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Determining the effects of non-native brook stickleback (Cualea inconstans) on the lentic systems at Turnbull National Wildlife Refuge, Cheney, WA

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DETERMINING THE EFFECTS OF NON-NATIVE BROOK STICKLEBACK (*CUALEA INCONSTANS*) ON THE LENTIC SYSTEMS AT TURNBULL NATIONAL WILDLIFE REFUGE, CHENEY, WA

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Approval Sheet

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MASTER'S THESIS

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ABSTRACT

The introduction of non-native fishes can cause trophic cascades in freshwater habitats; these effects may be amplified in ephemeral/temporary habitats. Non-native brook stickleback fishes (*Culaea inconstans*) were first documented on the Turnbull National Wildlife Refuge (WA) in 1999 and are now present in many portions of the refuge. The consequences of their presence on the refuge's perennial/permanent and temporary habitats are poorly understood. Therefore, the purpose of my project was to determine if brook stickleback are affecting habitat characteristics that are important for waterfowl nesting success. From April – August 2015, I compared the macroinvertebrate and macrophyte community characteristics, fingernail clam population and stress metrics, and water quality parameters in twelve lentic systems on the refuge; those that contain brook stickleback or are fish free, and are either temporary or permanent. The fish free, permanent lentic systems had more macroinvertebrate and macrophyte taxonomic/species variety, more macroinvertebrates and macrophyte dried biomass (abundance), the highest fingernail clam condition index, and the highest clam brood sizes. Macroinvertebrate taxonomic assemblages were additionally influenced by lentic system category and size. The macrophyte abundance and diversity was influenced more by the permanent or temporary status. Fingernail clam condition index, clam length, chlorophyll, transparency, nitrate, and pH were influenced by the compounding effects of brook stickleback presence and permanent or temporary status. Overall, it appears that the presence of brook stickleback most likely affects habitat quality characteristics in the lentic systems that are temporary, especially those that also are smaller in size. This is of particular concern for the refuge because these changes, if they persist, may impact waterfowl nesting success.

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PURPOSE

The purpose of my project was to determine if brook stickleback are affecting habitat characteristics in the lentic systems at the Turnbull National Wildlife Refuge, with a focus on those characteristics that are important for waterfowl nesting success.

INTRODUCTION

According to the U.S. Fish and Wildlife Service, viable wetland habitat has decreased 53% from the estimated 221 million wetland acres that were present when settlers first arrived in what would become the continental United States (Brinson and Malvarez 2002, U.S.F.W. 2015). The management and conservation of healthy, stable, and sustainable wetlands is important for maintaining habitat productivity and biodiversity (Rapport et al. 1998, Alcamo et al. 2004). Wetlands are important for industrial production, irrigation, recreation, transportation, waste disposal, and keeping fish, waterfowl, and invertebrate populations sustainable and diverse (Jackson et al. 2001, Brinson and Malvarez 2002). A majority of the wetlands in the United States that are not privately owned are protected by 205 national wildlife refuges, consisting of about 3.5 million acres (Brinson and Malvarez 2002, U.S.G.S. 2013). Many of the wildlife refuges that contain wetlands and other types of lentic systems have management priorities that focus on maintaining habitat that is suitable for waterfowl. Unfortunately, lentic habitats, both within and outside of refuges, are becoming increasingly degraded by land use conversions, eutrophication, toxicity, fire, and an array of human-aided introductions, particularly fish introductions (Brinson and Malvarez 2002, Gozlan et al. 2010, Strayer 2010). The introduction of fish species is a widespread problem, especially in the Western U.S., where almost 25% of the fish species are considered invasive (Marchetti et al. 2004).

Non-native species can have detrimental economic effects, can alter ecosystems, and decrease productivity and biodiversity (Lovell and Stone 2005). The negative consequences of fish introductions are of regional concern given the presence of non-native fish at the Turnbull National Wildlife Refuge (Turnbull NWR) in Spokane County, WA.

Turnbull NWR consists of approximately 18,217 acres of channeled scablands, 3,000 of which are wetlands made from the ice age floods that moved through eastern Washington 15,000 years ago (Weis and Newman 1989). The habitats at Turnbull NWR consist of ponderosa pine forests, shrub-steppe grassland, and marshes. The marshes and wetlands at Turnbull NWR provide essential habitat for 29 waterfowl species and up to 100,000 birds, both year round and migratory species (Curry et al. 2007). The more prominent waterfowl species at Turnbull NWR are the Ruddy Duck (*Oxyura jamaicensis*), Redhead (*Aytha americana*), Mallard (*Anas platyrhynchos*), and the Blue-winged teal (*Anas discors*) (Curry et al. 2007).

One of the non-native fish species at Turnbull NWR, brook stickleback (*Culaea inconstans*), was first found in the Rock Creek watershed portion of the refuge in 1999 (Scholz et al. 2003). Prior to the study by Scholz et al. (2003), brook stickleback had not been found in Washington State or even west of the continental divide. A recent study by Walston et al. (2016) concluded that brook stickleback now exist in additional portions of the refuge (Cow Creek watershed). Considering that brook stickleback appear to be spreading through the refuge and Turnbull NWR is managed for waterfowl, it is critical to understand whether the quality of the lentic habitats at the refuge are impacted by the presence of the non-native fish.

Non-native fish species, especially omnivorous species like sticklebacks, can negatively impact habitat quality (Table 1) by altering the invertebrate prey, nutrient availability, chlorophyll levels, water turbidity, and macrophyte abundance (Bouffard and Hanson 1997). High consumption rates of macroinvertebrates and zooplankton can alter water quality, particularly nutrients; this phenomenon is called a trophic cascade (Harmon et al. 2009). For example, intense predation on zooplankton by brook stickleback results in decreased consumption of detritus, and increased phytoplankton abundance and blue-green algae blooms (Spencer and King 1984). The increased abundance of phytoplankton, algal blooms, and detritus can lead to an increase in turbidity and a decrease in macrophyte abundance (Vierssen and Prins 1985). Work with two related stickleback species, three-spined stickleback (*Gasterosteus aculeatus*) and nine-spined stickleback (*Pungitius pungitius*), which are invasive species in some parts of North America and Europe illustrates the full trophic cascade phenomeon (which has not been demonstrated for brook stickleback), including alterations in chlorophyll levels, turbidity, and nutrient levels (Daldorph and Thomas 1995, Jakobsen et al. 2003, Feuchtmayr et al. 2007). The increases in turbidity and chlorophyll levels that typically accompany invasive fish presence can in turn alter nutrient levels, conductivity, water temperature, pH and dissolved O_2 levels (Table 1; Erickson 1985, Bayley and Prather 2003, Beekey and Karlson 2003, Morgan et al. 2010). It is clear that the presence of various stickleback species can have adverse effects on freshwater habitats, primarily through the alteration of macroinvertebrate and zooplankton communities (Spencer and King 1984, Daldorph and Thomas 1995, Jakobsen et al. 2003)

Given that stickleback species likely alter water quality parameters through consumption of invertebrates and macrophytes, the presence of non-native stickleback is particularly problematic for freshwater habitats that are managed for waterfowl. Brook stickleback will consume anything from aquatic and terrestrial invertebrates, crustaceans, various eggs and larvae, mollusks, and macrophytes (Table 2; Stewart et al. 2007). There is substantial diet overlap between brook stickleback and a diversity of waterfowl species (Hornung and Foote 2006, Wieker et al. 2016), perhaps reaching a 50% overlap at Turnbull NWR (Bridges 2011). Both diving and dabbling waterfowl species consume vascular plants and angiosperm seeds as well as a variety of invertebrates within Mollusca, Chironomidae, and Diptera (Kenow 1996, Sanchez et al. 2000, Dessborn et al. 2011, Tidwell et al. 2013). Macrophytes play important roles in lentic habitats, not just because they are a major food source for fish and waterfowl, but also because they are refugia for invertebrates and breeding habitat for waterfowl (Vierssen and Prins 1985, Hornung and Foote 2006). Similarly, benthic macroinvertebrates such as fingernail clams, snails, and crustaceans are vitally important for duckling growth and survival, especially during the spring and summer months (de Szalay et al. 2003). The loss of macroinvertebrates and macrophytes due to the presence of non-native fish species, particularly omnivorous species, proves challenging for waterfowl reproduction, specifically for brooding pairs and ducklings (Joyner 1980, Bouffard and Hanson 1997, Richman and Lovvorn 2009, Epners et al. 2010). Competition for food is not the only problem facing waterfowl wetland habitats that contain non-native fish. Considering that the presence of brook stickleback increases turbidity, waterfowl species richness may

decrease because waterfowl prefer less turbid waters for breeding and molting (Epners et al. 2010).

A component of the work proposed herein focuses on freshwater clams (Corbiculoidea suborder) for two reasons: (1) they are an abundant and important food source for waterfowl (Joyner 1980, Sanchez et al. 2000, Mackie 2007, Richman and Lovvorn 2009), and (2) they can serve as indicators of habitat quality, can be sensitive to changes in water quality (Dussart 1979, Kilgour and Mackie 1991, Joyner-Matos et al. 2007, Roy and Williams 2007, Joyner-Matos et al. 2011), and can alter water quality through the consumption of phytoplankton (Vaughn and Hakenkamp 2001, Mackie 2007, Sousa et al. 2008, Foster et al. 2012). A full characterization of a clam population involves traditional population ecology measures such as abundance, size/frequency distributions and fecundity (Avolizi 1976, Mackie 1978b, Dussart 1979, Kilgour and Mackie 1991, Beekey and Karlson 2003, Guralnick 2004b, Mackie 2007, Roy and Williams 2007, Joyner-Matos et al. 2011), as well as stress-related metrics such as condition index and RNA: DNA (Crosby and Gale 1990, Chicharo and Chicharo 1995, Norkko and Thrush 2006, Joyner-Matos et al. 2007).

Objectives

The objective of my project was to determine if the presence of brook stickleback in the lentic systems at Turnbull NWR is associated with altered macroinvertebrate and submerged macrophyte community characteristics, fingernail clam parameters and condition metrics, and altered water quality parameters. I conducted a field study in which I compared lentic systems that contain brook stickleback with those that are (as yet) free of brook stickleback (Figure 1a; Table 3). I referred to these two categories of

lentic systems as “YesFish” or “NoFish”, but note that brook stickleback are not recognized as an invasive species in the state of Washington (no economic and/or ecological harm has been formally acknowledged). In anticipation of the likely complication of drought during the 2015 field season and the inherent variation across the twelve lentic systems, I complemented the field study with a short-term mesocosm experiment to test hypotheses that link the presence of brook stickleback with alterations in water quality.

The field study contained one additional component, relative differences in hydroperiod. As 2014 was a very dry year, four of the YesFish lentic systems and one of the NoFish systems dried up (Table 4). As the consequences of this shortened hydroperiod are unknown, I compared four lentic systems categories (NoFish-Wet, NoFish-Dry, YesFish-Wet, and YesFish-Dry) to determine whether there are interacting effects of brook stickleback presence and drought. According to M. Rule (Refuge Biologist, Turnbull NWR), we expected that the dry lentic systems would be repopulated with brook stickleback during the winter/spring of 2015, though mechanisms are unknown. Considering that we could not add brook stickleback to the lentic systems at Turnbull NWR (unlike McParland and Paszkowski 2006), the number of lentic systems per category was constrained and unbalanced.

Hypotheses

I addressed several aspects of lentic system community composition that are important for waterfowl, including benthic macroinvertebrate populations, macrophyte abundance and diversity, and fingernail clam population dynamics. I hypothesized that several factors would be decreased in the presence of brook stickleback, including submerged

macrophyte abundance (biomass) and diversity, macroinvertebrate abundance and diversity, and clam abundance and size. I also hypothesized that these factors would be lower in temporary/dry lentic systems than in permanent/wet systems. I hypothesized that stress metrics (lower condition, RNA: DNA, and smaller relative brood size) would be most apparent in clams from fish-containing lentic systems.

To address whether water quality varied across lentic system categories, I measured the following parameters: chlorophyll, transparency, dissolved O_2, conductivity, water temperature, pH, ammonia, nitrate, and phosphate. I hypothesized that the water quality of the lentic systems with brook stickleback in them would be significantly different than those without brook stickleback. Specifically, I hypothesized that chlorophyll, pH, transparency, ammonia, nitrate, and phosphate would be higher in the lentic systems with brook stickleback than in those without brook stickleback. Additionally, I predicted that dissolved O_2 and conductivity would be lower in the YesFish lentic systems. I also hypothesized that the differences in water quality parameters (i.e., the differences between lentic system categories) would be greater if the water bodies experienced drought (lower or absent water levels) during the last spring/summer (2014).

In the laboratory portion of this study, I tested whether the nine water quality parameters listed above were altered by the presence/abundance of brook stickleback. I hypothesized that the water quality measurements would follow similar trends with those from the field study (chlorophyll, pH, transparency, ammonia, nitrate, and phosphate would be higher, and dissolved O_2 and conductivity would be lower). I predicted that

changes in water quality parameters would be greater in the mesocosms that have higher densities of brook stickleback.

METHODS

Sampling Design

In the field study, I collected data from twelve lentic systems, eight of which contain brook stickleback and were categorized as "YesFish", and four that do not have brook stickleback ("NoFish"; Figure 1a). The number of sampling sites and sampling events per lentic system are listed in Table 3. Several characteristics of the lentic systems including size and drainage are presented in Table 4. All sample collections were taken Monday - Friday between 7 am and 5 pm. The twelve lentic systems were pooled together and then randomly distributed over the course of three weeks; this sampling distribution was repeated the next three weeks from April through August 2015 (approximately one sampling per month), coinciding with the Ruddy Duck, Redhead, Mallard and Blue-winged teal breeding and fledging periods (Curry et al. 2007).

Brook Stickleback Presence/Absence

Prior to all measurements and sample collections, baited minnow traps that contained 1 cup of Meow Mix cat foot/trap were set for 24 hours and were used to confirm presence/absence of brook stickleback in each lentic system. No estimates of catch per unit effort were made. Walston et al. (2016) lists the presence of pumpkinseed (*Lepomis gibbosus*) in Blackhorse Lake, Cheever Lake, Turnbull Slough, and West Tritt Lake, speckled dace (*Rhinichthys osculus*) in Windmill Pond, and brown bullhead (*Ameriurus nebulosus*) in Cheever Lake. As I did not find any of these other fish species in any of the minnow traps, methodological descriptions will only refer to brook stickleback.

Selection of Study Sites

The sampling site locations and number of sampling sites per lentic system were determined using stratified-randomized, modified intervals of equal width (EWI) method (U.S.G.S. 2006), and with the use of a global positioning system (GPS) and ArcGIS/ArcMap (Figure 1b). To calculate the area in hectares of each lentic system the "measure" application on the "draw" tool bar in ArcMap was used. For approximately every 1.4 hectares there were three sampling sites (maximum of five sites at a given water body, Norlin et al. 2006). The number of sampling sites were tripled to obtain the number of transect increments. Each lentic system's width was divided by its individualized number of transect increments to obtain the equal width distance between each transect (U.S.G.S. 2006). ArcMap was used to stratify and randomize the previously determined number of sampling site locations by choosing every other or every fourth transect increment location (depending on number of sampling sites and water body size).

Sampling sites were confirmed using GPS points and were marked with flagging tape ($5 \leq$ sampling sites/lentic system ≥ 3). All measurements were taken within the first two meters of water from the shore line because brook stickleback tend to prefer near shore littoral habitats for feeding (Gray et al. 2005). As lentic systems dried up and the shorelines changed, we moved straight in from the shoreline until the new shoreline (initial contact with pond/lake water) was found. When the distance between the two shorelines was less than four meters, all measurements were taken halfway between the two shorelines. Each time macroinvertebrate and macrophyte samples were collected we moved either one meter to the right or left of the previously sampled location at each

sampling site. Table 3 lists the number of study sites per lentic system and the change in the number of sample sites per lentic system over time.

Submerged Macrophytes

At each sampling site within a lentic system, one macrophyte sample was collected between the hours of 7 - 10 am. Within near shore habitats (first 2 m from the shore line) macrophytes were collected to the left or right of the macroinvertebrate sampling location by doing one sweep (sweeping one-meter length) across the benthic material using a standard metal (14 prong) gardening rake. While the sample was still on the rake, the sample was lightly agitated in the water to remove as much sediment as possible. The raked sample was then placed inside a gallon Ziploc bag, put into a 5 gallon bucket and then transported to EWU at room temperature.

The abundance of macrophytes was calculated as the dried biomass in grams (Bayley and Prather 2003, Gray et al. 2005, Norlin et al. 2006). Macrophytes were rinsed with dechlorinated water and processed within hours of collection. All macrophytes were sorted and identified to species, and then placed on lunch trays to dry out at room temperature for 24-48 hours. Once the macrophyte samples were completely dried, the weights (g) were recorded for each species at each site. Appendix 1 contains the 'keys' for the genus and species of macrophytes that we counted and weighed.

Macroinvertebrates

At each sampling site within a lentic system on the same day that macrophytes were sampled, macroinvertebrates were collected within near shore habitats (first 2 meters from the shore line). Macroinvertebrates were collected by a maximum of two standardized sweeps (sweeping one-meter length, a meter stick was used to measure this

distance) across the benthic material using a D-frame dip net (500 µm-mesh, Gray et al. 2005, Hornung and Foote 2006, Norlin et al. 2006, Wieker et al. 2016). If the first sweep was too vigorous (dip net was more than half way full of sediment) or too light (less than a handful of sediment), a new, second sweep was done one meter to the left or right of the original sweeping location. The bottom of the dip net was flush with the benthic sediment. The water level in which we sampled was no more than 1 meter in depth, and no less than the height of the dip net frame (13 cm), therefore, the depth sampled was consistent from the bottom up, but varied from the top down. The sweep (while still in the net) was agitated with our hands to remove as much excess sediment as possible. The sweep net sample was then placed inside a gallon Ziploc back, and water was added to the bag until the sediment and water levels were flush. Macroinvertebrates samples were placed in a 5 gallon bucket and then transported to EWU at room temperature.

The macroinvertebrate samples were diluted with dechlorinated water and processed within hours of collection. All macroinvertebrates visible to the naked eye were separated from any macrophytes and debris and then identified to class or order (Gray et al. 2005, Hornung and Foote 2006, Norlin et al. 2006). Appendix 2 contains the 'keys' that we used for the macroinvertebrate taxa that we counted.

Fingernail Clams

All fingernail clams (*Musculium* spp.) collected during the macroinvertebrate sample sorting were set aside for additional processing. The shell length (from anterior to posterior margin, or adductor to adductor) of each clam was determined by measuring with calipers (Avolizi 1976, Dussart 1979, Kilgour and Mackie 1989, Guralnick 2004b, Joyner-Matos et al. 2011). These clams are ovoviviparous, internally brooding shelled

larvae and extruding shelled juveniles. Counts of larvae and/or juveniles are considered reliable measures of fitness (Mackie 1978a, Martin 1998, Joyner-Matos et al. 2007, Mackie 2007). Adult clams (≥ 5 mm shell length) were dissected and the numbers of brooded larvae were counted as a measure of fecundity (Avolizi 1976, Mackie 1978b, Dussart 1979, Beekey and Karlson 2003, Guralnick 2004b, Roy and Williams 2007). Brood counts included information on the number of brood sacs and the number of larvae per sac that were visible at 15X magnification.

After the number of brooded larvae were recorded, we blot-dried the tissue and weighed the samples (including adult tissue and offspring, no adult shell). The ratio of wet mass (g) to shell volume (shell length x width x height, cm^3, Viergutz et al. 2012) was used to calculate condition index (Bayne et al. 1979, Crosby and Gale 1990, Cataldo et al. 2001, Beekey and Karlson 2003, Norkko and Thrush 2006). The feet from groups of five similarly-sized clams per sampling day/lentic system were pooled, flash-frozen in liquid N_2, and stored at -80°C to be used for RNA: DNA as an indicator of physiological condition.

RNA: DNA

The RNA: DNA was used as an indicator of physiological condition and as a steady-state indicator of population health (Chicharo and Chicharo 1995, Dahlhoff 2004, Norkko and Thrush 2006, Joyner-Matos et al. 2007, Chicharo and Chicharo 2008, Yan et al. 2010).

Nucleic acids were extracted and quantified using standard procedures. Briefly, each pooled tissue sample (containing the foot tissue of five clams), was weighed and then put into the Eppendorf tubes that contained 500 µl of Tris buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA, 2% SDS, pH 8) and five 2 mm glass beads (Bio Spec Products).

The clam samples were incubated for 5 minutes at room temperature with three burst-vortexing events of 45 second each. We then added 500 µl of phenol:chloroform:isoamyl alcohol (PCI, 25:24:1, saturated with 10 mM Tris, pH 8, 1.0 mM EDTA, manufactured by Sigma Life Sciences) to each tube and incubated the tubes for 5 minutes at room temperature with multiple burst-vortexing events of 10 seconds each. Samples then were centrifuged at 14,000x for 10 minutes at 4°C.

The clear supernatant, which contains the nucleic acids, was transferred to new tubes and supplemented with 500 µl of PCI Supernatants, which were then incubated for 5 minutes at room temperature with multiple burst-vortexing events of 10 seconds each. Then, the supernatants were centrifuged at 14,000x for 5 minutes at 4°C. The clear supernatant was transferred to pre-weighed tubes, which were weighed again after the supernatant had been added to allow an estimate of the supernatant volume.

Samples were maintained on ice until nucleic acids were quantified on a Qubit Fluorometer (Life Technologies). RNA samples were diluted 1:10 in TE (10 mM Tris and 1 mM EDTA, stored at room temp) prior to analysis. We used the DNA broad range and RNA high specificity, and followed the manufacturer's instructions.

Water Quality in Field Study

All water sampling and quality measurements were conducted in the afternoon between the hours of 1 - 4 pm. Measurements and samples were taken from a canoe to decrease the chances of altering transparency levels. If there was not enough water in the lentic system to float the canoe, then water measurements were taken in the littoral zone using waders. All measurements were obtained within the first two meters of the shore line, within the vicinity of where macroinvertebrate and macrophyte samples had been

collected earlier that day. Conductivity (μS), temperature (°C), and dissolved O_2 (mg/L) measurements were taken once per site for each sampling day using a YSI model 85 probe provided by Dr. Ross Black (Araujo and Williams 2000, Morgan et al. 2010). A single water sample from each site was obtained for quantifying nitrate, ammonium, phosphate, pH, chlorophyll, and transparency by obtaining one 500 ml water sample after the YSI data was recorded. The 500 ml bottle was rinsed three times in the pond water before the actual water sample was obtained to avoid contamination. The water samples were then transported to EWU in a shaded container at room temperature. Appendix 3 contains detailed protocols for the nutrient analyses.

The 500 ml water samples from each site were shaken and then distributed into multiple containers. A 45 ml water sample to be used for nutrient analysis was filtered, frozen, and stored at -20°C (see below). One volume of approximately 50 ml was used for pH measurement (Fisher Scientific Accumet AB15 Basic pH Meter) and then discarded. Approximately 8-9 ml of water was poured into each of three glass test tubes (6 x 50 mm tube) for triplicate estimates of transparency (based on phytoplankton and inorganic particle abundance). The samples were shaken vigorously immediately before being read in a Turbidimeter (Biolog Turbidimeter Model 21907); the absorbance was recorded to the nearest percentage and then samples were discarded. High values indicate transparent water; low values indicate more turbid samples. As the values are graphed as percentages, these data are identified as "transparency." Then, water was poured into three cuvettes (each approximately ¾ of the way full or about 2.5 ml poured into the 3.5 ml cuvette) to be processed for chlorophyll. Each cuvette was cleaned and dried using a kimwipe, then carefully placed in the fluorometer (provided by Dr. Camille McNeely),

read in RFU (relative fluorometric unit) due to time constraints as a measurement of Chlorophyll (Harmon et al. 2009), and then discarded. Due to time constraints the fluorometer was later calibrated by Dr. Camille McNeely to read chlorophyll in µg/l (a standard unit of chlorophyll) rather than in RFU. As a result, an equation ($y = 0.0656x + 3.9942$) was used to convert the RFU chlorophyll readings into µg/l of chlorophyll.

The remaining water (approximately 50 ml) was filtered and frozen to be later analyzed for nitrate, ammonia, and phosphate levels. For each site, a 45 ml water sample was filtered slowly (1 ml/sec) through a Gelman A/E filter (47 nm, Taylor Scientific and Pall Corporation) contained in a syringe holder that was attached to a 50 ml plastic syringe. The water sample was filtered into a labeled 50 ml plastic tube, and then capped and sealed closed with parafilm. The water sample was stored at -20°C. Any remaining water was poured down the sink.

We used a Flow Solution 3100 (OI Analytical) flow analyzer to quantify nitrate, ammonia and phosphate levels (Bakker et al. 2010). Briefly, nitrate was converted to nitrite through a reduction reaction in the presence of cadmium. To produce a colored dye that was detected at a 450 nm wavelength, the nitrite (both the newly formed nitrite and what was in the sample originally) were mixed *N*-(1-naphthyl) ethylenediamine dihydrochloride and sulfanilamide. The assay was then repeated without the cadmium step to quantify the nitrite that was originally in the sample and then used to calculate the nitrate level.

Phosphate levels were determined using the orthophosphate procedure. Briefly, orthophosphate, molybdenum (VI), and antimony (III) were mixed in acidic conditions;

the mixture was reduced with ascorbic acid, forming a colored solution with an absorbance that was quantified at 880 nm wavelength.

Ammonia was quantified by the Total Kjeldahl Nitrogen method. Briefly, samples were boiled at a very high temperature with sulfuric acid, potassium sulfate, and a copper-containing compound; in these conditions, ammonia was converted to ammonium sulfate. The sample was brought to a pH of 11 and ammonia gas was trapped in an alkaline hypochlorite solution (forming chloramine). Chloramine was mixed with salicylate to form a solution that was blue; absorbance was measured at 660 nm wavelength.

Mesocosm Experiment

The mesocosm experiment was conducted from June through July at the Turnbull Laboratory for Ecological Studies (TLES), Cheney, WA. The mesocosm design was based upon studies that explored changes in water quality parameters with the addition of three-spined stickleback (Beklioglu and Moss 1998, Stephen et al. 2004, Harmon et al. 2009, Sorf et al. 2015). The established mesocosm tanks (100 Gallon Rubbermaid Stock Tanks) were kept at replicate conditions approximately 30 feet uphill from the TLES Pond. There were three treatment levels of brook stickleback in each set of 100 Gallon mesocosm tanks, with 10 replicate mesocosms per treatment (Stephen et al. 2004, Harmon et al. 2009): no fish, low fish (4 fishes) and high fish (8 fishes). Each tank was randomly assigned a treatment level of brook stickleback.

Mesocosms were filled with water from TLES Pond (approx. 80 L/tank) by use of a pump with a mesh filter (500 µm) attached that was placed in the deepest portion of the pond. At the end of the pump where the water was poured into the tanks, a finer mesh

filter (200 µm) was attached. The pump ran for approximately 7 min at each tank, filling the tanks with approximately 80 L of pond water.

On the day that the tanks were filled, two stove pipe samples that contained sediment, macroinvertebrates and macrophytes were added to each tank from within the first two meters from the shoreline of the TLES pond (20 cm of sediment, Beklioglu and Moss 1998, Chase 2003, Stephen et al. 2004, Harmon et al. 2009, Sorf et al. 2015). Stove pipe samples were collected by lightly digging a hollow 20 gallon round Rubbermaid Brute plastic trashcan into the water/sediment. Then, a D-frame net (500 µm-mesh) was dragged in a circular motion around the inside of the trashcan to scoop up all of the macroinvertebrates, sediment, and macrophytes. The scoop was then transferred to a 2.5 gallon bucket, and the scooping process was repeated to ensure that all organisms were collected. The stove pipe was then moved to a new location within the pond for another stovepipe sample/scoop (which was added to the same 2.5 gallon bucket). One, 2.5 gallon bucket containing two compete stovepipe samples/scoops was added to each mesocosm tank. The mesocosms containing water, sediment, macroinvertebrates, and macrophytes were allowed to settle for 1 week before the zooplankton were added to them (Beklioglu and Moss 1998, Harmon et al. 2009).

Zooplankton samples were obtained from the middle of the TLES Pond, off the side of a canoe, using 200 µm-mesh plankton nets with a diameter of 0.5 m and tow length 0.75 m. A total of two plankton tows were added to each 2.5 gallon bucket. One bucket of zooplankton was randomly added to each tank. The tanks were allowed to establish for another week. Individual zooplankton were not counted.

Initial water quality measurements (transparency, temperature, pH, nutrients, conductivity, and dissolved O_2) were conducted after zooplankton were established for one week. Water quality measurements were conducted as described above in the field study, with temperature, conductivity, and dissolved O_2 measured by YSI meter, and chlorophyll, transparency, nutrients, and pH measured in a sample of 50 ml collected from each mesocosm.

Brook stickleback were added after initial water quality measurements were conducted. Brook stickleback were obtained from Cheever Lake through the use of baited minnow traps and transported to the mesocosms (minnow traps were set out with approximately 1 cup of Meow Mix cat food for 12 hours). Cages were set in the evening the day before the fish were to be sorted and placed in their mesocosm tanks. Only the apparently healthy and similarly-sized fishes (snout to tail length) were used for this experiment. Once the fish were added, water quality measurements were measured once per week over four weeks at mid-depth (pH, transparency, chlorophyll, ammonia, nitrate, and phosphate) or at surface level (temperature, conductivity, and dissolved O_2) within the mesocosm tanks (Beklioglu and Moss 1998, Chase 2003, Stephen et al. 2004, Harmon et al. 2009, Sorf et al. 2015). Any fish that died in the mesocosm tanks were replaced with freshly caught (from Cheever Lake), similarly-sized fish (Stephen et al. 2004, Harmon et al. 2009). The experiment was started on 6/6/2015 and concluded on 7/27/2015. The fish were present for only four weeks as the water levels were dropping rapidly in late July and fish mortality was occurring.

Statistical Analyses

Macrophyte and macroinvertebrate abundance and number of taxa were compared across lentic system categories by repeated measures analysis of variance (RM-ANOVA) with

Tukey HSD posthoc tests. Count data were log(x+1) transformed prior to analysis. These analyses were conducted with the lme4, effects, and multicomp library packages in R/R-Studio (version 3.0).

Non-metric multidimensional scaling (NMDS) analyses were conducted in R to characterize the lentic system categories on the basis of macroinvertebrate data (untransformed counts) or water quality data (excluding ammonia, nitrate, and phosphate). NMDS was not conducted with macrophyte data because of the large number of zero values. In the macroinvertebrate NMDS, the raw data were untransformed abundances for each sampling site/time combination; the analysis accounted for pseudo replication within lake and repeated measures. The ordination with the lowest final stress value was selected. NMDS scores were analyzed by RM-ANOVA and Tukey HSD tests.

Clam abundance was analyzed by RM-ANOVA in SigmaPlot (version 11.0) with Holm-Sidak pairwise multiple comparisons. Most of the remaining clam datasets failed the assumptions of normality and/or equal variance and thus were analyzed by Kruskal-Wallis ANOVA in SigmaPlot with the Dunn's Method multiple comparison procedure, which does not include an adjustment for ties. Relationships between clam brood size and adult shell length were characterized by linear regressions (SigmaPlot). Size-frequency distributions were compared across lentic system categories by Chi square analysis with 10,000,000 simulations in R.

Comparisons in water quality data among lentic system categories were characterized by RM-ANOVA in R. Relationships between lentic system size, select water quality and macroinvertebrate metrics were analyzed by Spearman Rank Order Correlation, and by Best Subset Regression in SigmaPlot. Differences between the two

main watersheds (Cow Creek and Rock Creek) were assessed with t-tests or Mann Whitney Rank Sum tests (SigmaPlot). Water quality data from the mesocosm study were analyzed by Kruskal-Wallis ANOVA in SigmaPlot.

RESULTS

Samplings and Brook Stickleback Presence

The number of sampling sites and visits for the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories throughout the 2015 field season can be found in Table 3.

Brook stickleback were found throughout the 2015 field season in Kepple Lake, Cheever Lake, Windmill Pond, 30 Acre Lake, Blackhorse Lake, Turnbull Slough (Upper), and West Issacson Lake. Previous surveys by Walston et al. (2016) found brook stickleback in all of the previously mentioned lentic systems except for West Issacson Lake, and confirmed brook stickleback presence in West Tritt Lake. Walston et al. (2016) also lists the presence of pumpkinseed (*Lepomis gibbosus*) in Blackhorse Lake, Cheever Lake, Turnbull Slough, and West Tritt Lake, speckled dace (*Rhinichthys osculus*) in Windmill Pond, and brown bullhead (*Ameriurus nebulosus*) in Cheever Lake. However, my survey during the 2015 field season did not find any other fish species besides brook stickleback in the 12 lentic systems.

Macrophyte Collection and Identification

When averaged across all lentic system categories, the submerged macrophyte dried biomass (g) increased towards the end of the 2015 field season (Figure 2; RM-ANOVA, $p < 0.0001$). The NoFish-Wet lentic systems had the greatest abundance of dried macrophyte biomass (Figure 3; RM-ANOVA, $p < 0.001$ vs. YesFish-Dry; $p < 0.002$ vs. YesFish-Wet; $p < 0.003$ vs. NoFish-Dry). The average dried biomass of macrophytes for

each of the four lentic system categories, when averaged across sampling date and water body, were: NoFish-Dry, 0 g macrophytes; NoFish-Wet, 65 g macrophytes; YesFish-Dry, 25 g macrophytes; and YesFish-Wet, 38 g macrophytes (sampling sizes for each category are in Table 5).

The NoFish-Wet lentic systems had significantly more macrophyte species than did any other lentic system category (Figure 4 and Table 5; RM-ANOVA, $p < 0.0004$ vs. YesFish-Dry; $p < 0.002$ vs. YesFish-Wet; and $p < 0.0003$ vs. NoFish-Dry). The YesFish-Dry systems had fewer macrophyte species than did the YesFish-Wet systems (RM-ANOVA, $p < 0.008$).

Within the NoFish-Wet category, the two larger water bodies, Campbell – Lasher and Long Lake, had at least double, if not triple, the macrophyte biomass than did TLES Pond (Tables 5 and 6). The dominant species in Long Lake was *Ceratophyllum demersum* (Coontail) and in Campbell – Lasher Lake was *Vallisneria americana* (Wild Celery). In the YesFish-Dry category (Table 7), study sites at 30 Acre Lake had substantially lower macrophyte biomass than the other lentic systems, which was solely composed of *Potamogeton pectinatus* (Sago Pondweed). The other three water bodies in this category were dominated by Wild Celery and Sago Pondweed. The YesFish-Wet category (Tables 8a and 8b) had two lentic systems at opposite ends of the spectrum of macrophyte biomass, with Kepple Lake averaging 100 g of macrophyte biomass (predominantly Coontail) whereas Windmill Pond averaged 3.8 g (predominantly *Elodea canadensis*, or Waterweed). The two lentic systems with intermediate macrophyte biomass, Cheever Lake and Windmill Pond, were dominated by Waterweed (and *Potamogeton richardsonii*, Richardson's Pondweed in Cheever).

Macroinvertebrate Collection and Identification

When averaged across all lentic system categories, the macroinvertebrate abundance increased towards the end of the 2015 field season (Figure 5; RM-ANOVA, $p < 0.0002$). Throughout the season, the NoFish-Wet lentic systems had a greater abundance of macroinvertebrates than did the YesFish-Dry and YesFish-Wet lentic systems (Figure 6 and Table 9; RM-ANOVA, $p < 0.001$ and $p < 0.004$, respectively). The number of total macroinvertebrates for each of the four lentic system categories, when averaged across sampling date and water body, were: NoFish-Dry, 124 macroinvertebrates; NoFish-Wet, 242 macroinvertebrates; YesFish-Dry, 93 macroinvertebrates; and YesFish-Wet, 129 macroinvertebrates.

