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## **Invasive fish effects on the invertebrate constituents of wetland communities**

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# **Invasive Fish Effects on the Invertebrate Constituents of Wetland Communities**

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A Thesis  
Presented To  
Eastern Washington University  
Cheney, Washington

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In Partial Fulfillment of the Requirements  
for the Degree of  
Master of Science in Biology

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By  
Levi Bridges  
2011

Thesis of Levi W. Bridges approved by

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Advisor, Graduate Study Committee

Date

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Member, Graduate Study Committee

Date

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Member, Graduate Study Committee

Date

## Master's Thesis

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## Abstract

The purpose of this research was to assess the impacts of invasive fishes on wetland communities. Within this study we explored fish effects on the invertebrate constituents of these wetlands, and possible niche overlap of fish and dabbling waterfowl using stable isotope analysis. Brook stickleback (*Culaea inconstans*) and pumpkinseed (*Lipomis gibbosus*) pose a potentially growing threat to temperate Pacific drainages of North America due to their ability to outcompete native fauna. Our study compares and contrasts the invertebrate constituents, their diversity, abundances, and stable isotope ecology among fish invaded and non-invaded wetlands. We found that brook stickleback and pumpkinseed are having limited effects on the invertebrate communities of these wetlands, however stickleback appear to have a greater effect on these communities than pumpkinseed. When comparing stickleback and pumpkinseed ponds collectively against fish free ponds we find only three invertebrate taxa are significantly affected, branchiopods, coenagrionids, and baetid mayflies. However when we compare the presence of stickleback versus no stickleback we find several differences among taxa abundance and evenness. Evenness among invertebrate communities within stickleback and no stickleback ponds was significantly different while diversity and richness were comparable among ponds. Fish free ponds exhibited higher evenness values ( $E=0.6$ ) among taxa than stickleback invaded ponds ( $E=0.4$ ). Among the 20 most common littoral taxa, bivalves, chironomids, *Chaoborus*, oligochaetes, coenagrionids, and baetid mayflies were found to significantly vary in abundance. Our stable isotope analysis found evidence to suggest potential niche overlap between waterfowl and stickleback; assessment of  $\delta^{13}\text{C}$  ratios between stickleback, sampled duck feathers, and duck blood were significantly different indicating different dietary sources. Analysis of  $\delta^{15}\text{N}$  between stickleback and waterfowl feather samples show that these two species occupy similar trophic levels; however p-values suggest these tissues are not highly alike. When comparing  $\delta^{15}\text{N}$  among stickleback and duck blood samples we see these two species occupy significantly different trophic levels. However, sticklebacks appear to be generalists in their diets, showing evidence of both pelagic (depleted  $\delta^{13}\text{C}$ ) and littoral (enriched  $\delta^{13}\text{C}$ ) carbon sources. This generalist diet allows for some degree of potential overlap and possible competition effects with waterfowl. Pumpkinseeds show potential niche overlap with waterfowl as they possess similar carbon resource signatures. Pumpkinseed tissues compared to duck feather and blood samples were not found to vary significantly for  $\delta^{13}\text{C}$ . Duck blood samples for  $\delta^{15}\text{N}$  proved to be significantly different from pumpkinseed tissues. Nitrogen analysis between waterfowl feathers and pumpkinseed show no significant differences, indicating that waterfowl and pumpkinseed share similar trophic levels as well as diets and the potential for competition exists.

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## Introduction

This study aimed to investigate some of the potential impacts of brook stickleback (*Culaea inconstans*) and pumpkinseed (*Lepomis gibbosus*) on the invertebrate and waterfowl constituents of wetland communities. Fish free wetland communities provide excellent habitat and refuge for migratory waterfowl as food resources are abundant while competitors for these resources are lacking. Dabbling waterfowl require diverse and abundant invertebrate prey communities to reduce foraging effort while nesting, promote rapid growth of young, and provide a diet that supports a metabolically expensive migration (McNally 2004, Jonathan et al. 2005, Thongwittaya 2007).

Among lakes perhaps the single greatest driving force on aquatic invertebrate assemblages is the presence or absence of fishes (O'Brien 1987, Lazzaro et al. 2009, Schulze 2011). Brook stickleback are a small bodied (5-9 cm) predaceous spiny fish that inhabit shallow, cool, vegetated, small ponds and backwaters (Stewart et al. 2007). The brook stickleback is a successful fish with a native distribution throughout most of the upper latitudes of the United States including the Great Lakes and their tributaries as well as extending up into Canada (Rachel 2007). Historically these fish have only been found in Atlantic drainages; currently the Rock Creek and TNWR invasion are one of five invasions west of the Continental divide (Scholz et al. 2003). They feed on many aquatic invertebrates, predominantly oligochaetes and branchiopods (Jonathan and Lee Foote 2005). Size of prey items consumed by fishes is often dependent on the gape size or maximum size of mouth opening. Stickleback are large size selective, gape limited (< 2mm), visual predators; meaning they are visually dependent with large eyes that tend to seek out larger size prey more readily and will eat the largest prey size that can be fit into their mouths (Wootton 1976). Food fighting, where multiple fish tear apart and

consume a single prey item, allows *C. inconstans* to consume larger size prey than those that could typically be fit within their gape (Reisman and Cade 1967). They can live up to three years, are highly resilient, and have a population doubling time estimated at less than fifteen months (Magnuson et al. 1985). Brook stickleback have the potential to severely alter small pond macro-invertebrate assemblages, reducing available prey abundances and diversity (Tompkins and Gee 1983).

Pumpkinseeds are also gape limited (< 10mm), and like stickleback we expected them to consume and target the largest prey items that can be consumed given their gape limitation. Pumpkinseeds eat primarily aquatic insects located in the benthic regions of the water body. The difference in gape sizes between *L. gibbosus* and *C. inconstans* allow pumpkinseed to consume larger prey sizes and may reduce competition between these fishes. Pumpkinseeds are also known to eat fish eggs, small vertebrates, as well as invertebrates off submerged vegetation (Declerck et al 2002). They, unlike brook stickleback, tend not to be heavy planktivores except during juvenile stages, where they tend to feed mostly in the littoral regions (Keiffer and Colgan 1991). Pumpkinseeds are highly invasive to the Pacific Northwest, and like the brook stickleback originate east of the continental divide (Wydoski and Whitney 2003). Pumpkinseed are listed as being among the top 10 most damaging invasive fish species to native fauna, such as other fishes, predatory macro-invertebrates, and higher order organisms like waterfowl (Casal 2005). Studies show that invasive fishes can alter pond organism trophic relationships in three ways: 1) their presence may significantly decrease the amount of prey available to native species; 2) there may be niche overlap between native and invasive species; and 3) they may disrupt the behavior and dynamics of native prey adapted to native predators (Benigno 2001). Small bodied predatory fish like the brook stickleback and pumpkinseed typically inhabit eutropic, hypoxia-

prone small ponds and potholes where invertebrates are abundant (McParland and Paszkowski 2006).

A typical visual predator effect on wetland constituents is the elimination or significant reduction of large-sized (body length) zooplankton. For example, in a study by Brooks and Dodson 1965, mean zooplankton body lengths were significantly reduced by the presence of a visual predator, shad (*Alosa spp.*). Planktivory by the alewife completely rearranged plankton and invertebrate communities to only include very small size individuals and species following their introduction. Sunfish have a similar effect on their macro-invertebrate prey, for example, blue gill (*Lepomis macrochirus*) have been shown to cause shifts in the size structure of larval odonate populations. Mean head width, a common measure of body size for odonates, was 1.3mm in the absence of fish; while in the presence of blue gill odonate mean head width was reduced to 0.8mm with the complete loss of all sizes larger than 1.5mm (Butler 1989).

Elimination of larger prey types can cause increased competition among species such as waterfowl (Jonathan and Lee Foote 2005). Many studies have demonstrated the predatory effects of fishes upon zooplankton and macro-invertebrate communities in freshwater systems; as these invertebrates serve as an important link between basal energy resources and higher order consumers, and can serve as indicators for system health and function (Heatherly et al. 2005). The presence of planktivorous or predatory fishes have been shown to alter the diversity, abundances, and distributions of many zooplankton and invertebrate species among water bodies (Grosholz and Gallo 2006, Wissenger et al. 2006, Beisner and Peres-Neto 2009).

Stable isotopes are often used to provide insight on the trophic position, spatial foraging habitat, and dietary sources of a given organism under observation (Minagawa and Walda 1984, France 1995). Literature shows that increases in  $\delta^{15}\text{N}$  values indicate an organism feeding from

higher trophic levels providing some insight to the trophic position or place in the ecosystem food web. These values look at the direction and flow of energy within an ecosystem. Depleted  $\delta^{13}\text{C}$  (more negative values) ratios are used to determine general diet and the spatial source of this diet within a given ecosystem, for aquatic systems this is typically pelagic or littoral sources. In freshwater systems pelagic algae show less  $\delta^{13}\text{C}$  fractionation during carbon fixation than those of benthic algae and terrestrial sources. These primary producers' different fixation rates can then be followed through the food chain and up through the trophic levels as depleted  $\delta^{13}\text{C}$  ratios stay relatively fixed throughout the food web; and can be used to indicate the source of species diet (Fry 2006).

Stable isotope analysis of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  can be used to determine links among species of a given community or system, identify potential niches, as well as niche dimensions, and niche overlap among these species (Newsome et al. 2007, Flaherty 2010). The ratios of heavy and light naturally existing isotopes are used to examine species specific diet and flow of energy through the food web (Fry 2006). Stable isotope analysis is especially practical for water drainages and wetlands (DeNiro and Epstein 1978, DeNiro and Epstein 1981). In this study stable isotope analysis was used to examine the potential for niche overlap between the invasive fish species brook stickleback, pumpkinseed, and dabbling waterfowl at TNWR.

This project aims to confirm fish presence (+/-) in sampled wetlands as well as test the following five hypotheses: 1) there will be no fish effect on the abundance, richness, diversity, and evenness of aquatic invertebrates among fish-present and fish-absent wetlands; 2) there will be no fish effect on invertebrate mean body lengths among fish-present ponds and absent wetlands; 3) there will be no differences in  $\delta^{13}\text{C}$  among fishes and waterfowl; 4) no differences in  $\delta^{15}\text{N}$  among fishes and waterfowl; and 5) no differences among  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between

waterfowl inhabiting fish-present and absent wetlands.

As shown in other studies involving the presence or absence of insectivorous and planktivorous fishes we expected that the general abundances, diversity, richness, and evenness, of macro-invertebrate and zooplankton communities will be significantly lower in fish-present ponds than fish-absent ponds (Wootton 1976, Tompkins and Gee 1983, Thorp 1988, Stewart et al. 2007). Based on the research of Brooks and Dodson 1965 and Butler 1989, as well as other studies regarding prey size reductions, we expected that brook stickleback and pumpkinseed will selectively feed on larger sizes and species of invertebrates, and thus expected to see significant size differences among prey items between fish-present and absent ponds. We also predicted that stable isotope analysis will show similar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values among fishes and waterfowl, as we expected they are dependent on similar resources for their diet as shown in previous studies (Jonathan and LeeFoote 2005, McParland and Paszkowski 2006). Following that logic we expected waterfowl to have slightly varying  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios between fish-present and absent ponds as a result of increased foraging effort and reduction of potential prey items causing potential shifts in dominant dietary sources.

