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Differentiating the effects of two non-native fish in the Turnbull National Wildlife Refuge

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Differentiating the Effects of Two Non-Native Fish in the Turnbull National Wildlife Refuge.

A Thesis

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By Sarah Richardson

Winter 2022

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Abstract

Differentiating the Effects of Two Non-Native Fish on the Invertebrates of Turnbull National Wildlife Refuge.

By

Sarah Richardson

Winter 2022

This study seeks to determine the individual effects of two non-native fish, the brook stickleback, *Culaea inconstans*, and pumpkinseed, *Lepomis gibbosus*, on the aquatic invertebrates and food webs of Turnbull National Wildlife Refuge (TNWR). Ten ponds were analyzed and compared with stable isotope analysis and invertebrate abundance data. Three ponds were chosen to represent each of the following categories: fishless, brook stickleback invaded (BS), and coinvaded, along with a single pumpkinseed (PS) pond. Overall, $\delta^{13}C$ and $\delta^{15}N$ values increased in invaded ponds, indicating shifts in the zooplankton community structure and the carbon source of predatory invertebrates. Mean $\delta^{13}C$ was the highest among coinvaded ponds at 6.35‰ greater than mean fishless $\delta^{13}C$, reflecting a shift towards benthic carbon sources. Mean $\delta^{15}N$ of BS ponds was 2.85‰ greater than fishless, which may indicate a greater amount of omnivory among invertebrate predators within BS ponds. Mean zooplankton length decreased by 104% in the presence of BS ponds and 79% in coinvaded ponds. The addition of pumpkinseed was found to be the primary factor effecting zooplankton abundance and biomass, where abundance was 662.3% greater in coinvaded ponds than in fishless ponds and biomass was 328.6% greater than fishless ponds. Stickleback alone was not found to be a significant factor in abundance or biomass. The increase in abundance and biomass in coinvaded ponds were a result of an increase in small zooplankton species, especially Chydoridae species (17-fold greater abundance), and reduction of larger branchiopods. Invertebrate predators such as Hirudinae and Coleoptera demonstrated enriched $\delta^{13}C$ and $\delta^{15}N$, likely due to increased omnivory in the presence of fish. The largest amount of variation in isotopic values and zooplankton community structure was seen among coinvaded ponds and the possible explanatory factor of relative fish abundance (CPUE) was explored but undetermined due to the inability to rule out other factors such as seasonal effects. Further research is needed to differentiate the effects of these fish, particularly that of pumpkinseed. These insights will aid managers at TNWR in implementing effective management plans designed to address the specific effects of both species.

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Introduction

Non-native fish have been the focus of much research not only for their ecological impacts but also for the associated economic costs that often directly correlate with the magnitude of ecological impacts (Vila et al. 2010). In 2005, the impacts of non-native fish were estimated to cost upwards of $5.4 billion USD annually in the United States alone, highlighting the need for mitigation efforts (Pimentel et al. 2005). Like many other species, non-native fish can have a wide range of effects depending on multiple factors (Miller and Crowl 2006, Preston et al. 2017), further complicating the difficult task of predicting the outcome of a new invasion from both a management and economic perspective (Charles and Dukes 2008, Ricciardi et al. 2013, Preston et al. 2017).

Eutrophic systems, such as wetlands, characterized by high nutrient and low oxygen availability, provide a prime example of the varying degree to which a non-native fish can impact native organisms. Aquatic invertebrates, such as zooplankton and insects, are of particular interest due to their complex interactions within the food web of a wetland (Murkin and Wrubleski 1988). These invertebrates, comprising the majority of the macrobiotic diversity within wetlands, play important roles in the overall health of the system by serving as a link in the flow of nutrients between primary producers and organisms located at the top of the food web (Batzer 2013, Zimmer et al. 2000). Therefore, the diversity and abundance of the invertebrate communities are of dire importance in the overall health of a wetland.

Food webs serve as a representation of the predator-prey relationships within a system by illustrating detailed interactions such as energy flow among species (Vander Zanden et al. 2016). Energy flow refers to the flow of matter, such as nutrients, from one trophic level to another through the consumption of food (Sharma 2009). When a new fish species invades a system, shifts in food web structure can alter the flow of energy as a result of many factors (Jardine et al. 2003). In highly productive systems, such as wetlands, non-native fish can alter the composition of the invertebrate community through predation and resource competition, causing shifts in the flow of energy, especially if these wetlands were fishless prior to invasion (Hanson & Riggs 1995). Stable isotope analysis (SIA), is commonly used to trace paths of energy flow in aquatic food webs by comparing the mean ratios of ^{15}N:^{14}N and ^{13}C:^{12}C (denoted as $\delta^{15}N$ and $\delta^{13}C$) of each species (Jardine et al. 2003). These isotopes are found in the tissues of every organism and are obtained through their diet resulting in an isotope ratio that reflects the isotope ratios of their prey (Fry 2006a, 2006b). The amount of $\delta^{15}N$ indicates the trophic position that an organism occupies, while $\delta^{13}C$ indicates the primary production sources of an organism's diet (Vander Zanden et al. 1999, Black et al. 2003). Organisms that derive more energy from benthic sources contain greater amounts of $\delta^{13}C$ and those that derive more energy from pelagic sources contain less $\delta^{13}C$ (Jardine et al. 2003). Researchers often illustrate these data on scatterplots as seen in Figure 1. These scatterplots can visualize predictable patterns in the levels of $\delta^{13}C$ and $\delta^{15}N$ that can help differentiate pathways within a food web. In a single pathway, an increase in trophic level is generally indicated by a stepwise trophic enrichment of 1‰ $\delta^{13}C$ and 3.4 ± 1.1‰ $\delta^{15}N$, resulting in a mean slope of about 3.4 for the plotted values (Minagawa & Wada 1984, Vander Zanden et al. 1999, Black et al. 2003). So, by plotting the mean ratios of $\delta^{13}C$ and $\delta^{15}N$ of each species in a food web, inferences can be made about predator-prey interactions, making it particularly useful in analyzing the impacts of non-native fish (Vander Zanden et al. 1999).

Since 2000, the Aquatic Ecology Laboratory at Eastern Washington University under Dr. Ross Black has utilized SIA for a wide range of applications, thoroughly demonstrating its reliability as a tool for analyzing food webs. Earlier studies focused on seasonal variation and anthropogenic effects on food webs of Washington lakes (Barlow 2000, Black et al. 2003). More recently, the Aquatic Ecology Laboratory has shifted its focus from seasonal and anthropogenic effects to that of introduced fish on aquatic food webs. A 2011 thesis by Bridges, analyzed the effects of brook stickleback and pumpkinseed on the invertebrate communities of TNWR wetlands. Since then, continued efforts to research the effects of non-native fish at TNWR have identified additional factors in need of study, providing a unique opportunity to utilize SIA in illustrating shifts in the food web as a response to non-native fish.

The refuge has undergone multiple invasions since the late 1990’s, making it an excellent model for studying invasion impacts (Wieker et al. 2016). Within the refuge, the presence of two non-native fish, brook stickleback and pumpkinseed, have been reported within several ponds and lakes, sometimes coinvading the same waterbodies (Scholz et al. 2003, Bridges 2011, Walston et al. 2015, Gunselman & Spruell 2019). These fish are known to consume many of the same invertebrates as waterfowl in the refuge (Krapu & Reinecke 1992, Bridges 2011). Waterfowl heavily rely upon benthic macroinvertebrates when nesting and breeding, so the degree of impact of non-native insectivorous fish could have significant implications on the future of migratory waterfowl within TNWR. (Bouffard & Hanson 1997; US Fish and Wildlife Service 2007).

Overall, we have limited knowledge of these fish and their impacts on TNWR. Research by Levi Bridges in 2011 investigated eight lakes at the refuge to determine if stickleback or pumpkinseed were having an impact on the invertebrate community composition and if these fish were directly competing with waterfowl for invertebrate prey. Four of these lakes were fishless, two were invaded by brook stickleback, and two were invaded by pumpkinseed. The study found that invertebrate community composition of stickleback lakes was significantly different for several invertebrate taxa, while pumpkinseed lakes were not significantly different from that of fishless lakes. In addition to this, SIA samples were collected and used to create a food web representing the mean isotope ratios of all sampled organisms across all eight waterbodies (Figure 2). Both stickleback and pumpkinseed were found to have some niche overlap with waterfowl, implying that they are indeed competing for the same resources. Even though pumpkinseed occupied the upper trophic position they did not appear to significantly impact invertebrate abundance. Further unpublished research by Black et al. from 2012 suggests that brook stickleback may be impacting invertebrate communities. Comparisons of stickleback present and stickleback absent ponds (n = 4 ponds of both types) revealed significant differences in the densities of common zooplankton and macroinvertebrate orders. Several groups, such as *Daphnia*, *Diaptomus,* Ostracoda, Conchostraca, Hemiptera, and Odonata occurred at significantly lower densities in the presence of stickleback, while others occurred at significantly higher densities, such as *Diacyclops*.

Some variation was seen in the results of these studies. Certain invertebrate taxa, such as branchiopods and copepods did not react to stickleback presence in the same manner. In the study by Bridges, branchiopods and copepods did not occur at significantly different abundances in the presence or absence of stickleback. However, in Black et al. 2012, Branchiopods occurred at drastically lower numbers in the presence of stickleback. Copepods were identified and separated into two families, revealing that one family increased while another decreased in the presence of stickleback. The lack of difference in Copepod abundance in the Bridges study may be a result of the combined effects of the two families, resulting in a negligeable change in abundance for the whole order. This variation indicates that there is much we do not know about the effects of stickleback on native invertebrates and highlights the need for additional replicates and the inclusion of coinvaded waterbodies. To date, coinvaded waterbodies have not been analyzed nor has the food web structure of individual waterbodies across the refuge been reconstructed through SIA for comparison. By increasing the number of replicate sites and investigating the food webs of individual waterbodies, including coinvaded sites, it may be possible to clarify the effects of stickleback and illuminate the more subtle effects of pumpkinseed. In this situation, where multiple species have invaded a system, addressing the effects of a single non-native fish is difficult, as each fish can cause an array of direct or indirect effects on the food web (Preston et al. 2012). By further investigating the individual impacts of brook stickleback and pumpkinseed at TNWR, the following study may aid managers in effectively mitigating the impacts of multiple non-native fish on native macroinvertebrates and waterfowl.

