2020

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Recommended Citation

Wainright, Charles, "Food Web Effects of Lake Trout (Salvelinus namaycush) Invasion in Northwestern Montana" (2020). *Graduate Student Theses, Dissertations, & Professional Papers*. 11585.
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FOOD WEB EFFECTS OF LAKE TROUT (*SALVELINUS NAMAYCUSH*) INVASION IN NORTHWESTERN MONTANA

By

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Thesis
presented in partial fulfillment of the requirements
for the degree of

Systems Ecology, Aquatic Ecology

The University of Montana
Missoula, MT

May 2020

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Abstract

WAINRIGHT, CHARLES, M.S., May 2020 Systems Ecology, Aquatic Ecology

Food Web Effects of Lake Trout (*Salvelinus namaycush*) Invasion in Northwestern Montana

Chairperson: Shawn Devlin, Ph.D.

Around the turn of the 20th century, lake trout (*Salvelinus namaycush*) were widely introduced in several lakes and reservoirs outside their native range in western North America. Since then, lake trout have become problematic in many lakes where they were introduced, causing significant declines in popular sport fishes and native species, most notably federally protected bull trout (*Salvelinus confluentus).* Despite evidence that invasive fish can cause cascading trophic effects in aquatic communities, the impacts of lake trout introduction / invasion on aquatic food webs remain poorly understood. Moreover, native fish restoration programs tend to focus on suppression of invasive fish and rarely examine the broader food web effects of remediation efforts. In this study, I used stable isotope analysis to examine the food web effects of lake trout invasion and remediation (e.g., gillnetting suppression) in 12 lakes (four uninvaded, five invaded, and three remediated) to which bull trout are native in northwestern Montana. Although bull trout and lake trout had higher $\delta^{15}N$ than other fishes, lake trout had higher $\delta^{15}N$ than bull trout in all invasion categories, indicating bull trout may both compete with and be preyed upon by lake trout. Analyses of bull trout diets revealed bull trout consumed low proportions of pelagic fish in remediated lakes. In contrast, bull trout consumed relatively high proportions of pelagic prey in uninvaded or invaded lakes. Bayesian standard isotope ellipse area indicated that remediated lakes had uniquely disorganized food web structures compared to invaded and uninvaded lakes, suggesting that remediated lakes may be at an intermediate stage of food web succession. Isotope niche overlap between bull trout and lake trout was symmetric in remediated lakes and asymmetric in invaded lakes, suggesting suppression may diminish lake trout impacts on bull trout. Finally, space-for-time substitution revealed that it takes about 70 years for lake trout to displace bull trout in the study region, indicating many of these bull trout populations may soon be functionally extinct. My results show that lake trout invasion causes significant food web structural changes and that suppression activities may remain the requisite cornerstone of a multi-faceted bull trout restoration effort.

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Chapter 1 : Food Web Effects of Invasive Lake Trout and Implications for Management

Abstract

Lake trout (*Salvelinus namaycush*) have been widely introduced outside their native range. Introduced lake trout are now understood to be a cause of native species declines in lakes and reservoirs in western North America, most notably federally protected bull trout (*Salvelinus confluentus*). Despite an understanding that invasive species can cause cascading trophic effects, native species restoration programs tend to focus on invasive species suppression and rarely examine the food web effects of these invasions. In this chapter, I used stable isotope analysis to examine the food web effects of lake trout invasion in twelve lakes (four uninvaded, five invaded, and three remediated) in northwestern Montana, USA. Although bull trout and lake trout had higher $\delta^{15}N$ than other fishes, lake trout had higher $\delta^{15}N$ than bull trout in all invasion categories, indicating bull trout may both compete with and be preyed upon by lake trout. Our results suggest food web disorder was associated with lake trout invasion. Remediated lakes had the highest standard isotope ellipse area, suggesting high fish diet variability in remediated lakes. Bull trout diet and isotope niche overlap also suggested food web disorder in remediated lakes. These findings suggest remediated lakes may be an unstable intermediate stage in food web succession and emphasize the role of lake trout suppression in bull trout conservation.

Introduction

Humans have introduced invasive species worldwide (Vitousek et al., 1996). These introductions have had broad ecological effects including declines of native species and loss of ecosystem function (Lodge, 1993; Moyle and Marchetti, 2006). The wide-ranging effects of invasive species introductions are commonly attributed to complex interactions between biota in natural food webs (Byrnes et al., 2007; Jackson et al., 2012). Modern analytical and statistical methods allow ecologists to examine food web interactions like niche shifts (Jackson et al., 2011; Layman et al., 2007) and diet composition (Phillips et al., 2014; Stock et al., 2018) in detail.

Fish invasions are known to affect structure and function of food webs. Fish invasions can change predation and competition for resources (Ellis et al., 2011), and alter niche width (Layman et al., 2007; Olsson et al., 2009), trophic structure (Syväranta and Jones, 2008; Vander Zanden et al., 1999, 2003, 1997), primary production, and microbial community function (Devlin et al., 2017), food chain length (Post et al., 2000), and many other aspects of food webs. Despite general agreement that invasive fish can produce cascading trophic effects, quantitative food web assessments after fish invasions are rare. A growing body of literature suggests restoring aquatic food web characteristics could be important to fisheries restoration outcomes (Cross et al., 2013; Vander Zanden et al., 2010).

Fisheries restoration programs traditionally focus on invasive fish removal to restore imperiled species or those with high recreational value. For example, managers have implemented various strategies to reduce invasive lake trout in several lakes in the western United States (Martinez et al., 2009). Similarly, managers are actively suppressing Asian carp in several tributaries in the Great Lakes region to reduce impacts to native species (Tsehaye et al., 2013). However, the assumption that removing an invasive fish will equate to restoring a native ecosystem has been called into question (Propst et al., 2015; Syslo et al., 2013). In some cases, removal of invasive fish has enabled re-establishment of native fish (e.g., Lepak et al., 2006; Weidel et al., 2000). In other cases, invasive fish removal was unsuccessful (Donkers et al., 2012) or insufficient as a sole means for restoration after invasive fish become established (Weber et al., 2016). Thus, fisheries managers are increasingly integrating ecosystem-level restoration measures, such as using salmon carcasses to restore macroinvertebrate productivity (Wipfli and Baxter, 2010), when rehabilitating native fishes after species invasion (Kitchell et al., 2000).

Lake trout (*Salvelinus namaycush*) are relatively large, long-lived piscivores native to deep, cold, oligotrophic lakes of Canada and the northern United States (Crossman 1995). However, lake trout have been widely introduced outside their native range in the western United States and have expanded to more than 200 waters through dispersal and unauthorized translocations (Martinez et al., 2009). Despite their ecological value as a top-level predator in lakes where they are native (Kitchell et al., 2000) and recreational value lake trout afford (Hansen et al., 2016), they have had negative effects on native and sport fishes in many lakes where they are introduced (Ellis et al., 2011; Tronstad et al., 2010). Due to declines in native species, lake trout are now being suppressed in many lakes and reservoirs (Martinez et al., 2009). For example, large-scale lake trout suppression efforts have been implemented to conserve native fishes in Yellowstone Lake, Wyoming (Syslo et al., 2011), Lake Pend Oreille, Idaho (Hansen et al., 2008), Priest Lake, Idaho (Ng et al., 2016), Blue Mesa Reservoir, Colorado (Pate et al., 2014), Quartz and Logging lakes, Montana (Fredenberg et al. 2017), and Flathead Lake, Montana (Hansen et al., 2016). In most cases, gill netting to suppress lake trout and restore imperiled native fish has been challenging (Martinez et al., 2009; Syslo et al., 2011).

Bull trout (*Salvelinus confluentus*) have declined in most North American lakes where lake trout have been introduced and lake trout are presumed to be the primary cause of bull trout declines due to the likelihood of competition and predation between these species (Fredenberg, 2002; Guy et al., 2011; Hansen et al., 2016, 2008; Propst et al., 2015). Severe bull trout declines led to the species being protected under the U.S. Endangered Species Act in 1998 (USFWS, 1998). Lake trout suppression is a primary focus for bull trout restoration in parts of western North America (Downs et al., 2013; U.S. Fish and Wildlife Service, 2015). For example, lake trout suppression has been ongoing in Glacier National Park's Quartz and Logging Lakes for nearly a decade (NPS, 2013) and Swan Lake hosted a suppression program for several years until its discontinuation in 2017 (Smalley, 2018). Northwest Montana has long been recognized as excellent habitat for bull trout and contains one-third of the remaining lake-dwelling bull trout habitat in the United States (Fredenberg et al., 2007). Despite the prevalence of excellent habitat in this region, bull trout have become imperiled in recent decades (Fredenberg et al., 2007).

Lake trout were introduced into Flathead Lake, a large lake in northwest Montana, in 1905 (USFWS, 2010), where they remained in low abundance for several decades (Ellis et al., 2011). Between 1968 and 1975, the opossum shrimp, *Mysis diluviana*, was introduced into lakes upstream of Flathead Lake (Devlin et al., 2017; Spencer et al., 1991) and was subsequently documented in Flathead Lake in 1981 (Ellis et al., 2011). Once established, *Mysis* became an important food for juvenile lake trout thereby alleviating a lake trout recruitment bottleneck and causing catastrophic changes in Flathead Lake's food web (Ellis et al., 2011; Spencer et al., 1991). Flathead Lake's flourishing lake trout population is believed to be the center of a regional diaspora of lake trout throughout northwest Montana (Fredenberg et al., 2007; Meeuwig et al., 2011; Muhlfeld et al., 2012).

In this study, we examined the impacts of lake trout invasion and suppression in northwest Montana, USA. Northwest Montana is an ideal setting to examine the impacts of lake trout on food webs because the region contains lakes without lake trout (i.e., reference), lakes with lake trout (i.e., invaded), and lakes where managers have implemented lake trout suppression programs (i.e., remediated). We used stable carbon and nitrogen isotopes to examine the food web structure of our study lakes. Despite stable isotopes being widely to study invasive species and food webs, we believe this is the first application of stable isotope ecology to evaluate both fish invasion and invasive fish remediation by various state, federal and tribal management agencies. The findings from this study will provide feedback to stakeholders about the effects of those lake trout removal programs and inform future decisions about controlling invasive fish to restore native fish.

Methods

Study system

We studied the food web structure of 12 waterbodies (11 lakes and one reservoir; herein referred to as "lakes") west of the continental divide in northwest Montana, USA (Appendix: Table 1.2 and Figure 1.7). These lakes are oligotrophic, dimictic lentic waterbodies in largely forested and undeveloped watersheds. Study lakes fit into three categories based on their history of lake trout existence and remediation: 1) reference, 2) invaded, and 3) remediated (Appendix: Table 1.2). Reference lakes have a native fish assemblage and have no lake trout. Invaded lakes have sympatric bull trout and lake trout populations and do not have a lake trout gill net suppression program. Remediated lakes also have sympatric bull trout and lake trout populations, but these lakes have current or past lake trout suppression programs (Downs et al., 2013; Syslo et al., 2013; U.S. Fish and Wildlife Service, 2015).

Sample collection

We collected 19 fish species (Appendix: Table 1.3) concurrently with government agency fisheries surveys in summer and fall 2017, 2018, and 2019. Fish were collected using mono- and multi-filament gill nets, littoral fyke nets, benthic hoop nets, hook and line, and backpack electrofishing. Sinking monofilament gill nets consisted of 38 m long by 2 m deep panels of 38 to 101 mm bar mesh. The number of gill nets, mesh sizes, and soak times depended on agency fish survey goals and permitting requirements to minimize bull trout bycatch. Fyke nets had 8 m leads and 4 m hoop sections with one 75 mm vertical trapping pane, one 90 mm throat, and black 6 mm stretch mesh. Benthic hoop nets were 4 m long with two 90 mm throats and black 6 mm stretch mesh. Fyke and hoop nets were generally deployed in twelve-hour increments, depending on permitting requirements. Electrofishing was conducted in shallow water along lake shores using a Smith-Root LR-24 (Smith-Root, Inc. Vancouver, WA).

All collected fish were identified to species and measured for length and weight (total length, mm; wet weight, g). Hybridization between rainbow (*Oncorhynchus mykiss*) and cutthroat trout (*Oncorhynchus clarkii spp*.; Muhlfeld et al., 2017) and bull and brook trout (*Salvelinus fontinalis*; Kanda et al., 2002) have been documented in northwest Montana. For this study, we identified fish to species by phenotype. Therefore, this study does not account for phenological nor ecological differences resulting from hybridization. A subsample of collected fish were biopsied for stable isotope (δ^{15}N and δ^{13}C) analysis. Only bull trout and lake trout presumed to be piscivorous (total length ≥ 200 mm; McPhail & Baxter, 1996) were biopsied. Each fish in this biopsy subsample was anesthetized with MS-222 (Popovic et al., 2012; Sladky et al., 2001) and a 4-mm soft tissue biopsy sample (Integra Miltex 336; Integra Life Sciences, Princeton, NJ, USA) was extracted (4 mm diameter by 4 mm long) from the dorsal white muscle. Dorsal white muscle is ideal for stable isotope food web studies because it has lower within-tissue isotope variance than other tissues, like red muscle (Pinnegar and Polunin, 1999). Next, biopsy wounds were cleaned and sealed using tissue glue (Wildgoose, 2000). Finally, biopsied fish were resuscitated and released. Muscle samples were stored in 100% industrial ethanol (95% ethanol, 5% methanol) while afield and stored in a -10ºC freezer for later processing.

Lab methods

Fish muscle samples were further subsampled to generate a representative and comparable analytical dataset for each lake. The analytical subset of fish muscle tissue is as follows: (1) all available bull trout; (2) 10 lake trout; and (3) five of all other sampled fish species. Samples were dried in a 60ºC oven for 72 hours and homogenized to a powder using a mortar and pestle (Jardine et al., 2003). 1 mg (± 0.1 mg) of fish tissue was loaded into a tin cup (5x9 mm, Costech 41077). Stable isotope analyses were conducted at University of California at Davis on a 20-20 Europa Scientific mass spectrometer. Stable isotopes can be used to infer food web structure. The ratio of heavy isotope to light isotope (^{15}N:^{14}N or ^{13}C:^{12}C) in a sample can be compared to an analytical standard (Fry, 2006). The difference in isotope concentration between the sample and standard can then be expressed in ‰ to produce the sample's $\delta^{15}N$ and $\delta^{13}C$ value (Fry, 2006). The ratio of stable nitrogen isotopes, $\delta^{15}N$, is higher (approximately 3-4 ‰) in predators than their prey and is thereby used to infer consumer trophic position (Fry, 2006). Conversely, the ratio of stable carbon isotopes, $\delta^{13}C$, changes very little (<1 ‰) between predators and prey and is thereby used to track patterns of biomass production (Fry, 2006). Herein, isotopic ratios are expressed in standard delta "δ" notation relative to Vienna PeeDee Belamnite ($\delta^{13}C$) and atmospheric nitrogen ($\delta^{15}N$) and following Sharp (2017).

Fish functional groups

Fish species were assigned to five functional groups for analyses: 1) bull trout, 2) lake trout, 3) littoral forage fish, 4) generalist fish, and 5) pelagic forage fish (Appendix: Table 1.3). Functional groups (i.e., littoral and pelagic forage fish and generalist fish) aggregated presumed prey fishes based on habitat (Neverman and Wurtsbaugh, 1994; Page and Burr, 2011) and trophic position relative to lake trout and bull trout (Meeuwig et al., 2011). Littoral forage fish, like redside shiners (*Richardsonius balteatus*), occupy nearshore habitat (Page and Burr, 2011). Generalist fish, like cutthroat trout (*Oncorhynchus clarkii lewisi*), may move between habitats regularly and consume a variety of prey (Page and Burr, 2011). Pelagic forage fish, like mountain whitefish (*Prosopium williamsoni*), occupy offshore habitat (Page and Burr, 2011).

Data analysis

We used 95% confidence interval ellipses to visualize our $\delta^{13}C$ and $\delta^{15}N$ data (Jackson et al., 2011). Next, we used linear mixed effects models to examine magnitude, direction, and statistical significance of changes in $\delta^{13}C$ and $\delta^{15}N$ between lake trout invasion categories (Bates et al., 2015). We included lake as a random effect in these models to account for among-lake isotope signature variation. We neither transformed nor corrected isotope data for preservation and model fit and residual normality was confirmed using residual plots. All data analyses were completed in R (R Core Team, 2018).

We calculated a posterior distribution of population mean and variance (μ and σ^2) using R package SIBER's markov chain monte carlo (mcmc) and uninformative priors as detailed in Jackson et al. (2011). Then we generated a distribution of isotope ellipses based on posterior parameter distributions (Jackson et al., 2011). Next, we calculated Bayesian standard ellipse area (SEA.b) based on posterior-derived ellipses (Jackson et al., 2011). We used SEA.b to probabilistically account for sample mean uncertainty associated with small sample size and characterize isotope niche area, an ecologically important aspect of food web structure (Jackson et al., 2011). As diet specificity increases, ellipse area decreases (Jackson et al., 2011). Therefore, SEA.b is a probabilistic approach to measuring niche width and height (Jackson et al., 2011).

We used R package nicheROVER (Lysy et al., 2014) to investigate the symmetry of isotope niche overlap between bull trout and lake trout. Pairwise comparison of isotope niche overlap describes directionality of overlap, which is useful for examining the likelihood of competitive exclusion (Swanson et al., 2015). Asymmetric overlap, where one species is likely to be in another species' isotope niche but the opposite is not likely, can suggest competitive exclusion (Swanson et al., 2015). Conversely, symmetric overlap, where both species are likely to exist in each other's isotope niche, can suggest resource partitioning (Swanson et al., 2015).

We used R package MixSIAR (Stock et al., 2018) to model bull trout diets in each lake trout invasion category. MixSIAR allows researchers to probabilistically estimate diet proportions given more than two isotope sources and only two biotracer isotopes (Stock et al., 2018). We used MixSIAR's mcmc, as described in Stock et al. (2018), to produce a posterior distribution of proportions of three presumed prey fish groups: generalist fish, littoral forage fish, and pelagic forage fish.

Results

Summary

Despite near-constant mean δ^{15}N and δ^{13}C values, (Figure 1.2 and 1.3), the 95% confidence interval ellipses around fish functional group δ^{15}N and δ^{13}C means were noticeably larger in remediated lakes than either reference or invaded lakes (Figure 1.1). Isotope niche ellipse area reflects diet variability (Layman et al., 2007), so elevated niche area in remediated lakes suggests that food web disorder was the hallmark of remediated lakes. Markov chain monte carlo estimation confirmed that ellipse area was elevated in remediated lakes for four of five fish functional groups (Figure 1.4). Isotope niche overlap between bull trout and lake trout was symmetric in remediated lakes and asymmetric in invaded lakes (Figure 1.5), suggesting lake trout suppression may alleviate part of the presumed competitive advantage lake trout have over bull trout. Modelling of bull trout diet revealed bull trout diet shifted from a generalist piscivore diet composed of large proportions of pelagic prey in reference and invaded lakes to two specialist diets with low reliance on pelagic prey (Figure 1.6).