The NoFish-Wet lentic systems had significantly more macroinvertebrate taxa than did the other categories (Figure 7 and Table 9; RM-ANOVA, $p < 0.003$ vs. YesFish-Dry; $p < 0.001$ vs. YesFish-Wet; and $p < 0.008$vs. NoFish-Dry). The YesFish-Wet systems had more macroinvertebrate taxa than did the YesFish-Dry systems (RM-ANOVA, $p < 0.007$).

Within the NoFish-Wet category (Table 10), the largest lentic system, Long Lake, had at least double, if not triple, the number of macroinvertebrates than did Campbell – Lasher Lake and TLES Pond. The most abundant taxa in Long Lake were amphipods and chironomids. For example, the number of amphipods found during the fourth sampling cycle at Long Lake was 1,494 individuals. Campbell – Lasher Lake had the highest number of macroinvertebrate taxa, the most abundant of which were ephemeropterans, chironomids and amphipods. In the YesFish-Dry category (Table 11), the Turnbull Slough had substantially higher numbers of macroinvertebrates than the other lentic

systems, with the highest counts in ephemeropterans, corixids, and chironomids. In the second sampling cycle, Turnbull Slough contained 1,308 chironomids. In contrast, West Issacson and 30 Acre Lake were dominated by clams (Sphaeriidae) and chironomids. Blackhorse Lake had the fewest taxa; the most abundant were corixids and chironomids. The YesFish-Wet category (Table 12a and 12b) had two lentic systems (Kepple Lake and West Tritt Lake) that contained about twice as many macroinvertebrates as the other two systems (Cheever Lake and Windmill Pond). Kepple Lake was dominated by ephemeropterans, chironomids, and clams and West Tritt Lake by ephemeropterans and chironomids. Both Cheever Lake and Windmill Pond were dominated by chironomids and snails (Hygrophila).

The NMDS for macroinvertebrate community composition is presented with the four categories and the vectors in Figure 8a, and the categories with the lentic systems identified in Figure 8b. Boxplots for the axis scores are in Figures 9a and 9b. The proportion of the error variance that was explained by lentic system ID and the repeated nature of the sampling was 76.2%. The NoFish-Wet lentic systems had significantly more positive scores on the MDS1 axis than did the two YesFish lentic system categories (RM-ANOVA; $p < 0.001$ vs. YesFish-Dry; $p < 0.002$ vs. YesFish-Wet). Positive scores on the MDS1 axis are associated with amphipods, zygopterans and notonectids. The relationship between notonectid abundance and this axis is almost entirely driven by one sampling event in Long Lake; the other two macroinvertebrate taxa were abundant in all three lentic systems.

On the MDS2 axis, the YesFish-Wet lentic systems had significantly more negative scores than did the YesFish-Dry (RM-ANOVA; $p < 0.002$) and NoFish-Wet

systems (RM-ANOVA; $p < 0.001$). This partly reflects the lower abundance of "other" beetles (not diving beetles) in the YesFish-Wet systems; patterns in hydracarina and chironomid abundance were not consistent across lentic system category.

Clam Characterization and RNA: DNA

Clam abundance—In all lentic systems in which we found clams, abundance decreased over time (Figure 10). Clam abundance differed significantly across category (RM-ANOVA, $p = 0.003$) when Stubblefield Lake (NoFish-Dry) was included in the model. In the full model, the 'zero clam' results for the NoFish-Dry were significantly different from each of the other three categories. When the model was run without Stubblefield Lake, clam abundance differed across the remaining three categories (Figure 11; RM-ANOVA, $p = 0.032$), with clam abundance significantly higher in the NoFish-Wet lentic systems than in the YesFish-Wet systems. There was variation within category (Table 13); TLES Pond had significantly higher clam abundance than either Long Lake or Campbell Lake (RM-ANOVA, $p < 0.001$).

Clam Length—When we consider all clams collected, clam length differed significantly across the three lentic system categories (Figure 12a; K/W ANOVA, $p < 0.001$), with the longest clams found in the YesFish-Dry, and followed by the YesFish-Wet category. However, when we focus on adult clams (≥ 5 mm shell length), the clams from the YesFish-Wet lentic systems were significantly larger than those in the other two lentic system categories (Figure 12b; K/W ANOVA, $p < 0.009$).

Condition Index— Condition index in clams is calculated as the tissue mass divided by the shell length; this was determined only for adult clams. The adult clams in the NoFish-Wet lentic systems had the highest condition index (Figure 13; K/W ANOVA, $p < 0.001$;

Table 14). Clams from the YesFish-Dry systems had a lower condition index than did clams from the YesFish-Wet systems (K/W ANOVA, $p < 0.001$).

RNA: DNA—The ratio of RNA to DNA (RNA: DNA) in the tissue of adult clams did not differ significantly across lentic system category when analyzed as a 'raw' variable (ng/mg tissue; Figure 14a; K/W ANOVA, $p = 0.236$) nor when corrected for clam size ((ng/mg)/length in mm; Figure 14b; K/W ANOVA, $p = 0.216$).

Brooded Larvae—Clams from the NoFish-Wet lentic systems had significantly larger brood sizes than did clams from fish-containing systems (Figure 15; K/W ANOVA, $p < 0.05$). This pattern remained the same when the brood sizes were corrected for adult shell length (Figure 16; K/W ANOVA, $p < 0.05$).

When summed across all lentic system categories, the number of brooded larvae was positively related to the adult clam length (Figure 17; N = 306, $y = 3.28x - 12.01$, $R^2 = 0.299$, $p < 0.001$). There was one very large clam that had a large brood which collected from West Issacson Lake; removing this clam from the regression (data not shown) did not alter the relationship between the variables (N = 305, $y = 3.14x - 11.26$, $R^2 = 0.269$, $p < 0.001$).

We examined the relationship between brood size and adult clam size separately for each lentic system category. There was no relationship between the two variables in clams from the YesFish-Wet lentic systems (specifically Kepple Lake; Figure 18; N = 82, $y = 1.2222x - 0.0792$, $R^2 = 0.025$, $p = 0.1545$). There was a positive relationship between brood size and adult clam length within the YesFish-Dry lentic systems (Figure 19; West Issacson Lake, N = 115, $y = 3.1872x - 11.6406$, $R^2 = 0.24$ $p < 0.0001$; 30 Acre Lake, N = 51, $y = 4.0492x - 18.0905$, $R^2 = 0.425$, $p < 0.0001$). There was a positive relationship

between brood size and adult clam length in clams collected from TLES Pond (NoFish-Wet lentic system; Figure 20; N = 44, y = 4.1228x – 15.9059, R^2 = 0.604, p < 0.0001), but not in clams from Campbell-Lasher Lake (N = 14, y = 0.4907x + 6.0462, R^2 = 0.010, p = 0.733).

Size-Frequency Distributions – The size-frequency distribution of clams in the three lentic system categories differed significantly (Figure 21; Chi Square, p < 0.001). The YesFish-Wet lentic systems tended to have the highest proportion of adult clams and the NoFish-Wet systems tended to have the lowest proportion of adult clams. The size-frequency distributions are presented in Figures 22a – 22c grouped by lentic system category but graphed independently for each lentic system. The graphs for most of the lentic systems illustrate the growth of a single cohort of clams from recently-extruded juveniles (~ 1 mm in shell length) to reproductively mature adults (≥ 5 mm shell length). In some lentic systems, such as TLES Pond, Campbell - Lasher Lake, and Kepple Lake, the collection of 1 – 2 mm clams at later sampling points, after at least some clams had reached adult size, potentially indicates the production of a second cohort of individuals.

Water Quality Parameters

Water collected from lentic systems in the YesFish-Wet category had lower chlorophyll (µg/L) than did water collected from the YesFish-Dry lentic systems (Figure 23 and Tables 17 and 18; RM-ANOVA, p = 0.031). There were no significant differences in chlorophyll content among the other three categories.

The water collected from lentic systems in the YesFish-Wet category were less turbid (higher transparency or % absorbance based on phytoplankton and inorganic particle abundance) than did the water collected from the YesFish-Dry and NoFish-Dry

system categories (Figure 24; RM-ANOVA, $p < 0.002$ and $p < 0.044$, respectively). There was no significant difference between the two perennial categories nor between the two "Dry" lentic system categories.

There were no significant differences in dissolved O_2 level (mg/L) across the four lentic system categories (Figure 25; RM-ANOVA, $p = 0.405$).

Water conductivity (μS) tended to be the lower in the NoFish-Dry lentic system (Figure 26; RM-ANOVA, $p < 0.002$) than in the NoFish-Wet and YesFish-Dry systems. The YesFish-Dry category tended to have the highest conductivity levels and NoFish-Dry had the lowest (Tables 15 and 16; RM-ANOVA, $p < 0.002$); YesFish-Dry systems had significantly higher conductivity level than the YesFish-Wet (RM-ANOVA, $p < 0.026$). NoFish-Wet systems had higher conductivity levels than the NoFish-Dry systems (RM-ANOVA, $p < 0.022$).

There were no significant differences in water temperature (°C) among the four lentic system categories (Figure 27; RM-ANOVA, $p = 0.274$).

The NoFish-Dry lentic system category had lower pH than the NoFish-Wet and YesFish-Dry systems (Figure 28; RM-ANOVA, $p < 0.007$and $p < 0.022$, respectively).

The NMDS for water quality (excluded ammonia, nitrate, and phosphate) is presented with just the four categories and with the vectors in Figure 29a, and the categories with the lentic systems identified in Figure 29b. Boxplots for the axis scores are in Figures 30a and 30b. The proportion of the error variance that was explained by lentic system ID and the repeated nature of the sampling was 88.6%. The lentic system category scores did not differ significantly on the MDS1 axis; no single factor showed any obvious correlation with the MDS1 axis. On the MDS2 axis, the NoFish-Dry lentic

system category had significantly more positive scores than did either Wet category (RM-ANOVA; $p = 0.032$ vs. NoFish-Wet; $p = 0.028$ vs. YesFish-Wet); this pattern is likely due to the lower conductivity in Stubblefield Lake than in any other system.

Ammonia levels (ppt) did not differ significantly among the four lentic system categories (Figure 31; RM-ANOVA, $p = 0.973$). Water from the dry lentic systems tended to have lower nitrate (ppt) than the wet systems within each fish category; this difference was significant within the NoFish category (Figure 32; RM-ANOVA, $p = 0.002$). Water from the NoFish-Dry lentic system had the highest phosphate level (ppt) (Figure 33; RM-ANOVA, $p < 0.001$).

Watershed and Size Patterns

As Stubblefield Lake does not contribute to the Cow Creek or Rock Creek watersheds, it was not included in comparisons between watersheds (Table 4). The Cow Creek watershed, which is on the western half of the refuge, contains lentic systems in the three remaining categories; the Rock Creek watershed, which is on the eastern half of the refuge, only contributes to the YesFish categories. Without respect to lentic system category, Cow Creek watershed was characterized by larger lentic systems (t-test, $p = 0.006$), higher conductivity (Rank Sum, $p = 0.028$), and higher macroinvertebrate abundance (Rank Sum, $p = 0.017$). The macroinvertebrate abundance result likely is due to the high number of ephemeropterans (Rank Sum, $p = 0.017$), chironomids (Rank Sum, $p = 0.03$), and amphipods (Rank Sum, $p = 0.009$) in the Cow Creek watershed.

I next examined relationships between lentic system size (including Stubblefield Lake, excluding TLES Pond as its size is not reported by the refuge), water quality and macroinvertebrates using best subset regression and Spearman Rank correlations (as we

could not evaluate normality in the best subset regression), which gave somewhat similar results. The best subset regression modelled factors related to the size of lentic system because preliminary analyses indicated that many watershed-level or category-level differences were driven by Upper Turnbull Slough and Long Lake (which are large) versus 30 Acre Lake and Windmill Pond (which are small). The best subset regression developed three models for factors related to lentic system size in which most variance inflation factors were ≤ 1.5. The first model contained only mean conductivity ($R^2 = 0.725$, $p < 0.001$); the second model ($R^2 = 0.868$) contained conductivity ($p = 0.002$) and macroinvertebrate abundance ($p = 0.019$); the third model ($R^2 = 0.945$) contained conductivity ($p = 0.002$; VIF = 1.529), macroinvertebrate abundance ($p = 0.006$) and chlorophyll level ($p = 0.016$).

The Spearman Rank Correlations were assessed with an acceptable error rate of $p \leq 0.001$; variables were selected based on their contribution to the best subset regression, the NMDS, or the watershed-level differences. The following variables positively correlated with lentic system size: conductivity ($r = 0.809$, $p = 0.0009$), macroinvertebrate abundance ($r = 0.836$, $p < 0.0001$), ephemeropteran abundance ($r = 0.773$, $p = 0.0037$), and chironomid abundance ($r = 0.809$, $p = 0.0009$). The following variables were positively correlated with conductivity: temperature ($r = 0.783$, $p = 0.0014$) and ephemeropteran abundance ($r = 0.846$, $p < 0.0001$). Unsurprisingly, given their high abundance, chironomid abundance was significantly correlated with macroinvertebrate abundance ($r = 0.895$, $p < 0.0001$).

Mesocosms

The following water quality measures did not differ among the three categories: chlorophyll level (Figure 34; K/W ANOVA, $p = 0.591$); transparency (Figure 35; K/W ANOVA, $p = 0.155$); dissolved O_2 (Figure 36; K/W ANOVA, $p = 0.989$); conductivity (Figure 37; K/W ANOVA, $p = 0.333$); temperature (Figure 38; K/W ANOVA, $p = 0.530$).

The HighFish mesocosm category had the highest pH (Figure 39; K/W ANOVA, $p < 0.001$ and $p = 0.007$ respectively). None of the three nutrients differed significantly across mesocosm category (ammonia: Figure 40, K/W ANOVA, $p = 0.623$; nitrate: Figure 41, K/W ANOVA, $p = 0.165$; phosphate: Figure 42, K/W ANOVA, $p = 0.245$).

DISCUSSION

The overall goal of this project was to determine if the presence of brook stickleback in the lentic systems at Turnbull NWR is associated with altered habitat characteristics that are important for waterfowl breeding success, as the refuge provides essential habitat for breeding, migratory and wintering waterfowl (Curry et al. 2007). Those habitat characteristics include water quality parameters, macroinvertebrate and macrophyte community characteristics, and fingernail clam population and stress metrics. The presence of brook stickleback might be problematic for waterfowl as both brook stickleback and waterfowl consume similar prey items such as, vascular plants and angiosperm seeds, and various macroinvertebrates such as Mollusca, Chironomidae, and Diptera (Kenow 1996, Sanchez et al. 2000, Dessborn et al. 2011, Tidwell et al. 2013). I conducted a field study in which I collected data from 12 lentic systems, of which eight contained brook stickleback and four did not. Within each fish category (with or without

brook stickleback), the lentic systems were further subdivided into two groups, temporary (dry) or permanent (wet). Within each of the four lentic system categories I quantified the macrophyte abundance (biomass) and species diversity, macroinvertebrate abundance and taxonomic diversity, water quality metrics, and fingernail clam population data. I tested whether each of these datasets differed among the four lentic system categories or across the two main watersheds on the refuge. I also explored relationships among metrics and relationships with lentic system size.

Overall, there were clear differences between watersheds, with larger lentic systems, higher conductivity levels, higher macroinvertebrate abundances (specifically larger abundances of ephemeropterans and chironomids) in the Cow Creek watershed (western half of the refuge) compared to the Rock Creek watershed (eastern half). This pattern is likely due, in part, to the positive relationships between lentic system size, conductivity, and macroinvertebrate abundance (more chironomids).

Although watershed ID and lentic system size were influential, we nonetheless were able to detect some differences across lentic system categories. In general, the NoFish-Wet category had more macroinvertebrate and macrophyte taxa/species diversity, more macrophyte dried biomass, the highest fingernail clam condition index, and the highest clam brood sizes. The NoFish lentic systems had more abundant macroinvertebrates, particularly amphipods. The additional patterns in individual macroinvertebrate taxa were influenced by lentic system category and size. The wet/dry classification, rather than the presence/absence of brook stickleback, strongly influenced the number of macrophyte species and macroinvertebrate taxa. There appeared to be synergistic effects between the presence of brook stickleback and dry status for fingernail

clam condition index, clam length, chlorophyll, transparency, nitrate levels, and pH. Overall, it appears that the presence of brook stickleback most likely affects habitat quality characteristics in lentic systems that are temporary (dry), especially those that also are smaller in size. Therefore, if the refuge managers aspire to take action about the brook stickleback presence, they might make the strongest impact by eliminating brook stickleback from isolated, temporary (dry) systems.

In 2015, 85% of Washington State experienced at least a D3 drought, or extreme drought, by the end of August (drought scale ranges from D0 – D4), whereas in 2014, 0% of the state was at least a D3 and only 20% was at least a D2 or severe drought (Fuchs 2016). The 2015 drought is evident in my study, as lentic systems that were classified by the refuge as permanent were not accessible for the entire season. I was able to visit two of the lentic systems that were characterized as "wet", Campbell – Laser Lake and TLES Pond, only five times. Additionally, West Tritt Lake had a limited collection in the sixth collection cycle. As for those lentic systems characterized as "dry", Stubblefield Lake was dry after the second collection, and Blackhorse and 30 Acre Lake were dry after the fourth collection cycle. As these unusually hot and dry conditions likely altered water quality and macroinvertebrate patterns, this study should be repeated in a more typical or even a 'wet' year. This is especially important for the evaluation of the apparent synergism between brook stickleback presence and temporary/permanent (dry/wet) status, as this synergism may have been exaggerated by the 2015 drought.

Stubblefield Lake differed in many parameters from other lentic systems, likely due to its history and geography. Stubblefield Lake was the only lentic system on the refuge that was not drained for agricultural purposes in 1910-1912 (Curry et al. 2007).

Additionally, it is in its own drainage (Philleo Lake) and has no surface outlet (Curry et al. 2007). Stubblefield Lake is a playa lakes, which are characterized as temporary, found in semiarid regions, recharged by ground water, and belong to closed drainage basins, which means there is no outflow to rivers (Gurdak and Roe 2010). Stubblefield Lake had some unique characteristics, which included a low pH and high phosphate level; these factors may relate to the geochemistry of playa lakes. Neither pH nor phosphate levels varied consistently across either of the fish or wet/dry categorizations. In addition, it was difficult to characterize Stubblefield Lake because the drought only allowed two sampling cycles. Surprisingly, I observed the highest waterfowl abundance of any lentic system on Stubblefield Lake when the water was present. Stubblefield Lake was excluded from clam analyses because no clams were found in it. In addition, it had no submerged macrophytes. However, to be conservative, I included the 'zero' data points for macrophytes in the RM-ANOVA's, which may have skewed the influence of this category (NoFish-Dry) in the analyses.

Overall, in most lentic systems, macroinvertebrate abundance increased over time, with the exception of the YesFish-Dry category, which had irregular abundances. This variation with the YesFish-Dry category could reflect the compounded effects of brook stickleback presence and seasonality, in that more permanent ponds tend to have more macroinvertebrates (Brooks 2000). The factor that was most consistently related to the presence/absence of brook stickleback (with little to no contribution from hydroperiod) was the macroinvertebrate abundance. The greater abundance of macroinvertebrates within the NoFish lentic systems than in the YesFish systems was primarily driven by amphipod abundance. This is reasonable considering brook stickleback consume

amphipods (Stewart et al. 2007, Wieker et al. 2016). The consumption of amphipods by brook stickleback is problematic because migrating, breeding and nesting ducks and ducklings feed on amphipods (Sanchez et al. 2000, Epners et al. 2010, Anteau et al. 2011). Amphipods are also important to the lentic systems as they are detritivores, meaning they consume organic matter, algae, and bacteria (Strong 1972, Anteau et al. 2011).

The NoFish category also had larger abundances of ephemeropterans, zygopterans, and fingernail clams than did the YesFish categories. Although the abundance of ephemeropterans is greatest in the NoFish categories, this is likely a function of lentic system size as ephemeropteran abundance was higher in larger lentic systems, regardless of brook stickleback presence/absence. The maximum number of ephemeropterans across all categories was in West Tritt Lake (YesFish-Wet). The abundances are also likely influence by watershed identity (more in Cow Creek), which is similarly influenced by lentic size as most of the larger lentic systems are within the Cow Creek watershed. The largest abundances of ephemeropterans were in larger lakes, which is somewhat surprising as ephemeropterans prefer running water to standing water; however, they are capable of thriving in poor water quality conditions (Ulfstrand 1968), which may explain their high abundance in West Tritt Lake. The abundance of ephemeropterans also may be related to the length of the hydroperiod because water temperature warms throughout the season or as the water level drops. Ephemeropteran egg development rate increases with elevated water temperature, shortening the time the population spends in the egg stage and ultimately increasing the abundance of nymphs (Ulfstrand 1968). The potential relationship between brook stickleback presence and

ephemeropteran abundance is unclear as brook stickleback do not typically consume ephemeropterans, with the exception of Wieker et al. (2016), who observed consumption of ephemeropteran nymphs by brook stickleback in artificial, laboratory conditions. Even though brook stickleback and waterfowl typically do not consume ephemeropterans, various fish and other birds do consume them (Thorp and Rogers 2015). Potential decreases in ephemeropteran populations at the refuge also is problematic as ephemeropteran nymphs are detritivore/herbivores, and occasionally feed on chironomids (Edmunds et al. 1976).

Similarly to that of the ephemeropterans, zygopterans tended to be very low to absent in YesFish categories; this may be indirectly related to the presence/absence of brook stickleback as brook stickleback do not typically eat zygopterans. Zygopteran abundance was directly related to lentic system size, except in the YesFish-Dry category. The lower abundance of zygopterans in the YesFish systems may in part be due to the absence of waterweed, because waterweed decreases the chances of predation of zygopterans by fish (Manatunge et al. 2000). The presence of waterweed in TLES Pond, one of the smaller lentic systems, may reduce predation on zygopterans from waterfowl. Finally, zygopterans play an important role in lentic systems as they consume chironomids, ephemeropterans, and amphipods (Thompson et al. 2000).

The second strongest factor related to brook stickleback presence/absence was macrophyte dried biomass, as it was higher in the NoFish-Wet category than any other category. Macrophyte biomass is somewhat consistent with lentic system size, where biomass was highest in the largest lentic systems (including a YesFish lentic system, Kepple Lake, but excluding the YesFish system, Turnbull Slough). In contrast to my

results, a study by Norlin et al. (2005) found that shallow-water wetlands that contain brook stickleback had more macrophyte biomass.

Coontail was present and the most abundant in all three lentic systems in the NoFish-Wet category. Coontail is less abundant in lentic systems that contain fish because fish consume macroinvertebrates, which increases the abundance of phytoplankton and ultimately limits the amount of transparent sunlight that is used for photosynthesis (Williams et al. 2002). Coontail abundance was high in Kepple Lake (YesFish-Wet); otherwise, the abundance of Coontail was low to absent in all other lentic systems in the YesFish categories, especially in YesFish-Dry.

The abundance of wild celery is likely related to lentic system size, as the abundance of wild celery increases with size (McFarland and Shafer 2008). The larger systems in the NoFish-Wet category, Campbell – Lasher Lake and Long Lake, had greater abundance of wild celery, and West Issacson Lake, a large system in the YesFish-Dry category, had a high abundance of wild celery. However, the other large lentic system within the YesFish-Dry category, Turnbull Slough, and a large system in YesFish-Wet, West Issacson, had a low abundance of wild celery, possibly because they are in the same drainage. The greater abundance of wild celery might also be contributed to lentic system size because it is better pollinated in deeper, stratified water, allowing longer photoperiods (McFarland and Shafer 2008).

Another strong factor relating to brook stickleback presence/absence was fingernail clam brood size, which was lower in both YesFish categories. Ovoviviparous clams, like fingernail clams, will alter brood size in response to a number of factors in addition to adult shell length (Mackie 2007), including substrate type (Mackie and Qadri

1978), parasitism by trematodes (Mackie 1976), season (Dietz and Stern 1977), and dissolved O_2 content (Joyner-Matos et al. 2011). Fingernail clam brood sizes tended to be larger in marshes and ponds than in large lakes (Guralnick 2004a) and larger in permanent ponds than in temporary ponds (Hornbach et al. 1980). The water chemistry components that are most closely tied to brood sizes are bicarbonate and phosphate ions and the univalent and divalent metal ions (Dussart 1979). The relationship between brood size and hydroperiod depends upon species, as some continue to develop brood while estivating(McKee and Mackie 1983) while others halt brood development or even reabsorb brooded larvae during periods of desiccation (Mackie 2007). To our knowledge, no previous study has linked brood size or trade-offs between brood size and somatic growth to a biotic factor like predation (brook stickleback consumed clams in lab conditions, Wieker et al. 2016).

Surprisingly, brood size did not increase with lentic system size, possibly due to uneven sampling size of clams in each lentic system or seasonality, as clams enter a period of high reproductive activity later in the year, and I did not sample late enough in the season (Guralnick 2004a). Clams were typically smaller in the lentic systems that were about to dry up (typically within last sampling cycle clams were found), as the most successful clams at 'over-wintering' or surviving in sediment are the young, 1 mm shelled juveniles (Guralnick 2004a).

Although the sample sizes are too low to make concrete statements, it is possible that the clams are making a trade-off between somatic growth and reproduction, depending on whether they are in lentic systems that contain brook stickleback and depending upon the availability of water. At the height of reproductive activity, a clam

may have up to 60-70% of its tissue mass as developing larvae (Dietz and Stern 1977); this necessarily requires compensatory decreases in glycogen (stored carbohydrate reserves) and total protein. The active brood production must be supported by high rates of respiration and feeding; as these clams were most often found clinging to submerged vegetation, they are vulnerable to predation (from waterfowl or fish) if in the water column. Compared to clams in the YesFish-Wet lentic systems, clams in the YesFish-Dry systems tended to have longer shell lengths, lower condition index (tissue mass: shell length), smaller broods, somewhat stronger relationships between adult size and brood size, and lower RNA:DNA (when corrected for shell length). Although not all of these comparisons were significant, collectively they hint at a trade-off between somatic growth, which could indicate evasion from predators and/or heightened ability to survive through the dry period, and brood production in conditions in which extruded juveniles may not survive. This relationship could be tested with reciprocal transplants and/or common garden experiments that are at least three months long (to allow for brood cycle completion). The persistence of robust clam populations (robust in terms of abundance, condition index, and reproductive success) is critical to habitats that support waterfowl because fingernail clams are an important food source for diving ducks and ducklings, especially during the spring and summer months (Joyner 1980, Sanchez et al. 2000, de Szalay et al. 2003, Mackie 2007, Richman and Lovvorn 2009).

Although brook stickleback presence is a strong contributor to lentic system "quality", the *a priori* expectations were that we would see synergistic effects between fish presence and shortened hydroperiod/ephemeral status. Not including Stubblefield Lake, where I did collect any submerged macrophytes, the YesFish-Dry lentic systems

had the lowest macrophyte diversity (lowest number of macrophyte species). Although brook stickleback do directly alter macrophyte communities through consumption, brook stickleback can alter macrophyte communities indirectly as well (Stewart et al. 2007). Brook stickleback and other stickleback species can alter macrophyte communities by consuming zooplankton, which lowers predation on phytoplankton and algae, thus decreasing the transparency of the water (Spencer and King 1984, Vierssen and Prins 1985, Daldorph and Thomas 1995, Jackson et al. 2001). Other factors that influence macrophyte growth and success include the availability of nitrogen and phosphorous, which, if high, can decrease zooplankton, thus increasing phytoplankton abundance and decreasing macrophyte growth (Beekey and Karlson 2003). However, hydroperiod can also influence macrophyte communities, where temporary ponds typically have few macrophyte species than permanent ponds (Nicolet 2001), which I also observed.

The NoFish-Dry category (Stubblefield Lake) had the lowest number of macroinvertebrate taxa, which may be related to its short hydroperiod, as taxa typically increased with hydroperiod (Brooks 2000). Stubblefield Lake's unique historic and geographic characteristics (Curry et al. 2007), and its lower pH, also may limit macroinvertebrate diversity. Amongst the other three categories, macroinvertebrate taxonomic diversity tended to be lower in the dry lentic systems than in the wet, and lower in the presence of brook stickleback. As I did not identify macroinvertebrates to the species level, I cannot speak to species richness or community composition; it is possible that there is diversity in the YesFish lentic systems that I did not note when identifying at such high taxonomic levels. Alternatively, if major taxa are identified to species, we may

find that there are even more significant differences in macroinvertebrate diversity across lentic system categories than what we observed here.

Although the number of macroinvertebrate taxa increased with hydroperiod, the presence of brook stickleback may be a confounding factor in the taxonomic variance across the remaining three lentic system categories. The abundance of corixids, for example, was higher in the YesFish-Dry than YesFish-Wet category; this was the case for most systems except for Windmill Pond (YesFish-Wet), which had greater abundance of corixids than any YesFish-Dry systems. Quick-moving invertebrates like corixids tend to prefer temporary systems (Nicolet 2001, Stewart et al. 2007). The greater abundance of corixids in YesFish-Wet systems could be because corixids are not consumed by stickleback until later in the season (Ravinet et al. 2013).

Several other macroinvertebrate taxa were rare or absent within the YesFish-Wet category, and were even less abundant in the YesFish-Dry systems; those taxa include anisopterans, zygopterans, trichopterans, notonectids, and dyticids. Although brook stickleback and other stickleback species consume the trichopterans, notonectids, and dyticids, the low abundances of these macroinvertebrates is also likely due to the hydroperiod lengths as these invertebrates are typically earlier season residents and prefer temporary ponds (Schilling et al. 2009, Bischof et al. 2013). The low abundance of anisopterans and zygopterans in the YesFish-Dry systems is likely due to their preference for permanent ponds and competition with brook stickleback for their main sources of food, chironomids (Lillie 2003, Smith et al. 2003).

Some water quality parameters, such as chlorophyll, transparency, nitrate, and pH appear to be influenced by ephemeral/perennial status, sometimes in combination with

brook stickleback presence/absence. Chlorophyll was lower in the YesFish-Wet category than in the YesFish-Dry (the NoFish categories were intermediate of both YesFish categories). The lower chlorophyll levels in the YesFish-Wet category may be due to compounding effects from the presence of brook stickleback (through consumption of zooplankton) and the larger sizes of the more permanent ponds within that category. The presence of brook stickleback or three-spined stickleback have been associated with decreased abundance of zooplankton, increasing the abundance of phytoplankton, but with more macrophytes chlorophyll can decrease (Spencer and King 1984, Norlin et al. 2006). Phytoplankton abundances, and lower chlorophyll levels, also typically increase with the surface area of the lentic systems (Wetzel 2001). The shorter photoperiods in ephemeral ponds can increase chlorophyll levels due to the shortened photoperiod for phytoplankton or algal growth (Williams 2006). There was no evidence of a direct causal relationship between brook stickleback presence and chlorophyll level in the mesocosm experiments, indicating that linking changes in chlorophyll level to brook stickleback presence, can only be accomplished within the contexts of ephemeral/perennial status and lentic system size and not in small mesocosms. The short duration of the mesocosm experiment also could explain the insignificant results, as other mesocosm experiments with significant changes in chlorophyll were for 7 – 10 weeks (Stephen et al. 2004, Sorf et al. 2015).

There tended to be higher turbidity (lower transparency) in the ephemeral lentic systems than in the perennial systems; this effect was strongest in the YesFish systems. I expected to see an effect of brook stickleback presence on turbidity, especially in the mesocosms, because the presence of brook or three-spined stickleback has been

associated with increases in turbidity as phytoplankton and algal blooms increase (Spencer and King 1984, Jakobsen et al. 2003). However, the mesocosm experiment was probably too short, given that other experiments with significant differences in turbidity were around 7 – 10 weeks long (Stephen et al. 2004, Sorf et al. 2015). Some of the variation in turbidity levels within and across lentic system categories could be due to the inability to use the more accurate Secchi disk method of measuring turbidity as see in Webster et al. (2007). The turbidity was likely higher in the ephemeral/temporary ponds because the short hydroperiod increases nutrient circulation and phytoplankton and algal blooms, thus increasing turbidity or lowering water transparency (Wetzel 2001, Williams et al. 2002). Higher turbidity is problematic for the refuge as waterfowl prefer less turbid waters for breeding and molting (Epners et al. 2010). Additionally, I expected to see a relationship between chlorophyll and turbidity across the lentic systems. These two factors were negatively related, as predicted, but the relationship was not significant ($r = -0.502$, $p = 0.089$; data not shown). This relationship between chlorophyll and turbidity was observed in the larger lentic systems, in which the sampling date for max chlorophyll was the same date for max turbidity (lowest transparency), and vice versa, for the Turnbull Slough, Long Lake, Campbell – Lasher Lake, and smaller 30 Acre Lake. This pattern was similar but not exactly the same for Kepple Lake.

There was less nitrate in the ephemeral lentic systems than in the perennial systems; this pattern was significantly stronger in the NoFish categories, but also present in the YesFish categories when considering the median values. When ephemeral/temporary lentic systems experience more drastic water level fluctuations than permanent systems, there is an increase in the amount of nitrogen that is release from the

sediment, which increases the number forms of nitrogen (nitrate) available for bacteria and plants (Wetzel 2001, Williams 2006). Similarly to that of the chlorophyll and turbidity trends, there was no change in nitrate levels within the mesocosm experiment, indicating there is no clear effect of brook stickleback presence.

As noted above, pH was significantly lower in the NoFish-Dry category (Stubblefield Lake) than in most lentic systems in all other categories. 30 Acre Lake was the only lentic system with a lower pH (minimum value, not median) than Stubblefield Lake. Most of this low pH pattern may be attributed to Stubblefield Lake's geographic characteristics as a playa lake (Gurdak and Roe 2010) as it is receiving an inflow of acidic-saline groundwater (Long et al. 1992). Unlike the other water quality parameters, pH was the only parameter that differed in the mesocosm experiments. The HighFish tanks had high pH than either the NoFish or LowFish tanks, indicating a possible causal relationship between pH and brook stickleback presence. However, this relationship to brook stickleback presence and high pH was not supported by the field experiment. This high pH in the mesocosm experiments could be due to higher ammonia levels as higher pH aids bacterial configuration of ammonium into ammonia, but I did not detect significant differences in ammonia (Wetzel 2001). The difference in pH are more likely due to the water hardness, as the hardness decreases the fluctuations in pH become more severe (Wetzel 2001), unfortunately, hardness was not measured in either of my studies.

There was no clear relationship between other water quality parameters in the field study and either the presence/absence of brook stickleback or the ephemeral/perennial status. Those water quality parameters include water temperature, dissolved O_2, ammonia, and phosphate. Similarly, there was no clear relationship

between those other water quality parameters in the mesocosm study. The short duration of the mesocosm study could be the issue with most of the parameters being insignificant. As for the field study, the lack of significant water quality results could be due to the compounding effects of the varying lentic system: (1) sizes, because the surface area is related to chlorophyll and phytoplankton abundance, influencing ammonia and phosphate levels, (2) depth, because stratification influences temperature and dissolved O_2 (Wetzel 2001, Bayley and Prather 2003).