The objective of this study was to investigate the impacts of *C. inconstans* and *L. gibbosus* on native species through the comparison of waterbodies at TNWR that either have remained fishless, have been invaded by a single species, or have been coinvaded by both species. The following null hypotheses were tested: in fishless, brook stickleback, pumpkinseed, and coinvaded waterbodies (1) there will be no differences in the mean isotope signatures of each pond's constituents and (2) there will be no difference in zooplankton abundance, diversity, or mean body length. To address these hypotheses, the food web of each waterbody was compared using two metrics: zooplankton community composition and the analysis of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. Both stickleback and pumpkinseed are known to be visual, size-selective predators (Tompkins & Gee 1983, Bridges 2011). However, stickleback are highly opportunistic planktivores, consuming a wide range of prey within the pelagic zone where light easily penetrates, while pumpkinseed, which are primarily benthic predators, are often found among macrophytes where low visibility can potentially limit their prey choice (Confer & Blades 1975, Matthews 1998, Bridges 2011). Because of this, it is expected that food webs invaded by stickleback will greatly differ from those invaded by pumpkinseed and will have a greater impact on invertebrate composition.

Methods

Site Description

All sampling for this project was conducted within TNWR in the summer of 2020 beginning with preliminary sampling in late July. The 20,000 acres of TNWR, containing about 130 individual lakes and ponds, are located five miles south of Cheney, WA within the Channeled Scablands (US Fish and Wildlife Service 2007). The unique geological formation of the Channeled Scablands originated near the end of the last Ice Age, when the ice dams of Glacial Lake Missoula breached multiple times between 21-13,000 years ago, releasing massive floodwaters (Hanson et al. 2012). These floods carved deep depressions into the landscape of eastern Washington, and as these depressions filled with water, an abundance of productive wetlands and lakes emerged, providing food and refuge to migratory waterfowl. When settlers arrived in the late 1800s, the need for agricultural land necessitated the drainage of many of these wetlands, leading to a sharp decline in the total number of wetlands in the region (US Fish and Wildlife Service 2007). Conservationists began to recognize that the remaining wetlands represented some of the last quality breeding grounds for waterfowl in eastern Washington and by 1937, President Franklin D. Roosevelt signed an executive order to establish the Turnbull Migratory Waterfowl Refuge (later named the Turnbull National Wildlife Refuge) as a sanctuary for migratory waterfowl (US Fish and Wildlife Service 2007).

Recent fish assemblage surveys suggest that invasions and die-offs occur frequently within the refuge (Walston et al. 2015, Gunselman & Spruell 2019). Preliminary surveys were conducted to confirm the presence or absence of brook stickleback and pumpkinseed in ponds throughout the refuge. The goal of these surveys was to identify three ponds of each of the following categories: fishless, brook stickleback invaded (BS), pumpkinseed invaded (PS), and coinvaded. We aimed for three replicate sites per category so that any variation observed among waterbodies within each category could be quantified and compared to the observed differences between categories. We anticipated the dominant factor influencing variation among food webs is the presence or absence of fish, as suggested by Wieker et al. (2016), who found a 10-fold reduction in macroinvertebrate density in ponds with stickleback.

Fish Abundance

Evidence suggests that the abundance of a non-native fish may have a non-linear relationship with their degree of impact (Kornis et al. 2014, Jackson et al. 2015) and may be a cause of variation among ponds grouped together based on presence/absence data alone. Because of this, relative fish abundance was estimated for each site in an effort to account for some within-category variation. Relative fish population abundance was estimated using the commonly used metric, catch per unit effort (CPUE). Five minnow traps were submerged in each pond at randomly selected sites for a duration of 20-24 hours. Sites were selected by overlaying a grid on a map of each pond and numbering the quadrants that overlapped the littoral zone. Five random numbers were selected, indicating which quadrants would be used. CPUE was calculated by dividing the number of captured fish per minnow trap by the number of hours each trap was submerged. By coupling presence/absence data with CPUE, the fish assemblage of each waterbody may be better characterized (MacRae & Jackson 2006).

SIA Samples

Invertebrate samples for SIA included taxa known to dominate pumpkinseed, brook stickleback, and waterfowl diets as well as those shown in past studies to be heavily affected by the presence of these fish (Krapu & Reinecke 1992, Bridges 2011, Wieker et al. 2016). Primary consumers were collected to serve as a baseline or indicator of each pond's primary production. Daphnia represented pelagic primary production while Amphipods and Ephemeroptera represented benthic primary production. Substituting primary producers for primary consumers is a common practice in studies that utilize stable isotopes to analyze lake food webs (Black et al. 2003, Mahoney 2012).

In early August, fish and invertebrate samples were collected from each lake over the course of one month. Five fish of each species were collected in minnow traps per waterbody. Benthic macroinvertebrates were collected with dip nets and zooplankton were collected with plankton tow nets. All invertebrates were subsequently pooled by taxonomic order in the laboratory. Due to low invertebrate numbers at this time of year, sampling for invertebrates often required multiple trips over several days to meet the minimum weight requirement for SIA samples of 1 mg each. Invertebrates were kept alive for one day at the laboratory where they were sorted by taxonomic order and then frozen for storage. Samples were prepared according to the protocol of the University of California, Davis (UC Davis) Stable Isotope Facility where $\delta^{13}C$ and $\delta^{15}N$ content of all samples were analyzed (UC Davis Stable Isotope Facility 2020). Sample preparations began by thawing and rinsing invertebrates to remove any clinging algae and detritus before oven drying at 60°C for about 24 hours. Mortar and pestles were used to grind samples into a fine homogenous powder. Small invertebrates were pooled together and divided into three 1 mg samples, while larger invertebrates such as dragonflies and beetles were kept separate as individual samples. All samples were folded into tin capsules and organized into 96-well trays for shipping. All equipment utilized in this process were washed overnight in a HCL solution to prevent carbon contamination between samples.

Zooplankton Community Composition

To assess the zooplankton community composition of each site, the abundance, diversity, and mean body-length of each taxonomic group were estimated. Beginning on August 19 samples were collected from five randomly selected sites within the littoral zones of each waterbody, using a plankton tow net. Random sites were selected with the same method used to select sites for fish abundance estimates. Vertical plankton tows provide a measure of invertebrate abundance per volume that can be extrapolated for the entire waterbody. The plankton tow net consisted of a 19.4 cm opening diameter and was used to collect a single vertical tow from each site. From the edge of the selected site, we would wade towards the center of the pond until a depth of about 1m was reached as measured by a meter stick. Being careful not to disturb sediments or algae, the net was lowered, face up, until completely flat against the substrate. At this point, the net was quickly pulled upwards until out of the water. All net contents were washed into a labeled Nalgene bottle. The contents of the bottles were later poured through a 200 µm sieve to concentrate the contents. The sieve and its contents were set in a tray of 95% ethanol to quickly euthanize zooplankton, then backwashed into a bottle and stored in 70% ethanol (Black and Dodson 2003). All zooplankton were counted, measured for total body-length (mm), and identified to the lowest taxonomic level possible in the laboratory. Zooplankton abundance was estimated as mean count/L, using the volume extrapolated from corresponding vertical tow samples. Biomass was estimated as mean µg/L. The dry weights for biomass estimates of zooplankton were obtained from species specific length-weight regression equations as outlined by Dumont et al. 1975.

Statistical Analysis

All data analysis was performed in R version 4.1.2, with Vegan (version 2.5-7) and Stats (version 4.1.2) packages. All figures were rendered using ggplot2 (version 3.3.5), Effects (version 4.2-0), and Interactions (version 1.1.2) packages, and R script provided by Brian Hayden (2021) as a part of his Analyzing Stable Isotope Data video series.

To detect differences among pond categories, mean invertebrate isotope signatures were compared with one-way ANOVAs, where ponds were grouped by invasion status of fishless, BS, PS, and coinvaded. Two-way ANOVAs were used to detect which factors, stickleback or pumpkinseed presence/absence and their degrees of interaction, are influencing invertebrate isotope variations. Similarly, multiple linear regressions were used to determine if stickleback or pumpkinseed abundance and their degrees of interaction are influencing mean invertebrate isotope signatures (Quinn & Keough 2002).

The Shannon Index (Shannon & Weaver 1949) was used to calculate the zooplankton community indices of richness, evenness, and diversity. Similar to SIA data analysis, one-way ANOVAs were used to detect if invasion status influenced differences in community indices, abundance, and mean body-length. Two-way ANOVAs were used to analyze the effects of fish presence/absence and their degrees of interaction. Multiple linear regressions were used to analyze the effects of relative fish abundance and their interactions on zooplankton community indices, abundance, and mean body-length (Quinn & Keough 2002).

Results

Study Sites

Preliminary sampling for fish presence was conducted in 14 sites, however, only 10 of these ponds were utilized for this study (Table 1). Delays in sampling occurred due to Washington State mandated COVID-19 restrictions, until July 20th when peak invertebrate productivity had likely passed (Stankavich 2013). In August, several sites were found to be insufficient in macroinvertebrate abundance, thus reducing the number of ponds utilized for this study. The remaining 10 ponds included three replicates of fishless ponds (Helm's Marsh, Middle Findley, Upper Findley), BS ponds (Kepple, Windmill pond, Winslow pool), and coinvaded ponds (Long Lake, Middle Pine, McDowell), while only one pumpkinseed invaded pond was found (Lower Findley).

Fish Abundance

The ponds in this study exhibited a broad range of fish abundance as can be seen in Table 2. Each trap was submerged for about 24hrs with some variation in total time (22-24.5hrs). As a result, the time each trap was submerged was standardized to a 24hr interval, where (total number of fish captured) / (total submersed hours) * 24. The highest CPUE was seen in Kepple at 20.77 fish/trap/24hr and the lowest was seen in Lower Findley at 2.00 fish/trap/24hr. Between pond types, less variation was seen. Mean fish abundance for coinvaded ponds was 12.12 fish/trap/24hr and 13.17 fish/trap/24hr for BS ponds. Stickleback were the dominate species in our study with a mean abundance of 8.95 fish/trap/24hr, while pumpkinseed mean abundance was 2.18 fish/trap/24hr. Of the three coinvaded ponds in this study, Middle Pine had the greatest total fish abundance at

17.78 fish/trap/24hr, which was 76% greater than Long Lake at 10.10 fish/trap/24hr. McDowell exhibited the lowest total abundance for both species at 8.50 fish/trap/24hr.

Stable Isotope Analysis

The expected trophic enrichment of 1‰ $\delta^{13}C$ and 3.4 ± 1.1‰ $\delta^{15}N$ was not seen in any of our study ponds (Table 3, Figure 3). There was a negative slope for several ponds (Helm's Marsh, Middle Findley, Long Lake, McDowell, Middle Pine) indicating a depletion of $\delta^{13}C$ for increasing trophic level. For all other ponds, trophic enrichment resulted in 0.005 - 1.2‰, far below that of 3.4‰, indicating a low enrichment of $\delta^{15}N$ for each increase in trophic level. Biplots of fishless, BS, and coinvaded ponds can be found in Figures 4-6.