Confidence interval ellipses and linear mixed effects models

The mean δ^{15}N of bull trout, lake trout, littoral forage fish, and generalist fish did not statistically significantly change depending on invasion category (Figure 1.2 and Table 1.1). This indicates these fish groups neither increased nor decreased in trophic position in correlation with lake trout invasion and remediation. However, the δ^{15}N of pelagic forage fish was statistically significantly higher in remediated lakes than either reference or invaded lakes (Figure 1.2 and Table 1.1). An increase in δ^{15}N correlates with an increase in trophic position and can suggest switching from low trophic position prey, like invertebrates, to higher trophic position prey, like fish (Fry, 2006). The mean δ^{13}C of all fish functional groups did not change significantly based on invasion category (Figure 1.3

and Table 1.1). This indicates these fish did not switch prey along a pelagic-littoral gradient as the result of lake trout invasion and remediation.

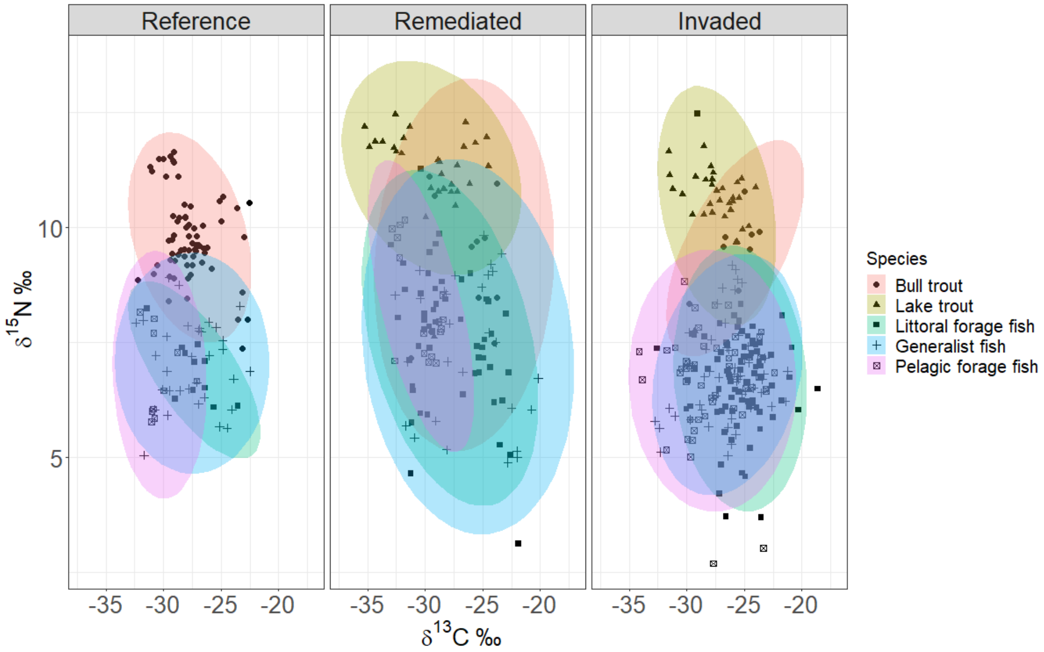

Figure 1.1. 95% confidence interval ellipses of fish δ^{13}C and δ^{15}N in reference, remediated, and invaded lakes in northwest Montana, USA.

Table 1.1. Summary table of fish δ^{13}C and δ^{15}N in reference, invaded, and remediated lakes from northwest Montana, USA. Data are presented as mean ± standard error. For all fish groups besides lake trout, linear mixed effects model p-values compare the mean isotope value of a fish group to its mean value in reference lakes. Lake trout p-values compare the mean isotope value of invaded lakes to remediated lakes because lake trout are not present in reference lakes.

	n	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**Total Length (mm)**	**$\delta^{13}C$ p-value**	**$\delta^{15}N$ p-value**
Invaded	**226**					
Bull trout	8	-25.78 ± 0.69	9.55 ± 0.27	387 ± 47	0.54	0.45
Lake trout	33	-27.39 ± 0.35	10.48 ± 0.17	527 ± 19	0.08	0.49
Littoral forage fish	82	-25.35 ± 0.26	6.71 ± 0.13	179 ± 15	0.59	0.56
Generalist fish	52	-26.47 ± 0.36	6.80 ± 0.14	164 ± 15	0.40	0.83
Pelagic forage fish	51	-27.78 ± 0.40	6.67 ± 0.16	231 ± 18	0.86	**0.03**
Reference	**122**					
Bull trout	68	-27.72 ± 0.26	9.79 ± 0.11	451 ± 15	-	-
Littoral forage fish	11	-27.33 ± 0.61	6.91 ± 0.20	288 ± 30	-	-
Generalist fish	33	-27.51 ± 0.46	7.05 ± 0.16	255 ± 26	-	-
Pelagic forage fish	10	-30.34 ± 0.44	6.80 ± 0.29	223 ± 28	-	-
Remediated	**136**					
Bull trout	10	-26.62 ± 0.82	9.20 ± 0.43	360 ± 68	0.43	0.78
Lake trout	30	-29.44 ± 0.55	11.30 ± 0.16	393 ± 31	-	-
Littoral forage fish	52	-27.83 ± 0.42	7.60 ± 0.20	226 ± 23	0.38	0.06
Generalist fish	16	-26.30 ± 0.67	7.41 ± 0.30	222 ± 29	0.89	0.15
Pelagic forage fish	28	-30.35 ± 0.43	8.30 ± 0.29	229 ± 11	0.86	0.07
Total	**484**					

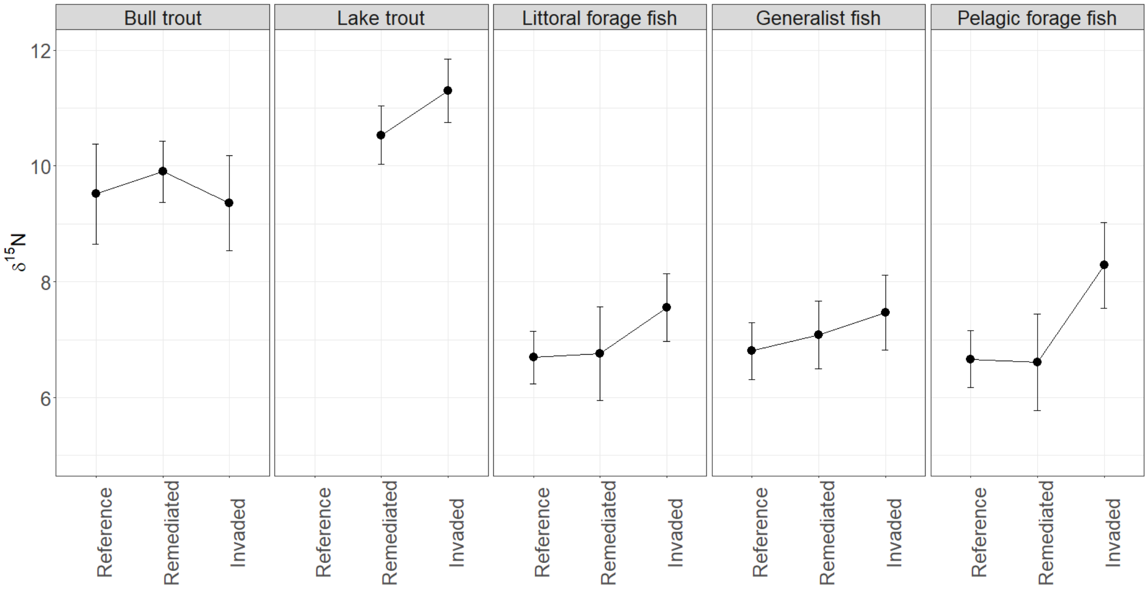

Figure 1.2. $\delta^{15}N$ linear mixed effects model results. Error bars show the 95% confidence interval around mean $\delta^{15}N$ (‰) values.

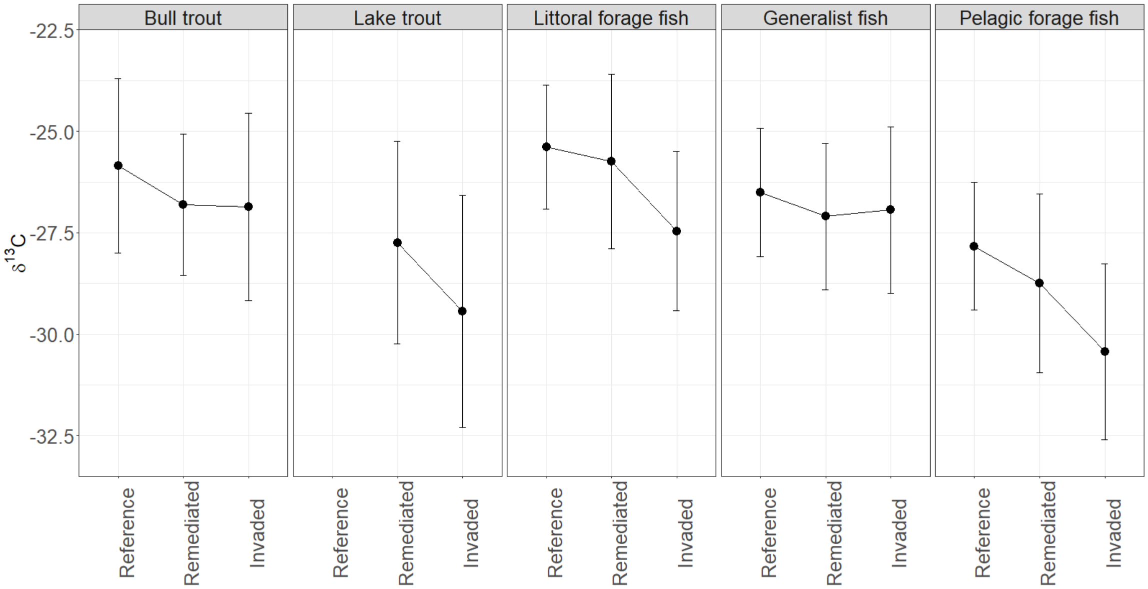

Figure 1.3. $\delta^{13}C$ linear mixed effects model results. Error bars show the 95% confidence interval around mean $\delta^{13}C$ (‰) values.

Isotope ellipse area

Isotope ellipse area was highest in remediated lakes for four of five fish functional groups (Figure 1.4). In contrast, pelagic forage fish isotope ellipse area was highest in invaded lakes. Isotope ellipse area increases with increasing diet variability from consumption of new prey or consumption of the same prey in different proportions (Jackson et al., 2011). Ellipse area alone cannot distinguish between these possibilities (Jackson et al., 2011). Further, changes in isotope niche area can reflect disturbance (Karlson et al., 2018). Therefore, it is likely elevated isotope ellipse area reflects the transient intermediate food web stages resulting from ongoing lake trout invasion.

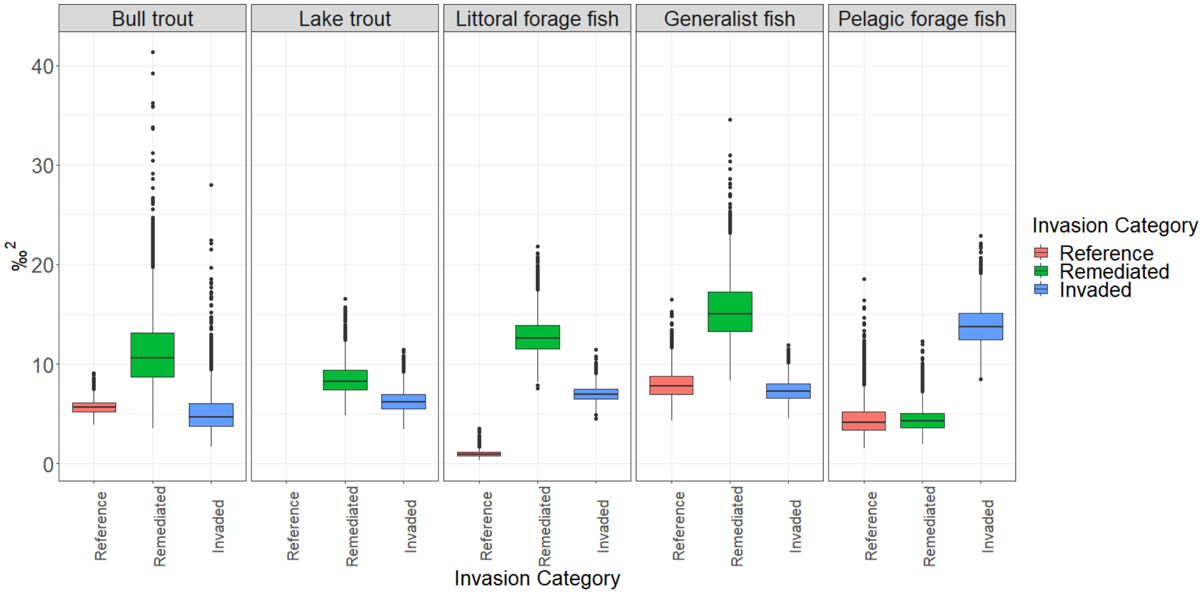

Figure 1.4. Boxplots showing median and interquartile range (boxes), minimum and maximum (whiskers), and outliers (dots) of Bayesian posterior estimates of standard isotope ellipse area (SEA.b) of five fish functional groups in three lake trout invasion categories (red: reference lakes; green: remediated lakes; blue: invaded lakes) collected in northwest Montana 2017-2019.

Asymmetric isotope niche overlap

Isotope niche overlap between bull trout and lake trout was asymmetric in invaded lakes but was symmetric in remediated lakes. On average, in invaded lakes, 84% of bull trout isotope niche overlapped lake trout isotope niche (Figure 1.5A), while only 41% of lake trout isotope niche overlapped bull trout isotope niche (Figure 1.5B). Given isotope niche overlap credible intervals in Figures 1.5A and 1.5B, isotope niche overlap between bull trout and lake trout was highly asymmetric in invaded lakes. In contrast, in remediated lakes, an average of 42% of bull trout isotope niche overlapped lake trout isotope niche (Figure 1.5C) and 56% of lake trout isotope niche overlapped bull trout isotope niche (Figure 1.5D). Given isotope niche overlap credible intervals in Figures 1.5C and 1.5D, isotope niche overlap between bull trout and lake trout was highly symmetric in remediated lakes. Symmetric isotope niche overlap can suggest resource partitioning, whereas asymmetric isotope niche overlap can suggest competitive exclusion (Swanson et al., 2015). Thus, since lake trout suppression correlated with increasing symmetry of bull trout and lake trout isotope niche overlap, lake trout suppression may diminish lake trout's presumed competitive exclusion of bull trout.

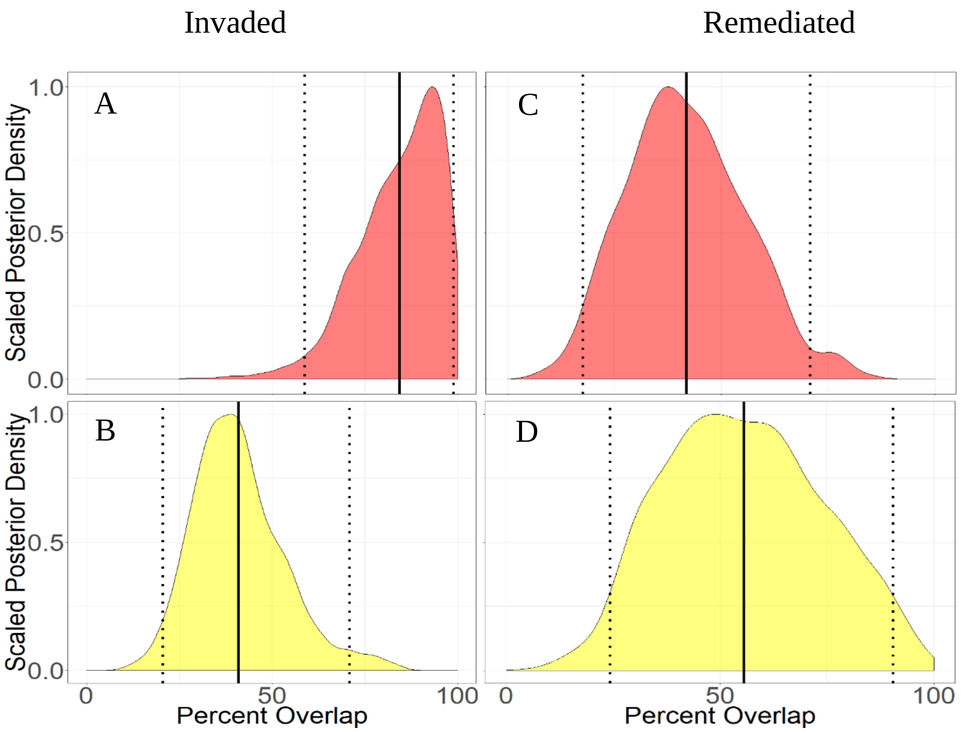

Figure 1.5. Scaled posterior density of percent isotope niche ellipse overlap. Red: percent of bull trout isotope niche overlapping lake trout isotope niche. Yellow: percent of lake trout isotope niche overlapping bull trout isotope niche. Solid line: mean overlap percentage. Dotted lines: 95% credible interval overlap percentage. 1.5A: Percent of bull trout isotope niche overlapping lake trout isotope niche in invaded lakes. 1.5B: Percent of lake trout isotope niche overlapping bull trout isotope niche in invaded lakes. 1.5C: Percent of bull trout isotope niche overlapping lake trout isotope niche in remediated lakes. 1.5D: Percent of lake trout isotope niche overlapping bull trout isotope niche in remediated lakes.

Bull trout diet

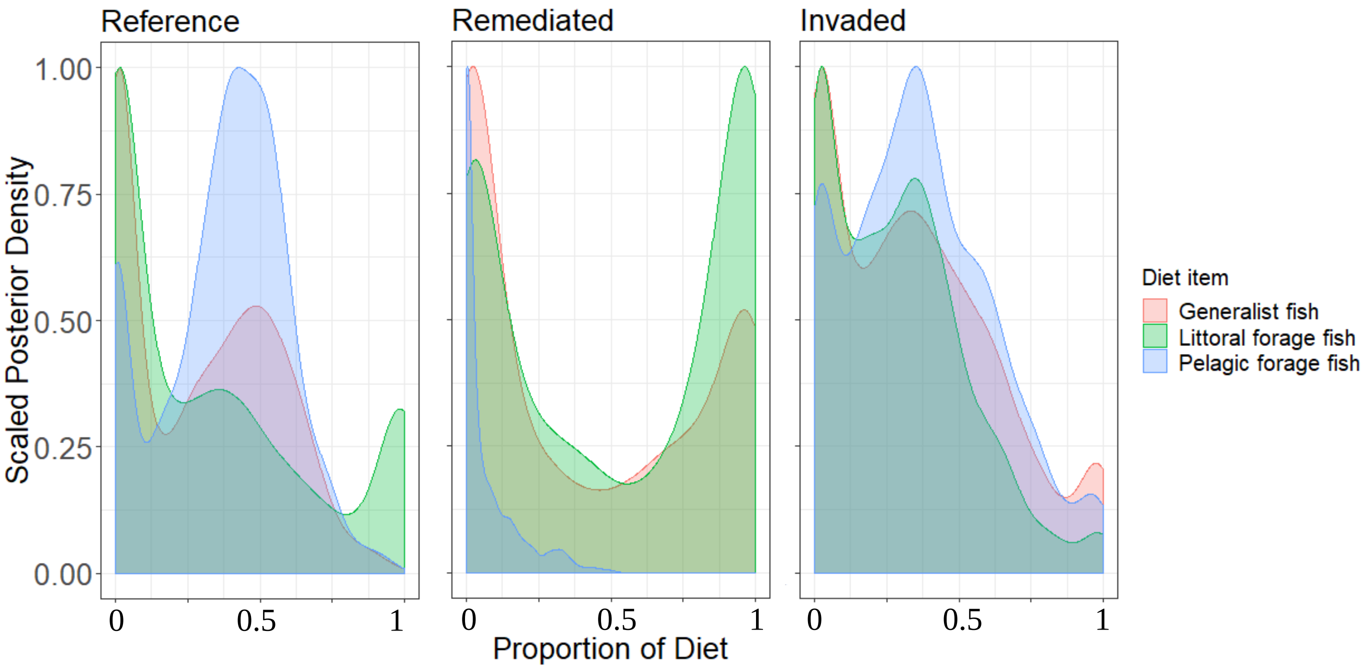

Figure 1.6. Scaled posterior density of proportional contribution of three prey fish functional groups to bull trout diet in reference, remediated, and invaded lakes in northwest Montana.