Conductivity is an indicator of dissolved solids/ions (related to salinity and electrical conductance, Green et al. 2015), and is especially important to monitor in freshwater ecosystems that are not exposed to high levels of salt. Conductivity was the strongest parameter influencing the water quality NMDS. Conductivity was the only other water quality metric that had significant differences between lentic system categories, however its patterns were unique, especially because it was the only water quality parameter that differed across watershed identity (higher levels of conductivity in the Cow Creek watershed). The size of the lentic systems and water temperature were also related to conductivity, where the larger lentic systems and warmer water had higher conductivity levels. Larger lentic systems tend to be deep enough to thermally stratify, which allows for mixing in the epilimnion (surface water, where I measured), which increased the amount of dissolved solids/ions (Green et al. 2015). However, smaller lentic systems can have high conductivity levels because the water mixes throughout the entire water column, interacting with the sediment and releasing more solids that can be dissolved (Green et al. 2015). As conductivity increases, the abundance of ephemeropterans also increased, possibly a consequence of the strong relationship

between lentic system size and ephemeropteran abundance. The greater abundance of ephemeropterans in high conductivity systems could also be related to their tolerance of poor water quality (Menetrey et al. 2008), as most macroinvertebrates are unable to tolerate high conductivity levels. Other tolerant macroinvertebrates include copepods and chironomids (James et al. 2003); chironomids were abundant throughout the Cow Creek watershed (I did not measure copepods). There was no clear relationship between conductivity and the presence/absence of brook stickleback or hydroperiod.

Recommendations for Refuge Management

The refuge could eradicate brook stickleback by increasing the brook stickleback predators, such as water beetle larvae, dragonfly nymphs, or garter snakes, which have been known to eat three-spined stickleback (Bell and Haglund 1978, Stewart et al. 2007). Additional eradication techniques include preventing the flow of brook stickleback back into those small lentic systems that dry up, or adding rotenone as a fish poison (Brown and Ball 1943). Considering the refuge was historically fishless (Curry et al. 2007), it is not necessarily a negative consequence if the other fish die, but rotenone treatments also affect macroinvertebrates (Mangum and Madrigal 1999) and thus could affect waterfowl nesting success. The data presented herein should be evaluated within the context of the refuge's waterfowl data (brooding pair counts) to determine if the differences that I detected are related to differences in waterfowl lentic system use. The thesis research by Bridges (2011), in which a stable isotope analysis suggests that waterfowl are shifting their diet and consuming different prey items at the lentic systems with brook stickleback present, is additional evidence that waterfowl nesting/breeding success may be affected

by brook stickleback presence. Any of the eradication measures should be used as a last resort, and only after gathering evidence that waterfowl nesting success is impaired.

Prior to the refuge taking action to eradicate the brook stickleback, most of the parameters reported here should be measured again but during a more typical or wet year to determine if the trends across brook stickleback presence/absence, ephemeral/perennial, watershed and lentic system size are consistent. This repeated field study should be accompanied by a longer-term mesocosm experiment conducted during cooler months and/or during a more typical "wet" year to determine whether there is a causal relationship between brook stickleback presence and altered water quality parameters.

Future studies also should take a closer look at the relationship between water quality and macroinvertebrate parameters and the drainages contained within watersheds that were not included in these analyses. The Kaegle drainage in the Rock Creek watershed was one of the drainages not explored in theses analyses and it might be interesting as it contains a variety of differently-sized lentic systems, and it does not contain brook stickleback according to Walston et al. (2016). Inclusion of the Kaegle drainage would allow larger sample sizes in both of the NoFish categories. Last but not least, the quality of the water entering refuge in the Cow Creek drainage originates from dairy farms, and has previously had high phosphorus and nitrogen levels (Curry et al. 2007). Although I did not detect these nutrients in high levels, the sampling should be repeated in more lentic systems in Cow Creek to determine whether the water quality has improved over time. If additional research confirms my findings, the refuge should then

consider taking actions to remove brook stickleback from the smaller, ephemeral lentic systems that appear to have poor macroinvertebrate and macrophyte communities.

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TABLES

Table 1. Changes in invertebrate and macrophyte composition and water quality parameters in studies with and without stickleback fish (NF = no fish); * indicates non-significant results and – indicates the study did not measure that variable (Macroinverts=macroinvertebrates)

Stickleback Species	Brook[1]	Brook[2]	Nine-spined[3]	Three-spined[4]	Three-spined[5]	NF[6]	NF[7]	NF[8]	NF[9]	NF[10]
Zooplankton	↓	-	↓	↓	-	-	-	-	↓	-
Phytoplankton	↑	-	↑	↑	-	↑	-	↑	↑	-
Macroinverts	-	↓	-	-	-	-	-	-	-	↓
Macrophytes	↓	-	*	-	↑	↓	-	↓	↑	-
Temperature	-	-	*	-	↑	-	↑	-	-	-
Conductivity	-	-	*	-	-	↓	-	-	-	↓
Chlorophyll	-	-	↑	↑	-	↑	-	-	↑	-
Phosphorus	-	-	↑	-	-	↑	-	-	↑	↑
Nitrate	-	-	↑	-	-	↑	-	-	↑	↑
pH	-	-	-	-	-	↓	↑	-	↑	↓
Dissolved O_2	-	-	*	-	↓	-	↓	-	-	-
Ammonia	-	-	↑	-	-	↑	↑	-	↑	-
Turbidity	↑	-	-	↑	-	↑	-	-	-	-

[1](Spencer and King 1984); [2](Wieker et al. 2016); [3](Daldorph and Thomas 1995); [4](Jakobsen et al. 2003); [5](Morgan et al. 2010); [6](Bayley and Prather 2003); [7](Erickson 1985); [8](Vierssen and Prins 1985); [9](Bakker et al. 2010); [10](Parsons et al. 2010)

Table 2. Diet choices brook stickleback and the four most common waterfowl species, with corresponding months that the waterfowl use habitats at Turnbull NWR.

Species	Month	Diet Choices	Citation
Mallard (*Anas platyrhynchos*)	Year Round	Seeds, Naididae, Mollusca, Daphnia, Diptera, Coleoptera, Chironomidae and Gastropoda	(Dessborn et al. 2011, Tidwell et al. 2013)
Blue-Winged Teal (*Anas discors*)	Year Round	Seeds, Planorbidae, Chironomidae, Mollusca, Corixidae and Odonata	(Dessborn et al. 2011, Tidwell et al. 2013)
Redhead (*Aythya americana*)	March-Oct. (April-Sept.)	Chronomidae, Mollusca, Gastropoda and Angiosperm Seeds	(Sanchez et al. 2000)
Ruddy Duck (*Oxyura jamaicensis*)	March-Sept.	Plant Material, Chironomidae, Gastropods, Cladocera, Hemiptera, Diptera and Angiosperm Seeds	(Kenow 1996)
Brook Stickleback (*Culaea inconstans*)	N/A	Algae and Plant Material, Fish Eggs and Larvae, Mollusca, Chironomidae, Amphipoda, Diptera, Crustacea, and Naididae/Oligochaeta	(Stewart et al. 2007)

Table 3. Lentic systems categorized by whether brook stickleback presence was noted in the original publication or newest, relative dryness for the 2014 field season, and the corresponding number of sampling sites.

No Fish Present and Wet in 2014 ("NoFish/Wet")	No Fish Present and Dry in 2014 ("NoFish/Dry")	Fish Present and Wet in 2014 ("YesFish/Wet")	Fish Present and Dry in 2014 ("YesFish/Dry")
Long Lake	Stubblefield Lake	Kepple Lake	30 Acre Lake
– 5 sampling sites	– 3 sampling sites	– 4 sampling sites	– 3 sampling sites
– 6 sampling cycles*	– 2 sampling cycles	– 6 sampling cycles	– 4 sampling cycles
TLES Pond		Cheever Lake	Blackhorse Lake
– 3 sampling sites		– 3 sampling sites	– 3 sampling sites
– 5 sampling cycles		– 6 sampling cycles	– 4 sampling cycles
Campbell - Lasher Lake		Windmill Pond	Turnbull Slough (Upper)
– 4 sampling sites		– 3 sampling sites	– 5 sampling sites
– 5 sampling cycles		– 6 sampling cycles	– 5 sampling cycles
		West Tritt Lake	West Issacson Lake
		– 3 sampling sites	– 3 sampling sites
		– 6 sampling cycles	– 5 sampling cycles

Fish presence in West Tritt, Kepple Lake, Cheever Lake, Windmill Pond, 30 Acre Lake, Blackhorse Lake, and the Turnbull Slough water bodies was confirmed in Walston et al. (2016); fish presence in West Issacson Lake was confirmed by the current study and by personal communication, Mike Rule.

* One complete "sampling cycle" refers to sampling all measurements within all lentic systems that have water before going back to the lentic systems for the next sampling cycle

Table 4. Lentic system relative dryness for the 2015 field season, total size in acres (further categorized by TNWR as large, medium, and small), watershed and drainage. Information for all lentic systems except TLES Pond size were provided by M. Rule.

Category	Lentic System	Relative Dryness	Size in Acres (Lg., Med., Sm.)	Watershed	Drainage ("Sub-watershed")
NoFish-Dry	Stubblefield Lake	Dried in 5/2015	72.9 (Lg.)	N/A	Philleo Lake
NoFish-Wet	Campbell – Lasher Lake	Mostly dry in 7/2015	107 (Lg.)	Cow Creek	Company
	Long Lake	Wet	236 (Lg.)	Cow Creek	Company
	TLES Pond	Mostly dry* in 7/2015	3.72 (Sm.)	Cow Creek	Company
YesFish-Dry	Blackhorse Lake	Dried in 7/2015	33.7 (Med.)	Rock Creek	Kepple
	Turnbull Slough (Upper)	Mostly dry in 7/2015	312 (Lg.)	Cow Creek	Company
	West Issacson Lake	Dried in 7/2015	41.1 (Med.)	Rock Creek	Issacson
	30 Acre Lake	Dried in 7/2015	25.9 (Med.)	Rock Creek	Kepple
YesFish-Wet	Cheever Lake	Wet	71.8 (Lg.)	Rock Creek	Pine Creek
	Kepple Lake	Wet	103 (Lg.)	Rock Creek	Kepple
	West Tritt Lake	Mostly dry in 8/2015	139 (Lg.)	Cow Creek	Company
	Windmill Pond	Wet	3.14 (Sm.)	Rock Creek	Pine Creek

* "mostly dry" refers to when a lentic system has too low of a water level (less than approximately 10 cm deep) to be able to sample all measurements, thus all future sampling cycles for the field season are terminated

Table 5. Average (STDEV) macrophyte dried biomass (g) and number of macrophyte species, averaged over time, min, and max per lentic system.

Category	Lentic System		Ave (SD)	Min. [Date]	Max. [Date]
NoFish-Dry	Stubblefield Lake	Biomass	0	0	0
		No. species	0	0	0
NoFish-Wet	Campbell – Lasher Lake	Biomass	69.8 (81.9)	2.0 [1]	195.6 [4]
		No. species	3 (1)	2 [1,3]	4 [2,4]
	Long Lake	Biomass	90.2 (39.3)	46.1 [2]	148.6 [4]
		No. species	4 (0)	4	4
	TLES Pond	Biomass	30.3 (19)	8.2 [1]	50.7 [4]
		No. species	4.2 (0.8)	3 [2]	5 [3,5]
YesFish-Dry	Blackhorse Lake	Biomass	29.7 (25.4)	2.0 [1]	62.2 [4]
		No. species	2.5 (0.6)	2 [1,2]	3 [3,4]
	Turnbull Slough	Biomass	18.6 (14.2)	0.9 [1]	37.2 [4]
		No. species	2.2 (0.4)	2 [1,3-5]	3 [2]
	West Issacson Lake	Biomass	45.4 (51.1)	2.0 [1]	131.7 [4]
		No. species	3 (0.7)	2 [1]	4 [4]
	30 Acre Lake	Biomass	1.2 (2.4)	0 [1,3,4]	4.7 [2]
		No. species	0.3 (0.5)	0 [2-4]	1 [1]
YesFish-Wet	Cheever Lake	Biomass	29.4 (17.8)	8.2 [4]	56.4 [3]
		No. species	4.7 (1.2)	3 [4]	6 [1,2]
	Kepple Lake	Biomass	99.9 (92.5)	11.5 [1]	265.9 [6]
		No. species	3.5 (0.5)	3 [1,2,6]	4 [3-5]
	West Tritt Lake	Biomass	18.4 (15.4)	1.1 [1]	43.2 [4]
		No. species	3 (0.6)	2 [1]	4 [4]
	Windmill Pond	Biomass	3.8 (1.9)	1.8 [3]	6.8 [5]
		No. species	1.8 (1)	1 [1,3,4]	3 [2,6]

Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 6. Total macrophyte dried biomass (grams) and total number of macrophyte species within each lentic system at a given sampling cycle during the 2015 field season for the NoFish-Wet category (Note: no plants were found in Stubblefield Lake).

	Campbell – Lasher Lake					Long Lake						TLES Pond				
	1	2	3	4	5	1	2	3	4	5	6	1	2	3	4	5
Hydrocharitaceae																
Elodea canadensis (Waterweed)	0	0	0	0	0	0	0	0	0	0	0	0.9	0.1	1.1	6.3	1.02
Vallisneria americana (Wild Celery)	1.9	6.1	16.2	81.3	131	16.8	14.9	32.5	39.2	6.8	4.01	0	0	0.4	5.5	1.1
Potamogetonaceae																
Potamogeton natans (Broad-Leaf Pondweed)	0	0	0	0	0	0	0	0	0	0	0	0	0	4.9	0	5.8
P. pectinatus (Sago Pondweed)	0	0.01	0	11.8	46.7	0	0	3.63	18.3	2.11	18.7	1.1	11.4	18.4	13	21.5
P. richardsonii (Richardson's Pondweed)	0	0	0	0	0	0.1	0	0	0	0	0	6.2	0	0	0	0
Ceratophyllaceae																
Ceratophyllum demersum (Coontail)	0	2.9	18.2	13.5	18.1	21.6	22.1	57.6	70.1	54.0	43.8	0.02	2.5	22.2	26	2.4
Haloragaceae																
Myriophyllum spicatum (Milfoil)	0.2	2.2	0	1.4	0	13.0	8.1	25.2	21.0	21.2	25.5	0	0	0	0	0
Total dried biomass (g)	2.0	11.1	34.4	108	196	51.4	46.1	119	149	84.2	92	8.2	14.0	47.0	51	31.8
Number of species	2	4	2	4	3	4	4	4	4	4	4	4	3	5	4	5

Sampling cycle 1 was 4/14 – 5/1, 2 was 5/5 – 5/22, 3 was 5/26 – 6/12, 4 was 6/23 – 7/10, 5 was 7/14 – 7/31, and 6 was 8/5 – 8/20.

Table 7. Total macrophyte dried biomass (grams and identified to species) and total number of macrophyte species for all sampling sites within each lentic system at a given sampling cycle during the 2015 field season for the YesFish-Dry category.

	Blackhorse Lake				Turnbull Slough					West Issacson Lake					30 Acre Lake			
	1	2	3	4	1	2	3	4	5	1	2	3	4	5	1	2	3	4
Hydrocharitaceae																		
Elodea canadensis (Waterweed)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vallisneria americana (Wild Celery)	0.8	0	0.4	60	0	0	0	0	0	1.4	11	28	45	126	0	0	0	0
Potamogetonaceae																		
Potamogeton natans (Broad-Leaf Pondweed)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. pectinatus (Sago Pondweed)	1.2	18	33	0.2	0	5.7	20	37	19	0	0	0	0.4	4.5	4.9	0	0	0
P. richardsonii (Richardson's Pondweed)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratophyllaceae																		
Ceratophyllum demersum (Coontail)	0	2.7	0.6	1.9	0.1	2.4	6.8	0	0	0	1.4	1.1	0	0.6	0	0	0	0
Haloragaceae																		
Myriophyllum spicatum (Milfoil)	0	0	0	0	0.8	0.9	0	0.2	0.4	0.6	1.8	4.0	0.7	0.7	0	0	0	0
Total dried biomass (g)	2.0	20	34	62	0.9	9.0	26	37	19	2.0	14	33	46	132	4.9	0	0	0
Number of species	2	2	3	3	2	3	2	2	2	2	3	3	3	4	1	0	0	0

Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 8a. Total macrophyte dried biomass (grams and identified to species) and total number of macrophyte species for all sampling sites within each lentic system at a given sampling cycle during the 2015 field season for the YesFish-Wet category.

	Cheever Lake						Kepple Lake					
	1	2	3	4	5	6	1	2	3	4	5	6
Hydrocharitaceae												
Elodea canadensis (Waterweed)	5.6	2.5	48.2	0.47	15.5	6.4	0	0	0	0	0	0
Vallisneria americana (Wild Celery)	1.5	2.9	3.6	3.9	1.3	0.72	0	2.25	20.4	13.3	38.0	78.0
Potamogetonaceae												
Potamogeton natans (Broad-Leaf Pondweed)	0	0	0	0	0	0	0	0	0	0	0	0
P. pectinatus (Sago Pondweed)	1.6	5.07	0.20	0	0	0	0.01	0	0.87	17.0	11.9	0
P. richardsonii (Richardson's Pondweed)	0.51	6.01	0.47	3.6	25.5	20.0	0	0	0	0	0	0
Ceratophyllaceae												
Ceratophyllum demersum (Coontail)	1.1	5.8	3.9	0.20	0	2.6	10.5	26.0	30.3	70.8	44.9	150
Haloragaceae												
Myriophyllum spicatum (Milfoil)	4.5	2.8	0	0	1.3	0	1.02	1.4	4.05	6.74	12.6	38.4
Total dried biomass (g)	14.8	25.0	56.4	8.2	42.3	29.8	11.5	29.6	58.2	127	107	266
Number of species	6	6	5	4	3	4	3	3	4	4	4	3

Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 8b. Total macrophyte dried biomass (grams and identified to species) and total number of macrophyte species for all sampling sites within each lentic system at a given sampling cycle during the 2015 field season for the YesFish-Wet category.

	West Tritt Lake						Windmill Pond					
	1	2	3	4	5	6	1	2	3	4	5	6
Hydrocharitaceae												
Elodea canadensis (Waterweed)	0	0	0	0	0	0	5.6	2.8	1.8	3.1	3.0	2.1
Vallisneria americana (Wild Celery)	0	0.03	0.28	0	1.6	1.7	0	0	0	0	0	0.28
Potamogetonaceae												
Potamogeton natans (Broad-Leaf Pondweed)	0	0	0	0	0	0	0	0	0	0	0	0
P. pectinatus (Sago Pondweed)	0	0	0	25.0	13.3	26.9	0	0.33	0	0	3.8	0
P. richardsonii (Richardson's Pondweed)	0	0	0	0	0	0	0	0	0	0	0	0.12
Ceratophyllaceae												
Ceratophyllum demersum (Coontail)	0.36	5.6	9.9	17.4	3.2	0.32	0	0	0	0	0	0
Haloragaceae												
Myriophyllum spicatum (Milfoil)	0.77	2.2	1.3	0.81	0.09	0	0	0	0	0	0	0
Total dried biomass (g)	1.1	7.8	11.5	43.2	18.1	28.9	5.6	3.3	1.8	3.1	6.8	2.5
Number of species	2	3	3	3	4	3	1	3	1	1	2	3

Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 9. The number of macroinvertebrates and number of macroinvertebrate taxa, averaged over time, min, and max per lentic system.

Category	Lentic System		Ave (SD)	Min. [Date]	Max. [Date]
NoFish-Dry	Stubblefield Lake	No. Macroinv.	371 (274)	177 [1]	564 [2]
		No. taxa	7.5 (3.5)	5 [1]	10 [2]
NoFish-Wet	Campbell – Lasher Lake	No. Macroinv.	585 (217)	353 [3]	872 [4]
		No. taxa	12.6 (1.1)	11 [1]	14 [3]
	Long Lake	No. Macroinv.	1,691 (739)	907 [1]	2,897 [4]
		No. taxa	12.5 (0.5)	12 [1,4,6]	13 [2,3,5]
	TLES Pond	No. Macroinv.	495 (226)	216 [2]	802 [4]
		No. taxa	12 (1.7)	9 [2]	13 [1,3,4]
YesFish-Dry	Blackhorse Lake	No. Macroinv.	213 (76)	100 [1]	257 [2]
		No. taxa	10 (2)	9 [1,2,3]	13 [4]
	Turnbull Slough	No. Macroinv.	718 (468)	352 [5]	1,506 [2]
		No. taxa	10 (2)	7 [5]	12 [3]
	West Issacson Lake	No. Macroinv.	156 (86)	50 [1]	288 [3]
		No. taxa	9.6 (1.1)	8 [2]	11 [3]
	30 Acre Lake	No. Macroinv.	146 (65)	97 [3]	240 [4]
		No. taxa	10.3 (2.2)	7 [2]	12 [4]
YesFish-Wet	Cheever Lake	No. Macroinv.	147 (159)	33 [6]	428 [1]
		No. taxa	5.3 (1.4)	4 [4]	8 [1]
	Kepple Lake	No. Macroinv.	564 (314)	152 [3]	942 [6]
		No. taxa	10.2 (2)	8 [2,3]	13 [4]
	West Tritt Lake	No. Macroinv.	496 (291)	180 [5]	913 [5]
		No. taxa	8.6 (2.1)	5 [2]	11 [3]
	Windmill Pond	No. Macroinv.	296 (301)	36 [3]	713 [6]
		No. taxa	7.3 (1)	6 [3]	9 [1]

Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 10. Total number of macroinvertebrates and taxa found within each lentic system at a given sampling cycle (2015) for the NoFish-Wet and NoFish-Dry (Stubblefield L.) categories.

	Campbell – Lasher Lake					Long Lake						TLES Pond					Stubblefield	
	1	2	3	4	5	1	2	3	4	5	6	1	2	3	4	5	1	2
Insecta																		
Ephemeroptera[1]	172	138	28	245	143	59	169	62	381	312	255	12	7	14	4	10	0	3
Odonata																		
Anisoptera[1]	11	5	3	9	6	5	3	4	12	21	21	3	5	3	9	2	0	1
Zygoptera[1]	13	7	1	54	57	158	126	71	156	105	51	17	8	12	56	79	1	1
Trichoptera[2]	7	3	0	0	0	22	9	10	89	9	11	5	0	0	7	1	1	0
Coleoptera																		
Dytiscidae[2]	1	4	2	3	4	0	1	3	2	0	0	1	8	5	1	1	2	27
Other	0	1	6	4	13	6	6	8	0	6	2	2	2	5	2	5	0	1
Hemiptera																		
Corixidae	1	14	11	7	3	4	13	61	9	7	4	5	0	14	22	8	0	31
Notonectidae	0	1	1	0	3	103	1	0	7	12	1	0	0	0	0	0	0	2
Diptera																		
Chironomidae[2]	171	119	87	284	286	356	195	827	727	645	855	69	25	78	329	281	172	484
Other[1]	0	0	19	4	0	0	0	8	0	2	6	2	0	5	0	0	1	1
Crustacea																		
Amphipoda	62	25	134	226	223	168	395	572	1,494	80	553	46	41	226	267	188	0	0
Hydracarina	18	10	4	14	24	9	7	3	8	4	0	15	8	10	3	8	0	0
Hirudinea	0	0	11	4	0	0	4	0	2	21	2	0	0	3	4	1	0	13
Mollusca																		
Sphaeriidae	64	97	34	3	1	8	2	3	0	0	0	168	112	140	89	5	0	0
Hygrophila	5	6	12	15	8	10	0	48	10	20	5	2	0	4	9	0	0	0
Total Number	525	430	353	872	744	907	931	1,680	2,897	1,966	1,766	347	216	519	802	589	177	564
Number of taxa	11	13	14	13	12	12	13	13	12	13	12	13	9	13	13	12	5	10

[1]Nymph; [2]Larva; Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 11. Total number of macroinvertebrates (identified to family or class, and further categorized as nymphs or larva) and macroinvertebrate taxa for all sampling sites within each lentic system at a given sampling cycle during the 2015 field season for the YesFish-Dry category.

	Blackhorse Lake				Turnbull Slough					West Issacson Lake					30 Acre Lake			
	1	2	3	4	1	2	3	4	5	1	2	3	4	5	1	2	3	4
Insecta																		
Ephemeroptera[1]	16	2	10	69	229	236	68	57	29	1	0	1	16	8	1	0	0	22
Odonata																		
Anisoptera[1]	1	2	4	5	1	6	1	23	0	2	3	1	1	0	1	0	3	4
Zygoptera[1]	0	6	7	39	0	1	8	1	1	0	0	1	0	3	0	0	1	1
Trichoptera[2]	1	0	0	0	0	1	0	0	0	2	2	0	0	0	2	0	1	0
Coleoptera																		
Dytiscidae[2]	0	5	0	7	12	0	5	0	0	1	0	0	0	0	6	7	5	7
Other Coleoptera	2	1		4	6	2	4	8	5	0	0	4	5	2	2	2	6	4
Hemiptera																		
Corixidae	11	151	173	53	15	41	123	32	20	1	12	32	0	4	1	17	26	5
Notonectidae	0	0	0	1	0	0	0	5	0	0	0	0	0	0	0	0	0	1
Diptera																		
Chironomidae[2]	8	64	43	34	95	1,308	268	621	291	12	20	66	63	102	21	38	20	33
Other Dipteran[1]	0	1	0	6	0	2	28	1	0	0	0	5	1	1	0	0	0	1
Crustacea																		
Amphipoda	0	0	2	24	7	1	11	5	1	0	0	8	10	2	1	0	0	0
Hydracarina	49	25	3	1	50	3	23	12	5	4	9	0	11	2	5	4	3	87
Hirudinea	0	0	0	2	0	0	2	0	0	0	1	2	1	1	0	0	3	0
Mollusca																		
Sphaeriidae	2	0	0	0	4	0	0	0	0	25	106	96	21	0	86	36	20	36
Hygrophila	10	0	3	3	0	5	6	1	0	2	1	12	29	3	13	3	9	39
Total Number	100	257	248	248	419	1,506	547	766	352	50	154	288	158	128	139	107	97	240
Number of taxa	9	9	9	13	9	11	12	11	7	9	8	11	10	10	11	7	11	12

[1]Nymph; [2]Larva; Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 12a. Total number of macroinvertebrates (identified to family or class, and further categorized as nymphs or larva) and macroinvertebrate taxa for all sampling sites within each lentic system at a given sampling cycle during the 2015 field season for the YesFish-Wet category (Cheever Lake and Kepple Lake).

	Cheever Lake						Kepple Lake					
	1	2	3	4	5	6	1	2	3	4	5	6
Insecta												
Ephemeroptera[1]	5	1	1	0	0	1	50	57	4	85	89	240
Odonata												
Anisoptera[1]	1	0	1	2	0	0	12	12	2	8	34	119
Zygoptera[1]	0	0	0	0	0	0	0	0	0	16	18	78
Trichoptera[2]	0	0	0	0	0	0	2	2	0	5	0	6
Coleoptera												
Dytiscidae[2]	0	1	0	0	0	0	0	0	0	0	0	0
Other Coleoptera	1	0	0	0	1	0	0	0	1	4	6	14
Hemiptera												
Corixidae	2	0	0	0	1	1	0	0	3	1	4	0
Notonectidae	0	0	0	0	0	0	1	0	0	0	0	14
Diptera												
Chironomidae[2]	353	34	44	187	4	10	148	173	100	654	420	430
Other Dipteran[1]	0	0	0	0	0	0	0	0	0	17	0	3
Crustacea												
Amphipoda	0	0	0	0	0	0	3	0	0	4	0	5
Hydracarina	60	21	14	3	1		4	14	2	8	6	7
Hirudinea	0	0	0	0	0	0	2	1	0	1	3	1
Mollusca												
Sphaeriidae	2	0	0	0	0	0	174	86	39	46	60	0
Hygrophila	4	9	16	52	27	17	12	2	1	35	8	7
Total Number	428	66	76	244	34	33	408	347	152	884	648	942
Number of taxa	8	5	5	4	5	5	10	8	8	13	10	12

[1]Nymph; [2]Larva; Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 12b. Total number of macroinvertebrates (identified to family or class, and further categorized as nymphs or larva) and macroinvertebrate taxa for all sampling sites within each lentic system at a given sampling cycle during the 2015 field season for the YesFish-Wet category (West Tritt Lake and Windmill Pond).

	West Tritt Lake						Windmill Pond					
	1	2	3	4	5	6	1	2	3	4	5	6
Insecta												
Ephemeroptera[1]	179	364	662	72	113	254	1	0	0	0	0	0
Odonata												
Anisoptera[1]	7	4	2	5	1	5	1	0	0	0	0	0
Zygoptera[1]	8	8	11	8	3	53	0	1	0	1	0	1
Trichoptera[2]	0	0	0	0	0	0	0	0	1	0	0	0
Coleoptera												
Dytiscidae[2]	0	0	1	0	0	0	0	0	0	0	0	0
Other Coleoptera	0	0	0	0	1	5	0	0	0	0	2	2
Hemiptera												
Corixidae	0	0	7	7	0	1	27	2	0	18	36	121
Notonectidae	1	0	0	0	0	2	0	0	0	0	0	0
Diptera												
Chironomidae[2]	168	310	170	28	52	230	7	4	7	122	329	309
Other Dipteran[1]	0	0	23	0	0	2	0	0	0	0	239	0
Crustacea												
Amphipoda	0	0	1	34	4	25	3	1	0	2	1	2
Hydracarina	14	29	4	15	3	0	23	50	4	3	0	4
Hirudinea	1	0	5	2	3	0	6	3	6	3	24	4
Mollusca												
Sphaeriidae	2	0	0	2	0	0	21	0	14	0	0	0
Hygrophila	1	0	27	37	0	0	52	11	4	20	15	270
Total Number	381	715	913	210	180	577	141	72	36	169	646	713
Number of taxa	9	5	11	10	8	9	9	7	6	7	7	8

[1]Nymph; [2]Larva; Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 13. Average (STDEV) clam abundance in each lentic system.

Category	Lentic System	Clam abundance, Ave (SD)
NoFish-Dry	Stubblefield Lake	0.0 (0.0)
NoFish-Wet	Long Lake	0.39 (0.53)
	TLES Pond	34.27 (20.73)
	Campbell – Lasher Lake	9.95 (10.27)
YesFish-Wet	Cheever Lake	0.11 (0.27)
	Kepple Lake	16.88 (14.82)
	Windmill Pond	1.94 (3.10)
	West Tritt Lake	0.22 (0.34)
YesFish-Dry	30 Acre Lake	14.83 (9.56)
	Blackhorse Lake	0.17 (0.33)
	Turnbull Slough	0.16 (0.36)
	West Issacson Lake	16.53 (16.0)

Table 14. Average (STDEV) clam condition index (Bayne et al.), averaged over time per lentic system.

Category	Lentic System	Clam CI Ave (SD)
NoFish-Wet	Campbell – Lasher Lake	2.84 (0.69)
NoFish-Dry	Stubblefield Lake	2.74 (1.28)
YesFish-Wet	Kepple Lake	2.07 (0.81)
YesFish-Dry	30 Acre Lake	1.84 (0.74)
	Blackhorse Lake	1.58 (0.60)

Table 15. Average (STDEV) chlorophyll, transparency, dissolved O_2, conductivity, temperature, pH, ammonia, nitrate, and phosphate levels, averaged over time, min, and max per lentic system within the NoFish-Dry category.

Lentic System		Ave (SD)	Min. [Date]	Max. [Date]
Stubblefield Lake	Chlorophyll (µg/L)	38 (19)	17.9 [1]	64 [2]
	Transparency (%)	85 (17)	54 [2]	97 [1]
	Dissolved O_2 (mg/L)	8.1 (4.7)	3.8 [2]	13 [1]
	Conductivity (µS)	173 (73)	128 [1]	258 [1]
	Temperature (°C)	22 (5.4)	16 [1]	27 [2]
	pH	7.9 (0.47)	7.4 [2]	8.6 [1]
	Ammonia (ppt)	0.06 (0)	0.06 [2]	0.06 [2]
	Nitrate (ppt)	0.17 (0.3)	0.03 [2]	0.05 [1]
	Phosphate (ppt)	0.38 (0.11)	0.28 [1]	0.57 [2]

Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 16. Average (STDEV) chlorophyll, transparency, dissolved O_2, conductivity, temperature, pH, ammonia, nitrate, and phosphate levels, averaged over time, min, and max per lentic system within the NoFish-Wet categories.

Lentic System		Ave (SD)	Min. [Date]	Max. [Date]
Campbell – Lasher Lake	Chlorophyll (µg/L)	34 (15)	22 [1]	65 [5]
	Transparency (%)	92 (6.7)	71 [5]	97 [1]
	Dissolved O_2 (mg/L)	11 (4)	5.8 [2]	17 [3]
	Conductivity (µS)	695 (157)	450 [5]	1,070 [5]
	Temperature (°C)	23 (6.8)	13 [1]	32 [4]
	pH	8.9 (0.8)	7.9 [2]	10 [5]
	Ammonia (ppt)	0.06 (0.3)	0.03 [3]	0.15 [5]
	Nitrate (ppt)	0.72 (0.04)	0.69 [2]	0.85 [5]
	Phosphate (ppt)	0.01 (0.01)	0.002 [3]	0.04 [5]
Long Lake	Chlorophyll (µg/L)	43 (19)	22 [4]	90 [1]
	Transparency (%)	93 (2.82	86 [1]	97 [4]
	Dissolved O_2 (mg/L)	9.6 (2.7)	4.8 [5]	17 [3]
	Conductivity (µS)	766 (181)	575 [1]	1,238 [5]
	Temperature (°C)	23 (6.2)	15 [2]	33 [4]
	pH	9.1 (0.48)	8.2 [1]	10 [4]
	Ammonia (ppt)	0.08 (0.07)	0.01 [5]	0.24 [6]
	Nitrate (ppt)	0.63 (0.2)	0.02 [1]	0.73 [5]
	Phosphate (ppt)	0.03 (0.03)	0.01 [3]	0.12 [4]
TLES Pond	Chlorophyll (µg/L)	29 (13)	17 [2]	63 [5]
	Transparency (%)	89 (7.8)	69 [1]	96 [4]
	Dissolved O_2 (mg/L)	8.0 (3.5)	2.7 [3]	13 [1]
	Conductivity (µS)	359 (64)	277 [1]	488 [5]
	Temperature (°C)	20 (5.6)	11 [1]	32 [5]
	pH	9.1 (0.71)	7.8 [3]	10 [5]
	Ammonia (ppt)	0.02 (0.01)	0.02 [3]	0.03 [2]
	Nitrate (ppt)	0.43 (0.34)	0.04 [3]	0.71 [4]
	Phosphate (ppt)	0.02 (0.01)	0.01 [2]	0.03 [3,4]

Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 17. Average (STDEV) chlorophyll, transparency, dissolved O_2, conductivity, temperature, pH, ammonia, nitrate, and phosphate levels, averaged over time, min, and max per lentic system within the YesFish-Dry category.

