et al. 2011, Hanisch et al. 2013). Against this background, trout stocking may have no greater effect than other stochastic events, such as winterkill (e.g., Hanisch et al. 2013) and other small lake-to-lake differences.

Conclusions

Stocked trout had minimal impacts on the overall structure of food webs relative to unstocked lakes and after stocking in a focal lake. Although a few vulnerable taxa (e.g., chaoborids, trichopterans) were consumed in lesser quantities in long-stocked lakes, others (e.g., anisopterans, notonectids) were still consumed frequently in long-stocked lakes. Forage fishes appeared to show the greatest response to trout presence, either increasing or decreasing use of littoral habitat, depending on the lake. Many invertebrate taxa contained high percentages of littoral carbon across all lakes; primarily inhabiting the littoral zone should allow them to benefit from the refuge provided by dense macrophyte beds present in my lakes and marked habitat shifts would not be required. Stocking productive lakes such as those in this study may thus provide a viable alternative to the long-standing practice of stocking alpine and oligotrophic lakes, where effects of trout are generally strong and negative (Dunham et al. 2004, Eby et al. 2006).

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Table 2-1. Study design, limnological characteristics, native small-bodied fish communities, and estimated trout stocking densities for ten boreal foothills lakes. For treatment, U= unstocked, U/S= unstocked then stocked (see methods), S= stocked, and FL= fishless. For fish communities D= dace (*Chrosomus spp.*), F= Fathead Minnow (*Pimephales promelas*), B= Brook Stickleback (*Culaea inconstans*), P= Pearl Dace (*Margariscus margarita*), I= Iowa Darter (*Etheostoma exile*)

Lake (Abbreviation)	**Beaver (BE)**	**Dog Leg (DL)**	**Fiesta (FI)**	**Gas Plant (GP)**	**Teal (TE)**	**Ironside (IR)**	**Mitchell (MI)**	**Strubel (ST)**	**Yellowhead (YE)**	**Dog Paw (DP)**
Treatment	S	U	U/S	U	U	S	S	S	S	FL
Years Sampled	2008 - 2010	2006 - 2009	2006 - 2010	2006 - 2009	2006 - 2009	2006 - 2010	2006 - 2010	2006 - 2010	2006 - 2007	2006 - 2007
Fish species	D,F,B	D,F,B	D,F,B,P	D,F,B	D,F,B	D	D	D,S	D,F,B,P,I	—
Trout stocking density (fish ha^{-1})	110 - 112	—	83 - 288	—	—	76 - 76	67 – 80 / 93 – 267**	734 –768	743***	—
Date of first stocking	1999	—	2007	—	—	1977–1987; 2005	1950	1950	1983	—
Forage fish density (fish ha^{-1})	Not estimated	3240 - 8738	7362 - 17876	3614 - 4402	3354*	17766*	3419 - 8178	2335*	2619	—
Surface area (ha)	31.2	6.7	6.6	17.5	16.6	3.3	15	25.9	24.5	3
Secchi depth (m)	2.4 - 3.9	1.9 - 2.2	1.9 - 2.7	1.3 - 1.7	2.0 - 2.3	3.8 - 3.9	3.1 - 3.5	5.5 - 6.0	2.5 - 2.7	1.8 - 2.1
Maximum depth (m)	10	5	7.1	3.9	9	13	6	12.5	12.2	6
Chl a (μg L^{-1})	2.3 - 8.4	4.5 - 14.5	1.9 - 12.5	6.3 - 11.3	3.4 - 10.7	1.9 - 2.9	2.7 - 3.3	0.8 - 1.7	3.0 - 4.9	6.0 - 6.9
Total phosphorus (μg L^{-1})	33 - 34	30.5 - 52.4	24.5 - 37.4	39.2 - 51.3	23.7 - 32.6	13.0 - 15.7	14.5 - 15.7	7.5 - 7.7	15.5 - 15.9	36.2 - 46.5
Total nitrogen (μg L^{-1})	498 - 606	829 - 1030	612 - 929	744 - 1096	680 - 898	634 - 658	930 - 1072	480 - 523	741 - 754	967 - 1055
pH	6.6 - 7.0	6.9 - 7.7	7.6 - 7.8	7.4 - 7.9	6.7 - 7.8	7.7 - 8.1	7.3 - 7.2	8.3 - 8.3	7.9 - 8.4	6.7 - 7.4

Range of yearly means
*2006 only
** Brown Trout / Rainbow Trout
*** 2008 only

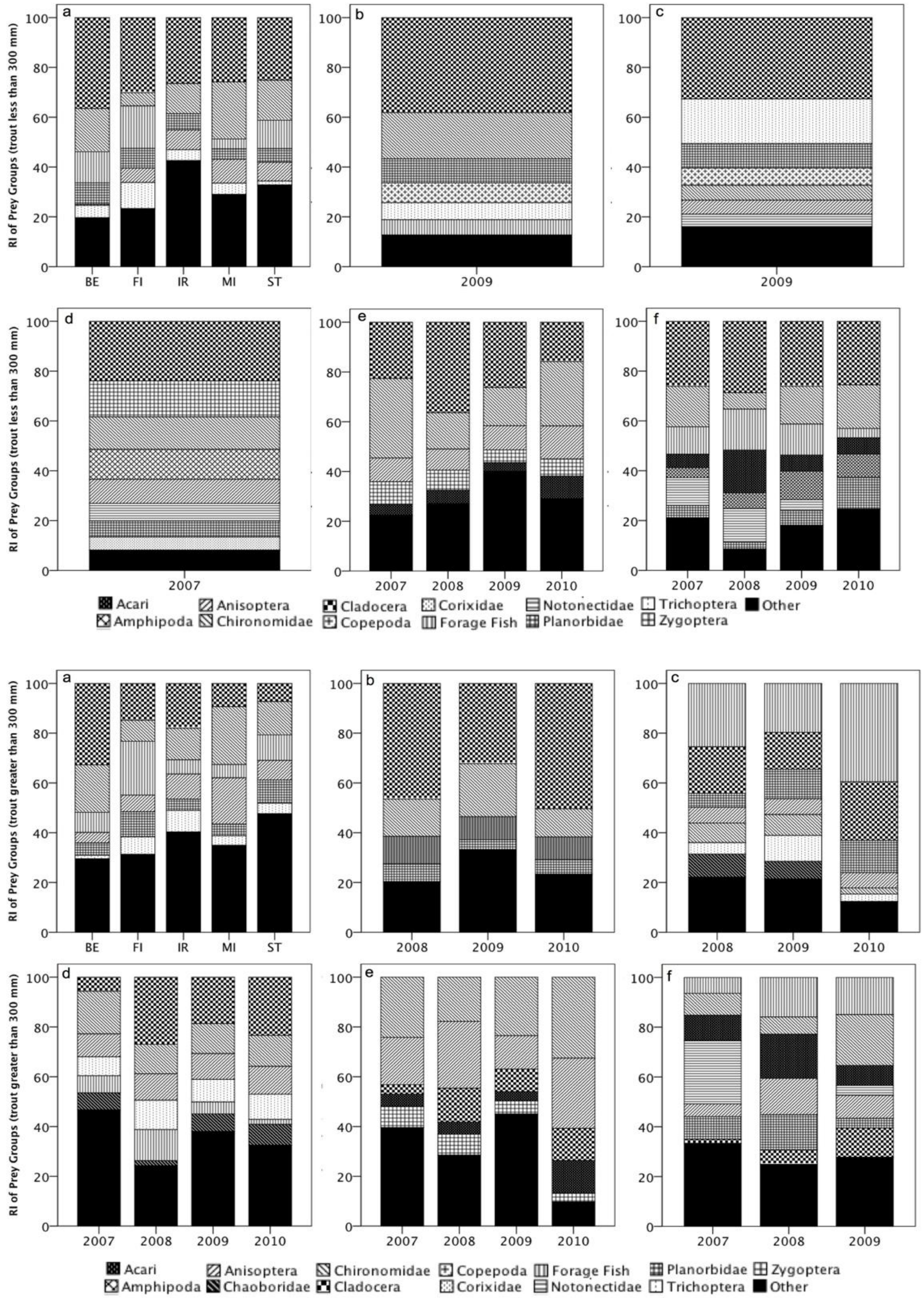

Figure 2-1. Index of relative importance for prey of small trout < 300 mm (top panel) and large > 300 mm (bottom panel) trout across all lake*years for each study lake (a) and for each year for Beaver Lake (BE, b), Fiesta Lake (FI, c), Ironside Pond (IR, d), Mitchell (MI, e) and Strubel Lake (ST, f). Sample sizes ranged from 16 – 138 across size classes in each lake*year. No small trout were caught in some lake*years.

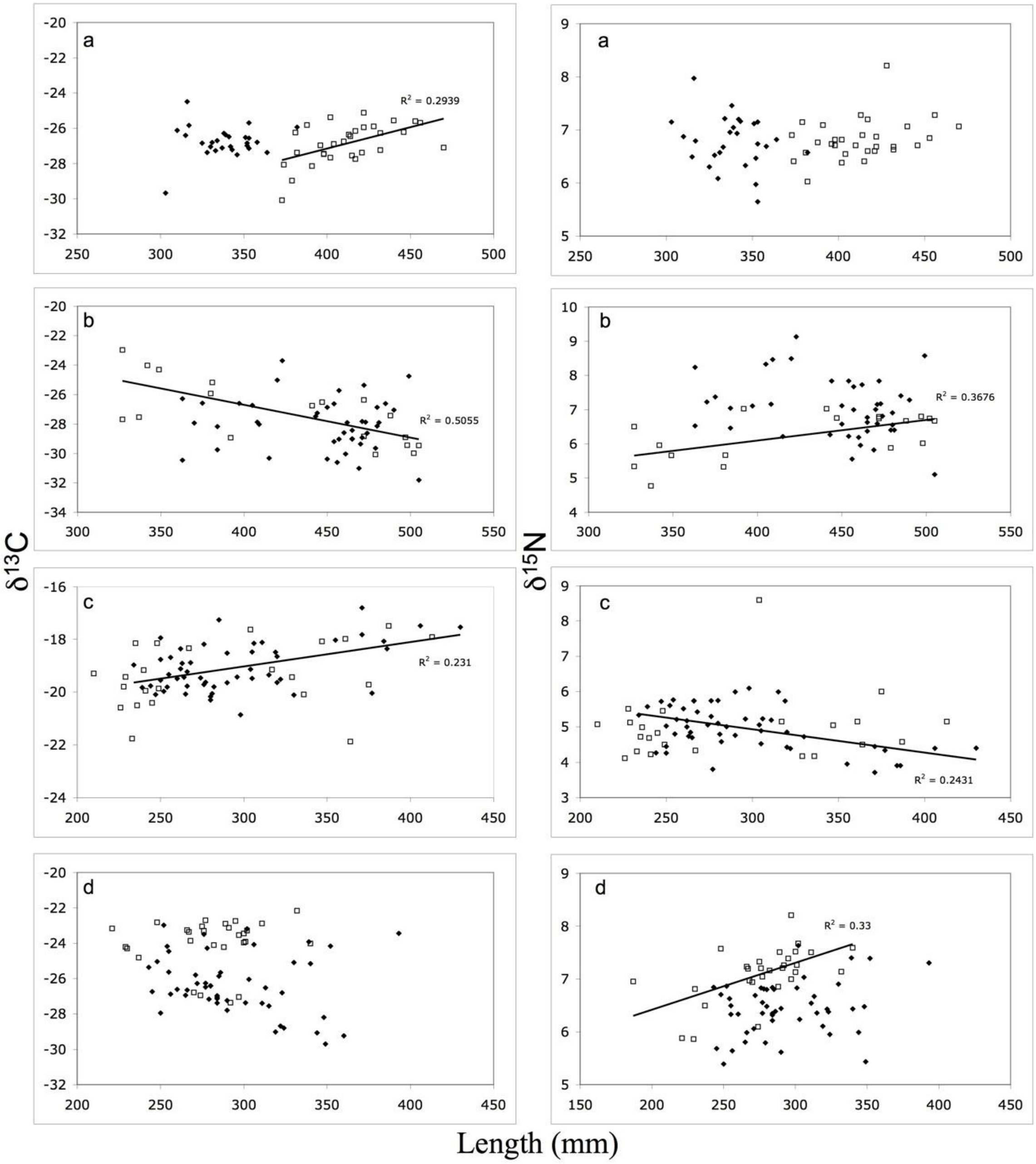

Figure 2-2. Plots of trout length vs $\delta^{13}C$ (left) and $\delta^{15}N$ signatures (right). For Fiesta (a) and Ironside (b) lakes, filled diamonds are the first post-stocking year (2008 and 2007, respectively) and open squares are the second year post-stocking (2009 and 2008, respectively). For long-stocked Mitchell (c) and Strubel (d) lakes, dark diamonds are 2007 and black squares are 2008. Regression lines and r^2 values are displayed for significant ($p< 0.05$) regressions. Sample sizes ranged from 5 – 52, depending on lake*year.

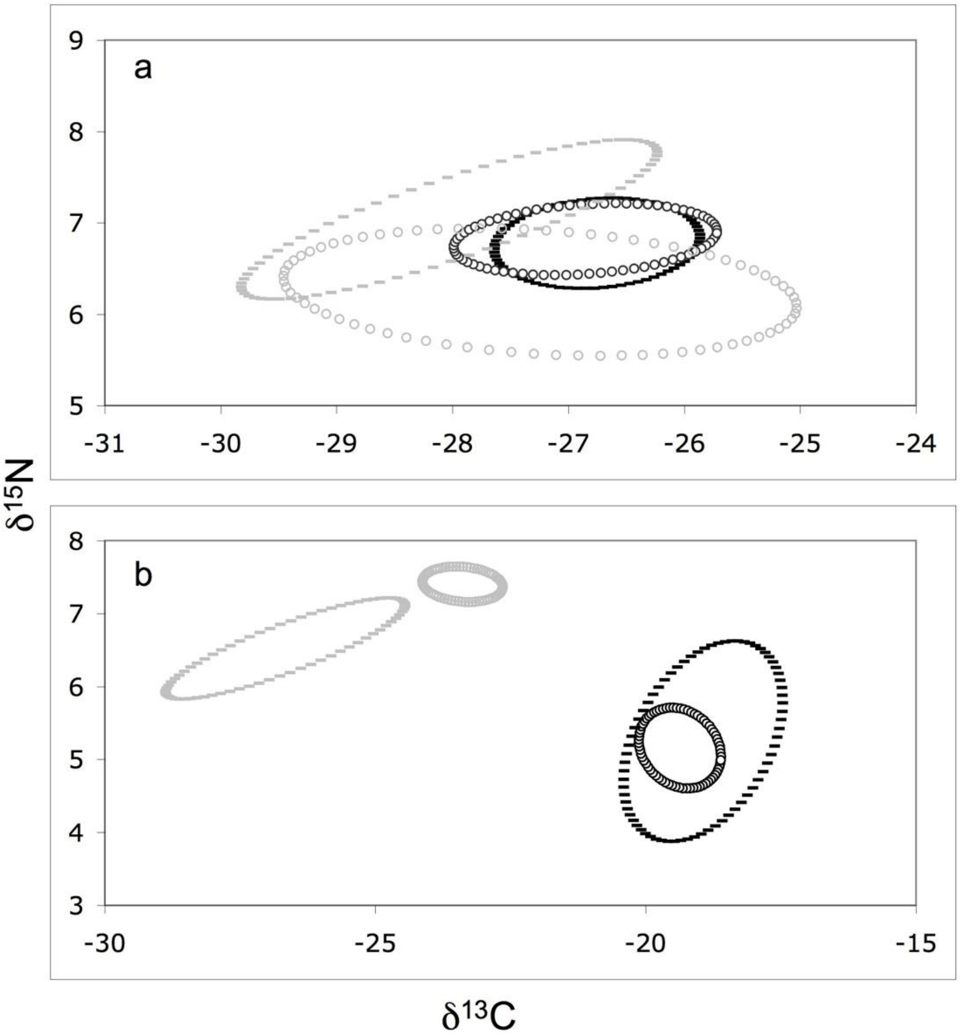

Figure 2-3. Stable isotope ellipses for large trout (> 300 mm) in (a) recently stocked lakes (Fiesta, black; Ironside; grey) and (b) lakes with a long history of stocking (Mitchell, black; Strubel, grey). For (a), lines represent the first post-stocking year (2008 and 2007, respectively) and open circles represent the second year post-stocking (2009 and 2008, respectively). For (b), lines represent 2007 and open circles represent 2008.

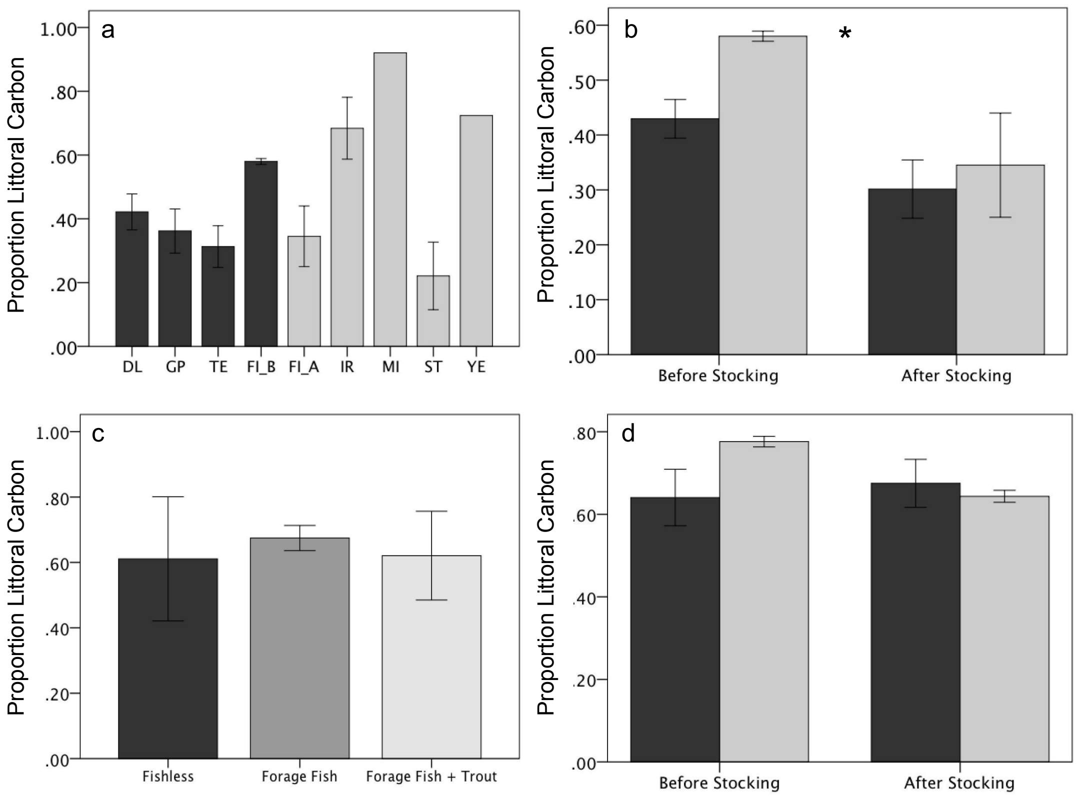

Figure 2-4. Results of mixing models to estimate the mean (±SE) proportion of littoral carbon in forage fishes (a,b) and mobile, predatory invertebrates (c,d) in different lake types. (a) Results for each lake, with unstocked lakes in dark bars and stocked lakes in light bars. See Table 2-1 for abbreviations. Fiesta Lake was considered unstocked in 2006 and 2007 (FI_B) and stocked in 2008 and 2009 (FI_A). (b) BACI results for control lakes (dark bars) and Fiesta Lake (light bars). (c) Results for each treatment (fishless: dark bars, unstocked: dark gray bars, stocked: light gray bars). (d) BACI results for control lakes (dark bars) and Fiesta Lake (light bars). *:$p < 0.10$

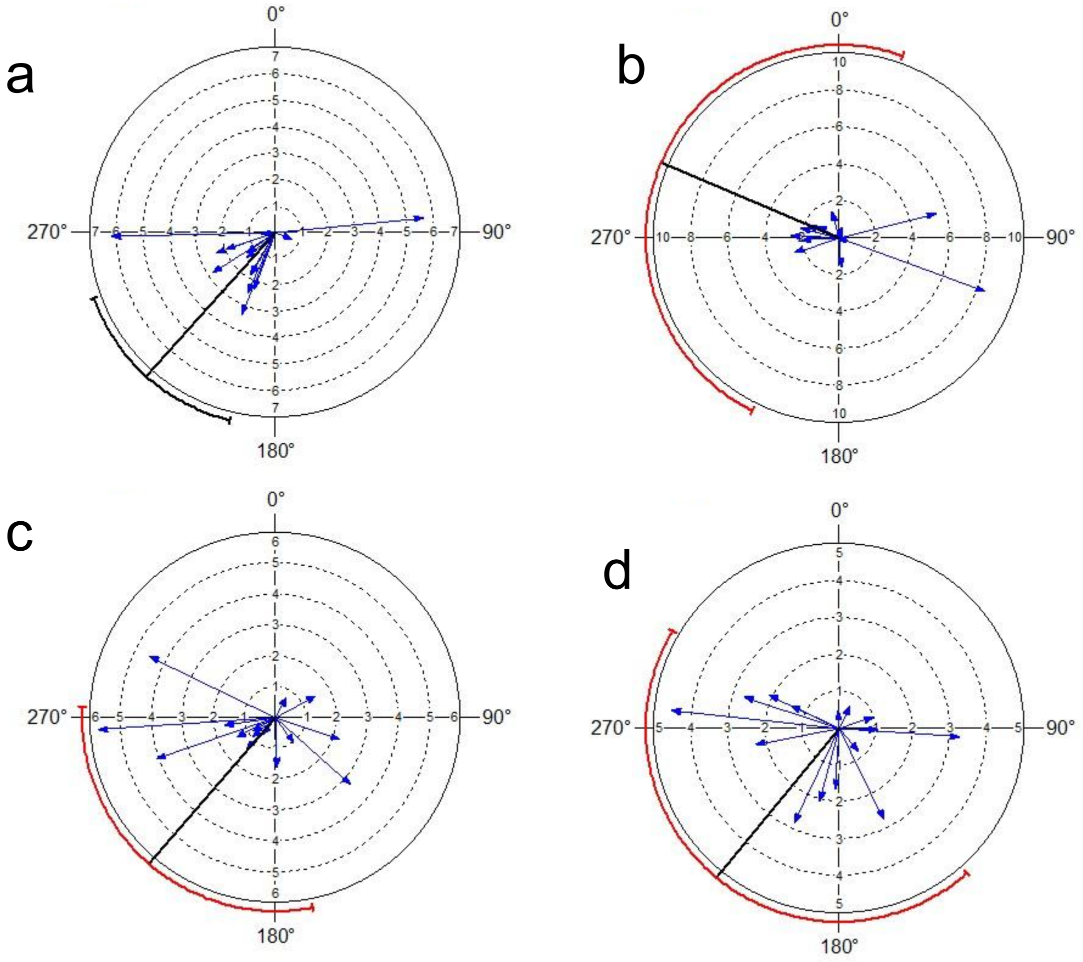

Figure 2-5. Circular graphs indicating angle (arrows) and magnitude (length of arrows) of change in isotope signatures for taxa in the "after stocking" time period relative to the "before stocking" time period in Dog Leg (a), Teal (b), Gas Plant (c) and Fiesta (d). Also represented are the mean angle of change (long dark line) and 95% confidence interval of the angle (light arc outside each circle). Shifts in carbon signatures (e.g., habitat use) are indicated by species vectors oriented toward 90 degrees (enriched in ^{13}C; littoral) or 270 degrees (depleted in ^{13}C; pelagic). Shifts in nitrogen signatures (e.g., trophic position) are indicated by species vectors oriented toward 0 degrees (higher trophic position) or 180 (lower trophic position).