In reference and invaded lakes, isotope data suggest bull trout ate a varied piscivorous diet consisting of all three diet items: generalist fish, littoral forage fish, and pelagic forage fish. The proportions of each diet item varied slightly between invaded and reference lakes, with bull trout relying more heavily on pelagic fish in reference lakes than invaded lakes. In contrast, the diet of remediated bull trout was markedly different than either the invaded or reference bull trout. Bull trout in remediated lakes relied mostly on generalist fish or littoral forage fish and had a uniquely low probability of consuming pelagic forage fish (Figure 1.6).

Discussion

Summary

Lake trout invasion caused significant food web disruption in our study system. Food webs were highly disordered in remediated lakes, relative to invaded or reference lakes. Increasing disorder suggests remediated lakes may be a disturbed intermediate successional stage between two relatively stable states: reference lakes and invaded lakes. Interestingly, lake trout invasion generally did not produce simple, directional shifts in the isotope signature of fish in our study system, as was expected based on literature review. This finding suggests that lake trout suppression could be especially important to prevent further food web transformation from reference toward fully invaded lakes.

Isotope signature

Meeuwig et al. (2011), a previous isotope ecology study from our study region, found that lake trout generally had higher $\delta^{15}N$ and lower $\delta^{13}C$ than bull trout. Our data mirror these findings further suggesting lake trout may prey upon bull trout and prefer pelagic prey fish. Additionally, Meeuwig et al. (2011) found partial diet overlap between these bull trout and lake trout. Our data corroborate partial diet overlap between lake trout and bull trout, especially in invaded lakes. However, our analyses further probed niche overlap between these species and showed that there was significantly more symmetric niche overlap between lake trout and bull trout in remediated lakes than invaded lakes. Meeuwig et al. (2011) noted the challenge of attributing observed changes in bull trout $\delta^{15}N$ and $\delta^{13}C$ to lake trout invasion because "…bull trout food habits often differ among lakes…" We used mixed effects linear modelling to account for among-lake diet differences for all sampled fish and generally found no significant effect of invasion category on fish $\delta^{15}N$ and $\delta^{13}C$. Meeuwig et al. (2011) also noted their study lacked reference lakes against which food web structure comparisons could be based. Our study included three reference lakes, most notably Big Salmon Lake, which is, according to Montana Fish, Wildlife, and Parks, among the best-preserved reference bull trout lakes in Montana (Rosenthal, 2019). Therefore, our study addressed some of the lingering questions posed in Meeuwig et al. (2011) with replicate reference lakes to maximize the strength of our inferences.

Niche overlap

Our data (and data from Meeuwig et al. (2011)) show piscivorous bull trout and lake trout in our study system have partial niche overlap along both the $\delta^{15}N$ and $\delta^{13}C$ axes. These findings suggest selection pressure among these species may operate in both the habitat-foraging axis ($\delta^{13}C$) and trophic level axis ($\delta^{15}N$). Our pairwise niche overlap modelling showed there was more symmetric isotope niche overlap between lake trout and bull trout in remediated lakes than invaded lakes in our study area. Increasing symmetry of niche overlap suggests increasing diet similarity between bull trout and lake trout after remediation, which is promising evidence gill net suppression of lake trout may even the playing field for bull trout.

Trophic displacement from fish invasion

Surprisingly, our data showed little evidence that lake trout caused simple trophic displacement of bull trout in our study systems. Instead of straightforward, directional trophic displacement (e.g., decreasing bull trout $\delta^{15}N$ correlating with lake trout invasion), as has been documented after fish invasion in other study systems (Lake Superior: Schmidt et al., 2009; Canadian lakes: Vander Zanden et al., 1999), lake trout invasion in our study system correlated with increasing food web disorder. Highly ordered trophic linkages increase ecosystem stability (Madigan et al., 2012), diet plasticity becomes increasingly evolutionary costly as ecosystems stabilize (Bolnick et al., 2003; Chavarie et al., 2016; Snorrason and Skúlason, 2004), and well-defined trophic linkages, the result of diet specialization, are known to produce small isotope niche area (Martínez del Rio et al., 2009). Therefore, the small fish isotope ellipse area of reference and invaded lakes suggests these lakes may be relatively stable ecosystem states. However, the large fish isotope ellipse areas in remediated lakes indicates low ecosystem stability in remediated lakes, suggesting remediated lakes may be an unstable transition state.

Bull trout diet

Since bull trout trophic position and signature were consistent in our three invasion categories, either a) bull trout ate the same diet (prey type and amount) regardless of the presence of lake trout or b) bull trout simply ate less (smaller amounts) of the same prey in the presence of lake trout. Given precipitous declines in bull trout abundance corresponding with establishment of lake trout (Fredenberg et al., 2017), the latter option is more plausible and bull trout may simply be inflexible in their diet and unable or unwilling to feed in the presence of lake trout. This explanation is supported by isotope ellipse area data and diet modelling data.

In many locations, adult bull trout are piscivorous and eat a variety of prey fish depending on prey availability (Donald and Alger, 1993; Guy et al., 2011; Schoby and Keeley, 2011; Wilhelm et al., 1999). Our diet models confirm this trend for bull trout in reference and invaded lakes. However, our diet models suggest divergence from this trend in remediated lakes. Instead, our diet models suggest bull trout from remediated lakes exhibit one of two specialist diets: high reliance on generalist fish, like northern pikeminnow (*Ptychocheilus oregonensis*), and high reliance on littoral fish, like redside shiners.

Diet and fecundity: a plausible mechanism for bull trout declines

That bull trout and lake trout overlap in isotope niche and in space and time suggests competition for food is plausible between bull trout and lake trout. Isotope niche overlap and likelihood of interaction are not enough to prove competition (Meeuwig et al., 2011). However, food competition between bull trout and lake trout could cause declining bull trout abundance via starvation or starvation-induced reductions in fecundity. Bull trout are not known to be adaptable (Jones et al., 2014; Selong et al., 2017) and there is little evidence that bull trout exhibit trophic polymorphism, or adaptation to consume a variety of prey (Dunham et al., 2008). Therefore, it is plausible bull trout are simply unable to feed or must reduce feeding in the presence of lake trout. Partial starvation could cause reduced fecundity in bull trout because bull trout fecundity is size-dependent and larger females produce more eggs (Johnston and Post, 2009). Therefore, if competition with lake trout caused bull trout to partially starve, bull trout size-at-age and fecundity could decrease after invasion. Over time, reduced fecundity could yield lower recruitment and a decline in bull trout abundance. The evidence presented here shows competition for food between bull trout and lake trout is plausible and the results of that competition could produce the bull trout abundance declines measured in our study system. Future studies should compare our findings against other study systems with comparable species invasions and removal programs and examine bull trout body condition factors to investigate the plausibility of starvation as a mechanism for bull trout abundance declines.

Management implications

Our findings show the effects of lake trout invasion on food webs may be more substantial than originally thought. Lake trout caused noticeable food web disorder in our study system and this disorder appears to be the precursor to bull trout displacement. This finding emphasizes the potential benefits of lake trout suppression for bull trout conservation. Suppression, when applied at adequate fishing pressure, may be effective at causing lake trout population collapse (Hansen et al., 2016) which seems to be the only long-term solution for bull trout conservation. Given our findings, it seems that lake trout suppression may remain the cornerstone of tributary spawning (adfluvial) bull trout conservation.

Isotope ecology's role in restoration and conservation

Our findings emphasize the need for a broad understanding of ecological responses to disturbance. This theme transfers to nearly any study system or focal species because humans have introduced species worldwide (Vitousek et al., 1996) and controlling introduced species is a priority in many species restoration plans (Ruiz-Jaen and Mitchell Aide, 2005). Isotope ecology offers unique and valuable insights into the effects invasive species establishment and remediation can have on food webs. Those insights can inform management and policy decisions in a broad array of circumstances and enhance restoration outcomes in a variety of ecological contexts.

Limitations

Care must be taken when inferring an animal's diet based exclusively on stable isotope data (Phillips et al., 2014) because isotopic niche is closely correlated with, but not equivalent to ecological niche (Jackson et al., 2011). Therefore, it is important to incorporate error into diet inferences to account for subtle niche differences. Our models account for error structure inherent with inferring diet preference from isotope data.

Additionally, as the number of diet sources (i.e., prey) increases, uncertainty around diet inferences increases (Stock et al., 2018). To maximize certainty of our inferences, we used three diet sources, only one more than a traditional weighted average two-end-member isotope mixing model.

Acknowledgements

This research was funded by the United States Geological Survey (USGS) and University of Montana Flathead Lake Biological Station, and conducted in collaboration with the National Park Service, USGS, Montana Fish Wildlife & Parks, US Forest Service, US Fish and Wildlife Service, and University of Montana Flathead Lake Biological Station. T. Bansak of Flathead Lake Biological Station collaborated with the Biological Station's donor network to raise funds for sample analysis. V. D'Angelo of USGS in West Glacier, MT provided technical support and guidance. L. Rosenthal and S. Bourret of Montana Fish Wildlife & Parks in Kalispell, MT contributed data, samples, and manuscript guidance. C. Downs and J. McCubbins of the National Park Service in West Glacier, MT contributed data and samples. J. Vanderwall, B. Weber, and Z. Ren of Flathead Lake Biological Station in Polson, MT provided field support.

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Appendix

Table 1.2. Study lakes in northwestern Montana, USA.

Lake Name	**Surface Elevation (m)**	**Surface Area (ha)**	**Max Depth (m)**	**Invasion Phase**
Kintla	1221	1039	120	Invaded
Bowman	1228	910	77	Invaded
Lower Quartz	1277	80	19	Invaded
McDonald	961	2760	144	Invaded
Lindbergh	1369	329	36	Invaded
Grace	1208	52	30	Reference
Trout	1189	114	50	Reference
Big Salmon	1340	393	42	Reference
Hungry Horse Reservoir	1112	9630	149	Reference
Logging	1161	581	60	Remediated
Swan	940	1335	43	Remediated
Quartz	1345	352	84	Remediated

Table 1.3. Fish species found in study lakes.

Common name	**Scientific name**	**Non-native species**	**Native Species**	**Functional group**
Bull trout	*Salvelinus confluentus*		X	Bull trout
Cutthroat trout	*Oncorhynchus clarkii lewisi*		X	Generalist
Mountain whitefish	*Prosopium williamsoni*		X	Pelagic
Longnose sucker	*Catostomus catostomus*		X	Littoral
Largescale sucker	*Catostomus macrocheilus*		X	Littoral
Peamouth	*Mylocheilus caurinus*		X	Pelagic
Northern pikeminnow	*Ptychocheilus oregonensis*		X	Generalist
Slimy sculpin	*Cottus cognatus*		X	Generalist
Redside shiner	*Richardsonius balteatus*		X	Littoral
Lake trout	*Salvelinus namaycush*	X		Lake trout
Lake whitefish	*Coregonus clupeaformis*	X		Pelagic
Kokanee	*Oncorhynchus nerka*	X		Pelagic
Bluegill	*Lepomis macrochirus*	X		Littoral
Northern pike	*Esox lucius*	X		Littoral
Central mudminnow	*Umbra limi*	X		Littoral
Yellow perch	*Perca flavescens*	X		Littoral
Rainbow trout	*Oncorhynchus mykiss*	X		Generalist
Brook trout	*Salvelinus fontinalis*	X		Littoral
Yellowstone cutthroat trout	*Onchorhynchus clarkii bouveri*	X		Generalist

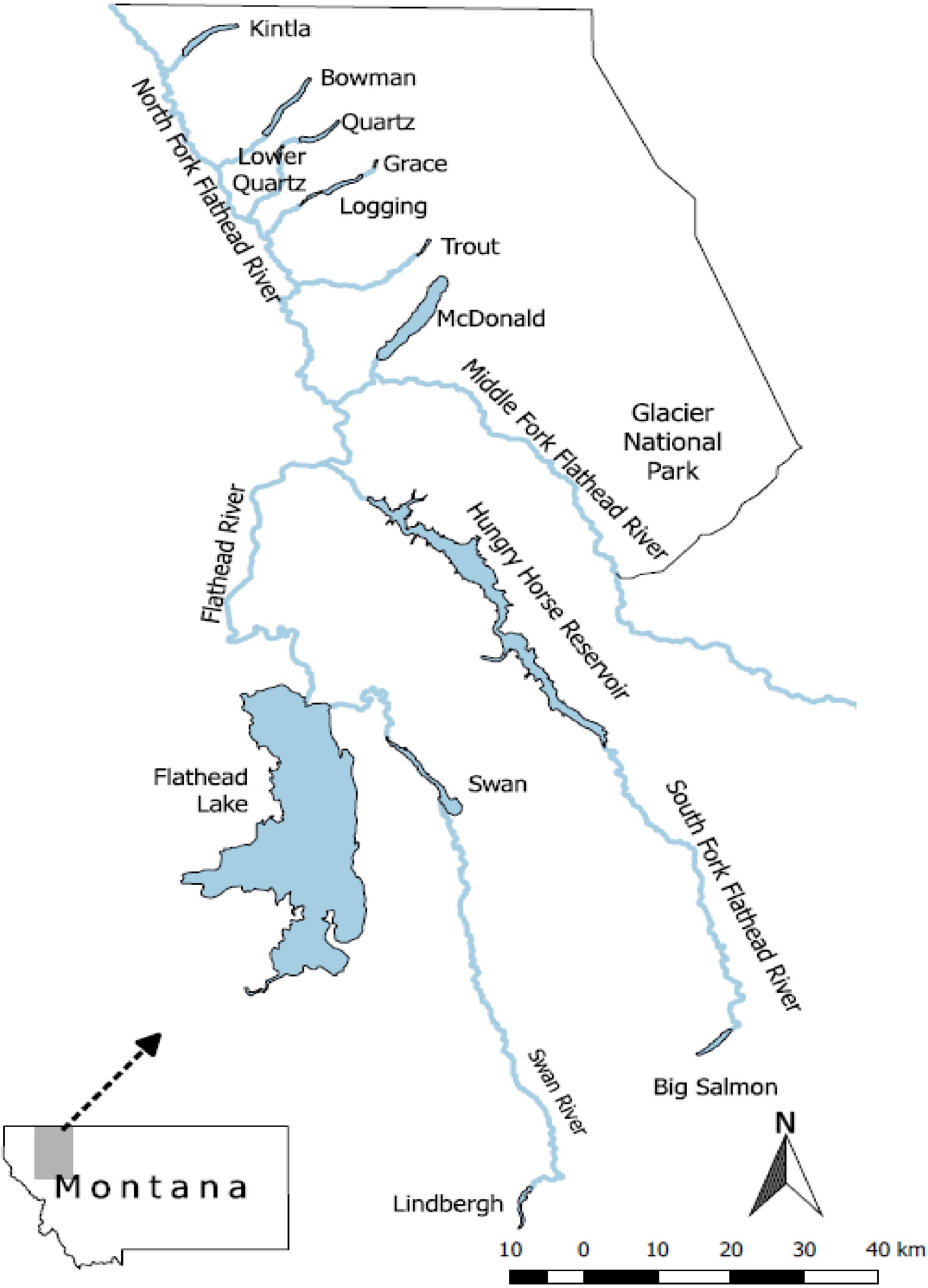

Figure 1.7. Map of study system in northwest Montana, USA.

Table 1.4. Lake invasion category, lake name, species, total sample size (N), and total length of the total sample of fish collected from each lake and isotope sample size (n), total length, and $\delta^{13}C$ and $\delta^{15}N$ of fish used for stable isotope analysis. Values are reported as mean ± standard error.