Lentic System		Ave (SD)	Minimum [Date]	Maximum [Date]
Blackhorse Lake	Chlorophyll (µg/L)	36 (16)	18 [3]	68 [4]
	Transparency (%)	86 (7.4)	73 [4]	95 [2]
	Dissolved O_2 (mg/L)	13 (4.1)	6.6 [2]	20 [1]
	Conductivity (µS)	565 (47)	514 [3]	639 [1]
	Temperature (°C)	23 (5.3)	19 [1]	33 [4]
	pH	9.8 (0.75)	8.2 [1]	11 [4]
	Ammonia (ppt)	0.08 (0.04)	0.03 [1]	0.13 [4]
	Nitrate (ppt)	0.15 (0.23)	0.01 [2]	0.70 [1]
	Phosphate (ppt)	0.05 (0.09)	0.004 [1]	0.29 [4]
Turnbull Slough	Chlorophyll (µg/L)	56 (25)	26 [1]	113 [5]
	Transparency (%)	84 (16)	31 [5]	97 [1]
	Dissolved O_2 (mg/L)	9.7 (4.2)	2.2 [4]	20 [3]
	Conductivity (µS)	1,503 (558)	480 [4]	2,750 [5]
	Temperature (°C)	26 (4.5)	18 [1]	33 [4]
	pH	9.3 (0.40)	8.8 [1]	10 [3]
	Ammonia (ppt)	0.08 (0.04)	0.02 [1]	0.15 [5]
	Nitrate (ppt)	0.62 (0.24)	0.07 [3]	0.78 [4]
	Phosphate (ppt)	0.02 (0.03)	0.001 [3]	0.11 [4]
West Issacson Lake	Chlorophyll (µg/L)	31.9 (9.8)	24 [3]	54 [5]
	Transparency (%)	94.6 (1.3)	92 [5]	97 [2]
	Dissolved O_2 (mg/L)	10.3 (4.6)	3.2 [2]	17 [4]
	Conductivity (µS)	497 (62)	404 [1]	593 [5]
	Temperature (°C)	25 (4.9)	17 [1]	31 [4]
	pH	8.8 (0.95)	7.5 [2]	10 [4]
	Ammonia (ppt)	0.03 (0.01)	0.02 [2]	0.04 [4]
	Nitrate (ppt)	0.16 (0.28)	0.01 [3]	0.71 [3]
	Phosphate (ppt)	0.01 (0.01)	0.004 [1,2]	0.02 [5]
30 Acre Lake	Chlorophyll (µg/L)	36 (9.5)	19 [1]	48 [3]
	Transparency (%)	91 (3)	85 [3]	95 [1]
	Dissolved O_2 (mg/L)	6.4 (3.6)	2.5 [3]	12 [1]
	Conductivity (µS)	115 (25)	75 [1]	148 [3]
	Temperature (°C)	15 (2.6)	11.6 [1]	19 [3]
	pH	6.9 (0.25)	6.5 [3]	7.2 [2]
	Ammonia (ppt)	0.02 (0)	0.02 [3]	0.02 [3]
	Nitrate (ppt)	0.03 (0.02)	0.01 [1,3]	0.05 [2]
	Phosphate (ppt)	0.01 (0.004)	0.01 [1]	0.02 [2]

Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

Table 18. Average (STDEV) chlorophyll, transparency, dissolved O_2, conductivity, temperature, pH, ammonia, nitrate, and phosphate levels, averaged over time, min, and max per lentic system within the YesFish-Wet category.

Lentic System		Ave (SD)	Minimum [Date]	Maximum [Date]
Cheever Lake	Chlorophyll (µg/L)	43.2 (20)	18 [3]	81 [6]
	Transparency (%)	90 (7.2)	75 [1]	96 [1]
	Dissolved O_2 (mg/L)	10 (2.9)	4 [2]	13 [1]
	Conductivity (µS)	236 (54)	211 [1]	368 [3]
	Temperature (°C)	21 (4.2)	16 [1]	26 [3]
	pH	9.6 (0.31)	9.1 [1]	10 [6]
	Ammonia (ppt)	0.03 (0.01)	0.02 [4]	0.03 [1]
	Nitrate (ppt)	0.09 (0.1)	0.01 [1]	0.28 [1]
	Phosphate (ppt)	0.01 (0.01)	0.01 [1]	0.03 [4]
Kepple Lake	Chlorophyll (µg/L)	28 (12)	17 [1]	71.3 [6]
	Transparency (%)	95 (3.2)	84 [6]	99 [5]
	Dissolved O_2 (mg/L)	8.6 (4.3)	4.7 [6]	19 [5]
	Conductivity (µS)	560 (184)	419 [3]	882 [6]
	Temperature (°C)	24 (6.6)	12 [1]	32 [4]
	pH	8.5 (0.28)	8.1 [3]	9.5 [3]
	Ammonia (ppt)	0.18 (0.36)	0.001 [3]	0.95 [6]
	Nitrate (ppt)	0.7 (0.01)	0.69 [6]	0.72 [3]
	Phosphate (ppt)	0.01 (0.01)	0.004 [1,2]	0.02 [6]
West Tritt Lake	Chlorophyll (µg/L)	33 (16)	18 [2]	68 [5]
	Transparency (%)	92 (4.6)	83 [4]	97 [1,3]
	Dissolved O_2 (mg/L)	11 (4.4)	4 [3]	17 [1]
	Conductivity (µS)	1,047 (305)	637 [1]	1,588 [6]
	Temperature (°C)	24 (6.3)	13 [1]	31 [4]
	pH	9.2 (0.53)	8.5 [3]	10 [6]
	Ammonia (ppt)	0.03 (0.01)	0.02 [3]	0.04 [4]
	Nitrate (ppt)	0.22 (0.3)	0.05 [6]	0.88 [3]
	Phosphate (ppt)	0.01 (0.01)	0.004 [1,2]	0.02 [5]
Windmill Pond	Chlorophyll (µg/L)	36 (23)	8 [3]	76 [5]
	Transparency (%)	95 (2.3)	91 [6]	99 [1]
	Dissolved O_2 (mg/L)	13 (5.3)	5.4 [3]	20 [4]
	Conductivity (µS)	188 (86)	169 [1]	251 [6]
	Temperature (°C)	16 (4.3)	8.9 [1]	21 [4]
	pH	8.3 (0.89)	7.2 [3]	9.5 [6]
	Ammonia (ppt)	0.03 (0.02)	0.02 [1]	0.06 [6]
	Nitrate (ppt)	0.04 (0.04)	0.002 [5]	0.07 [5]
	Phosphate (ppt)	0.03 (0.03)	0.01 [4]	0.08 [1]

Sampling cycle 1 was 4/14–5/1; 2 was 5/5–5/22, 3 was 5/26–6/12, 4 was 6/23–7/10, 5 was 7/14–7/31, and 6 was 8/5–8/20.

FIGURE LEGENDS

Figure 1a. Map of lentic systems and sampling sites at Turnbull National Wildlife Refuge, Cheney, WA.

Figure 1b. Example of method used to stratify and randomize samplings sites of equal distance.

Figure 2. The macrophyte dried biomass (g) for the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories over the six sampling cycles throughout the 2015 field season (Mid. April – Mid. August). Data are presented as line/scatter plots with the shape points representing the average macrophyte dried biomass (g) and the error bars representing ± 1 standard deviation (sampling sizes are in Table 5).

Figure 3. The average number of macrophyte dried biomass (g) for each of the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. All data points for each site (N = 202) are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (sampling sizes for each category are in Tables 6, 7, 8a, and 8b).

Figure 4. The total number of macrophyte species for each of the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. All data points for lentic system (N = 60) are presented as box and whisker plots with the line indicating the median, the box

indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (sampling sizes are in Tables 6, 7, 8a, and 8b).

Figure 5. The average number of macroinvertebrates for the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories over the six sampling cycles throughout the 2015 field season (Mid. April – Mid. August). Data are presented as line/scatter plots with the shape points representing the average number of macroinvertebrates and the error bars representing ± 1 standard deviation (sampling sizes for each category are in Table 9).

Figure 6. The number of macroinvertebrates for each of the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. All data points for each site (N = 202) are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (sampling sizes for each category are in Tables 10, 11, 12a and 12b).

Figure 7. The total number of different macroinvertebrate taxa (family, order) for each of the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. All data points for lentic system (N = 60) are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots. The same letters indicate there is no

significant difference between groups (sampling sizes for each category are in Tables 10, 11, 12a and 12b).

Figure 8. NMDS plots of macroinvertebrates by lentic system category and individual lentic systems. Figure 8a contains just lentic system categories with vectors, and Figure 8b contains lentic system categories and individual lentic systems. The darkest gray circles represent NoFish-Dry category, next lightest circles are NoFish-Wet, followed by YesFish-Dry, and the YesFish-Wet as the lightest circles. The squares with X symbols represent Stubblefield Lake, plus signs are Campbell – Lasher Lake, upside down triangles are Long Lake, and the asterisks are TLES Pond. Blackhorse Lake is represented by the triangle symbols, the diamonds with X's are Turnbull Slough, the circles with plus signs are West Issacson Lake, and the open circles are 30 Acre Lake. The X symbols represent Cheever Lake, diamonds are Kepple Lake, triangles with over lapping upside down triangles are West Tritt Lake, and the squares with plus signs are Windmill Pond.

Figure 9. The average macroinvertebrate NMDS scores for MDS axis 1 (Figure 9a) and MDS axis 2 (b), per lentic system category. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as hollow or white dots. The same letters indicate there is no significant difference between groups.

Figure 10. The average clam abundance for the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories over the six sampling cycles throughout the 2015 field season (Mid. April – Mid. August). Data are presented as line/scatter plots with the shape points representing the average abundance of clams and the error bars representing ± 1 standard deviation (sampling sizes are in Table 13).

Figure 11. The average clam abundance for the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots (sampling sizes for each category are in Table 13). The same letters indicate there is no significant difference between groups.

Figure 12a. The average length (adductor to adductor, in mm) of all fingernail clams in the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (NoFish-Wet N = 711, YesFish-Dry N = 431, and YesFish-Wet N = 389).

Figure 12b. The length (adductor to adductor, in mm) of adult clams (length > 5mm) in the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating

the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (NoFish-Wet N = 65, YesFish-Dry N = 170, and YesFish-Wet N = 86).

Figure 13. The average condition index (CI, (g tissue/mm length)*10^3) for all adult clams (> 5 mm in length) for each of the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (sampling sizes for each category are in Table 14).

Figure 14a. The average ratio of RNA to DNA from three or more pooled adult fingernail clam (*Musculium* spp.) feet in the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots. The sample sizes for the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic systems are 5, 16, and 12 respectively.

Figure 14b. The ratio of RNA to DNA/length from 3 or more pooled adult clam (lengths > 5 mm) feet in the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots. The bars represent the standard deviations.

The sample sizes for the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic systems are 5, 16, and 12 respectively.

Figure 15. The average, total number of brooded larvae in the brood sacs of all adult fingernail clams (*Musculium* spp.) in the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots. Boxplots with nonmatching letters are significantly different (NoFish-Wet N = 61, YesFish-Dry N = 168, and YesFish-Wet N = 86).

Figure 16. The average, total number of brooded larvae/length (adductor to adductor, in mm) within all adult clams (length > 5 mm) in the NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (NoFish-Wet N = 61, YesFish-Dry N = 168, and YesFish-Wet N = 86).

Figure 17. The total number of brooded larvae for each clam length (adductor to adductor, in mm) across all lentic system categories. Data are presented as scatter plots with the dots representing the data points for the number of brooded larvae/lengths and the line as the linear regression.

Figure 18. The total number of brooded larvae for each clam length (adductor to adductor, in mm) in the YesFish-Wet lentic system category, specifically Kepple Lake. Data are presented as scatter plots with the dots representing the data points for the number of juveniles/lengths and the solid black line as the linear regression.

Figure 19. The total number of brooded larvae for each clam length (adductor to adductor, in mm) in the YesFish-Dry lentic system category, specifically West Issacson and 30 Acre Lake. Data are presented as scatter plots with the dots representing the data points for the number of juveniles/lengths and the lines as the linear regressions. West Issacson Lake is represented by the hollow or white dots and the thin solid line, and 30 Acre Lake is represented by the black dots and the thick dashed line.

Figure 20. The total number of brooded larvae for each clam length (adductor to adductor, in mm) in the NoFish-Wet lentic system category, specifically Campbell-Lasher Lake and TLES Pond. Data are presented as scatter plots with the dots representing the data points for the number of brooded larvae/lengths and the lines as the linear regression. Campbell-Lasher Lake is represented by the hollow or white dots and the thin solid line, and TLES Pond is represented by the black dots and the thick dashed line.

Figure 21. The proportion of clams in each size class (shell length in mm) for the NoFish-Wet (N = 711), YesFish-Dry (N = 431), and YesFish-Wet (N = 399) lentic system categories.

Figure 22a. The proportions of clams in each size class (shell length in mm) for TLES Pond, Long Lake, and Campbell – Lasher Lake across the sampling cycles.

Figure 22b. The proportions of clams in each size class (shell length in mm) for Blackhorse Lake, West Issacson Lake, the Turnbull Slough, and 30 Acre Lake across the sampling cycles.

Figure 22c. The proportions of clams in each size class (shell length in mm) for Kepple Lake, Cheever Lake, West Tritt Lake, and Windmill Pond across the sampling cycles.

Figure 23. The average chlorophyll level (µg/L) for each of the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots (NoFish-Dry N = 6, NoFish-Wet N = 64, YesFish-Dry N = 56, and YesFish-Wet N = 67).

Figure 24. The average transparency level (% absorbance, inverse of turbidity) for each of the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system

categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (NoFish-Dry N = 6, NoFish-Wet N = 64, YesFish-Dry N = 56, and YesFish-Wet N = 68).

Figure 25. The average dissolved oxygen level (mg/L) for each of the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots (NoFish-Dry N = 6, NoFish-Wet N = 60, YesFish-Dry N = 56, and YesFish-Wet N = 68).

Figure 26. The average conductivity level (µS) for each of the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (NoFish-Dry N = 3, NoFish-Wet N = 59, YesFish-Dry N = 53, and YesFish-Wet N = 64).

Figure 27. The average water temperature (°C) for each of the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th

percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots (NoFish-Dry N = 6, NoFish-Wet N = 64, YesFish-Dry N = 56, and YesFish-Wet N = 68).

Figure 28. The average pH level for each of the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (NoFish-Dry N = 6, NoFish-Wet N = 64, YesFish-Dry N = 56, and YesFish-Wet N = 68).

Figure 29. NMDS plot of all water quality parameters, excluding ammonia, nitrate, and phosphate, by lentic system category and individual lentic systems. Figure 29a contains lentic system categories with vectors, and Figure 29b contains lentic system categories and individual lentic systems. The darkest gray circles represent NoFish-Dry category, next lightest circles are NoFish-Wet, followed by YesFish-Dry, and the YesFish-Wet as the lightest circles. The squares with X symbols represent Stubblefield Lake, plus signs are Campbell – Lasher Lake, upside down triangles are Long Lake, and the asterisks are TLES Pond. Blackhorse Lake is represented by the triangle symbols, the diamonds with X's are Turnbull Slough, the circles with plus signs are West Issacson Lake, and the open circles are 30 Acre Lake. The X symbols represent Cheever Lake, diamonds are Kepple Lake, triangles with over lapping upside down triangles are West Tritt Lake, and the squares with plus signs are Windmill Pond.

Figure 30. The average water quality (excluding ammonia, nitrate, and phosphate level) NMDS scores for MDS axis 1 (Figure 30a) and MDS axis 2 (30b), per lentic system category. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as hollow or white dots. The same letters indicate there is no significant difference between groups.

Figure 31. The average ammonia level (ppt) for each of the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots (NoFish-Dry N = 1, NoFish-Wet N = 37, YesFish-Dry N = 24, and YesFish-Wet N = 42).

Figure 32. The average nitrate level (ppt) for each of the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (NoFish-Dry N = 4, NoFish-Wet N = 44, YesFish-Dry N = 35, and YesFish-Wet N = 32).

Figure 33. The average phosphate level (ppt) for each of the NoFish-Dry, NoFish-Wet, YesFish-Dry, and YesFish-Wet lentic system categories. Data are presented as box and

whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (NoFish-Dry N = 6, NoFish-Wet N = 59, YesFish-Dry N = 55, and YesFish-Wet N = 68).

Figure 34. The average chlorophyll level (µg/L) for each of the NoFish, LowFish, and High Fish mesocosm categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots (NoFish N = 43, LowFish N = 50, and HighFish N = 50).

Figure 35. The average transparency level (% absorbance, inverse of turbidity) for each of the NoFish, LowFish, and High Fish mesocosm categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots (NoFish N = 45, LowFish N = 50, and HighFish N = 50).

Figure 36. The average dissolved O_2 level (mg/L) for each of the NoFish, LowFish, and High Fish mesocosm categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots (NoFish N = 45, LowFish N = 50, and HighFish N = 50).

Figure 37. The average conductivity level (µS) for each of the NoFish, LowFish, and High Fish mesocosm categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots (NoFish N = 45, LowFish N = 50, and HighFish N = 50).

Figure 38. The average water temperature (°C) for each of the NoFish, LowFish, and High Fish mesocosm categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots (NoFish N = 45, LowFish N = 50, and HighFish N = 50).

Figure 39. The average pH level for each of the NoFish, LowFish, and High Fish mesocosm categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers indicating the 10th – 90th percentile, and outliers indicated as dots. The same letters indicate there is no significant difference between groups (NoFish N = 45, LowFish N = 50, and HighFish N = 50).

Figure 40. The average ammonia level (ppt) for each of the NoFish, LowFish, and High Fish mesocosm categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25th to 75th percentiles, the whiskers

indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots (NoFish N = 38, LowFish N = 39, and HighFish N = 40).

Figure 41. The average nitrate level (ppt) for each of the NoFish, LowFish, and High Fish mesocosm categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots (NoFish N = 15, LowFish N = 17, and HighFish N = 22).

Figure 42. The average phosphate level (ppt) for each of the NoFish, LowFish, and High Fish mesocosm categories. Data are presented as box and whisker plots with the line indicating the median, the box indicating the 25^{th} to 75^{th} percentiles, the whiskers indicating the 10^{th} – 90^{th} percentile, and outliers indicated as dots (NoFish N = 40, LowFish N = 45, and HighFish N = 44).

FIGURES

Figure 1a

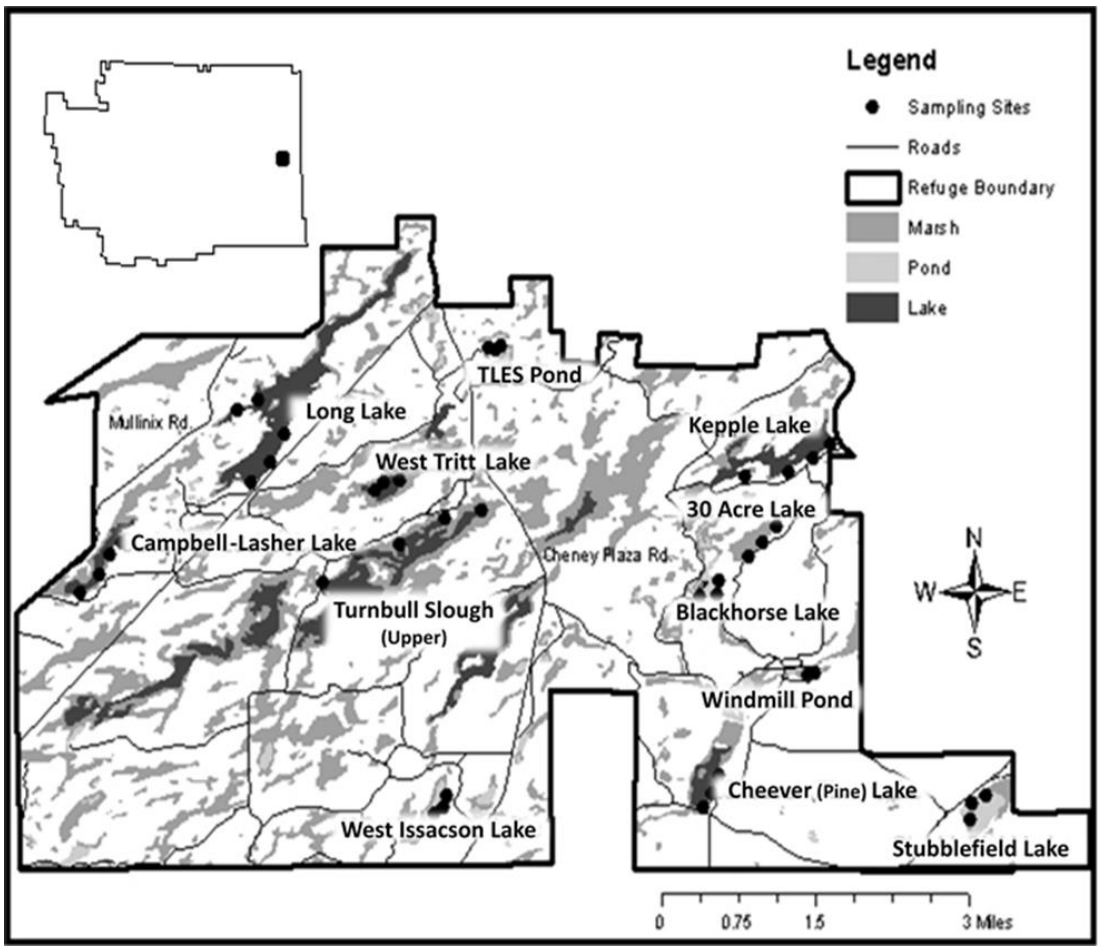
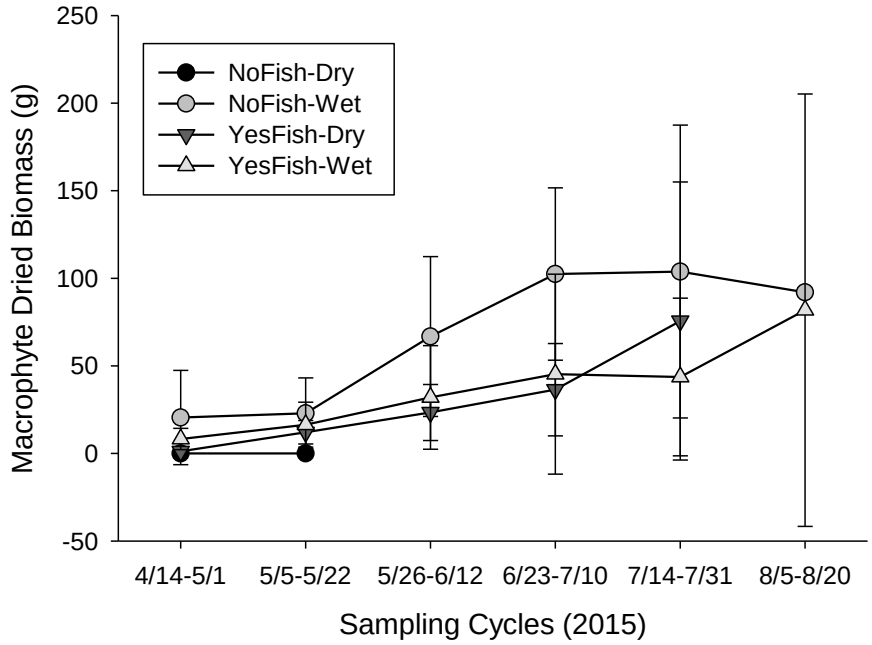


Figure 1b

Figure 2



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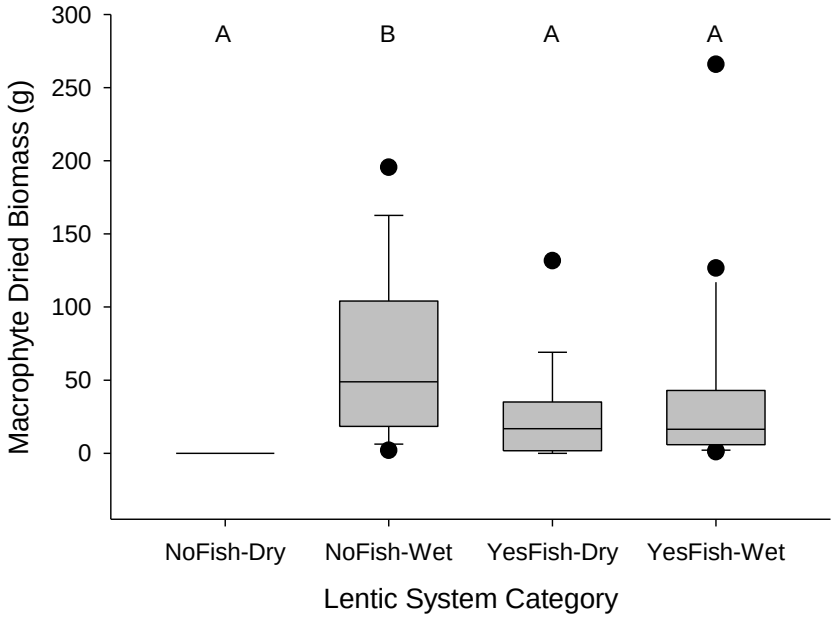

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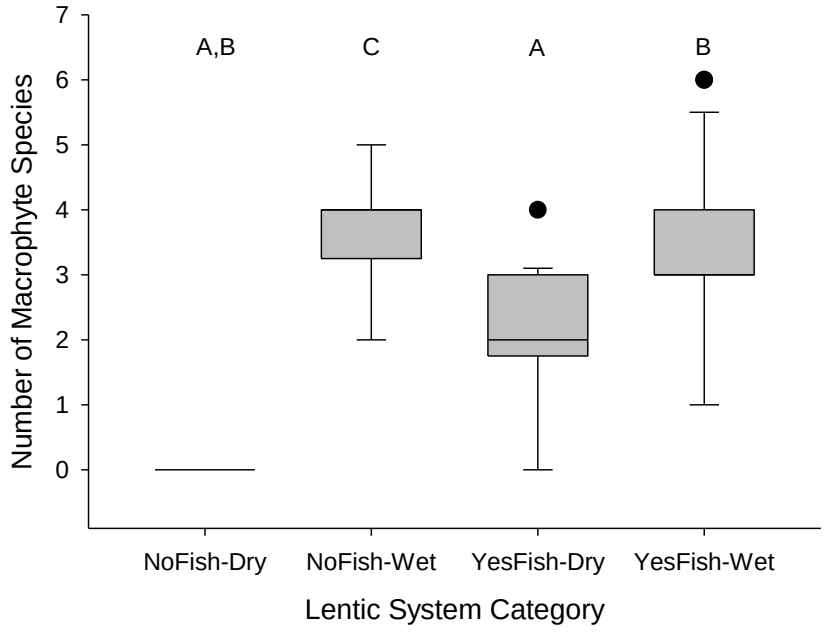
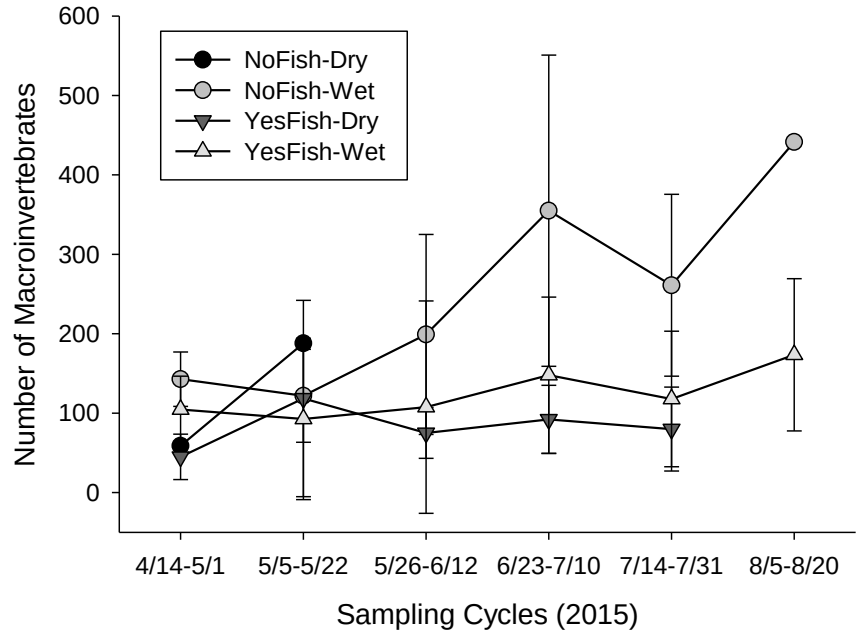


Figure 5



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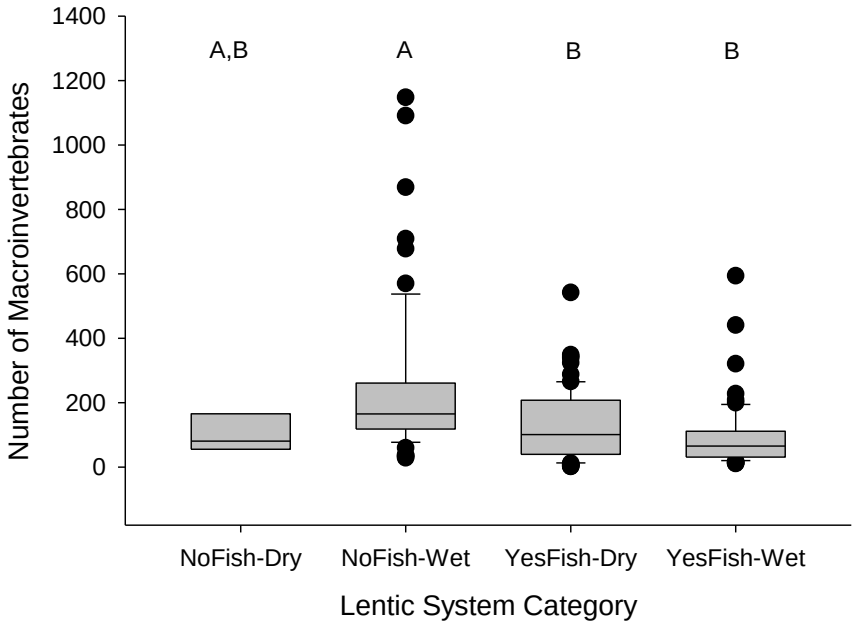


Figure 7

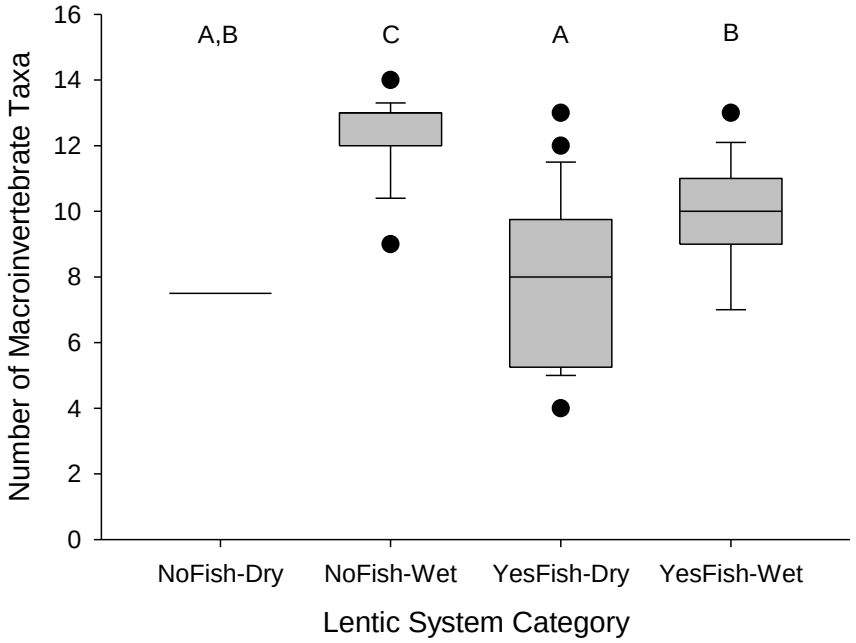


Figure 8a

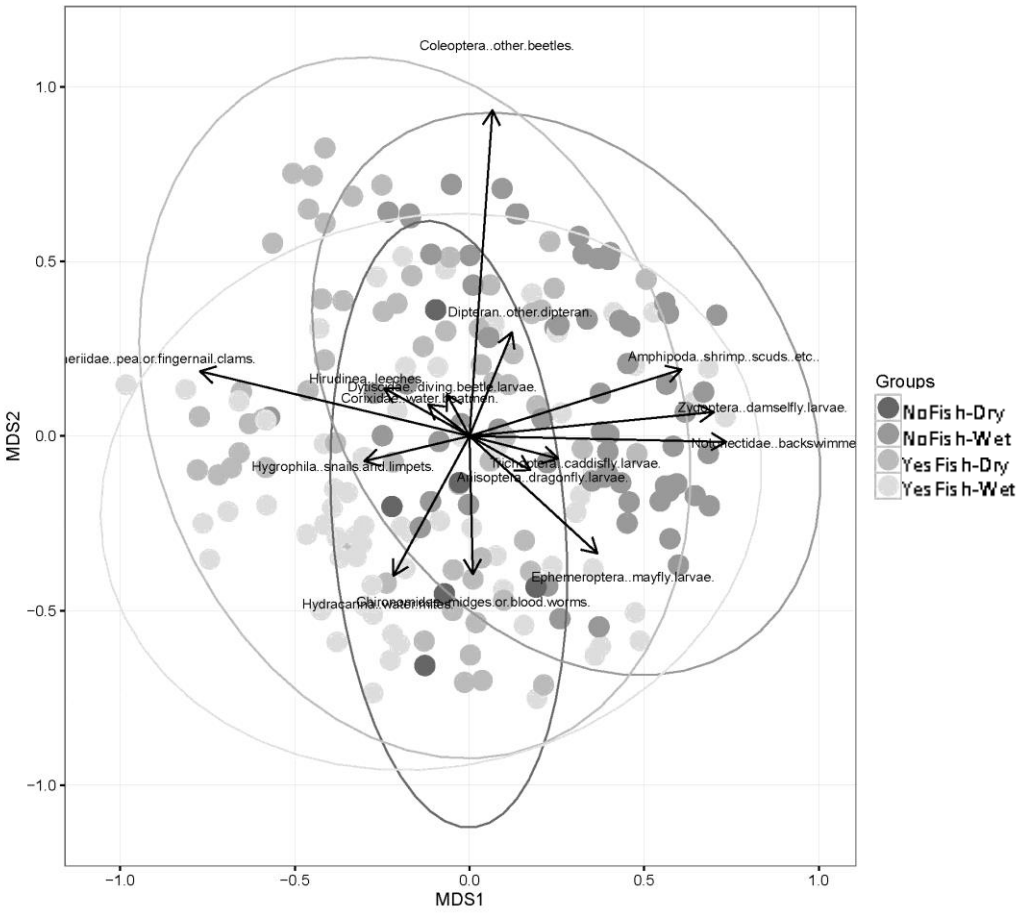

Coleoptera..other.beetles.
Dipteran..other.dipteran.
Amphipoda..shrimp..scuds..etc..
Hirudinea..leeches.
Dytiscidae..diving.beetle.larvae.
Corixidae..water.boatmen.
Zygoptera..damselfly.larvae.
Hygrophila..snails.and.limpets.
Trichoptera..caddisfly.larvae.
Anisoptera..dragonfly.larvae.
Ephemeroptera..mayfly.larvae.
Hydracarina..water.mites.
Chironomidae..midges.or.blood.worms.
MDS1
MDS2
1.0
0.5
0.0
−0.5
−1.0
Groups
NoFish-Dry
NoFish-Wet
Yes Fish-Dry
Yes Fish-Wet