## **Methods**

*Site Description:* Turnbull National Wildlife Refuge is found on the eastern edge of the Columbia Basin in Spokane County, Washington. The refuge inhabits the channeled scablands and wetlands that are a result of glacial retreat in this area, and was founded initially to provide and promote breeding, nesting, and brooding sites for many migratory bird species, most specifically migrating waterfowl. Mallard (*Anas platyrhynchos*), pintail (*A. acuta*), green and blue wing teal (*A. carolinensis* and *A. discors* respectively), cinnamon teal (*A. cyanoptera*),

gadwall (*A. strepera*), American widgeon (*A. americana*), canvas back (*Aythya valisineria*), red head (*Aythya americana*), ring necks (*Aythya collaris*), wood ducks (*Aix sponsa*), golden eye (*Bucephala clangula*), bufflehead (*Bucephala albeola*), and ruddy ducks (*Oxyura jamaicensis*), all inhabit TNWR seasonally. The 3,036 acres of wetlands at TNWR represent some of the last quality breeding habitat available in eastern Washington for waterfowl. Waterfowl have experienced population declines due to loss and degradation of North American wetlands (Duncan et al. 1999). The introduction of invasive fish species among many North American lakes has often led to severe negative impacts on invertebrate ecology and thus displacement of native fauna through competitive exclusion.

Historically, largemouth bass (*Micropterus salmoides*), brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), blue gill (*Lepomis macrochirus*), pumpkinseed (*Lepomis gibbosus*), brown bullhead (*Ameiurus nebulosus*), and brook stickleback (*Culaea inconstans*) were introduced into the surrounding lakes of TNWR and were at one point documented within the wetlands of the refuge itself (M. Rule, personal communication, April 29<sup>th</sup>, 2010). Due to unsuitable habitat most of these fish are no longer present. Today only pumpkinseed, brown bullhead, speckled dace (*Rhinichthys osculus*), redbelt shiner (*Richardsonius balteatus*), and brook stickleback can be found in several of the refuge wetlands. Among these, brook stickleback and pumpkinseed are the most abundant and voracious predators most likely to alter the invertebrate communities and ecology of these wetlands.

*Study Design:* Turnbull is comprised of approximately 130 different wetlands and vernal ponds. Most of these are connected in a series of multiple drainages, i.e. Pine Creek, Rock Creek, etc., making them susceptible to invasion. Of these, some of the major water bodies include Long Lake, West Tritt, Cambell-Lasher, Upper Turnbull Slough, Black Horse Lake, and its multiple

ponds including Swan Pond (Figure 1). These sites serve as the focus of this research as three of these wetlands were fish free (Long Lake, West Tritt, Cambell-Lasher) and three were stickleback or pumpkinseed inhabited. This changed over the course of the study as pumpkinseed invaded W. Tritt, previously a fish free wetland, during the spring of 2010. All sites are perennial wetlands and easily accessible via routine maintenance roads. These sites are typical of temperate semi-arid coulee wetlands in this area. Littoral vegetation includes bulrush (*Scirpus sp.*), reed canary (*Phalaris arundinacea*), and common mixed grasses; the surrounding uplands include ponderosa pine (*Pinus ponderosa*), savannah, and shrub-steppe communities. This area provides refuge for many local resident birds, as well as migratory birds, many local ungulates, and small mammals.

*Fish Abundance:* To better inform local wetland managers we attempted to quantify and estimate general fish abundance in inhabited waters. This allowed some inference to the level of impact by the abundance of these invasive fish species. We also surveyed non-inhabited sites to be sure they remained uninhabited by fish. While collecting fish samples for SIA using wire mesh minnow traps, we determined relative *C. inconstans* and *L. gibbosus* abundances by quantifying the number of fish/trap/24hrs period at systematically chosen littoral regions of each inhabited wetland. This was done during the spring of 2010, after the high water season. Five traps were set at each site and used to determine average fish/trap/unit effort.

*Fresh Water Invertebrate Abundance and Diversity:* At each of the previously mentioned sites, both inhabited and uninhabited by fishes, general aquatic invertebrate diversity and abundance both in the littoral and pelagic regions of the wetland were sampled and compared to determine fish effects on the invertebrate ecology of these wetlands. Diversity, richness, and evenness indices were calculated as per Shannon-Weaver (1948). We sampled macro-invertebrates from

each site/lake, identified them and measured total body length for most taxa; we examined nearly 1,250 macro-invertebrates per lake (Table 2). From these we calculated the total proportion (%) of each taxon by abundance to identify common and dominant taxa for these wetlands.

For littoral abundance and diversity, a stovepipe sampling method was chosen, in which a container of known volume is used to define a standardized volume or column of water from which to sample. Five sample sites from each water body were systematically selected by estimating the total circumference of the water body and dividing this value by five. The container or stovepipe is placed haphazardly along the littoral edge and pushed firmly into the substrate. The depth of water within the stovepipe was measured and a thorough back and forth sweep of the contained volume was made using a dip net, paying special attention to avoid digging into the substrate, so as to only sample water and vegetation above the sediment. Samples were then diluted through three water buckets to remove vegetation and knock off clinging invertebrates. The buckets containing the sample were then run through a 153 $\mu$ m sieve and samples were stored in 70% ethanol. Stovepipe samples were collected during the spring and summer of 2009.

Conical, 253 $\mu$ m, tow nets were used to capture zooplankton samples, we then identified, and quantified zooplankton and copepod abundance and size. These species are a major indicator of water quality and are a predominant constituent of *C. inconstans* and *L. gibbosus* diet. Zooplankton samples were run through a 300 $\mu$ m sieve and dipped in a 95% ethanol kill solution before being stored in 70% ethanol for similar analysis (Black and Dodson 2003). Samples were subdivided using a Folsom plankton splitter. Samples were taken in July of 2011; for pelagic zone sampling we included the addition of two more fish free ponds found within the refuge, Turnbull Lab Pond (TLP) and Eagle Pond (E.P.). The addition of these ponds allowed us to



compare 4 fish free and 4 fish inhabited ponds, for these sampling efforts we were testing only against the presence or absence of fish rather than the specific presence of stickleback versus no stickleback.

Invertebrates were counted and identified to lowest possible taxonomic level. This allowed us to quantifiably compare the diversity, abundance ( $\#/m^2$  for littoral samples and  $\#/L$  for pelagic zooplankton samples), size, and assemblage composition of invertebrates in fish-present and non-present waters.

*Niche Comparison:* We applied  $\delta^{15}N$  and  $\delta^{13}C$  stable isotope analysis (SIA) to our study to provide more information regarding the diets of brook stickleback and pumpkinseed, as well as their trophic position and their potential for niche overlap with waterfowl. It was predicted that there would be evidence of dietary niche overlap between brooding waterfowl, brook stickleback, and pumpkinseed for similar dietary resources (freshwater macro-invertebrates) within similar water bodies.

Samples for SIA were taken from fish inhabited water sources (Blackhorse Pond, Swan Pond, and Upper Turnbull) and non-inhabited sites (West Tritt, Cambell-Lasher, and Long Lake), to allow for direct comparison of diet and energy flow among stickleback, pumpkinseed, and waterfowl. Samples of littoral wetland invertebrates were also taken to give reference to source of diet among fishes and waterfowl.

Waterfowl samples were collected via littoral trapping and checked daily (M-F) over the brooding seasons. Upon capture, duck species were identified, sexed, aged, banded, and recorded as instructed by refuge personnel of U.S. Fish and Wildlife Services. Approximately 25 feather samples were collected ranging from all six sites, while only 12 blood samples were taken. Only first hatch year (FHY) dabbling (Anatinae) waterfowl were used for sampling purposes, these

waterfowl were crucial for our experiment because their diet consists primarily of freshwater macro-invertebrates and these birds are still flight restricted to a single water source, as primary flight feathers have not fully fledged (Szymanski et al. 2007). If chosen for sampling, five haphazardly selected breast feathers and one milliliter of blood obtained from the jugular vein were collected from each individual. Proper procedure for waterfowl blood sampling was instructed and approved by refuge personnel and the Institutional Animal Care and Use Committee (IACUC). Samples were then frozen and stored for SIA. Prior to being sent for SIA feather samples were rinsed in a 2:1 chloroform:methanol solution to rid feathers of natural oils which may contaminate results (Yohannes et al. 2005).

Freshwater littoral macro-invertebrates and zooplankton were captured using a combination of littoral dip netting and zooplankton net halves. The littoral grazers [mayflies (Ephemeroptera), caddisflies (Trichoptera), scuds (Amphipoda), and branchiopods] were collected from each pond because they are common constituents of dabbling waterfowl, pumpkinseed, and brook stickleback diet (McParland and Paszkowski 2007). A minimum of 20 µg of dried and homogenized sample were required for SIA (UC-Davis SIF 2008). Multiple samples were collected over five randomly selected sites per wetland for SIA. Samples were rinsed and stored in de-ionized water and frozen for storage prior to analysis.

Both *C. inconstans* and *L. gibbosus* were caught using wire mesh minnow traps in the littoral zones of the three chosen fish inhabited wetlands. A minimum of 5 stickleback and pumpkinseed tissue samples from each inhabited lake were collected for analysis. Upon capture, samples were processed immediately as freezing/thawing whole samples will contaminate tissues. Tissue samples were prepared for SIA by removing a small piece of muscle tissue (500-800 µg); the maximum size that can be processed is 75 mg (UC-Davis SIF 2008). It is important

to only remove muscle tissue and not cut into the digestive tract and contaminate the sample; this is most easily done by filleting the fish. Once tissues were removed they were rinsed and stored in de-ionized water and frozen for storage prior to analysis.

All materials were rinsed with de-ionized water during processing to reduce any possible error or contamination. Samples were then dehydrated at 60°C for 48 hours and homogenized. Samples were sent to Dr. Raymond Lee of Washington State University, School of Biological Sciences at Pullman, WA. and were analyzed on a Europa 20/20 mass spectrometer.

*Statistical Analysis:* Student's *t*-test comparison and Kolmogorov-Smirnov test were used to identify significant stickleback and pumpkinseed effects on the abundances, diversity, richness, evenness, and size distributions of aquatic macro-invertebrates. We ran analysis of two treatment types, fish (n=3) versus no fish (n=3), and then stickleback (n=2) versus no stickleback (n=4) and selected stickleback versus no stickleback comparisons for data presentation. This was in part due to the relative similarity between pumpkinseed and fish free ponds, the more pronounced and dramatic effect of stickleback on the invertebrate ecology of these wetlands, as well as due to the fact that our original treatment of 3 fish and 3 non fish wetlands changed over the course of the study with pumpkinseed coming to inhabit W. Tritt. We used a Wilcoxon Rank-Sum Test to compare duck versus stickleback, duck versus pumpkinseed, and fish free duck versus fish inhabited duck samples for similar basal carbon sources and trophic position using SIA. Systat software was used for statistical analysis.

## **Results**

*Fish Abundance:* Table 1 shows the distribution and relative abundance (fish/trap/unit effort) of *C. inconstans* and *L. gibbosus* across our study sites. Black Horse and Swan Pond are our only

stickleback ponds and the densities of fish in these two ponds are hugely different. Swan Pond has the highest density of stickleback with a total of 303fish/trap/24hrs, while black horse yielded only 19 fish/trap/24hrs. Relative density of pumpkinseed was consistent across study sites, 21fish/trap/24hrs. for W. Tritt and 36fish/trap/24hrs. at Upper Turnbull. These results are troubling as pumpkinseed have only recently invaded W. Tritt, a once fish free pond, and already appear to occupy this water body at similar densities to long time occupied Upper Turnbull.