Mean isotope values and standard deviation for brook stickleback in BS ponds were -26.83 ± 4.03‰ $\delta^{13}C$ and 11.34 ± 2.59‰ $\delta^{15}N$. For coinvaded ponds they were -24.67 ± 4.03‰ for $\delta^{13}C$ and 9.64 ± 2.58‰ for $\delta^{15}N$. Mean pumpkinseed isotope values in coinvaded ponds were -25.36 ± 4.86‰ for $\delta^{13}C$ and 9.47 ± 1.87‰ for $\delta^{15}N$. Within coinvaded ponds pumpkinseed did not differ with stickleback values, however, mean $\delta^{15}N$ values of pumpkinseed from coinvaded ponds were significantly less than stickleback in BS ponds at $P = 0.012$, indicating a lower trophic level for pumpkinseed. A similar range of difference was seen between stickleback in BS and coinvaded ponds in $\delta^{15}N$ values, but no significance was found likely due to greater variation among stickleback within each pond type (coinvaded SD: ± 2.58‰; BS SD: ± 2.59‰).

Mean invertebrate isotope ratios can be found in Table 1 for each pond. Substantial variation was seen within pond categories of mean invertebrate $\delta^{13}C$ and $\delta^{15}N$

values. Among fishless ponds, Helm's Marsh was significantly depleted in $\delta^{13}C$ compared to Middle Findley and Upper Findley (both at $P \leq 0.001$), while $\delta^{15}N$ values were enriched compared to Middle Findley at $P = 0.012$ but not Upper Findley. Among BS ponds, Kepple was significantly enriched in $\delta^{13}C$ and depleted in $\delta^{15}N$ values compared to Windmill ($\delta^{13}C$: $P = 0.025$; $\delta^{15}N$: $P = 0.005$) and Winslow ($\delta^{13}C$: $P = 0.002$; $\delta^{15}N$: $P = 0.006$). Among coinvaded ponds, Middle Pine was significantly enriched in $\delta^{13}C$ values compared to Long Lake and McDowell, both at (both at $P \leq 0.001$), and Long Lake was enriched in $\delta^{13}C$ compared McDowell at $P = 0.002$. Mean $\delta^{15}N$ was significantly enriched in Middle Pine compared to Long Lake ($P = 0.003$) and McDowell ($P = 0.0001$), however, Long Lake and McDowell did not significantly differ in mean $\delta^{15}N$ values.

Overall, there was a large amount of variation in isotopic signatures among invertebrates within each pond type (Figures 4-6). Several factors were found to influence invertebrate $\delta^{13}C$ values: invasion status ($P \leq 0.001$), stickleback presence ($P \leq 0.001$), and pumpkinseed presence ($P \leq 0.001$). All three variables contributed to $\delta^{13}C$ enrichment across each food web, indicating a shift towards benthic/littoral sources in the presence of fish. Mean $\delta^{13}C$ of coinvaded ponds was 6.35‰ greater than fishless ponds. For invertebrate $\delta^{15}N$ values, invasion status ($P = 0.014$) and stickleback presence ($P \leq 0.001$) were found to be influencing factors. In this case, mean $\delta^{15}N$ values were 2.85‰ greater in BS ponds than in fishless ponds, however, these values declined somewhat in coinvaded ponds, which was only 1.79‰ greater than mean fishless $\delta^{15}N$. Pumpkinseed presence was not found to be a significant factor influencing invertebrate $\delta^{15}N$ values. However, there was a significant interaction effect between pumpkinseed and stickleback abundance ($P \leq 0.001$), where $\delta^{15}N$ values decreased as pumpkinseed abundance increased dependent on increasing stickleback abundance (Figure 7).

The invertebrate taxa whose isotopic signatures were affected by fish presence were Amphipoda, Coleoptera, and Hirudinae. Amphipod $\delta^{13}C$ values were enriched in coinvaded ponds compared to fishless ($P = 0.013$) and BS ponds ($P = 0.026$), indicating a greater reliance on benthic carbon sources in coinvaded ponds. Pumpkinseed presence ($P = 0.006$) rather than stickleback presence was the main factor in the shift in amphipod $\delta^{13}C$ values, which was evidenced by their similarity between fishless and BS ponds. Likewise, Hirudinae $\delta^{13}C$ values were somewhat enriched in the presence of stickleback at $P = 0.070$. Coleoptera ($P = 0.035$) and Hirudinae ($P = 0.042$) $\delta^{15}N$ values all increased in the presence of stickleback, indicating higher trophic positions for these taxa in ponds with stickleback. Ephemeroptera and Chironomidae were the only invertebrates affected by fish abundance. Ephemeroptera $\delta^{15}N$ values increased with pumpkinseed abundance at $P = 0.031$. The strength of Pumpkinseed abundance on $\delta^{15}N$ values was found to be an interactive effect dependent on increasing stickleback abundance ($P = 0.040$). Finally, chironomid $\delta^{13}C$ values increased with increasing fish abundance at $P = 0.014$.

Zooplankton Community Structure

Values for Shannon diversity, richness, evenness, abundance, and biomass for each pond can be found in Table 4. Zooplankton evenness and diversity was influenced by stickleback presence at $P = 0.047$ and 0.027, respectively (Figure 8). Pumpkinseed had little to no impact on these indices, individually or as a co-variable. Zooplankton richness did not significantly differ across any metric.

Overall, zooplankton abundance was significantly impacted by invasion status (*P* = 0.037). Coinvaded ponds had nearly significantly greater abundance at 833.72 count/L ± 294.94, which was 662.3% greater than fishless ponds at 109.28 count/L ± 48.37 (*P* = 0.058) and 976.5% greater than BS ponds at 84.51 count/L ± 33.021 (*P* = 0.051). Pumpkinseed presence was found to be the primary factor influencing the increase in zooplankton abundance (*P* = 0.038), while brook stickleback was found to be an insignificant factor. Zooplankton biomass demonstrated weaker differences between pond types. Biomass was much larger in coinvaded ponds at 1599.32 µg/L ± 1204.54, which was 328.6% greater than fishless ponds at 373.19 µg/L ± 145.30, and 2112.6% greater than BS ponds at 72.28 µg/L ± 33.51, but lacked statistical significance (*P* = 0.332), likely due to a large variation in biomass across coinvaded ponds (Figure 9). Likewise, we failed to detect whether stickleback and pumpkinseed presence were influencing factors on zooplankton biomass. Bar graphs of zooplankton abundance and biomass can be found in Figures 9 and 10.

Several zooplankton taxa showed significant differences in abundance among pond types. Total *Daphnia* fell drastically from a mean of 22.25 individuals/L in fishless ponds to less than one individual/L in BS and coinvaded ponds but lacked statistical significance due to variation in fishless ponds. Helm's Marsh and Upper Findley contained 43.30 and 23.43 individuals/L, respectively, while Middle Findley contained similar amounts of *Daphnia* to BS and coinvaded ponds, which all contained less than one individual/L. In coinvaded ponds, Ostracoda (*P* = 0.035), Chydoridae (*P* = 0.018), and copepod nauplii (*P* = 0.007) occurred at significantly higher abundance than in fishless and stickleback ponds. Most noteably, Chydoridae occurred at 1689.8% greater abundance in coinvaded ponds versus fishless ponds. It is worth noting the zooplankton with nearly significant changes in abundance. Rotifer abundance was greater in coinvaded ponds than in fishless ponds at $P = 0.06164$, while Calanoida copepods were nearly non-existent in coinvaded ponds at $P = 0.069$ when compared to fishless ponds. Stickleback presence was found to be the main factor influencing both rotifer and calanoid abundance.

Fewer shifts in zooplankton biomass were seen as compared to abundance. Calanoid and copepod nauplii shifted in biomass across pond types. As mentioned before, calanoids were nearly non-existent in coinvaded ponds, resulting in $P = 0.046$ when compared to fishless ponds. Copepod nauplii biomass was lowest in fishless ponds and increased significantly in coinvaded ponds at $P = 0.005$. Although nauplii biomass was not significantly greater in stickleback ponds than in fishless ponds, the biomass in coinvaded ponds was significantly greater, 284.5%, than in BS ponds at $P = 0.007$. For calanoids, brook stickleback presence was the major influencing factor in biomass, while the main factor influencing nauplii biomass was the presence of both fish which were found to have an interactive effect at $P = 0.014$. Finally, ostracod biomass occurred at its highest levels in coinvaded ponds and was mainly affected by pumpkinseed presence at $P = 0.032$. Detailed results of zooplankton abundance and biomass for each taxon can be found in Tables 5 and 6.

Invasion status was a predictor of mean zooplankton length ($P = 0.034$). Zooplankton length was the greatest within fishless ponds at a mean of 0.678 mm ± 0.116 (Figure 11). Mean length for BS and coinvaded ponds was 0.331 mm ± 0.032 and 0.378 mm ± 0.051, respectively. When compared to BS ponds, the average length in fishless ponds was 104% greater (*P* = 0.040). Coinvaded ponds maintained a similar mean to BS ponds but were only 79% less than fishless ponds (*P* = 0.068). Stickleback presence was the influencing factor behind the shift in zooplankton length with *P* = 0.022. Lengths for taxonomic groups within each pond can be found in Table 7.

Ceriodaphnia and *Simocephalus* were the only two zooplankton that differed in average length across pond type. For *Ceriodaphnia*, average length was the greatest in BS ponds at 0.5 mm ± 0.025 compared to the average length in fishless ponds of 0.46 mm ± 0.019 (*P* = 0.014). Average length for *Simocephalus* was greatest in fishless ponds at 1.13 mm ± 0.137 and the lowest in BS ponds at 0.60 mm ± 0.130 (*P* = 0.035). Cyclopoid copepods had a nearly significant shift in average length between BS ponds, 0.76 mm ± 0.071, and fishless, 0.58 mm ± 0.028, ponds with *P* = 0.094.

Discussion

The intent of this study was to discern the individual impacts of two non-native fish within the TNWR wetlands. We structured this project to test whether there would be differences between fishless ponds and those invaded by stickleback, pumpkinseed, or both. We examined the isotopic signatures of native invertebrates across all study ponds and quantified the relative community structure using indices such as abundance, diversity, and mean body length. Originally, we intended to use a total of 12 ponds representing four invasion categories. However, we were unable to find more than one pond that was solely invaded by pumpkinseed. The lone PS pond, Lower Findley, was used in our analysis of relative fish abundance and presence/absence variables but was not used to represent PS ponds as a whole category. As a result, we refocused much of our analysis on differentiating fishless, BS, and coinvaded ponds.