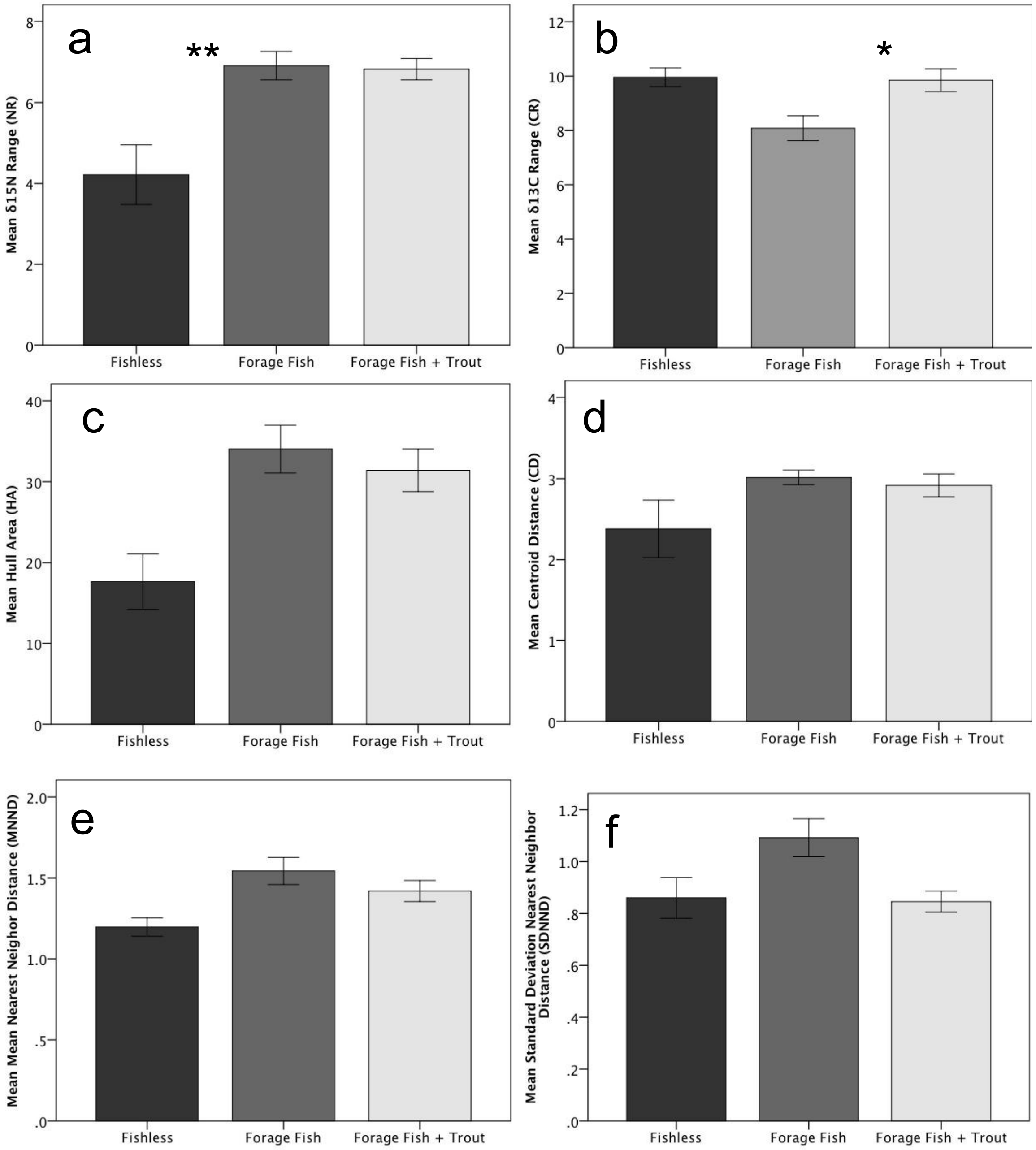

Figure 2-6. Mean (± SE) stable isotope distance metrics (a, NR; b, CR; c, HA; d, CD; e, MNND; f, SDNND) for fishless (dark bars), unstocked but fish-bearing (dark grey bars), and stocked lakes (light grey bars). Means are generated from lake*year means for each treatment. For difference between means, **: $p < 0.05$; *: $p < 0.10$.

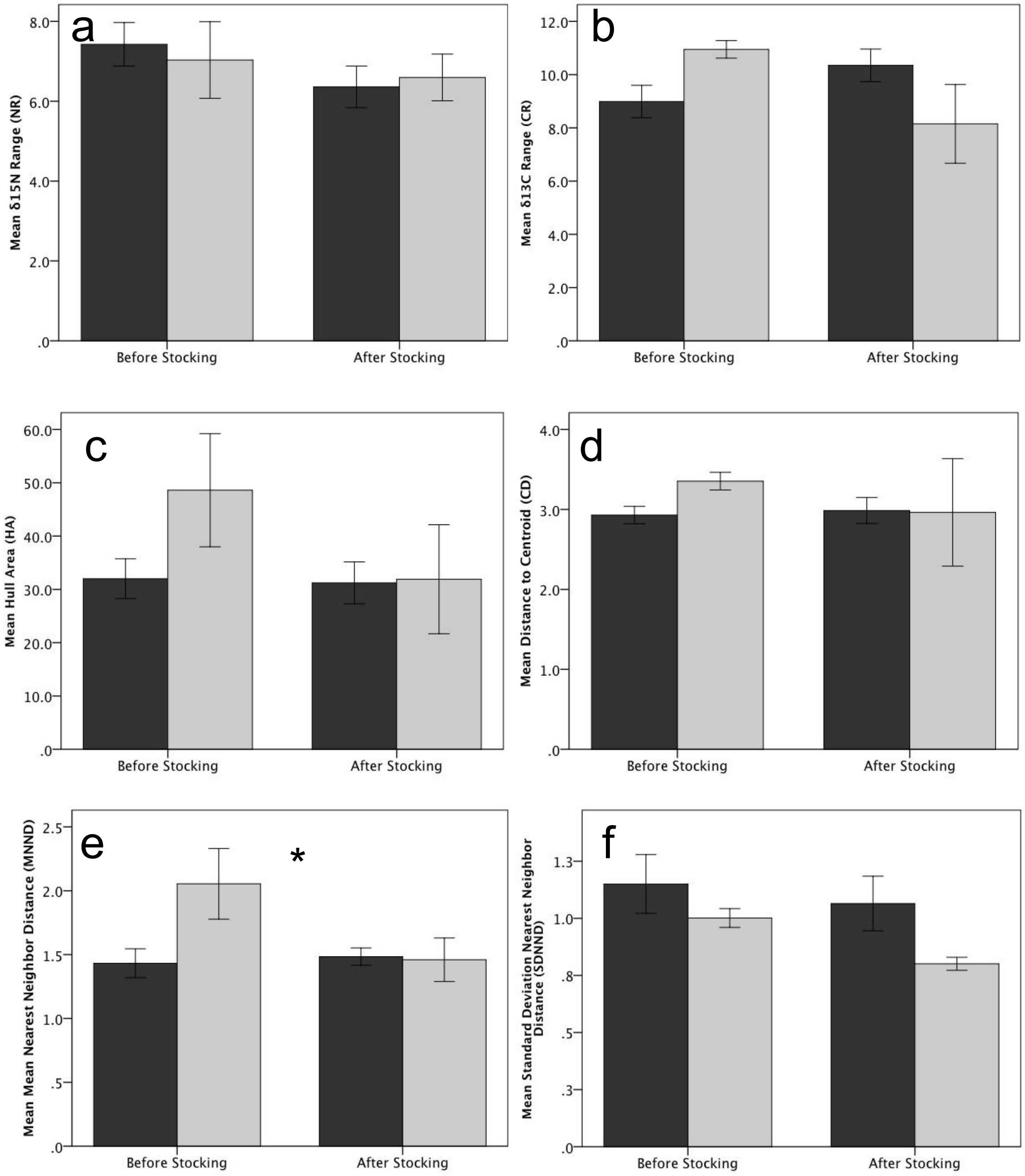

Figure 2-7. Mean (± SE) stable isotope distance metrics (a, NR; b, CR; c, HA; d, CD; e, MNND; f, SDNND) for unstocked control lakes (dark bars), and Fiesta Lake (light grey bars) before stocking and after stocking. Means are generated from lake*year means for each treatment. For time*treatment interaction (repeated measures ANOVA), *: $p< 0.10$.

Chapter 3. Complex littoral habitat influences the response of native minnows to stocked trout: evidence from whole lake comparisons and experimental predator enclosures

Abstract

Strong negative effects of introduced predatory fishes on native species are frequently reported but may not be universal. Recent research from productive lakes, for example, has documented few serious negative effects. Our objective was to determine how complex littoral habitat mediates the response of adult and young-of-year (YOY) native dace (*Chrosomus* spp.) and Fathead Minnow (*Pimephales promelas*) to the introduction of Rainbow Trout (*Oncorhynchus mykiss*) in productive lakes in Alberta, Canada. We first determined whether stocked trout affected the abundance or length of dace and Fathead Minnow in a single stocked lake relative to three unstocked controls. We then quantified inshore/offshore habitat use of native fish in stocked and unstocked lakes with heavily vegetated littoral zones. Finally, we manipulated the presence/absence of trout and densities of macrophytes within enclosures in an unstocked lake and assessed the behavioral response of native fish. Our whole-lake comparisons revealed that trout did not affect the abundance or length of native fishes, and that adult and YOY fishes occurred in vegetated inshore areas to a greater extent in stocked relative to unstocked lakes. In the enclosure experiment, native fishes did not respond to the introduction of trout at natural macrophyte densities, but dace significantly reduced their occupation of enclosures with reduced macrophytes once trout were added. Our results suggest that complex littoral macrophyte beds provide important refuge habitat for native fishes, which can potentially mitigate negative effects associated with introductions of a piscivorous predator.

Introduction

Predatory fishes have been introduced throughout North America for over 100 years, primarily to create and maintain recreational fisheries (Crawford and Muir 2008, Halverson 2008). These introductions can have wide-ranging ecological effects, including extirpation of prey (Anderson 1980, Goldschmidt et al. 1993, Penczak 1999), changes in nutrient cycling (Schindler et al. 2001), alterations of native food webs (Lepak et al. 2006), and even trophic cascades (Tronstad et al. 2010). Because introductions of predatory fishes, both accidental and intentional, are certain to continue, we require a greater understanding of the responses of native species across a diversity of ecosystem types.

Of the fishes introduced for recreational sport fishing, trouts (*Oncorhynchus, Salmo, Salvelinus*) are among the most commonly stocked (Halverson 2008). Extirpations of amphibians, invertebrates, and fish have all been reported after stocking trout (Schindler 2000, Eby et al. 2006), but the effects of trout appear to be most severe in headwater and alpine systems (Dunham et al. 2004). Such systems are typically characterized by low spatial habitat complexity and low native species richness, two characteristics thought to enhance the vulnerability of aquatic systems to negative effects of introduced species (Moyle and Light 1996). Consequentially, much of the research into the effects of stocked trout has been conducted in structurally simple, unproductive systems such as alpine lakes (reviewed in Dunham et al 2004, Eby et al. 2006). Recent research in productive, lower elevation lakes, however, has not found severe effects of stocked trout on native amphibians (Schank et al. 2011), invertebrates (Wissinger et al. 2006, Nasmith 2008), and fishes (Nasmith et al. 2010), and reviews have begun to question the belief that significant negative effects almost always accompany the introduction of predatory fishes (Gozlan 2008). Recent studies suggest that characteristics typical of productive lakes, including dense beds of littoral macrophytes, contribute to the limited effects. If native species can respond behaviorally to an introduced predator by using refuge habitat, direct population-level effects of the introduced predator may be minimized. Refugia can be particularly important as nursery areas for young, vulnerable age-classes (Dembski et al. 2008) or for species that remain vulnerable throughout their lives.

In aquatic systems, macrophytes provide important spatial refugia, and predation risk is often lower in macrophyte beds when compared to more open-water habitats (Sass et al. 2006, Dupuch et al. 2009). In the presence of predatory fish, several groups of potential prey, including amphibians (Walls 1995), macroinvertebrates (Luecke 1990, Harrison et al. 2005), zooplankton (Burks et al. 2002), and fish (Werner et al. 1983, Stuart-Smith et al. 2008) often show preference for complex habitats. However, not all prey species display equally effective avoidance behavior in the presence of predators, especially introduced predators (Cox and Lima 2006).

In productive lakes in the boreal foothills of Alberta, Nasmith et al. (2010) recently documented no differences in densities of native small-bodied fish between lakes with and without stocked trout, and similar to other studies, they proposed that complex littoral macrophyte beds buffer native fishes against negative effects of trout. After the study by Nasmith et al. (2010), one of the previously unstocked lakes, Fiesta Lake, was stocked with Rainbow Trout. This allowed us to investigate whether or not trout stocking affected forage fishes within this lake compared to unstocked control lakes. In this study, we examined whether the abundance and length of small-bodied fishes were affected in Fiesta Lake relative to three unstocked control lakes using a Multiple Before-After-Control-Impact (MBACI) design (Downes et al. 2002). We then determined whether native small-bodied fishes responded to the presence/absence of trout in a suite of stocked and unstocked lakes by adjusting their use of habitats with higher or lower densities of macrophytes in both natural lakes and experimental enclosures. In the recently-stocked Fiesta Lake, we predicted that stocked trout would not reduce the abundance or average length of native fishes. In the suite of stocked and unstocked lakes, a subset of those studied by Nasmith et al. (2010), we predicted that adult fish, and especially more vulnerable YOY fish, would occur in littoral macrophyte habitat more frequently in stocked relative to unstocked lakes. We similarly predicted that native fishes would reduce use of enclosures with reduced macrophyte densities after trout were introduced, but show little response to the presence of trout in enclosures with high macrophyte densities. The results of our research will advance the currently limited understanding of the effects of trout, ubiquitous introduced predators, in more productive ecosystems.

Methods

Study area

Research was conducted in six lakes in the vicinity of Caroline, Alberta, Canada (52°05'N, 114°44'W). Two study lakes, Ironside and Fiesta, have been stocked with Rainbow Trout (*Oncorhynchus mykiss*) since 2005 and 2007, respectively, and the third lake, Mitchell, has been stocked with both Rainbow and Brown trout (*Salmo trutta*) since 1950. Fiesta Lake is considered an "impacted" lake in the MBACI portion of this study and as a "stocked lake" in the comparisons of habitat use, where all data collection occurred after Fiesta Lake was stocked. All three unstocked lakes (Table 3-1) served as both the "control lakes" in MBACI analysis and as "unstocked" lakes in the comparisons of habitat use. All study lakes are small, mesotrophic, and have highly vegetated littoral margins, consisting primarily of *Potamogeton* and *Nuphar,* that dominate littoral habitat structure. In surveys of littoral zones, all quadrats in all six study lakes had at least 85% macrophyte coverage (Nasmith 2008). Native fish communities consist of a dace species complex (*Chrosomus eos*, *C. neogaeus*, and a parthenogenic hybrid between the two), Fathead Minnow (*Pimephales promelas*), Pearl Dace (*Margariscus nachtriebi*), and Brook Stickleback (*Culaea inconstans*). See Table 3-1 for the fish communities and limnological characteristics of each lake. Distinguishing parental *Chrosomus* individuals from hybrids is impractical under field conditions (Binet and Angers 2005), therefore, we grouped parental and hybrid individuals in one category, hereafter referred to as "dace" (this category excludes Pearl Dace). Because the *Chrosomus spp.* complex was ubiquitous and the most abundance native fish taxon in all six lakes (Nasmith et al. 2010), only this dace group was analyzed for habitat use by adult fishes. Due to identification challenges in the field, young-of-year (YOY) of all native fishes were grouped together for whole-lake analysis of habitat use, but were likely primarily dace. Both dace and Fathead Minnow were caught in sufficient quantities to be analyzed separately in the enclosure experiment and the MBACI portion of this study.

Before After Control Impact Analysis

Between 2006 – 2009, abundances of dace and Fathead Minnow in Fiesta Lake and unstocked controls were estimated using the catch-per-unit-effort procedure described below for habitat use of adult fish; however, in 2006 and 2007, minnow traps were not evenly distributed between inshore and offshore habitat. At least 100 individuals of each species were measured during each day of fish sampling to generate mean lengths for each species in each lake•year. Fiesta Lake was first stocked in September 2007 with 1,900 triploid Rainbow Trout (average fork length 15 cm), and an additional 1,100 and 550 individuals were stocked in May of 2008 and 2009, respectively (average fork length 26 cm; Alberta Sustainable Resource Development yearly stocking reports, http://mywildalberta.com/Fishing/StockingReports.aspx). Therefore, for MBACI analysis, Fiesta Lake was considered unstocked in 2006 – 2007 and stocked 2008 – 2009.

Habitat use by adult fish

In 2008 and 2009, we estimated the habitat use by adult (total length > 45 mm) dace in the six study lakes (three stocked, three unstocked). For 5 consecutive days in each lake, Gee-type minnow traps were deployed in 15 randomly chosen inshore and offshore locations on the lake bottom. Inshore locations were < 1 m deep in macrophyte beds consisting primarily of broad-leafed *Potamogeton* (*P. richardsonii* and *P. gramineus*) that extended throughout the water column. Offshore locations were in open water (i.e., no visible vegetation) > 1 m deep but rarely deeper than 4 m. The number of fish caught and duration of deployment (2 - 24 hours, based on Nasmith et al. 2010) were recorded for each trap, and an average catch-per-unit-effort (CPUE; fish•hour^{-1}) was determined each day for inshore and offshore traps.

Young-of-year fish sampling

Young-of-year native fishes were sampled in four of the study lakes (Table 3-1: Fiesta, Mitchell, Dog Leg, Gas Plant) in late July of 2009 and 2010. Five inshore and five offshore locations, as defined above, were randomly chosen in each lake, and each location was visited four times, including day and night. At each visit in each location, a subsurface tow net (30 cm diameter, 250μm mesh) was thrown approximately 8 m and retrieved at approximately 1 $m \bullet s^{-1}$. A small diameter net was used to facilitate tows in the shallow, vegetated littoral zones (e.g., Danylchuk and Tonn 2001). The number of fish captured per tow was recorded for a total of 40 tows per lake per year. Because catches per tow were not normally distributed, we used the sum of fish captured in each habitat in each lake and year as the response variable.

Enclosure experiment

We conducted an enclosure experiment in the summer of 2010 to test how habitat use by adult native fish was affected by macrophyte density (natural vs. reduced) in the presence and absence of trout. The experiment was conducted in an unstocked study lake, Dog Leg Lake (51°59’ N, 114°43’ W; Table 3-1). We installed six pairs of enclosures (1.8 m x 1.8 m) in the littoral zone (≤ 1.0 m in depth) in late May. Enclosures were constructed of vertical metal t-bars wrapped with 2.5 cm polyethylene mesh, which allowed native fish from the lake to pass unimpeded into and out of the enclosures. In each pair, one enclosure contained “natural” (75-100% cover) and one contained “reduced” (10-20% cover) macrophyte density. Density was defined as the percentage of the water surface covered by broad-leafed *Potamogeton* (primarily *P. richardsonii and P.*

gramineus) and *Nuphar* plants. Densities were standardized within treatments by transplanting *Potamogeton* to increase densities or clipping plants to reduce densities.

We obtained Rainbow Trout of the size used locally for stocking (mean total length ± 1 SE; 254 ± 5 mm; stocked trout range: 170 - 260 mm) from Alberta Sustainable Resource Development's Raven Brood Trout Station (10 km from Dog Leg Lake). On 15 August 2010, we transported the trout to the lake in aerated containers. After a series of partial water changes over 3 hours to acclimatize the hatchery trout to lake water, we added one trout to each enclosure. All trout were removed from enclosures with dip nets on 19 - 20 August, and any macrophytes that were disturbed during netting were returned to the enclosure.

To assess use of trout enclosures by adult dace and Fathead Minnow, we placed one Gee-type minnow trap inside each enclosure for three time periods: before trout addition (10 - 12 August 2010), concurrent with trout (16 - 18 August 2010), and after trout removal (20 - 22 August 2010). We also placed one "control" minnow trap outside, but within 10 m, of each enclosure in an area of similar macrophyte coverage during all three time periods. We determined the mean number of fish captured in each minnow trap during two, 24-hour sets. To rule out the possibility that enclosures acted as either attractants or repellants of fishes, we had earlier conducted 3 days (19 - 21 June) of paired 24-hour minnow trap sets. During these sets, one minnow trap was placed inside each enclosure and another trap was placed nearby, outside each enclosure in an area of similar macrophyte coverage.

Statistical Analysis

For MBACI analysis, we used linear mixed models (SPSS for Mac Version 17) with "year" as a repeated factor and "lake" as a random factor to test for a significant statistical interaction between treatment (Control vs. Impact) and time period (Before vs. After). For adult habitat use, we used 2-factor repeated linear mixed models to test for a treatment*location interaction. A significant interaction indicates a different pattern of inshore and offshore habitat use between stocked and unstocked lakes. Treatment and location were fixed factors, lake was a random factor, and year was treated as a repeated measure. Because YOY tow data were not normally distributed, we used 2 x 2 contingency table $\chi 2$ tests (Zar 1999) to determine if the number of fish caught in inshore and offshore tows differed between treatments in 2009 and in 2010.

For the enclosure experiment, each enclosure was used as the unit of replication, and the response variable was the mean number of fish caught from two consecutive, 24-hour minnow trapping events for each time period. We used analysis of variance (ANOVA) to test for differences in dace and Fathead Minnow catches across the three time periods (before, with, and after trout) in natural and reduced macrophyte treatments. If a difference was found, Fisher's least significant difference test (Zar 1999) was used for pairwise comparison. We used the same procedures to test for differences in catches in "control" traps placed outside of each enclosure during each time period. A paired t-test assessed differences in minnow traps placed inside and outside each enclosure during our test sampling in June. All data were analyzed in SPSS Version 17.0 for Mac. Results of statistical tests were considered significant when $p< 0.05$. Assumptions of normality and homoscedasticity for parametric tests were confirmed in SPSS.

Results

MBACI Analysis

There was no significant BA*CI interaction for the CPUE of dace ($F_{3,2.3}$= 2.53, p= 0.28) or Fathead Minnow ($F_{3, 2.6}$= 0.52, p= 0.70) of Fiesta Lake relative to the unstocked controls (Figure 3-1a-b). Similarly, there was also no significant BA*CI interaction for the length of dace ($F_{3,3.5}$= 0.79, p= 0.565) or Fathead Minnow ($F_{3, 7.9}$= 0.55, p= 0.67) in Fiesta Lake relative to the unstocked controls (Figure 3-1c-d).

Adult fish habitat use

Catch-per-unit-effort of dace was higher inshore (relative to offshore) in stocked lakes than in unstocked lakes (treatment x location interaction; $F_{1,14.4}$= 14.8, p= 0.002; Figure 3-2). Over both years, the ratio of inshore to offshore catch differed by a factor of 2.8 between lake types: 25:1 for stocked lakes but only 9:1 for unstocked lakes.

Young-of-year habitat use

We captured 891 YOY in our tows, ranging in length from 8.7 mm to 37 mm. In both 2009 and 2010, a higher proportion of the catch was inshore in stocked lakes relative to unstocked lakes (2009: χ^2_1= 13.0, p< 0.001; 2010; χ^2_1= 24.6, p< 0.001; Figure 3-3). Averaging over both years, the ratio of inshore to offshore catch was 13-times higher in stocked vs. unstocked lakes (52:1 and 4:1, respectively).

Enclosure experiment

Similar numbers of fish were captured in traps placed inside and outside of enclosures during our test sampling in June for both dace (2-tailed paired t-test; t_{11}= -

0.74, p= 0.47) and Fathead Minnow (2-tailed paired t-test; t_{11}= -0.33, p= 0.75). Mean (± 1 SE) inside catch for dace and Fathead Minnow was 109 ± 19 and 36 ± 13 individuals, respectively, whereas outside catch was 126 ± 13 and 39 ± 9 individuals, respectively.

During our trout manipulations in August, the number of dace captured in enclosures at natural macrophyte densities did not differ among the three time periods (before, during, and after trout; $F_{2,15}$= 0.51, p= 0.61, Figure 3-4a). In contrast, dace catch in the reduced macrophyte enclosures differed significantly among time periods ($F_{2,15}$= 6.89. p= 0.008, Figure 3-4a). Multiple comparisons showed that the catch of dace in enclosures before trout were added was higher than when trout were present (p= 0.003) and after trout were removed (p= 0.024). Catches between the latter two time periods did not differ (p= 0.29). During the same period, mean (± 1 SE) catches of dace in control traps set outside of enclosures did not differ for either natural ($F_{2,15}$= 0.842, p= 0.45; control before= 214 ± 35, control during= 183 ± 37, control after= 154 ± 25) or reduced ($F_{2,15}$= 0.239, p= 0.79; control before= 161± 37, control during= 134 ± 40, control after= 125 ± 39) macrophyte treatments.

For Fathead Minnow, catches were similar among time periods in both the natural ($F_{2,15}$= 0.134, p= 0.88) and reduced macrophyte treatments ($F_{2,15}$= 0.191, p= 0.83: Figure 3-4b). Catches of Fathead Minnows in control traps set outside of enclosures also did not differ among time periods for either natural ($F_{2,15}$= 0.945, p= 0.41; control before= 23 ± 11, control during= 17 ± 5, control after= 8 ± 4) or reduced ($F_{2,15}$= 0.538, p= 0.59; control before= 130 ± 64, control during= 108 ± 52, control after= 57 ± 29) macrophyte treatments.