Figure 8b

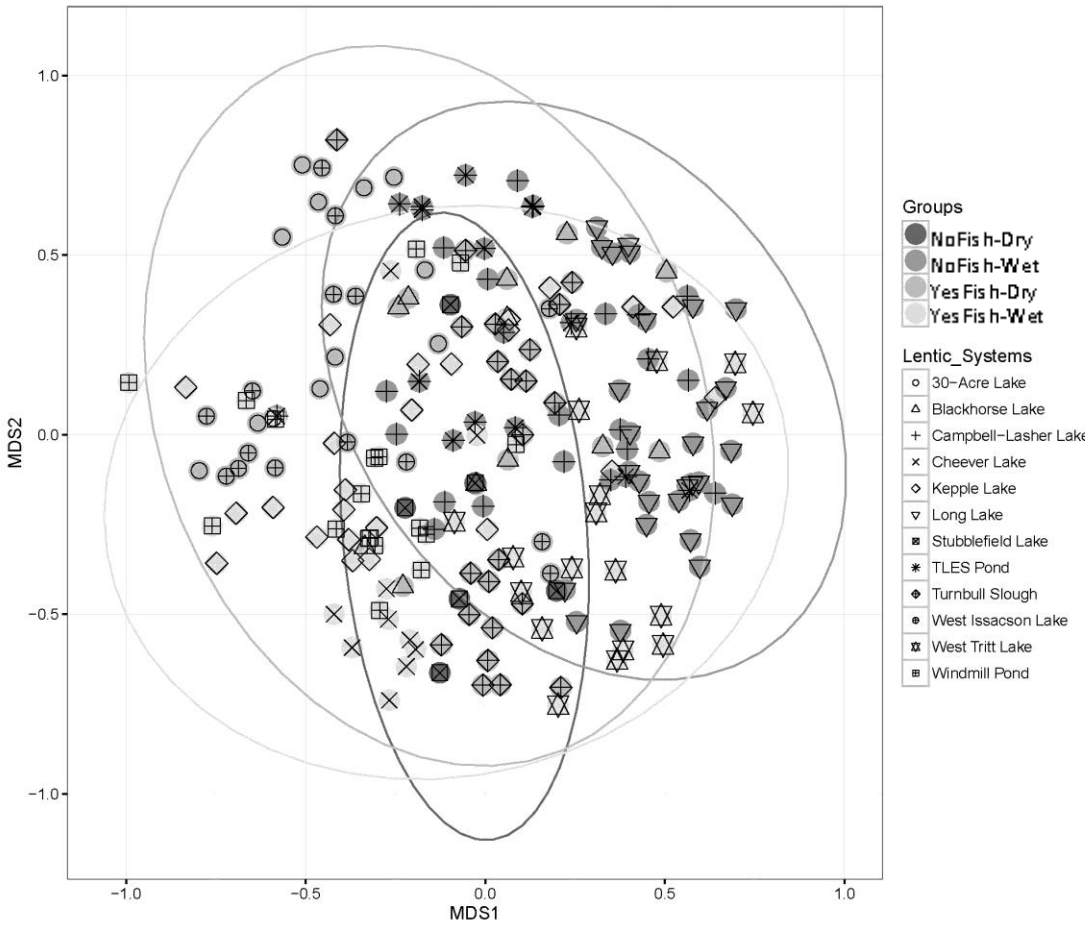

MDS2
MDS1
1.0
0.5
0.0
−0.5
−1.0
−1.0
−0.5
0.0
0.5
1.0
Groups
NoFish-Dry
NoFish-Wet
Yes Fish-Dry
Yes Fish-Wet
Lentic_Systems
30−Acre Lake
Blackhorse Lake
Campbell−Lasher Lake
Cheever Lake
Kepple Lake
Long Lake
Stubblefield Lake
TLES Pond
Turnbull Slough
West Issacson Lake
West Tritt Lake
Windmill Pond

Figure 9a

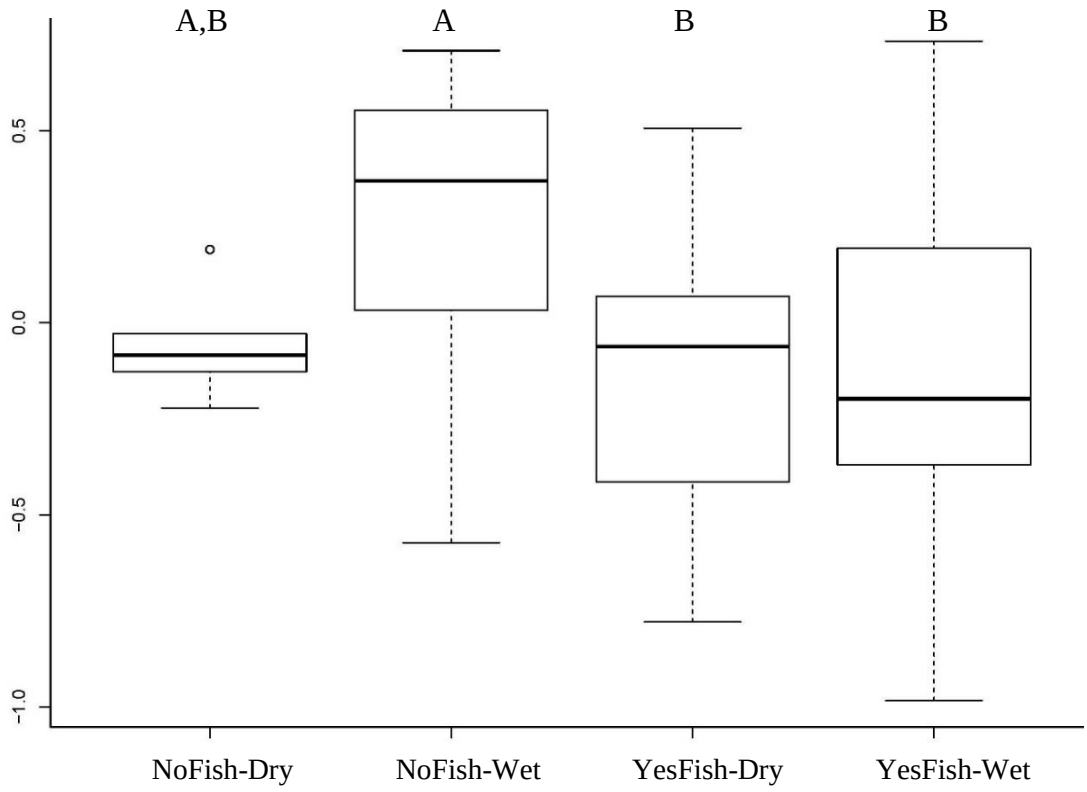

A,B
A
B
B
0.5
0.0
−0.5
−1.0
NoFish-Dry
NoFish-Wet
YesFish-Dry
YesFish-Wet

Figure 9b

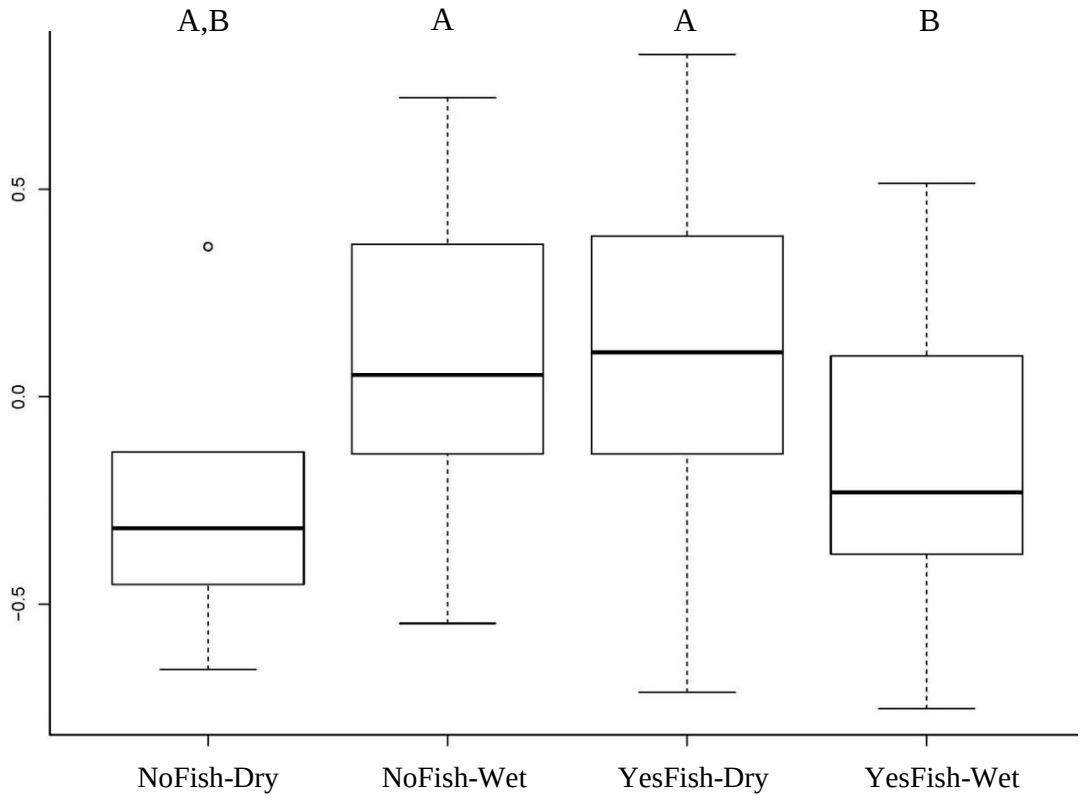


Figure 10

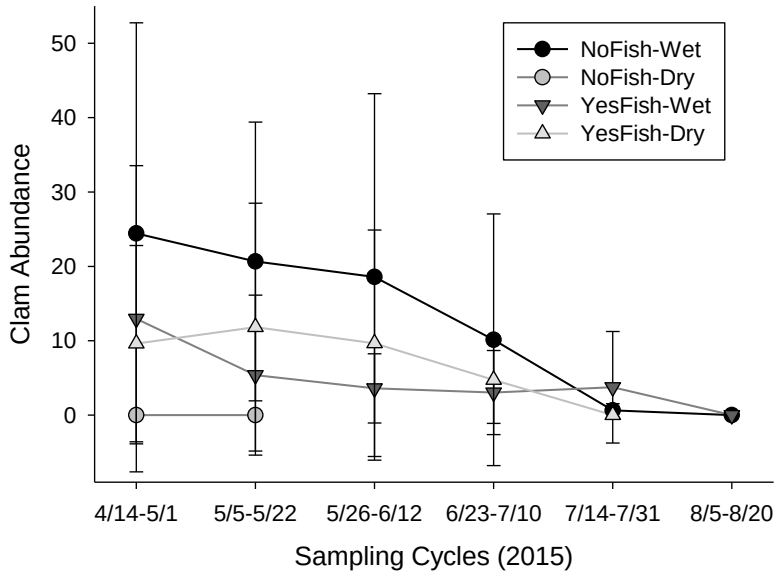


Figure 11

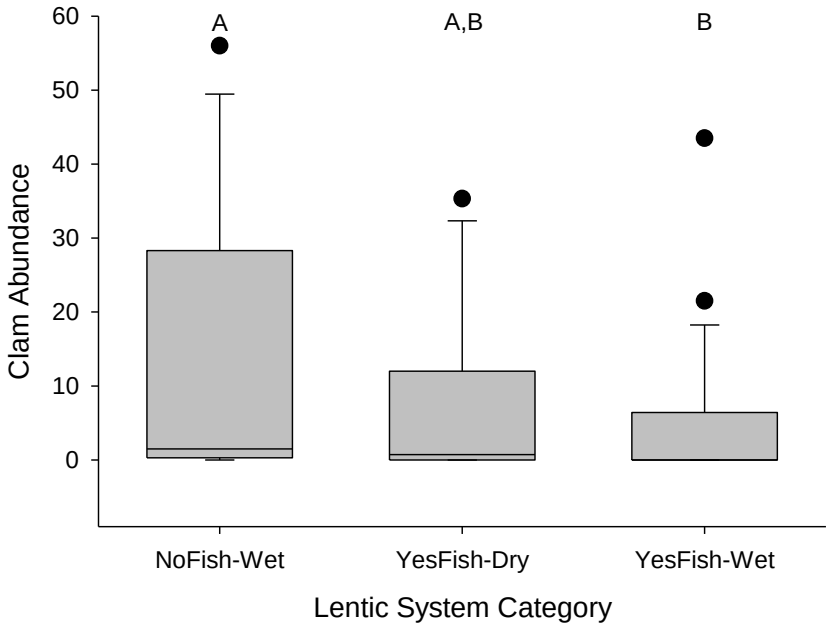


Figure 12a

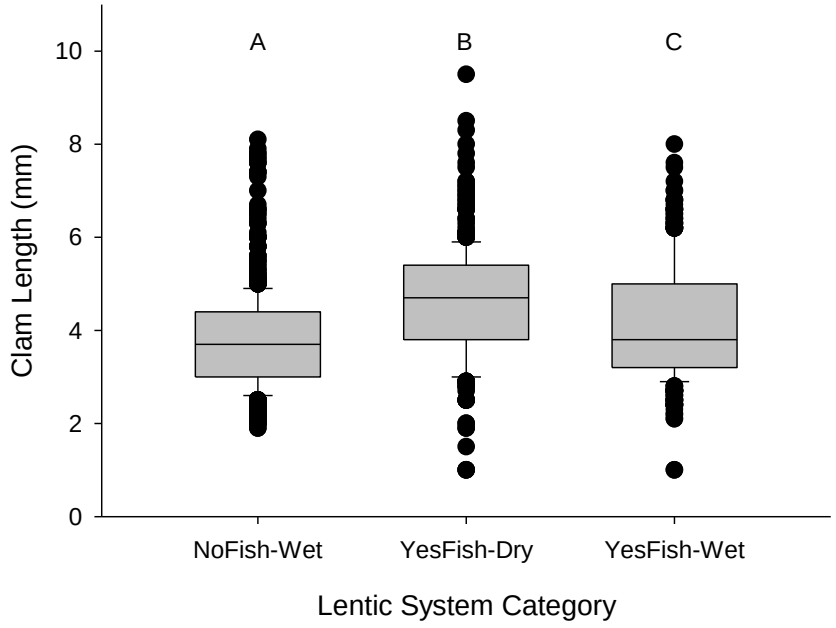


Figure 12b

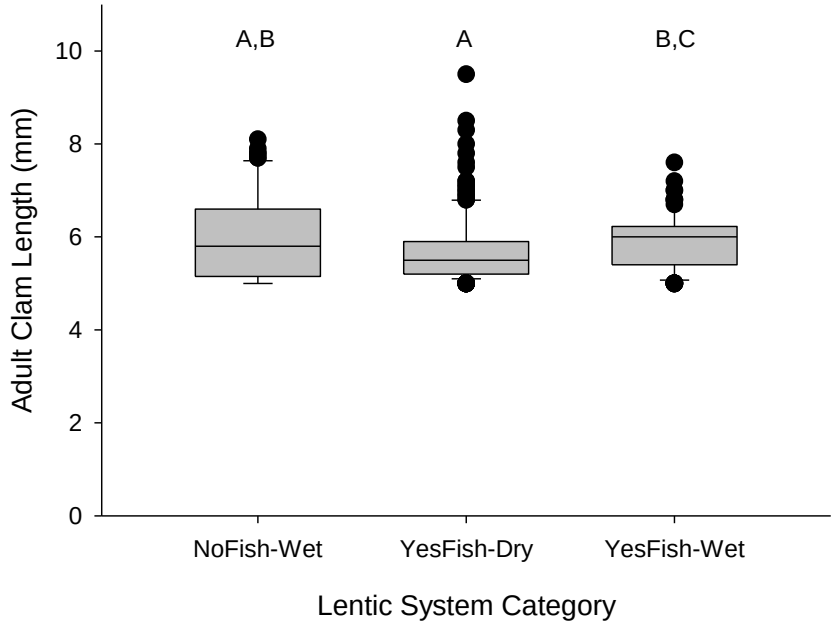


Figure 13

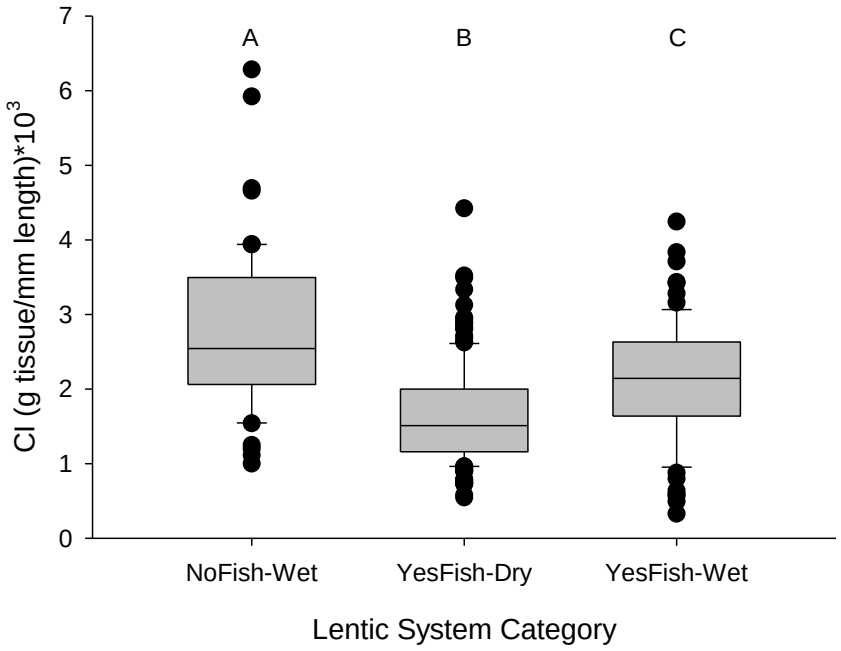


Figure 14a

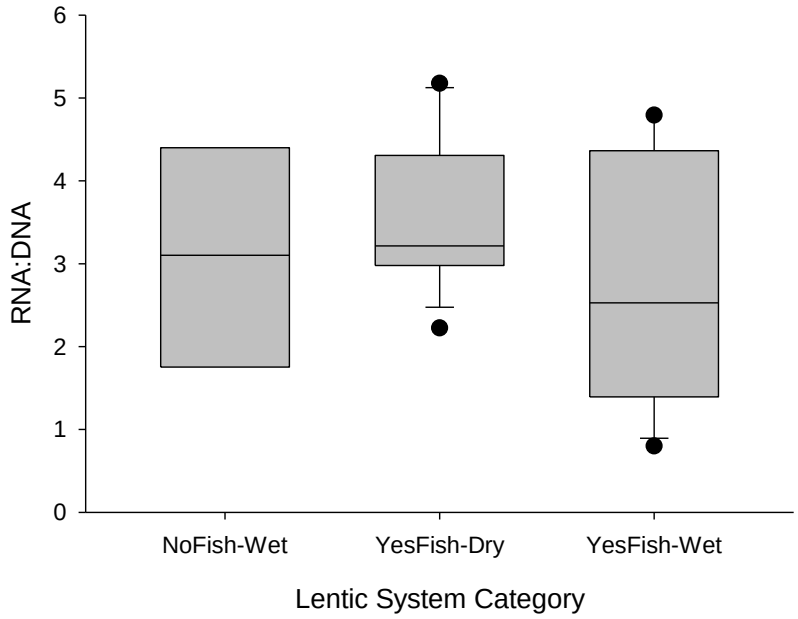


Figure 14b

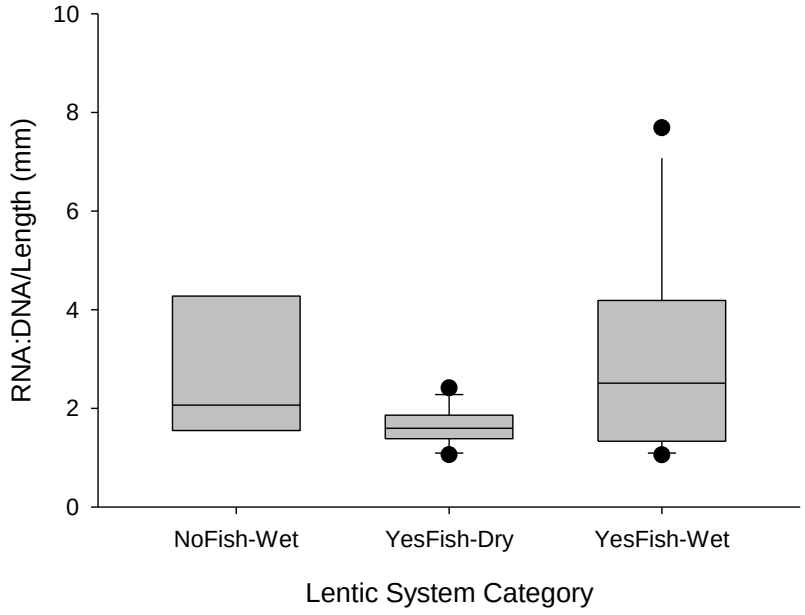


Figure 15

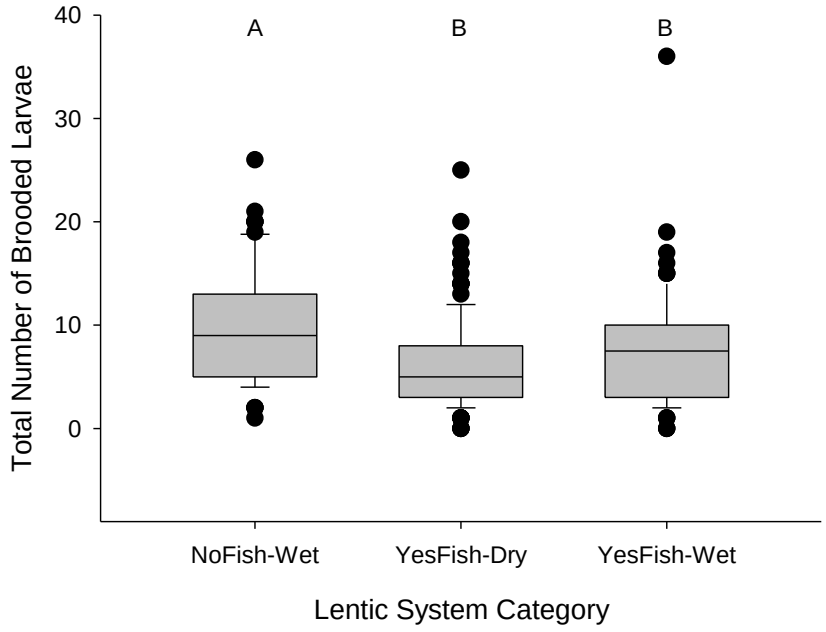


Figure 16

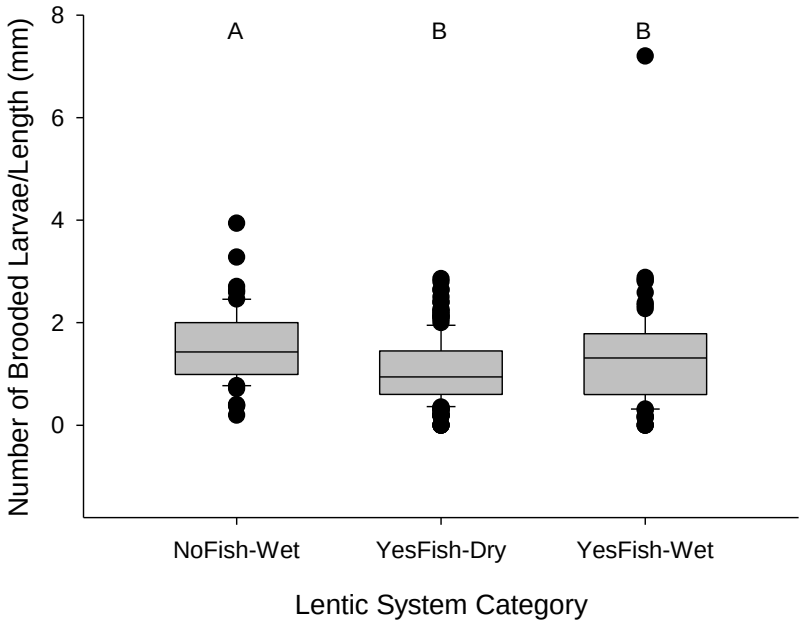


Figure 17

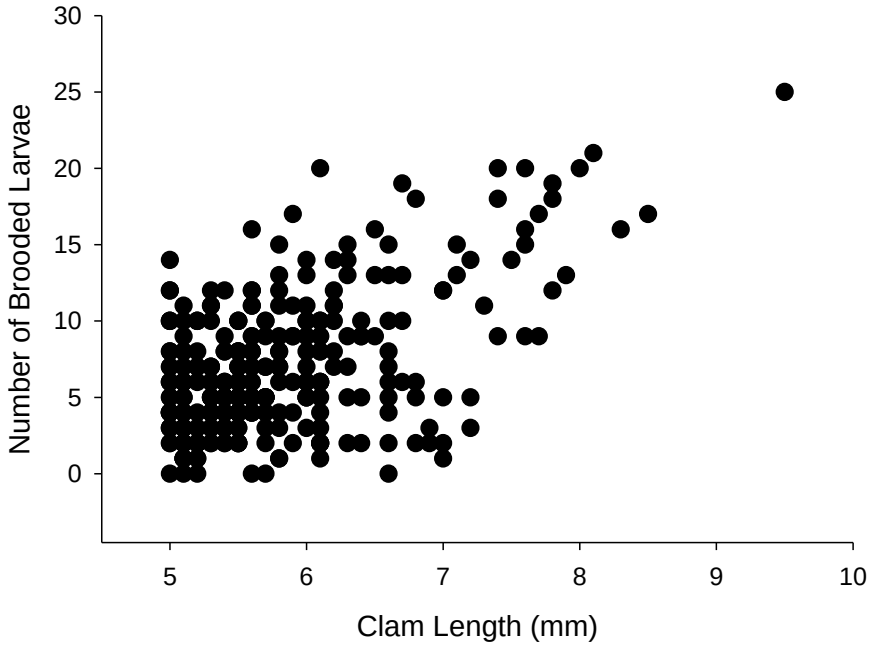


Figure 18

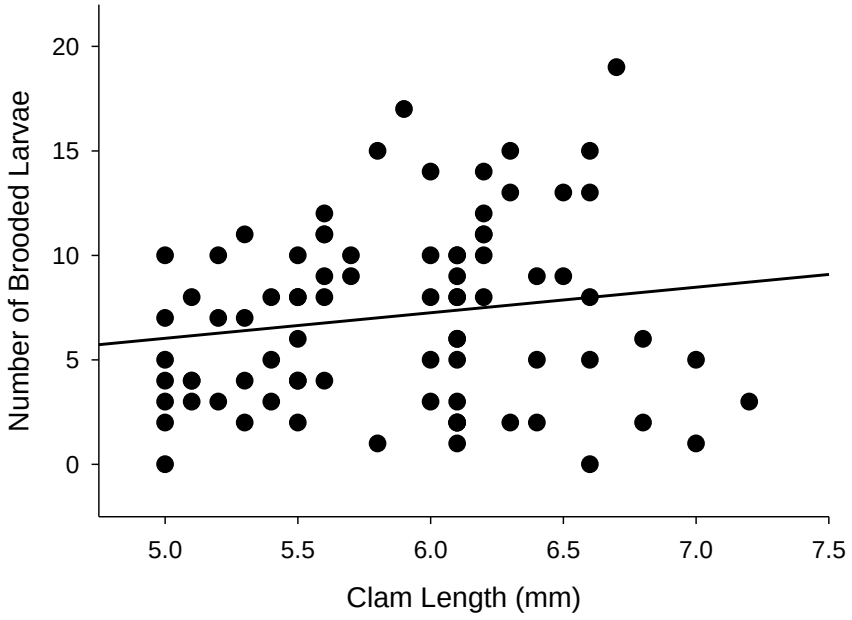
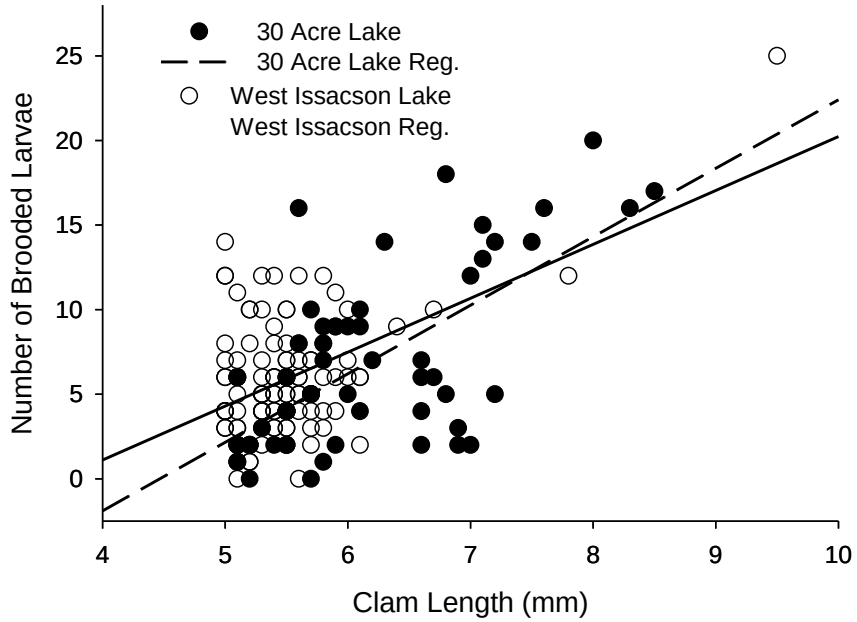


Figure 19



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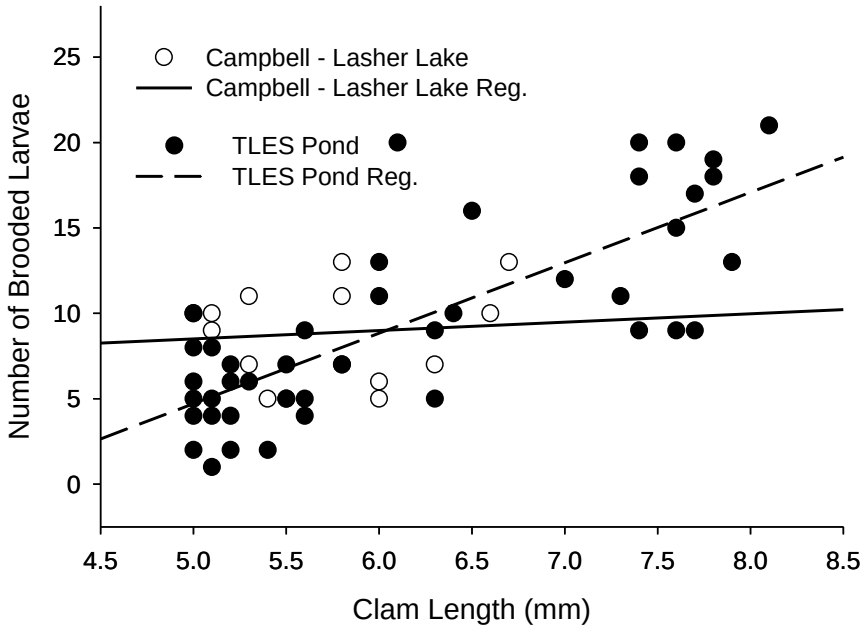

Campbell - Lasher Lake
Campbell - Lasher Lake Reg.
TLES Pond
TLES Pond Reg.
Number of Brooded Larvae
Clam Length (mm)
0
5
10
15
20
25
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5

Figure 21

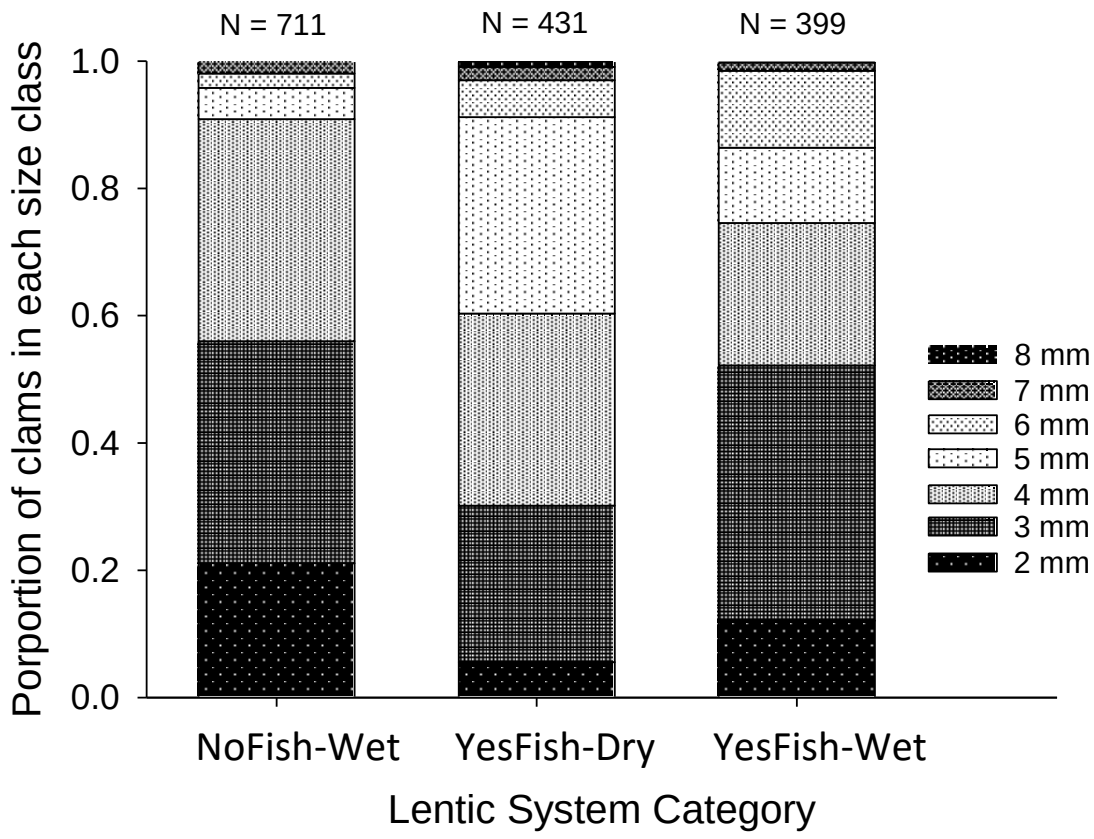

N = 711
N = 431
N = 399
Porportion of clams in each size class
1.0
0.8
0.6
0.4
0.2
0.0
8 mm
7 mm
6 mm
5 mm
4 mm
3 mm
2 mm
NoFish-Wet
YesFish-Dry
YesFish-Wet
Lentic System Category