Stable Isotope Analysis

Surprisingly, no significant differences were seen in stickleback and pumpkinseed isotopic signatures, which contrasts with previous work showing stickleback as more omnivorous with depleted $\delta^{13}C$ values compared to pumpkinseed (Bridges 2011). The same research collected SIA samples in June and early July, while samples for the present study were collected in August (Bridges 2011). The timing of pumpkinseed spawning is often documented as occurring in late spring to early summer (Zięba et al. 2010), and because SIA samples were collected in the late summer, the isotope ratios of pumpkinseed may reflect a population dominated by young of year which are known planktivores compared to littoral dwelling adults (Confer & Blades 1975, Hambright et al. 1992, García-Berthou & Moreno-Amich 2000). Alternatively, pumpkinseed may be relying on plant-based food resources in times of low food availability. Wieker et al. (2016) documented a sharp decline in macroinvertebrate abundance in TNWR ponds where fish were present. Although littoral invertebrate abundance was not quantified for this study, abundance in general was low enough to necessitate multiple sampling attempts in several ponds (Long Lake, McDowell, Upper Findley, Winslow) and some were found to be so insufficient of invertebrates for isotope analysis (Black Horse, Swan Pond, Cheever), that they were excluded entirely from this study. The low littoral invertebrate abundance may influence pumpkinseed foraging behavior, resulting in a reliance on zooplankton, algae, and other debris. (García-Berthou & Moreno-Amich 2000, Wieker et al. 2016).

Aquatic food webs invaded by nonnative species commonly experience shifts in their carbon basal sources (Vander Zanden et al. 1999, Ozersky et al. 2012,). In the case of invaded ponds in TNWR, we found a significant shift towards benthic sources, as indicated by enriched $\delta^{13}C$ values. This shift from pelagic sources was expected as stickleback are known planktivores (Confer & Blades 1975, Matthews 1998), and can greatly reduce the number of large zooplankton species in a system (Des Roches et al. 2013). Both stickleback and pumpkinseed presence had significant effects on overall mean invertebrate $\delta^{13}C$ values, and together their effects appeared to compound in coinvaded ponds where $\delta^{13}C$ values were highest in the study (Figure 12).

For $\delta^{13}C$ values, Amphipods showed the greatest sensitivity to fish presence of all invertebrates. Amphipods can have variable diets but are often described as herbivores and can be utilized as representatives of littoral primary producers in SIA studies since primary producers tend to be highly variable in their isotopic ratios (Zohary et. al 1994, Mahoney 2012). The $\delta^{13}C$ enrichment may indicate a shift from a variable diet to one primarily consisting of plant matter in the presence of pumpkinseed.

The general trend of $\delta^{15}N$ enrichment in the presence of stickleback was unexpected. Values of $\delta^{15}N$ have the potential to be highly variable especially for herbivores (Vander Zanden & Rassmussen 2001). While the exact reason for the $\delta^{15}N$ enrichment is unclear it may be a result of omnivory among invertebrates. In the context of food web ecology, omnivory refers to when an organism feeds on multiple trophic levels, and in the case of our invaded ponds, some invertebrates may be feeding on higher trophic levels in lieu of their preferred prey (Jones & Waldron 2003). Such may be the case for predatory Coleoptera and Hirudinae, whose δ^{13}C values also increased with stickleback presence (Figure 5).

As we originally anticipated, variation was seen within pond categories and our effort to quantify relative fish abundance in hopes that it may explain some of this variation was worthwhile. Although stickleback presence was found to be the main factor influencing invertebrate δ^{15}N enrichment, coinvaded ponds had a less significant δ^{15}N ($P = 0.297$) enrichment than BS ponds. As shown in Table 2, there was greater variation in δ^{15}N values among coinvaded ponds, but they still held to the general trend of δ^{15}N enrichment compared to fishless ponds. While presence/absence data alone was unable to explain the variation seen, relative fish abundance may provide a more accurate explanation. Of the three coinvaded ponds in this study, Middle Pine had the greatest δ^{15}N values and the lowest pumpkinseed abundance at 1.49 CPUE while Long Lake and McDowell had significantly lower δ^{15}N values and greater pumpkinseed abundance as 6.44 and 5.31 CPUE, respectively (Tables 1 & 2). Likewise, statistical analysis revealed that as pumpkinseed abundance increased within a pond, δ^{15}N values tended to decrease, although this effect was dependent on increasing stickleback abundance. While we hesitate to fully attribute this phenomenon seen in TNWR coinvaded ponds to variation in pumpkinseed abundance, due to lack of PS pond replicates, it may be that high pumpkinseed abundance are mitigating some stickleback effects. In contrast, among BS ponds, Kepple Lake was significantly depleted in δ^{15}N while containing the highest stickleback abundance. However, statistical analysis across all ponds revealed a general trend of δ^{15}N enrichment as stickleback abundance increases. This inconsistency may be a result of species-specific differences between stickleback and pumpkinseed, where limits in the effect of abundance exists. Previous work has demonstrated as abundance of a non-native fish population increases, intraspecific interactions can outweigh the interspecific interactions at high abundance, thus lowering their degree of impact (Kornis et al. 2014). Kepple Lake contained the most abundant stickleback population across all ponds and may represent a threshold over which the abundance of a stickleback population loses its effect on a food web. The data presented here are not enough to fully ascertain the nuance of stickleback and pumpkinseed abundance, and we encourage further exploration into this topic.

Zooplankton Community Composition

As predicted, coinvaded ponds differed greater than ponds invaded by stickleback alone, while the community composition of stickleback ponds appeared as intermediates between fishless and coinvaded ponds. The results of this study revealed zooplankton abundance to be the community index most sensitive to the introduction of non-native fish. The significantly greater zooplankton abundance found in coinvaded ponds was in large part due to a shift in zooplankton biomass from the larger branchiopods, such as *Daphnia* and *Ceriodaphnia*, to the smaller Chydoridae branchiopods and copepod nauplii. This shift was first evidenced in stickleback ponds where zooplankton data reflected a drastic decline in average length, through the reduction of *Daphnia* abundance. In coinvaded ponds, chydorids could be experiencing a release from predation and competition, allowing them to thrive in the presence of fish. Size-selective predators such as stickleback are less likely to feed on smaller chydorids when other, larger branchiopods, such as *Daphnia*, are available (Tiberti et al. 2014, Klemetsen et al.

2020). Other studies have shown similar shifts in branchiopod communities while in the presence of stickleback (Hornung et al. 2006, Helenius et al. 2016, Laske et al. 2016). A study by Laske et al. in 2017, observed the introduction of the ninespine stickleback, *Pungitius pungitius*, in two ponds within the Arctic Coastal Plain of Alaska. They found chydorids and other small zooplankton became the dominant species after larger branchiopods declined over the course of the study. Similar research with the threespine stickleback, *Gasterosteus aculeatus*, found increased abundance of small zooplankton when stickleback consumed large crustaceans, releasing the zooplankton from competition and predation (Helenius et al. 2016).

The invasion by stickleback alone, however, was not enough to alter zooplankton abundance within our study ponds. Instead, the addition of pumpkinseed appeared to be the influencing factor behind increased zooplankton abundance in coinvaded ponds where chydorid branchiopods flourished with a nearly 17-fold increase in abundance compared to fishless ponds. These results somewhat contrast with Bridges' work in 2011, showing little to no pumpkinseed effect and a strong stickleback effect on zooplankton abundance. There are two possible explanations for this difference. First, as mentioned earlier, seasonal effects resulting from the timing of sample collection could be the cause for low zooplankton response to stickleback in the present study. It is possible if samples had been collected in June-July, rather than August-September when peak invertebrate abundance had likely passed, greater zooplankton abundance may have allowed for the detection of subtle differences between ponds that were not found in this study (Stankavich 2013). Research by Wieker et al (2016) provides additional support of a seasonal effect on the invertebrate food web. They found Bridges' work most resembled their littoral invertebrate abundance samples from June-July and contrasted with their samples from August-September (Wieker et al. 2016). Second, previous work on stickleback effects in TNWR were not designed to investigate coinvasion impacts and instead considered ponds invaded by only one species (Bridges 2011, Wieker et al. 2016). Because of the differences in experimental design, the results presented here do not necessarily contradict past work, rather it may demonstrate that a pumpkinseed effect is contingent on the presence of an additional non-native fish. Other researchers have found similar evidence for such additive effects. Research by Fryxell et al. in 2016 revealed additive interactions between two invasive aquatic species, the benthic red swamp crayfish, *Procambarus clarkii*, and the pelagic western mosquitofish, *Gambusia affinis*. Although both species are known to consume snails, snail abundance was significantly reduced only in the presence of both species. When invading alone, Mosquitofish caused a small increase in snail abundance, while red swamp crayfish had little effect. The researchers speculated that a single invader may have facilitated snail abundance by releasing them from competition with other more preferrable invertebrate prey, while the combined invasion likely caused enough predation on snails to surpass the positive effects of reduced competition and ultimately reduced their numbers.

Conclusion

In the case of TNWR ponds, mean $\delta^{13}C$ enrichment of invaded ponds, particularly that of coinvaded ponds, is largely reflective of shifts in the zooplankton community structure and the diets of predatory invertebrates. The composition of the zooplankton community shifted to smaller species in the presence of a single non-native fish but was not enough to impact abundance and biomass between stickleback and fishless ponds. The addition of a second non-native fish, however, further exaggerated this shift in zooplankton community composition. The coinvaded communities maintained reduced mean zooplankton length but increased in both biomass and abundance, indicating an environment where small zooplankton species flourished under reduced competition and/or predation by other invertebrates. Concurrently, several large predatory invertebrates shifted to omnivorous diets in the presence of fish as indicated by an enrichment of $\delta^{15}N$ values. Increased competition with non-native fish for prey is potentially forcing invertebrate predators into a greater reliance on $\delta^{13}C$ enriched benthic sources. Finally, while the present study revealed some evidence of an abundance effect on invaded ponds, whether variation within pond types can be explained by relative fish population abundance has yet to be determined as other factors such as the seasonal effects of sampling in the late summer could not be ruled out. In general, the impacts of multiple species invasions are difficult to predict and attributing cause and effect to one species or the other is likely outside the scope of this study due to a lack of replicate PS ponds. Ultimately, this study highlights the difficulties faced by managers in determining a plan of action when faced with multiple species invasions and emphasizes the importance of exploring these issues as our understanding of the complex nature of multiple species invasions remains relatively limited.

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Tables and Figures

TABLE 1. A list of all sampled ponds with their corresponding invasion status, mean invertebrate $\delta^{13}C$ and $\delta^{15}N$ values, and Standard Deviation.