Discussion

The abundance and length of native populations of small-bodied fishes were not affected by trout after stocking in a single lake compared to unstocked control lakes, and both adult and young-of-year fishes exhibited different patterns of habitat use in lakes stocked with trout than in unstocked lakes. Studies documenting the effects of trout on native fishes in productive lakes are rare, but similar lack of impact on abundance and length of native fishes has been documented from our study lakes (Nasmith et al. 2010), where researchers have suggested that dense beds of macrophytes have ameliorated impacts of trout.

Indeed, in our lakes adult and especially YOY fishes were more concentrated in vegetated inshore habitat in the presence of trout. Elevated use of inshore habitat was strongest in YOY, with the ratio of inshore to offshore catch 13 times higher in stocked relative to unstocked lakes, compared to an adult inshore catch ratio that was 2.8 times higher in stocked lakes. In our experiment, adult dace (but not Fathead Minnow) decreased use of enclosures with reduced macrophyte densities in the presence of trout, but did not alter use of enclosures containing natural, high densities of macrophytes. Interestingly, dace continued to avoid enclosures for several days after trout were removed, but only enclosures where macrophyte densities were low. Consistent with our predictions, dace and Fathead Minnow abundance and length were not affected by trout after stocking, and dace were able to recognize and avoid the threat imposed by an introduced predator.

While other mechanisms, such as predator-induced mortality, might contribute to the observed differences in relative abundance of small-bodied fishes inshore vs. offshore in stocked vs. unstocked lakes, we believe that mortality was not responsible. Our

MBACI results and other research in our region documented no consistent differences in overall densities of native small-bodied fish in stocked vs. unstocked lakes (Nasmith et al. 2010). In the absence of detectable demographic shifts, avoidance is the most likely mechanism to explain the different patterns in habitat use of native fish between stocked and unstocked lakes.

Comparable patterns have also been seen in studies of habitat use by fish in the presence of native predators. For example, Werner et al. (1983), found juvenile Bluegill Sunfish (*Lepomis macrochirus*) to increase use of inshore habitat in the presence of Largemouth Bass (*Micropterus salmoides*), and Dupuch et al. (2009) found that adult *C. eos* increased their use of inshore habitat when predation risk from Brook Trout (*Salvelinus fontinalis)* offshore was high. Our results expand on these observations, as they demonstrate habitat shifts in two life stages (adult and young-of-year) and at two spatial scales (whole lake and enclosure) in response to an introduced (novel) predator. Additionally, although we did not explicitly test temporal patterns, our results suggest that antipredator behavioral shifts can be both rapid (as in enclosures) and sustained (as in our stocked lakes, with 1 - 58 years of trout presence). Shifts to complex habitat in the presence of a predator are commonly observed in laboratory and enclosure studies (Zimmerman and Vondracek 2002, Stuart-Smith et al. 2008, Kovalenko et al. 2010); however, such shifts at the whole-lake scale, after introduction of predatory fishes, have rarely been documented (but see Pink et al. 2007, Nasmith et al. 2010).

In contrast to the clear changes in the behavior of dace, Fathead Minnows did not alter their use of enclosures in the presence of trout. Indeed, dace frequently show antipredator behavior, including increased shoal size and use of inshore habitat (Pink et al. 2007, Dupuch et al. 2009). Fathead Minnow, however, appears to have limited capacity to make similar behavioral adjustments (Moody et al. 1983, Savino and Stein 1989) and is often found in allopatry with piscivorous species (Tonn and Magnuson 1982, Robinson and Tonn 1989). Nevertheless, in this study and in other lakes where they co-occur with trout, Fathead Minnows are found at densities comparable to nearby unstocked lakes (Nasmith et al. 2010). The mechanisms promoting this coexistence are unclear, especially because Fathead Minnows appeared not to respond behaviorally to trout presence in the exclosure experiment and were actually found in higher abundance in reduced macrophyte enclosures. It is possible that Fathead Minnows are relying on an alternative antipredator strategy in boreal lakes (e.g., short-term responses to alarm cues). Additionally, while Fathead Minnow may not actively choose vegetated habitat in the presence of trout, Fathead Minnows are typically found in shallow inshore habitat (Price et al. 1991). Because nearly 85% of the littoral zones of our boreal lakes are heavily vegetated (Nasmith 2008), many individual Fathead Minnows will, by chance, be found in macrophytes. Thus, whereas dace appears to benefit from actively choosing vegetated habitat, Fathead Minnow may passively benefit from dense macrophytes because so little of the littoral of our boreal lakes is unvegetated.

In reduced macrophyte enclosures, dace catch did not return to pre-stocking levels after trout were removed (although catch appeared to be increasing). Recent research has documented that anti-predator behaviors in fish persist longer when perceived predation risk is higher (Ferrari et al. 2010). Perceived predation risk for dace was likely highest when trout were present in reduced macrophyte enclosures; thus, it is possible that learned avoidance of the reduced density enclosures by dace persisted even after trout were removed. It is also possible that chemical alarm substances may have lingered in enclosures, causing the continued avoidance of enclosures by dace (e.g., Wisenden and Barbour 2005) and possibly explaining the small, but non-significant, reduction of dace caught in the natural macrophyte enclosures when trout were present.

Although habitat shifts may promote coexistence of predators and prey, refuges can sometimes be suboptimal foraging habitat for prey (e.g., Mittelbach 1981, Werner et al. 1983). Often, small-bodied fishes may move offshore to feed on zooplankton, which can be more abundant there (e.g., Biro et al. 2003). In 2009, cladocerans were more than twice as abundant (individuals per liter) offshore in stocked study lakes (mean ± 1 SE, 34.6 ± 11.0) relative to unstocked lakes (mean ± 1 SE, 13.0 ± 4.3; T. Donald, University of Alberta, unpublished data). If both YOY and adult fishes are restricted to littoral habitats in stocked lakes, there is potential for reductions in YOY growth rates due to increased density-dependent competition (e.g., Tonn et al. 1992). However, we did not see a reduction in the length of dace or Fathead Minnow in the first two years after stocking in Fiesta Lake relative to control lakes. Although dace in unstocked lakes were caught proportionally more often offshore (as compared to stocked lakes), the general lack of long-term effects on native fish populations accompanying trout stocking (Nasmith et al. 2010) indicate that shifts in habitat use do not have large population consequences.

Results of our study demonstrate that trout have not affected the abundance or average lengths of native fishes two years after trout were stocked and also show that habitat shifts by both adult and YOY fishes can occur immediately after stocking and such patterns may be sustained for years (e.g., Biro et al. 2003, Nasmith et al. 2010).

Because macrophyte refugia are generally available in productive lakes and because native fishes increase their use of this habitat in the presence of trout, it is likely that the availability of dense macrophyte beds as predator refuges contribute substantially to the lack of population-level effects of trout on native fishes and other potential prey taxa observed in several recent studies in productive lakes (Wissinger et al. 2006, Nasmith et al. 2010, Nasmith 2008, Schank et al. 2011). Macrophytes physically conceal prey (Savino and Stein 1989) and reduce the foraging efficiency of predators (Diehl et al. 1998, Padial et al. 2009), resulting in lower predation risk when compared to less complex habitat (Dupuch et al. 2009).

Our results have implications for both applied fisheries management and basic understanding of introduced species. Natural lakes bearing sustainable game fish populations are limited in many regions, including Alberta, and as a result, trout stocking is likely to remain an important management tool in such areas. Because refugia provided by macrophytes may promote the coexistence of stocked trout and their prey, managers may want to seek out lakes with well-vegetated littoral zones as targets for stocking if minimal impacts on native littoral-dwelling species are desired (e.g., Pearsons and Hopley 1999). Additionally, freshwater ecosystems are experiencing repeated introductions of exotic, predatory species (Rahel 2007). In particular, salmonids have become established outside of their native ranges throughout the world, yet their impacts are not always easy to predict (Fausch 2008). Not surprisingly, most research on the effects of stocked salmonids is conducted in areas where they have known or suspected negative effects (e.g., Leprieur et al. 2009, Korsu et al. 2010), and examples of introductions with negligible effects are much less common (but see Fausch et al. 2001, Marchetti et al. 2004, Gozlan 2008). Because of this, the proposition that stocked trout have significant negative effects on native communities is often regarded as a simple fact (Gozlan 2008). Despite this, trout are still stocked in large numbers (171.5 million in 2004 by the United States government alone; Halverson 2008) across a diverse range of ecosystems. Research on ecosystems exhibiting or suspected to exhibit negligible impacts should provide valuable and complementary insight into factors governing the outcome of introductions.

Although we agree that the precautionary principle should be applied before introducing a species into a novel ecosystem (e.g., Pearsons and Hopley 1999, Leprieur et al. 2009), some systems, such as the isolated productive lakes in our study, appear to have the capacity to support introduced trout with few effects on native species (amphibians: Schank et al. 2011; invertebrates: Wissinger et al. 2006, Nasmith 2008; small-bodied fishes: Nasmith et al. 2010). Research at sites with and without strong negative effects should together help elucidate factors controlling impacts of introduced species.

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Table 3-1. Fish communities, dace densities, and morphometric and water quality characteristics (range of values from 2008 - 2009) of stocked (n= 3) and unstocked (n= 3) lakes in the boreal foothills of west-central Alberta surveyed for native fish habitat use. For fish communities, D= dace (*Chrosomus* spp.), F= Fathead Minnow (*Pimephales promelas*), B= Brook Stickleback (*Culaea inconstans*) and P= Pearl Dace (*Margariscus margarita*).

Lake Name	Fiesta	Ironside	Mitchell	Dog Leg	Gas Plant	Gun Range
Treatment	Stocked	Stocked	Stocked	Unstocked	Unstocked	Unstocked
Fish Species	D,F,B,P	D	D	D,F,B	D,F,B	D,F,B
Dace density (Fish/ha)	10,655 - 12,150	17,081 - 18,390	5,788 - 8,178	3,184 - 5,399	2,103 - 3,149	1,175 - 4,170
Surface Area (ha)	6.6	3.3	15.0	6.7	17.5	5.9
Secchi depth (m)	2.4 - 2.6	4.3 - 4.4	3.9 - 4.3	1.9 - 2.2	1.5 - 1.7	2.8 - 3.6
Maximum Depth (m)	7.0	13.0	6.0	5.0	4.0	13.0
Chl a (µg L^{-1})	0.85 - 1.46	0.44 - 0.52	1.17 - 1.33	3.50 - 14.53	5.43 - 5.93	2.16 - 5.70
Total Phosphorus (µg L^{-1})	17 - 19	9 - 12	11 - 14	29 - 52	37 - 39	15 - 15
Total Nitrogen (µg L^{-1})	624 - 690	468 - 549	807 - 815	809 - 829	739 - 820	614 - 882
pH	7.6 - 7.8	7.4 - 7.5	7.3 - 7.4	7.2 - 7.5	7.4 - 7.6	7.5 - 7.6

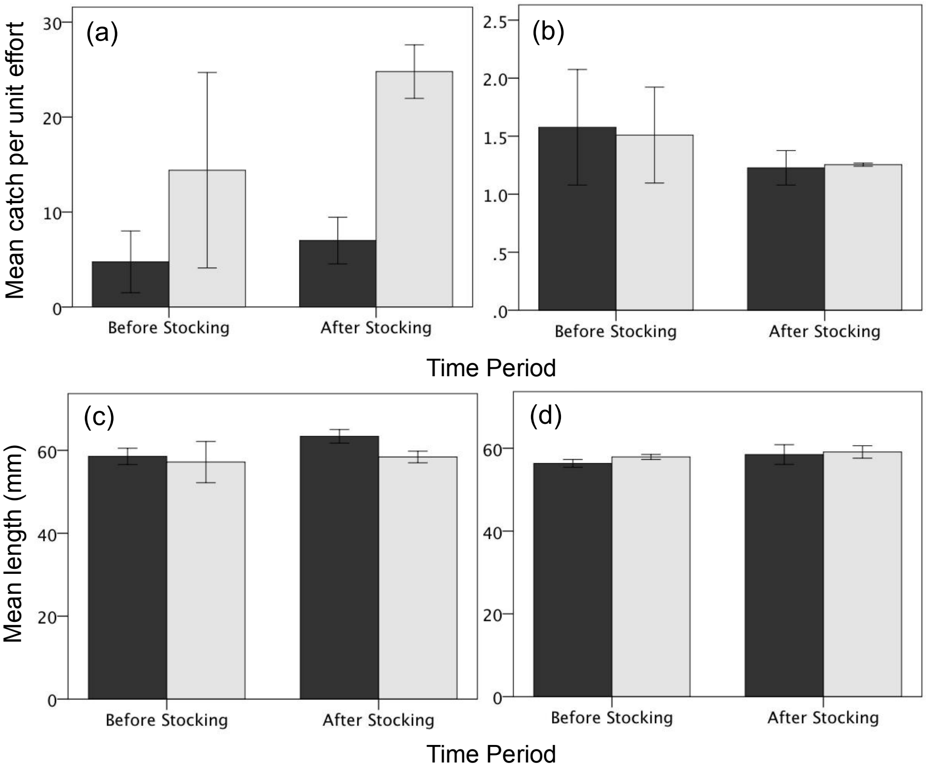

Figure 3-1. Mean (± 1 SE) catch-per-unit-effort (fish•hour^{-1}) and length of adult dace (CPUE, a; Length, c) and Fathead Minnow (CPUE, b; Length, d) from control lakes (dark grey) and Fiesta Lake (light grey) before (2006 -2007) and after (2008 – 2009) Fiesta Lake was stocked with trout.

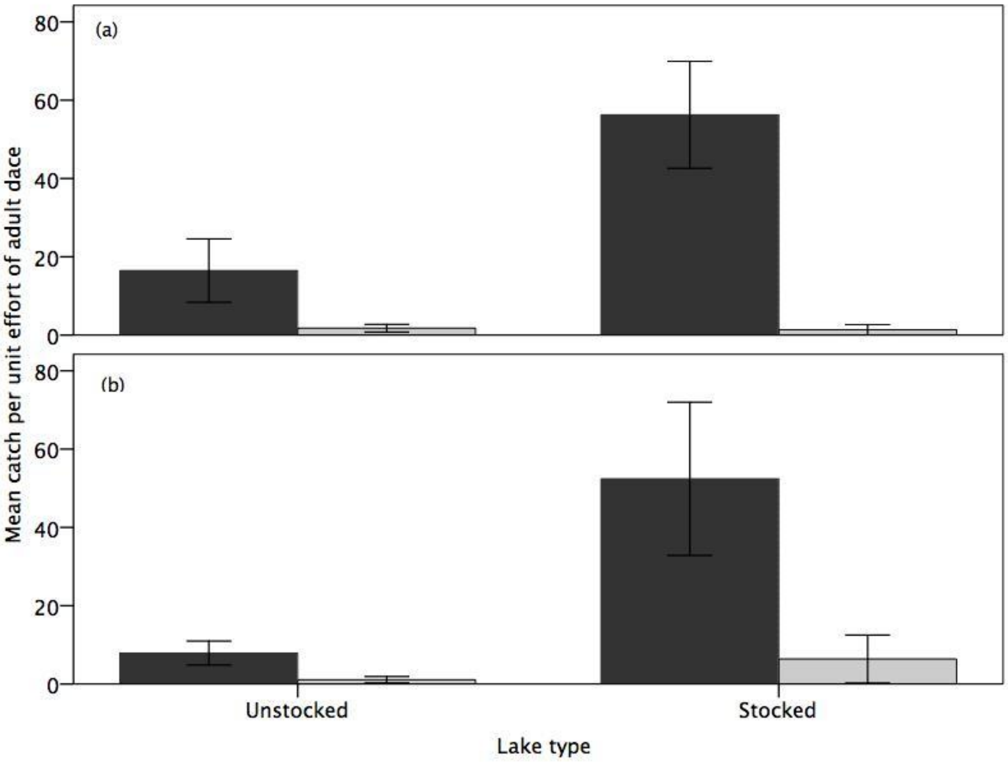

Figure 3-2. Mean (± 1 SE) catch-per-unit-effort (fish•hour^{-1}) with minnow traps of adult dace in inshore (dark grey) and offshore (light grey) habitats of stocked (n= 3) and unstocked (n= 3) lakes in the boreal foothills of Alberta in 2008 (a) and 2009 (b).

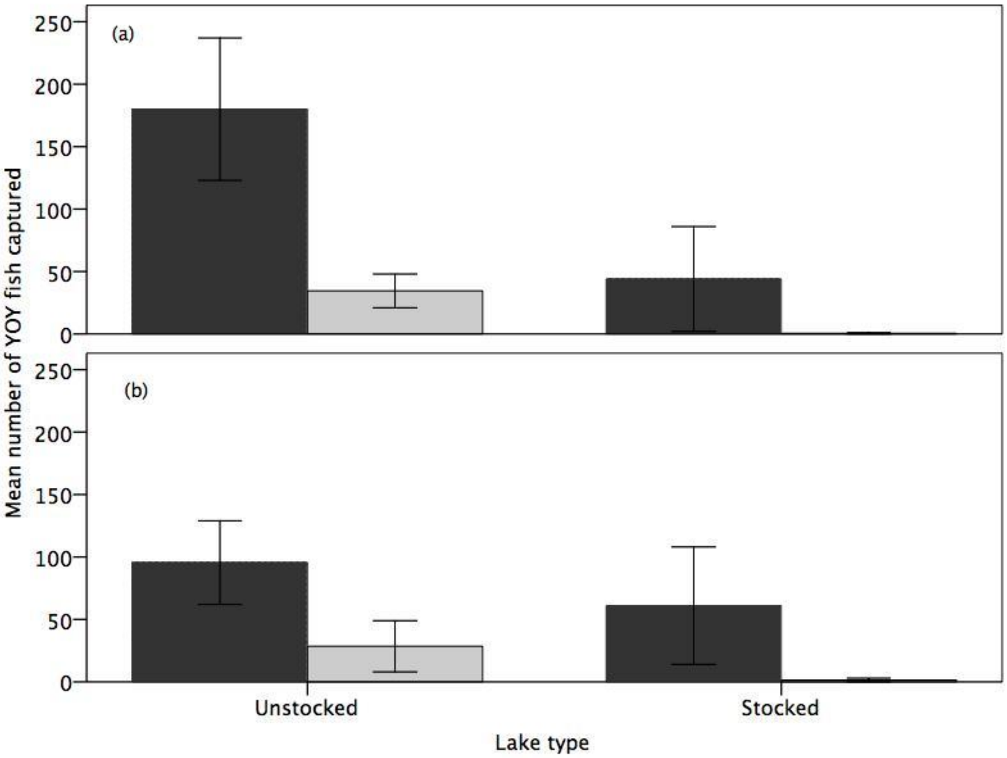

Figure 3-3. Mean (± 1 SE) total catch with a tow net of young-of-year native fishes in inshore (dark grey) and offshore (light grey) habitat of stocked (n= 2) and unstocked (n= 2) lakes in the boreal foothills of Alberta in 2009 (a) and 2010 (b).

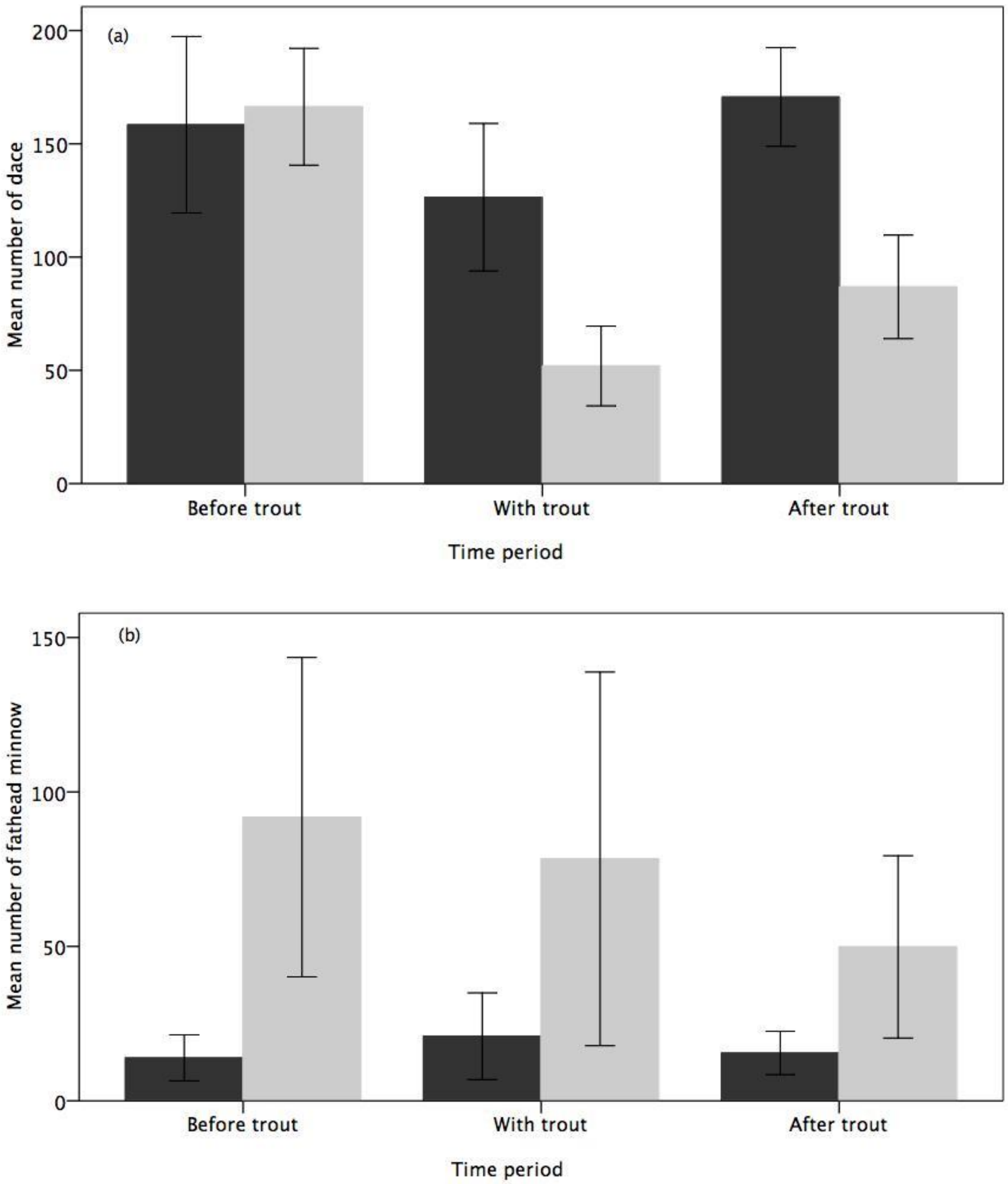

Figure 3-4. Mean (± 1 SE) catch of dace (a) and Fathead Minnow (b) with minnow traps from six enclosures with natural macrophyte densities (dark grey) and six enclosures with reduced macrophyte densities (light grey) at three time periods: before trout introduction, with trout present, and after trout removal. A single minnow trap was set in each enclosure for two 24-hour sessions for each of the three time periods, the value for each enclosure used in analysis was the mean catch for the two sessions.

Chapter 4. Stocked trout have minimal effects on littoral invertebrate assemblages of productive fish-bearing lakes: a whole-lake BACI study

Abstract

Rainbow Trout (*Oncorhynchus mykiss*) is commonly stocked as a sport fish throughout the world but can have serious negative effects on native species, especially in headwater systems. Productive fish-bearing lakes represent a frequently stocked yet infrequently studied system, and effects of trout in these systems may differ from those in headwater lakes. We used a Before-After Control-Impact (BACI) design to determine how stocked trout affected assemblage-level and taxon-level biomass, abundance, and average length of littoral invertebrates in a stocked lake relative to three unstocked control lakes in the boreal foothills of Alberta, Canada. Lakes were studied one year before and for two years after stocking. Because characteristics of productive fish-bearing lakes should buffer impacts of introduced fish, we predicted that trout would not affect assemblage-level structure of littoral invertebrates but might reduce the abundance or average length of large-bodied taxa frequently consumed by trout.

Relative to the unstocked control lakes, biomass, but not abundance, of the littoral invertebrate assemblage was affected indirectly by trout through increases of some taxa after trout stocking. At the individual taxon-level, trout stocking did not affect most (23 of the 27) taxa, with 4 taxa increasing in abundance or biomass after stocking. Only 1 taxon, Chironomidae, showed evidence of size-selective predation by trout, being consumed frequently by trout and decreasing significantly in average length after stocking. Our results contrast with the strong negative effects of trout stocking on invertebrate assemblages commonly reported from headwater lakes. A combination of factors, including large and robust native populations of forage fish, the generalized diet of trout, overwinter aeration, relatively high productivity, and dense macrophyte beds, likely work in concert to reduce potentially negative effects of stocked trout in these systems. As such, productive, fish-bearing lakes may represent a suitable system for trout stocking, especially where native sport fish populations are lacking.