	Total sample		Isotope sample			
	N	Total Length (mm)	n	Total Length (mm)	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**
INVADED	983		226			
Bowman	**310**		**47**			
Bull trout	4	314 ± 37	4	314 ± 37	-24.51 ± 0.39	9.48 ± 0.3
Cutthroat trout	5	222 ± 54	5	222 ± 54	-27.89 ± 0.29	6.73 ± 0.24
Lake trout	65	418 ± 14	10	499 ± 44	-27.28 ± 0.45	10.03 ± 0.5
Largescale sucker	7	93 ± 11	5	90 ± 10	-23.77 ± 0.18	5.45 ± 0.5
Longnose sucker	52	218 ± 13	5	247 ± 15	-24.71 ± 0.18	6.86 ± 0.21
Mountain whitefish	151	262 ± 5	4	273 ± 25	-28.38 ± 0.85	8.43 ± 0.13
Peamouth	4	78 ± 3	4	78 ± 3	-25.96 ± 0.67	6.8 ± 0.23
Redside shiner	5	51 ± 10	5	51 ± 10	-26.42 ± 0.34	6.54 ± 0.76
Slimy sculpin	17	53 ± 2	5	52 ± 3	-30.31 ± 0.94	5.69 ± 0.15
Kintla	**267**		**45**			
Bull trout	1	588	1	588	-26.65	9.79
Cutthroat trout	12	215 ± 17	5	194 ± 6	-25.75 ± 0.73	6.58 ± 0.09
Lake trout	45	491 ± 19	10	542 ± 38	-25.72 ± 0.36	10.35 ± 0.15
Largescale sucker	14	263 ± 16	5	220 ± 28	-24.24 ± 0.31	6.76 ± 0.25
Longnose sucker	39	169 ± 19	5	207 ± 32	-24.93 ± 0.48	6.82 ± 0.25
Mountain whitefish	106	254 ± 4	5	288 ± 26	-29.29 ± 0.6	7.08 ± 0.15
Peamouth	33	192 ± 2	5	208 ± 12	-26 ± 0.74	6.75 ± 0.24
Redside shiner	4	49 ± 1	4	49 ± 1	-26.66 ± 0.25	6.13 ± 0.45
Slimy sculpin	13	51 ± 2	5	54 ± 4	-26.08 ± 0.49	6.72 ± 0.56
Lindbergh	**128**		**39**			
Bull trout	1	490	1	490	-24.97	10.79
Lake trout	5	500 ± 85	5	500 ± 85	-30.77 ± 0.44	11.09 ± 0.16
Largescale sucker	3	302 ± 73	3	302 ± 73	-20.22 ± 0.91	6.25 ± 0.13
Longnose sucker	13	379 ± 15	5	436 ± 10	-28.63 ± 0.59	7.27 ± 0.23
Mountain whitefish	25	252 ± 4	5	264 ± 4	-29.78 ± 0.33	7.02 ± 0.13
Northern pikeminnow	52	230 ± 6	5	310 ± 7	-25.92 ± 0.29	8.93 ± 0.09
Peamouth	3	212 ± 10	3	212 ± 10	-23.35 ± 0.21	6.56 ± 0.1
Rainbow trout	1	343	1	343	-25.7	6.65
Redside shiner	12	66 ± 8	5	94 ± 7	-26.94 ± 0.83	6.17 ± 0.12
Slimy sculpin	1	46	1	46	-31.04	5.9
Yellow perch	12	132 ± 13	5	164 ± 6	-22.75 ± 0.74	7.19 ± 0.2
Lower Quartz	**65**		**35**			
Cutthroat trout	6	242 ± 14	5	252 ± 11	-29.58 ± 1.17	6.75 ± 0.39
Largescale sucker	7	305 ± 50	5	313 ± 68	-24.02 ± 0.72	6.63 ± 0.23
Longnose sucker	14	117 ± 25	5	58 ± 3	-25.6 ± 0.43	4.94 ± 0.43

Mountain whitefish	12	152 ± 17	5	109 ± 29	-30.17 ± 2.06	4.97 ± 0.93
Peamouth	6	68 ± 6	5	68 ± 7	-27.95 ± 1.21	6.52 ± 0.42
Redside shiner	7	60 ± 7	5	54 ± 9	-29.12 ± 1.14	7.17 ± 0.32
Slimy sculpin	13	48 ± 2	5	54 ± 2	-23.99 ± 0.91	6.55 ± 0.23
McDonald	**213**		**60**			
Brook trout	6	123 ± 12	6	123 ± 12	-25.48 ± 0.38	6.84 ± 0.23
Bull trout	2	380 ± 132	2	380 ± 132	-28.3 ± 1.48	8.97 ± 0.62
Cutthroat trout	5	238 ± 26	5	238 ± 26	-25.94 ± 0.17	6.15 ± 0.39
Lake trout	29	418 ± 18	8	508 ± 30	-27.5 ± 0.45	10.84 ± 0.19
Lake whitefish	12	486 ± 16	5	514 ± 10	-28.29 ± 1.5	7.08 ± 0.19
Largescale sucker	4	301 ± 75	4	301 ± 75	-23.88 ± 0.86	7.79 ± 0.17
Longnose sucker	20	348 ± 25	5	341 ± 43	-25.62 ± 0.75	7.6 ± 0.13
Mountain whitefish	35	279 ± 9	5	310 ± 10	-29.21 ± 0.4	5.65 ± 0.21
Northern pikeminnow	44	194 ± 5	5	200 ± 11	-24.78 ± 0.45	7.59 ± 0.28
Peamouth	24	168 ± 5	5	187 ± 20	-25.23 ± 1.13	6.84 ± 0.43
Redside shiner	17	42 ± 3	5	54 ± 7	-25.97 ± 1.02	7.65 ± 1.24
Slimy sculpin	15	44 ± 2	5	46 ± 2	-23.75 ± 0.89	6.55 ± 0.35
REFERENCE	267		122			
Big Salmon	**67**		27			
Bull trout	12	551 ± 66	12	493 ± 20	-29.47 ± 0.31	11.21 ± 0.13
Cutthroat trout	12	303 ± 13	5	321 ± 24	-27.62 ± 1.38	7.18 ± 0.42
Longnose sucker	24	123 ± 12	5	195 ± 20	-27.12 ± 0.95	6.66 ± 0.24
Mountain whitefish	19	97 ± 11	5	150 ± 27	-29.93 ± 0.87	7.57 ± 0.27
Grace	**15**		**13**			
Bull trout	12	289 ± 44	10	325 ± 43	-24.05 ± 0.54	9.08 ± 0.35
Yellowstone cutthroat trout	3	248 ± 102	3	248 ± 102	-26.3 ± 2.08	6.84 ± 0.52
Hungry Horse	**149**		**69**			
Bull trout	69	497 ± 21	43	468 ± 18	-28.19 ± 0.19	9.51 ± 0.07
Cutthroat trout	13	275 ± 26	5	366 ± 8	-28.36 ± 1.02	6.88 ± 0.31
Largescale sucker	11	274 ± 43	5	380 ± 18	-26.71 ± 0.42	6.88 ± 0.25
Longnose sucker	1	295	1	295	-31.42	8.24
Mountain whitefish	8	282 ± 9	5	297 ± 8	-30.75 ± 0.2	6.03 ± 0.12
Northern pikeminnow	20	155 ± 34	5	364 ± 70	-28.14 ± 0.59	7.84 ± 0.46
Slimy sculpin	27	39 ± 4	5	64 ± 4	-30.6 ± 0.34	6.33 ± 0.38
Trout	**36**		**13**			
Bull trout	3	463 ± 25	3	463 ± 25	-26.25 ± 1.29	10.46 ± 0.17
Cutthroat trout	12	313 ± 15	5	353 ± 6	-25.08 ± 0.57	7.76 ± 0.16
Slimy sculpin	21	54 ± 3	5	70 ± 4	-26 ± 1.01	6.45 ± 0.39
REMEDIATED	311		136			
Logging	**106**		**45**			
Bull trout	2	277 ± 38	2	277 ± 38	-24.66 ± 0.86	10.33 ± 0.63
Cutthroat trout	13	326 ± 10	5	361 ± 9	-27.29 ± 0.7	8.6 ± 0.19

Kokanee	1	178	1	178	-32.64	7.1
Lake trout	12	248 ± 23	10	268 ± 20	-26.53 ± 0.43	11.42 ± 0.18
Largescale sucker	12	277 ± 10	5	306 ± 6	-24.54 ± 0.5	8.16 ± 0.26
Longnose sucker	16	212 ± 50	5	262 ± 34	-26.16 ± 1.11	8.27 ± 0.37
Mountain whitefish	12	228 ± 11	5	264 ± 8	-28.6 ± 0.07	8 ± 0.14
Northern pikeminnow	12	259 ± 20	5	310 ± 34	-24.35 ± 0.31	9.25 ± 0.19
Redside shiner	24	53 ± 3	5	56 ± 6	-24.5 ± 0.83	5.86 ± 0.78
Slimy sculpin	2	57 ± 2	2	57 ± 2	-30.37 ± 1.29	5.74 ± 0.05
Quartz	**86**		**47**			
Bull trout	6	218 ± 7	6	218 ± 7	-25.52 ± 0.81	9.08 ± 0.47
Cutthroat trout	14	302 ± 14	5	336 ± 10	-26.81 ± 1.28	6.92 ± 0.4
Lake trout	13	281 ± 12	10	296 ± 10	-28.66 ± 0.25	10.55 ± 0.34
Largescale sucker	12	304 ± 23	5	377 ± 32	-27.61 ± 1.44	7.78 ± 0.47
Longnose sucker	12	229 ± 12	5	270 ± 4	-29.13 ± 0.6	8.18 ± 0.28
Mountain whitefish	11	233 ± 7	5	252 ± 3	-29.69 ± 0.2	7.26 ± 0.12
Redside shiner	12	69 ± 7	5	92 ± 5	-23.9 ± 0.46	6.26 ± 0.34
Slimy sculpin	6	59 ± 8	6	59 ± 8	-21.71 ± 0.42	5.64 ± 0.3
Swan	**119**		**44**			
Bluegill	5	59 ± 5	5	59 ± 5	-29.82 ± 0.19	8.25 ± 0.86
Bull trout	2	642 ± 57	2	642 ± 57	-30.13 ± 0.55	8.67 ± 1.23
Central mudminnow	2	78 ± 31	2	78 ± 31	-30.62 ± 0.54	6.95 ± 1.19
Kokanee	27	159 ± 7	5	181 ± 16	-32.3 ± 0.18	9.87 ± 0.14
Lake trout	39	601 ± 20	10	616 ± 25	-33.13 ± 0.42	11.93 ± 0.09
Longnose sucker	4	499 ± 8	4	499 ± 8	-31.91 ± 0.39	8.48 ± 0.71
Northern pike	12	247 ± 54	5	416 ± 86	-29.27 ± 0.29	8.49 ± 0.69
Rainbow trout	1	518	1	518	-30.48	9.07
Redside shiner	22	47 ± 4	5	37 ± 5	-30.62 ± 0.39	6.77 ± 0.58
Slimy sculpin	4	54 ± 14	4	54 ± 14	-30.68 ± 0.95	7.31 ± 0.79
Yellow perch	1	214	1	214	-30.54	7.42
GRAND TOTAL	**1561**		**484**			

Chapter 2 : Using Space-For-Time Substitution to Examine Lake Food Web Succession After Species Invasion

Abstract

In the early 1900s, lake trout (*Salvelinus namaycush*) were widely introduced in several lakes and reservoirs outside their native range in western North America. More recently, lake trout have become problematic in western North America because they are now understood to cause declines in popular sport fishes and native species, most notably federally protected bull trout (*Salvelinus confluentus)*. Despite literature asserting invasive fish can cause cascading trophic effects in aquatic communities, food web effects of fish invasions are rarely quantified and native fish restoration plans have traditionally neglected these food web effects. In this chapter, I combined stable isotope analysis with space-for-time substitution to quantify the effects of lake trout invasion on lake food webs in northwestern Montana, USA. I found that the isotope signature of several taxa showed significant isotope value changes and primarily shifted toward littoral carbon reliance on littoral-pelagic axis ($\delta^{13}C$) with time. $\delta^{13}C$ shifts were surprisingly pronounced in prey fish, especially largescale sucker (*Catostomus macrocheilus*), indicating lake trout invasion may be affecting prey directly through predation or indirectly through cascading trophic effects. In general, macroinvertebrate isotope signature did not correlate with time, but non-metric dimensional scaling ordination revealed littoral macroinvertebrate community composition changed considerably with time. Space-for-time substitution also revealed that it takes approximately 70 years for lake trout to displace bull trout in this study region, underscoring the importance of invasive species monitoring.

Introduction

Ecosystem transformations after species invasions are well documented (e.g., Case, 1990; Gamfeldt & Hillebrand, 2008; Ives & Carpenter, 2007; Ives et al., 2019; Layman, Quattrochi, et al., 2007; Rahel, 2000; Rieman et al., 2007; Steiner et al., 2005; Vander Zanden et al., 2004; Vitousek et al., 1996). Species invasions affect ecosystems in numerous ways, ranging from altered energy and nutrient dynamics (Walsh et al., 2016) to collapse of native species (Schmidt et al., 2009) or hybridization and declines in reproductive fitness for fish (Hitt et al., 2003; Muhlfeld et al., 2009). Despite copious examination of how species invasions affect ecosystems, the natural complexity of food webs makes predicting the timing and severity of these effects difficult (Chapin et al., 2011; Vander Zanden et al., 1999).

Studying food web shifts in response to invasion in real time is impractical because this process can take many years (Blois et al., 2013; Johnson & Miyanishi, 2008). Instead, studies investigating the temporal dynamics of food web response to species invasion have relied on historical specimens and written records of food web structure through time (e.g., Ellis et al., 2011; Schmidt et al., 2009; Vander Zanden et al., 2003). Historical records and specimens can provide useful insight. For example, Schmidt et al. (2009) found that Lake Superior supported native and invasive species because its ecological diversity promoted its food web stability. However, Ellis et al. (2011) documented how introduced opossum shrimp (*Mysis diluviana*) caused catastrophic food web changes in Flathead Lake and subsequent decline of native species. Despite the utility of historical records and specimens, this approach is limited by availability and quality of non-purpose-collected data.

To study food web succession in our study system (lakes of northwestern Montana responding to introduced lake trout), we combined space-for-time substitution with stable isotope analysis. Space-for-time substitutions, an approach popularized by terrestrial primary succession studies (e.g., Chapin et al., 1994), assume spatial and temporal variation in ecological succession are approximately equivalent and, therefore, different locations with different histories of disturbance may be used to represent temporal stages of succession (Pickett, 1989). Parameters like relative alien species abundance (R.A.S.A.), non-native species abundance as a proportion of community abundance (Catford et al., 2012), have recently become popular for quantifying invasion status. In this study, we adapt R.A.S.A to quantify invasion status of individual lakes and substitute lakes to represent successional stages in the trajectory of species invasion over time. Stable isotopes of nitrogen and carbon are the conventional analytical tools used to infer food web structure (Fry, 2006). Stable nitrogen isotope composition (^{15}N:^{14}N; $\delta^{15}N$) is enriched by

3-4 ‰ in predators relative to their prey and is used to estimated trophic position (Fry, 2006). Stable carbon isotope composition (^{13}C:^{12}C; $\delta^{13}C$), however, is consistent between predators and prey (<1‰ enrichment) and is used to identify patterns of production because periphyton is typically $\delta^{13}C$ enriched relative to phytoplankton in lakes (Fry, 2006).

Northwest Montana, USA, is an ideal location to use space-for-time substitution to study lake food web succession because Montana hosts many natural lakes in various stages of fish invasion and remediation. Lake trout (*Salvelinus namaycush*), a piscivorous fish native to the Great Lakes and Hudson Bay drainage, were widely introduced outside their native range in the early 20th century, including to Montana's Flathead Lake in 1905 (Hansen et al., 2016). Flathead Lake's lake trout existed in relatively low abundance and were mostly confined to Flathead Lake for the next several decades (Ellis et al., 2011). However, *Mysis diluviana*, a freshwater shrimp native to the Great Lakes and circumpolar region, were introduced in lakes of the Flathead River drainage in the late 1960s (Hansen et al., 2016). Following the Flathead River downstream, *Mysis* become established in Flathead Lake by the early 1980s (Devlin et al., 2017). Establishment of *Mysis* alleviated a lake trout recruitment bottleneck in Flathead lake (Ellis et al., 2011), enabling lake trout population growth and subsequent range expansion to lakes throughout northwest Montana (W. Fredenberg, 2002; Meeuwig et al., 2011). Lake trout invasion is presently ongoing and is implicated as a primary cause of declines in northwest Montana's native fish, including federally protected bull trout (*Salvelinus confluentus;* Ellis et al., 2011; Fredenberg et al., 2017; Hansen et al., 2016).

The objective of this study was to develop a relationship between time and invasion in our study system and apply that relationship, along with stable isotope data, to examine food web succession a theoretical lake would follow after lake trout invasion. This study empirically tested ecological principles of disturbance and succession using a series of whole-lake systems. Certainly, many studies have examined disturbance and succession in aquatic systems (e.g., Matsuda and Abrams, 2004; Propst et al., 2015; Vander Zanden et al., 2003). However, this is the first study to feature a complete set of intact lakes representing a gradient of successional stages ranging from uninvaded to highly invaded. Additionally, the mathematical relationship between time and invasion developed herein can be adapted to other systems for management and research purposes, like as an indicator of invasive fish suppression program effectiveness.

Methods

Study area

The data presented here were collected from 11 sites (10 natural lakes and one reservoir, hereafter referred to as "lakes", in northwest Montana, USA; Appendix Figure 1 and Table 1). These lakes are oligotrophic, dimictic, subalpine lentic water bodies west of the continental divide where bull trout are native and lake trout are non-native. The lakes average 1196 m surface elevation, 1364 ha surface area, 70 m maximum depth, 12.6º C summer surface-water temperature, and 8.9 m summer Secchi depth. These lakes are in heavily forested watersheds within national park, national forest, or state forest boundaries.

Study design

We selected lakes with comparable in biogeochemical states and varying lake trout abundance. Lake trout are known to displace bull trout over time in our study region (W. Fredenberg, 2002; Meeuwig et al., 2011) and, therefore, we used lake trout relative abundance as a proxy for time-since-invasion (Table 2.1; Catford et al., 2012). First, we used standardized gill net survey data (Equation 1; Table 2.1) to calculate each lake's 2019 (or most recent) conversion ratio (C). Conversion is the ratio of invasive species to native species plus invasive species (Equation 1; adapted from Catford et al., 2012). Then we developed a binomial generalized linear model (GLM) to relate conversion and time based on historical gill net survey data (Equation 2; Table 2.2). GLM fit was evaluated with residual plots. Finally, we used simple linear regression to quantify magnitude and direction of food web changes through time.

$$conversion\ (C) = \frac{n_L}{(n_L + n_B)} \quad (1)$$

For our study system, n_L is the number of lake trout caught in a given lake in a given year and n_B is the number of bull trout caught in the same lake in the same year.

$$C' = \frac{1}{1+e^{-(\beta o+\beta x)}} \qquad (2)$$

Predicted conversion (C') can be estimated for any timestep (x) in any study system in which the invasive-native species displacement relationship from Equation 1 holds and enough empirical survey data are available to produce an acceptable fit. The fitted line has two logit-link coefficients (β_o and β) that define the line's intercept and instantaneous rate of change, respectively. These coefficients may vary among study systems and can be determined using binomial linear regression.

Finally, we estimated the average detection period (Equation 3) and full conversion period (Equation 4) for our full set of lakes. Detection period is the number of years between initial invasive species colonization and detection by monitoring surveys. Detection period is the value of x when C' equals zero (Equation 3). Full conversion period is the number of years between detection and full displacement of the native species in the proportion from Equation 1. Full conversion period is the value of x when C' equals one (Equation 4).

$$detection\,period = \lim_{C' \to 0} = \frac{1}{1+e^{-(\beta o+\beta x)}} \qquad (3)$$

$$full\ conversion\,period = \lim_{C' \to 1} = \frac{1}{1+e^{-(\beta o+\beta x)}} \qquad (4)$$

Sample collection

From 2017 to 2019, samples were collected between June and October. Fish were collected using gill nets, fykes, hoop nets, seines, backpack electrofisher, and hook and line. Fish were identified to species, weighed, and total length recorded. From a subset of collected fish (Appendix Table 3), a dorsal muscle biopsy was collected using a 4-mm soft tissue biopsy punch (Integra Miltex 336; Integra Life Sciences, Princeton, NJ, USA) and preserved in 100% ethanol. Littoral macroinvertebrates were collected using a 500-µm D-net at 0.5, 1, and 1.5 m from seven sampling locations in each lake. Profundal macroinvertebrates were collected with a grab sampler dredge from depths exceeding twice the Secchi maxima at each lake and filtered through a 500-µm D-net. Macroinvertebrates were identified to family, depurated, and preserved in ethanol for isotope sample preparation. Bulk zooplankton were collected using a 100-µm tow net in the pelagic epilimnion of each lake (United States Environmental Protection Agency, 2012). Periphyton was collected from littoral rocks of each lake using a brush.