Name	**Invasion Status**	**$\delta^{13}C$**	**$\delta^{15}N$**
Lower Findley	Pumpkinseed	-22.53 ± 2.33	3.39 ± 1.97
Helm's Marsh	Fishless	-33.74 ± 2.58	4.93 ± 1.67
Kepple Lake	Brook Stickleback	-29.99 ± 2.77	3.56 ± 3.19
Long Lake	Coinvaded	-24.65 ± 2.39	4.52 ± 3.31
McDowell Lake	Coinvaded	-28.46 ± 2.21	3.38 ± 2.47
Middle Pine Lake	Coinvaded	-18.29 ± 1.37	9.07 ± 2.18
Middle Findley	Fishless	-27.73 ± 1.74	2.68 ± 1.04
Upper Findley	Fishless	-28.99 ± 1.88	3.99 ± 1.75
Windmill Pond	Brook Stickleback	-26.60 ± 3.08	8.24 ± 2.30
Winslow Pool	Brook Stickleback	-25.14 ± 2.35	8.34 ± 3.73

TABLE 2. CPUE values for each invaded pond. CPUE was calculated over a 24-hour period for five sites per pond. These values were averaged for both species to provide Mean CPUE for BS and/or PS. In coinvaded ponds (Long Lake, Middle Pine, and McDowell), Total mean CPUE was calculated by combining CPUE of both species. (BS = Brook Stickleback, PS = Pumpkinseed)

	Kepple	**Windmill**	**Winslow**	**Lower Findley**	**Long Lake**		**Middle Pine**		**McDowell**	
Site Number	**BS**	**BS**	**BS**	**PS**	**BS**	**PS**	**BS**	**PS**	**BS**	**PS**
1	31.03	13.86	5.96	1.16	1.06	2.13	1.05	0	5.30	4.42
2	44.55	0	47.70	3.12	0	4.29	78.24	2.11	0	8.81
3	14.60	1.06	1.98	4.64	1.07	10.72	0	3.19	4.86	2.65
4	8.40	0	15.82	0	15.03	12.89	0	0	2.67	5.33
5	5.30	5.33	1.98	1.10	1.09	2.19	2.15	2.15	3.12	5.34
Mean CPUE:	20.77	4.05	14.69	2.00	3.65	6.44	16.29	1.49	3.19	5.31
Total Mean CPUE:					10.10		17.78		8.50	

TABLE 3. Slope, Intercept, and R^2 of each pond's $\delta^{13}C$: $\delta^{15}N$ regression lines. See Figure 3 for a visual comparison of all ponds.

	Slope	Intercept	R^2
Helm's Marsh	-0.120	0.970	0.033
Middle Findley	-0.220	-3.300	0.130
Upper Findley	0.150	8.300	0.025
Lower Findley	0.005	3.501	4E-05
Kepple	0.110	6.800	0.009
Windmill	0.081	10.000	0.012
Winslow	1.200	38.000	0.540
Long Lake	-0.710	-13.000	0.260
McDowell	-0.140	-0.560	0.015
Middle Pine	-0.110	7.000	0.005

TABLE 4. Zooplankton community indices of all sampled ponds. Richness, Diversity (*H'*), and Evenness (*H'/H'_{max}*) were calculated according to the Shannon Index (Shannon & Weaver 1949). Biomass (Avg. μg/L) was calculated using dry weight estimates of zooplankton taxa provided by Dumont et al. 1975.

Site	Invasion Status	Richness	Diversity (H')	Evenness (H'/H'_{max})	Abundance (Avg. count/L)	Biomass (Avg. μg/L)
Helm's Marsh	Fishless	11	1.40	0.61	73.23	599.39
Middle Findley	Fishless	9	1.42	0.68	49.56	102.10
Upper Findley	Fishless	11	1.60	0.70	205.04	418.09
Kepple	Brook Stickleback	10	1.40	0.64	85.17	76.80
Windmill	Brook Stickleback	11	1.00	0.44	141.37	127.93
Winslow	Brook Stickleback	10	1.00	0.46	26.99	12.12
Lower Findley	Pumpkinseed	11	1.33	0.58	201.65	257.72
Long Lake	Co-invaded	10	1.12	0.51	479.36	334.15
McDowell	Co-invaded	10	1.14	0.52	602.50	456.44
Middle Pine	Co-invaded	9	1.24	0.60	1419.30	4007.36

TABLE 5. Mean branchiopod abundance (Avg. count/L), biomass (Avg. µg/L), and length (Avg. mm) for each pond type.

Pond Type		*Bosmina*	*Ceriodaphnia*	*Chydoridae*	*Daphnia*	*Simocephalus*	*Scapholebris*
Fishless	Abundance (Avg. count/L)	0.05	7.26	25.33	22.25	2.95	0.03
	Biomass (Avg. µg/L)	0.05	21.33	9.12	203.00	40.22	0.02
	Length (Avg. mm)	0.37	0.46	0.27	1.02	1.13	0.43
BS	Abundance (Avg. count/L)	0.23	0.06	39.55	0.23	1.57	0.69
	Biomass (Avg. µg/L)	0.39	0.18	15.68	0.76	6.36	0.83
	Length (Avg. mm)	0.36	0.50	0.24	0.70	0.60	0.39
Coinvaded	Abundance (Avg. count/L)	0.50	1.86	453.32	0.26	129.05	2.66
	Biomass (Avg. µg/L)	1.70	2.71	237.15	7.53	900.50	4.18
	Length (Avg. mm)	0.47	0.36	0.27	1.34	0.96	0.37

TABLE 6. Mean zooplankton (non-branchiopods) abundance (Avg. count/L), biomass (Avg. µg/L), and length (Avg. mm) for each pond type. Calanoid copepods were not found in Coinvaded ponds.

Pond Type	Metric	Calanoid	Copepod nauplii	Cyclopoid	Ostracod	Rotifers
Fishless	Abundance (Avg. count/L)	1.49	2.00	31.53	11.84	6.55
	Biomass (Avg. µg/L)	31.87	0.63	55.05	10.78	1.12
	Length (Avg. mm)	1.50	0.22	0.58	1.12	0.19
BS	Abundance (Avg. count/L)	0.55	2.27	17.10	9.15	15.38
	Biomass (Avg. µg/L)	4.43	0.79	33.61	6.99	2.27
	Length (Avg. mm)	1.15	0.36	0.76	0.85	0.19
Coinvaded	Abundance (Avg. count/L)	0.00	9.29	124.16	79.45	42.47
	Biomass (Avg. µg/L)	0.00	3.03	384.81	51.81	5.90
	Length (Avg. mm)		0.23	0.58	10.76	0.18

TABLE 7. Mean length (mm) of each zooplankton taxa across all ponds are presented in rows 2-12. Mean Zooplankton Lengths presented in row 13 represent the mean length of all zooplankton for each pond.

Zooplankton taxa	**Helm's Marsh**	**Middle Findley**	**Upper Findley**	**Kepple**	**Windmill**	**Winslow**	**Lower Findley**	**Long Lake**	**McDowell**	**Middle Pine**
Bosmina	0.450		0.291	0.402	0.380	0.300	0.283	0.422	0.513	
Calanoida	1.502	1.540	1.450	0.738	0.275	2.450	1.387			
Ceriodaphnia	0.429	0.459	0.495	0.475	0.525		0.425	0.362	0.330	0.393
Chydoridae	0.270	0.282	0.248	0.256	0.204	0.264	0.317	0.267	0.250	0.296
Copepod Nauplii	0.223	0.208	0.231	0.238	0.598	0.250	0.282	0.223	0.250	0.223
Cyclopoida	0.560	0.638	0.550	0.634	0.881	0.762	0.662	0.552	0.649	0.536
Daphnia	1.228	0.900	0.941	0.563	0.700	0.825	0.663	1.018	2.300	0.708
Ostracoda	1.082	1.234	1.035	1.188	0.243	1.123	0.439	7.396	22.121	2.758
Simocephalus	1.130	1.361	0.888	0.438	0.856	0.500	1.215	0.881	0.999	1.005
Scapholeberis	0.517		0.350		0.527	0.250	0.407	0.327	0.400	0.371
Rotifers	0.186	0.198	0.196	0.176	0.204	0.194	0.159	0.143	0.201	0.196
Mean Zooplankton Length:	0.903	0.518	0.613	0.360	0.366	0.268	0.417	0.306	0.352	0.476

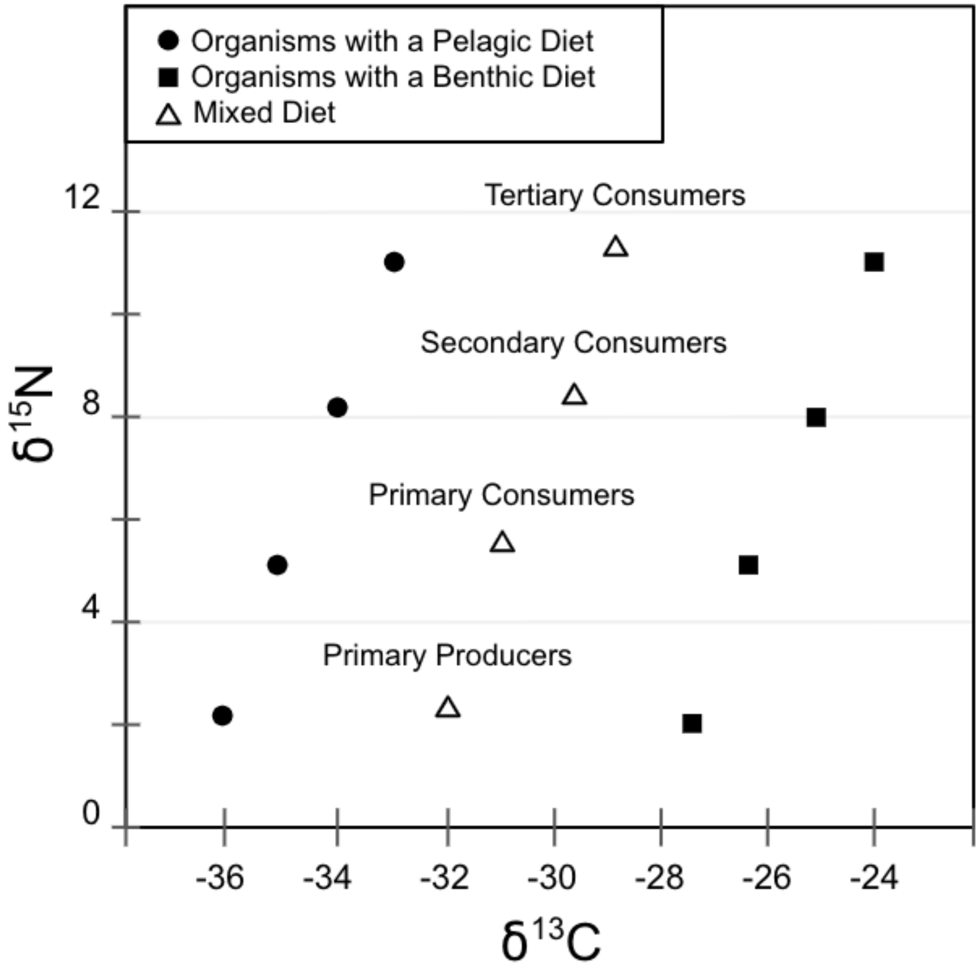

Figure 1. Stable isotope analysis can be used to compare the levels of $\delta^{13}C$ and $\delta^{15}N$ between organisms in a food web and are often visualized in scatter plots as seen here. The amount of $\delta^{15}N$ indicates the trophic position that an organism occupies, while $\delta^{13}C$ indicates the primary production sources of an organism's diet. Organisms that derive more energy from benthic sources contain greater amounts of $\delta^{13}C$ and those that derive more energy from pelagic sources contain less $\delta^{13}C$ (Vander Zanden, et al., 1999). Furthermore, a predictable pattern in the levels of $\delta^{13}C$ and $\delta^{15}N$ has been established for a typical food web. In general, with an increase in trophic level, there is a trophic enrichment of 3% $\delta^{15}N$ and 1% $\delta^{13}C$, resulting in a mean slope of three for the plotted values (Black et al. 2003)