Introduction

For over 100 years, introductions of salmonids into lakes and streams across North America have created or enhanced recreational fishing opportunities (Halverson 2008). Often, these introductions are into historically fishless headwater systems, where stocked trout can have serious negative effects on native species, including reduced abundance, biomass or even extirpations (Stoks & McPeek 2003; Schilling, Loftin & Huryn 2009). In such systems, large benthic and nektonic invertebrate taxa are most frequently affected by stocked trout (e.g., Carlisle & Hawkins 1998; Knapp, Matthews & Sarnelle 2001).

Because of these well-known serious negative effects, most research on the impacts of salmonid stocking has focused on fishless headwater lakes and streams (reviewed in Dunham, Pilliod & Young 2004; Eby*, et al.* 2006). These ecosystems possess characteristics (e.g., low species richness, low habitat complexity, native fauna naïve to fish predators, etc.) that are thought to facilitate negative impacts of introduced aquatic species (e.g., Moyle & Light 1996). Thus, it is not surprising that much of the research on stocked trout has emphasized negative effects on native biota (Knapp*, et al.* 2005; Gozlan 2008). However, trout are stocked in a broad range of ecosystems, and recent studies from non-headwater lakes suggest that negative effects are not universal. For example, in New Zealand, Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*) introduced into shallow, productive fish-bearing lakes negatively affected native galaxiid fishes (McIntosh et al. 2010), but had little detectable effect on benthic invertebrate assemblages (Wissinger, McIntosh & Greig 2006).

In the boreal region of Alberta, Canada, numerous small, productive lakes contain abundant small-bodied minnows and sticklebacks but lack larger-bodied sport fishes (Nelson & Paetz 1992). As a result, trout stocking is an important management tool used to expand angling opportunities that are otherwise limited due to climactic, limnological, and biogeographical factors (Nelson & Paetz 1992; Sullivan 2003). Recent research has found few differences in the populations and assemblages of native amphibians (Schank*, et al.* 2011), forage fish (Nasmith*, et al.* 2010), and littoral invertebrates (Nasmith*, et al.* 2012) between a suite of Alberta lakes stocked with trout and a set of nearby unstocked lakes.

As with research conducted in New Zealand, however, these Alberta studies were primarily comparative, focusing on lakes with a long history of trout stocking (up to 50 years). Although comparative studies can yield valuable information about the effects of trout stocking, especially when large numbers of lakes are included (e.g., Knapp*, et al.* 2001), such designs typically do not include data from the first few years before and after stocking. Additionally, lakes are often chosen to stock based on their perceived ability to support fish populations and are thus often inherently different from lakes that remain unstocked (Pope 2008). Our goal was to use a Before-After Control-Impact (BACI) design to determine post-stocking effects of trout on invertebrate assemblages. BACI, which provides a more rigorous approach than control-impact designs, is among the leading designs for detecting environmental impacts and is a powerful method for separating effects of an impact from natural variation (Downes*, et al.* 2002). We focused on one boreal foothills lake, monitored one year before and for two years after trout stocking, and compared its littoral invertebrates to those in three nearby unstocked control lakes over the same period. We tested for a statistical interaction between time (Before vs. After) and treatment (Stocked vs. Unstocked) contrasts on composition, abundance, biomass, and body sizes of littoral invertebrates. We also monitored trout diet within the newly stocked lake.

Based on the comparative studies of Wissinger*, et al.* (2006) and Nasmith*, et al.* (2012), we predicted that stocked trout would not significantly affect the overall assemblage-level structure (composition, biomass and abundance) of invertebrates in these boreal foothills lakes. However, we did expect a reduction in abundance and/or size of some large-bodied, active taxa frequently consumed by trout. Our investigation not only expands the small number of studies on the effects of stocked trout in systems other than those historically linked to negative impacts, it also applies the rigor of the BACI design to address concerns associated with the comparative nature of previous studies. As such, our study helps clarify the circumstances under which strong negative effects of non-native fish should or should not be expected (e.g., Leprieur*, et al.* 2009). Additionally, because our BACI experiment was conducted in a subset of lakes previously investigated in Nasmith*, et al.* (2012), our results serve as an experimental assessment of the patterns detected through their alternative, Control-Impact approach.

Methods

Study design, limnological and biological characteristics

The study was conducted using a Multiple Before-After Control-Impact (MBACI) design (Downes*, et al.* 2002), with one impacted (i.e., stocked with trout) lake (Fiesta Lake) and three control (i.e., unstocked) lakes, located in the boreal foothills near

Caroline, Alberta, Canada (52° 5′ 36″ N and 114° 44′ 22″ W). These mesotrophic study lakes are small, moderately deep, and are inhabited by several native small-bodied fish species (Table 4-1). The littoral zones of all lakes are heavily vegetated, primarily with *Potamogeton* and *Nuphar* beds (Nasmith, *et al.* 2012).

Stocking of Fiesta Lake

Fiesta Lake was first stocked in September 2007 with 1,900 triploid Rainbow Trout (average fork length 15 cm), and an additional 1,100 and 550 individuals were stocked in May of 2008 and 2009, respectively (average fork length 26 cm; Alberta Sustainable Resource Development yearly stocking reports, http://mywildalberta.com/Fishing/StockingReports.aspx). The lake is managed as a catch-and-release trophy fishery with no allowable harvest of trout. Since trout have been stocked, surface aeration has been used each winter (approximately November – March) to maintain open water and prevent periods of hypoxia that typically occur under the ice in foothills lakes.

Invertebrate samples

To sample invertebrate assemblages, ten littoral locations per lake were randomly chosen and one sweep sample was taken at each location each year. Sweeps were taken between 08:00 and 18:00 in late July of 2007 (before stocking) and 2008 - 2009 (after stocking). Upon arriving at each location, a specific sweep site was chosen within 1 m of the shoreline in an area less than 1 m deep so that the sweep would include both the lake bottom (i.e., sediment) and a bed of macrophytes. Samples were taken with a triangular

30 cm x 30 cm x 30 cm sweep net fitted with 500 μm mesh. Each sweep consisted of placing the net 5 cm into lake sediment and rapidly moving the net along the contour of the lake bottom and up through the stand of aquatic macrophytes (total distances ranged from 0.30 and 1.1 m), thereby sampling both the benthos and organisms living among the macrophytes. The total distance traveled by the sweep was recorded and later used to standardize abundance and biomass measurements per meter sweep. Samples were washed within the sweep net to remove excess sediment, and large pieces of plant matter were also rinsed in the net to remove adhering invertebrates. Net contents were kept fresh in coolers and preserved with approximately 80% ethanol later on the day of sampling.

In the laboratory, each sample was washed through a 2.0 mm sieve, and the organisms and detritus retained were preserved in ethanol. Organisms were identified to the lowest feasible taxonomic level (LFTL), typically family or genus for insects, genus for gastropods, and order or family for other taxa (Clifford 1991). Only the 2.0 mm fraction was processed and analyzed, as previous work in our study lakes found that including organisms from the 0.5 mm fraction (after passing through a 2.0 mm filter) had little effect on relative abundance or average length of taxa (Nasmith*, et al.* 2012); organisms smaller than 2.0 mm adhere to detritus retained by the 2.0 mm sieve (J. Hanisch, unpublished data). Additionally, trout select for the largest individuals of macroinvertebrate (e.g., Carlisle & Hawkins 1998; Luecke 1990) and zooplankton (Budy, Haddix, & Schneidervin 2011) taxa, so the organisms retained in our 2 mm sieve represent the size fractions most vulnerable to trout predation.

Body length, excluding antennae, cerci or other appendages, was measured (± 0.1 mm) for the first 20 individuals of each LFTL in each sample, with the exception of gastropods, for which we measured shell length. All subsequent individuals were counted. Length-to-mass relationships were used to generate biomass (dry weight in mg) from body and shell lengths (Wrona 1982; Leeper & Taylor 1998; Benke*, et al.* 1999; Baumgärtner & Rothhaupt 2003, P. Venturelli, University of Minnesota, personal communication). Abundance and biomass were calculated for each LFTL for each sample and divided by the distance of the sweep to generate response variables of individuals m^{-1} sweep (abundance) and mg m^{-1} sweep (biomass).

Trout stomach contents

In May - August of 2008 and 2009, the first two years after stocking Fiesta Lake, we angled to catch trout and obtain stomach contents. Immediately after capture, trout were briefly placed in an anesthetic mixture (approximately 50 mg/L of clove oil and water), and gastric lavage, which can extract nearly 100% of stomach contents from trout (Light, Adler & Arnold 1983), was used to remove recently ingested prey. Prey items were stored on ice in the field and then frozen until laboratory analysis. In the laboratory, prey items were identified to the LFTL and enumerated and measured using a procedure similar to that described above for invertebrate sweep samples. Lengths and abundances of trout prey were used to estimate predation pressure exerted by trout on the invertebrate assemblage of Fiesta Lake.

Statistical Analyses

From invertebrate sweeps, 95 LFTL groups were identified across all lakes and years. However, because many taxa were rare or occurred sporadically, we combined many groups to the family or order level, creating 36 categories. We used two multivariate approaches to analyze invertebrate assemblages. First, we used the Nonmetric Multidimensional Scaling (NMS) Scores procedure (Peck 2010) in PC-ORD (Version 6, McCune & Mefford 2011) to summarize and compare invertebrate assemblages based on abundance and biomass in Fiesta Lake and three control lakes through time. We plotted NMS scores of the three controls lakes and generated convex polygons to enclose the assemblages of the three control lakes in each year, creating an annual "control lake space." We then determined where Fiesta Lake's assemblage was located relative to the control lake space for each year. If Fiesta Lake's assemblage changed after stocking in a manner different than the control lakes' assemblages, Fiesta Lake should diverge from the control lakes' ordination space (e.g., Jones, Scrimgeour & Tonn 2008). Ordination plots for biomass and abundance were rotated using the Varimax procedure in PC-ORD (Peck 2010) to represent visually Fiesta Lake and control lakes most effectively. When a three-axis solution was recommended, we chose to represent graphically the pair of axes that explained the most variation and yielded the clearest visual representation.

Second, to quantify multivariate changes in Fiesta Lake assemblages relative to the three unstocked lakes, we used Redundancy Analyses in CANOCO (version 4.5, ter Braak & Similauer 2006) in a BACI framework. Specifically, we tested for a time*treatment interaction using abundance and biomass (e.g., Leps & Šmilauer 2003).

Abundance and biomass data were log(x+1) transformed before analyses in both PC-ORD and CANOCO.

To test univariate responses in abundance, biomass, and length of LFTL groups, we used linear mixed models (SPSS for Mac Version 17) with “year” as a repeated factor and “lake” as a random factor to test for a significant statistical interaction between treatment (Control vs. Impact) and time period (Before vs. After). Because occurrence and sample sizes for some LFTL were low, we tested only those groups that occurred in at least one year in each lake; thus 27 taxa were analyzed for abundance and biomass. For length, we excluded three additional taxa due to low sample size. We also used linear mixed models to compare changes in total abundance and biomass of all invertebrates, of “nektonic” and “benthic” invertebrates, and of “microarthropods” in Fiesta Lake vs. control lakes (for taxa assignments, see Table 4-3).

For trout stomach content samples, 2 x 2 χ^2 contingency table tests were used to determine if the occurrence of the top 10 prey items in trout diets differed between 2008 and 2009, and t-tests were used to determine if the lengths of the top 10 prey items from trout stomachs differed between the two years.

Statistical results were considered significant when $p< 0.05$ and marginally significant when $0.05 < p < 0.10$. We did not correct alpha values for the use of multiple univariate BACI tests to assess patterns of abundance and biomass. We felt that lowering the alpha value was too conservative when investigating human-mediated impacts known to be severe in other systems (Dunham et al. 2004, Eby et al. 2006), especially when data are analyzed at the whole-lake scale. Other studies investigating impacts of trout have taken a similar approach (Schilling et al. 2009, Drouin et al. 2011). Assumptions of normality and homogeneity of variance for linear mixed models were confirmed in SPSS 17.0 for Mac.

Results

Littoral invertebrate Assemblage Analyses

Using invertebrate abundance, NMS ordination recommended a three axes solution with a final stress level of 6.5 (Monte Carlo permutation, p= 0.02, Figure 4-1a). The three axes explained 84.7% of the variation in the data. In 2007, the unstocked Fiesta Lake fell within the ordination space of the three control lakes, but in 2008 (the first year after stocking), Fiesta Lake diverged from the control lake space, driven primarily by higher abundances of Corduliidae and *Gyraulus* (Figure 4-1b) in Fiesta Lake. However, in 2009, Fiesta Lake once again occurred within the ordination space of the three control lakes, indicating that its invertebrate assemblage was again similar to those in control lakes. Overall, the multivariate BACI analysis did not detect a significant time*treatment interaction for invertebrate assemblage composition (Monte Carlo permutation (n= 499), F= 1.25, p= 0.50).

For invertebrate biomass, a three axes solution was recommended with a final stress value of 4.2 (Monte Carlo permutation; p= 0.02, Figure 4-1a). The three axes explained 31% of the variation of the dataset. In 2007, Fiesta Lake was situated marginally outside of the control lake space, but in 2008, Fiesta Lake was located far from the ordination space of the control lakes. This distance decreased somewhat in 2009, primarily due to a shift in the position of the control lakes’ space (Figure 4-1). The multivariate analysis of the time*treatment interaction was significant for biomass

(Monte Carlo permutation (n= 499), F= 1.91, p= 0.038) and appeared to be driven primarily by post-stocking increases in biomass of phryganeid caddisflies and sphaeriid clams in Fiesta Lake (Figure 4-1b).

Our group-level BACI analyses found no significant (p> 0.10) treatment*time interactions for either total abundance or biomass for the entire invertebrate assemblages (Table 4-3). There were also no treatment*time interactions for abundance and biomass of nektonic or benthic littoral invertebrate habitat groups or the microarthropod group (Table 4-3).

Analyses of Individual Invertebrate Taxa

Three LFTL— erpopdellid leaches, lymneid snails, and *Lestes* damselflies— were present in Fiesta Lake samples before stocking but absent after stocking. However, each of these taxa was also present, then absent, in sweep samples during the same time period for at least one control lake.

Only two of 27 taxa showed significant treatment*time interactions for abundance (Table 4-3). Both valvatid snails and Zygoptera increased in abundance to a greater degree in Fiesta Lake after stocking compared to control lakes (Figure 4-2, Table 4-3). For biomass, sphaeriid clams and valvatid snails increased significantly more and phryganeid caddisflies increased marginally more in Fiesta Lake after stocking relative to control lakes (Figure 4-3, Table 4-3). The remaining 24 taxa showed no significant treatment*time interactions (Table 4-3).

Several of the 24 LFTL groups examined displayed significant or marginally significant time*treatment interactions for length (Table 4-3). Lengths of Copepoda, Sphaeriidae, Zygoptera, and Caenidae increased more, or decreased less, in Fiesta Lake after stocking relative to control lakes (Figure 4-3, Table 4-3). In contrast, Chironomidae, Haliplidae, *Hyalella*, and Notonectidae either decreased or showed less of an increase in Fiesta Lake after stocking relative to control lakes (Figure 4-3, Table 4-3).

Trout Abundance and Stomach Contents

We captured 64 trout from Fiesta Lake (mean total length= 331 mm, range= 242 - 382 mm) in 2008 and 88 trout (mean length= 380 mm, range= 263 - 470 mm) in 2009. These 152 trout consumed 8,384 invertebrates and 341 forage fish; only two stomachs were empty in 2008 and zero in 2009. Among invertebrate prey, the top 10 prey taxa by numbers comprised 98.2% of individuals consumed (Table 4-2). The most commonly consumed invertebrate prey, cladocerans, comprised 70.3% of all invertebrates consumed over both years, whereas the tenth-ranked prey, physid gastropods, comprised 0.7% of all invertebrates consumed (Table 4-2). The occurrence of some top prey items varied significantly between years, with Hirudinea ($\chi_1^2= 8.45$, $p< 0.01$) decreasing and Cladocera ($\chi_1^2= 4.58$, $p< 0.05$) and Planorbidae ($\chi_1^2= 6.28$, $p< 0.025$) increasing from 2008 to 2009 (Table 4-2). In addition, consumed chironomids were significantly smaller in 2009 relative to 2008 (two-tailed t-test, $t_{207}= 5.0$, $p< 0.01$) and planorbid snails were marginally smaller in 2009 relative to 2008 (two-tailed t-test, $t_{251}= 1.9$, $p= 0.063$; Table 4-2).

Of the eight taxa from invertebrate sweeps that displayed significant time*treatment interactions for length, only one, Haliplidae, was never found in trout stomachs. However, for the other seven taxa, the relative sizes in stomachs of trout in

Fiesta Lake were not always consistent with changes in average length of these taxa in the lake after stocking. For example, trout ate relatively larger individuals than those found in invertebrate sweeps for Zygoptera, Notonectidae, Chironomidae, and Caenidae, individuals relatively smaller than those in sweep samples for Copepoda, and individuals similar to the mean size for Sphaeriidae and *Hyalella* (Figure 4-3). Of these taxa, Chironomidae and Copepoda were also among the top 10 prey items consumed by trout (Table 4-2), and their changes in average length were consistent with size-selective predation by trout (Figure 4-3).

Discussion

Unlike the strong negative effects of trout commonly reported (reviewed in Dunham*, et al.* 2004; Eby*, et al.* 2006), stocking in our study lakes resulted in minimal negative effects on littoral invertebrates. Indeed, the few changes in littoral assemblages and taxa were typically driven by increases in abundance or biomass. Although this may initially be surprising, several aspects of productive, fish-bearing lakes likely buffer against the types of negative effects reported elsewhere.

In contrast to invertebrates from fishless lakes that are naïve and especially vulnerable to predation from introduced trout (Knapp*, et al.* 2005; Cox & Lima 2006), the invertebrate assemblage in Fiesta Lake was likely already shaped by native fishes before trout stocking. Small-bodied fishes are capable of structuring invertebrate assemblages (Vinebrooke*, et al.* 2001; Zimmer, Hanson & Butler 2001), and invertebrate populations that coexist with fish often exhibit antipredatory behavior (Stoks & McPeek 2003) and altered life histories (Wellborn 1994). Abundance estimates of native fishes in Fiesta

Lake during our study exceeded 100,000 individuals. Such large populations of native fish are not uncommon in our stocked study lakes (Nasmith*, et al.* 2010; Hanisch*, et al.* 2012), which have been chosen for stocking based partly on their ability to support fish populations (e.g., Pope 2008). Thus, trout introduced on top of preexisting native fish populations likely had little additional effect on native invertebrates. Amphibian populations from our study lakes also appeared to be structured by a more general "fish effect" rather than by a "trout effect" (Schank*, et al.* 2011). Similarly, in Maine, USA, effects of introduced Brook Trout (*Salvelinus fontinalis*) on invertebrate populations were less severe in lakes supporting naturalized populations of small-bodied fishes than in nearby fishless lakes (Schilling*, et al.* 2009).

Additionally, the generalized diet of trout in Fiesta Lake likely resulted in diffuse predation pressure across the invertebrate assemblage. Although the majority of trout prey items by number were cladocerans, trout in Fiesta Lake preyed on a variety of functional groups, including forage fish, pelagic, littoral, and benthic taxa, large and small-bodied taxa, as well as highly mobile and relatively sedentary groups. As in other systems where omnivory dampens effects on prey assemblages (e.g., Fagan 1997; Eriksson*, et al.* 2011), trophic generalization by trout in Fiesta Lake likely resulted in predation pressure spread across the prey community, with fewer significant effects on individual taxa or groups.

Unlike the majority of studies where abundance or biomass of invertebrates decreased in the presence of non-native trout (e.g., Luecke 1990; Schindler 2000; Martinez-Sanz, García-Criado & Fernández-Aláez 2010), the few taxa affected in our study increased in abundance and/or biomass after stocking relative to unstocked control lakes. The assemblage-level effect of trout on invertebrate biomass and abundance was most pronounced in the first summer after stocking and appeared to be driven primarily by increases in certain taxa. For abundance, this effect appears to have disappeared in the second post-stocking year, as the assemblage of Fiesta Lake once again fell within the ordination space of the control lakes. Although we found evidence that the effects of trout stocking on assemblage-level biomass may be sustained longer than for abundance, comparative studies involving productive fish-bearing lakes that have been stocked for many years suggest that convergence in biomass will eventually occur (Wissinger*, et al.* 2006; Nasmith*, et al.* 2012).

It is not immediately clear why the biomass or abundance of some taxa should increase after stocking. Because the few taxa that responded were not consumed in great numbers by trout, and two of these taxa (Valvatidae and Sphaeriidae) are primary consumers, it is possible that indirect effects of trout, including trophic cascades, triggered these responses (e.g., Potthoff*, et al.* 2008; Tronstad*, et al.* 2010). However, complex littoral habitats in productive lakes do not consistently support trophic cascades (Strong 1992; but see Jones and Sayer 2003). We also did not observe significant consumption by trout of likely invertebrate predators of these two mollusks, nor did the abundance of any predator decrease significantly. We cannot rule out, however, indirect effects on invertebrate behavior that could have resulted in the observed increase in abundance in littoral fauna (e.g., taxa concentrated within inshore refugia).

It is also possible that overwinter aeration, used in Fiesta Lake to increase oxygen levels and promote overwinter survival of trout, promoted the growth and survival of aquatic invertebrates, especially benthic forms with limited mobility like mollusks.

These productive foothills lakes can become hypoxic over winter, and low oxygen levels are known to reduce growth and survival of aquatic invertebrates, including leeches (Davies*, et al.* 1992), clams (Holopainen 1987), and gastropods (Liu*, et al.* 2011). Few studies have tested the effects of lake aeration on invertebrate assemblages (Dinsmore & Prepas 1997a, b), and some evidence exists that aeration alone can increase the abundance and/or biomass of native organisms (Nasmith*, et al.* 2010; Aku & Tonn 1999). Although we documented no strong negative effects on invertebrate biomass or abundance, trout predation may have altered the size structure of populations of some taxa. Eight taxa showed a treatment*time interaction, and two of these were among the ten taxa most frequently consumed by trout. Chironomid larvae showed the strongest evidence of size-selective predation by trout. Not only did the lengths of chironomids decrease after stocking in Fiesta Lake relative to control lakes, lengths of chironomids in trout stomachs decreased significantly in 2009 relative to 2008.

Trout stomach contents also suggested an additional potential direct effect of trout. Although we did not detect changes in the leech populations of Fiesta Lake after stocking relative to the control lakes, about half as many trout had leeches in their stomachs in 2009 relative to 2008 (20.5% vs. 43.8%, respectively). Leeches are especially vulnerable to fish predation (Venturelli & Tonn 2006), and the reduced consumption of leeches by trout in the second year of stocking may indicate decreased availability of leeches as food for trout.

Study designs for detecting impacts of introduced fishes

Conservation biologists use a diversity of study designs to evaluate ecological impacts, such as the effects of species introductions on native biota. We used a whole-lake BACI design with three unstocked "control" lakes and one "impact" lake to assess the effects of introduced trout on littoral macroinvertebrates over a three-year period. Using this BACI design, we identified relatively subtle effects comprising short-term changes in assemblage structure, increases in biomass/and or abundance of five taxa, and evidence of size-selective predation by trout on two taxa. Using a Control-Impact (CI) design in a suite of 11 lakes, including the then-unstocked Fiesta Lake and all other lakes in our study, Nasmith *et al.* (2012) also identified relatively subtle effects of trout on native benthos. Although both studies found relatively weak effects of trout, the specific taxa impacted and the directions of effect were not identical. Of 17 individual taxa tested by Nasmith *et al.* (2012), copepods and mites were more abundant in unstocked lakes, and Ephemeroptera, Brachycera, and Nematocera were more abundant in stocked lakes. Interestingly, however, Nasmith *et al.* (2012) also showed evidence of size-selective predation by trout on Chironomidae, indicating that in boreal foothills lakes, chironomids may be the taxon most susceptible to direct effects of trout. Taken together, these two studies indicate that initial effects of trout stocking may eventually give way to limited sustained effects on the abundance of a few taxa, which may not be the taxa initially affected by trout. Although the taxon-level results of the BACI and CI designs in our study lakes differed somewhat, both studies concluded that the impact of trout in boreal foothills lakes was minimal. Thus, well-designed CI studies offer a longer-term complementary approach to detecting impacts of introduced fishes.