*Littoral Invertebrate Abundances:* The proportions of invertebrate taxa varied between fish and no fish ponds. However, these values and their representative abundances varied to a greater degree among stickleback and no stickleback ponds. When we removed the initial pumpkinseed only pond (Upper Turnbull) and assessed only stickleback present (n=2) versus absent (n=4) ponds we found a greater effect on the littoral invertebrate abundances and evenness of species composition among stickleback inhabited wetlands. Littoral invertebrate abundances are presented for stickleback effects only rather than fish versus no fish effect.

Over all sampling locations, we found the following invertebrates represent the dominant taxa in respective order: chironomids, oligochaetes, amphipods, branchiopods, coenagrionids, caenids, ostracods, copepods, *Chaoborus*, baetid mayflies, and bivalves (see Table 3 for actual proportions). Other invertebrate taxa were observed but their presence was limited to less than 1% of the total abundances observed and thus left out of further analysis, these are shown on Table 3 as having 0.0 proportion values.

For all littoral stovepipe samples we separated our analysis into two size groups, individuals <20mm in total body length and those >20mm. This was done to separate likely potential prey items for *C. inconstans* and *L. gibbosus*, as well as to examine specific effects of smaller larval predation by fishes on larger adult classes of odonates. Anisopterans and

zygopterans were the only taxa observed in the >20mm category that represent potential prey items, all other taxa compared were from the <20mm group. Among stickleback present and absent ponds invertebrates >20mm did not significantly vary in abundance with  $p=0.62$  for anisopterans and  $p=0.07$  for zygopterans (Figure 5A & B). Zygopteran abundances were nearly significant in their variation between pond types, showing almost no presence in stickleback inhabited wetlands (Figure 5B).

We observed only two differences between the abundances of dominant littoral taxa in fish-present and fish-absent ponds; coenagrionids ( $p=0.01$ ) and baetid mayflies ( $p=0.03$ ), both showed a decreased abundance among fish inhabited ponds. Among the smaller invertebrates, oligochaetes ( $p=0.01$ ), chironomids ( $p=0.03$ ), and bivalves ( $p=0.01$ ) are all significantly higher in abundance among stickleback inhabited wetlands than their non-inhabited counterparts (Figure 2A-C). Chironomids in particular showed nearly a fivefold increase in their abundance among stickleback ponds as compared to other non inhabited sites (Figure 2B).

Stickleback had a limited effect on larger littoral macro-invertebrates; only coenagrionids ( $p=0.01$ ) and baetid mayflies ( $p=0.01$ ) were significantly less in abundance, while caenid mayflies ( $p=0.72$ ) and amphipod ( $p=0.22$ ) abundances appear similar in both stickleback present and absent ponds (Figure 4A-D). Both coenagrionid and baetid mayfly abundances were reduced to nearly zero individuals per square meter among stickleback ponds, suggesting they may be a preferred prey item of stickleback (Figure 4B & D).

*Zooplankton Abundances:* Among littoral zooplankton samples only *Chaoborus* midge abundances ( $p=0.04$ ) appear to be significantly decreased among stickleback ponds, while copepods ( $p=0.18$ ), ostracods ( $p=0.51$ ) and branchiopods ( $p=0.57$ ) showed no stickleback effect (Figure 3A-D). For pelagic zooplankton analysis we included the addition of two fish free

ponds, TLP and E.P., to strengthen our test results and provide more insight into zooplankton and fish effects. When comparing a fish (n=4) versus no fish (n=4) effect on pelagic zooplankton we saw no significant differences in branchiopod abundances, however there exists a trend towards reduced abundance with a nearly significant  $p=0.12$  among fish-present and absent ponds (Figure 6A). Branchiopods were nearly reduced to zero individuals per liter among fish inhabited sites while among fish free sites they averaged between 8-16 individuals per liter. Copepods also showed no significant difference ( $p=0.50$ ) among pelagic zone samples from either fish or fish free ponds and showed nearly equal abundances between pond types (Figure 6B).

*Invertebrate Size Distributions:* Our comparison of the average sizes of dominant taxa between stickleback present and absent ponds suggested that among stickleback inhabited wetlands only branchiopods were significantly reduced ( $p=0.04$ ) in mean body length from an average of approximately 1.3mm down to 0.8mm in stickleback ponds (Figure 7). Other taxa measured included chironomids ( $p=0.20$ ), caenids ( $p=0.27$ ), hyallelids ( $p=0.39$ ), and copepods ( $p=0.70$ ), whose mean body lengths were not found to significantly vary between pond types. However there exists a trend among all measured taxa that suggests a potential for reduction in total mean body length within stickleback inhabited wetlands (Figure 7).

*Shannon-Weaver Diversity Indices:* Diversity, richness, and evenness measurements of littoral invertebrate communities were taken among stickleback versus no stickleback ponds. Diversity and richness measurements yielded no significant differences between stickleback present and absent ponds,  $p=0.08$  for both measurements (Figure 8). However when we compare evenness among littoral invertebrate assemblage composition we found a significant difference between pond types ( $p=0.01$ ), showing a higher evenness value  $E=0.6$  among stickleback free wetlands

than stickleback inhabited wetlands  $E=0.4$  (Figure 8).

*Niche Comparison:* Figure 9 shows stickleback, pumpkinseed, and waterfowl,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic signatures as well as signatures for a variety of the invertebrate constituents. This figure represents taxa from all sampled lake sites to compare stickleback, pumpkinseed, and waterfowl diets across the refuge. Among sampled invertebrates we saw two distinct carbon signature groups, those more depleted in  $\delta^{13}\text{C}$  ( $> -30\text{‰}$ ), like ephemeroptera and zygoptera, and those more enriched ( $< -30\text{‰}$ ), amphipods, coleoptera, trichoptera, and anisoptera. Pumpkinseed and waterfowl appeared to be consuming more enriched carbon sources (above), indicating a more specific littoral based diet; while stickleback appeared as more of a generalist in their diet, consuming prey from both pelagic (depleted  $\delta^{13}\text{C}$ ) and littoral invertebrates sources, as stickleback  $\delta^{13}\text{C}$  assessment shows stickleback between both invertebrate groups along the x-axis.

Assessment of  $\delta^{13}\text{C}$  ratios between stickleback ( $-28.99\text{‰}$ ,  $\text{SE}=0.50$ ), sampled duck feathers ( $-23.53\text{‰}$ ,  $\text{SE}=0.34$ ), and duck blood ( $-21.20\text{‰}$ ,  $\text{SE}=1.56$ ) were significantly different ( $p<0.01$  and  $p<0.01$ ) suggesting little direct niche overlap. Analysis of  $\delta^{15}\text{N}$  between stickleback ( $8.12\text{‰}$ ,  $\text{SE}=0.17$ ) and waterfowl feather samples ( $6.99\text{‰}$ ,  $\text{SE}=0.54$ ) showed that these two species occupy similar trophic levels ( $p=0.09$ ). However, when comparing  $\delta^{15}\text{N}$  among stickleback and duck blood samples p-values ( $p<0.01$ ) suggested these two species occupy significantly different trophic levels, with stickleback approximately one trophic level above ducks.

Pumpkinseeds show some potential to compete with waterfowl as they possess similar carbon ratios, suggesting a similar proportion of pelagic versus littoral carbon. Pumpkinseed tissues ( $-23.54\text{‰}$ ,  $\text{SE}=3.60$ ) compared to duck feather and blood samples were found to not

significantly vary for  $\delta^{13}\text{C}$ ,  $p=0.45$  and  $p=0.41$ , respectively. Duck blood samples for  $\delta^{15}\text{N}$  proved to be significantly different from pumpkinseed tissues ( $8.61\text{‰}$ ,  $\text{SE}=0.37$ ) ( $p=0.01$ ). Nitrogen analysis between waterfowl feathers and pumpkinseed showed no significant differences ( $p=0.17$ ), indicating that waterfowl and pumpkinseed share similar trophic levels as well as diets and the potential for niche overlap exists. The discrepancy of  $\delta^{15}\text{N}$  between duck blood and feather tissues may be the result of a more recent diet shift which is manifest in the blood but not yet incorporated into more permanent tissues like feathers.

Lastly we compared  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  among blood ( $n=12$ ) and feather ( $n=21$ ) samples from FHY waterfowl from fish inhabited versus non-inhabited sites. Blood samples showed no significant difference ( $p=0.88$ ) in  $\delta^{15}\text{N}$  between fish inhabited ( $5.38\text{‰}$ ,  $\text{SE}=0.53$ ) and non-inhabited ( $5.24\text{‰}$ ,  $\text{SE}=0.69$ ) resident waterfowl. Feather samples tested for  $\delta^{15}\text{N}$  also showed no significant difference in their isotopic ratios,  $6.66\text{‰} \pm 0.59$  for fish inhabited and  $7.83\text{‰} \pm 1.16$  for non inhabited sites respectively ( $p=0.27$ ). Blood samples tested for  $\delta^{13}\text{C}$  showed a marginal difference between carbon sources ( $p=0.053$ ), which suggests there is some difference between the specific diets of waterfowl foraging from fish inhabited ( $-18.66\text{‰}$ ,  $\text{SE}=1.50$ ) and non-inhabited ( $-25.43\text{‰}$ ,  $\text{SE}=0.91$ ) wetlands. Feather samples tested for  $\delta^{13}\text{C}$  suggested strong evidence for significantly different diets between waterfowl feeding from fish-present ( $-22.98\text{‰}$ ,  $\text{SE}=0.32$ ) and absent ( $-24.93\text{‰}$ ,  $\text{SE}=0.56$ ) wetlands ( $p<0.01$ ). Waterfowl inhabiting fish present ponds exhibited more littoral based carbon utilization, while those that occupied fish free wetlands showed a more pelagic based carbon signature, suggesting that when sympatric with invasive fishes, waterfowl shift to a more littoral diet.

Stable isotope analysis yielded several surprising results that differed from our original hypotheses. We expected to see similar dietary sources ( $\delta^{13}\text{C}$ ) among stickleback and waterfowl



when in fact we find that stickleback appear to consume prey items from both littoral and pelagic sources. Pumpkinseed and waterfowl appear to be more specific and similar, feeding mostly from littoral sources. We found that stickleback, pumpkinseed, and waterfowl all share similar trophic level, between 6.9 and 8.6‰  $\delta^{15}\text{N}$ , supporting our original hypothesis for  $\delta^{15}\text{N}$  despite lacking evidence of direct competition between stickleback and waterfowl. Among waterfowl tissues sampled for  $\delta^{13}\text{C}$  from fish-present and absent ponds we found support of our original hypothesis of different foraging strategies or diets among waterfowl as a result of fish presence.

## Discussion

The purpose of this research was to assess the level of impact *C. inconstans* and *L. gibbosus* are having on the abundances and structure of aquatic invertebrate communities as well as their potential for niche overlap with native fauna such as waterfowl. The diverse and abundant communities of aquatic invertebrates that reside in the absence of fish are highly important to maintaining migrating and brooding waterfowl (Bouffard and Hanson 1997). Competition as a result of resource limitation and predation is a major force driving the ecology of biotic communities, especially “microcosm” like lakes and wetlands where planktivorous fish are present (O’Brien et al. 1984, Forbes 1887).

Initial invertebrate abundance and diversity data suggested that pumpkinseed ponds more closely resembled fish free ponds. This was not expected as literature shows these fish to be efficient predators and highly planktivorous (Casal 2005). The apparent limited effects of pumpkinseed on these aquatic invertebrate communities is likely due to the dramatically larger size and volume of pumpkinseed inhabited wetlands as well as pumpkinseeds limited abundances as compared to stickleback abundances in much smaller wetlands. No significant differences were found in the abundances of dominant littoral taxa among pumpkinseed and no

fish ponds. Stickleback ponds however, differed significantly for several invertebrate taxa from fish free and pumpkinseed wetlands.