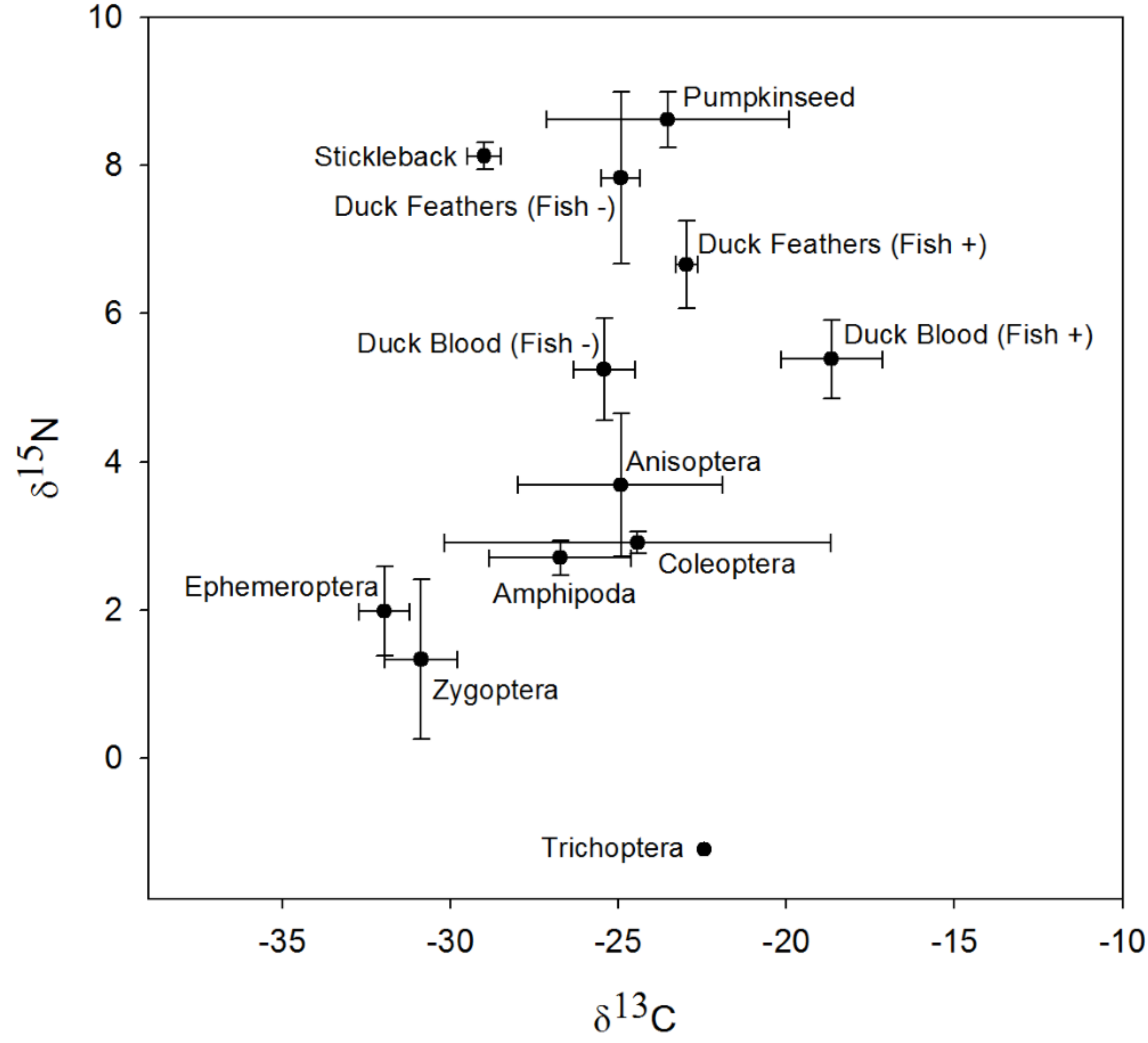

Figure 2. From Bridges 2011 - Mean isotope ratios representing the constituents of eight waterbodies found in Turnbull National Wildlife Refuge. Four waterbodies were fishless, two were invaded by stickleback, and two were invaded by pumpkinseed.

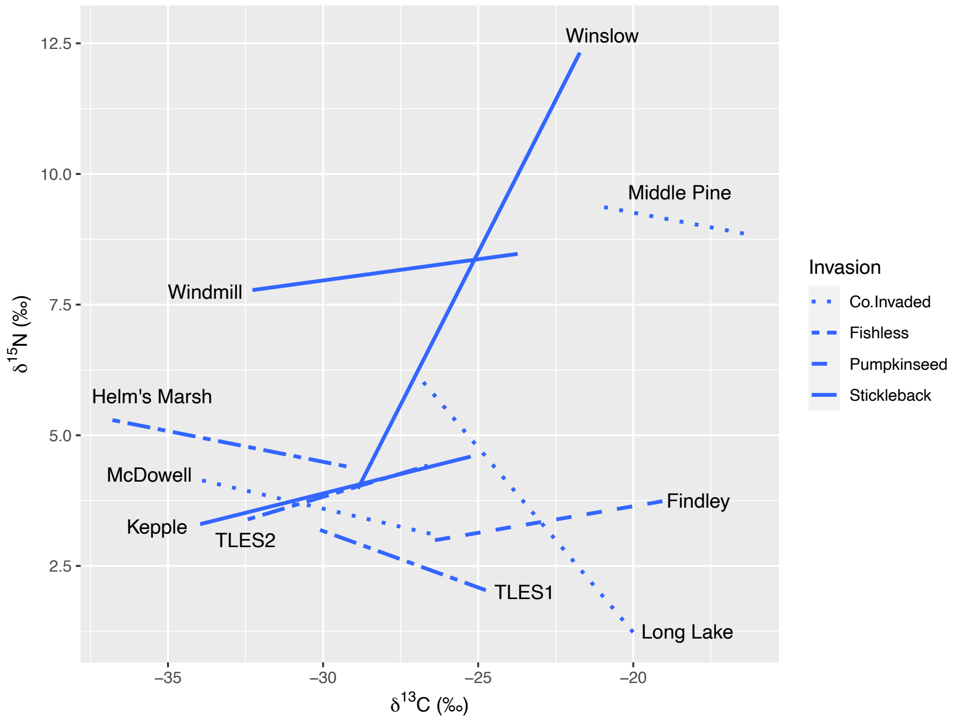

Figure 3. Regression lines of invertebrate isotopes for all 10 ponds. Slope, intercept and R^2 for each regression line can be found in Table 3.

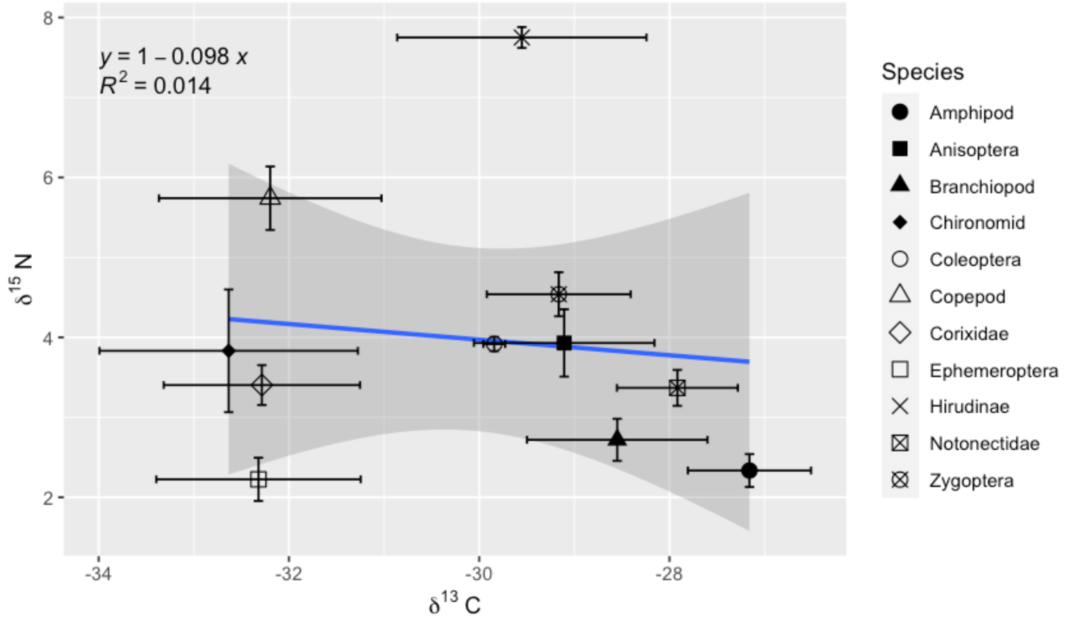

Figure 4. Mean isotopic signatures of invertebrates in fishless ponds (Helm’s Marsh, Middle Findley, Upper Findley) with standard deviation.