Lab

All samples were dried in a 60ºC oven for 72 hours. Samples were homogenized into a powder using a mortar and pestle and 1 mg (± 0.1 mg) of animal tissue or 10 mg (± 0.1 mg) of periphyton or plant material loaded into tin cups (Costech 5x9 mm). For all taxa, besides bull trout and lake trout, a maximum of five isotope samples per taxon per lake were prepared (Table 2.5). A maximum of ten lake trout and all available bull trout were processed for isotope analysis (Table 2.5). Based on this protocol, 484 fish muscle samples and 1131 macroinvertebrate samples for stable isotope analyses were prepared. Isotope samples were processed for $\delta^{13}C$ and $\delta^{15}N$ by isotope ratio mass spectrometer in the University of California at Davis Stable Isotope Lab. Isotope data are expressed in ‰ $\delta^{13}C$ and $\delta^{15}N$ relative to Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$.

Quantifying food web change

I used simple linear regression to determine the direction and magnitude of food web structural change through time. Linear regression of $\delta^{15}N$ quantifies changes in trophic structure through time, whereas linear regression of $\delta^{13}C$ quantifies changes in basal resource reliance (i.e., periphyton or phytoplankton) through time. R package SIBER (Jackson et al., 2011) was used to calculate Layman metrics. Layman metrics are multivariate point estimates of population- or community-level food web structure used to measure isotope niche spacing and trophic redundancy (Layman et al., 2007). $\delta^{15}N$ range (NR) and $\delta^{13}C$ range (CR) are arithmetic differences between the most enriched and deplete ($\delta^{15}N$ and $\delta^{13}C$, respectively) individual of a species or community and quantify isotope niche height and width (Jackson et al., 2011). Mean nearest neighbor distance (MNND or NND) is the arithmetic average Euclidean distance (in ‰) of an individual from the isotopic average of its conspecifics and quantifies species diet evenness (Layman et al., 2007). I used a threshold of $P < 0.05$ to establish statistical significance for each regression. Where possible, I calculated an effect size for each metric to compare metrics to a value of known ecological importance. Effect size was calculated as the value of the metric (e.g., $\delta^{15}N$ range) relative to trophic discrimination (e.g., $\Delta^{15}N$) from literature (McCutchan et al., 2003; Post, 2002).

Ordination

I used R package "vegan" (Oksanen et al., 2018) to calculate non-metric dimensional scaling (NMDS) ordinations to evaluate macroinvertebrate community similarity within and among lake trout invasion timesteps. I ordinated macroinvertebrate communities by sampling transect because transect is the lowest aggregation at which I expect independent macroinvertebrate communities in this study design. Next, I calculated NMDS scores to quantify macroinvertebrate community similarity each transect, grouped NMDS scores by timestep, and plotted NMDS scores with 95% confidence interval ellipses. Finally, I tested for community similarity among timesteps using permutational multivariate analysis of variance (permANOVA).

Results

Conversion

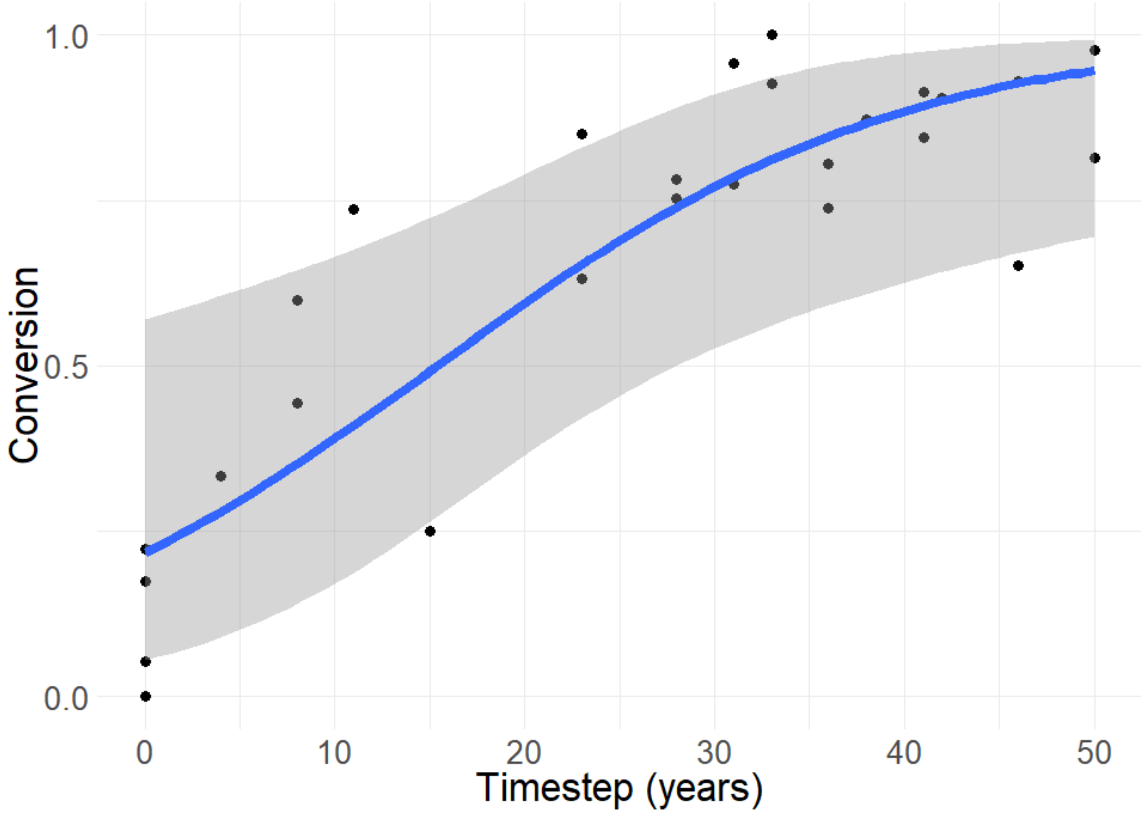

Figure 2.1. Binomial linear regression of conversion through time in northwest Montana. Empirical data are from 1969-2019 standardized gill net surveys in Glacier National Park, Montana, USA (McCubbins et al., In Prep.). In this figure, survey data have been normalized to timestep, or year since first survey year. Empirical conversion (n = 24; black points) and predicted conversion (blue line; $r^2 = 0.792$) with 95% confidence intervals (gray ribbon).

Predicted conversion (C')

We used binomial linear regression in R to fit a logistic growth model to predict conversion (C') given our study system's empirical conversion (C) at each timestep (x). For our study system, binomial linear regression coefficients were: $\beta_o = -1.41$ and $\beta = 0.087$.

Detection period

Detection period for our study system (Equation 3) averaged 18 years, indicating that lake trout were present in the study lakes for 18 years, on average, prior to gill net surveys detecting them.

Full conversion period

Full conversion period for our study system (Equation 4) averaged 51 years, indicating it will take about 51 years after detection, on average, for lake trout to fully displace bull trout in our study lakes, after detection.

Summing detection and full conversion periods provides an estimate of the invasion timeline. We estimate it takes roughly 69 years, on average, from initial lake trout colonization for lake trout to fully displace bull trout in our study area.

Quantification of food web changes

Bull trout mean δ^{15}N decreased though time from 9.77 ‰ ± 0.12 ‰ at 0 years to 9.33 ± 0.12 ‰ at 71.7 years (mean ± standard error; Figure 2.2; Table 2.3; n = 86; p = 0.11). This trend was not statistically significant given our sample size. However, a mean reduction of bull trout δ^{15}N by 0.43 ‰ represents 12.6 % of one trophic level, assuming mean trophic discrimination (Δ^{15}N) of 3.4 ± 0.45 ‰ δ^{15}N (mean ± se; Post, 2002; Table 2.3) or 14.8 % of one trophic level, assuming mean trophic discrimination of 2.9 ± 0.32 ‰ δ^{15}N (mean ± se; McCutchan et al., 2003; Table 2.3). Lake trout δ^{15}N did not change through time (Figure 2.2; n = 63; p = 0.12). The δ^{15}N of other fish species did change through time (Appendix Figure 2.11).

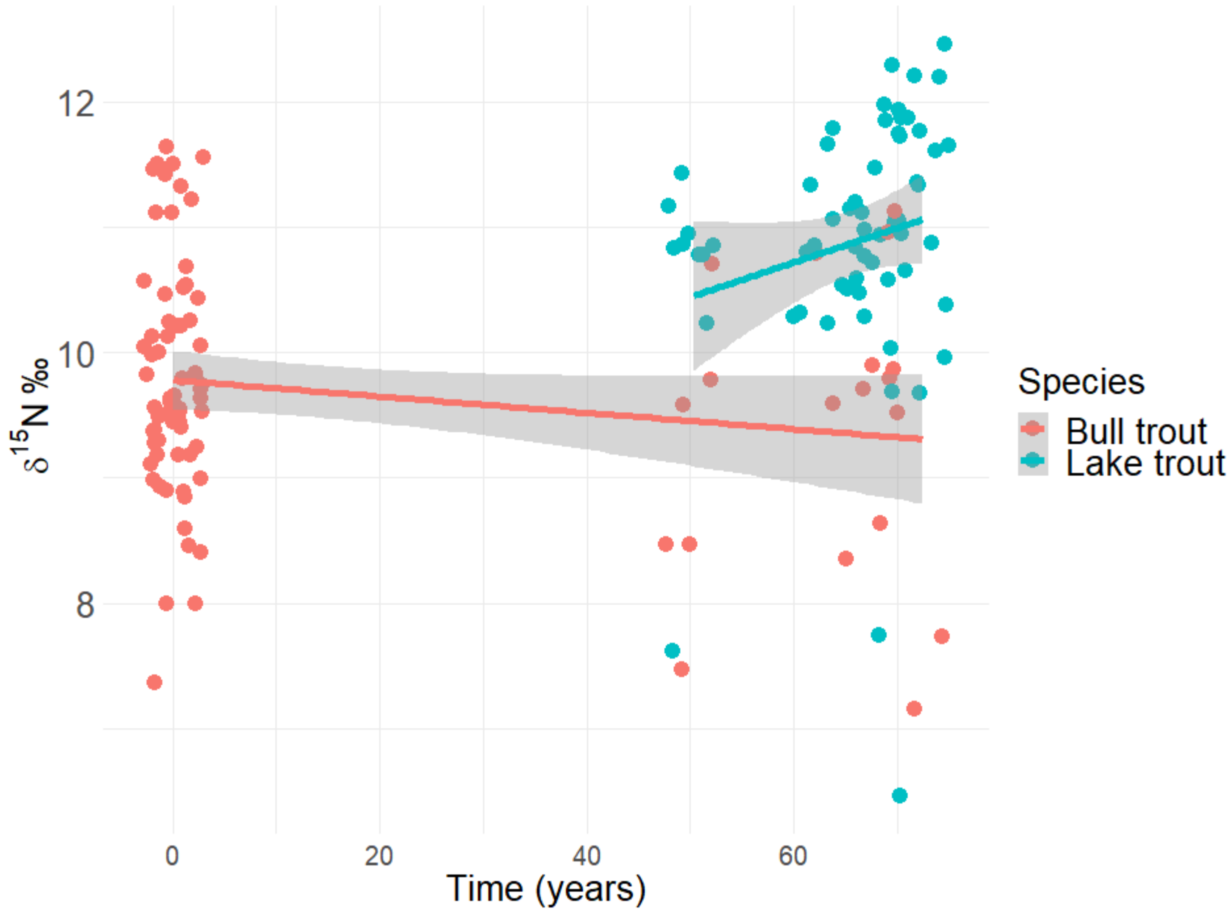

Figure 2.2. Linear models of bull trout and lake trout δ^{15}N through time. Points: empirical δ^{15}N values. Lines: linear model δ^{15}N mean ± 95% confidence intervals.

δ^{13}C values generally increased over time in the fish species studied. Bull trout mean δ^{13}C increased over time from -27.72 ± 0.27 ‰ at 0 years to -25.99 ± 0.27 ‰ at 71.7 years (mean ± se; Figure 3; Table 3; n = 86; p = 0.01). This 1.73 ‰ increase in bull trout δ^{13}C is more than four times the mean trophic discrimination (Δ^{13}C) of 0.39 ± 0.04 ‰ δ^{13}C (mean ± se; Post, 2002) or 1.33 times mean trophic discrimination of 1.3 ± 0.3 ‰ δ^{13}C (mean ± se; McCutchan et al., 2003). Lake trout δ^{13}C increased from -30.78 ± 0.66 ‰ at 50.4 years to -26.90 ± 0.66 ‰ at 71.7 years (Figure 3; Table 3; n = 63; p = 0.001). Largescale sucker, a benthic grazer, δ^{13}C increased from -27.23 ± 1.03 ‰ at 0 years to -23.89 ± 1.03 ‰ at 71.7 years (Figure 3, Table 3; n = 32; p = 0.01). Mountain whitefish, a zooplanktivore, δ^{13}C increased from -30.40 ± 0.36 ‰ at 0 years to -29.05 ± 0.36 ‰ at 71.7 years (Figure 3, Table 3; n = 32; p = 0.01)

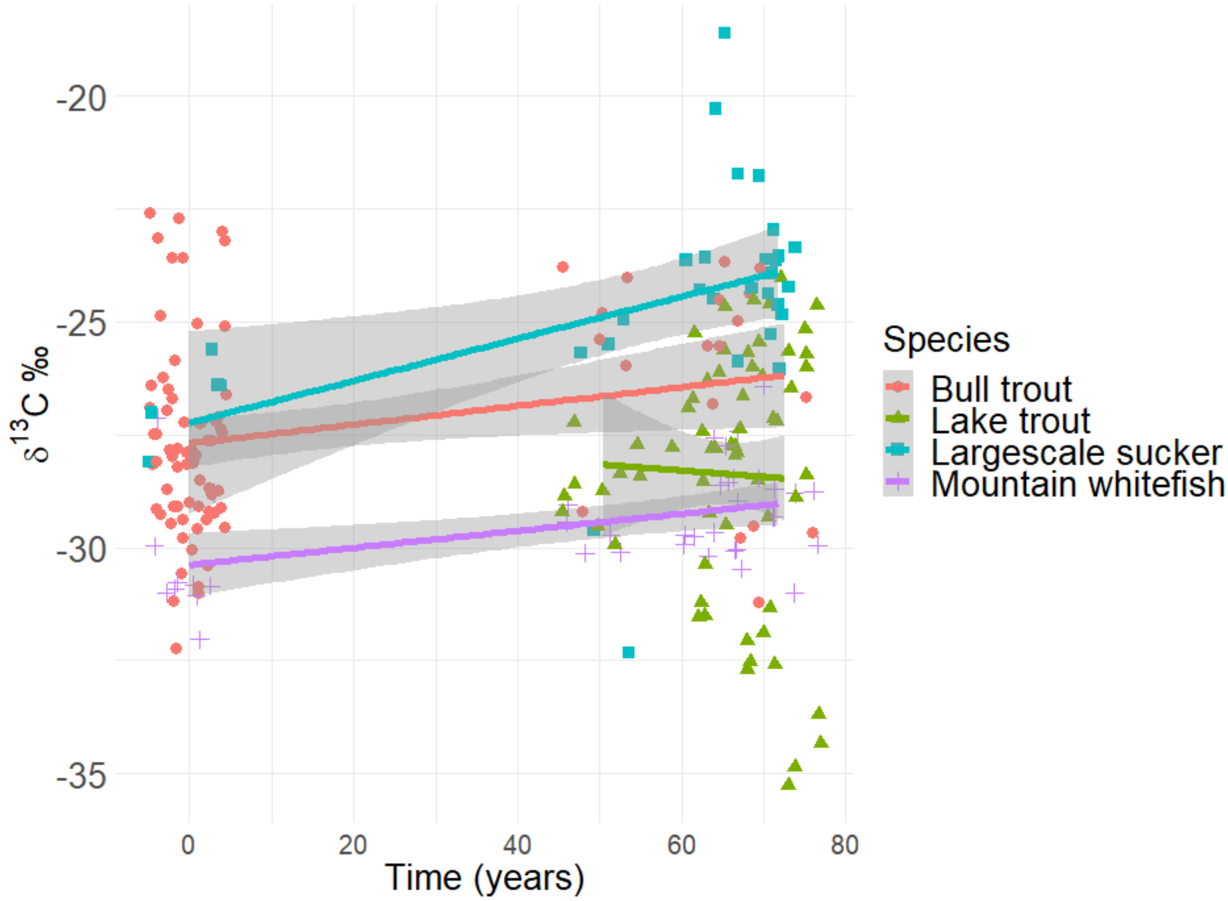

Figure 2.3. Linear models of fish δ^{13}C through time. Only prey species with statistically significant δ^{13}C changes are shown here. Points: empirical δ^{13}C values. Lines: linear model δ^{13}C mean ± 95% confidence intervals.

$\delta^{13}C$ of two major potential invertebrate prey families was correlated with time (Figure 2.4; Table 2.3). $\delta^{13}C$ in the Caenidae, this study's fourth-most abundant mayfly, increased from -27.70 ± 1.46 $\delta^{13}C$ ‰ to -22.50 ± 1.46 $\delta^{13}C$ ‰ (mean ± se; Figure 2.4; Table 3; n = 27; p = 0.01) while in the Limnephilidae, our study's most abundant caddisfly, $\delta^{13}C$ decreased from -22.68 ± 0.67 ‰ $\delta^{13}C$ to -25.74 ± 0.67 $\delta^{13}C$ ‰ (Figure 3; Table 2.3; n = 47; p = 0.001).

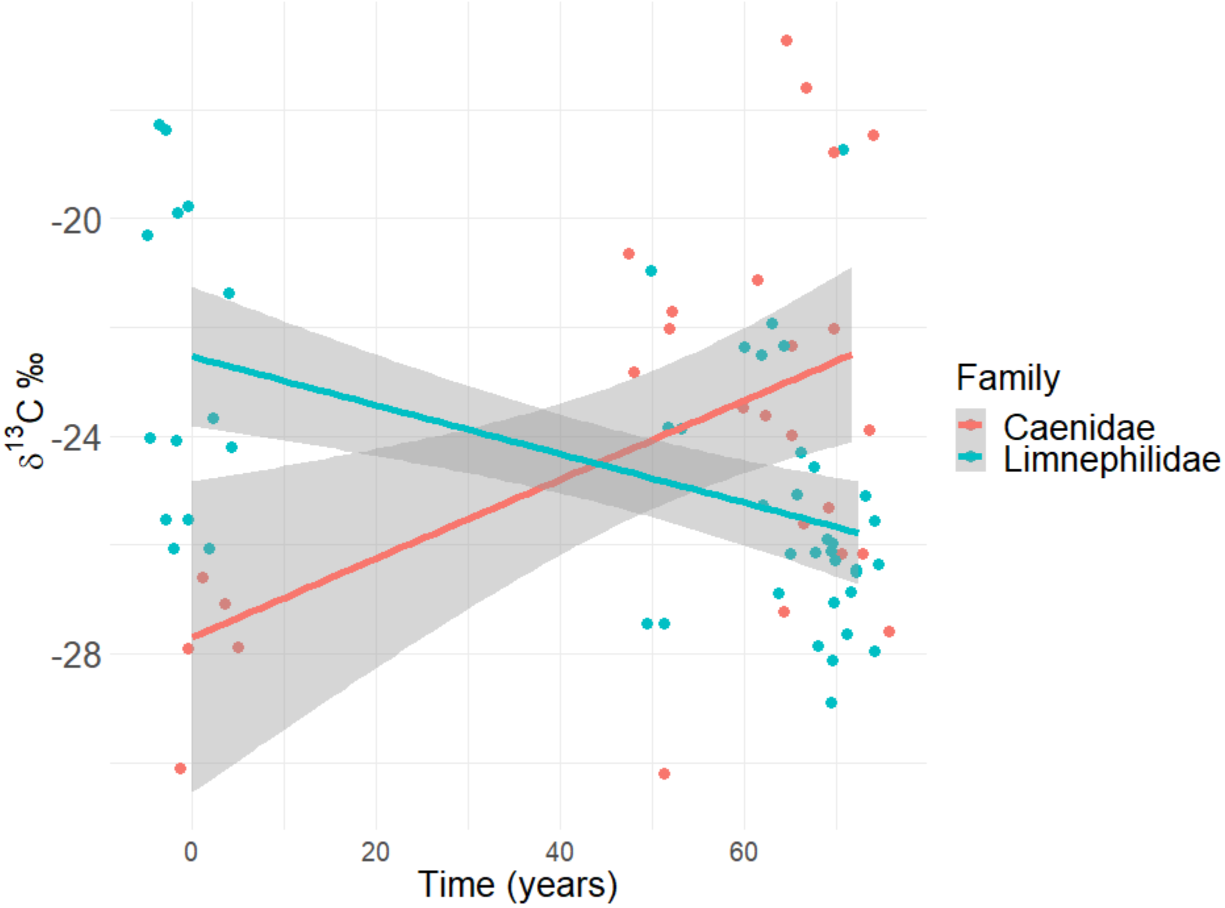

Figure 2.4. Linear models of invertebrate $\delta^{13}C$ through time. Macroinvertebrate families without statistically significant changes in $\delta^{13}C$ are excluded here. Points: empirical $\delta^{13}C$ values. Lines: linear model $\delta^{13}C$ mean ± 95% confidence intervals.