Figure 22a

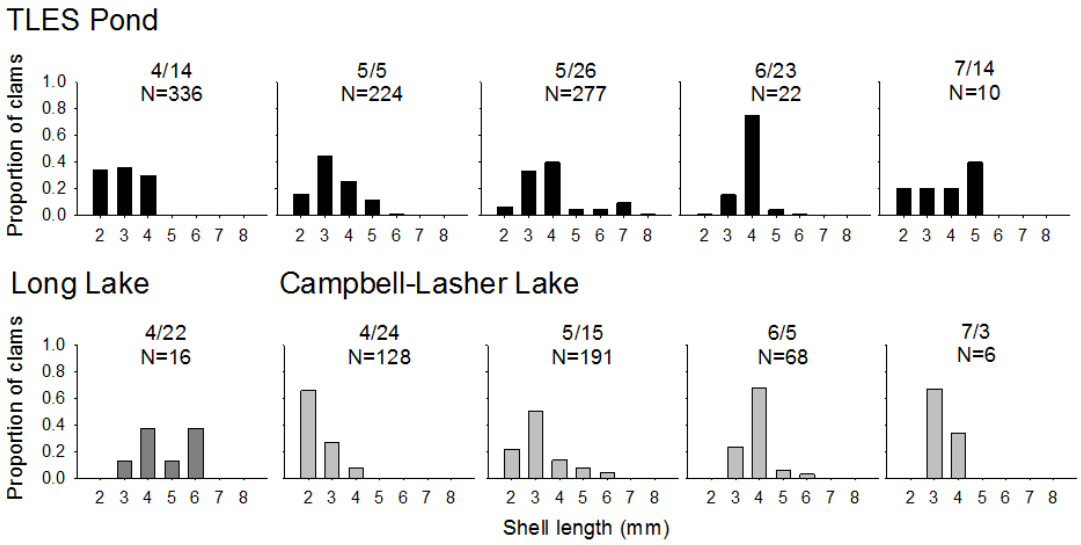


Figure 22b

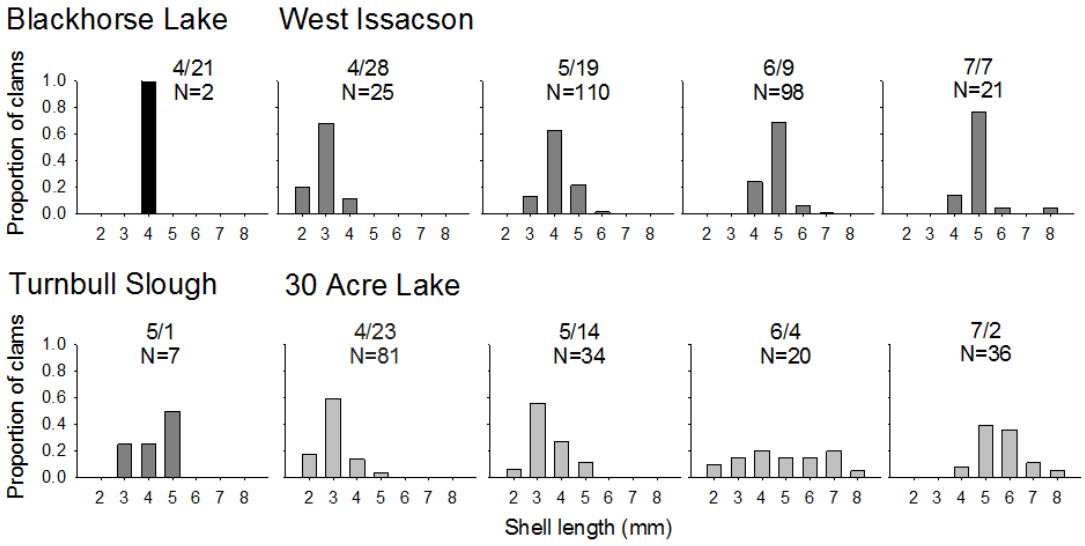


Figure 22c

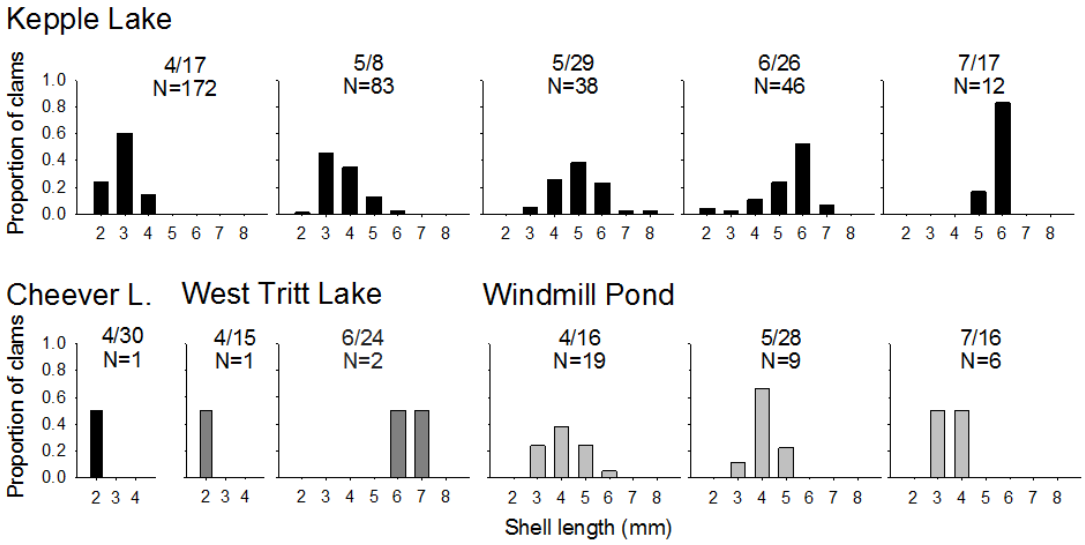


Figure 23

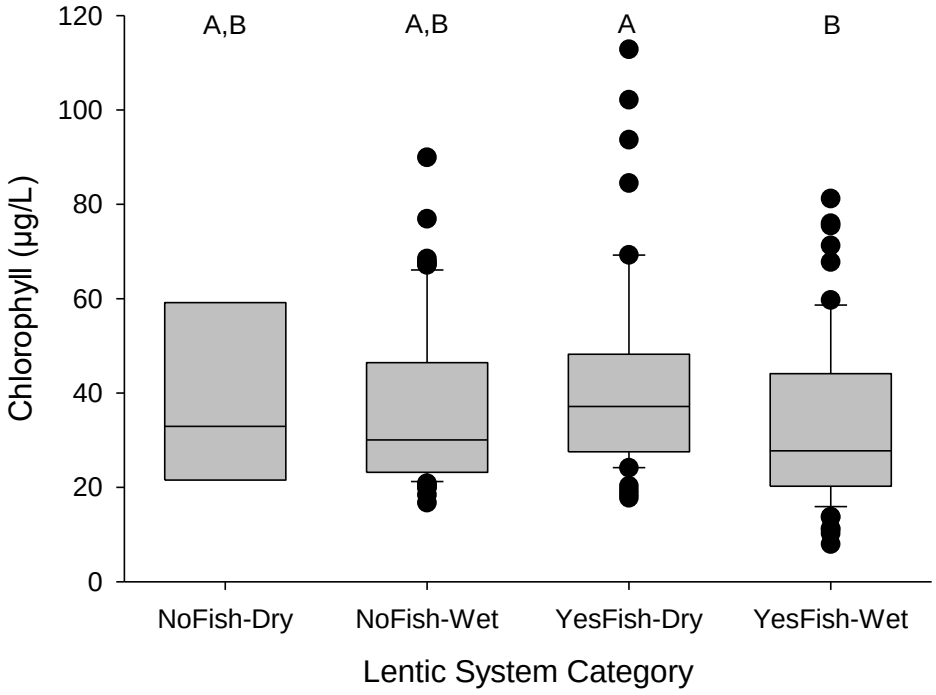


Figure 24

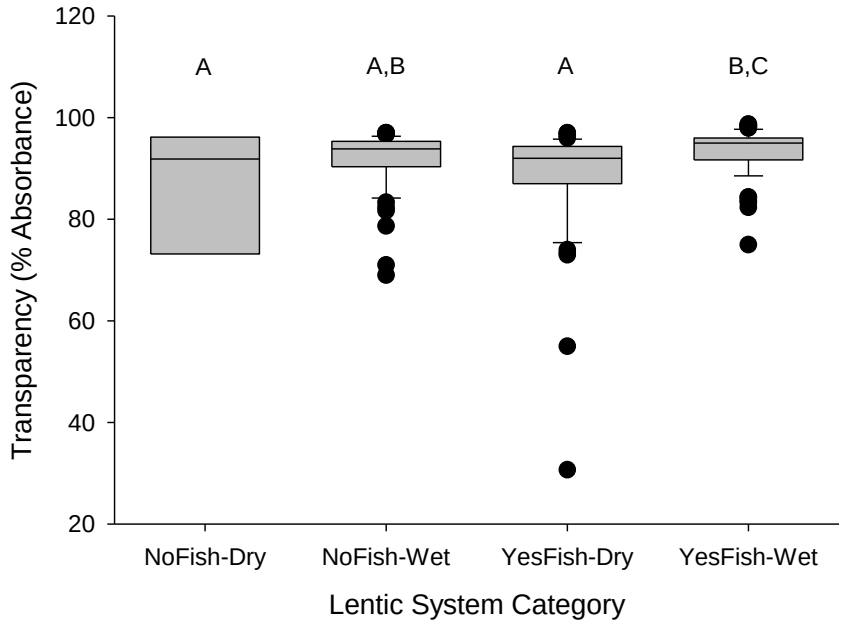


Figure 25

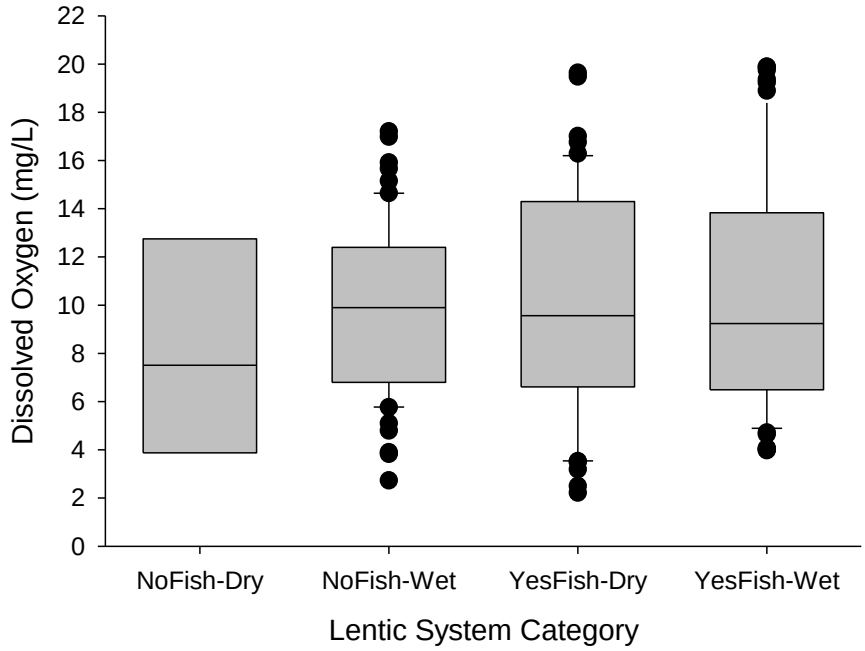


Figure 26

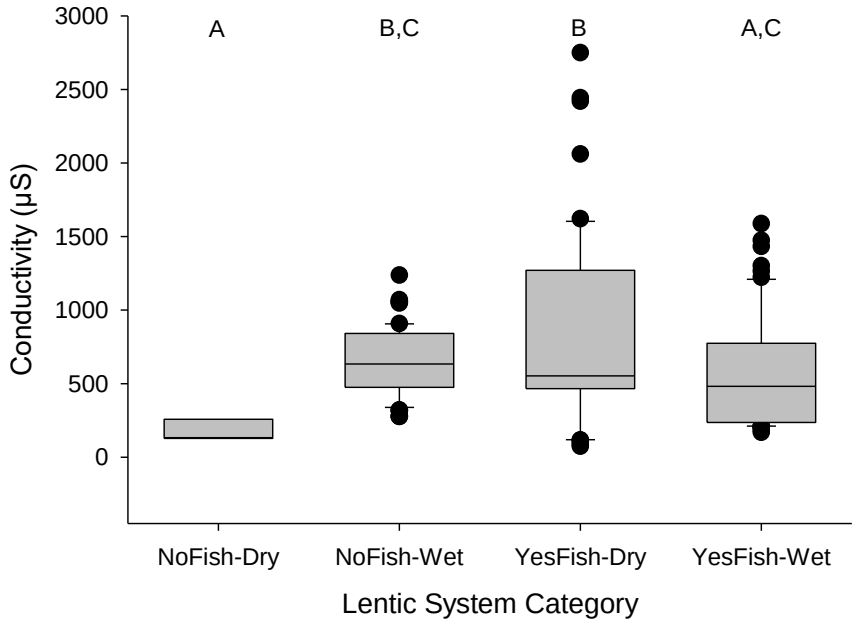


Figure 27

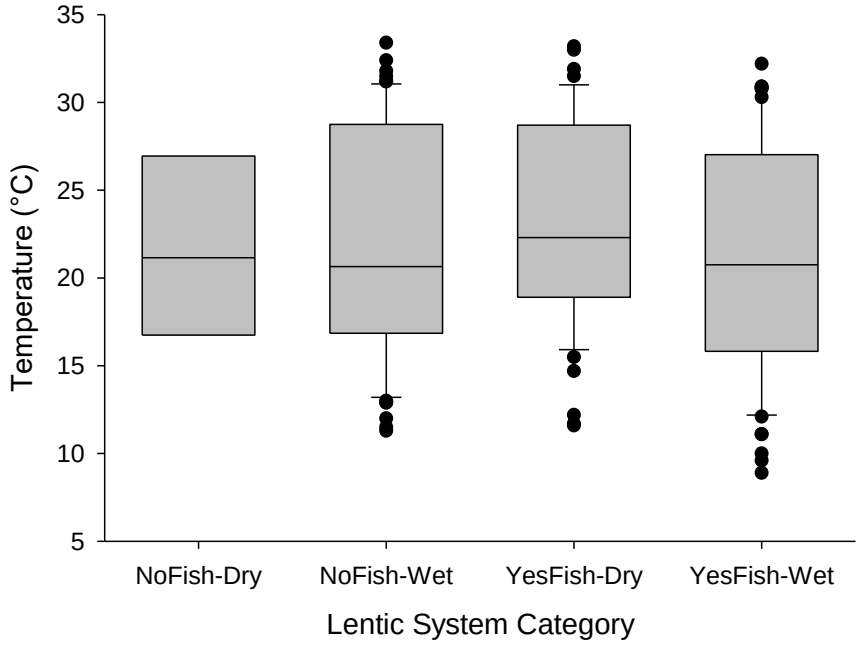


Figure 28

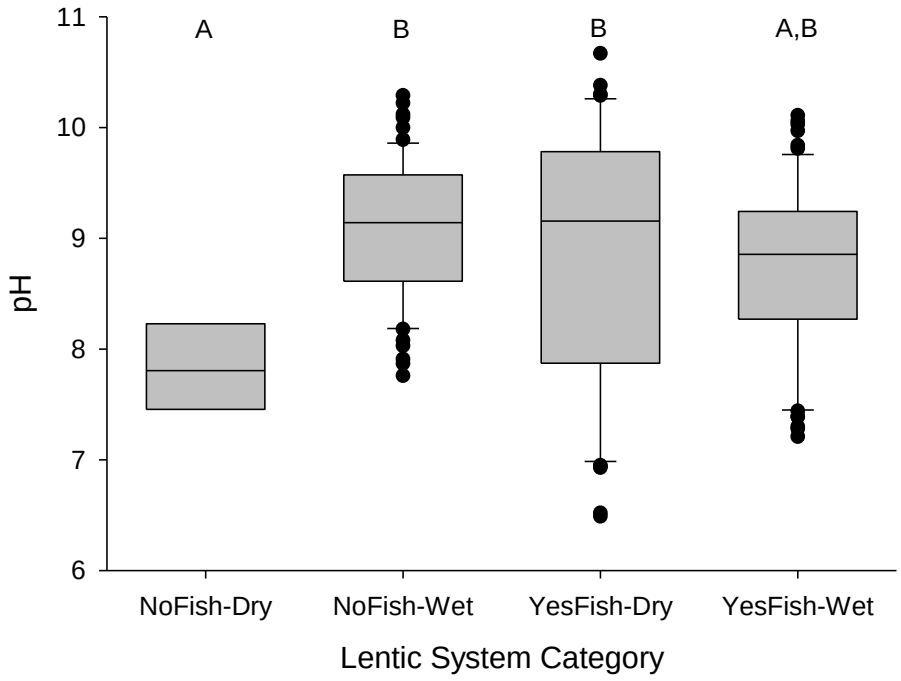

11
10
9
8
7
6
pH
A
B
B
A,B
NoFish-Dry
NoFish-Wet
YesFish-Dry
YesFish-Wet
Lentic System Category

Figure 29a

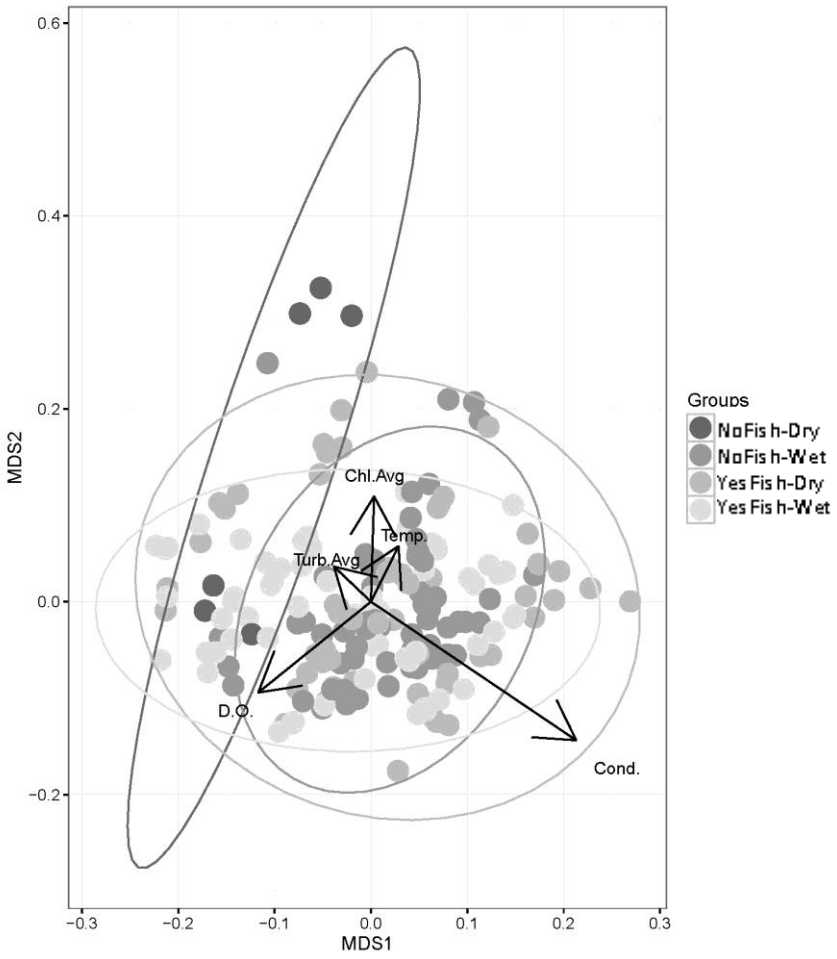

0.6
0.4
0.2
0.0
−0.2
MDS2
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3
MDS1
Chl.Avg
Temp.
Turb.Avg
D.O.
Cond.
Groups
NoFish-Dry
NoFish-Wet
Yes Fish-Dry
Yes Fish-Wet

Figure 29b

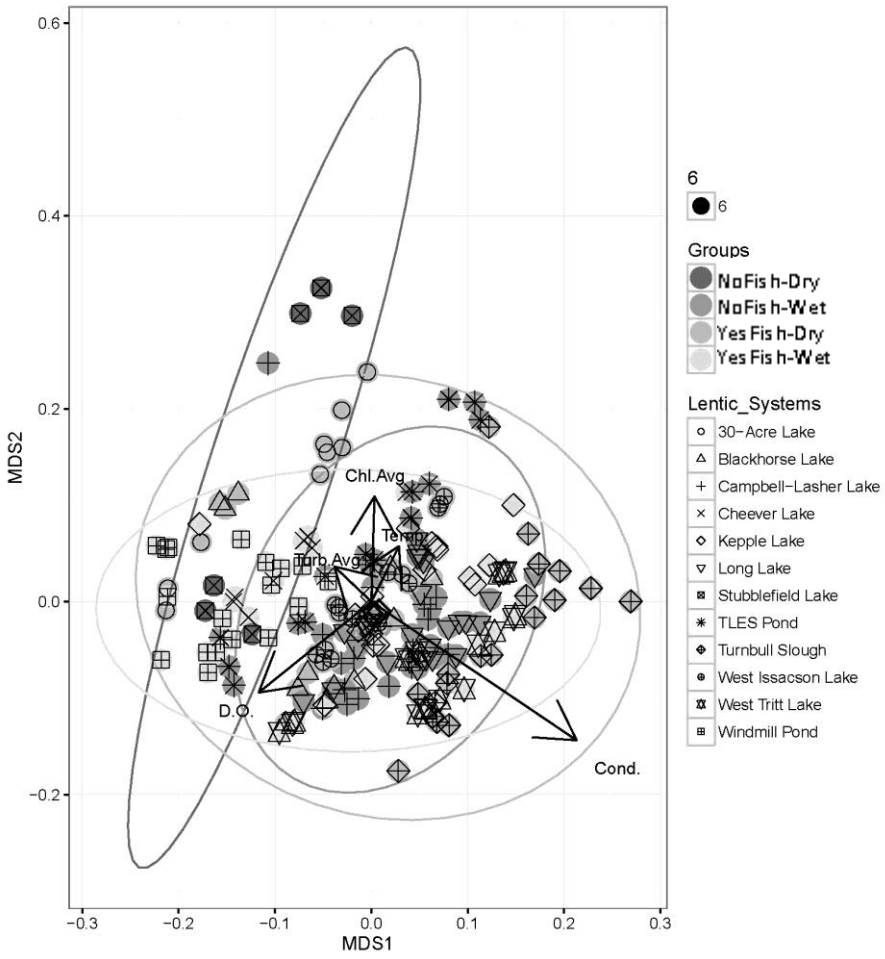

MDS2
MDS1
0.6
0.4
0.2
0.0
−0.2
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3
Chl.Avg.
Temp.
Turb.Avg.
D.O.
Cond.
6
6
Groups
NoFish-Dry
NoFish-Wet
Yes Fish-Dry
Yes Fish-Wet
Lentic_Systems
30−Acre Lake
Blackhorse Lake
Campbell−Lasher Lake
Cheever Lake
Kepple Lake
Long Lake
Stubblefield Lake
TLES Pond
Turnbull Slough
West Issacson Lake
West Tritt Lake
Windmill Pond