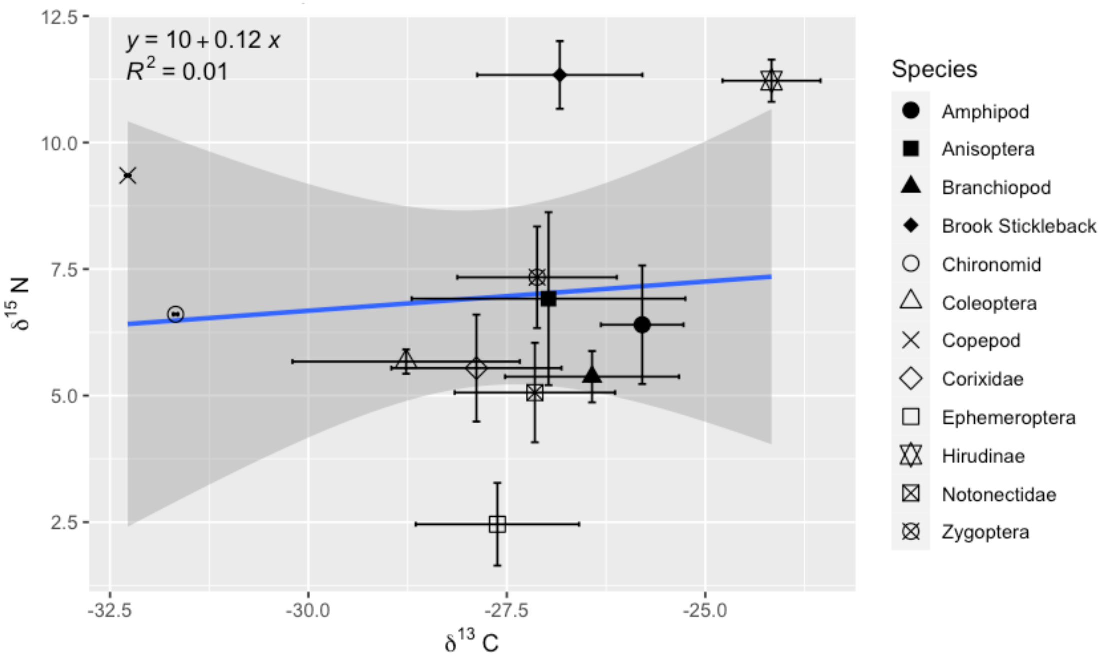

Figure 5. Mean isotopic signatures of invertebrates in BS ponds (Kepple, Windmill, Winslow) with standard deviation.

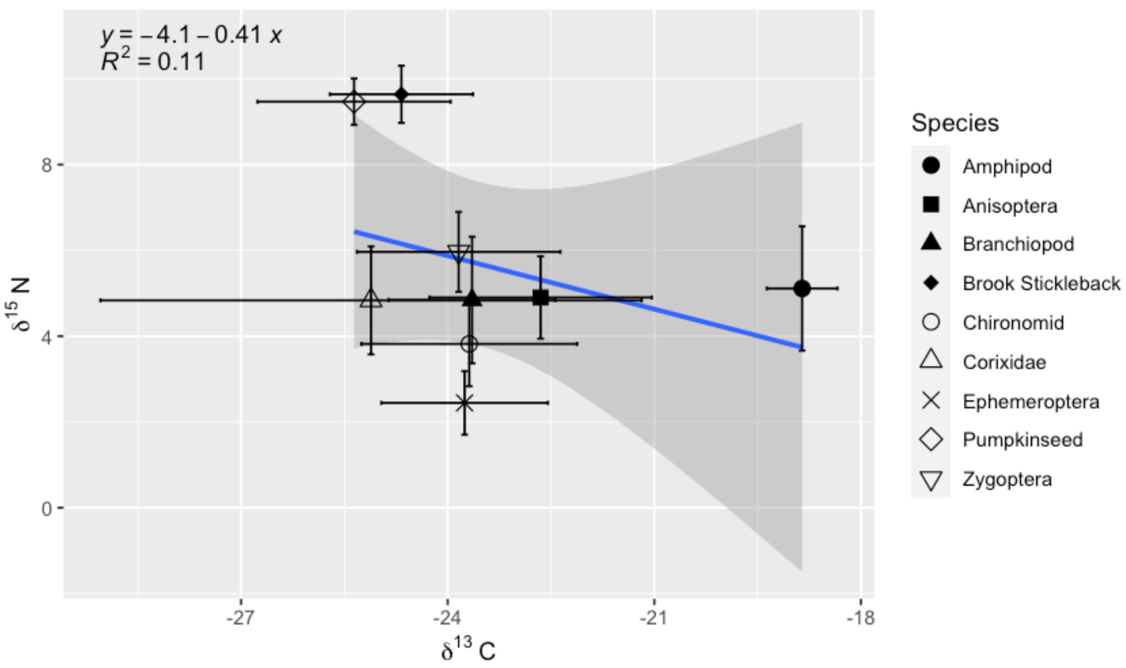

Figure 6. Mean isotopic signatures of invertebrates in coinvaded ponds (Long Lake, McDowell, Middle Pine) with standard deviation.

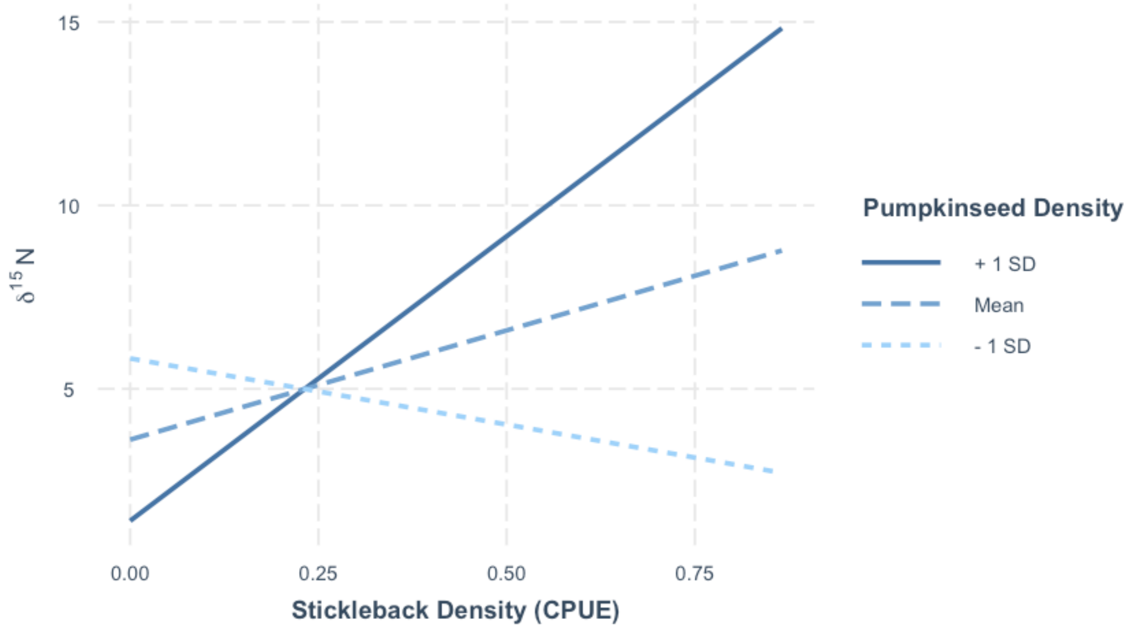

Figure 7. Interaction plot showing the effect of stickleback abundance (CPUE) on mean invertebrate δ^{15}N values at three levels of pumpkinseed abundance (CPUE): mean CPUE, and +1 and -1 standard deviation from the mean. As stickleback abundance increases, the effect of pumpkinseed abundance on invertebrate δ^{15}N values strengthens, causing δ^{15}N values to increase at higher abundance of pumpkinseed.

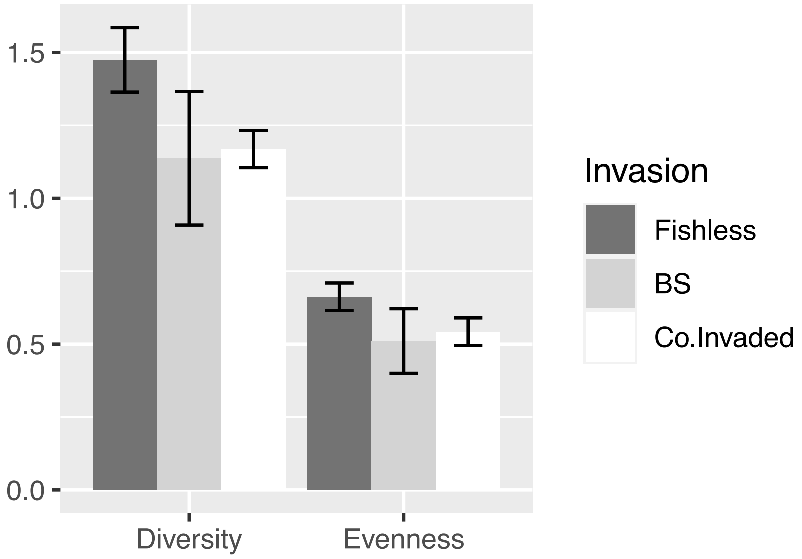

Figure 8. Mean zooplankton Diversity and Evenness for Fishless, BS, and coinvaded ponds. The presence of stickleback was the main factor causing zooplankton diversity (*P* = 0.0471) and evenness (*P* = 0.0269) to decline.

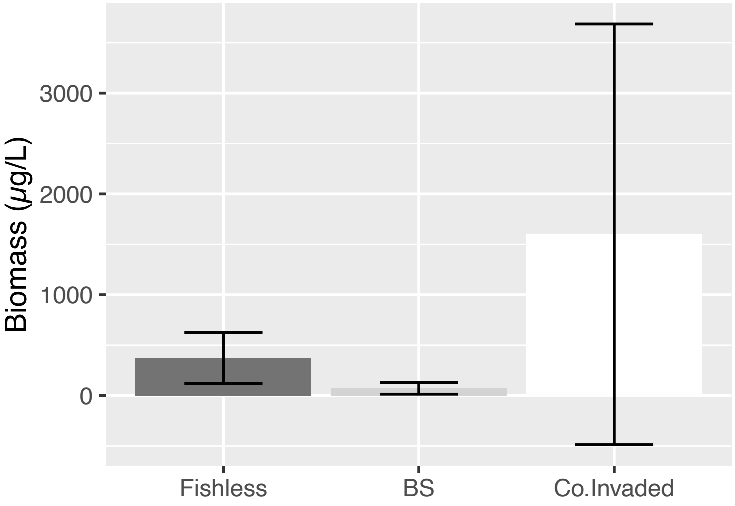

Figure 9. Mean zooplankton biomass (µg/L) and standard error for each pond type: Fishless, BS, and Coinvaded.