Layman metrics

Bull trout and lake trout $\delta^{15}N$ range, the arithmetic difference between maximum and minimum $\delta^{15}N$, were uncorrelated with time and one another. Bull trout $\delta^{15}N$ range increased from 1.88 ± 0.83 ‰ (mean ± se) at 0 years to 2.83 ± 0.83 ‰ at 67.5 years (n = 7; p = 0.42; Figure 2.5; Table 2.3). This 0.96 ‰ increase in mean bull trout $\delta^{15}N$ range represents 28 % of one trophic level, assuming mean trophic discrimination of 3.4 ± 0.45 ‰ $\delta^{15}N$ (mean ± se; Post, 2002; Table 2.3) or 33 % of one trophic level, assuming mean trophic discrimination of 2.9 ± 0.32 ‰ $\delta^{15}N$ (mean ± se; McCutchan et al., 2003; Table 2.3). In contrast, lake trout $\delta^{15}N$ range decreased from 2.94 ± 0.09 ‰ at 50.4 years to 1.84 ± 0.09 ‰ at 71.7 years (mean ± se; n = 7; p = 0.60; Figure 2.5; Table 2.3). This 1.10 ‰ decrease in mean lake trout $\delta^{15}N$ range represents 32 % of one trophic level, assuming mean trophic discrimination of 3.4 ± 0.45 ‰ $\delta^{15}N$ (mean ± se; Post, 2002; Table 2.3) or 38 % of one trophic level, assuming mean trophic discrimination of 2.9 ± 0.32 ‰ $\delta^{15}N$ (mean ± se; McCutchan et al., 2003; Table 2.3). $\delta^{15}N$ of bull trout and lake trout were uncorrelated (p = 0.46; Table 2.3).

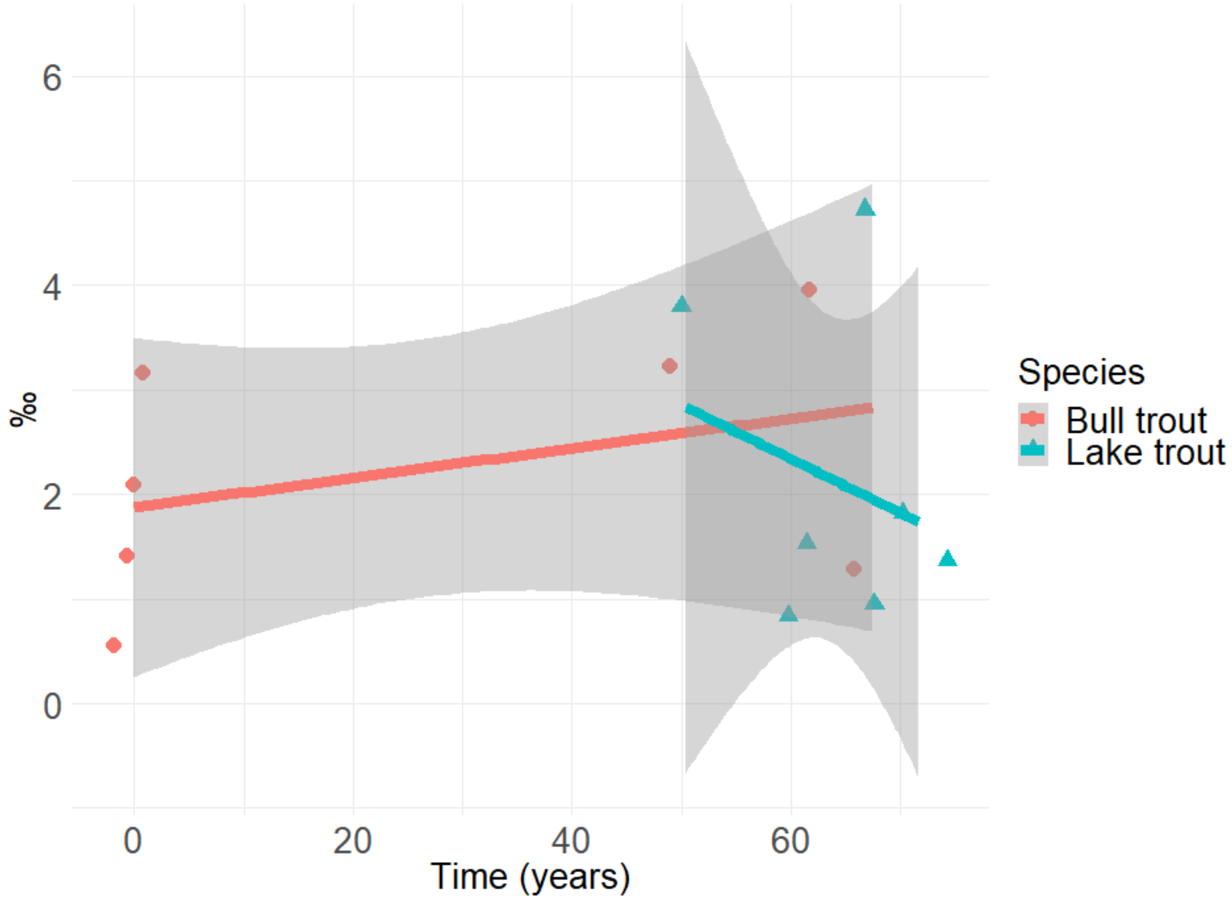

Figure 2.5. Linear models of bull trout and lake trout $\delta^{15}N$ range through time. Points: empirical $\delta^{15}N$ ranges. Lines: linear model $\delta^{15}N$ range mean ± 95% confidence intervals.

Bull trout and lake trout $\delta^{13}C$ range, the arithmetic difference between maximum and minimum $\delta^{13}C$, were uncorrelated with time and one another. Bull trout $\delta^{13}C$ range decreased from 4.82 ± 0.96 ‰ at 0 years to 2.57 ± 0.96 ‰ at 69.3 years (mean ± se; n = 7; p = 0.13; Figure 2.5; Table 2.3). This 2.25 ‰ increase in mean bull trout $\delta^{13}C$ range represents 5.8 times mean trophic discrimination of 0.39 ± 0.04 ‰ $\delta^{13}C$ (mean ± se; Post, 2002) or 1.7 times mean trophic discrimination of 1.3 ± 0.3 ‰ $\delta^{13}C$ (mean ± se; McCutchan et al., 2003). Lake trout $\delta^{13}C$ range increased from 2.80 ± 1.70 ‰ at 50.4 years to 4.42 ± 1.70 ‰ at 71.7 years (n = 7; p = 0.16; Table 2.3; Figure 2.5). The $\delta^{13}C$ range of bull trout and lake trout were uncorrelated (p = 0.17; n = 14; Table 2.3; Figure 2.5).

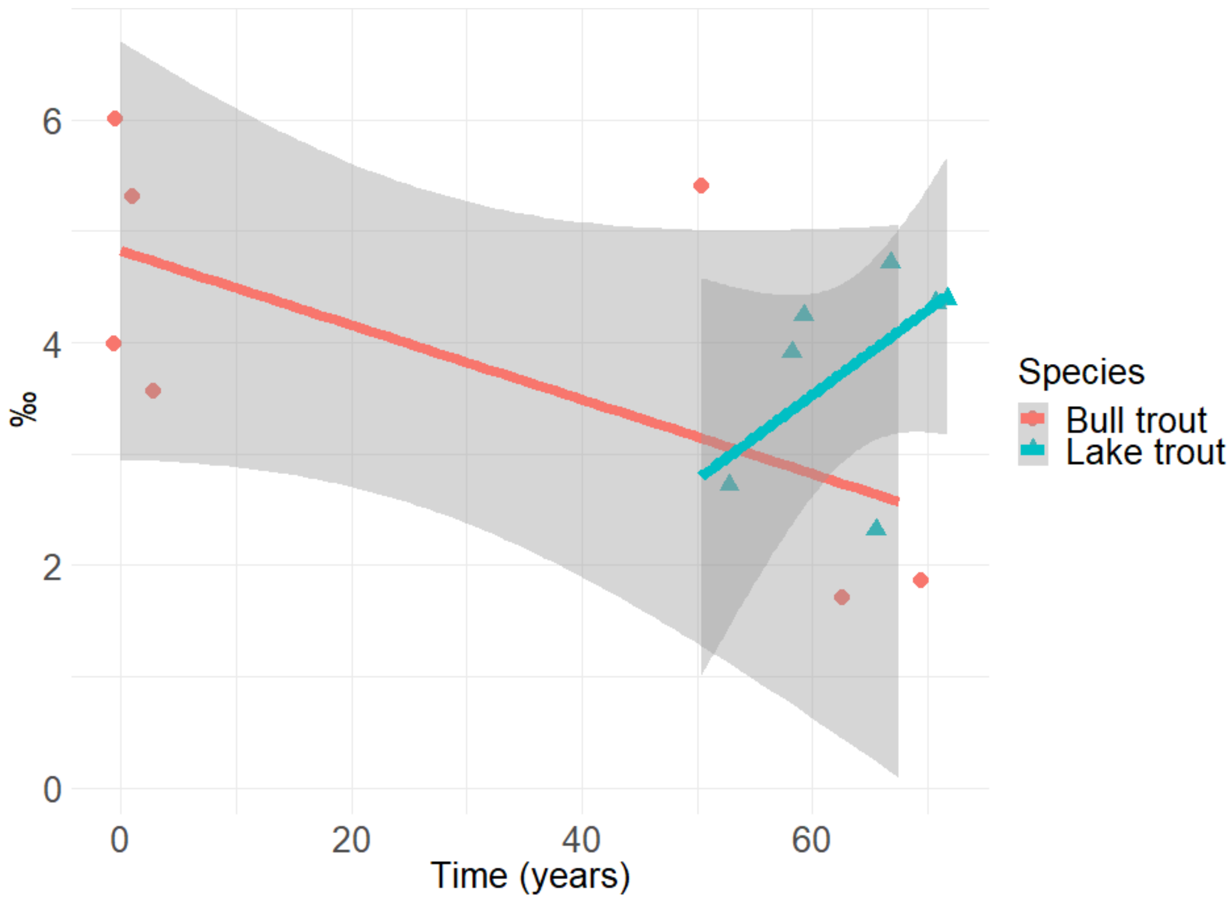

Figure 2.6. Linear models of bull trout and lake trout $\delta^{13}C$ range through time. Points: empirical $\delta^{13}C$ ranges. Lines: linear model $\delta^{13}C$ range mean ± 95% confidence intervals.

Bull trout and lake trout mean nearest neighbor distance (NND), the bivariate ($\delta^{13}C$ and $\delta^{15}N$) Euclidean distance between conspecifics in isotopic space, were uncorrelated with time and one another. Bull trout NND increased from 0.79 ± 0.43 ‰ at 0 years to 1.69 ± 0.43 ‰ at 69.3 years (mean ± se; n = 7; p = 0.26; Figure 2.7; Table 2.3). Lake trout NND increased from 0.64 ± 0.62 ‰ 50.4 years to 0.77 ± 62 ‰ at 71.7 years (mean ± se; n = 7; p = 0.64; Figure 2.7; Table 2.3).

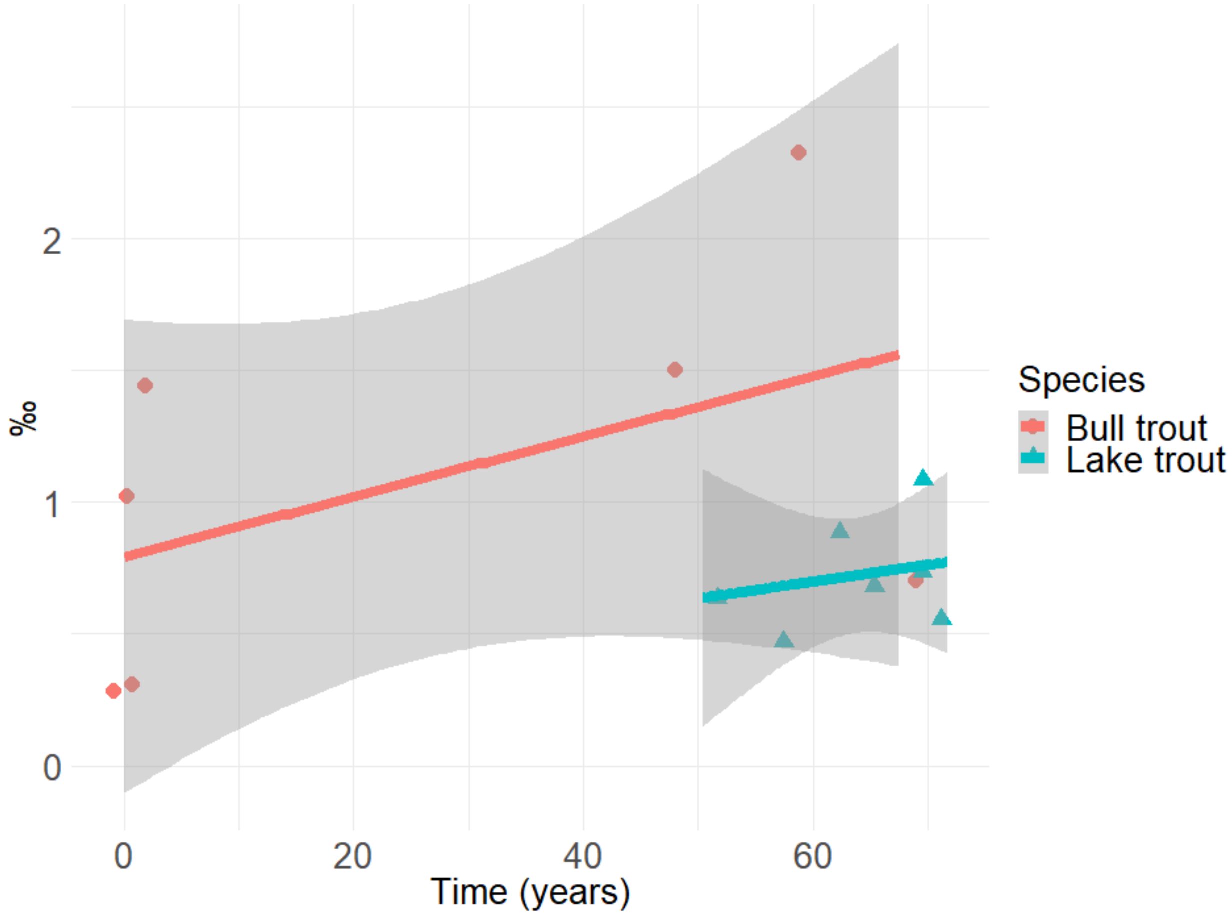

Figure 2.7. Linear models of bull trout and lake trout mean nearest neighbor distance through time. Points: empirical mean nearest neighbor distances. Lines: linear model mean nearest neighbor distance mean ± 95% confidence intervals.

Littoral macroinvertebrate community ordination

Non-metric dimensional scaling ordination revealed statistically significant differences in macroinvertebrate community composition depending on timestep (Figure 2.8; p = 0.04). Reference-timestep (x = 0 years) and mid-timestep (0 < x ≤ 60 years) communities overlapped considerably in ordination space, indicating high macroinvertebrate community similarity (Figure 2.8). In contrast, late-timestep (x > 60 years) communities were dispersed compared to reference- or mid-timestep communities, indicating late-timestep communities are relatively dissimilar to one another. Further, 17 of 35 late-timestep communities fell outside the confidence interval ellipses of the reference- or mid-timestep communities, suggesting the macroinvertebrate communities of late-timesteps diverged from reference- or mid-timestep communities.

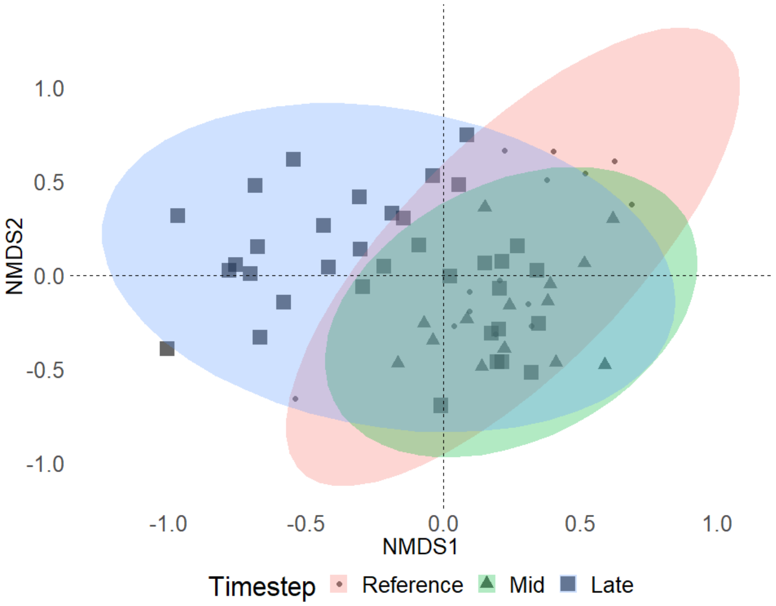

Figure 2.8. Non-metric dimensional scaling (NMDS) ordination of macroinvertebrate communities at three categories of timesteps in northwest Montana study lakes. Reference: timestep = 0 years; Mid: 0 < timestep ≤ 60 years; Late: timestep > 60 years. Data are presented with 95% confidence interval ellipses. Permanova: p = 0.04; ordination stress = 0.17.