Among stickleback inhabited ponds oligochaete, chironomid, and bivalve abundances increased significantly as was expected based upon previous research which suggested potential increases among certain profundal fauna within fish-present water bodies (Figure 2B & C) (McParland and Paszkowski 2007). Chironomid abundances increased nearly fivefold over fish free ponds in the presence of stickleback. Midges are a common constituent of stickleback prey but have been shown to increase in abundance in the presence of insectivorous/planktivorous fishes; this is likely the result of relief from chironomid predators or competitors by stickleback predation (Batzner 1998, Batzner et al. 2000). Table 2 shows Swan Pond having the highest densities of chironomids and relatively low densities of other invertebrates, likely the cause of low evenness values among stickleback ponds as was presented in the results (Figure 8). Swan pond is also the same wetland with the highest density of stickleback shown in Table 1. Bivalve and oligochaete abundances also increased in the presence of stickleback (Figure 2A & C), supporting current literature and suggesting that some profundal organisms tend to flourish in the presence of stickleback. We were slightly surprised by the increase in abundances of oligochaetes despite literature supporting the potential increases among benthic organism abundances. Jonathan and Lee Foote (2005) suggested that oligochaetes are a dominant prey item among brook stickleback. Our study suggests this may not be the case when stickleback are presented with an abundance of other prey items, as oligochaete abundances are four times greater among stickleback present wetlands than those non-inhabited.

Infaunal organisms appear to increase in the presence of stickleback while many water column invertebrates are significantly reduced in their abundances. *Chaoborus* abundances were

reduced among stickleback ponds (Figure 3D). This was expected as many zooplankton species are a common dietary constituent of planktivorous fishes and are easy targets for predation by visually dependent predators (Brooks and Dodson 1965). *Chaoborus* midges are suspended in the water column and are heavily affected by water viscosity, thus subject to easy predation. Our study also showed a trend towards reduced branchiopod abundances, though this was not statistically significant, while mean copepod abundances are relatively identical between stickleback present and absent ponds (Figure 3A & B). This is likely a direct result of the jumping motion associated with branchiopods or daphnids as compared to the more sporadic or suspended state of copepods. This was demonstrated in a Wright and O'Brien (1982) study where white crappie chose moving daphnids 80% of the time over still or suspended larger diaptomid copepods. This coupled with the results for increased abundance of sediment-dwellers among stickleback ponds confirms previous studies suggesting stickleback are visually dependent, predominantly water column foragers (Tompkins and Gee 1983).

Following similar logic for visually apparent prey we expected that among stickleback ponds baetid mayflies and coenagrionid damselflies would be reduced in their abundances while less mobile or substrate dwelling organisms like caenids would be less targeted. Our data confirms that while caenid mayflies are not significantly reduced, baetid mayfly and coenagrionid abundances are reduced (Figure 4A, B, & C). This is likely due to the substrate-dwelling nature of caenids as compared to the water column habitat and undulating swimming motion of baetids and coenagrionids. To a visual predator foraging in the water column, the baetid mayflies and coenagrionids are easy targets for visually dependent predators. Wright and O'Brien (1982 & 1984) demonstrated planktivorous fish had much higher predation success rates based on detection of a moving or undulating prey item than still or suspended prey. While

moving prey are obviously harder to consume than still prey they are more easily detected and thus find themselves more frequently the targets of predation events despite their potential for evasion.

In small eutrophic lakes and ponds visually feeding predators like the brook stickleback and pumpkinseed pose a significant threat to macro-invertebrate and zooplankton communities (Vinyard and O'Brien 1976). Predation events or the "predation cycle" involve several distinct steps to complete a successful predation event. For a visual predator such as brook stickleback and pumpkinseed this includes location of prey items, pursuit of prey, attack, and retention of said prey items (O'Brien 1979). Visual stimulus and detection are a major determinant of vulnerability for zooplankton and small macro-invertebrates in the presence of visually feeding fish. Larger sized zooplankton and other aquatic insects can be located at greater distances than smaller subjects of similar taxa and are thus more readily detected and selected for predation events. For example, Brooks and Dodson (1965) showed that only small bodied zooplankton remained in the presence of heavy planktivory by fish. This is a residual artifact of predation on larger sized and more readily detected prey by the predator. Smaller species and smaller individuals among those species tend to dominate invertebrate assemblage composition in fish-present lakes (Brooks and Dodson 1965, Threlkheld 1979); especially in the presence of gape limited predators like *C. inconstans* and *L. gibbosus* (Zerat 1980). This was true for our data as well, while we did not see significant reductions among branchiopod abundances as was expected (Figure 3C and Figure 6A), significant reductions in mean body lengths were evident, concurrent with literature review. Branchiopods showed a nearly one half mean body length reduction among stickleback inhabited wetlands (Figure 7). Like Brooks and Dodson (1965) this is a direct result of a predator preferentially selecting larger sized prey items until the larger

species are significantly less abundant or removed from the community altogether.

As most planktivores and insectivorous fishes tend to select and target large prey, we expected invertebrates like anisopterans and zygopterans (which commonly exceed 20 mm in body length) to be significantly reduced in their abundances. However, anisoptera and zygopterans (>20 mm) showed no statistical differences in their abundances between stickleback present and absent wetlands (Figure 5A & B). This is likely due to the relatively small gape size of brook stickleback which does not typically allow feeding upon organisms of this size. Stickleback may avoid potential prey items like anisopterans, as dragonfly nymphs have been shown to feed on smaller fishes such as brook stickleback (Stewart 2007). Despite lacking statistical significance, zygopterans do exhibit a trend towards reduced abundances among stickleback wetlands. Smaller instars may be vulnerable to predation and thus potentially decrease overall abundance. Reduced abundances could also be due to a feeding strategy exhibited by brook stickleback known as “food fighting”; where several fish actively work together to tear apart a larger than normally consumed prey (Reisman and Cade 1967).

While we did not expect the increase in abundance of oligochaetes specifically, we were expecting increases among substrate or infauna organisms like chironomids. The dramatic increase of these organisms when sticklebacks are present is directly responsible for the discrepancies between stickleback and non-stickleback evenness values among observed aquatic invertebrate assemblages. We were expecting that stickleback and pumpkinseed would have a greater effect on diversity indices than was observed. Literature shows that in the presence of insectivorous/planktivorous fish diversity, richness, and evenness are commonly less among fish-present waters (Butler 1989). However, in our study only species evenness was affected by the presence of stickleback. Shannon-Weaver evenness was significantly less among stickleback

ponds as a result of the increases among infauna benthic organism abundances. This is a problem as both chironomids and oligochaetes are benthic dwellers and are not common constituents of waterfowl diet. While more pelagic or water column-inhabiting species like mayflies, midges, and zygopterans are significantly less among stickleback ponds. These prey items are more likely to be among the diets and habitat of feeding waterfowl, as such are more likely to impact the feeding ecology of waterfowl on these wetlands.

When comparing the abundances, sizes, and diversity indices of these wetlands and their respective aquatic invertebrate communities, stickleback clearly have a negative effect on individual constituents as well as community evenness. Further, our SIA data suggests niche overlap with waterfowl. Ducks and pumpkinseed appear to utilize similar proportions of littoral and pelagic carbon. Waterfowl and stickleback each utilize some degree of pelagic carbon. While pumpkinseed and stickleback appear to occupy very similar trophic levels, 8.12 and 8.61‰  $\delta^{15}\text{N}$  respectively, they tend to feed from very different carbon sources. Waterfowl appear to occupy lower trophic levels (6.99‰  $\delta^{15}\text{N}$ ) than pumpkinseed or stickleback. However, they share very similar  $\delta^{13}\text{C}$  signatures as a result of feeding from a more specific littoral invertebrate diet with a more enriched  $\delta^{13}\text{C}$  signature.

Stickleback appear to negatively affect water-column inhabiting invertebrates that exhibit some motion or dynamic that allows them to be easily detected more readily than benthic or infauna invertebrates. Infauna or sediment dwelling invertebrates tend to flourish in the presence of stickleback while branchiopod, *Chaoborus* midge, baetid mayfly, and coenagrionid damselfly communities all appear at risk of significant reductions in their abundances. Branchiopods are also at risk of significant size reductions as a result of stickleback targeting and feeding predominantly on larger sized individuals. These findings are consistent with literature review;

however the influx of infauna organisms in the presence of stickleback is somewhat controversial. Though stickleback do not appear to share exact niches with waterfowl or pumpkinseed there is some level of overlap as at least 50% of their diet is similar. It is also very important to note the varying feeding strategies among waterfowl occupying fish present and absent wetlands. It is clear that waterfowl feeding from fish present wetlands feed on a more littoral based carbon diet, while waterfowl feeding from fish free wetlands consume more pelagic invertebrate based food sources. These findings suggest that stickleback affect the ecology of wetlands, and that while they may not appear to occupy exactly the same niche as waterfowl and pumpkinseeds, there remains the potential for a negative effect on waterfowl.

## Works Cited

- Batzer, D.P. (1998) "Trophic interactions among detritus, benthic midges, and predatory fish in a fresh water marsh." *Ecology* 79: 1688-1698
- Batzer, D.R., C.R. Pustateri, and R. Vetter. (2000) "Impacts of fish predation on marsh invertebrates: Direct and indirect effects." *Wetlands* 20: 307-312
- Beisner, B, and P Peres-Neto. (2009) "Seasonal trophic dynamics affect zooplankton community variability." *Freshwater Biology* 54.11: 2351-2363.
- Benigno, E. (2001) "Identification of non-native freshwater fishes." *Convention on the Conservation of European Wildlife* 21: 1-35.
- Black, A.R. and S. Dodson. (2003) "Ethanol: A better preservation technique for daphnia." *Limnological Oceanography Methods*, 1: 45-50
- Bouffard, S.H. and M.A. Hanson. (1997) "Fish in waterfowl marshes: Waterfowl managers' perspective." *Wildlife Society Bulletin* 25: 146-157
- Butler, M. (1989) "Community responses to variable predation: Field studies with sunfish and freshwater macro-invertebrates." *Ecological Monographs* 59.3: 311-328.
- Brooks, J.L, and S. Dodson. (1965) "Predation, body size, and composition of plankton." *Science* 150: 28-35.
- Casal, C. (2005) "Global documentation of fish introductions: The growing crisis and recommendations for action." *Biological Invasions* 8.1: 3-11.
- Declerck, S, G Louette, T Bie, and L Meester. (2002) "Patterns of diet overlap between populations of non-indigenous and native fishes in shallow ponds." *Journal of Fish Biology* 61.5: 1182-1198.
- DeNiro, M.J., and S. Epstein (1978) "Influence of diet on the distribution of carbon isotopes in animals." *Geochimica et Cosmochimica Acta* 42: 495-506.
- DeNiro, M.J., and S. Epstein. (1981). "Influence of diet on the distribution of nitrogen isotopes in animals." *Geochimica et Cosmochimica Acta* 45: 341-351.
- Flaherty, Elizabeth. (2010) "Overlap and partitioning of the ecological and isotopic niches." *Oikos* 119.9: 1409-1416
- Forbes, S.A. (1887) "The lake as a microcosm." *Bulletin of Science Association of Peoria* 1887: 77-87.