Predicting the impacts of introduced species in freshwaters is notoriously difficult (Vander Zanden & Olden 2008). Our understanding of the responses of native fauna to trout presence is incomplete, and studies sometimes identify effects that cannot be readily explained (e.g., increases in copepod length after stocking in this study). Such "surprising" results could be real, perhaps indirect, effects of trout introduction, or "false positives," that coincided with, but were not caused by, trout introduction. Whole-lake experiments like ours are effective for detecting realistic impacts (e.g., Schindler 1998) but are not necessarily designed to pinpoint specific mechanisms underlying observations. Meta-analysis of the strengths and consistency of responses of all taxa included in ecosystem-level studies of fish introduction may offer a valuable tool for investigating mechanisms underlying patterns detected by such studies (e.g., Kornis, Mercado-Silva, and Vander Zanden 2012), especially when paired with smaller-scale experiments (e.g., Hanisch et al. 2012).

Productive boreal foothills lakes are characterized by natural variability, including considerable interannual variation in amphibian (Schank et al. 2011), fish (Nasmith et al. 2010) and invertebrate populations (Nasmith et al. 2012). Similarly, our control lakes also exhibited interannual variation in both abundance and biomass of invertebrates. For the effects of stocked trout to be significant (both statistically and biologically), their impact has to be large and additive beyond the range of natural variability. In the instances where we did not detect significant BACI effects, any "undetected impact" of trout can be considered as no greater than the natural background level of variation inherent to the system. To potentially increase detectability of impacts at smaller effect sizes in similarly variable environments, researchers and management agencies could conduct MBACI-style experiments with multiple newly stocked lakes and appropriately paired unstocked control lakes.

As noted, our results contrast with the strong negative effects of stocked trout on invertebrate assemblages commonly reported from headwater lakes (Carlisle & Hawkins 1998; Anderson 1980; Epanchin, Knapp & Lawler 2010). We suggest that a combination of factors, including robust populations of native fishes, trophic omnivory of trout, overwinter aeration, relatively high productivity, and ample littoral refuge in the form of dense macrophyte beds, likely work in concert to reduce potentially negative effects of stocked trout in these systems (e.g., Nasmith*, et al.* 2010; Hanisch*, et al.* 2012). Documenting the effects of trout in a system like ours provides valuable insights into factors controlling impacts of introduced aquatic species (e.g., Gozlan 2008; Leprieur*, et al.* 2009).

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Table 4-1. Native fish communities, estimated trout and dace densities, and limnological characteristics (range of monthly values from 2007 - 2009) of boreal foothills lakes sampled one year before (2007) and for two years after (2008 - 2009) Fiesta Lake was stocked with Rainbow Trout (*Oncorhynchus mykiss*). For fish communities, D= dace (*Chrosomus* spp.), F= Fathead Minnow (*Pimephales promelas*), B= Brook Stickleback (*Culaea inconstans*) and P= Pearl Dace (*Margariscus margarita*). *Values from 2008 - 2009. **Value from 2006.

Lake Name (Abbreviation)	Fiesta (FI)	Dog Leg (DL)	Gas Plant (GP)	Picard (PI)
Treatment	Stocked	Unstocked	Unstocked	Unstocked
Fish species	D,F,B,P	D,F,B	D,F,B	D,F,B
Trout stocking density (fish ha^{-1})	77 - 268*	-------	-------	-------
Forage fish density (fish ha^{-1})	15,772 - 17,876	3240 - 8737	3614 - 4402*	4602**
Surface area (ha)	6.6	6.7	17.5	8.7
Secchi depth (m)	2.2 - 2.6	1.9 - 2.2	1.5 - 1.9	2.5 - 2.7
Maximum depth (m)	7.0	5.0	4.0	4.0
Chl a (µg L^{-1})	0.85 - 2.42	3.50 - 14.53	5.43 - 7.5	1.89 - 4.66
Total phosphorus (µg L^{-1})	17 - 22	29 - 52	37 - 43	23 - 26
Total nitrogen (µg L^{-1})	624 - 847	809 - 1080	739 - 916	868 - 1080
pH	7.6 - 7.8	7.2 - 7.5	7.4 - 7.6	7.5 - 7.8

Table 4-2. Number of organisms consumed and percentage of trout diet based on abundance for the top ten invertebrate prey of 152 Rainbow Trout in Fiesta Lake sampled during the first two years after stocking (2008 - 2009). Also presented are frequency of occurrence (%) and average lengths (mm, ± 1 SE) of invertebrate prey consumed in 2008 (n= 64 trout) and 2009 (n= 88 trout). χ^2 contingency table tests and t-tests were used to test for differences in occurrence and average lengths, respectively, between years. Results in ***bold/italic*** indicate significant results ($p< 0.05$), and length in **bold** indicate marginally significant results ($p< 0.10$).

	First two years after stocking (2008 - 2009)		Frequency of Occurrence (%)		Average Length (mm) in trout stomachs	
Taxa	Number	Percentage of total	2008	2009	2008	2009
Cladocera	5898	70.3	***25.0***	***43.2***	1.5 ± 0.0	1.6 ± 0.0
Chaoboridae	794	9.5	31.3	25.0	8.7 ± 0.1	8.2 ± 0.1
Planorbidae	663	7.9	***20.3***	***40.9***	**7.1 ± 0.2**	**6.0 ± 0.2**
Chironomidae	388	4.6	40.6	38.6	***8.4 ± 0.3***	***6.3 ± 0.2***
Trichoptera	107	1.3	18.8	29.5	19.3 ± 1.7	21.4 ± 0.7
Hydrachnidia	106	1.3	10.9	17.0	0.4 ± 0.1	0.3 ± 0.1
Hirudinea	83	1.0	***43.8***	***20.5***	60.1 ± 3.3	53.9 ± 4.9
Copepoda	77	0.9	9.4	19.3	1.4 ± 0.1	1.6 ± 0.2
Anisoptera	75	0.9	23.4	19.3	18.7 ± 1.2	15.2 ± 1.1
Physidae	57	0.7	10.9	3.4	11.1 ± 0.3	11.2 ± 1.6

Table 4-3. Results of univariate BACI analyses of abundance and biomass of total (all groups combined), habitat groups (combined taxa found in benthic and open water habitats), one microarthropod group, and individual taxonomic groups of littoral macroinvertebrates from Fiesta vs. three control lakes (Treatment) and before vs. after stocking (Time). Significant ($p < 0.05$) Treatment*Time interactions are in ***bold/italic*** and marginally significant ($0.05 < p < 0.10$) interactions are in **bold**. Dashed lines indicate taxa not analyzed in a particular comparison (see Methods).

Taxa	Habitat/Group	Abundance	Biomass	Length
Total of All Taxa	F	$_{3,4.9}$= 0.38, p= 0.77 F	$_{3,2.6}$= 5.2, p= 0.12	-----------
Total Benthic	F	$_{3,3.8}$= 0.48, p= 0.72 F	$_{3,3.0}$= 2.4, p= 0.25	-----------
Total Nektonic	F	$_{3,3.0}$= 4.3, p= 0.75 F	$_{3,4.5}$= 1.7, p= 0.20	-----------
Total Microarthropod	F	$_{3,4.2}$= 0.47, p= 0.72 F	$_{3,4.6}$= 1.1, p= 0.44	-----------
Naididae	Benthic F	$_{3,5.8}$= 1.2, p= 0.40 F	$_{3,6.1}$= 0.60, p=0.64 F	$_{3.3.6}$= 0.60, p= 0.67
Glossiphoniidae	Nektonic F	$_{3,5.2}$= 1,5, p= 0.32 F	$_{3,4.3}$= 2.4, p= 0.20 F	$_{3,6.1}$= 0.50, p=0.70
Sphaeriidae	Benthic F	$_{3,4.9}$= 2.3, p= 0.20	***F$_{3,5.1}$= 9.1 p=0.02***	***F$_{3,2.6}$= 34.9, p= 0.01***
Physidae	Benthic F	$_{3,2.4}$= 1.4= 0.43 F	$_{3,6.2}$= 2.1, p=0.20 F	$_{3,4.6}$= 1.8, p=0.28
Gyraulus	Benthic	F$_{3,6.0}$= 1.2, p=0.38	F$_{3,6.1}$= 0.89, p=0.50	F$_{3,3.5}$= 0.41, p=0.75
Helisoma	Benthic	F$_{3,2.3}$= 3.2, p=0.23	F$_{3,2.2}$= 3.6, p= 0.21	F$_{3,6.0}$= 0.75, p= 0.56
Valvatidae	Benthic	***F$_{3,5.6}$= 46.1, p<0.001***	***F$_{3,2.4}$=127.4, p=0.004*** F	$_{3,2.7}$= 2.1, p=0.79
Hydrachnidia	Microarthropod F	$_{3,5.9}$= 0.32, p=0.81 F	$_{3.4.9}$= 0.18, p=0.90 F	$_{3,4.9}$= 3.3, p=0.11
Cladocera	Microarthropod F	$_{3,4.8}$= 0.36, p=0.79 F	$_{3,5.3}$= 0.44, p=0.74 F	$_{3,4.0}$= 0.36, p= 0.79
Copepoda	Microarthropod F	$_{3,4.9}$= 0.34, p=0.79 F	$_{3,5.3}$= 0.99, 0.47	***F$_{3,3.}$2= 17.5, p=0.018***
Hyalella	Benthic F	$_{3,4.4}$= 0.16, p=0.30 F	$_{3,4.2}$= 3.27, p= 0.14	**F$_{3,3.3}$= 7.02, p=0.06**
Caenidae	Benthic F	$_{3,3.4}$= 1.9, p= 0.30 F	$_{3,5.5}$= 1.1, p=0.44	**F$_{3,2.0}$= 14.5, p=0.06**
Siphlonuridae	Benthic F	$_{3,3.9}$= 0.59, p=0.65 F	$_{3,2.1}$= 0.66, p=0.65 F	$_{3,3.4}$= 0.66, p=0.62
Aeshnidae	Benthic F	$_{3,3.2}$= 0.73, p= 0.60 F	$_{3,4.9}$= 2.5, p=0.18 F	$_{3,3.0}$= 5.2, p=0.11
Corduliidae	Benthic F	$_{3,3.7}$= 2.9, p= 0.17 F	$_{3,5.7}$= 0.78, p=0.97 F	$_{3,5.8}$= 1.3, p= 0.35
Libellulidae	Benthic F	$_{3,3.0}$= 1.8, p= 0.31 F	$_{3,4.7}$= 0.59, p=0.68 F	$_{3,4.0}$=0.34, p=0.80
Zygoptera	Benthic	***F$_{3,4.1}$= 26.7, p=0.004*** F	$_{3,3.8}$= 2.9, p=0.83	**F$_{3,5.8}$= 3.7 p= 0.08**
Notonectidae	Nektonic F	$_{3,5.3}$= 2.8, p=0.14 F	$_{3,3.4}$= 2.2, p=0.26	**F$_{3,2.8}$= 10.1, p=0.07**
Gerridae	Nektonic F	$_{3,5.6}$= 0.96, p=0.48 F	$_{3,6.1}$= 1.7, p= 0.26	-----------
Corixidae	Nektonic F	$_{3,2.3}$= 1.1, p= 0.49 F	$_{3,2.4}$= 0.85, p= 0.57 F	$_{3,4.4}$= 1.80, p=0.28
Leptoceridae	Benthic F	$_{3,5.1}$= 1.1, p=0.44 F	$_{3,4.2}$= 0.79, p=0.56	-----------
Limnephilidae	Benthic F	$_{3,7.8}$= 1.0, p= 0.48 F	$_{3,3.9}$= 1.7, p= 0.31 F	$_{3,2.3}$= 2.57, p= 0.27
Phryganeidae	Benthic F	$_{3,2.9}$= 5.4, p= 0.10	**F$_{3,3.1}$= 6.8, p=0.07**	-----------
Dytiscidae	Nektonic F	$_{3,5.1}$= 2.1, p= 0.21 F	$_{3,4.8}$= 0.07, p= 0.97 F	$_{3,3.8}$= 3.94, p= 0.11
Haliplidae	Benthic F	$_{3,4.9}$= 0.49, p=0.70 F	$_{3,3.37}$= 1.03, p= 0.47	**F$_{3,5.2}$= 4.5, p=0.06**
Chironomidae	Benthic F	$_{3,4.4}$= 0.68, p= 0.61 F	$_{3,5.2}$= 1.49, p=0.32	**F$_{3,6.0}$= 4.2, p= 0.06**
Ceratopogonidae	Benthic F	$_{3,5.1}$= 0.18, p=0.90 F	$_{3,5.1}$= 3.63, p= 0.98 F	$_{3,6.7}$= 0.14, p=0.93

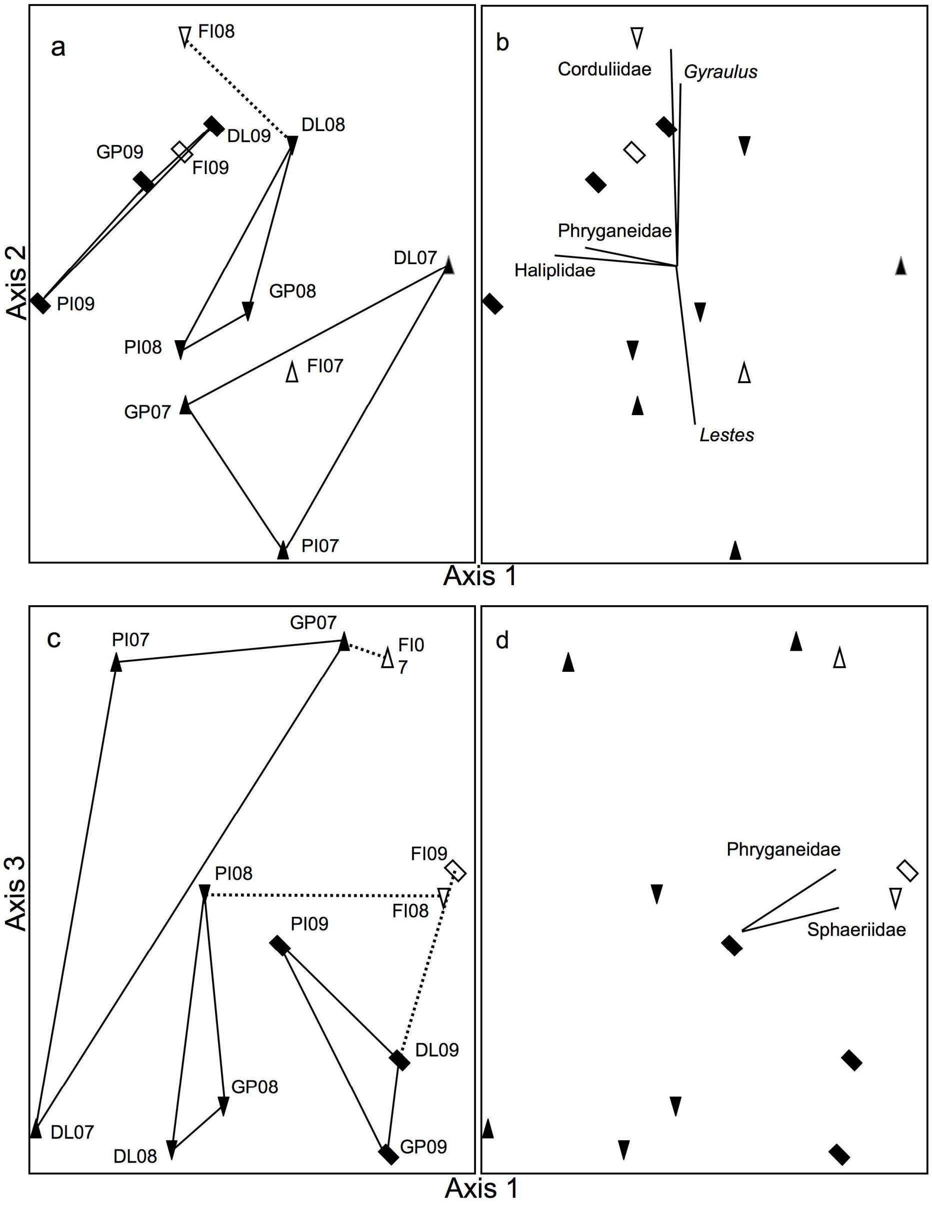

Figure 4-1. Nonmetric multidimensional scaling joint plots for littoral macroinvertebrate assemblages, based on abundance (a,b) and biomass (c,d), from three control lakes (closed symbols; polygons) and Fiesta Lake (open symbols) before trout stocking (2007; upward-pointing triangles) and after stocking (2008; downward pointing triangles, 2009; rectangles). Polygons in (a,c) represent "control lake space" and dashed lines indicate distance of the Fiesta Lake assemblage from control lake space in each year. Vectors in (b,d) indicate the direction and extent of increasing abundance of invertebrate taxa having $r^2 > 0.50$. Abbreviations: FI= Fiesta, DL= Dog Leg, GP= Gas Plant, PI= Picard; 07= 2007, 08= 2008, 09= 2009. Axes 1 and 2 (a,b) represent 65% of the variation in the data. Axes 1 and 3 (c,d) represent 22% of the variation in the data.

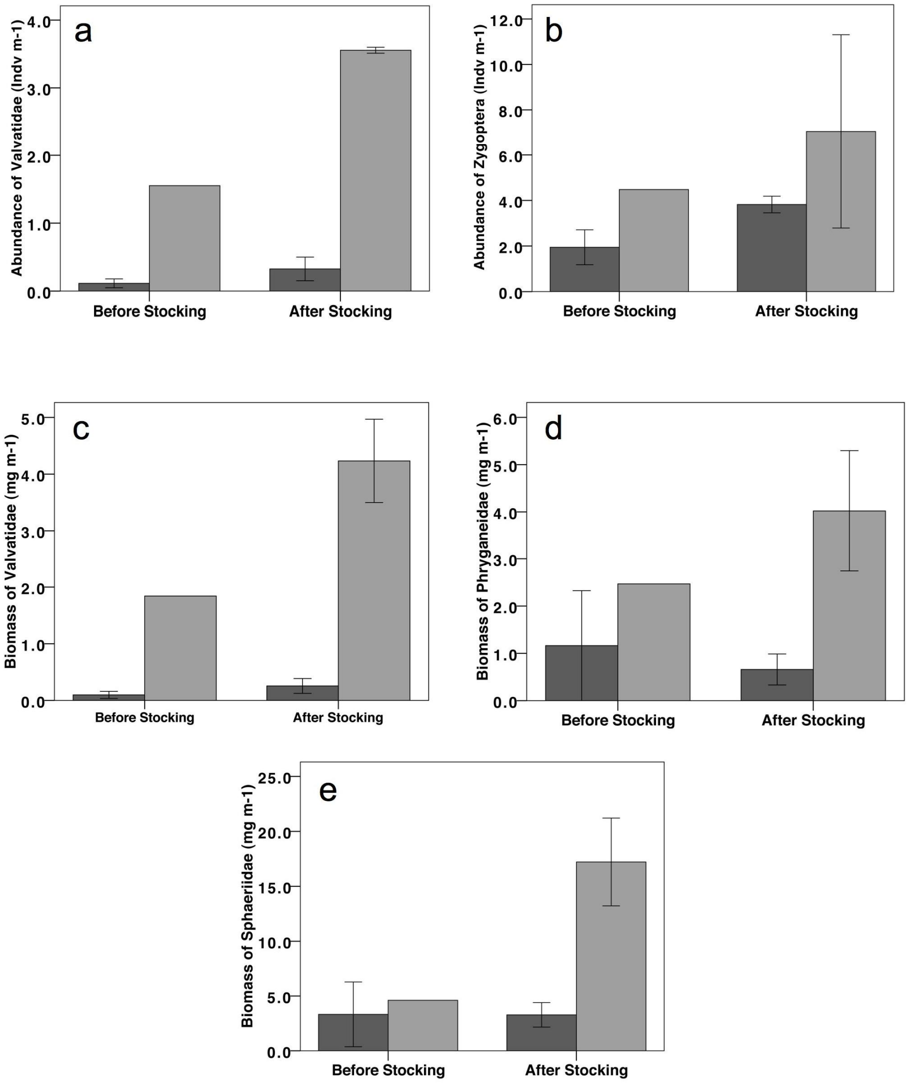

Figure 4-2. Mean (± 1 SE) abundance per 1 m sweep of Valvatidae (a) and Zygoptera (b) and mean (± 1 SE) biomass per 1 m sweep of Valvatidae (c), Phryganeidae (d), and Sphaeriidae (e) before (2007) and after (2008, 2009) trout stocking in Fiesta Lake (light bars) and three control lakes (dark bars). Standard errors represent variation around lake year means. Only taxonomic groups with significant or marginally significant time*treatment interactions are presented.

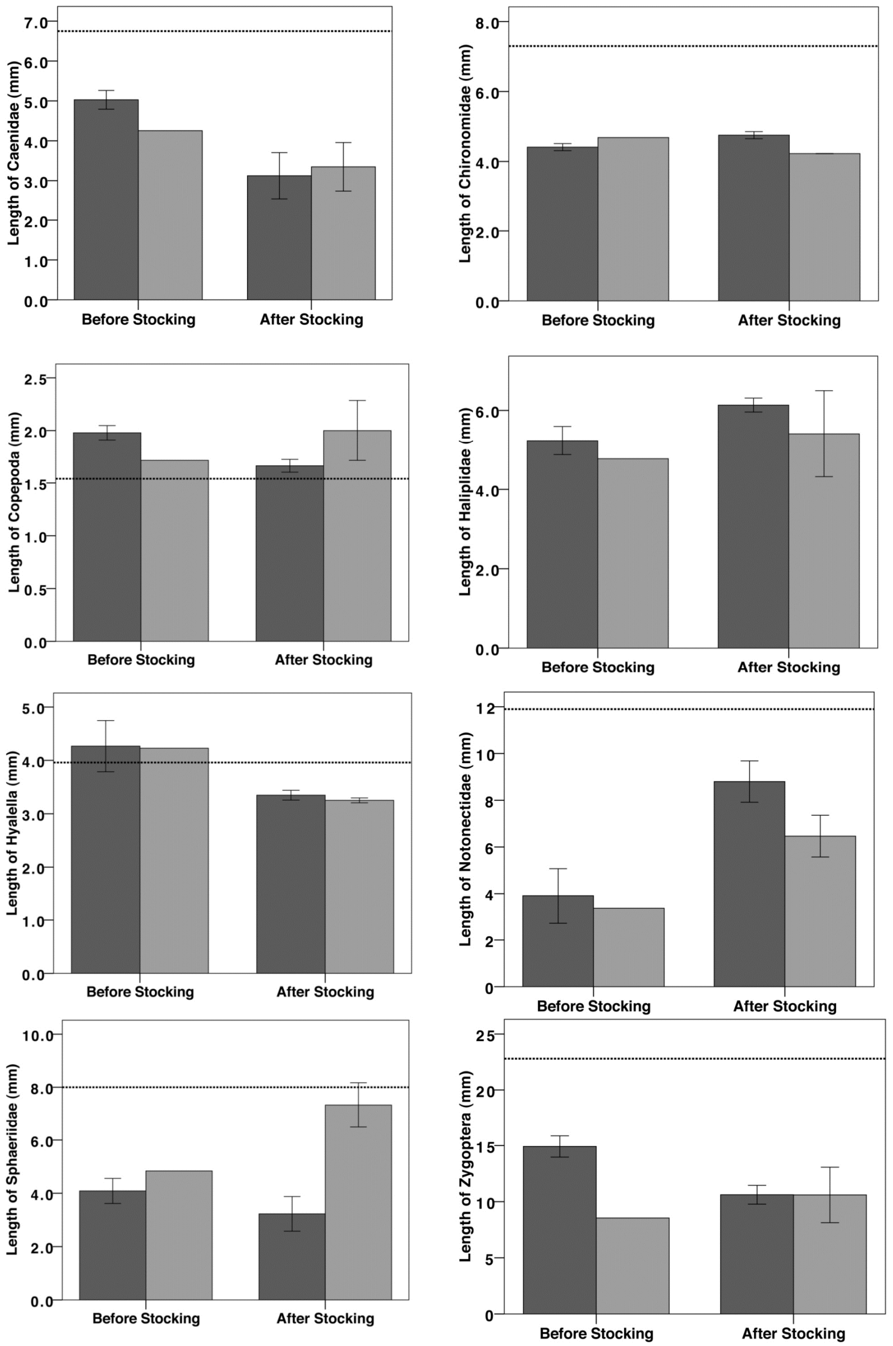

Figure 4-3. Mean (± 1 SE) lengths of eight taxa from before (2007) and after (2008, 2009) trout stocking in Fiesta Lake (light bars) and three control lakes (dark bars). The dashed horizontal line corresponds to the average length of the taxon from Fiesta Lake trout stomachs. No Haliplidae were found in trout stomachs. Standard errors represent variation around lake year means. Only taxonomic groups with significant or marginally significant time*treatment interactions are presented.