Discussion

Summary

In this study, we used stable isotopes to infer food web structure and examined food web structural changes through time in a space-for-time substitution. Food web structure changed noticeably through time in our study system. Several animals that showed significant isotope value changes shifted toward littoral carbon reliance on littoral-pelagic axis ($\delta^{13}C$) with time. These shifts were especially pronounced in prey groups, indicating lake trout invasion may be directly affecting prey through predation or indirectly through cascading trophic effects. In general, macroinvertebrate isotope signature did not correlate with time. However, macroinvertebrate community composition changed considerably with time. These findings demonstrate that lake trout invasion has affected food web structure in our study area which may have important implications for ecosystem processes like diversity changes (Paine, 1980), biomass regulation (Hairston et al., 1960) and community stability (Vander Zanden et al., 1999).

Conversion

Our study provides some of the first estimates of the time scales of ecological disruption caused by lake trout establishment. We estimated that lake trout were present in our study lakes for 18 years, on average, before they were detected by fisheries surveys and that it takes 69 years, on average for lake trout to displace bull trout. Fisheries surveys, like the standardized gill net surveys we used in this study, are a common monitoring tool used in fisheries management. However, our calculations show invasive fish can evade detection for many years. Therefore, gill net surveys may be inadequate as a sole means of invasive fish detection. That it took 18 years, on average, to detect lake trout in our study system is of concern for fisheries management as eighteen years is more than 25% of the total time required for lake trout to fully displace bull trout in our study system. Given the success of invasive fish suppression increases with early detection and action (Simberloff, 2003), routine fisheries monitoring may be even more important to native species conservation than previously thought and it may be worthwhile to explore additional methods of invasive species detection, like eDNA monitoring.

The relationship between conversion and time we developed here is applicable beyond our study system. For example, lake trout abundance is inversely correlated with Yellowstone cutthroat trout in Yellowstone Lake, USA (Ruzycki et al., 2003). Given empirical relative abundance data for both species in Yellowstone Lake, one could apply the regression methods described in this paper to determine that system's coefficients β and β_o (Equation 2). Then one could determine predicted conversion, detection period, or full conversion period. Doing so could provide a metric by which to assess the effectiveness of suppression approaches with results integrated into a decision-support framework to prioritize competing management efforts.

Quantification of food web structure response to lake trout invasion

Bull trout $\delta^{15}N$ decreased only by 0.43 ‰ or ~14% of one trophic level through our modelled study time period. Based on past studies, we expected bull trout $\delta^{15}N$ to decrease with invasion, but the magnitude of this change was smaller than expected. The small magnitude of bull trout mean $\delta^{15}N$ change in our study systems may be a function of prey fish availability or prey species richness because the magnitude of the $\delta^{15}N$ shift after invasion should correlate with the presence of available prey fish (Vander Zanden et al., 1999). Without alternative prey fish and only prey of lower trophic levels available, the magnitude of this shift would be larger (Vander Zanden et al., 1999) because when alternative prey fish are not present, piscivorous fish may eat larger proportions of lower trophic level invertebrates, producing relatively large $\delta^{15}N$ shifts (Vander Zanden, et al., 1999). In our study lakes, both littoral and pelagic prey fish species were present. Therefore, it is plausible that, during and after lake trout invasion, bull trout switched from preying on pelagic forage fish to littoral forage fish, producing only a relatively small change in $\delta^{15}N$.

Bull trout mean $\delta^{13}C$ increased through time in our study area. Given lake trout are known pelagic piscivores (Vogel & Beauchamp, 1999), we expected lake trout to displace bull trout in the pelagic zone of study lakes and bull trout to increasingly rely on littoral organic carbon, which would increase bull trout $\delta^{13}C$. The statistical insignificance ($P > 0.05$) of our observed bull trout $\delta^{13}C$ shift is likely due to our small sample size. However, since the effect size of this δ^{13}C shift was 1.3 to 4.4 times Δ^{13}C and there is little (< 1 ‰) ^{13}C enrichment from predator-prey interaction (Fry, 2006), an effect size of this magnitude is ecologically meaningful despite its p-value and suggests considerable prey switching for bull trout. Since bull trout shifted noticeably in δ^{13}C but not δ^{15}N through time, it is likely bull trout maintained piscivory but switched from pelagic to littoral prey fish. To increase reliance on littoral prey, bull trout would likely need to increase roving behavior and, correspondingly, increase energy expenditure for swimming (Moyle & Cech, 2004). Such behavior could also cause bull trout to spend more time in relatively warm near-shore water, incrementally increasing the respiratory burden on these coldwater fish (Moyle & Cech, 2004) and contributing to their decline in abundance. This supposition is neither confirmed nor disconfirmed by our data and additional research would be needed to test this hypothesis.

Interestingly, our data show both lake trout and bull trout increasingly rely on littoral carbon as invasion progresses. If prey fish abundance decreases as lake trout displace bull trout in our study lakes as has occurred in similar fish invasions (e.g., Vander Zanden et al., 1999), pelagic prey limitation could cause lake trout to increasingly rely on littoral prey, which would produce the observed trend of increasing δ^{13}C over time. Our dataset cannot answer this question because we did not quantify relative abundance nor catch rate of prey fishes and further study would be needed to test this hypothesis.

Two prey fishes, largescale sucker (*Catostomus macrocheilus*) and mountain whitefish (*Prosopium williamsoni*), showed statistically significant increases in δ^{13}C over time. The magnitude of this δ^{13}C shift for largescale sucker, approximately 2.6 to 8.6 times Δ^{13}C, was remarkably large and was the largest $^{\delta 13}$C shift of any animal sampled in our study area. Increasing δ^{13}C in fish suggests increasing reliance on littoral carbon (Fry, 2006). Therefore, it appears largescale suckers are shifting considerably from relying on pelagic (or profundal; Vander Zanden & Rasmussen, 1999) to littoral carbon. If lake trout are increasing predatory pressure in the offshore region of our study lakes, prey fish may seek nearshore refuge, which would produce the δ^{13}C trend demonstrated by largescale suckers and mountain whitefish. Thus, the diet shifts that we document extend beyond bull trout and lake trout and suggest the possibility of trophic cascading effects (Vander Zanden et al., 1999). Historically, our study region had relatively low fish diversity (Ellis et al., 2011) and, therefore, relatively short food chain length. Short food chain length correlates with high susceptibility to trophic cascade (Vander Zanden et al., 1999). Therefore, it is possible isotope shifts measured in our study system's prey represent cascading trophic effects from lake trout invasion.

Limnephilidae and Caenidae $\delta^{13}C$ were inversely correlated with one another and changed through time. The inverse correlation of Limnephilidae and Caenidae $\delta^{13}C$ could be explained by depth-specific variation in periphyton (Devlin et al., 2013) and changing predatory pressure as predatory fish increasingly rely on littoral foods (as described above). Increased predatory pressure in the littoral zone, from spatially displaced fishes as described above, would likely disproportionately negatively affect survival of Caenidae, a mayfly with no protective case, over Limnephilidae, a caddisfly with a protective case. If Caenidae were increasingly preyed upon, especially in our study's deeper littoral transects, 1.5 m, this trend could open niche space for Limnephilidae. If Limnephilidae increasingly fed on deeper-water $\delta^{13}C$ deplete foods, Limnephilidae $\delta^{13}C$ would decrease. Meanwhile, if Caenidae could only survive in the shallowest of our study's littoral transects, 0.5 m, Caenidae $\delta^{13}C$ would increase because these mayflies would be forced to feed on $\delta^{13}C$-enriched shallow-water periphyton. This trend would reflect the depth-specific $\delta^{13}C$ relationship of periphyton, the benthic photoautotroph, where periphyton $\delta^{13}C$ decreases with increasing water depth (Devlin et al., 2013). Additional research would be needed to confirm the cause of the observed Limnephilidae and Caenidae $\delta^{13}C$ changes.

The range of $\delta^{15}N$ values among consumers increases with increasing prey trophic variability (Layman et al., 2007). Bull trout and lake trout $\delta^{15}N$ range were uncorrelated with time, with bull trout $\delta^{15}N$ range statistically insignificantly increasing through time while lake trout $\delta^{15}N$ range slightly decreased through time. That bull trout $\delta^{15}N$ range slightly increased while bull trout $\delta^{15}N$ decreased and $\delta^{13}C$ increased suggests that, as invasion progressed, bull trout ate more prey from lower trophic levels (e.g., littoral invertebrates). However, given the small magnitude of $\delta^{15}N$ range increase and $\delta^{15}N$ decrease, it is unlikely bull trout diet is shifting to incorporate large proportions of invertebrates, probably due to the availability of littoral prey species as discussed earlier.

Likewise, decreasing lake trout $\delta^{15}N$ range coupled with near-constant lake trout $\delta^{15}N$ and increasing $\delta^{13}C$ suggests lake trout maintained high levels of piscivory. The weak inverse correlation between bull trout and lake trout $\delta^{15}N$ range suggests that, even when these species are shifted toward littoral prey, lake trout became increasingly piscivorous while bull trout became more omnivorous or ate somewhat smaller prey fish.

Ordination

In our study system, littoral macroinvertebrate community ordination, not $\delta^{13}C$ and $\delta^{15}N$, correlated with time-since-invasion. This trend was expected, given littoral macroinvertebrates should be precluded from exhibiting diet plasticity along the $\delta^{13}C$ and $\delta^{15}N$ axes due to the likelihood they will be eaten if they enter open water (Thorp & Bergey, 1981) and their reliance on periphyton (Zah et al., 2001). This correlation suggests a shift in macroinvertebrate community structure, like taxonomic identity or relative abundance. Most of our macroinvertebrate sampling transects had our study system's most abundant taxa, like chironomids, scuds, limnephilid caddisflies, and caenid mayflies (Appendix: Figure 2.10). However, the presence or absence of comparatively rare taxa, like damselflies and stoneflies, were more commonly found in reference- and mid-timestep lakes than late-timestep lakes (Appendix: Figure 2.10).

Study limitations

Our study has two main limitations: ontogeny and bull trout sample size. Ontogeny, especially diet changes resulting from life history stage, are known to affect the isotope values of fish (Jensen et al., 2012). Ontogeny could partially explain our results because we did not correct fish isotope values for fish total length and lake trout and bull trout range included in our analyses vary in length. Instead of correcting isotope values, we followed protocols used in previous studies (e.g., Meeuwig et al., 2011) and sampled bull trout and lake trout of total length > 200 mm, fish presumed piscivorous based on literature review, to minimize the effect of ontogenetic diet shifts on our results. Further, we collected both larger (> 300 mm) and smaller (≤ 300 mm) bull trout and lake trout in all lakes so ontogenetic diet trends should be evenly spread among lakes rather than biasing our findings. To determine the possible effect that ontogenetic, or life history, diet changes could have on our findings, we evaluated the relationship between bull trout and lake trout total length and $\delta^{15}N$ and $\delta^{13}C$ in our dataset (Figure 2.9). In short, it is unlikely ontogeny alone would explain the trends shown in this paper.

Of our total bull trout sample of 86 individuals, 68 were from timestep-0 lakes and 18 were from all other lakes (Table 2.5). Thus, our sample size is skewed toward reference lakes and our ability to detect changes in isotope values is lower at later timesteps. Compliance with Endangered Species Act section-10 requirements and the federally protected status of bull trout necessitated this sampling approach and it would be imprudent to thoroughly sample bull trout from late invasion stages. Our sample size is comparable with previous studies in our study region (e.g., Meeuwig et al., 2011) and our sample size does not compromise our findings.

Summary and broader implications

Our data showed lake trout invasion had significant effects on the structure of food webs in our study system and that these impacts are dynamic and ongoing. Bull trout, our study system's native top predator fish, increasingly relied on littoral prey as lake trout invasion and establishment progresses. Surprisingly, our data showed lake trout, our study system's invasive fish, also increasingly relied on littoral prey as invasion progresses. Our data also indicated that the effects of lake trout invasion were not limited to direct interactions between bull trout and lake trout. Instead, indirect interactions reflecting changes in top-down pressure from lake trout affected prey animals. The isotope signatures of two prey fish species, mountain whitefish and largescale sucker, and two highly abundant macroinvertebrate families, Limnephilidae and Caenidae, and macroinvertebrate community composition were also strongly correlated with time since lake trout invasion. In short, our data quantified time-integrated food web structural changes as a function of time since lake trout invasion. These findings can be used to evaluate effectiveness of invasive fish suppression, guide future research, and prioritize and define management objectives in our study area and elsewhere.

The work of Vander Zanden et al. (1999) on food web response to smallmouth bass (*Micropterus dolomieu*) invasion is a useful comparison for our study. In many ways, our findings corroborate trends described in Vander Zanden et al. (1999), such as directional shifts in the diet of the native top-predator after invasion and the integration of littoral and pelagic production to support changing consumer diets. Our study also provides empirical evidence to support Vander Zanden et al. (1999)'s hypothesis that the magnitude of consumer trophic shifts inversely correlates with prey availability. Our space-for-time substitution, however, provides novel insight about the timing of food web response to fish invasion. Specifically, our approach provides a means by which to predict the rate of food web changes continuously through time after invasion. Additionally, we expand on previous fish-focused studies by incorporating macroinvertebrate isotope data to provide evidence for indirect trophic effects in our study system.

Predicting the magnitude and direction of food web response to species invasion can be a daunting task for ecologists due to the abundance of confounding factors within and among ecosystems. However, the combination of tools applied in this study has allowed us to reconstruct food web response through time. Our study demonstrates that modern stable isotope analyses and space-for-time substitution can provide strong inference about the direction, magnitude, and rate of change of ecosystem-level disturbance. These inferences may be useful to quantify and predict ecosystem response to disturbance to conserve resources for posterity.

Acknowledgements

This research was funded by the United States Geological Survey (USGS) and University of Montana Flathead Lake Biological Station, and conducted in collaboration with the National Park Service, USGS, Montana Fish Wildlife & Parks, US Forest Service, US Fish and Wildlife Service, and University of Montana Flathead Lake Biological Station. T. Bansak of Flathead Lake Biological Station collaborated with the Biological Station's donor network to raise funds for sample analysis. V. D'Angelo of USGS in West Glacier, MT provided technical support and guidance. D. Six of the University of Montana provided laboratory space in Missoula, MT. L. Rosenthal and S. Bourret of Montana Fish Wildlife & Parks in Kalispell, MT contributed data, samples, and manuscript guidance. C. Downs and J. McCubbins of the National Park Service in West Glacier, MT contributed

data and samples. J. Vanderwall, B. Weber, and Z. Ren of Flathead Lake Biological Station in Polson, MT provided field support.

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Appendix

Table 2.1. Summary table of sampling lakes in northwest Montana.

Lake Name	Surface Elevation (m)	Surface Area (ha)	Max Depth (m)	Current conversion (y)	Timestep (years; mean ± 95% confidence interval)	Fisheries survey year	Current conversion fisheries survey source
Grace	1208	52	30	0	0	2019	(Muhlfeld et al., In Prep)
Trout	1189	114	50	0	0	2018	(Wainright et al., 2020)
Big Salmon	1340	393	42	0	0	2019	(Wainright et al., 2020)
Hungry Horse Reservoir	1112	9630	149	0	0	2019	(Rosenthal & Bourret, In Prep)
Quartz	1345	352	84	0.611	50.4 ± 8	2019	(Downs & McCubbins, 2019; McCubbins et al., In Prep.)
Swan	1000	1335	43	0.711	59.9 ± 8	2019	(Rosenthal & Bourret, In Prep)
McDonald	961	2760	144	0.814	62.2 ± 8	2019	(McCubbins et al., In Prep.)
Lindbergh	1369	329	36	0.857	64.7 ± 8	2019	(Rosenthal & Bourret, In Prep)
Bowman	1228	910	77	0.905	67.5 ± 8	2019	(McCubbins et al., In Prep.)
Logging	1161	581	60	0.937	69.3 ± 8	2019	(Muhlfeld et al., In Prep)
Kintla	1221	1039	120	0.978	71.7 ± 8	2019	(McCubbins et al., In Prep.)

Table 2.2. Standardized gill net survey data from northwest Montana study lakes for binomial linear regression of predicted conversion. N = 24.

Lake name	**Survey Year**	**Timestep**	**Lake trout**	**Bull trout**	**Conversion**
Logging	1977	0	0	6	0.00
	2000	23	12	7	0.63
	2005	28	25	7	0.78
	2010	33	42	0	1.00
McDonald	1969	0	8	38	0.17
	1977	8	8	10	0.44
	2000	31	24	7	0.77
	2005	36	33	8	0.80
	2010	41	33	6	0.85
	2015	46	15	8	0.65
	2019	50	35	8	0.81
Bowman	1977	0	0	41	0.00
	2000	23	57	10	0.85
	2005	28	52	17	0.75
	2010	33	64	5	0.93
	2015	38	41	6	0.87
	2019	42	67	7	0.91
Kintla	1969	0	3	54	0.05
	1977	8	18	12	0.60
	2000	31	45	2	0.96
	2005	36	34	12	0.74
	2010	41	32	3	0.91
	2015	46	53	4	0.93
	2019	50	44	1	0.98

Table 2.3. Summary statistics of linear relationships between δ^{15}N, δ^{13}C, δ^{15}N Range, δ^{13}C Range, and Mean nearest neighbor versus Timestep. Metrics are presented as mean ± standard error (se). P-values are derived from linear regression. Effect size is the arithmetic difference relative to trophic discrimination (Δ^{13}C or Δ^{15}N) as detailed in Post (2002) or McCutchan et al. (2003).

Taxon	**Metric**	**n**	**Metric at earliest timestep (mean ± se; ‰)**	**Metric at latest timestep (mean ± se; ‰)**	**Arithmetic difference of means (‰)**	**Linear model p-value**	**Effect size (Post)**	**Effect size (McCutchan et. al.)**
Bull trout	δ^{15}N	86	9.77 ± 0.12	9.33 ± 0.12	-0.43	0.13	0.13	0.15
Bull trout	δ^{13}C	86	-27.72 ± 0.27	-25.99 ± 0.27	1.73	0.01	4.4	1.3
Lake trout	δ^{13}C	63	-30.78 ± 0.66	-26.90 ± 0.66	3.88	0.0001	9.9	3.0
Largescale sucker	δ^{13}C	32	-27.23 ± 1.03	-23.89 ± 1.03	3.34	0.001	8.6	2.6
Mountain whitefish	δ^{13}C	39	-30.40 ± 0.36	-29.05 ± 0.36	1.35	0.01	3.5	1.0
Zooplankton	δ^{13}C	57	-32.08 ± 0.81	-31.55 ± 0.81	0.53	0.64	1.4	0.4
Physidae	δ^{13}C	28	-21.64 ± 1.25	-21.60 ± 1.25	0.04	0.98	0.1	0.03
Caenidae	δ^{13}C	27	-27.70 ± 1.46	-22.50 ± 1.46	5.20	0.01	13.3	4.0
Limnephilidae	δ^{13}C	47	-22.68 ± 0.67	-25.74 ± 0.67	-3.06	0.001	7.8	2.4
Bull trout	δ^{15}N Range	7	1.88 ± 0.83	2.83 ± 0.83	0.95	0.42	0.28	0.33
Lake trout	δ^{15}N Range	7	1.84 ± 1.58	2.94 ± 1.58	1.10	0.60	0.32	0.38
Bull trout vs lake trout	δ^{15}N Range	14				0.46		
Bull trout	δ^{13}C Range	7	4.82 ± 0.96	2.57 ± 0.96	-2.25	0.13	5.8	1.7
Lake trout	δ^{13}C Range	7	2.80 ± 1.70	4.42 ± 1.70	1.62	0.16	10.1	1.3
Bull trout vs lake trout	δ^{13}C Range	14				0.17		
Bull trout	Mean nearest neighbor	7	0.79 ± 0.43	1.69 ± 0.43	0.77	0.26		
Lake trout	Mean nearest neighbor	7	0.64 ± 0.62	0.77 ± 0.62	0.14	0.64		
Bull trout vs lake trout	Mean nearest neighbor	14				0.26		

Table 2.4. Fish species found in study lakes in northwest Montana.