Figure 30a

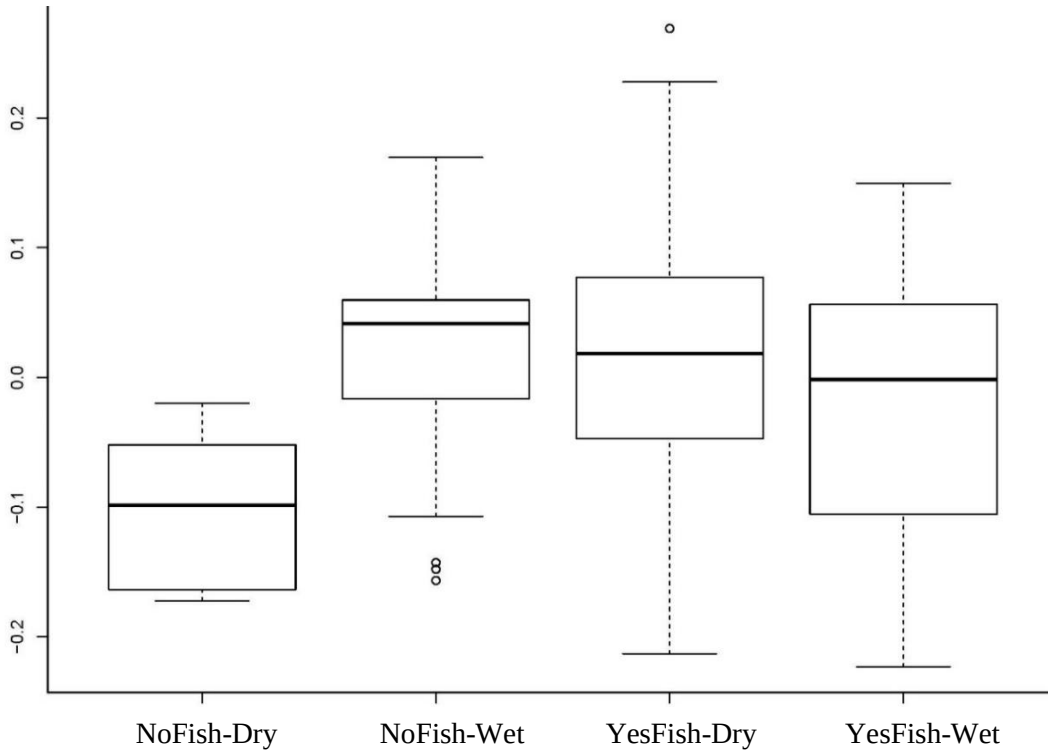


Figure 30b

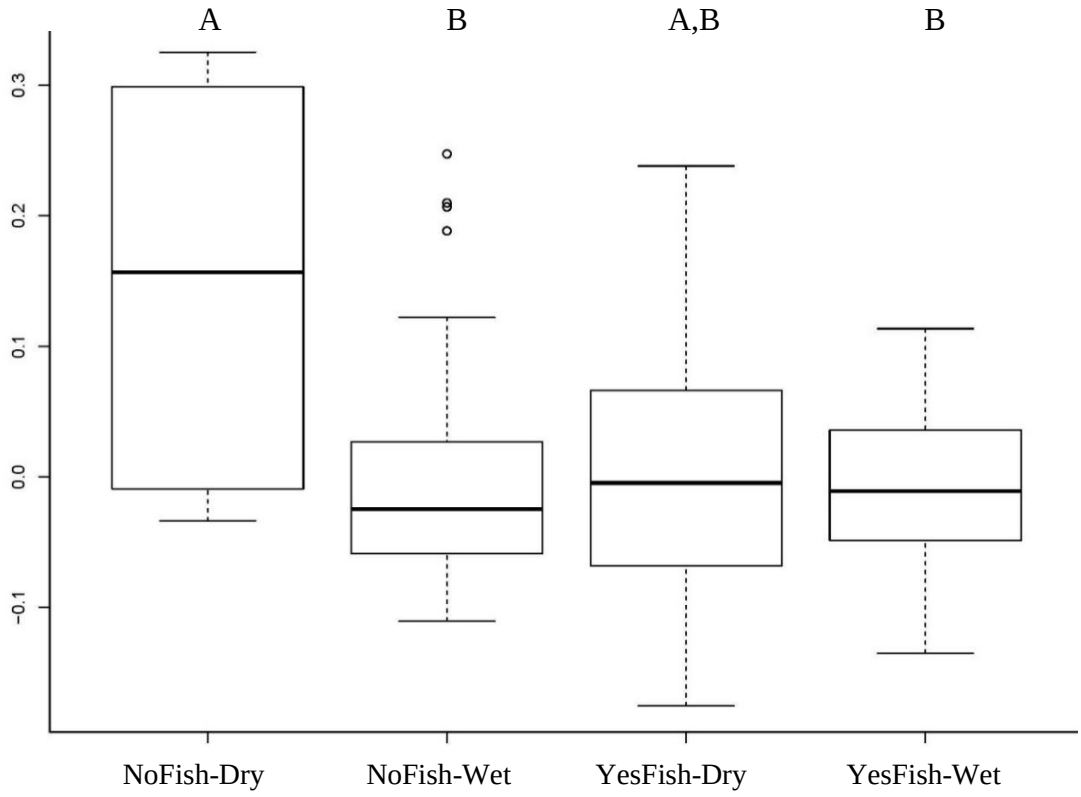

A
B
A,B
B
0.3
0.2
0.1
0.0
−0.1
NoFish-Dry
NoFish-Wet
YesFish-Dry
YesFish-Wet

Figure 31

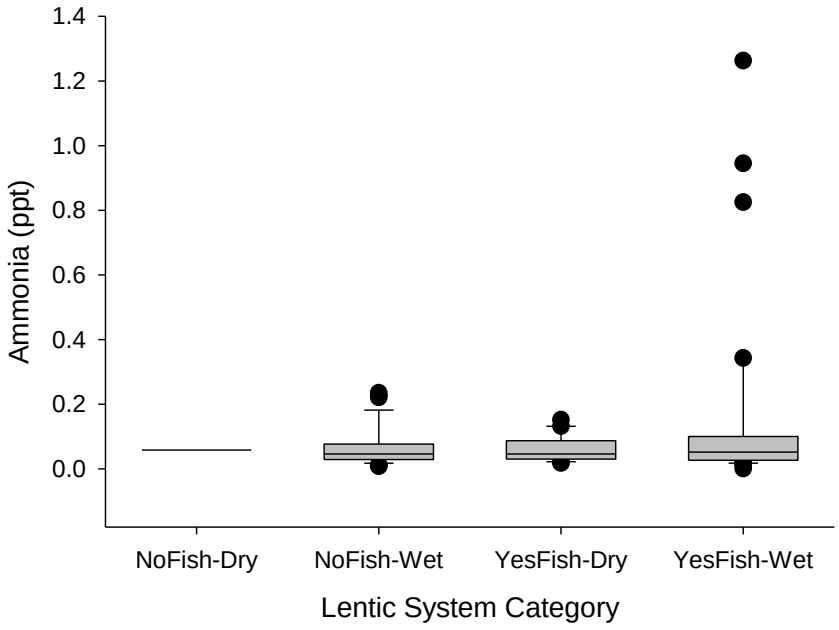


Figure 32

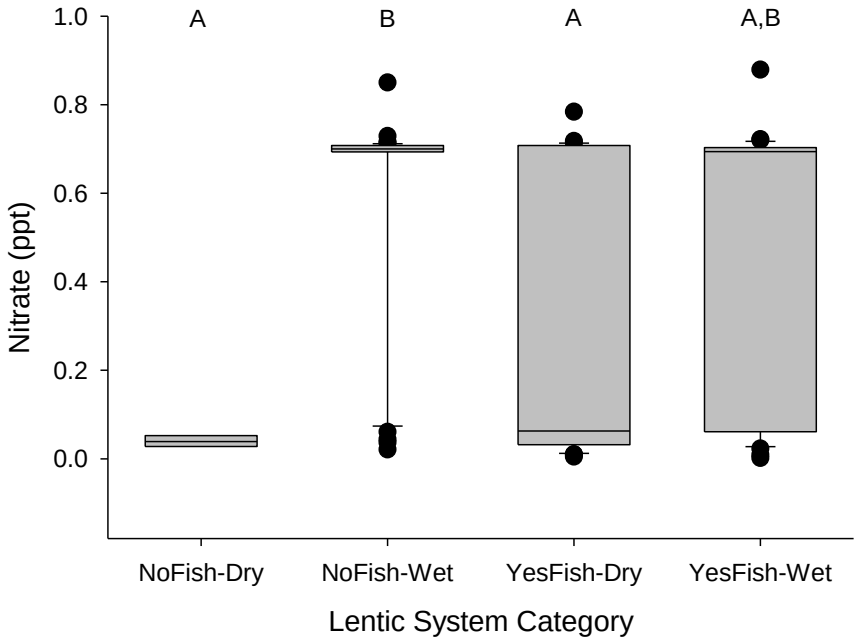


Figure 33

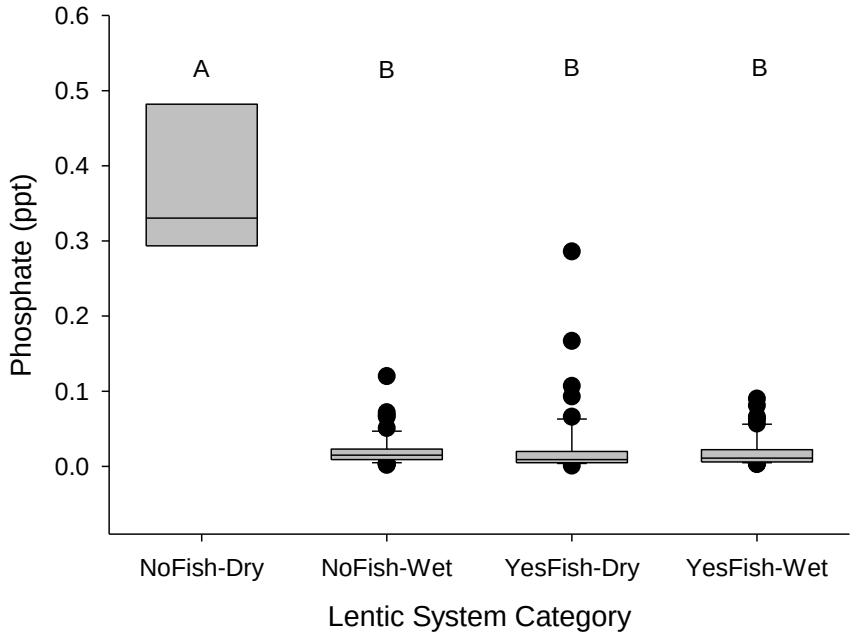


Figure 34

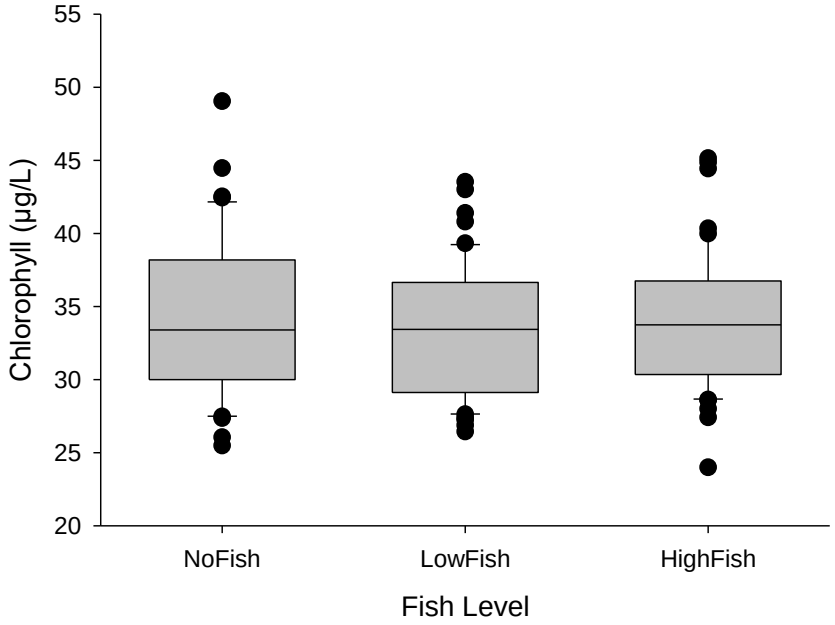


Figure 35

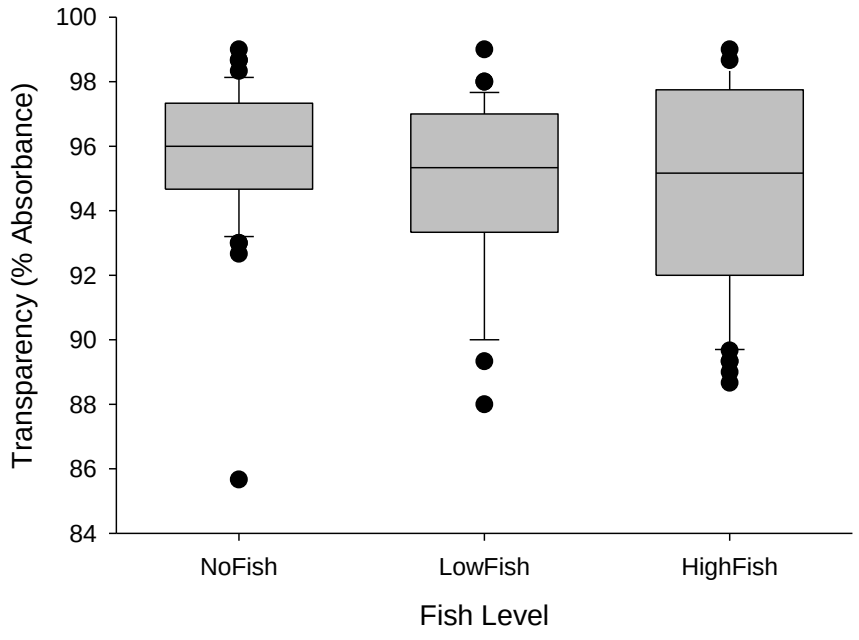


Figure 36

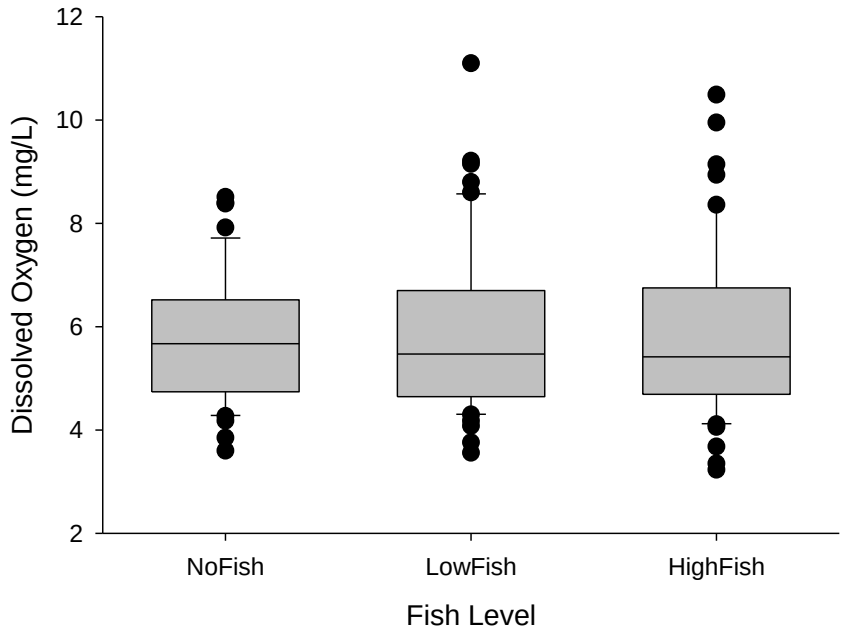


Figure 37

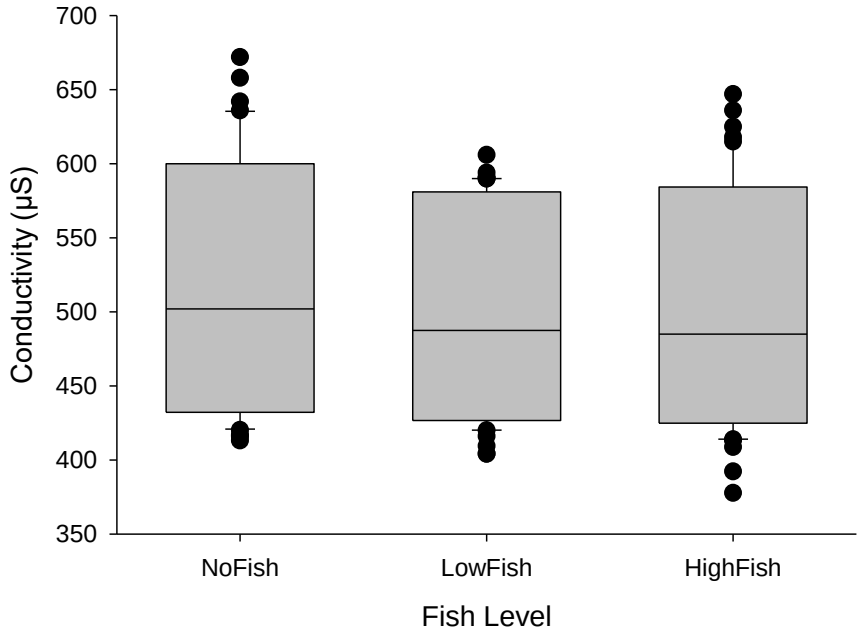


Figure 38

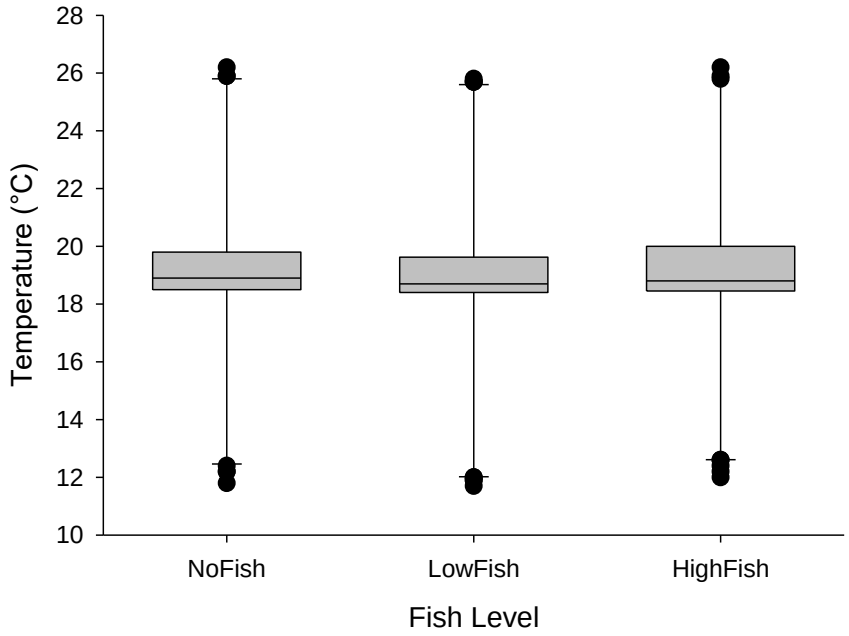


Figure 39

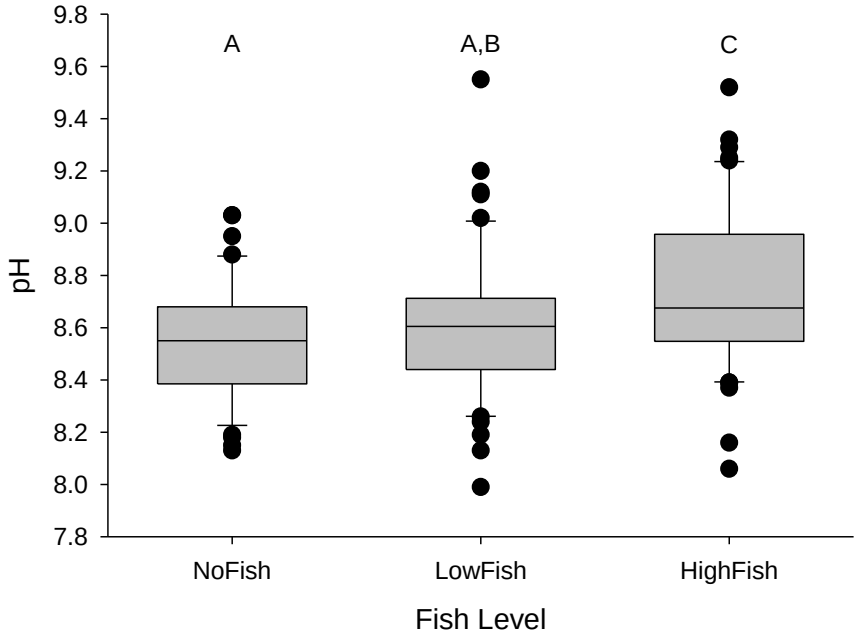


Figure 40

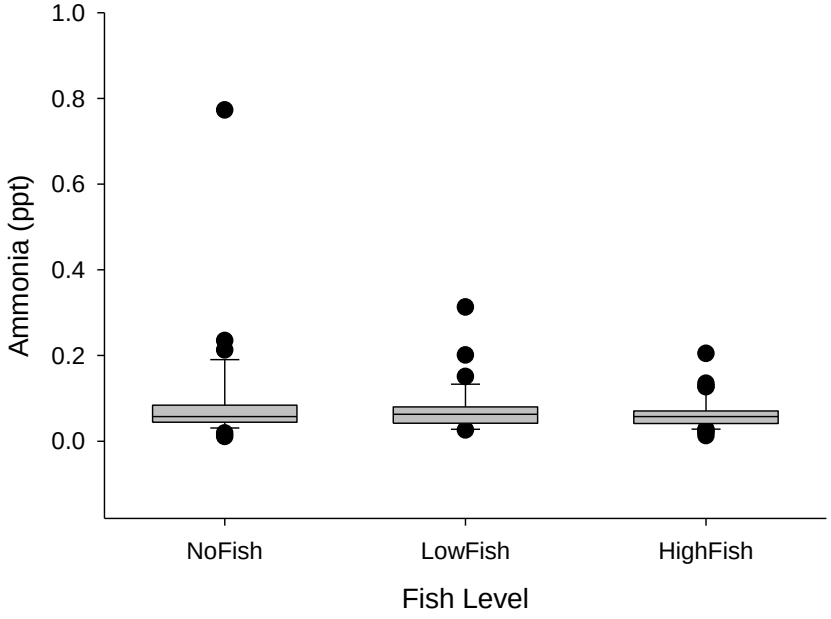


Figure 41

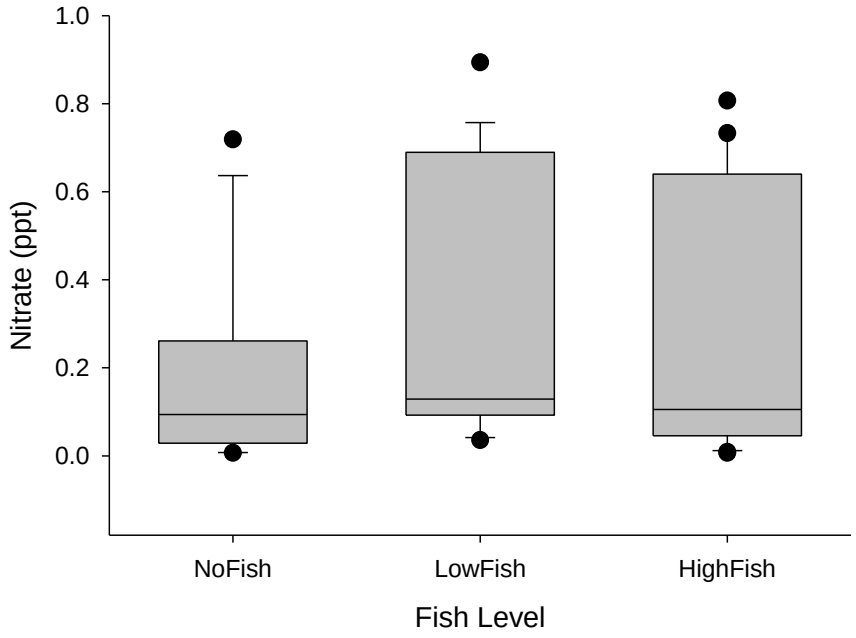
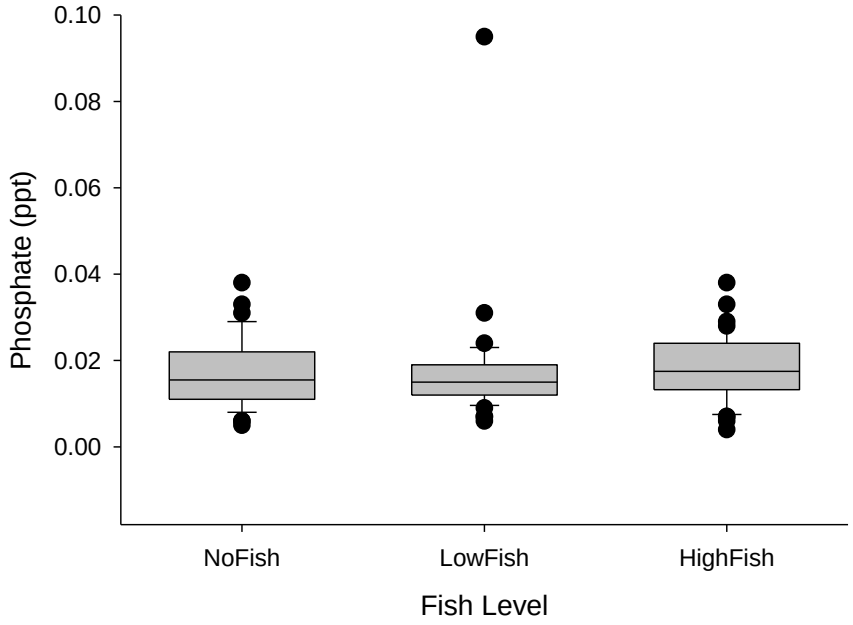


Figure 42



APPENDIX 1

Key to Submerged Aquatic Macrophytes (Genus and Species for TNWR)

Note: these are our informal comments, they do not take the place of using formal keys

Coontail

- Family: Ceratophyllaceae
- Genus: *Ceratophyllum*

Coontail or Hornwort (*Ceratophyllum demersum*): leaves are 1.5-4 cm long and forked, stiff and crunchy leaves, and groups of 5-12 leaves around stem.

Milfoil

- Family: Haloragaceae
- Genus: *Myriophyllum*

Spiked Water-Milfoil (*Myriophyllum spicatum*): leaves are about 15-35 mm long and in groups of 4 around the stem. Lots of branching (feather like) on each leaf.

Waterweed

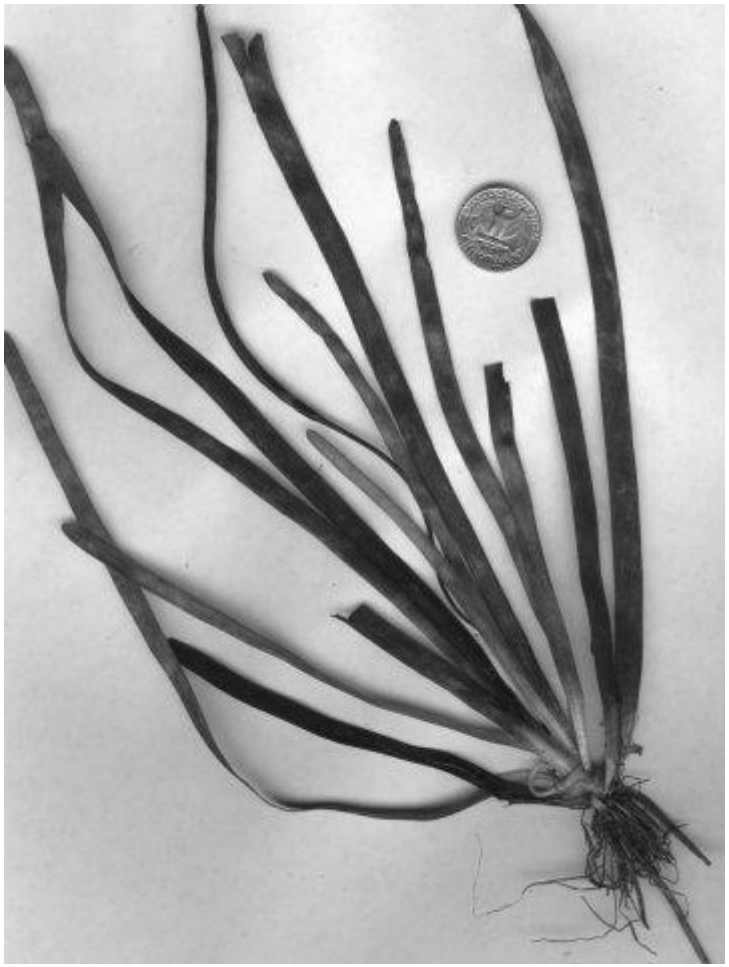
- Family: Hydrocharitaceae
- Genus: *Elodea*

Rocky Mountain Waterweed (*Elodea canadensis*): groups of 3 green leaves around stem, seaweed like texture, leaves are 6-15 mm long

Wild Celery

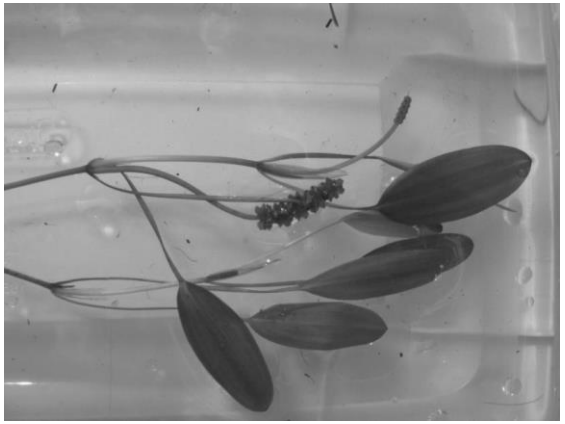
- Family: Hydrocharitaceae
- Genus: *Vallisneria*

American Wild Celery (*Vallisneria americana*): Leaves are about 1 cm in width and 1 long green stripe (vein) runs down center of leaves, ribbon like leaves have blunt end



Pondweed

- Family: Potamogetonaceae
- Genus: *Potamogeton*



Ribbon-leaved pondweed (*Potamogeton epihydrus*): leaves oblong, narrower and ribbon-like, green and about 2-22 cm in length

Broad-leaved pondweed (*potamogeton natans*): Leaves are oblong/egg shaped, dark green (sometimes reddish brown) and leathery, 5-10 cm long.

Pondweed

- Family: Potamogetonaceae
- Genus: *Potamogeton*

(Potamogeton zosteriformis): leaf blade width (2-5 mm) with 3-5 veins. Long slender leaf blades.

Sago Pondweed (Potamogeton pectinatus): leaves are 2-15 cm long and about 1 mm wide with pointed tips. Lots of branching (almost feather like).

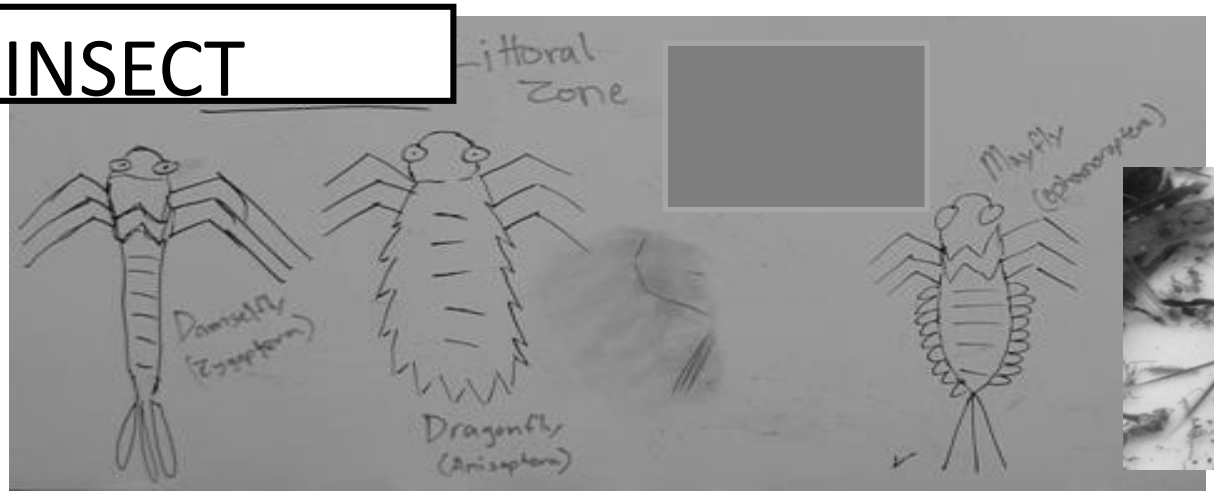
Richardson's pondweed (Potamogeton richardsonii): leaves alternate on stem and are 16-130 mm long (leaves feel like seaweed, plant looks like a fern).

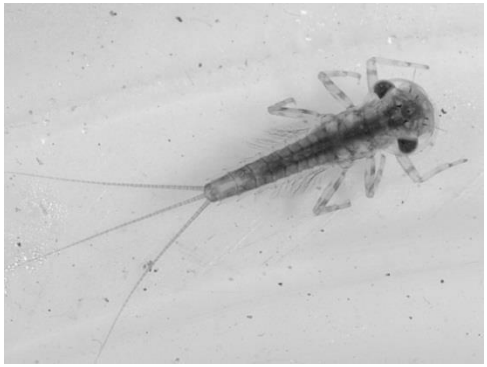
APPENDIX 2

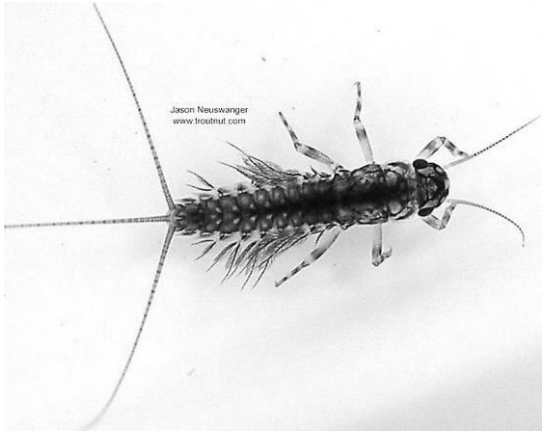
Key to Aquatic Macroinvertebrate Orders

Note: these are our informal comments, they do not take the place of using formal keys

INSECT



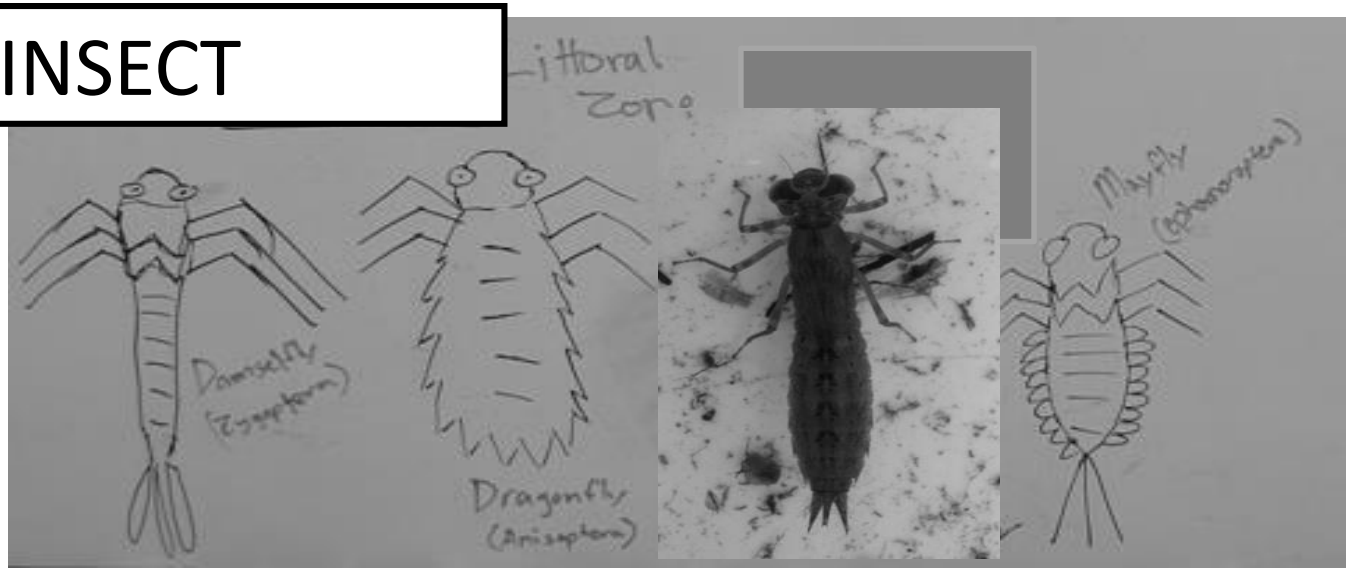




Mayfly larvae – Ephemeroptera

- Size is variable, usually smaller than other insect larvae
- Three caudal appendages - thin
- Gills along abdomen – delicate
- Can be very abundant! Can be very small!

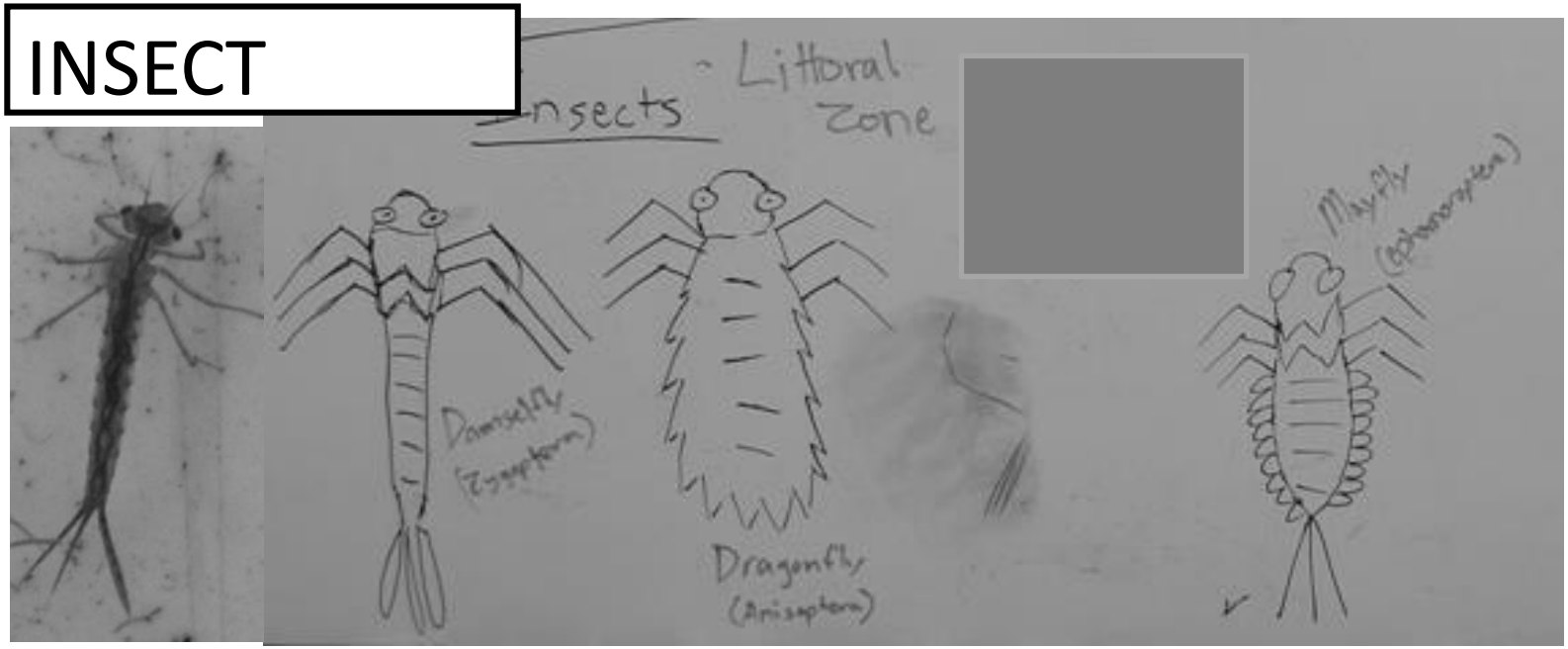
INSECT



Dragonfly larvae – Anisoptera

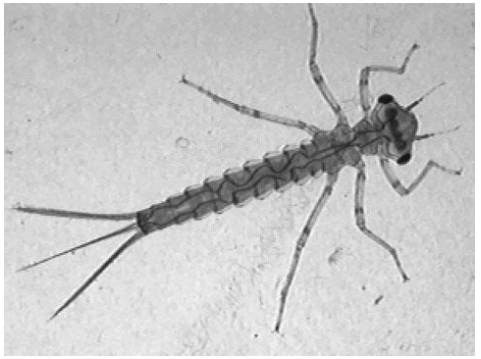
- Body is stout, head usually narrower than thorax and abdomen
- Five short, stiff appendages at tip of abdomen
- Big eyes, large mouthparts
- Relatively rare

INSECT

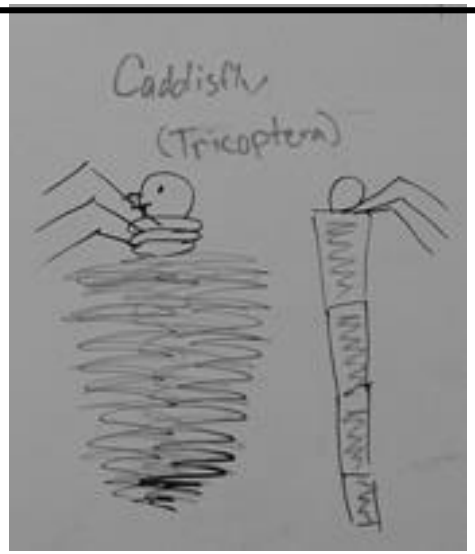


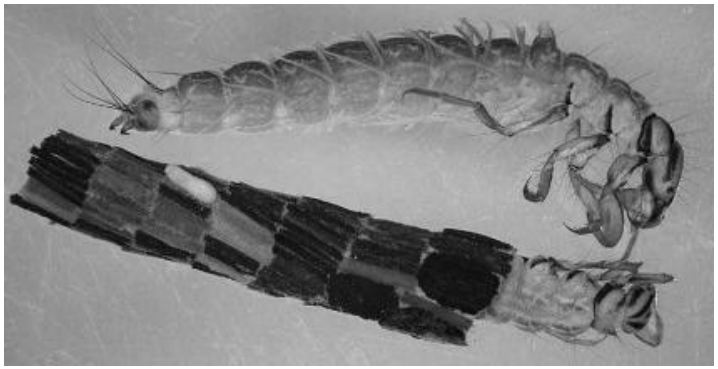
Damselfly larvae – Zygoptera

- Slender body, head wider than thorax and abdomen
- Three long, caudal gills at tip of abdomen
- Can be very abundant!



INSECT LARVAE

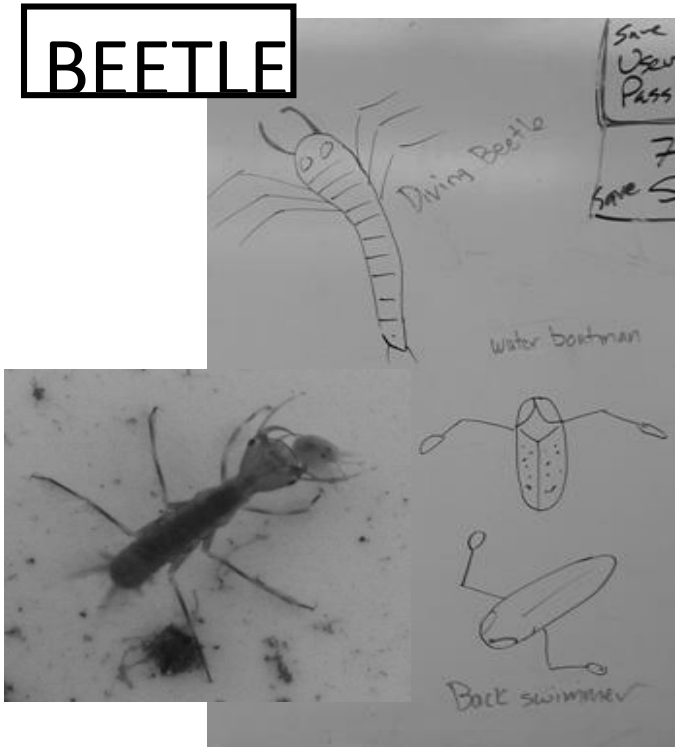




Caddisfly larvae – Tricoptera

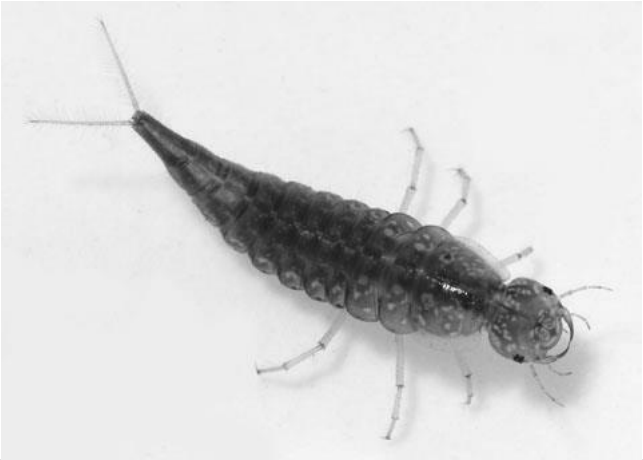
- Usually find them in their casings, may find them out of casings too
- Most common casings are thin pieces of leaves or big woven (fuzzy) cases
- Can be very abundant! Can be very small!

BEETLE



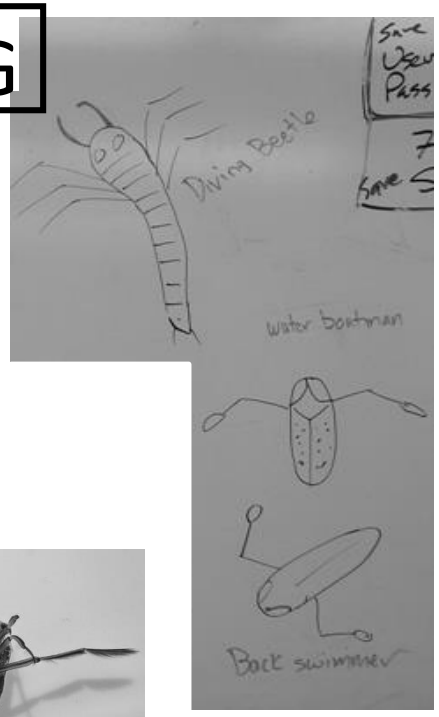
Diving beetle larvae – Dytiscidae

- Head often triangular shaped, with large mandibles
- Long and relatively slender body
- Rare!



All other beetles – if not diving beetles (and not boatmen or backswimmers), call "coleoptera"

BUG



Water boatmen – Corixidae

- Beetle-shaped body, almost like a bullet
- Swim on their bellies, have dark backs
- Front pair of legs scoop-shaped
- Usually smaller than backswimmers
- Can be rare

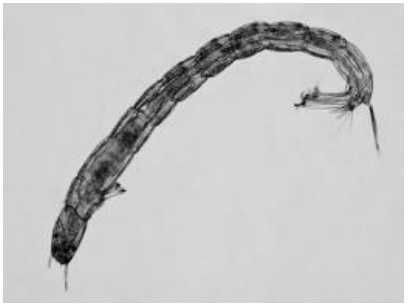
Backswimmer – Notonectidae

- Elongate body form, slender and oval
- Hind legs long and like oars
- Swim belly-up, dark bellies
- Can be rare

SMALL THINGS

Amphipoda

- Looks like a little shrimp
- Very small
- Moves fast
- Extremely abundant!