- France, R. (1995) "Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes." *Limnology and Oceanography* 40: 1310-1313.
- Fry, Brian. (2006) *Stable Isotope Ecology*. New York, NY: Springer, Print.
- Grosholz, E, and E Gallo. (2006) "The influence of flood cycle and fish predation on invertebrate production on a restored California floodplain." *Hydrobiologia* 568.1: 91-109.
- Heatherly, T., M. Whiles, D. Knuth, and J. Garvey. (2005) "Diversity and community structure of littoral zone macro-invertebrates in southern Illinois reclaimed surface mines." *American Midland Naturalist* 154.1: 67-77.
- Jonathan , P, J. Hornung, and A. Lee Foote. (2005) "Aquatic invertebrate responses to fish presence and vegetation complexity in western boreal wetlands, with implication for waterbird productivity." *Wetlands* 10: 1-12.
- Keiffer, J, and P Colgan. (1991) "Individual variation in learning by foraging pumpkinseed sunfish, *Lepomis gibbosus*: The influence of habitat." *Animal Behavior*. 41.4: 603-612.
- Lazzaro, X., G. Lacroix, B. Gauzens, J. Gignoux, and S. Legendre. (2009) "Predator foraging behavior drives food-web topological structure." *Journal of Animal Ecology* 78.6: 1307-1317.
- Magnuson, J, A.L. Beckel, K. Mills, and S.B. Brandt. (1985) "Surviving winter hypoxia: Behavioral adaptations of fishes in a northern Wisconsin winterkill lake." *Environmental Biology of Fishes* 14: 241-250.
- McNally, B. (2004) "Duck Diets." *Outdoor Life* 211.7: 12-14.
- McParland, C., and C.A. Paszkowski. (2006) "Effects of small-bodied fish on invertebrate prey and foraging patterns of waterbirds in Aspen Parkland wetlands." *Hydrobiologia* 567: 43-55.
- McParland, C., and C.A. Paszkowski. (2007) "Waterbird assemblages in the Aspen Parkland of western Canada: The influence of fishes, invertebrates, and the environment on species composition." *Ornithological Science* 6: 53-65
- Miller, R.S. (1967) "Patterns and processes in competition." *Advances in Ecology Research* 4: 1-74
- Minagawa, M. and Wada E. (1984) "Stepwise enrichment of  $^{15}\text{N}$  along food chains: Further evidence and the relation between  $^{15}\text{N}$  and animal age." *Geochimica et Cosmochimica Acta* 48: 1135-1140
- Newsome, S.D., C. Martinez del Rio, S. Bearhop, and D.L. Phillips. (2007) "A niche for isotopic ecology." *Frontiers in Ecology and the Environment*. 5.8: 429-436

- O'Brien, W.J. (1979) "The predator-prey interaction of planktivorous fish and zooplankton." *American Scientist* 67.5: 572-581
- O'Brien, W.J., B. Evans, and C. Luecke. (1984) "Apparent size choice of zooplankton by planktivorous sunfish: Exceptions to the rule." *Environmental Biology of Fish* 13: 225-233.
- O'Brien, W.J. (1987) "Planktivory by Freshwater Fish: Thrust and parry in the pelagia." In: Kerfoot, W.C., Sih, Andrew. Eds. *Predation: Direct and indirect impacts on aquatic communities*. University Press of New England: 3-16.
- Rachel, F. (2007) "Biogeographic barriers, connectivity and homogenization of freshwater faunas: It's a small world after all." *Freshwater Biology* 52: 696-710.
- Reisman, H.M., and T.J. Cade. (1967) "Physiological and behavioral aspects of reproduction in the brook stickleback, *Culaea inconstans*." *The American Midland Naturalist* 77.2: 257-295.
- Scholz, A.T., B.Z. Lang, A.R. Black, H.J. McLellan, and R.W. Peck. (2003) "Brook stickleback established in Washington." *Northwest Science* 77: 110-115.
- Schulze, P.C. (2011) "Evidence that fish structure the zooplankton communities of turbid lakes and reservoirs." *Freshwater Biology* 56.2: 352-365
- Shannon, Claude E. and Warren Weaver. (1949) *The Mathematical Theory of Communication*. The University of Illinois Press, Urbana, Illinois.
- Stewart, D.B., T.J. Charmichael, C.D. Sawatzky, N.J. Mochnacz, and J.D. Reist. (2007) "Fish diets and food webs in the Northwest Territories: Brook stickleback (*Culaea inconstans*)." *Can. Manuscript. Rep. Fish. Aquatic Science*: 2798.
- Szymanski, M., A. Afton, and K. Hobson. (2007) "Use of stable isotope methodology to determine natal origins of mallards at a fine scale within the upper Midwest." *Journal of Wildlife Management* 71.4: 1317-1324.
- Thongwittaya, N. (2007) "Substitution of plant protein for fish meal in the diet of laying ducks." *Animal Science* 78: 351-355.
- Thorp, J. (1988) "Patches and the responses of lake benthos to sunfish nest-building." *Oecologia*. 76.2: 168-174.
- Threlkheld, S.T. (1979) "The midsummer dynamics of two *Daphnia* species in Wintergreen Lake, Michigan." *Ecology* 60: 165-179.

- Tompkins, A, and J Gee. (1983) "Foraging behavior of brook stickleback, (*Culaea inconstans*, Kirtland): Optimization of time, space, and diet." *Canadian Journal of Zoology* 61: 2482-2490.
- Vinyard, G.L., and W.J. O'Brien. (1976) "Effects of light and turbidity on the reactive distance of bluegill sunfish (*Lepomis macrochirus*). *Journal of Fisheries Resources Board Canada*. 33: 2845-2849
- Wissinger, S, A McIntosh, and H Greig. (2006) "Impacts of introduced brown and rainbow trout on benthic invertebrate communities in shallow New Zealand lakes." *Freshwater Biology* 51.11: 2009-2028.
- Wootton, R.J. (1976) "The Biology of Sticklebacks." *Academic Press, N.Y.*
- Wright, D.I., and W.J. O'Brien. (1982) "Differential location of *Chaoborus* larvae and *Daphnia* by fish: The importance of motion and visible size." *American Midland Naturalist* 108.1: 63-73
- Wright, D.I. and W.J. O'Brien. (1984) "The development and field test of a tactical model of the planktivorous feeding of white crappie (*Pomoxis annularis*)." *Ecological Monographs* 54: 65-98
- Wydoski, R.S., and R.L. Whitney. (2003) *Inland Fishes of Washington*. 2nd Ed. Seattle, WA. University of Washington Press, 169-170.
- Yohannes, E, K Hobson, D Pearson, and L. Wassenaar. (2005) "Stable isotope analyses of feathers help identify autumn stopover sites." *Journal of Avian Biology* 36: 235-241.
- Zaret, Thomas. (1980) "Predation and freshwater communities." Yale University Press, 3-11.

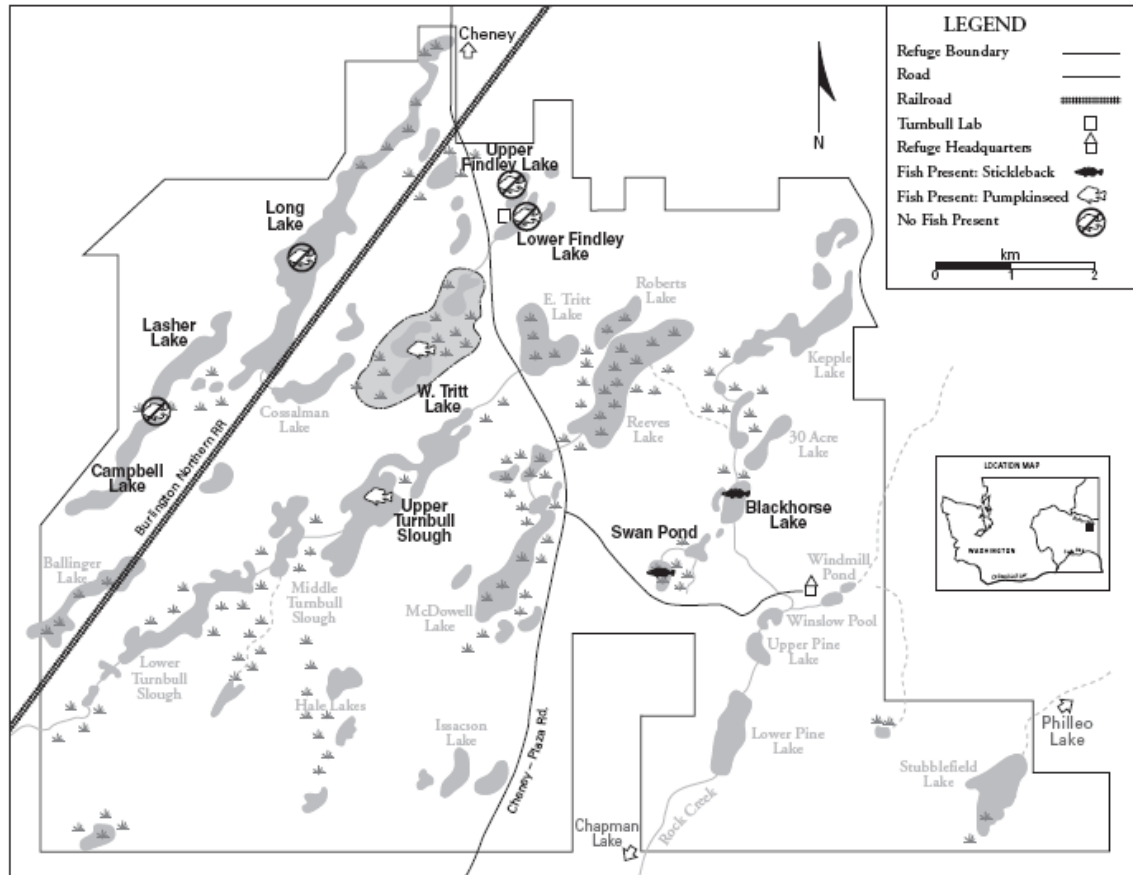


Figure 1: Turnbull National Wildlife Refuge; includes sample sites in bold and treatment types for each of the sampled ponds. Cheney, WA. (Modified from Scholz et al. 2003)

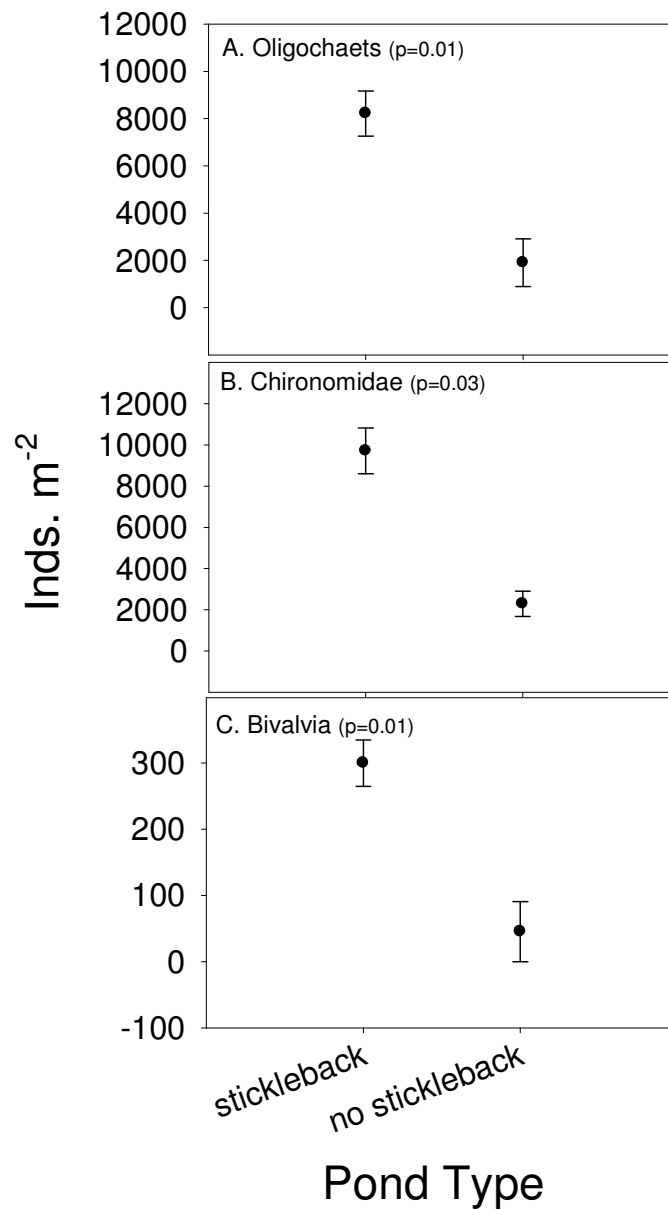


Figure 2. Mean ( $\pm 1$  SE) abundance of micro-invertebrates collected from littoral stove pipe samples, for each of stickleback present (n=2) and stickleback absent (n=4) ponds. Alpha error values are provided when abundances were determined to be significantly different. TNWR, Cheney, WA. June-July 2009