Common name	**Scientific name**	**Non-native species**	**Native Species**
Bull trout	*Salvelinus confluentus*		X
Westslope cutthroat trout	*Oncorhynchus clarkii lewisi*		X
Mountain whitefish	*Prosopium williamsoni*		X
Pygmy whitefish	*Prosopium coulterii*		X
Longnose sucker	*Catostomus catostomus*		X
Largescale sucker	*Catostomus macrocheilus*		X
Peamouth	*Mylocheilus caurinus*		X
Northern pikeminnow	*Ptychocheilus oregonensis*		X
Slimy sculpin	*Cottus cognatus*		X
Redside shiner	*Richardsonius balteatus*		X
Lake trout	*Salvelinus namaycush*	X	
Lake whitefish	*Coregonus clupeaformis*	X	
Kokanee	*Oncorhynchus nerka*	X	
Bluegill	*Lepomis macrochirus*	X	
Northern pike	*Esox lucius*	X	
Central mudminnow	*Umbra limi*	X	
Yellow perch	*Perca flavescens*	X	
Rainbow trout	*Oncorhynchus mykiss*	X	
Brook trout	*Salvelinus fontinalis*	X	
Yellowstone cutthroat trout	*Onchorhynchus clarkii bouveri*	X	

Table 2.5. Summary table of isotope samples from northwest Montana and included in this manuscript. Statistics are reported as mean ± standard error.

Lake	**Taxon**	**n**	**Total length (mm)**	**δ^{13}C (‰)**	**δ^{15}N (‰)**
Big Salmon	Bull trout	12	493 ± 20	-29.47 ± 0.31	11.21 ± 0.13
	Caenidae	5	NA	-27.91 ± 0.6	0.9 ± 0.1
	Limnephilidae	5	NA	-20.48 ± 1.44	0.1 ± 0.24
	Mountain whitefish	5	150 ± 27	-29.93 ± 0.87	7.57 ± 0.27
	Physidae	2	NA	-22.09 ± 0.52	0.64 ± 0.45
	Zooplankton	5	NA	-36.37 ± 0.49	4.06 ± 0.33
Bowman	Bull trout	4	314 ± 37	-24.51 ± 0.39	9.48 ± 0.3
	Lake trout	10	499 ± 44	-27.28 ± 0.45	10.03 ± 0.5
	Largescale sucker	5	90 ± 10	-23.77 ± 0.18	5.45 ± 0.5
	Limnephilidae	5	NA	-26.91 ± 0.22	0.7 ± 0.55
	Mountain whitefish	4	273 ± 25	-28.38 ± 0.85	8.43 ± 0.13
	Physidae	1	NA	-28.19 ± NA	2.09 ± NA
	Zooplankton	3	NA	-32.59 ± 2.81	3.11 ± 0.71
Grace	Bull trout	10	325 ± 43	-24.05 ± 0.54	9.08 ± 0.35
	Limnephilidae	4	NA	-22.81 ± 1.2	0.54 ± 0.61
	Physidae	4	NA	-24.06 ± 0.87	1.57 ± 0.35
	Zooplankton	5	NA	-29.64 ± 0.62	0.87 ± 0.36
Hungry Horse	Bull trout	43	468 ± 18	-28.19 ± 0.19	9.51 ± 0.07
	Largescale sucker	5	380 ± 18	-26.71 ± 0.42	6.88 ± 0.25
	Mountain whitefish	5	297 ± 8	-30.75 ± 0.2	6.03 ± 0.12
	Zooplankton	3	NA	-35.91 ± 0.52	4.61 ± 0.33
Kintla	Bull trout	1	588	-26.65 ± NA	9.79 ± NA
	Caenidae	4	NA	-26.31 ± 0.47	-0.12 ± 0.01
	Lake trout	10	542 ± 38	-25.72 ± 0.36	10.35 ± 0.15
	Largescale sucker	5	220 ± 28	-24.24 ± 0.31	6.76 ± 0.25
	Limnephilidae	5	NA	-26.27 ± 0.17	0 ± 0.12
	Mountain whitefish	5	288 ± 26	-29.29 ± 0.6	7.08 ± 0.15
	Physidae	5	NA	-24.53 ± 0.5	0.72 ± 0.05
	Zooplankton	3	NA	-33.9 ± 1.95	3.19 ± 0.48
Lindbergh	Bull trout	1	490	-24.97 ± NA	10.79 ± NA
	Caenidae	3	NA	-20.06 ± 1.71	0.57 ± 0.26
	Lake trout	5	584 ± 16	-30.77 ± 0.44	11.09 ± 0.16
	Largescale sucker	3	302 ± 73	-20.22 ± 0.91	6.25 ± 0.13
	Limnephilidae	4	NA	-24.83 ± 0.86	1.63 ± 0.96
	Mountain whitefish	5	264 ± 4	-29.78 ± 0.33	7.02 ± 0.13
	Physidae	3	NA	-22.83 ± 0.5	1.43 ± 0.22
	Zooplankton	3	NA	-30.46 ± 1.41	2.45 ± 0.2
Logging	Bull trout	2	277 ± 38	-24.66 ± 0.86	10.33 ± 0.63

	Caenidae	5	NA	-20.15 ± 1.2	1.92 ± 0.99
	Lake trout	10	268 ± 20	-26.53 ± 0.43	11.42 ± 0.18
	Largescale sucker	5	306 ± 6	-24.54 ± 0.5	8.16 ± 0.26
	Limnephilidae	5	NA	-24.12 ± 1.36	1 ± 0.6
	Mountain whitefish	5	264 ± 8	-28.6 ± 0.07	8 ± 0.14
	Physidae	5	NA	-17.75 ± 1.88	1.25 ± 0.28
	Zooplankton	10	NA	-30.44 ± 0.42	2.26 ± 0.16
McDonald	Bull trout	2	380 ± 132	-28.3 ± 1.48	8.97 ± 0.62
	Caenidae	5	NA	-24.78 ± 0.72	0.5 ± 0.32
	Lake trout	8	508 ± 30	-27.5 ± 0.45	10.84 ± 0.19
	Largescale sucker	4	301 ± 75	-23.88 ± 0.86	7.79 ± 0.17
	Limnephilidae	5	NA	-23.24 ± 0.65	1.12 ± 0.55
	Mountain whitefish	5	310 ± 10	-29.21 ± 0.4	5.65 ± 0.21
	Physidae	3	NA	-21.46 ± 0.95	1.36 ± 0.11
	Zooplankton	6	NA	-35 ± 0.83	3.41 ± 0.31
Quartz	Bull trout	6	218 ± 7	-25.52 ± 0.81	9.08 ± 0.47
	Caenidae	5	NA	-23.48 ± 1.72	0.43 ± 0.17
	Lake trout	10	296 ± 10	-28.66 ± 0.25	10.55 ± 0.34
	Largescale sucker	5	377 ± 32	-27.61 ± 1.44	7.78 ± 0.47
	Limnephilidae	5	NA	-24.71 ± 1.24	0.8 ± 0.37
	Mountain whitefish	5	252 ± 3	-29.69 ± 0.2	7.26 ± 0.12
	Physidae	2	NA	-17.84 ± 1.25	-0.11 ± 0.2
	Zooplankton	10	NA	-30.36 ± 1.36	1.7 ± 0.2
Swan	Bull trout	3	699 ± 57	-30.13 ± 0.55	8.67 ± 1.23
	Lake trout	10	616 ± 25	-33.13 ± 0.42	11.93 ± 0.09
	Limnephilidae	4	NA	-28.21 ± 0.24	1.42 ± 0.15
	Zooplankton	3	NA	-30.04 ± 0.66	6.15 ± 0.7
Trout	Bull trout	3	463 ± 25	-26.25 ± 1.29	10.46 ± 0.17
	Limnephilidae	5	NA	-24.71 ± 0.46	0.81 ± 0.11
	Physidae	3	NA	-18.83 ± 0.84	1.82 ± 0.06
	Zooplankton	6	NA	-29.07 ± 0.73	-0.21 ± 0.27

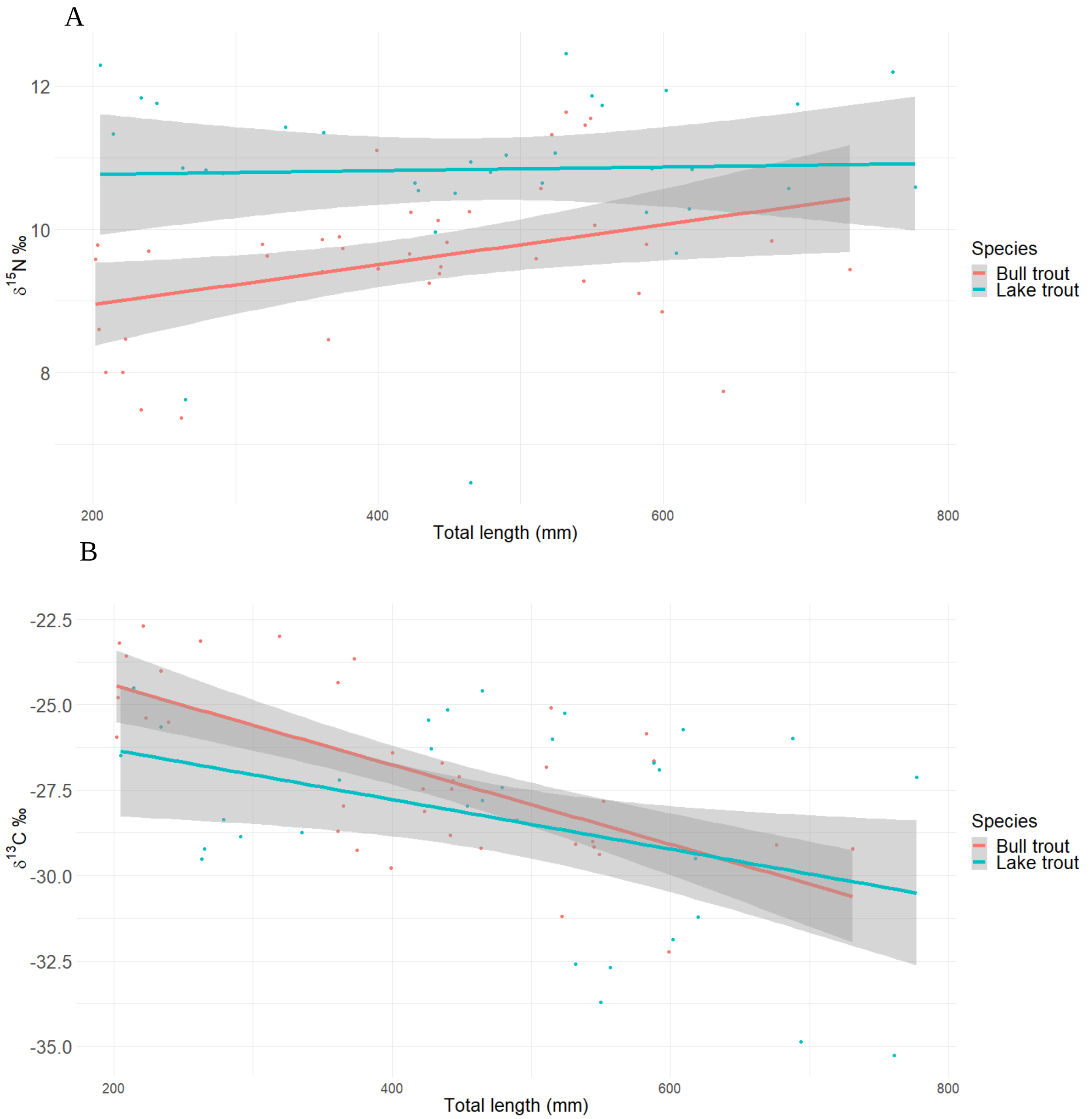

Figure 2.9. A. Linear regression of bull trout and lake trout δ^{15}N (bull trout: $p = 0.17$; lake trout: $p = 0.55$) and B. δ^{13}C (bull trout: $p << 0.05$; lake trout: $p = 0.003$) versus fish total length.

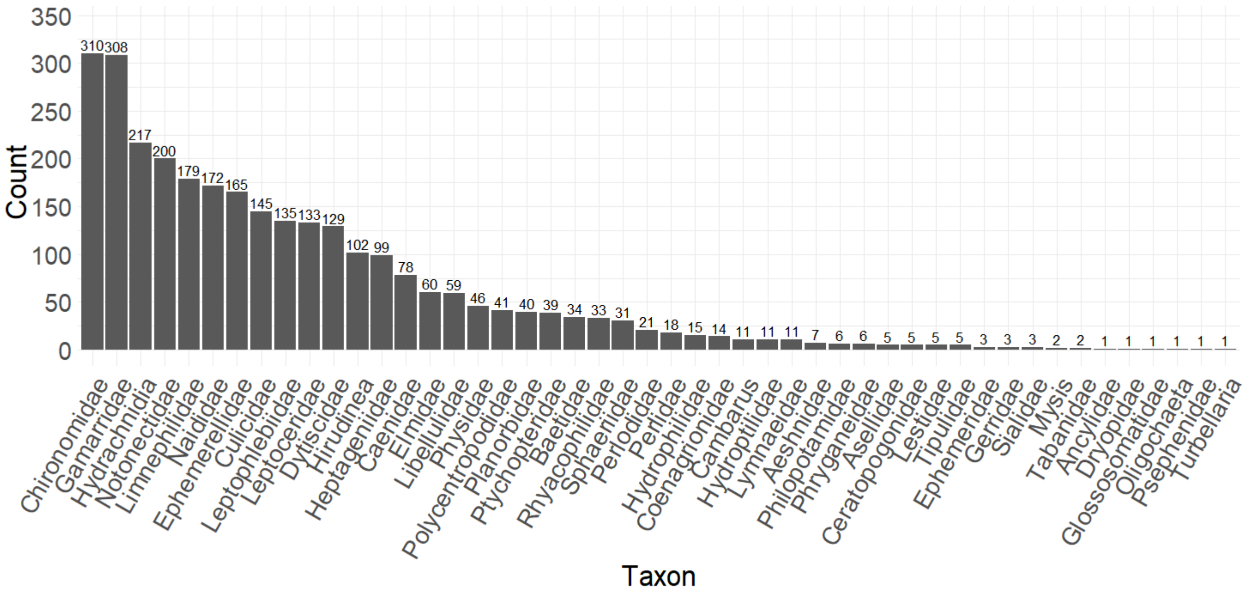

Figure 2.10. Littoral macroinvertebrate relative abundance as surveyed in northwest Montana lakes from 2018 to 2019. N = 2914.

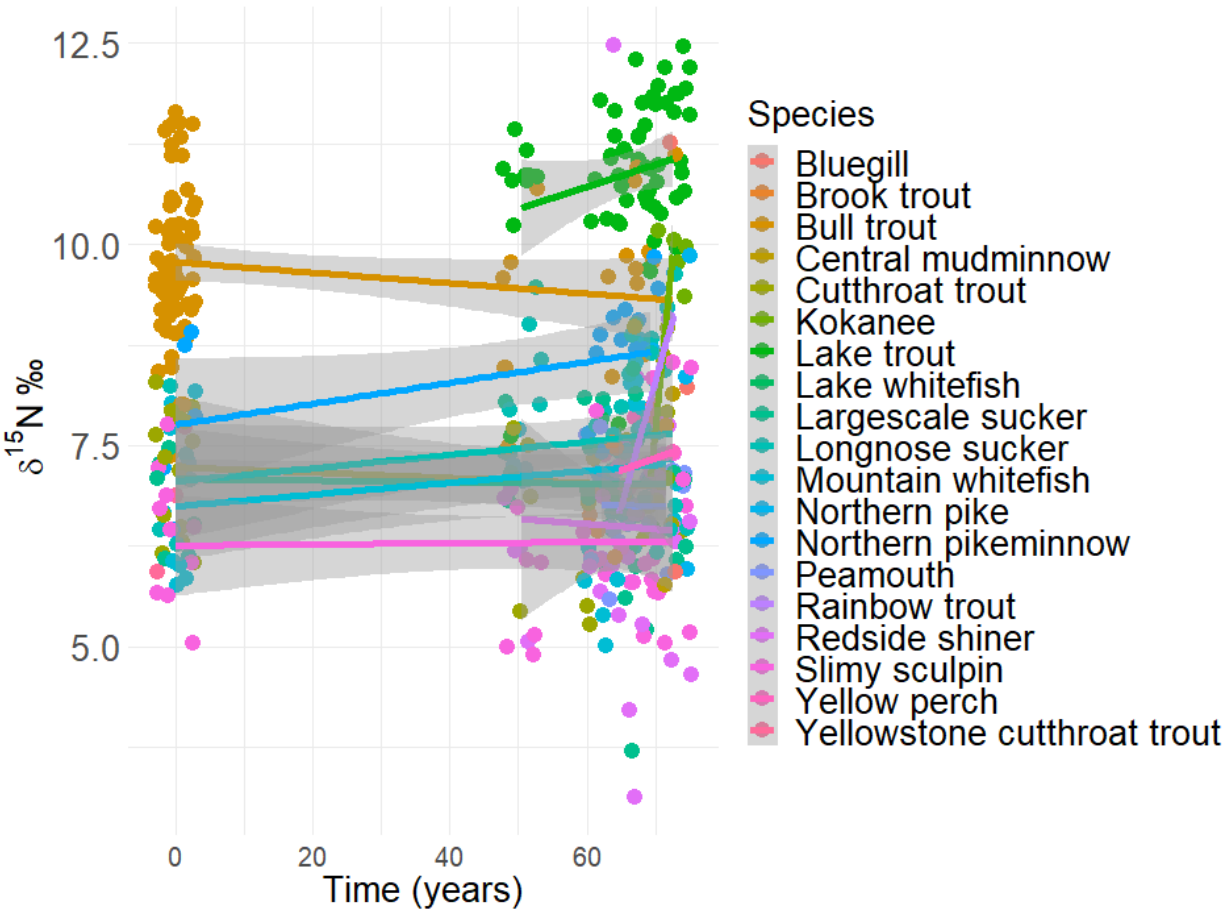

Figure 2.11. Linear modelling of δ^{15}N of nineteen fish species versus time. Points are empirical isotope data collected from northwestern Montana, USA 2017-2019. Lines are linear relationships between δ^{15}N and time for each fish species. Gray ribbons are 95% confidence intervals for linear relationships.