Chapter 5. An experimental study of the use of macrophyte beds by aquatic invertebrates as a function of plant density and the presence of small-bodied fish

Abstract

Aquatic macrophytes and native forage fishes each play important roles in structuring communities of aquatic invertebrates. Recently, both macrophytes and native fishes have been cited as ameliorating factors that potentially reduce impacts of stocked trout in lakes by providing refuge for native species and by "pre-structuring" invertebrate populations before the introduction of a novel fish predator. To evaluate the ability of macrophytes and forage fish to structure invertebrate populations of productive lakes, I designed a fully-crossed mesocosm experiment that included two macrophyte density treatments (natural and reduced densities) and two forage fish treatments (fish present and fish absent). Invertebrates were sampled from benthic and midwater habitats within each mesocosm, and the effects of both fish presence and macrophyte density were examined for the abundance and length of invertebrate taxa. Invertebrates were frequently found in higher abundance in natural density macrophyte mesocosms independent of fish presence, especially for taxa from midwater samples. Fish presence occasionally affected invertebrate abundance, with taxa typically found in higher abundance in fish-absent mesocosms. Neither macrophytes nor fish had an impact on length of individual invertebrate taxa. Both macrophytes and forage fishes were able to shape the abundance of invertebrates in a boreal foothills lake, and this "pre-structuring" effect may help explain the minimal impacts of trout stocked into productive, fish-bearing lakes.

Introduction

Aquatic macrophytes play important roles in structuring communities of aquatic organisms (Carpenter and Lodge 1986). In particular, the complex habitat that littoral macrophytes provide lowers the risk of predation relative to more open-water habitats for many organisms (Sass et al. 2006, Dupuch et al. 2009); consequently, macrophytes function as spatial refuge for many taxa, including amphibians (Walls 1995), macroinvertebrates (Luecke 1990, Harrison et al. 2005), zooplankton (Burks et al. 2002), and fish (Stuart-Smith et al. 2007).

The effectiveness of macrophytes as refuge can be illustrated by studies involving stocked trout. Salmonids, and especially Rainbow Trout (*Oncorhynchus mykiss*), are commonly stocked outside of their native ranges to create or enhance recreational angling opportunities (Halverson 2008). However, strong negative impacts of trout on native species are frequently observed after stocking, especially in alpine lakes lacking dense macrophyte cover (e.g., Dunham et al. 2004, Knapp et al. 2005, Eby et al. 2006). Research into potential effects of trout in other systems, such as lower-elevation, productive lakes, has documented limited effects of trout (e.g., Wissinger et al. 2006, Schank et al. 2011, Nasmith et al. 2012, Hanisch et al. 2013). These multi-lake studies, as well as those focused on lakes stocked with a predator (e.g., Shriver et al. 1995, Sass et al. 2006, Stuart-Smith et al. 2007), suggest that macrophytes buffer effects of trout by providing native organisms refuge from predation.

As research on impacts of stocked trout has expanded beyond highly vulnerable ecosystems, other factors that structure prey populations, and thus alter the intensity of trout effects, have been identified (Schank et al. 2011). For example, effects of trout stocked into naturally fishless lakes have more severe effects than those in similar lakes with native small-bodied fishes (Schilling et al. 2009a), suggesting that those native fishes may “pre-structure” prey populations prior to the introduction of trout (Nasmith et al. 2012, Hanisch et al. 2013). However, because macrophytes and native forage fish are typically not independently distributed among lakes, teasing apart the effects of habitat structure and native predators on prey species via multi-lake surveys is difficult. Experimental studies at meaningful ecological scales are often required to gain insights under such circumstances (e.g., Schriver et al. 1995, Kovalenko et al. 2010).

To investigate the structuring role of macrophytes and forage fish on littoral invertebrate populations, I designed a mesocosm experiment that examined the effects of manipulating forage fish presence and macrophyte densities. Specifically, I created a fully-crossed experiment with two macrophyte density treatments (natural and reduced densities) and two forage fish treatments (fish present and fish absent). I analyzed the abundance and length of invertebrate taxa from mesocosms as well as the composition of invertebrate communities based on abundance. If macrophytes provide refuge from predation by fish, I predicted that abundance of invertebrates should be greater in natural macrophyte-density treatments relative to reduced macrophyte-density treatments. Similarly, if forage fish structure invertebrate populations, I predicted that invertebrate abundance would be greater in fishless treatments than in fish-present treatments. For community composition, I predicated that significant differences would occur between both macrophyte treatments and fish treatments. I also predicted that when fish significantly affect the size structure of invertebrate populations, invertebrates would be relatively smaller in treatments with fish present (e.g., Vinebrooke et al. 2001, Zimmer et al. 2001).

A statistical interaction between treatments would indicate that the responses of invertebrates to fish presence are not constant across macrophyte treatments. When interactions occur, I predicted that they would be caused by fish presence having relatively stronger effects in reduced macrophyte density treatments relative to natural density treatments.

Results from this research should elucidate relationships between invertebrate populations, native small-bodies fishes, and macrophytes and therefore help evaluate the hypothesis that macrophytes and forage fish can structure invertebrate populations in productive lakes (e.g., Wissinger et al. 2006, Nasmith et al. 2012, Hanisch et al. 2013) and influence the effects of stocked trout on native species. Thus, my results will inform fisheries practices in areas where stocking lakes with trout or other top predators is being considered.

Methods

Study area

Dog Leg Lake, located near Caroline, Alberta, Canada (52°05'N, 114°44'W), was identified by the provincial government prior to my study as a candidate lake for trout stocking. Dog Leg Lake is small and mesotrophic (Table 5-1), with highly vegetated littoral margins (≥85% macrophyte coverage), consisting primarily of *Potamogeton* and *Nuphar*; macrophytes provide the majority of littoral cover and other structure, such as coarse woody debris, is rare (Nasmith 2008). The native fish community consists of a dace species complex (*Chrosomus eos*, *C. neogaeus*, and a parthenogenic hybrid between the two), Fathead Minnow (*Pimephales promelas*), and Brook Stickleback (*Culaea* *inconstans*). Distinguishing parental *Chrosomus* individuals from hybrids is impractical under field conditions (Binet and Angers 2005); therefore, I grouped parental and hybrid individuals in one category, hereafter referred to as “dace.” This dace complex was ubiquitous in area lakes and the most abundant fish in Dog Leg Lake (Nasmith et al. 2010); a winterkill in the winter/spring (2009/2010) prior to my experiment had eliminated almost all sticklebacks from Dog Leg Lake.

Experimental Design

I conducted the experiment using 24, 1.8 m x 1.8 m x 1.5 m tall mesocosms constructed of metal t-bars and plastic mesh distributed amongst four treatments. Among the four treatments, two were at “natural” macrophyte densities (75-100% coverage, hereafter abbreviated as “NM”), while two were at “reduced” macrophyte densities (10-20% coverage, hereafter abbreviated as “RM”). I defined macrophyte density as the percentage of the water surface covered by broad-leafed *Potamogeton* (primarily *P. richardsonii and P. gramineus*) and *Nuphar* plants. I standardized densities within treatments by transplanting or clipping plants as necessary. For both macrophyte treatments, half of the mesocosms allowed fish unimpeded access in and out of the mesocosm (25 mm mesh, hereafter abbreviated as “FP” (fish present)) and half were designed to exclude fish > 20 mm total length (3 mm mesh), hereafter after abbreviated as “FA” (fish absent)); young-of-year fish were observed in all mesocosms once they hatched in July. Thus, the experiment included four treatments (Appendix 5-A)—NM-FP, NM-FA, RM-FP, and RM-FA—each of which was replicated six times throughout Dog Leg Lake.

I constructed mesocosms between 17 and 19 May 2010. Littoral zone sites for mesocosm placement were approximately 1 m deep; fish treatments were assigned in an alternating pattern and macrophyte treatments were randomly assigned immediately after mesocosms were constructed (Figure 5-1). Mesocosm bottoms were left open to the natural substrate (with a ca. 30-cm skirt of mesh driven into the sediment around the bottom perimeter to "seal" each mesocosm) and tops were left open to the air. Seals with the lake bottom were manually inspected and additional substrate was used to close any gaps. I placed paired minnow traps inside and outside each FP mesocosm over 18-19 June to establish whether fish were present at similar densities inside and outside mesocosms. During this time, minnow traps were also placed within FA mesocosms to remove fish trapped during the construction of the mesocosms.

Invertebrate sampling

After 11 weeks, each mesocosm was sampled between 13th – 14th August 2010. I took one benthic sample and one midwater sample from each mesocosm in each month with a 30-cm triangular sweep net (500 µm mesh). For benthic sweeps, the net was placed approximately 5 cm into the lake sediment and rapidly swept horizontally for 1 m, thereby collecting benthos and organisms living within the macrophytes ca. 25 cm above the lake bottom. For midwater sweeps, I placed the net just below the lake surface and rapidly swept horizontally for 1 m, thereby collecting invertebrates living within the macrophytes and water column. Samples were washed within the sweep net to remove excess sediment; I also rinsed large pieces of plant matter into the net to remove adhering invertebrates. Net contents were kept fresh in coolers and preserved with 80% ethanol later on the day of sampling.

Laboratory Analysis

Midwater samples were dominated by smaller-bodied invertebrates and were washed through a 0.5 mm sieve. Benthic samples were dominated by larger-bodied individuals and a considerable volume of detritus and were washed through a 2 mm sieve. After washing, I preserved retained organisms in 95% ethanol. Invertebrates were identified to the lowest practical taxonomic level (LPTL; Clifford 1991), which was typically genus for insects and molluscs, and order or family for zooplankton and other arthropods. I measured body length, excluding antennae, cerci or other appendages, (± 0.1 mm) for the first 20 individuals of each LPTL in each sample, with the exception of gastropods, for which I measured shell length. All subsequent individuals were counted. I calculated abundance of each LPTL for each sample, recorded as number of individuals m^{-1} sweep. I identified 112 LPTL groups across all samples. However, because many taxa occurred in very low abundance or sporadically, I combined LPTL groups into 34 higher-level taxonomic groups, typically genus or family.

Statistical Analysis

Using the abundance data, I investigated multivariate patterns in the mesocosm invertebrate communities using Nonmetric Multidimensional Scaling (NMS) ordinations in PC-ORD (McCune and Medford 2011). I conducted separate ordinations for benthic and midwater taxa, using the four-mesocosm types as grouping variables. Ordinations were plotted for benthic and midwater samples, and complex polygons were generated for each mesocosm type. When a three-axis solution was recommended, I focused on the two axes that explained the greatest amount of variation in the dataset. The Multi-response Permutation Procedure (MRPP; McCune and Grace 2002) was used to determine whether significant differences in invertebrate assemblages occurred among mesocosm types across all treatments for the two habitat types.

Two-factor Analyses of Variance (ANOVA) tests were used to determine if macrophyte density and fish presence affected the abundance and length of each LPTL. I tested both main factors and their interaction within each habitat type (i.e., benthic and midwater). An LPTL was excluded from analysis if it did not occur in at least five of 24 mesocosms within a given habitat. This resulted in 17 and 24 individual LPTL tested for abundance and 12 and 24 LPTL tested for length in midwater and benthic habitats, respectively. I excluded additional taxonomic groups from the analysis of length if < 3 individuals were present within a given sample. A single average value for abundance and length for all invertebrate taxa combined in each mesocosm was also included in analysis. This "all-taxa" average was included to detect any small but cumulative effects on individual taxa potentially not detected when analyzing each taxon individually. I evaluated assumptions of normality and homoscedasticity prior to analysis. Assumptions were occasionally violated for some comparisons; however, the F-test used in two-factor ANOVA's is generally robust to deviations from these assumptions, especially when designs are balanced (Harwell 2003, Bathke 2004, Schmider et al. 2010). Thus, analyses were conducted on untransformed data. I considered results of ANOVA's significant when $p < 0.05$.

Multiple, related statistical tests, such as the ANOVA's performed here, present a risk of falsely rejecting a proportion of null hypotheses due to chance alone. This "False Discovery Rate" (rate of Type I errors) can be estimated by applying the Benjamini-Hochberg (B-H) procedure, which calculates the likely proportion of erroneously-rejected null hypotheses from the results of multiple, related tests (Benjamini and Hochberg 1995). I applied the B-H procedure separately to results for abundance and length, assuming an FDR of 20%. The procedure identified two of 30 significant (i.e., uncorrected $p< 0.05$) results for abundance as potential false discoveries and 10 of 11 significant (i.e., uncorrected $p< 0.05$) results for length as potential false discoveries. I have presented results from all significant ANOVA's (i.e., $p< 0.05$) in Tables 5-2 and 5-3; however, only those results considered significant after the B-H procedure are discussed below and represented graphically.

Preliminary analysis that used both abundance and biomass to assess responses of invertebrates in NMS ordinations and for individual taxa yielded similar results. Thus, I chose to present only results for abundance. Data were summarized with line graphs to aid in the interpretation of both main effects and interactions.

Results

There was no significant difference in the number of fish captured in minnow traps placed inside and outside of FP mesocosms during paired samplings for both dace (2-tailed paired t-test; $t_{11}= -0.74$, $p= 0.47$) and Fathead Minnow (2-tailed paired t-test; $t_{11}= -0.33$, $p= 0.75$). Mean (± 1 SE) catch inside mesocosms for dace and Fathead Minnow was 109 ± 19 and 36 ± 13 individuals, respectively, whereas outside catch was 126 ± 13 and 39 ± 9 individuals, respectively. Trapping also confirmed that FA mesocosms were free of fish after the initial removal period until young-of-year cyprinids (< 20 mm total length) were observed in all mesocosms in July.

Multivariate Analysis

For benthic invertebrates, NMS ordination recommended a three-axis solution with a final stress value of 8.51 (Monte Carlo permutation, $p= 0.004$). The three axes explained 70.8% of the variation in the data. Vectors were plotted for taxa where $r^2 > 0.50$, which included Chironomidae, *Hyalella*, and Hydrachnidia. The NM and RM mesocosms overlapped considerably within each fish treatment (Figure 5-2 a), but FA polygons showed much less overlap with FP polygons, especially RM-FP. Across all treatments, however, there was no significant difference in invertebrate composition (MRPP, $t= -1.125$, $p= 0.11$).

For midwater invertebrates, NMS ordination recommended a two-axis solution with a final stress value of 6.13 (Monte Carlo permutation, $p= 0.004$). The two axes explained 93.4% of the variation in the data. Vectors were plotted for taxa where $r^2 > 0.75$, which included Cladocera, *Hyalella*, and Hydrachnidia. Vectors for these taxa showed an increasing abundance along Axis 2, toward both NM mesocosms. Several other taxa (Caenidae, Chironomidae, Copepoda, Notonectidae, and Ostracoda) also showed increasing abundance along Axis 2, toward NM mesocosms, for $0.50 < r^2 < 0.75$; however, these taxa were not plotted to avoid obscuring polygons for NM mesocosms. There was a clear separation along Axis 2 between assemblages in NM and RM mesocosms and also between RM-FP and RM-FA mesocosms, driven by the abundances of the eight taxa listed above. Indeed, MRPP analysis revealed significant differences among treatments (t= -8.35, p< 0.0001). In pairwise comparisons, all treatments differed significantly from each other (p< 0.05), with the exception of NM-FA and NM-FP (t= -0.72, p= 0.21).

Univariate Analysis

Abundance

Benthic samples

Of 24 taxa tested from benthic samples, seven showed significant treatment effects (Table 5-2). Corixidae, Phryganeidae, Sphaeriidae, and Zygoptera were more abundant in FA treatments, and Caenidae, *Hyalella*, and Hydrachnidia were more abundant in NM treatments. Haliplidae displayed a significant interaction between treatments driven primarily by the absence of individuals from RM-FA mesocosms (Figure 5-3). The average abundance across all invertebrate taxa was significantly greater in mesocosms with natural macrophyte density but was unaffected by the presence of forage fish (Figure 5-3).

Midwater samples

Of 17 taxa tested from midwater samples, 16 taxa showed significant treatment effects (Table 5-2). All 16 were more abundant in NM treatments, and fish also affected two taxa. Planorbidae was more abundant in FA treatments, whereas Naididae was more abundant in FP treatments (Figure 5-4a). Naidiade also showed a significant interaction, driven by relatively higher abundance in NM-FP mesocosms compared to NM-FA mesocosms (Figure 5-4a).

Length

Of 24 comparisons for benthic taxa and 12 comparisons for midwater taxa, only a single comparison was significant after the B-H procedure (Table 5-3). The average length of all midwater taxa combined was significantly greater in FA treatments relative to FP treatments (Figure 5-4b).

Comparisons of habitat and main effects

Overall, the abundances of invertebrate taxa were affected by fish presence and macrophyte density much more frequently (25 of 41 taxa) than were their lengths (one of 36 analyses) ($X^2_1 = 29.03$, $p < 0.001$). For abundance, midwater taxa were affected more frequently (16 of 17 taxa) than were benthic taxa (9 of 24 taxa) ($X^2_1 = 13.41$, $p < 0.001$) (Table 5-2). In the midwater habitat, individual taxa were more frequently affected by macrophyte density than by fish presence or their interaction ($X^2_1 = 35.4$, $p < 0.001$). In the benthic habitat, there was no difference in the frequency of taxa affected by either main effect or interaction ($X^2_1 = 2.29$, $p = 0.32$).

Discussion

Relative effects of macrophytes and fish

Macrophytes had a broader role in structuring invertebrate populations than did fish presence in my mesocosm experiment, especially for midwater taxa. Similar results have been documented in other productive lakes with naturally-high macrophyte cover (Paukert and Willis 2003). As predicted, when invertebrates responded significantly to manipulations in macrophyte density, abundances were higher in NM relative to RM treatments. Similarly, macrophyte density was a stronger driver than fish presence for assemblage-level patterns of invertebrate abundance uncovered via multivariate NMS, and again was most pronounced for midwater taxa. Benthic taxa appeared to be shaped more strongly by fish presence; however, this pattern was not significant. Predation risk is often higher in open water habitat relative to the complex habitat created by macrophyte stems (Schriver et al 1995, Sass et al. 2006, Dupuch et al. 2009), thus macrophytes can function as a refuge from predation in the water column, where other forms of refuge, such as benthic sediments, are not available (Gilinsky 1984, Sass et al. 2006, Stuart-Smith et al. 2007). It is likely, therefore, that invertebrates were using NM mesocosms for both the physical habitat provided (e.g., as foraging substrate, resting substrate, shelter from waves, etc.; Kovalenko et al. 2012) and to reduce encounters with predators, whether or not fish were actually present (e.g., Crowder and Cooper 1982, Gilinsky 1984). High densities of macrophytes also provide predation refuge from other invertebrate predators; however, at lower densities, macrophytes may actually increase the risk of predation by providing substrate for predators (Burks et al. 2001). This could explain the absence of several taxa from midwater samples (which generally comprised earlier, smaller instars of most taxa)—including Ceratopogonidae, Polycentropidae, Sciomyzide, and Zygoptera—from both RM treatments regardless of fish presence.

Although macrophytes more frequently affected invertebrate abundance, fish also elicited significant responses from some taxa, with the majority (five of six) having lower abundances in FP mesocosms. Fish presence can alter invertebrate populations through direct consumption (Vinebrooke et al. 2001, Zimmer et al. 2001, McParland and

Paszkowski 2006) and through indirect effects such as avoidance and shifts in habitat use (Dalesman et al. 2007, Greig et al. 2013). Given that invertebrates could pass freely through mesocosms, and many can detect and avoid fish through both chemical (e.g., kairomones; Pestana et al. 2009, Szokoli et al. 2015) and visual cues (McGuffin and Baker 2011), indirect effects of fish could have been responsible for some of the reduced abundances. Indeed, some highly-mobile invertebrate taxa, e.g., corixids, were never captured in benthic samples in FP mesocosms. However, all of the taxa with reduced abundance in the FP mesocosms—Corixidae, Planorbidae, Phryganeidae, Sphaeriidae, Zygoptera—have also been recorded in dace stomachs from nearby lakes (Mee et al. 2013). Although no diet data for fish were collected in this study, it is probable that direct consumption reduced the abundance of the affected invertebrate taxa in FP treatments, regardless of macrophyte density.

An alternate explanation for the relatively weaker effects of fish in experimental mesocosms is that native fishes have already structured the invertebrate assemblage in Dog Leg Lake prior to the construction of mesocosms. Invertebrate assemblages show pronounced differences between naturally fishless and fish-bearing lakes (Schilling et al. 2009b) and abundances of many taxa are typically reduced when small-bodied fishes are experimentally introduced into naturally fishless systems (Zimmer et al. 2001, McParland and Paszkowski 2006). Thus, I had predicted that structuring effects of native fishes would be revealed by significant differences in invertebrate abundance between FP and FA mesocosms, and indeed, such effects were documented. However, I saw fewer differences than expected, and because Dog Leg Lake is naturally fish-bearing, it is possible that the potential "scope of change" to be exhibited by invertebrates to fish presence/absence in mesocosms was limited by the continuous, lake-wide structuring effects already exerted by native fish. Similar results have been seen in a whole-lake experiment, where invertebrate communities did not change significantly after fish were eliminated from productive, previously fish-bearing lakes (Pope and Hannelly 2013). As well, zooplankton (Holmes 2012) and amphibians (Schank et al. 2011) populations in nearby lakes showed differences between fishless lakes and lakes with native fishes, but not additional strong effects when trout were stocked into fish-bearing lakes. Thus, the relatively weak effects of the presence of small-bodied fishes in my manipulations may mirror the relatively weak effects of stocked trout observed in naturally fish-bearing lakes; invertebrates have already been structured by the historical presence of fish predation (e.g.,Vinebrooke et al. 2001, Zimmer et al. 2001).

Neither macrophyte density nor fish presence affected average length for any individual invertebrate taxon after B-H correction, only the length of the midwater all-taxa average was significantly smaller in FP relative to FA mesocosms. This likely represents the cumulative effect of size-selective predation by fish on larger individuals of midwater taxa (e.g., Rincón and Lobón-Cerviá 1995, Carlisle and Hawkins 1998, Schilling et al. 2009), the magnitude of each being too small to be detected at the level of an individual taxon. As well, chemical cues from fish can slow growth rates of invertebrates or prompt larger, more mobile invertebrates to leave FP mesocosms, which may also have contributed to the smaller sizes of individuals inside FP mesocosms (Dalesman et al. 2015).

Overall, statistical interactions between the two factors manipulated were limited, with only two significant comparisons for abundance (Haliplidae and Naididae). For

Haliplidae, the interaction was driven by the absence of benthic individuals in the RM-FA mesocosms, whereas for Naididae, there was a relatively higher abundance of individuals in NM-FP relative to NM-FA mesocosms. In a detailed study of the diets of Fathead Minnow and dace (*Chrosomus sp*.), neither Naididae nor Haliplidae were consumed by these cyprinids (Litvak and Hansell 1990), and Naididae were also absent from dace stomachs in nearby lakes (Mee et al. 2013). Manipulating fish presence can alter the abundance of invertebrates over multiple trophic levels (e.g., Crowder and Cooper 1982), and it is possible that cascading effects were responsible for this unexpected pattern of greater abundance of a taxon in mesocosms where fish were present.

Contrary to my predictions, interactions did not arise because fish reduced the abundance of individual invertebrate taxa to a greater degree in RM relative to NM mesocosms. A partial explanation may be that, as discussed above, native fishes may have structured invertebrate populations prior to my experimental manipulations. However, this predicted pattern was evident in NMS ordinations for midwater taxa. For these taxa, fish presence did not elicit a significant difference in NM mesocosms, but the RM-FP mesocosms differed significantly from the RM-FA mesocosms, driven primarily by lower abundances of Cladocera, *Hyalella*, and Hydrachnidia in the presence of fish. This suggests that predation risk was highest when fish were present and macrophyte density was reduced (e.g., Sass et al. 2006). Although fish presence and macrophyte density often act independently on invertebrate populations (e.g., Lucena-Moya and Duggan 2011), the interactions I did encounter demonstrated that the structure of invertebrate populations can, in part, be a product of multiple factors acting simultaneously.

Benthic vs. Midwater Taxa

Individual taxa from midwater samples were affected more frequently than benthic taxa, and when significant effects occurred, midwater taxa were affected much more frequently by macrophytes than were benthic taxa. This difference is also evident in the results of NMS ordinations where macrophyte density (and fish presence, to a lesser extent) were responsible for differences between mesocosm types for assemblages of midwater but not benthic taxa. The habitat available to benthic taxa was relatively more diverse than for midwater taxa; benthic taxa had both lake sediments *and* macrophytes as potential refuges, whereas midwater taxa had only macrophytes. The smaller-bodied taxa captured in midwater samples are especially vulnerable to predation by smaller, gape-limited fish such as the cyprinids found in Dog Leg Lake (e.g., Litvak and Hansell 1990, Rincón and Lobón-Cerviá 1995), and are also vulnerable to predation by benthic invertebrate taxa (Burks et al. 2001). Thus, high densities of macrophytes were likely more important to these vulnerable midwater taxa (e.g., Kovalenko et al. 2012). This strong response to macrophyte density coupled with the general absence of significant fish effects also provides evidence that forage fishes may have structured midwater taxa at a whole-lake scale prior to my experimental manipulations by conditioning these vulnerable taxa to inhabit areas with high densities of macrophytes.