Midges – Chironomidae

- Can be clear, yellowish, brownish, reddish
- Look like flattened worms
- Pretty small
- Can be small

Water mites - Hydracarina

- Small, red, circular
- Remind you of ticks

Leeches - Hirudinea

(brown thing in picture)

- Long or short
- Flattened body
- Tend to attach to everything!
- Also swim - flattened

APPENDIX 3: PROTOCOLS

Sampling Design

In the field study, I collected data from twelve lentic systems, eight of which contain brook stickleback and were categorized as "YesFish", and four that do not have brook stickleback ("NoFish"; Table 3). All sample collections were taken Monday - Friday between the hours of 7 am and 5 pm. The twelve lentic systems were pooled together and then randomly distributed over the course of two weeks; this sampling distribution was continuously repeated the next two weeks from March through August 2015 (approximately one samplings per month).

Stickleback Presence/Absence

Prior to all measurements and sample collections, baited minnow traps (containing 1 cup of Meow Mix cat foot/trap), set for 24 hours, and were used to confirm presence/absence of brook stickleback in each lentic system. No estimates of catch per unit effort were made.

Selection of Study Sites

1) The sampling site locations and number of sampling sites per lentic system were determined using stratified-randomized, modified intervals of equal width (EWI) method and with the use of a global positioning system (GPS) and ArcMap, accessed through EWU's virtual labs.
    a) To calculate the area in hectares of each lentic system the "measure" application on the "Draw" tool bar in ArcMap was used.

b) For approximately every 1.4 hectares there were three sampling sites (maximum of five sites at a given water body). The number of sampling sites were tripled to obtain the number of transect increments.

c) Each lentic system's width was divided by its individualized number of transect increments to obtain the equal width distance between each transect.

d) ArcMap was used to stratify and randomize the previously determined number of sampling site locations by choosing every other or every fourth transect increment location (depending on number of sampling sites and water body size).

2) Sampling sites were confirmed using GPS points and marked with flagging tape ($5 \leq$ sampling sites/lentic system ≥ 3).

3) All measurements were taken within the first two meters of water from the shore line.

4) As some of the lentic systems dried up and the shorelines changed, we moved straight in from the shoreline until the new shoreline (initial contact with pond/lake water) was found. When the distance between the two shorelines was less than four meters all measurements were taken halfway between the two shorelines.

Macroinvertebrates

1) At each sampling site within a lentic system macroinvertebrates (along with macrophytes) were collected between the hours of 7-10 am.

2) Within near shore habitats (first 2 meters from the shore line, used a meter stick to measure this distance) macroinvertebrates were collected by a maximum of two standardized sweeps (sweeping one-meter length, used meter stick to measure this distance) across the benthic material using a dip net (500 μm-mesh).
3) If the first sweep was too vigorous (dip net was more than half way full of sediment) or too light (less than a handful of sediment), a new second sweep was done one meter to the left or right of the original sweeping location.
4) The sweep (while still in the net) was then swished back and forth or agitated with our hands to remove as much excess sediment as possible.
5) The sweep net sample was then placed inside a gallon Ziploc back (labeled with site number, lentic system name, and date), and enough water was added to the bag until the sediment and water levels were flush in order to keep the invertebrates alive.
6) Bagged macroinvertebrates samples were placed in a 5 gallon bucket for easy of carrying, transported back to EWU and kept at room temperature for further processing.
7) To sort the invertebrates, a silver dollar-sized scoop of the sweep sample was placed in a white dissecting tray and diluted with dechlorinated water until the tray had ¼ or ½ of an inch of water in it.
8) Macroinvertebrates were separated from the debris using plastic pipettes or spoons (separate sorting trays or "dump" buckets were used when appropriate to put the counted invertebrates), counted and identified to class or order.
9) Steps 7 and 8 were repeated until the entire sweep sample for the first sight had been processed.

10) Steps 7-9 were repeated for all sites.
11) For statistical analyses we calculated the mean number of macroinvertebrates/volume (diameter of net * tow length * water height in the entrance of the frame) of water sampled for each lake.
    a) Volume of water sampled = (25 cm * 1,000 cm * 13 cm) = 325,000 cm^3
12) See following 'keys' for the major taxa of invertebrates that we counted (see documents attached).

Macrophytes

1) One macrophyte sample was collected at each sampling site within a lentic system.
2) Within near shore habitats (first 2 m from the shore line) macrophytes were collected to the left or right of the macroinvertebrate sampling location by doing one sweep (sweeping one-meter length) across the benthic material using a standard metal (14 prong) gardening rake.
3) While the sample of macrophytes were still on the rake, the sample was lightly swished back and forth or agitated with our hands in the water to remove as much sediment as possible.
4) The raked sample was then placed inside a gallon Ziploc back (labeled with site number, lentic system name, and date).
5) Bagged macrophyte samples were then placed in a 5 gallon bucket for easy of carrying and then transported back to EWU for further processing.
6) The abundance and diversity of macrophytes was calculated as proportional or percentage of dried biomass of each species.

7) Each macrophyte bag was emptied onto a white dissecting tray. Tap water was added to the tray until the water filled the tray up half way.
8) The macrophytes were then swirled and agitated in the dissecting tray in order to remove as much sediment as possible. The muddy tap water was dumped and refilled as necessary as it is difficult to sort and identify macrophyte species in muddy water. Wearing gloves was not required but recommended due to the possibility of having leaches in the samples.
9) Once most of the sediment was removed the macrophytes were identified, sorted by species and placed on lunch trays to dry out at room temperature for 24-48 hours.
10) Steps 2-4 were repeated for each site's macrophyte sample bag (the dissecting trays were rinsed with tap water in between each site).
11) Once the macrophyte samples were completely dried, the weights (g) were recorded for each species at each site.
12) See following 'keys' for the genus and species of macrophytes that we counted and weighed (see documents attached).

Water Quality

1) All water sampling and quality measurements were conducted in the afternoon between the hours of 1 and 4 pm.
2) Meas**u**rements and samples were taken from a canoe to decrease the chances of altering transparency (inverse of turbidity) levels.
3) If there was not enough water in the lentic system to float the canoe, then water measurements were taken at least 1 m in front of someone who carefully waded into the water.

4) All measurements and samples (up to five samples per lentic system, see table 1) were obtained within the first two meters of the shore line, within the vicinity of where macroinvertebrate and macrophyte samples had been collected earlier that day.
5) Conductivity (μS/cm), temperature (°C) and dissolved O_2 (mg/L) measurements were also taken at once per site for each sampling day using a YSI model 85 probe provided by Dr. Ross Black.
6) The nitrate, ammonium, phosphate, chlorophyll, and transparency (inverse of turbidity) measurements were taken by first obtaining one 500 ml water sample at each site after the YSI data were recorded.
    a) The 500 ml bottle was rinsed three times in the pond water before obtaining the actual water sample to avoid contamination.
    b) The water samples were then transported to EWU for analysis in a shaded container at room temperature.

YSI Model 85

1) The YSI required approximately 15 min to warm up and calibrate prior to the days sampling.
    a) Prior to calibration the sponge inside the instrument's calibration chamber needed to be wet (used deionized water for wet the sponge).
    b) After wetting the sponge and inserting the probe into the chamber, the pH meter was then turned ON.
    c) Then the MODE button was pressed until the dissolved oxygen measurement was displayed in either mg/L or %.

d) Then we waited approximately 15 (as previously stated) for the reading to stabilize. Once the reading was stable, we pressed down on the UP ARROW and DOWN ARROW at the same time.

e) The pH meter then asked for the appropriate altitude (feet), and then we hit ENTER.

f) Once the dissolved oxygen was stable again we pressed ENTER again to save and complete the calibration.

g) After using the YSI, the probe was stored in a beaker or jar of deionized water (until the next sampling/calibration day) in order to keep the probe membrane wet.

2) Gently swirl the YSI probe in the water until reading remain relatively constant.

3) The conductivity (μS/cm), temperature (°C) and dissolved O_2 (mg/L) readings then were recorded.

Transparency (inverse of turbidity)

1) Once the 500 ml water samples were brought back to EWU, the water bottle was shaken and the water was poured into three glass test tubes (6 x 50 mm tube), which were then capped. This was repeated for each site within a lentic system.

2) One test tube was filled with deionized water as a standard.

3) The outside of the test tubes were then cleaned and dried using kimwipes.

4) The Turbidimeter (Biolog Turbidimeter Model 21907) was obtained from the EWU Biotechnology lab.

5) The standard test tube was placed inside the Turbidimeter machine (by holding onto the cap in order to not add finger prints to the side of the test tube) and then the absorbance knob was turned to 100%.
6) The standard tube was removed, and the first triplicate tube from the first site was shaken vigorously and then placed in the Turbidimeter using previous methods.
7) The first time the needle on the Turbidimeter stabilized, that absorbance was recorded to the nearest percentage. The first stable reading was taken quickly due to possible particle settling within the water of the test tube, thus altering the samples transparency.
8) Steps 1-5 were repeated for each triplicate test tube sample for each site. The water was then poured down the sink.

Chlorophyll

1) After shaking the 500 ml water samples, water from the first site's 500 ml sample was poured into three cuvettes (each approximately ¾ of the way full).
2) Each cuvette was cleaned and dried using a kimwipe, then carefully placed in the fluorometer (provided by Dr. Camille McNeely) and read in RFU (relative fluorometric unit) due to time constraints* as a measurement of Chlorophyll.
    a) *time constraints refer to the inability of Jenae Yri and Dr. McNeely to find a similar time prior to sampling to calibrate the fluorometer, therefore it was calibrated after samples were collected.
3) Steps 7-8 were repeated for each site, with the cuvettes rinsed with deionized water in between sites. The water within the cuvettes could be poured down the sink after a reading was obtained.

4) Due to time constraints the fluorometer was later calibrated to read chlorophyll in µg/l (a standard unit of chlorophyll) rather than in RFU later by Dr. McNeely. As a result an equation (y=0.0656x + 3.9942) was used to convert my RFU chlorophyll readings into µg/l of chlorophyll.

pH

1) Calibration of the pH meter (Fisher Scientific accument AB15 Basic pH Meter) in the Joyner-Matos Lab was necessary (following standard pH calibration protocols). The pH meter was calibrated using the 7 and 10 pH standards.
2) For one sampling site, approximately 80 ml of water from the 500 ml bottle was poured into a 100 ml glass beaker.
3) An appropriately sized stirring rod was placed within the beaker of water, placed on the stirring plate underneath the pH meter, and then the stirring plate was turned on.
4) After the stable pH reading for the water sample was recorded the water sample was poured down the sink and the beaker and stir rod were rinsed with deionized water.
5) Steps 2-4 were repeated for the remaining water samples from each site.

Nitrate/Ammonia/Phosphate

1) The remaining water was filtered and frozen to be later analyzed for nitrate, ammonia and phosphate levels.
    a) A 50 ml plastic syringe was filled from the 500 ml sample bottle.
    b) This 50 ml sample of water was then slowly (1 ml/sec) pushed out of the syringe and through a syringe filter holder containing one Gelman A/E filter (47 nm, Taylor Scientific and Pall Corporation).

c) The water was filtered into a labeled 50 ml plastic tube up until the 45 ml line (to account for the water expanding when frozen).

d) Parafilm was then placed on top of the tube and the tube was capped and sealed closed (with the parafilm still on it) and then the entire tube was stored in the -20°C freezer.

2) Step 1 was repeated for each site, using a new Gelman filter for each site and rinsing the filter holder and syringe with deionized water in between each site.

3) Any remaining water was poured down the sink.

4) Next a Flow Solution 3100 (OI Analytical) flow analyzer was used to quantify nitrate, ammonia and phosphate levels. Each of these analyses used the standard protocols for the analyzer (see documents attached).

5) Nitrate was converted to nitrite through a reduction reaction in the presence of cadmium. The nitrite (both the newly formed nitrite and what was in the sample originally) were mixed with two chemicals, sulfanilamide and *N*-(1-naphthyl) ethylenediamine dihydrochloride; in combination, these chemicals produce a colored dye that is detected at a 540 nm wavelength.

6) The assay was then repeated without the cadmium step to quantify the nitrite that was originally in the sample and then used to calculate the nitrate level.

7) Phosphate levels were determined using the orthophosphate procedure. Briefly, orthophosphate, molybdenum (VI) and antimony (III) were mixed in acidic conditions; the mixture is reduced with ascorbic acid, forming a colored solution with an absorbance that was quantified at 880 nm wavelength.

8) Ammonia was quantified by the Total Kjeldahl Nitrogen method. Briefly, samples were boiled at a very high temperature with sulfuric acid, potassium sulfate and a copper-containing compound; in these conditions, ammonia was converted to ammonium sulfate. The sample was brought to a pH of 11 and ammonia gas was trapped in an alkaline hypochlorite solution (forming chloramine). Chloramine was mixed with salicylate to form a solution that was blue; absorbance was measured at 660 nm wavelength.

Mesocosms

After running a preliminary set of mesocosms throughout the Limnology course at EWU, the methods were optimized for thesis research. The mesocosm experiment for thesis research was then conducted from June through August at the Turnbull Laboratory for Ecological Studies (TLES), Cheney, WA. The established mesocosm tanks (100 Gallon Rubbermaid Stock Tanks) were kept at replicate conditions approximately 30 feet uphill from the TLES Pond. Various numbers of brook stickleback were added, and water quality metrics were measured weekly.

1) There were three treatment levels of brook stickleback in each set of 100 Gallon mesocosm tanks, with 10 replicate mesocosms per treatment: no fish, low fish (4 fishes) and high fish (8 fishes). Each tank was randomly assigned at treatment level of brook stickleback.
2) Mesocosms were filled with water from TLES Pond (approx. 80 L/tank).
    a) A pump with a mesh filter attached was placed in the deepest portion of the pond. The power cord and battery to operate the pump was stored in an unoccupied canoe that was anchored next to the pump. The power cord was then strung up on

wooden stakes above the water (to avoid shocking), and fed across the pond to the TLES building.

b) At the end of the pump where the water was poured into the tanks, another finer mesh filter was attached.

c) The pump ran for approximately 7 min at each tank (starting with the tank in the south west most corner, continuing to the left and then on to the next south west tank in the next row and so on. This pattern was kept the same throughout the experiment) filling them with approximately 80 L of pond water.

3) Next, two stove pipe samples of sediment, macroinvertebrates and macrophytes were added to each tank from the first two meters of water within the TLES pond.

a) Stove pipe samples were collected using a Rubbermaid Brute round 20 gallon plastic trash can (the bottom of the container was cut off).

b) While carefully standing within the first two meters of water (littoral zone) the trash cans or "stove pipes" were randomly and lightly dug into the water/sediment.

c) Using the same mesh D-frame net used for macroinvertebrates field sample collections, the net was placed inside the trashcan and was carefully dragged across the sediment/water in a circular pattern.

d) The scoop was then dumped into a 2.5 gallon bucket, and the scooping process was repeated one more time to insure all of the bugs, plants and sediment within that stovepipe were collected.

e) The stove pipe was then picked up and randomly placed down in a different location within the littoral zone; steps a-d were repeated once more (the sample was placed in the same 2.5 gallon bucket).

f) The 2.5 gallon bucket was then dumped into a mesocosm tank.

g) Steps a-f were repeated for each mesocosm tank.

4) The mesocosms containing water, sediment, macroinvertebrates and plants were allowed to settle for 1 week.

5) Zooplankton samples were obtained from the middle of the TLES Pond, off the side of a canoe, using 200 micron mesh plankton nets with a diameter of 0.5 m and tow length 0.75 m.

a) A total of two plankton tows were added to a 2.5 gallon bucket.

b) One bucket of zooplankton was randomly dumped into each tank. The tanks were allowed to establish for another week. Individual zooplankton were not counted.

6) Initial water quality measurements (turbidity, temperature, pH, nutrients, conductivity and dissolved O_2) were conducted before the brook stickleback were added.

a) Water quality measurements were conducted as described above, with temperature, conductivity and dissolved O_2 measured by YSI meter and transparency, nutrients, and pH measured in a sample of 50 ml that was collected from each mesocosm and taken to the lab at EWU.

7) Brook stickleback were then added to the corresponding treatment level tanks (step 1).

a) Brook stickleback were obtained from Cheever Lake through the use of baited minnow traps and transported to the mesocosms (minnow traps were set out with

approximately 1 cup of Meow Mix cat food for 12 hours). Cages were set in the evening the day before the fish were to be sorted and placed in their mesocosm tanks.

b) Only the apparently healthy and similarly sized fishes (snout to tail) were used for this experiment.

8) All water quality measurements were measured weekly over six weeks at mid depth within the mesocosm tanks (YSI measurements were taken at surface level depth).
9) Any fish that died in their mesocosm tanks throughout the entire experiment were replaced with freshly caught (from Cheever Lake), similarly-sized fish.
10) At the end of the experiment, remaining alive and dead stickleback were collected from the mesocosm tanks.
11) The water, sediment, macroinvertebrates, and macrophytes from the mesocosms were dumped out within the general vicinity of the TLES pond but not directly into the pond.
12) We watched carefully for any missed fish as the tanks were dumped out.
13) All fish were sacrificed and disposed of at the end of the experiment using IACUC-approved methods (see below, #2015-02-06).

a) IACUC Approved Methods: Brook stickleback were euthanized by exposure to tricaine methane sulfonate, or MS-222. The MS-222 stock solution of 10 g/L was made; sodium bicarbonate was added to saturation (with a pH ranging from 7.0 to 7.5). The stock solution was diluted so that the MS-222 concentration was at least 80 mg/L (considering stickleback are so small). The fish were left in this solution for at least ten minutes until all movement ceased. Once the fish

were fully anaesthetized, they were killed using direct spinal transection behind the head (severing their spinal column). The fish were then dumped in a field high above the mesocosm tanks (away from the pond) at TLES.

Fingernail Clams

1) While sorting the macroinvertebrate samples the abundance of clams were estimated as catch per unit effort (CPUE).
2) The size of each clam was determined by measuring shell height (from the umbo to ventral margin) and length (from anterior to posterior margin, or adductor to adductor) with calipers.
3) The clams with shell lengths ≥ 6 mm, indicating adults, were dissected and the numbers of brooded larvae were counted.
4) Brooded larvae were counted and grouped according to relative size for each brood sac (see examples below):
    a) 6<7 means that there were 13 larvae within that one clam but that 6 of them (all similarly sized) were from one brood sac and were smaller than 7 larvae (all similarly sized) from another brood sac.
    b) 6=6 means that there were 12 larvae within that one clam but that there were two brood sacs both containing 6 larvae that are all the same relative size within and across brood sacs.
    c) (1<3)<5 means that there were 9 larva within that one clam but that there were two brood sacs and that one of them contained 4 larvae, one that was smaller than

the other three (all similarly sized) larvae, which were all smaller than the 5 other larvae in the second brood sac (all similarly sized).

5) After the number of offspring had been recorded, we blot dried the tissue on kimwipes and weighed the samples (including adult tissue and offspring, no adult shell). The ratio of wet mass (g) to shell volume (shell length x shell width x shell height, cm^3 (Viergutz et al. 2012) were used to calculate condition index.
6) The feet from groups of five similarly-sized clams per sampling day/lentic system were pooled together, flash-frozen in liquid N_2 and stored at -80°C to be used for RNA/DNA.

RNA/DNA

1) The phenol:chloroform:isoamyl alcohol (PCI, 25:24:1, saturated with 10 mM Tris, pH 8, 1.0 mM EDTA, manufactured by Sigma Life Sciences) bottle was inverted 3 times to mix, then allowed to rest so that the overlaying buffer liquid does not contaminate the phenol-ethanol
2) The bench and fume hood were cleaned with RNase spray, and the utensils and pipettes were cleaned with 70% ethanol.
3) Three sets of sampling tubes were labeled; these are either 1.5 or 2 ml Eppendorf tubes, autoclaved.
    a) When quantifying 8 samples, 8 tubes were labeled as such **A**:1-8, 8 tubes **B**:1-8, and 8 tubes **C**:1-8, totaling 24 tubes
4) One, 1.5-2ml tube was obtained and labeled TRIS for every 2 sampling tubes; then 1,100 µl of TRIS-SDS buffer (stored at room) was aliquoted into each “TRIS” tube
    a) TRIS-SDS buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA, 2% SDS, pH 8) :

i) 50 ml of autoclaved distilled water was added to 100 ml glass beaker and placed on a stir plate with a stir bar and turned on

ii) Then 0.6057g of 0.05 M Tris was added to the water (mixed for 5 min)

iii) Then 0.584g of 0.1 M NaCl was added (mixed to 5 min)

iv) Then 10 ml of EDTA (0.01 M, pH 8.0) was added (mixed for 5 min)

v) The pH of the solution was checked (HCl was slowly added to bring pH up to 8.0)

vi) Then 20 ml of SDS was added (mixed for 5 min)

vii) The pH of the solution was checked (HCl was slowly added to bring pH up to 8.0)

viii) The solution was then poured into a 100 ml volumetric flask and autoclaved distilled water was added until the volume of the solution was exactly 100 ml

5) Auto-calved forceps were rinsed in 70% ethanol, and allowed to air dry on a kimwipe
6) Five, 2mm glass beads (Bio Spec Products) were put into each of the A tubes using the forceps
7) 500 µl of TRIS-SDS buffer was added to each **A** tube
8) Then, clam sample tubes were obtained from the -80 °C freezer and put into a liquid nitrogen container (no gloves)
9) A bucket of ice and the vortex from biotech were also obtained
10) Tissue samples were weighed using a frozen spatula to scrape tissues onto weigh paper and then into their corresponding **A** tube (no gloves, avoid letting the tissues thaw, refreeze spatula in between samples)

11) **A** tubes were incubated for 5 minutes at room temperature while vortexing each sample for 45 second intervals (each sample was vortexed at least 3 times)
12) The **A** tubes were then transferred to the fume hood, and 500 µl of PCI was added to each **A** tube (we made sure the pipette tip went below the liquid barrier inside of the PCI container)
13) **A** tubes then incubated for 5 minutes at room temperature while being vortexed for 10 second intervals (vortexed each sample as many times as we could within the 5 minutes, all vortexing was done under the fume hood)
14) **A** tubes were centrifuged at 14,000x for 10 minutes at 4°C (in biotech)
15) The clear supernatant was removed from the **A** tubes and put it into the **B** tubes (using p200 at 100 µl and p20 at 10 µl)
16) The **B** tubes were transferred to the fume hood and 500 µl of PCI was added to the **B** tubes
17) **B** tubes incubated for 5 minutes at room temperature while being vortexed for 10 second intervals (vortexed each sample as many times as we could within the 5 minutes, all vortexing was done under the fume hood)
18) **B** tubes were centrifuged at 14,000x for 5 minutes at 4°C (in biotech)
19) The **A** tubes were placed at 4°C until after RNA/DNA numbers were quantified, just in case any steps need to be repeated (if quantification went well A tubes were thrown away)
20) All empty **C** tubes were weighed to the nearest mg.
21) The clear supernatant phase was removed from the **B** tubes and put into the corresponding **C** tubes

22) **C** tubes were reweighed after the supernatant had been added

23) An optional 1:10 dilution into TE (100 µl of 1.0 M Tris, 100 µl of 0.01 M EDTA, 10 ml of autoclaved dH_20, stored at room temp) was done if RNA yields from the original **C** tubes were too high

    a) The same number of tubes as previously used for the **C** tubes were obtained but label CR:1-8 instead of **C**: 1-8

    b) 90 µl of TE was added to each CR tube

    c) 10 µl of each sample from the **C** tubes were added into their corresponding CR tube (we pipetted up and down to mix)

24) The **C** tubes (and CR tubes if we had any) were then placed directly into the ice bucket

25) **C** samples were the read on the Qubit Fluorometer (Life Technologies) in the L. Matos Lab. Sample preparation required several Qubit-specific supplies/reagents, which were in the L. Matos Lab: 'Qubit tubes', which were optically clear, 0.5 ml tubes; the DNA broad range kit (contained buffer, reagent dye, and standard) and the RNA high specificity kit (buffer, reagent dye, and standard).

    a) The following items were brought with to the L. Matos Lab:

        i) **C** tube samples on ice (and CR tubes, if necessary)

        ii) p1000, p200, and p10 (and tip boxes)

        iii) 2, 15 ml tubes (one labeled RNA and the other labeled DNA)

        iv) Sharpies, gloves, kimwipes and lab notebook

26) DNA quantification

a) The same number of Qubit tubes as samples plus two extra for the kit's standards were obtained and labeled on the top
b) The 'reaction buffer' contained buffer plus reagent dye and was made in the 15 ml DNA tube with the following:
    i) 100 µl/sample (plus 100 µl for slop) of the DS DNA Broad Range Buffer
    ii) 1 µl/sample (plus 1 µl for slop) of the DS DNA Broad Range Reagent (we made sure the reagent went into the buffer, the solution was then swirled to mix)
c) 190 µl of the reaction buffer was pipetted into each of the standard Qubit tubes (Standard 1=S1 and Standard 2=S2)
d) 195 µl of the reaction buffer was added into each of the Qubit tubes
e) 10 µl of the DNA standard 1 was added to the S1 Qubit tube and 10 µl of the DNA standard 2 was added to the S2 Qubit tube
f) The **C** tube samples were then inverted 2 times and then 5 µl of samples from the **C** tubes were added to their corresponding Qubit tubes
g) All Qubit tubes were briefly vortexed (2 seconds)
h) The Qubit tubes were incubated at room temperature for 2 minutes
i) Qubit tubes were centrifuged for 5 seconds, and then kimwiped before being read on the Qubit
j) The Qubit was then plugged in and turned on by pressing the HOME button
k) Selected ds DNA BR (hit go)
l) Selected run new calibration (hit go)

m) Inserted S1 (hit go), when the Qubit said COMPLETE we inserted S2 (hit go), when Qubit said COMPLETE for S2 then the first Qubit tube was inserted

n) Inserted the first Qubit tube (hit go), and recorded the amount of DNA

o) Selected calculate concentration (hit go), selected 5 µl (hit go), and recorded the concentration

p) Inserted the next Qubit tube and then repeated steps n and o for all Qubit tubes

27) RNA quantification

a) All RNA steps were repeated but using the RNA Buffer and Reagent, and the RNA Standard 1 and Standard 2

b) When we read the Qubit tubes on the Qubit, we selected RNA (not RNA BR) and proceeded to run a new calibration with our RNA Standards 1 and 2

c) If the RNA readings were too high then we repeated the steps using the 1:10 TE diluted samples (the CR Qubit tubes)

Curriculum Vitae

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Education

Present **Current Masters Graduate Student, Dept. of Biology, Eastern Washington University (EWU)**. Thesis: Determining the effects of non-native Brook Stickleback (*Culaea inconstans*) on the lentic systems at Turnbull National Wildlife Refuge (TNWR), Cheney, WA.

Present **Graduate Certificate in GIS, EWU**.

2013 **B.S. in Biology, EWU.** Emphasis on wildlife management and conservation.

2011 **Associates in Applied Science, Everett Community College (EvCC).** High School Running Start, Ocean Research College Academy Program (ORCA).

Employment

2015-present Teaching Assistant, Dept. of Biology, EWU. Duties include trapping fish using nets and minnow traps, housing fish and amphibians, collecting and housing aquatic and terrestrial plants, growing and housing plants in a green house, managing undergraduate students in a variety of courses, grading, and lecturing. As well as assisting students with microbiology studies pertaining to the gut, oral and skin microbiomes of mice and rats.

2014-2015 Research Assistant, Dept. of Biology, EWU. Duties include supervising over 12 undergraduate students, managing laboratory supplies and safety compliance, in addition to conducting research related to glycogen content (pipetting in 96-well plates, mixing solutions, and using acids, bases, and alcohols).

2013-2014 Outfitter, Retail Sales, Cabelas.

2011-2013 Cook, Dinning Services, EWU.

Teaching Assignments

2015-2016 Teaching Assistant for Biology I (BIOL 171), Biological Investigation (BIOL 270), Biology Capstone: Microbiome (BIOL 490), Fundamentals

of Genetics (BIOL 310), Biology Capstone: Animal Eco-Physiology (BIOL 490), Principles of Animal Physiology (BIOL 351), EWU.

2012-2013 Teaching Assistant for Biology I (BIOL 171), EWU.

Selected Awards

2016 EWU Deans Travel Grant ($100)
2013 EWU Deans List Fall Quarter
2011-2012 EWU General Scholarship ($3,300)

Membership in Professional Societies

2015-present Northwest Scientific Association (NWSA)

Funding

2016 EWU Student Provost Travel Grant ($500)
2015 Swartz-Werschler Graduate Scholarship ($3,600)
2015 Swartz Biotechnology Graduate Scholarship ($3,500)
2015 NWSA Student Research Grant Competition ($800)
2015 EWU Biology Department Mini Grant ($500)

Other Service/Outreach

2016 Cheney May Fest Volunteer, Cheney, WA (TNWR/EWU's Invasive Fish Booth).
2016 Science Olympiad Assistant Supervisor Volunteer, EWU.
2016 NWSA Volunteer at 2016 Conference in Bend, Oregon.
2015 TNWR Flowers, Feathers and Floods Festival Volunteer, Cheney, WA (TNWR/EWU's Invasive Fish Booth).
2015 Volunteer Moderator for 2014 NCUR, EWU.
2014 WDFW Volunteer, North West Trek Wildlife Park, Eatonville, WA (Frog Adult and Egg Surveys in Freshwater Streams, Ponds, and Wetlands).
2011 Northwest Organization for Animal Help Volunteer, Stanwood, WA.

Publications/Conferences

2016 USFW. Report on Permit # TBL – 15 – 002r. Project Title: Is the presence of brook stickleback (*Culaea inconstans*) problematic for the refuge? Turnbull National Wildlife Refuge, Cheney, WA.
2016 Schoonover, C. M., Wieker, J. Pope, R., Brown, C., Cooper, E., DeWitt, J., Gunselman, S., Jensen, C., Stevens, W., Yri, J., Nezat, C., and Joyner-Matos, J. Development of functional trait biomarkers for trace metal exposure in freshwater clams (*Musculium* spp.). 2016. *In print* at Comparative Biochemistry and Physiology, Part A.

2016 Main Author for a Poster at the NWSA 87th Annual Conference, March 23rd-26th, 2016 in Bend, Oregon.

2015 Co-author for a Poster at the Second International Conference on Oxidative Stress in Aquatic Habitats, November 11-14th, 2015 in La Paz, BCD.

Relevant Coursework/Experience

2016 **GIS Portfolio (GEOG 549):** Presenting all finial GIS maps in a paper and online portfolio created throughout the GIS certificate courses with the addition of maps created for my thesis and two maps published in papers by Dr. Joyner-Matos.

2016 **Geographic Information Systems III (GEOG 548):** GIS internship creating maps for my thesis (sampling/survey sites) that can be used by the USFW and Turnbull National Wildlife Refuge.

2016 **Current Topics in Ecophysiology (BIOL 599):** Discussion of current topics in ecology and physiology (particularly aquatic environments and non-native/invasive species).

2016 **Directed Study Integrating GIS and R (GEOG 599):** Integrating GIS (ArcMap) and R/R-Studio.

2016 **Geographic Information Systems (GIS) II (GEOG 538):** Using ArcINFO workspace to create and extract GIS data and maps, in addition to configuring maps from workspace into ArcMap.

2015-2016 **Research Seminar (BIOL 500):** Presenting thesis research as posters and power points in a professional setting.

2015 **Desktop Mapping (GEOG):** Integrating ArcMap with desktop programs and websites to design more public friendly maps.

2015 **Seminar Programing (BIOL 501):** Assisting with set up and program distribution for the EWU Biology Department Graduate seminars.

2015 **Limnology (BIOL 505):** Water quality analyses (temperature, pH, dissolved O2, conductivity, turbidity, chlorophyll, ammonia, nitrate, and phosphate using YSI, Turbidimeter, Secchi disk, flow analyzer, fluorometer, water sampler, etc.), instrument calibration, mesocosm experiments, aquatic insect collection and identification, zooplankton tows, and zooplankton identification and preservation.

2015 **Plant/Microbial Interactions (BIOL 596):** Discussion of plant immune systems, plant/microbe interactions and the role of chloroplasts (freshwater and terrestrial ecosystems).

2015 **Biological Research Methods II (BIOL 511):** Statistical analysis using R/R Studio.

2015 **Current Topics in Ecology/Evolution (BIOL 514):** Discussion of current research articles in ecology/evolution (invasive and non-native fish and plants).

2014-2016 **Thesis Research (BIOL 600):** Conduct water quality analyses (temperature, pH, dissolved O2, conductivity, turbidity, chlorophyll, ammonia, nitrate, and phosphate using YSI, Turbidimeter, Secchi disk,

flow analyzer, fluorometer, etc.), aquatic insect/invertebrate sweep net sample collections and identification, aquatic submerged macrophyte collections and identification, mesocosm experiments involving the housing of non-native brook stickleback fish, fingernail clam dissection, CPUE, RNA and DNA extraction and quantification all within 12 lentic systems (wetlands, ponds, and lakes) that contain or do not contain brook stickleback fish between the months of March and August, 2015.

2014 **Geographic Information Systems (GIS) I (BIOL 528):** Use of ArcGIS for projections, spatial modeling, cartographic and thematic map design.

2014 **Biological Research Methods I (BIOL 510):** Learn principles of experimental design, information literacy, and proposal writing.

2014 **Current Topics in Cell/Molecular Biology (BIOL 513):** Group discussion of current research articles in cell/molecular biology.

2013 **Senior Capstone: Animal Physiology and Lab (BIOL 490):** IACUC proposal, research project using heart rate monitors and blood glucose on rats.

2013 **Stream Ecology and Lab (BIOL 445):** Nutrient cycling, stream nutrient uptake, discharge volumes, aquatic invertebrate collection and identification, determining pool/riffle rations.

2013 **Wildlife Management (BIOL 443):** Discussion of management practices and mark-recapture using "Mark".

2013 **Field Botany and Lab (BIOL 411):** Pacific Northwest plant species identification and collection.

2013 **Vertebrate Zoology and Lab (BIOL 304):** Vertebrate anatomy and species identification (salmon and other fish species).

2013 **Fundamentals of Genetics (BIOL 310):** Discussion of DNA synthesis, the human genome and genetic testing.

2013 **Ornithology and Lab (BIOL 454):** Northwest bird species identification, hawk next survey, taxidermy and anatomy.

2012 **Invertebrate Zoology and Lab (BIOL 303):** Invertebrate species identification (primarily aquatic insects), collection and dissection.

2012 **Ichthyology and Lab (BIOL 462):** Collecting fish using minnow traps, nets, and electro shocking, species identification (including salmon species), mark-recapture survey, and CUPE.

2012 **Animal Behavior and Wildlife Field Studies Lab (BIOL 399**): Elk telemetry, GPS use, fencing and predator urine treatments.

2012 **Ecology and Ecology Lab (BIOL 440 and 441):** Invasive plant surveys, conifer species richness survey and nutrient supplementations.

2012 **GIS for Environmental Science (ENVS 323):** Manipulation of data and use of ArcGIS for wildlife and environmental science implications.

2012 **Cell Biology (BIOL 436):** Discussion of eukaryotic cell structure, function and growth.

2012 **Biology Series (BIOL 171-173):** Fundamentals of biology including, ecology, cell structure and function, metabolism, evolution, etc.

2012 **Chemistry Series (CHEM 151-153):** Fundamentals of chemistry including, nomenclature, atomic bonding, states of matter, solutions, acids, bases, titrations, organic chemistry, stoichiometry, etc.

2011 **Intro to Statistics (MATH 380):** Discussion of visualizations, probability and regressions.

2011 **Intro to Oceanography (Transfer Course from EvCC ORCA):** silt and sediment sample collection and analysis, ocean chemistry, surface and subsurface circulation, etc.

2011 **Marine Biology of the Pacific Northwest (Transfer Course from EvCC ORCA):** marine organism ecology and anatomy, classification and identification (aquatic organisms, including salmon species, and marine mammals and birds).