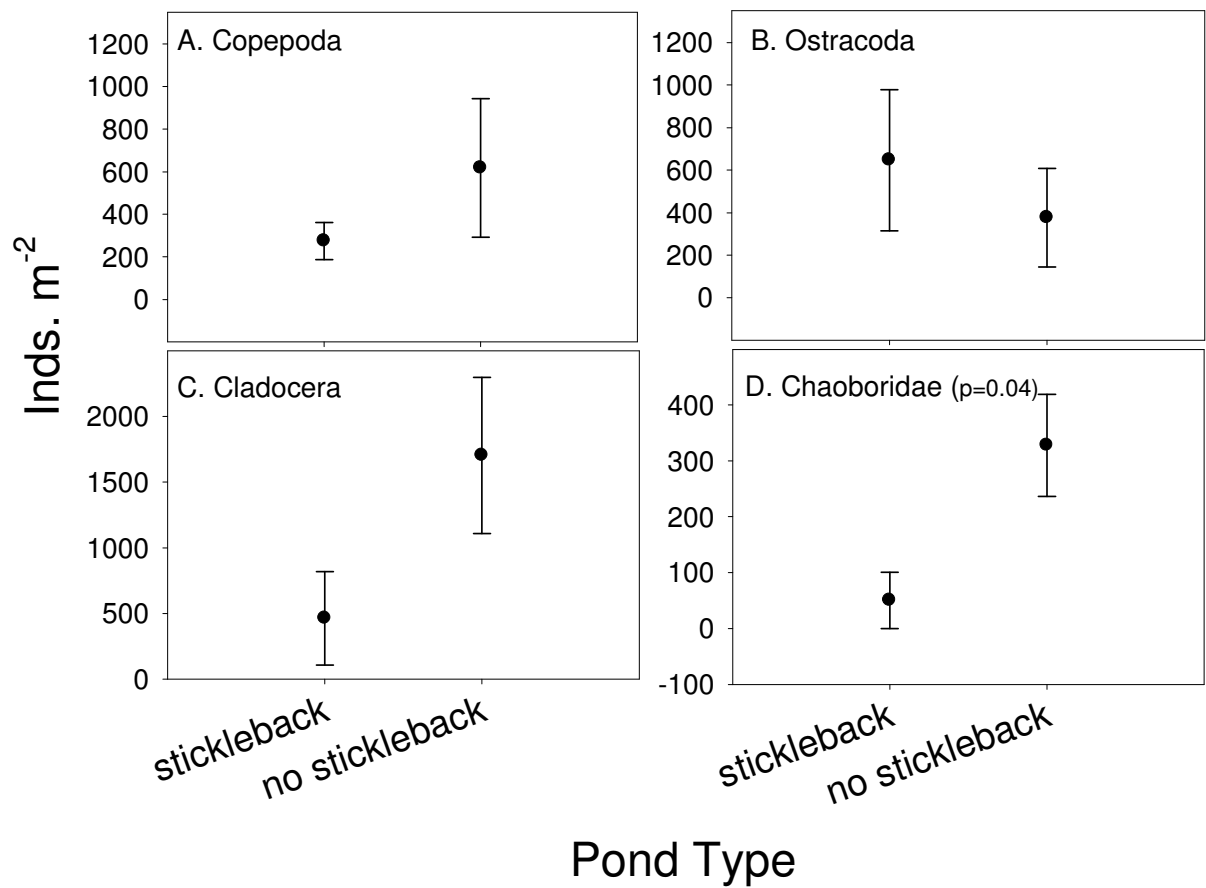


Figure 3. Mean ( $\pm 1$  SE) abundance of zooplankton collected from littoral stove pipe samples, for each of stickleback present (n=2) and stickleback absent (n=4) ponds. Alpha error values are provided when abundances were determined to be significantly different. TNWR, Cheney, WA. June-July 2009

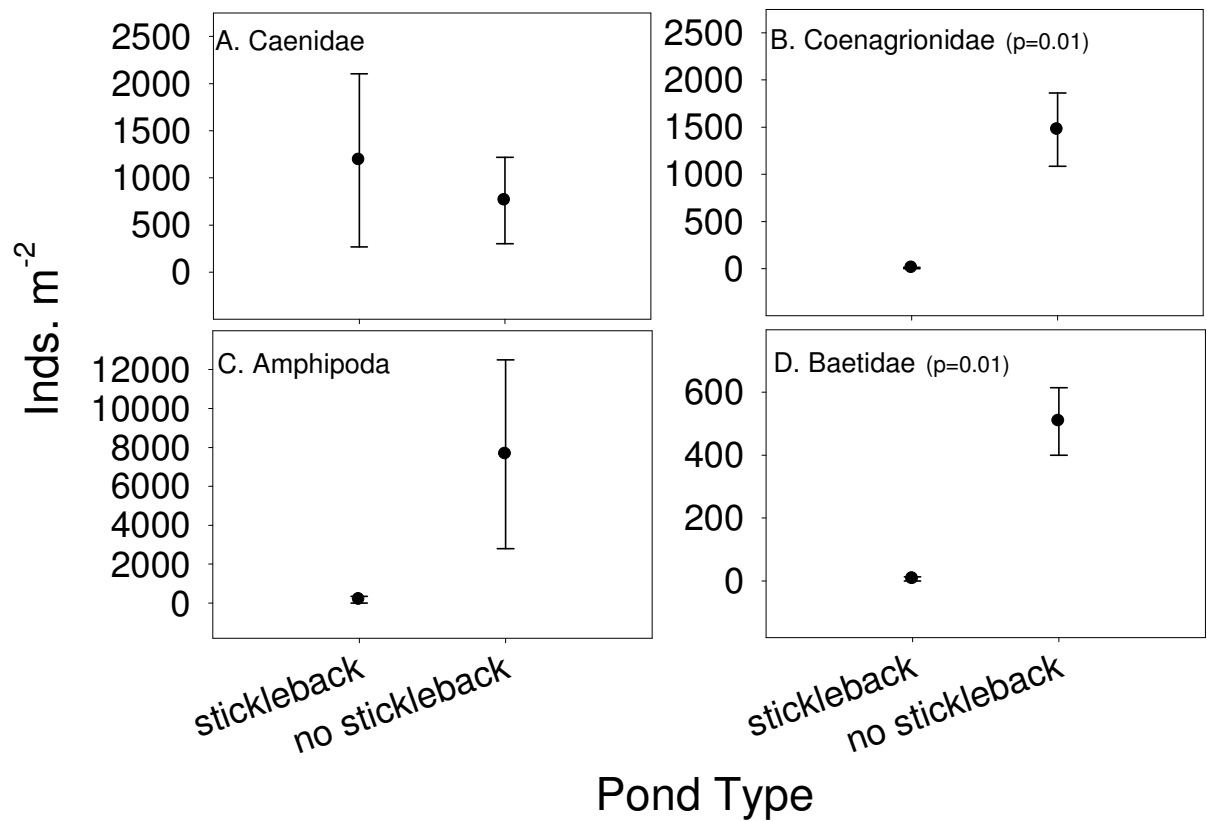


Figure 4. Mean ( $\pm 1$  SE) abundance of macro-invertebrates collected from littoral stove pipe samples, for each of stickleback present (n=2) and stickleback absent (n=4) ponds. Alpha error values are provided when abundances were determined to be significantly different. TNWR, Cheney, WA. June-July 2009

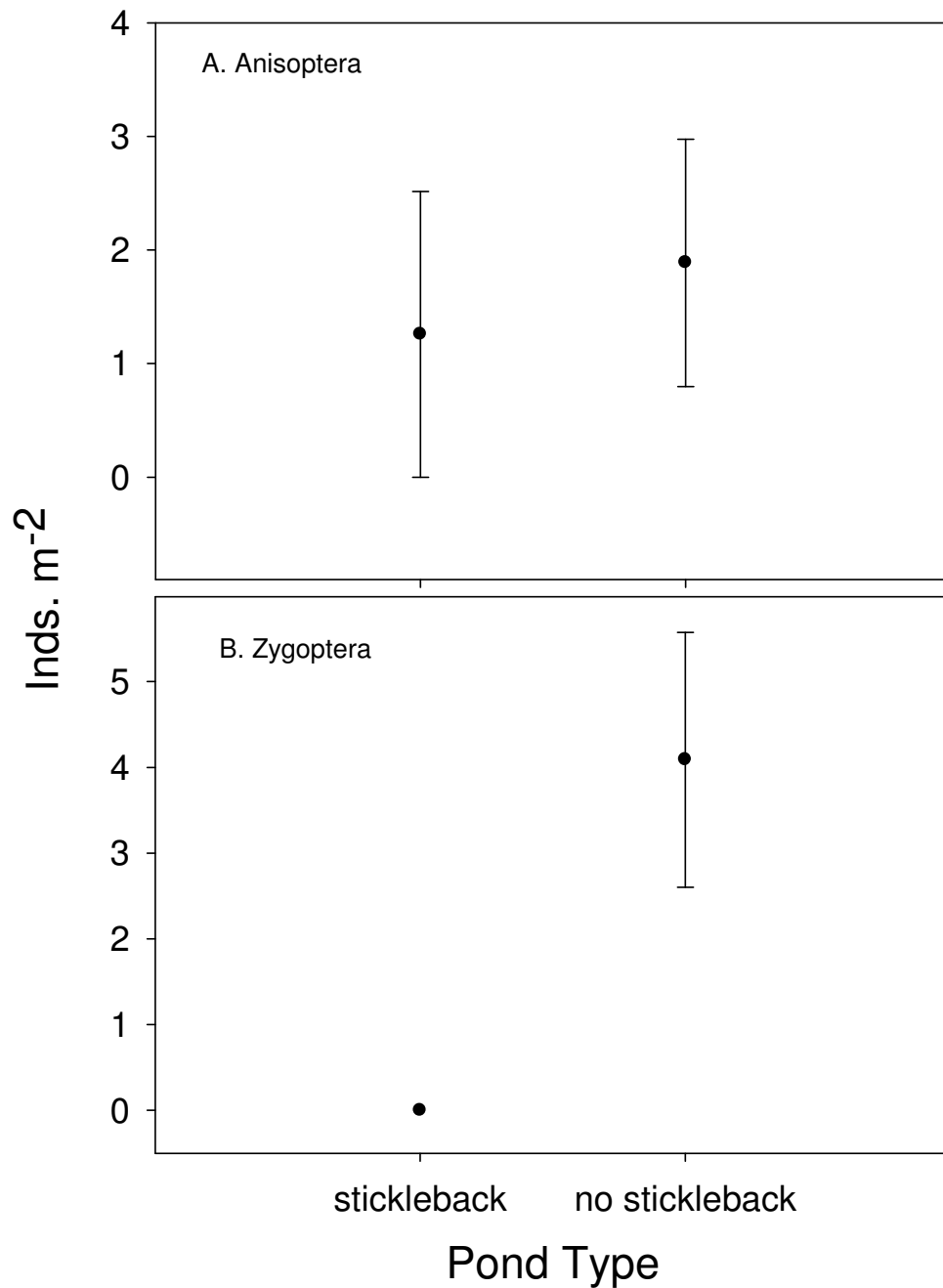


Figure 5. Mean ( $\pm 1$  SE) abundance of macro-invertebrates  $>20$ mm collected from littoral stove pipe samples, for each of stickleback present ( $n=2$ ) and stickleback absent ( $n=4$ ) ponds. Alpha error values are provided when abundances were determined to be significantly different. TNWR, Cheney, WA. June-July 2009