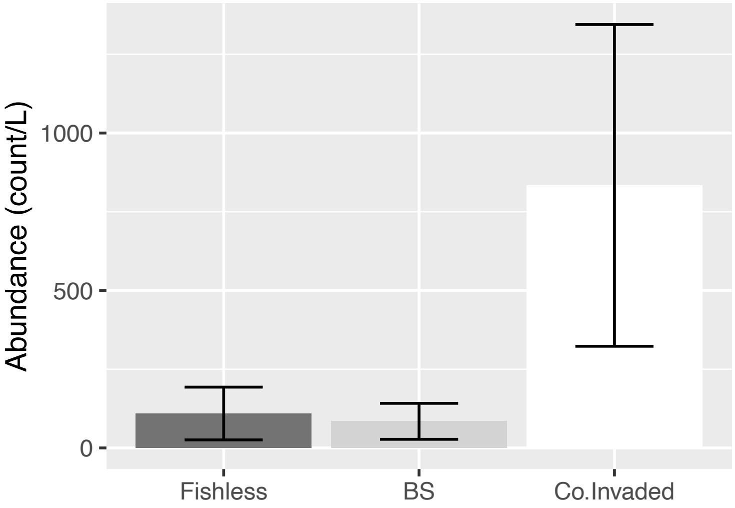

Figure 10. Mean zooplankton abundance (count/L) and standard error for each pond type: Fishless, BS and Coinvaded.

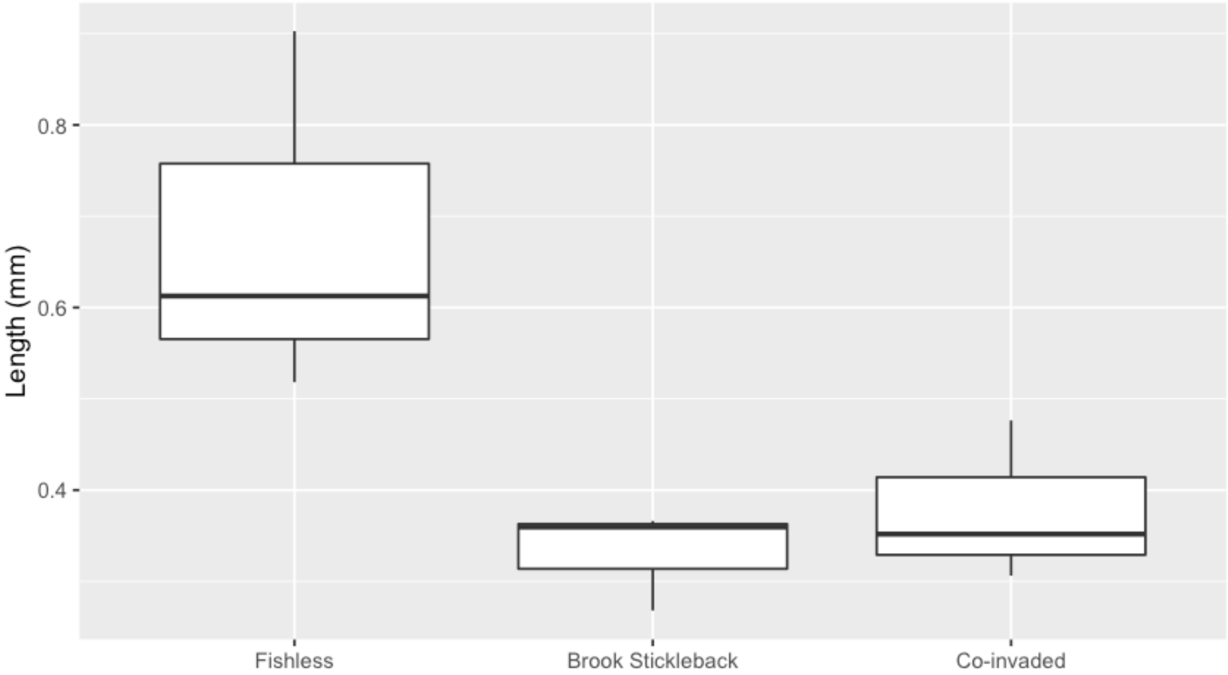

Figure 11. Mean zooplankton length (mm) and standard error for each pond type: Fishless, Brook Stickleback, and Coinvaded.

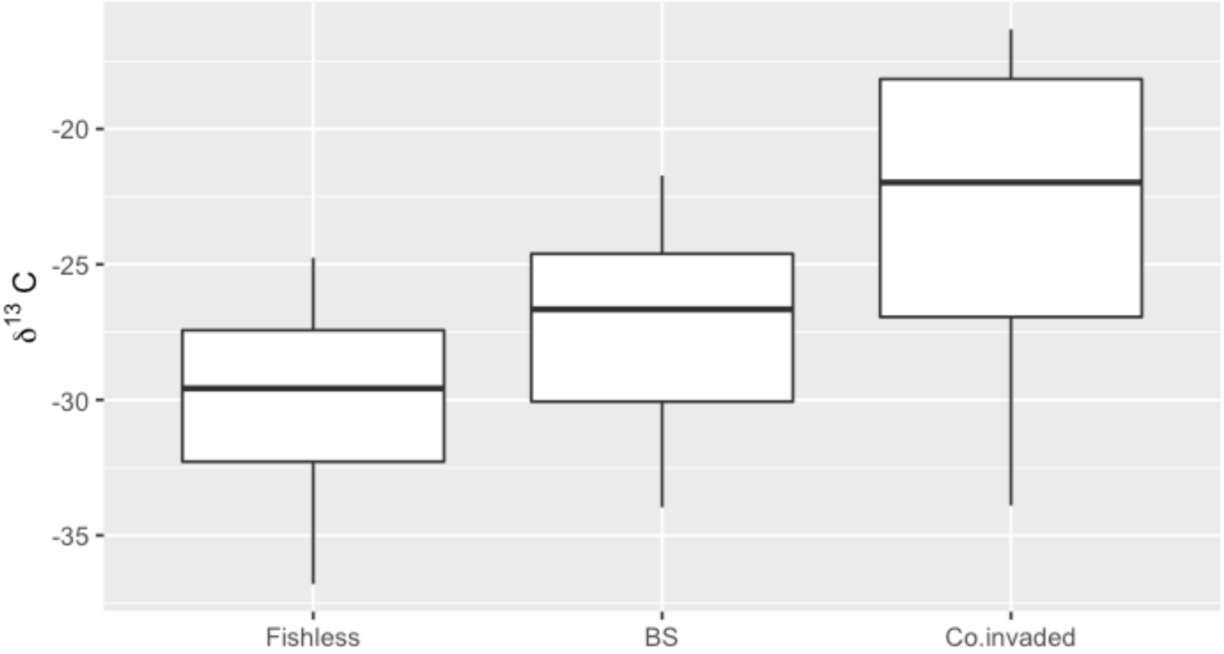

Figure 12. Mean invertebrate δ^{13}C values and standard error of each pond type: Fishless, BS, and Coinvaded.

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EDUCATION

M.S. Biology, Eastern Washington University — March 2022
Cheney, Washington
Advisors: Dr. A. Ross Black and Dr. Paul Spruell

B.S. Biology, Western Kentucky University — December 2011
Bowling Green, Kentucky

AWARDS

2019-2021 Graduate Service Appointment, Eastern Washington University, Biology Department

RELEVANT EXPERIENCE

Fisheries Technician III — June 2021 – October 2021
Kalispel Tribe of Indians

- Provided in-field leadership for the Eastern Brook Trout suppression project on Sullivan Creek. This project was designed to suppress non-native trout populations well as provide comprehensive biological data such as catch, effort, mortality, species composition, life history, abundance, distribution, movement, age, and growth to inform fishery management decisions for the Kalispel Natural Resource Department and Seattle City Lights.
- Independently coordinated work efforts with other technicians and biologists involved in the project. Led a small crew of Technicians II to complete daily tasks as assigned by head biologists.
- Captured native and non-native trout with backpack electrofishing and collected appropriate biological data. Ensured data entry and performed quality control of collected data.

Teaching Assistant — September 2019 – June 2021
Eastern Washington University

- Weekly duties included grading, prepping laboratories for class, proctoring exams and quizzes, and leading hour-long discussion classes.
- Assisted students with the compilation and refinement of biological data from

Saltwater Inc — May 2014 – August 2019
North Pacific Groundfish Observer Program

- Collected biological data for the National Marine Fisheries Service (NMFS) for in-season fishery management and establishment of sustainable fishing quotas for future seasons.
- Watched for compliance to fishing regulations by vessel crew and documented violations.
- Collaborated with vessel crew to obtain and independently verify all data on fishing activities such as dates, times, and GPS coordinates on the set and retrieval of every haul, and estimated catch and discard sizes.

- Incorporated effective sampling methods according to NMFS' sampling protocols to collect biological data on the species composition of every haul.

Oregon Department of Fish and Wildlife October 2018 - January 2019
Experimental Biology Aide

- Conduct spawning ground surveys and creel surveys for Elk River Hatchery on the Elk River to determine population estimates on both wild and hatchery raised chinook salmon within the river.
- Collect biological samples such as snouts and scales for genetic testing and keep accurate written records on all samples from both creel and spawning ground surveys.
- Navigate the river for spawning ground surveys by drift boat and swimming during high water winter months.

Oregon Department of Fish and Wildlife June - September 2018
Aquatic Invasive Species Program

- Inspected watercraft entering the state of Oregon for aquatic invasive species, particularly for zebra and quagga mussels, by following established protocols for inspection and decontamination of affected watercraft.
- Collected data by interviewing the watercraft owner of each inspected vessel to help determine history and subsequent risk each individual watercraft poses on Oregon waters.
- Occasionally coordinated with Oregon State Police, Oregon State Marine Board, county law enforcement, and other department staff to isolate any watercraft contaminated with quagga or zebra mussels or other aquatic invasive species.

Long Live the Kings April - May 2016
Glenwood Springs Hatchery

- Prepared salmon for release by clipping off the adipose fin.
- Anesthetized fish to be tagged and clipped using a MS-222 solution.
- Operated equipment to insert coded wire tags into the snouts of the fish.

Gulf Coast Research Laboratory May - August 2011
Shark Lab Internship under Jill Hendon

- Tagged and released various species of elasmobranchs caught off the coast of Mississippi on small longline research vessel.
- Collected vertebrae, blood, and fin clips as well as measured and recorded lengths.
- Data entry, cleaning and maintaining research equipment such as acoustic telemetry receivers.
- Assisted with shark biology course by setting up stations within the lab before classes began and by helping students with longline equipment during class field trips.