Across all comparisons, a wide variety of taxa exhibited significant responses to fish presence or macrophyte density, including zooplankton (e.g., both copepods and cladocerans), primary consumers (e.g., gastropods and bivalves), detritivores (e.g., amphipods and corixids), predatory taxa (e.g., odonates), and relatively large-bodied invertebrates (e.g., Phryganeidae). Of these, Caenidae, *Hyalella*, Hydrachnidia, and Zygoptera appeared most affected by experimental manipulations, as they showed significant treatments effects in both benthic and midwater treatments. Typically, smaller-bodied taxa responded more strongly to manipulations in macrophytes and larger-bodied taxa responded more strongly to fish presence/absence. However, five taxa (Erpobdellidae, Glossiphoniidae, Leptoceridae, *Ferrissia*, and Valvatidae) showed no significant effects of either treatment. These taxa were infrequently or never consumed by dace in nearby lakes (Mee et al. 2013) and are generally either larger-bodied predators that could likely escape forage fish (former two taxa) or relatively cryptic (latter two taxa). Interestingly, Erpobdellidae and Glossiphoniidae were consumed by trout in relatively large quantities in some of my study lakes (e.g., Hanisch et al. 2013) and have been negatively impacted elsewhere by trout predation (e.g., Macan 1966, Hemphill and Cooper 1984, Carlisle and Hawkins 1998). These taxa appear to have experienced the weakest "structuring effect" of macrophytes and forage fishes, which may contribute to their vulnerability to stocked trout.

Conclusions

This mesocosm experiment demonstrated that macrophytes and native small-bodied fishes can shape the abundance of many invertebrate taxa, the length of some taxa, and also affect the overall community structure of invertebrates. Littoral invertebrates were frequently affected by macrophyte density, especially in midwater samples, and were occasionally affected negatively by native fish, especially in benthic habitats. Interestingly however, the all-taxa average abundance of benthic invertebrates responded to the macrophyte treatment, indicating that macrophytes may have had a more important effect than revealed by the isolated analyses of individual taxa. These results are consistent with previous research that demonstrate both macrophytes (e.g., Carpenter and Lodge 1986, Pope and Hannelly 2015) and small-bodied fishes (e.g., Vinebrooke et al. 2001, Zimmer et al. 2001, González-Bergonzoni et al. 2014) can structure invertebrate populations, and that the structuring role of macrophytes or other complex structure may appear stronger than fish (Paukert and Willis 2003, Pope and Hannelly 2013). Similar to my study, Pope and Hannelly (2013) found that habitat variables were a stronger driver than fish presence/absence when fish (stocked Rainbow and Brook trout) were excluded from productive lakes in which they had been present for many decades. It is possible that invertebrates in these lakes also had a limited capacity to respond to fish manipulations due to a pre-structuring effect; indeed, abundance, biomass, and body size of invertebrates did not increase in these lakes after fish were removed.

In the absence of direct behavioral observations associated with the mesocosms, it is difficult to determine whether significant results were due to: 1) invertebrates actively seeking refuge habitat or avoiding fish, 2) taxa passively benefitting from inhabiting macrophyte stands, or 3) significant consumption of some invertebrates within FP mesocosms. Several of the taxa most frequently affected in this study—such as cladocerans and *Hyalella*—have shown active avoidance of predators (Lauridsen et al. 2001, Dalesman et al. 2007; Bool et al. 2011, Greig et al. 2013) but are also vulnerable to predation by dace (Litvak and Hansell 1990, Mee at al. 2013). Regardless of the mechanism(s), macrophytes and forage fish are clearly able to shape invertebrate abundance, both independently and sometimes in tandem.

I was unable to address the potential impacts of some predatory invertebrates within my mesocosms. For example, invertebrates that rely on non-visual cues for predation (e.g., microturbellarians) may have had greater success in NM mesocosms, where the dense stands of macrophytes conceal them (e.g., Compte et al. 2015). As well, mobile, large-bodied predatory invertebrates, such as dytiscids and belostomatids, were observed in mesocosms but were not captured in numbers large enough to permit analysis. Like fish, dytiscids select for larger-bodied invertebrate prey (Tate and Hershey 2003). If dytiscids occurred in greater abundance in FA mesocosms, they may have exerted predation pressure in these mesocosms similar to that exerted by fish in FP mesocosms.

In boreal foothills lakes with natural populations of small-bodied fishes, such as Dog Leg Lake and other lakes from my study system (Nasmith et al. 2012, Hanisch et al. 2013), invertebrate populations have likely developed anti-predator strategies—such as the use of refuge habitat and avoidance behaviors—that have allowed them to coexist with fish in general, not just small-bodied forage fishes. These "anti-predator toolkits" developed to deal with native fish species should also be effective against introduced fish predators such as trout (e.g., Rehage et al. 2009). Thus, as suggested by previous research within my study lakes (Nasmith et al. 2012, Schank et al. 2011, Hanisch et al. 2013, Holmes 2012), it is likely that forage fishes have the ability to "pre-condition" invertebrate populations to fish predation, especially given the naturally high densities of macrophytes that characterize these lakes. Thus, lakes containing natural populations of small-bodied fishes and dense stands of littoral macrophytes may be more resilient to stocking and therefore better candidates for this management strategy than lakes lacking these biotic features.

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Table 5-1. Characteristics of Dog Leg Lake. Data are ranges of yearly averages from 2008 – 2009, just prior to the mesocosm experiment. D= Dace (*Chrosomus* spp), F= Fathead Minnow (*Pimephales promelas*), B= Brook Stickleback (*Culaea inconstans*)

Surface area (ha)	Maximum depth (m)	Secchi depth (m)	Chl a (µg L^{-1})	Total phosphorus (µg L^{-1})	Total nitrogen (µg L^{-1})	pH	Forage fish species	Forage fish density (fish ha^{-1})
6.7	5	1.9 - 2.2	3.50 - 14.53	29 - 52	809 - 1080	7.2 - 7.5	D,F,B	3240 - 8737

Table 5-2. Results of two-way ANOVA's for abundance of taxa from benthic and midwater samples. Significant (after Benjamini-Hochberg; B-H) main effects for fish presence ("Fish") and macrophyte density ("Macrophyte") and fish x macrophyte interactions ("Interaction") are presented with test statistics in **bold**. Statistics in *italics* were identified as potential "false discoveries" after B-H. A dashed line (---) indicates non-significant results. "NA" indicates a taxon was not analyzed because of its relative rarity within and between mesocosms.

	Benthic			**Midwater**		
Taxa	Fish	Macrophytes	Interaction	Fish	Macrophytes	Interaction
Baetidae	…	…	…	…	**$F_{1,20}$= 11.08, p= 0.003**	…
Caenidae	…	**$F_{1,20}$= 4.69, p= 0.043**	…	…	**$F_{1,20}$= 15.22, p= 0.001**	…
Ceratopogonidae	…	…	…	…	**$F_{1,20}$= 13.85, p= 0.001**	…
Chironomidae	…	…	…	…	**$F_{1,20}$= 13.85, p= 0.001**	…
Cladocera	…	…	…	…	**$F_{1,20}$= 21.81, p< 0.001**	…
Copepoda	…	…	…	…	**$F_{1,20}$= 28.71, p< 0.001**	…
Corduliidae	…	…	…	NA	NA	NA
Corixidae	**$F_{1,20}$= 5.00, p= 0.037**	…	…	NA	NA	NA
Erpobdellidae	…	…	…	NA	NA	NA
Ferrissia	…	…	…	NA	NA	NA
Glossiphoniidae	…	…	…	NA	NA	NA
Haliplidae	…	…	**$F_{1,20}$= 5.43, p= 0.030**	NA	NA	NA
Hyalella	…	**$F_{1,20}$= 5.96, p= 0.024**	…	…	**$F_{1,20}$= 69.23, p< 0.001**	…
Hydrachnidia	…	**$F_{1,20}$= 5.29, p= 0.032**	…	…	**$F_{1,20}$= 69.23, p< 0.001**	…
Hydroptilidae	…	…	…	…	**$F_{1,20}$= 4.92, p= 0.038**	*$F_{1,20}$= 4.35, p= 0.05*
Leptoceridae	…	…	…	NA	NA	NA
Naididae	…	…	…	**$F_{1,20}$= 5.17, p= 0.034**	**$F_{1,20}$= 12.85, p= 0.002**	**$F_{1,20}$= 5.62, p= 0.027**
Ostracoda	NA	NA	NA	…	**$F_{1,20}$= 6.32, p< 0.001**	…
Phryganeidae	**$F_{1,20}$= 9.82, p= 0.005**	…	…	…	…	…
Planorbidae	…	…	…	**$F_{1,20}$= 5.15, p= 0.034**	**$F_{1,20}$= 21.36, p< 0.001**	*$F_{1,20}$= 4.44, p= 0.048*
Polycentropodidae	…	…	…	…	**$F_{1,20}$= 7.19, p= 0.014**	…
Sciomyzidae	NA	NA	NA	…	**$F_{1,20}$= 5.43, p= 0.030**	…
Sphaeriidae	**$F_{1,20}$= 5.33, p= 0.032**	…	…	NA	NA	NA
Valvatidae	…	…	…	NA	NA	NA
Zygoptera	**$F_{1,20}$= 7.80, p= 0.011**	…	…	…	**$F_{1,20}$= 6.05, p= 0.023**	…
All Taxa Average	…	**$F_{1,20}$= 4.67, p= 0.043**	…	…	**$F_{1,20}$= 61.43, p< 0.001**	…

Table 5-3. Results of two-way ANOVA's for length of taxa from benthic and midwater samples. Significant (after Benjamini-Hochberg; B-H) main effects for fish presence ("Fish") and macrophyte density ("Macrophyte") and fish x macrophyte interactions ("Interaction") are presented with test statistics in **bold**. Statistics in *italics* were identified as potential "false discoveries" after B-H. A dashed line (---) indicates non-significant results. "NA" indicates a taxon was not analyzed because its relative rarity within and between mesocosms.

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	Benthic			Midwater		
Taxa	Fish	Macrophytes	Interaction	Fish	Macrophytes	Interaction
Baetidae	…	…	…	…	…	…
Caenidae	…	*$F_{1,17}$=8.09, p= 0.011*	…	…	*$F_{1,12}$=9.17, p= 0.011*	…
Ceratopogonidae	…	…	…	NA	NA	NA
Chironomidae	*$F_{1,19}$=4.44, p= 0.049*	…	…	…	…	…
Cladocera	…	…	…	…	…	…
Copepoda	…	…	…	…	…	…
Corduliidae	*$F_{1,12}$=5.88, p= 0.032*	…	…	NA	NA	NA
Corixidae	…	…	…	NA	NA	NA
Erpobdellidae	…	…	…	NA	NA	NA
Ferrissia	…	…	…	NA	NA	NA
Glossiphoniidae	…	…	…	NA	NA	NA
Haliplidae	…	…	…	NA	NA	NA
Hyalella	…	*$F_{1,20}$=6.63, p= 0.018*	…	…	*$F_{1,14}$=10.27, p= 0.006*	*$F_{1,14}$=5.64, p= 0.032*
Hydrachnidia	…	…	…	…	…	…
Hydroptilidae	…	…	…	…	…	…
Leptoceridae	…	…	…	NA	NA	NA
Naididae	…	…	…	…	…	…
Ostracoda	NA	NA	NA	…	…	…
Phryganeidae	…	…	…	NA	NA	NA
Planorbidae	…	…	…	…	…	…
Polycentropodidae	…	…	*$F_{1,9}$=7.61, p= 0.022*	NA	NA	NA
Sphaeriidae	…	…	…	NA	NA	NA
Valvatidae	…	…	…	NA	NA	NA
Zygoptera	…	*$F_{1,17}$=5.59, p= 0.030*	*$F_{1,17}$=6.02, p= 0.025*	NA	NA	NA
All Taxa Average	…	…	…	**$F_{1,20}$=16.56, p= 0.001**	…	…

Figure 5-1. Spatial distribution of mesocosms in Dog Leg Lake. Natural density macrophyte treatments are represented by green squares and reduced macrophyte treatments by gray squares; fish-absent treatments are solid colour and fish-present treatments are hatched. Inset: the location of Dog Leg Lake in Alberta, Canada. Figures are modified from Google Earth imagery.

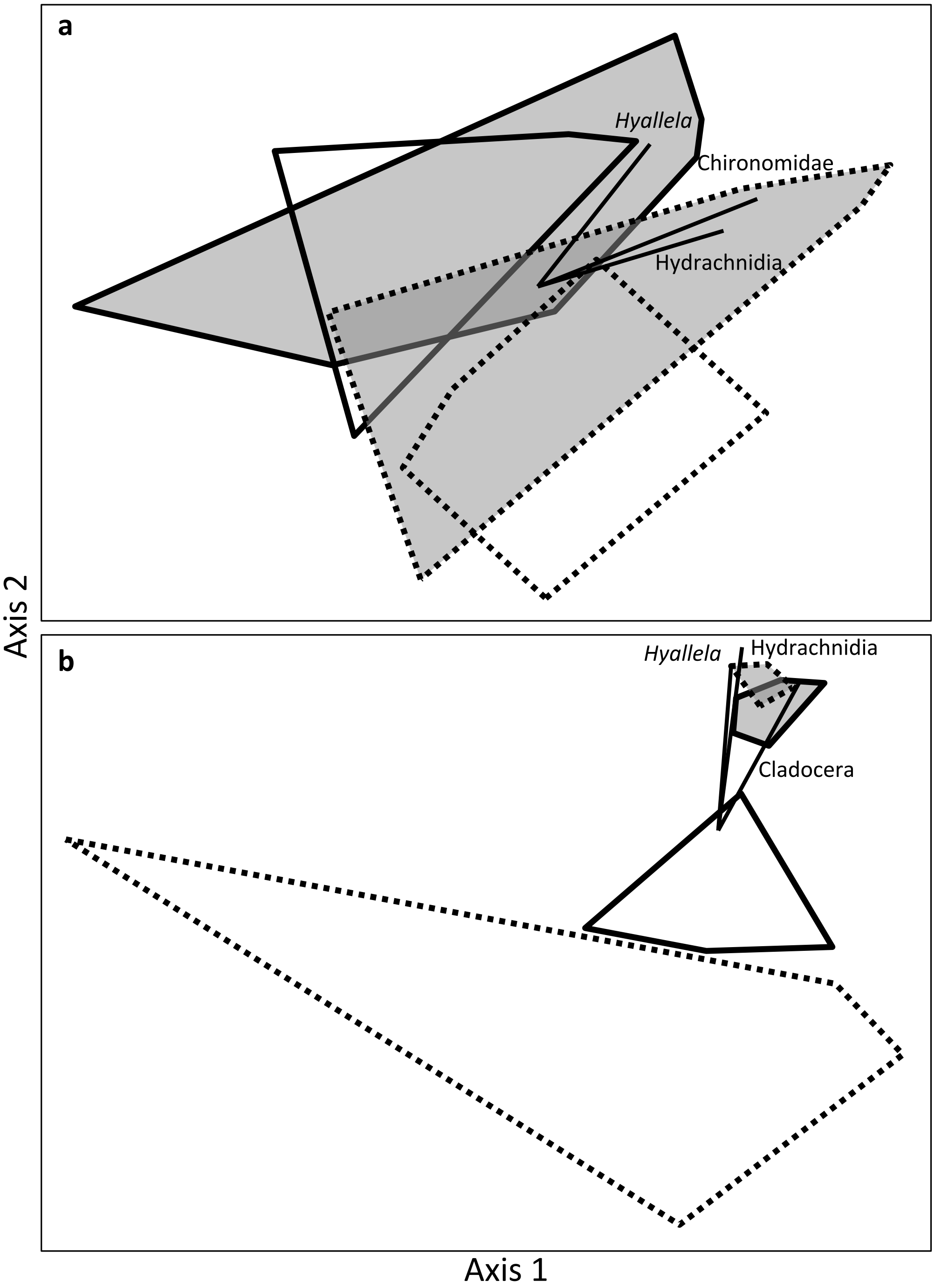

Figure 5-2. Non-metric multidimensional scaling joint plots, based on abundance, for invertebrate assemblages in mesocosms from benthic (a) and midwater (b) samples for 25 and 17 taxa, respectively. Polygons represent invertebrate assemblages for each of the four treatments. Macrophyte density is represented by shading (gray shading= natural, clear= reduced) and fish treatments are represented by lines (solid= fish absent, dashed= fish present). Vectors indicate the

direction and extent of increasing abundance of invertebrate taxa having $r^2 > 0.50$ (a) and $r^2 > 0.75$ (b). Caenidae, Chironomidae, Copepoda, Notonectidae, and Ostracoda also showed increasing abundance along Axis 2 in (b) for $0.50 < r^2 < 0.75$; however, these taxa were not plotted to avoid obscuring NM polygons. Axes 1 and 2 represent 75.8% of the variation for benthic taxa (a) and of the 93.4% of the variation for midwater taxa (b).

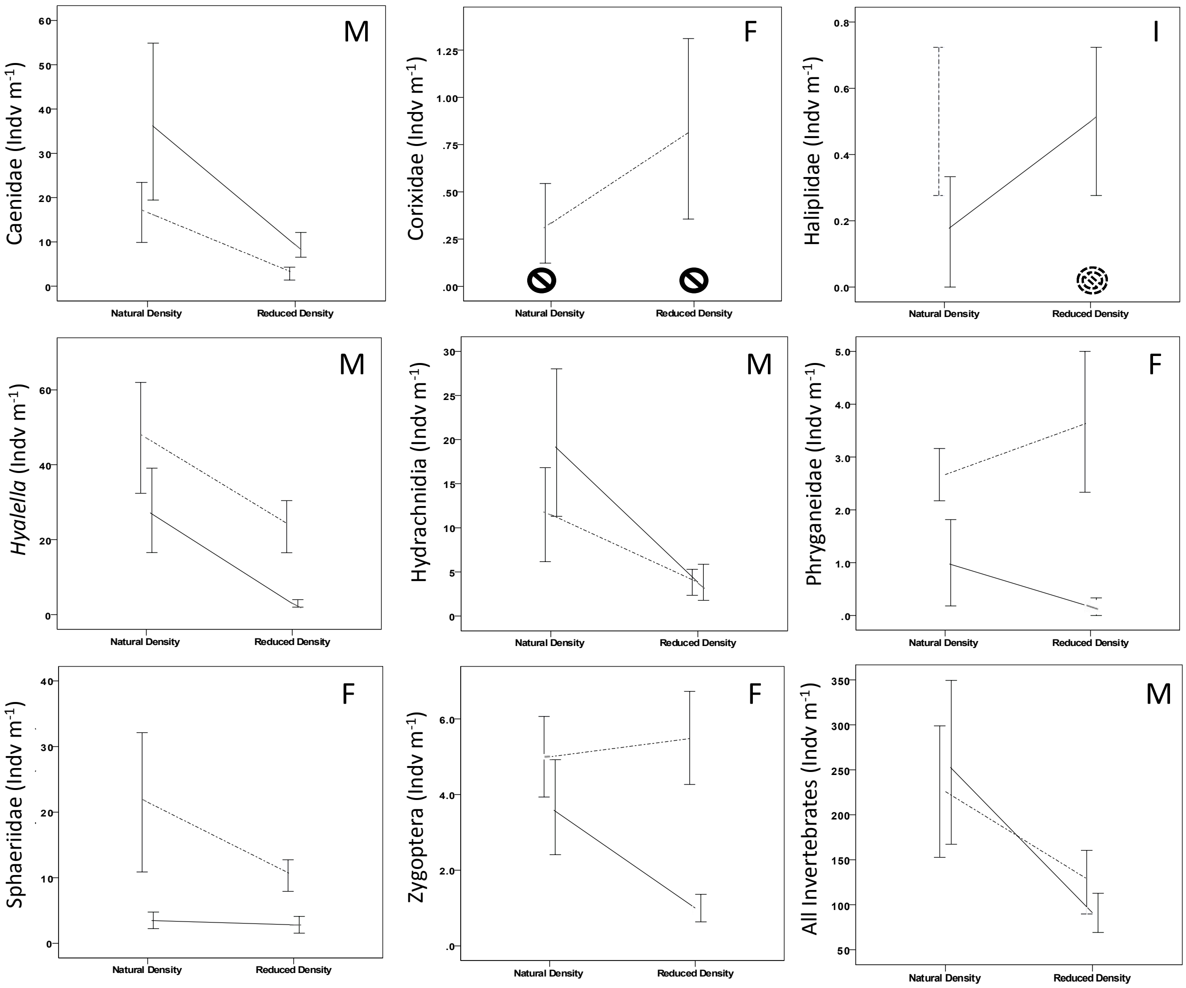

Figure 5-3. Mean abundance of benthic taxa that show significant (after B-H correction) effects of fish and/or macrophyte density in mesocosms. A null symbol (⦸) indicates no individuals of the taxon were caught in that treatment type. Solid lines and null symbols indicate the fish-present treatment and dashed lines and null symbols indicate the fish-absent treatment, at natural and reduced densities of macrophytes. F= significant effect of fish, M= significant effect of macrophytes, I= significant interaction. Error bars represent 1 standard error.

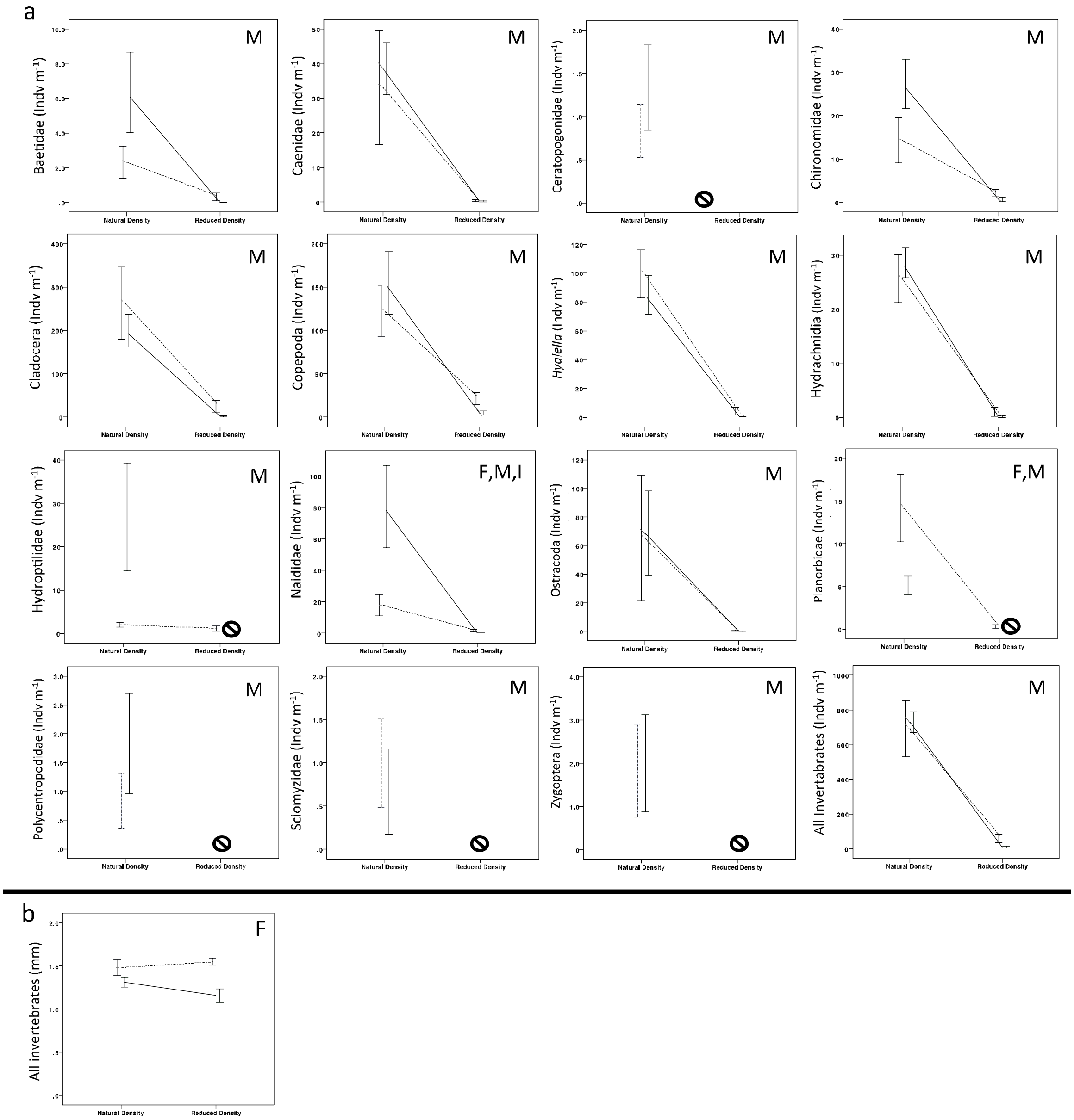

Figure 5-4. Mean abundance (a) and length (b) of midwater taxa that show significant (after B-H correction) effects of fish and/or macrophyte density in mesocosms. A null symbol (⃠) indicates no invertebrates were caught in that treatment type. Solid lines and null symbols indicate the fish-present treatment and dashed lines and null symbol indicate the fish-absent treatment, at natural and reduced densities of macrophytes. F= significant effect of fish, M= significant effect of macrophytes, I= significant interaction. Error bars represent 1 standard error.