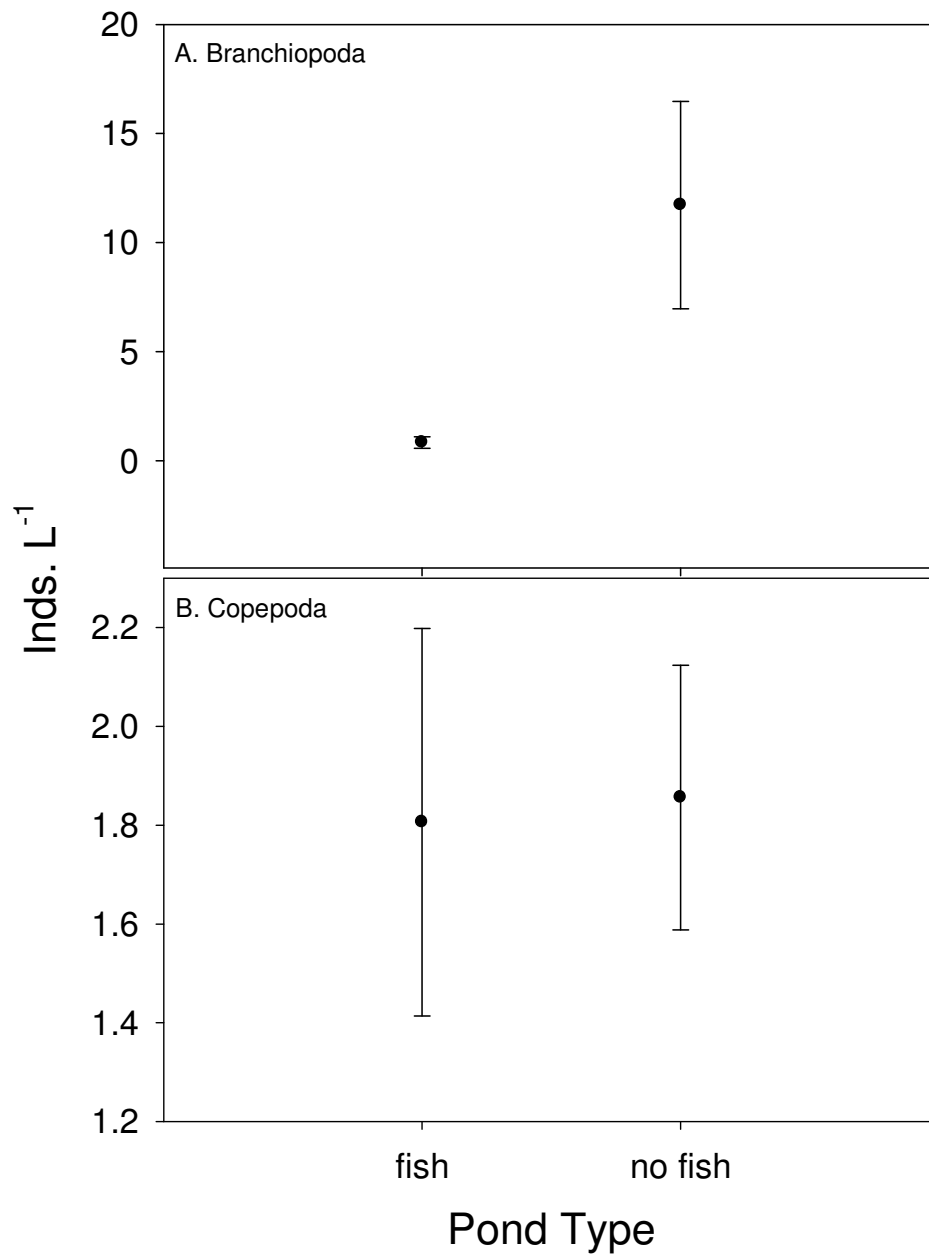


Figure 6. Mean ( $\pm 1$  SE) abundance of zooplankton collected from pelagic zooplankton net haul samples, for each of fish-present ( $n=4$ ) and fish-absent ( $n=4$ ) ponds. Alpha error values are provided when abundances were determined to be significantly different. TNWR, Cheney, WA. July 2011

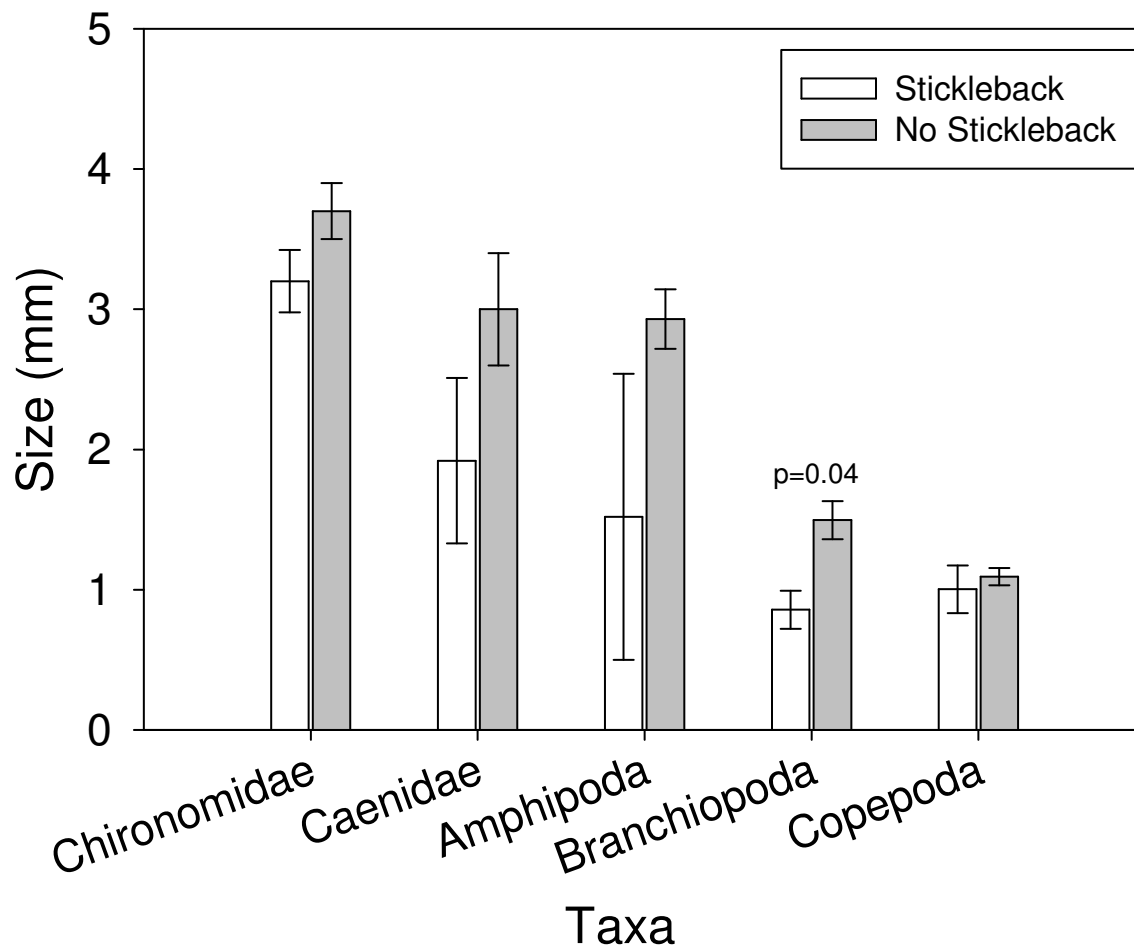


Figure 7: Average size estimates  $\pm$  SE of dominant invertebrates < 20mm sampled (50cm diameter stovepipe) from the littoral zone of 2 stickleback and 4 non-stickleback ponds (5 replicates/pond), showing significantly smaller ( $p=0.04$ ) branchiopods among stickleback ponds. TNWR, Cheney, WA. June-July 2009

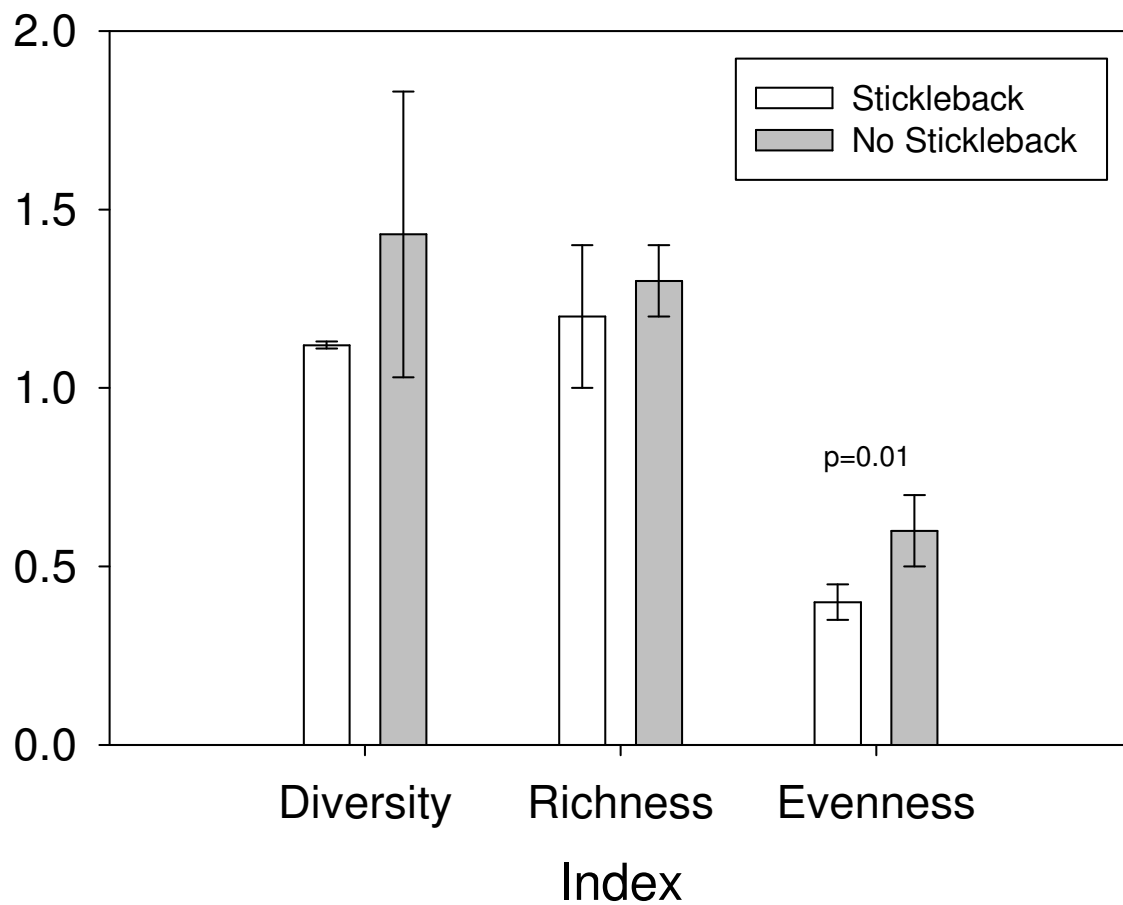


Figure 8: Average Shannon Weaver diversity ( $p=0.08$ ), richness ( $p=0.08$ ), and evenness ( $p=0.01$ ) indices of the littoral invertebrate communities of ponds populated by stickleback ( $n=2$ ) versus those with no stickleback ( $n=4$ ). TNWR, Cheney, WA. June-July 2009