Chapter 6. General Conclusion

Trout have been stocked across North America for over 100 years (Halverson 2008) and are among the most commonly stocked fishes in the world (Rahel 2007). Impacts of these introduced predators have been well-documented in some ecosystem types (e.g., alpine and oligotrophic lakes; Dunham et al. 2004, Eby et al. 2006) but are relatively unknown in others (e.g., productive, lower-elevation lakes). This thesis used multiple experimental and comparative methods at different temporal and spatial scales to investigate the potential impacts of stocked Rainbow Trout (*Oncorhynchus mykiss)* on native organisms in lower elevation lakes, a habitat type that is frequently stocked but infrequently studied (e.g., Halverson 2008). I have presented the results from comparisons between stocked and unstocked lakes, from Before-After-Control-Impact (BACI) analyses using a single stocked lake and several unstocked control lakes, and from experimental manipulations of macrophytes and native small-bodied fishes within an unstocked lake.

In Chapter 2, I documented few differences between food webs of stocked and unstocked lakes, and between the recently-stocked Fiesta Lake and unstocked controls. The structure of food webs in stocked lakes was generally unaffected by trout when compared to food webs of unstocked lakes, and only small-bodied fishes showed some evidence of increased consumption of littoral-derived carbon in stocked relative to unstocked lakes. Stable isotopes revealed that native organisms in all lakes frequently inhabited littoral habitat and may therefore have benefited—either passively or actively—from the refuge from predation by trout provided by aquatic macrophytes (Moyle and Light 1996).

In Chapter 3, I looked specifically at effects of trout on the abundance, length, and habitat use of small-bodied fishes using both whole-lake comparisons and experimental mesocosms. Stocked trout did not affect the abundance or average lengths of native fishes, but dace (*Chrosomus* spp.) were captured more frequently inshore, in vegetated habitat in stocked lakes relative to unstocked lakes. This difference was especially pronounced for young-of-the-year dace; the ratio of inshore:offshore catch was 13 times greater for juvenile fish but only 2.8 times greater for adult fish in stocked relative to unstocked lakes. I proposed that forage fishes were actively driving these patterns by avoiding open-water habitat in the presence of trout and taking advantage of the refuge from predation provided by macrophyte beds (e.g., Sass et al 2006). To test this assumption, I manipulated densities of macrophytes (natural vs. reduced densities) within mesocosms, stocked each mesocosm with trout, and monitored the use of each mesocosm by native fish before, during, and after trout stocking. Dace avoided mesocosms with reduced densities of macrophytes in the presence of trout, but did not adjust their use of mesocosms containing natural macrophyte densities before, during, or after trout were present. This behaviour has likely helped facilitate their coexistence with trout in my study lakes with few negative effects (Werner et al. 1983, Duluth et al. 2009, Nasmith et al. 2010).

In Chapter 4, I used a Before-After-Control-Impact (BACI) design to determine how stocked trout affected assemblage-level composition and taxon-level biomass, abundance, and average length of littoral invertebrates in a single stocked, Fiesta Lake, lake relative to three unstocked control lakes. Based on multivariate analyses of abundance and biomass, invertebrate community composition in Fiesta Lake appeared to be affected by trout only in the first year after stocking and became similar to communities of unstocked control lakes by the second year after stocking. Similarly, there were few taxon-level impacts of trout in Fiesta Lake after stocking relative to control lakes, and when effects occurred, they were generally due to some taxa *increasing* in abundance in Fiesta Lake after stocking. Although stocked trout often have severe negative effects on invertebrate populations (Knapp et al. 2005; Cox & Lima 2006), a weak impact of trout in Fiesta Lake was not surprising given the overall lack of a detectable effects of trout on food webs documented in Chapter 2, and similar comparative results from other research conducted in my study lakes (Nasmith et al. 2012) and in other productive lakes (Wissinger et al. 2006).

Results from Chapters 2 through 4, and from other studies (Wissinger et al. 2006, Nasmith et al. 2012), have suggested that macrophyte beds and native forage fishes have likely “pre-structured” invertebrate communities in productive lakes creating buffers against the impacts of larger stocked fish. To test these assumptions, I conducted a mesocosm experiment within an unstocked lake that was designed to investigate the effects of forage fishes on invertebrate communities at two densities of macrophytes (Chapter 5). Results from the experiment demonstrated that both macrophytes (e.g., Carpenter and Lodge 1986) and small-bodied fishes (e.g., Vinebrooke et al. 2001, Zimmer et al. 2001) can drive community composition and taxon-level abundance of invertebrates. For those taxa showing a response, abundance was generally higher in mesocosms with natural (i.e., high) densities of macrophytes and in fishless mesocosms, and these effects were most pronounced for midwater taxa, such as cladocerans and amphipods. Macrophyte density affected invertebrate populations more frequently than did fish, with higher abundances of many taxa found within mesocosms containing natural densities of macrophytes (e.g., Lucena-Moya and Duggan 2011). However, there was also evidence that forage fish likely pre-structured invertebrate populations prior to the start of my experiment, which may have limited the potential responses of invertebrates to experimental manipulations of fish.

My thesis demonstrates that the effects of stocked trout on the native fauna are not always negative (e.g., Gozlan 2008) and that native forage fishes and/or dense beds of macrophytes—characteristics typically absent from lakes where strong effects of trout have been documented—appear to buffer negative impacts of trout. Macrophytes have been previously shown to provide important refuge habitat for amphibians (Walls 1995), macroinvertebrates (Luecke 1990, Harrison et al. 2005), zooplankton (Burks et al. 2002), and fishes (Werner et al. 1983, Stuart-Smith et al. 2008); the value of macrophytes as refuge against trout predation for forage fishes (Chapter 2 and 3) and macroinvertebrates (Chapters 5) was evident in my research. Additionally, food web data (Chapter 2) indicated that forage fishes and stocked trout were exploiting similar invertebrate resources in stocked lakes and that forage fishes alone (Chapter 5) can structure invertebrate populations in productive lakes. Thus, stocked trout may simply represent another predatory fish to which native invertebrate species are not naive.

I used several different investigative pathways to explore and refine the initial observations of weak impacts of trout in my study lakes (Nasmith et al. 2010, Schank et al. 2011, Nasmith et al. 2012). Building on the work of these previous researchers, I used whole-lake comparisons of unstocked lakes vs. stocked lakes (with stocking history ranging from 1 to 60+ years), took advantage of the stocking of trout in one of my previously unstocked lakes to conduct a Before-After-Control-Impact (BACI) analysis, and used experimental manipulations within mesocosms to explore mechanisms hypothesized to shape the responses of native species to trout. Investigating impacts of trout at multiple spatial and temporal scales helped to reveal the sequence of events that occur after trout are stocked and the characteristics of receiving lakes that have allowed trout and native species to co-exist. For example, few differences in the demographics of fish and invertebrate populations were observed when comparing currently-stocked and unstocked lakes using a Control Impact design in this thesis and in the work by Nasmith et al. (2010, 2012). However, BACI analyses of food webs and littoral invertebrate communities revealed that some changes occur in the first year post-stocking, but then diminish in the second and subsequent years after trout stocking. My experimental manipulations of fish presence and macrophyte density within mesocosms showed that native organism can actively respond to introduced trout and may benefit passively by an inherent tendency to inhabit macrophyte beds. Habitat shifts of forage fish observed in mesocosms were also evident at the whole-lake scale in two life stages of native fish. Thus, the results from mesocosms appear to scale up well and explain patterns observed at the whole-lake level (e.g., Biro et al 2003), and these behaviours likely help explain why few long-term impacts of trout were observed at the scales of individual taxa, assemblages, and food webs in my study lakes. Such scalable results are often elusive when conducting mesocosm experiments (Schindler 1998), and while there was not perfect concordance in results from mesocosm, BACI, and CI portions of my thesis, all three designs helped to identify and then refine understanding of impacts of stocked trout and potential mechanisms mediating their effects.

My results have implications for both applied fisheries management and the basic understanding of introduced species. Natural lakes bearing sustainable populations of game fish are limited in many regions, and trout stocking is an important management tool in these areas (e.g., Halverson 2008). Because refugia provided by macrophytes appear to promote the coexistence of stocked trout and their prey, managers may want to seek out lakes with well-vegetated littoral zones as targets for stocking if minimal impacts on native littoral-dwelling species are desired (e.g., Pearsons and Hopley 1999). Freshwater ecosystems are experiencing repeated introductions of exotic, predatory species (Rahel 2007). In particular, salmonids have become established outside of their native ranges throughout the world, yet their impacts are not always easy to predict (Fausch et al 2001, Fausch 2008). Not surprisingly, most research on the effects of stocked salmonids is conducted in ecosystems where there are known or suspected negative effects (e.g., Leprieur et al. 2009, Korsu et al. 2010), and documented examples of introductions with negligible effects are much less common (but see Fausch et al. 2001, Marchetti et al. 2004, Gozlan 2008). Thus, my research provides a detailed case study of the characteristics of a system where the effects of introduced trout have not been strongly negative, even within lakes that have been stocked for greater than 60 years. Lessons learned from systems with few impacts should help us to predict under what conditions introduced species do not have strongly-negative effects on receiving ecosystems (e.g., Marchetti et al. 2004). Although the precautionary principle should be applied before introducing a species into a novel ecosystem (e.g., Pearsons and Hopley 1999, Leprieur et al. 2009), some systems, such as the isolated productive lakes that I studied in the Alberta foothills, appear to have the capacity to support introduced trout with few effects on native forage fishes and invertebrates.

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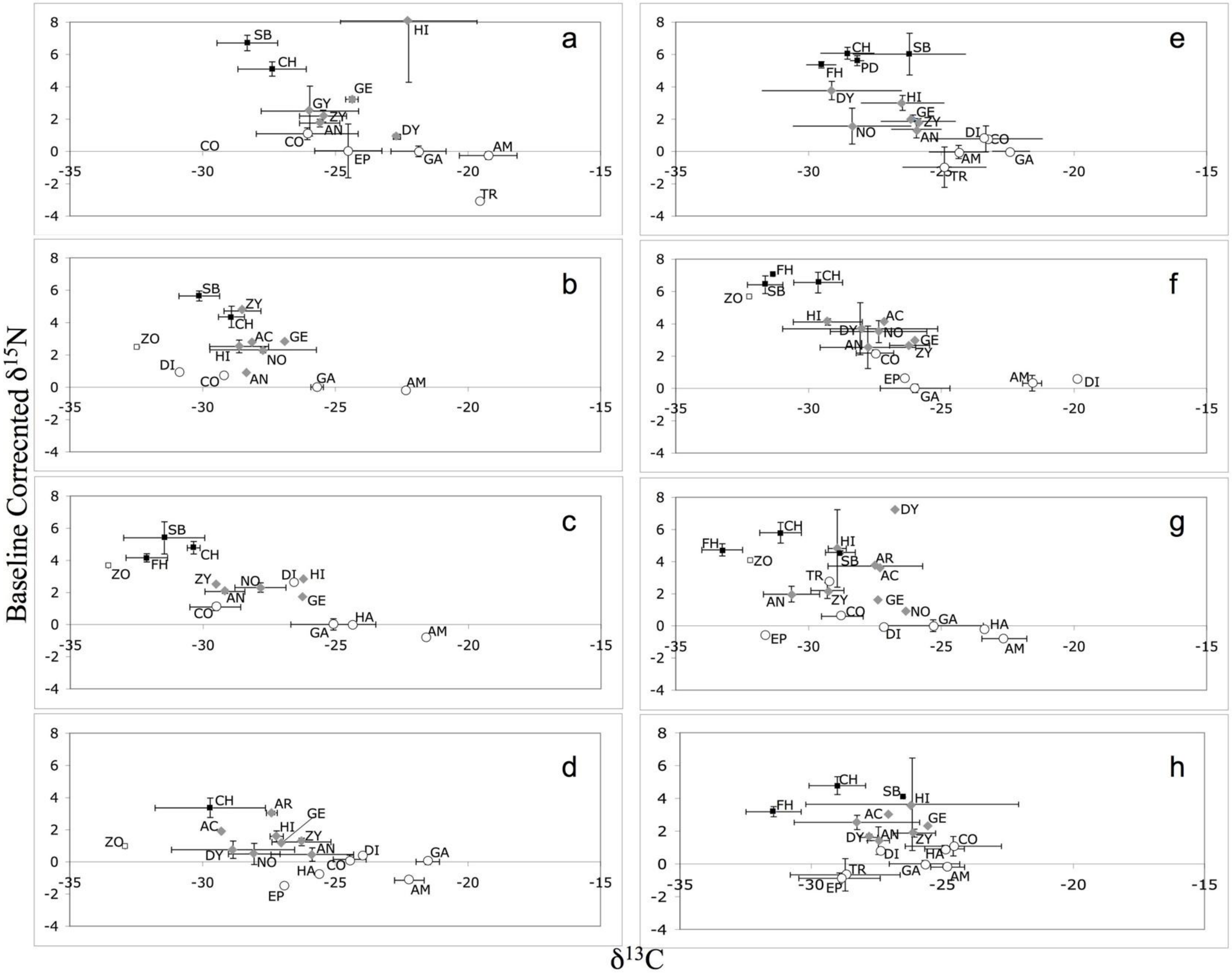
Appendices

Appendix 2-A. Stable isotope biplots (δ^{15}N vs. δ^{13}C) for Dog Leg (a-d; 2006 - 2009), Gas Plant (e-h; 2006 - 2009), Teal (i-l; 2006 - 2009), Fiesta (m-p; 2006 - 2009), Ironside (q-r; 2006 - 2007), Mitchell (s-t; 2006 - 2007), Strubel (u-v; 2006 - 2007), Yellowhead (w-x; 2006 - 2007), and Dog Paw (y-z; 2006 - 2007) lakes. Years are lettered consecutively within each lake. Symbols represent mean values of each taxonomic group, and lines indicate one standard error. Dark squares: forage fish taxa, Gray Squares: predatory invertebrates, White Circles: primary consumers, White boxes: zooplankton. AM, Amphipoda; AN, Anispotera; AC, Acari; AR, Aranaea; BE, Belostomatidae, BRT, Brook Trout, CH; *Chrosomus*; CO, Corixidae; DI, Diptera; DY, Dytiscidae; EP, Ephemeroptera; FH, Fathead Minnow; GA, Gastropoda; GE, Gerridae; GY, Gyrinidae; HA, Haliplidae; HI, Hirudinea; NO, Notonectidae; PD, Pearl Dace, RBT, Rainbow Trout, SB, Stickleback; SP, Sphaeriidae; TR, Trichoptera, ZY; Zygoptera, ZO, Zooplankton


Baseline Correcnted δ15N
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GY
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b
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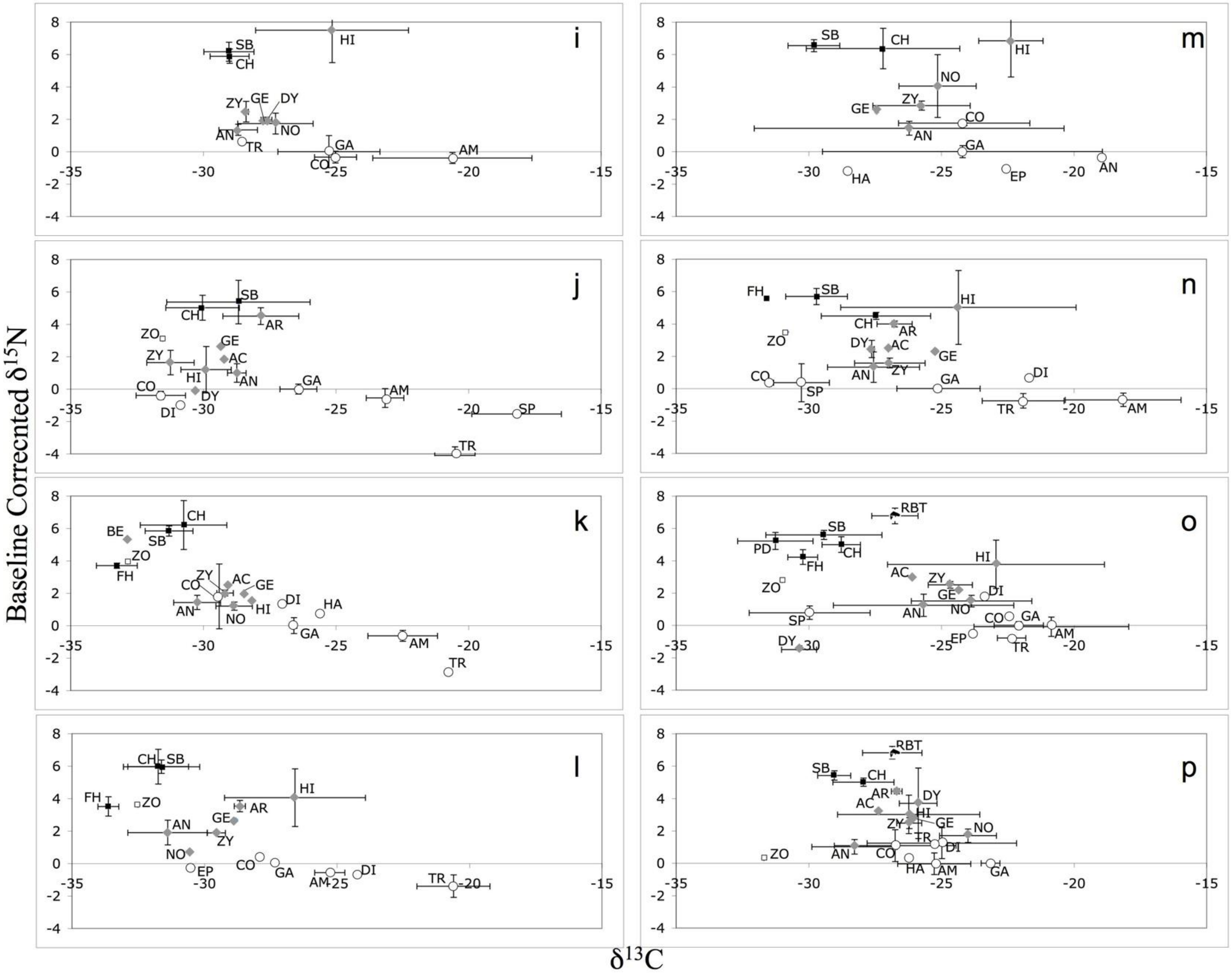
Baseline Correcnted δ15N
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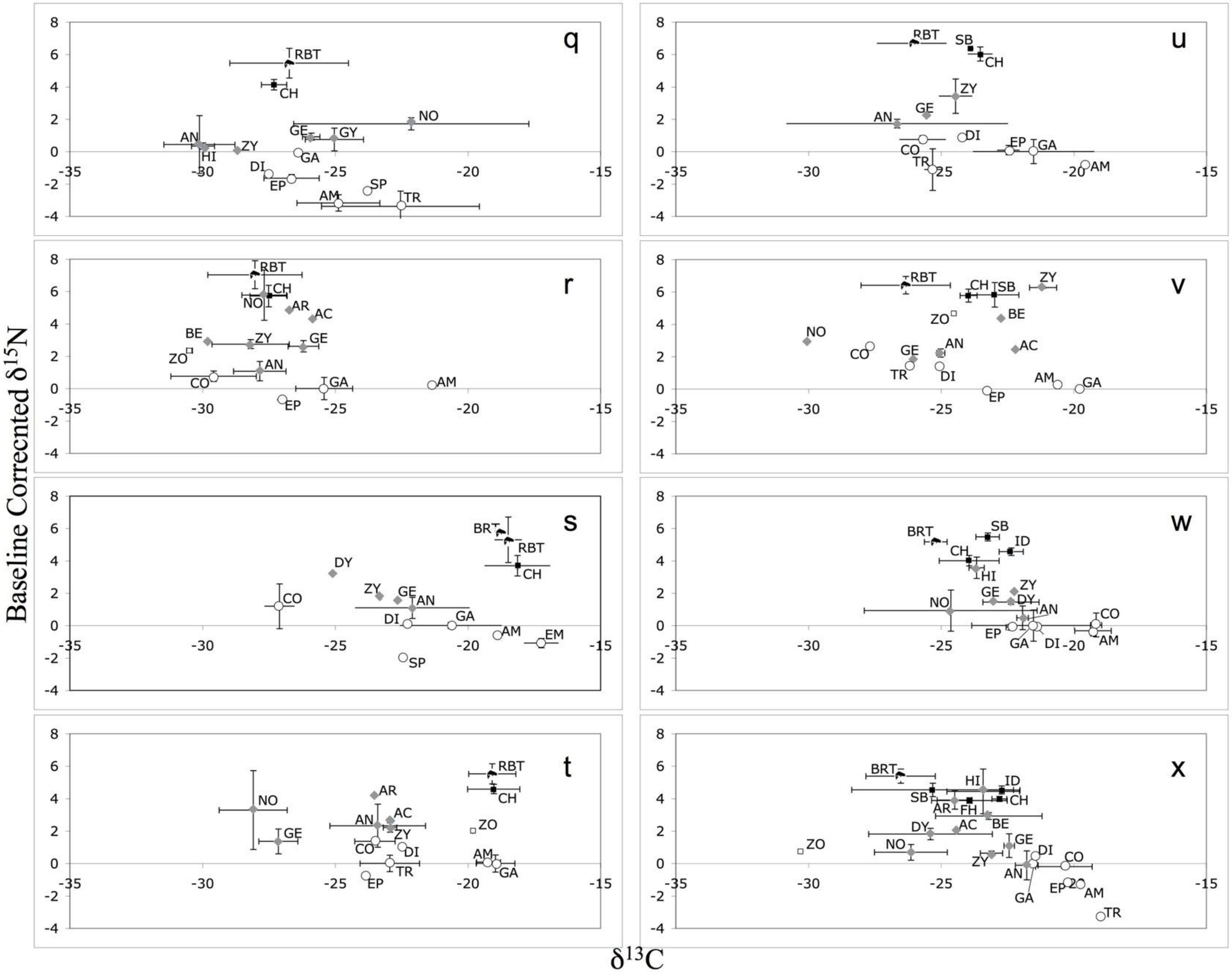
Baseline Correcnted δ15N
δ13C
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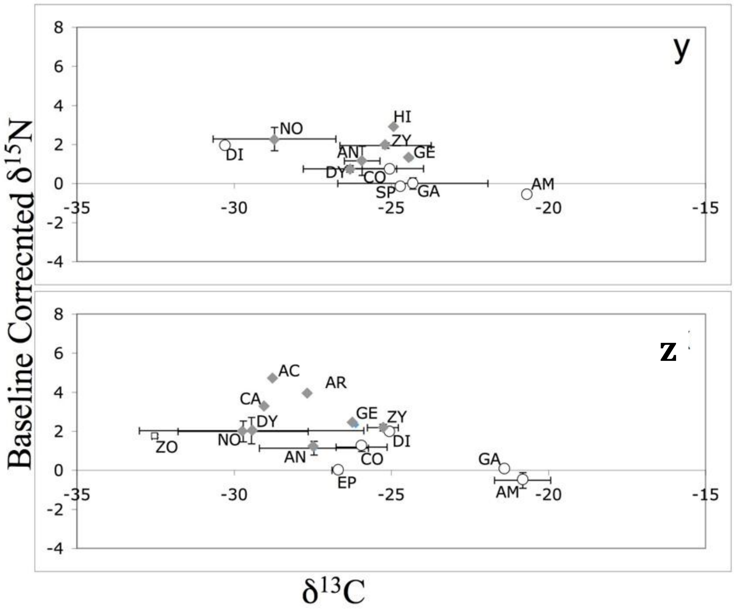
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Baseline Correcnted δ15N
δ13C
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Appendix 5-A. Representative photos of mesocosm types, including (a) Natural Macrophyte Density-Fish Absent (NM-FA), (b) Natural Macrophyte Density-Fish Present (NM-FP), (c) Reduced Macrophyte Density-Fish Absent (RM-FA), and (d) Reduced Macrophyte Density-Fish Present (RM-FP). Note the finer mesh of the FA mesocosms, which excluded adults of the native fishes present in Dog Leg Lake.