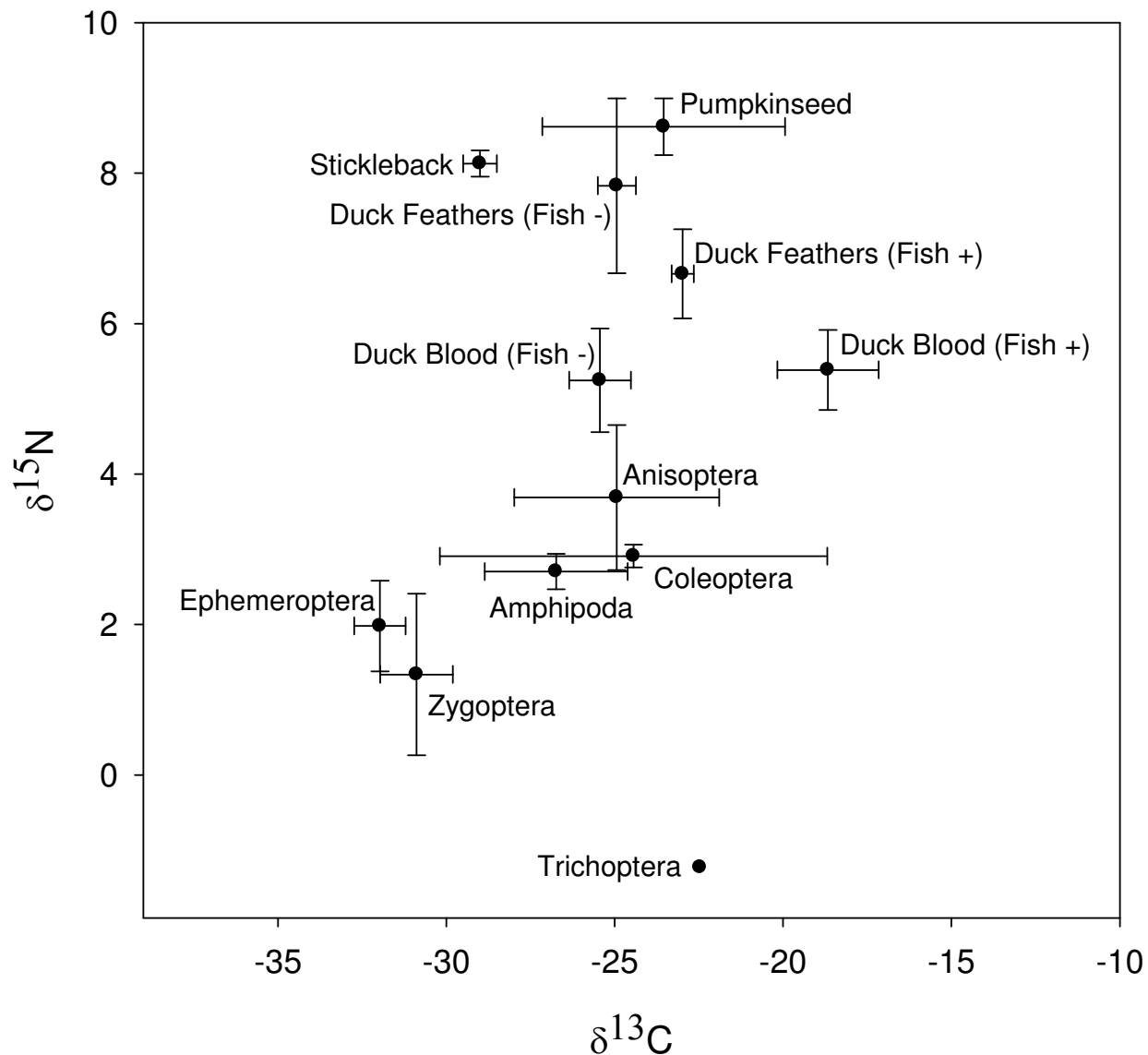


Figure 9: Mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C} \pm \text{SE}$  of all sampled specimens across all lakes, showing stickleback feeding from a lower basal carbon resource group (damselflies and mayflies), being more of a generalist than pumpkinseed and dabbling waterfowl, which tend to feed from higher basal carbon resources (scuds, caddis flies, and dragonflies).

Table 1: Results of Fish/Unit Effort 24hr. relative abundance measurements using wire mesh minnow traps at all pond sample sites. TNWR, Cheney, WA. July-August 2009

Site	Stickleback		Pumpkinseed		No Fish	
	Blackhorse	Swan Pond	West Tritt	Upper Turnbull	Campbell	Long lake
1	0	95	2	2	0	0
2	1	19	3	3	0	0
3	3	53	7	4	0	0
4	8	59	9	24	0	0
5	7	77	0	3	0	0
Totals	19	303	21	36	0	0

Table 2: Number of each taxon observed per lake and total number of all macro-invertebrates sampled per lake.

	Chaoboridae	Culicidae	Chironomidae	Caenidae	Baetidae	Coenagrionidae	Lestidae	Aeshnidae
Black Horse	0	1	641	157	1	1	0	0
Swan Pond	13	0	1137	37	0	0	0	0
W. Tritt	50	0	293	20	47	67	1	0
Upper Turnbull	15	0	69	12	18	58	0	0
Long Lake	3	0	55	62	22	71	1	1
Cambell Lasher	30	1	284	42	30	155	1	3
	Amphipoda	Cladocera	Copepoda	Ostracoda	Bivalvia	Oligochaeta	Hirudinea	Total/Lk
Black Horse	26	61	27	73	25	684	1	1698
Swan Pond	2	9	26	47	14	690	0	1975
W. Tritt	69	140	23	93	14	356	0	1173
Upper Turnbull	508	85	23	5	0	13	0	806
Long Lake	605	102	45	5	0	16	3	991
Cambell Lasher	121	38	29	12	0	189	1	936

Table 3: Proportion (%) of each taxon observed in fish versus no fish ponds, with refuge average from which we chose dominant taxa.

	Fish	No Fish	average
<i>Chaoboridae</i>	0.01	0.03	0.02
Culicidae	0.00	0.00	0.00
Chironomidae	0.41	0.20	0.31
Caenidae	0.05	0.04	0.04
Baetidae	0.00	0.03	0.02
Coenagrionidae	0.01	0.09	0.05
Lestidae	0.00	0.00	0.00
Aeshnidae	0.00	0.00	0.00
Amphipoda	0.12	0.26	0.19
Branchiopoda	0.03	0.09	0.06
Copepoda	0.02	0.03	0.02
Ostracoda	0.03	0.04	0.03
Bivalvia	0.01	0.00	0.01
Oligochaeta	0.31	0.18	0.25
Hirudinea	0.00	0.00	0.00

## **Levi Bridges Curriculum Vitae (2010)**

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Masters Candidate, Department of Biology, Eastern Washington University, Cheney, WA.

### **Education:**

-M.S. Biology, Eastern Washington University

Thesis Research: Invasive Fish Effects on the Invertebrate Constituents of Wetland Communities. Advisor: Dr. A.R. Black

-2008 B.S. Biology, Eastern Washington University

### **Grants and Fellowships:**

Pending- Carlisle T. and Charles P. Barry Memorial Research Grant, Eastern Washington University, Cheney, WA. (\$700)

2009-2010 EWU Graduate Fellowship, Eastern Washington University, Cheney, WA. Award includes graduate tuition and nine month stipend.

2008 EWU Graduate Mini Grant Award, Eastern Washington University, Cheney, WA. (\$500)

### **Research:**

2008-Present: M.S. Thesis Research, EWU Biology Department. "Invasive Fish Effects on the Invertebrate Constituents of Wetland Communities." L. Bridges and A.R. Black

- A study assessing the impacts of an invasive fish, brook stickleback (*Culaea inconstans*), on the invertebrate constituents of local wetlands and waterfowl refuge Turnbull National Wildlife Refuge.
- Plan to submit to Hydrobiologia and Fresh Water Biology journals for publication. Expected submission date: June 2011

2008-Present: Wildlife Biologist Assistant for Mike Rule of U.S. Fish and Wildlife Service, Turnbull National Wildlife Refuge, Cheney, WA. Responsibilities: Annual refuge wide waterfowl trapping and banding which includes recording and identifying species, sex, and age of all waterfowl trapped. Monitor invasive fish distribution throughout the refuge using wire mesh minnow traps.

2006-2008: Graduate Assistant for Kevin Mahoney, EWU Biology Department: "A Stable Isotope assessment of the food web position and energy utilization of the fishes within 23 Eastern Washington lakes." Responsibilities: Field sampling assistant and sample preparation for stable isotope analysis.



**Teaching Experience:**

2005-Present: Teaching Assistant for EWU Biology Department Courses: Introduction to Biology (Bio 100), Investigating Biology (Bio 115), Human Anatomy and Physiology for non Majors (Bio 232), Biological Investigation (Bio 270), and Ecology Lab (Bio 441)

2008-2009: Laboratory Assistant for Honors Natural Science Lab (Honors 104), EWU Biology department, Heather McKean, Instructor.

2007-2009: Guest Lecturer for Introduction to Biology (Bio 100), EWU Biology department, Dr. L Flaherty, Instructor. Topics: Genetics, Respiratory and Circulatory Systems

**Presentations:**

2010 L. Bridges, A. Hodgson, A.R. Black. "Invasive Fish Effects on the Invertebrate Constituents of Wetland Communities." North American Benthological Society and American Society of Limnology and Oceanography Joint Meeting, Santé Fe, NM. June 2010

**Relevant Courses:**

EWU, Undergraduate: Evolution, Botany, Field Botany, Field Ecology, Riparian Ecology, Vertebrate Zoology, Animal Physiology, Cellular Biology, Microbiology, Investigative Biology, and Biological Teaching Methods.

EWU, Graduate: Current Topics in Evolution and Ecology, Stream Ecology, Wetland Sciences, Limnology, Ichthyology, Conservation Biology, and Freshwater Invertebrate Zoology.

**Relevant Experience:**

Geographic Information Systems (GIS)/Environmental Systems Research Institute (ESRI) certificate of proficiency through EWU Geography Department, Cheney, WA. Expected Completion Date: Dec. 2010

2007-2010 Biology/Ecology Event Supervisors for Pacific Northwest Regional Science Olympiad

**Professional Memberships:**

Ducks Unlimited (2003-Present)

Trout Unlimited (2008-Present)

North American Benthological Society (2009-Present)

American Society of Limnology and Oceanography (2009-Present)

Rocky Mountain Elk Foundation (2003-Present)

**Interests:** Waterfowl, aquatic ecology, limnology, fish behavior and ecology, invasive species, evolution, population and life histories ecology, wetland management and restoration.

**References:**

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