Summer 2018

The Associations of Epiphytic Macroinvertebrates and Aquatic Macrophytes in Canyon Lake, WA

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The Associations of Epiphytic Macroinvertebrates and Aquatic Macrophytes in Canyon Lake, WA

By

Jesse Tyler Klinger

Accepted in Partial Completion

of the Requirements for the Degree

Master of Science

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MASTER'S THESIS

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Jesse Tyler Klinger

Date: 27 June 2018

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A Thesis

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The Faculty of

Western Washington University

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Abstract

Macroinvertebrate abundances on six dominant macrophytes taxa were compared in Canyon Lake, Washington to determine whether there were patterns of association with macrophyte type or among assemblages of macroinvertebrates. Macrophytes and associated epiphytic macroinvertebrates were collected during August 2016. The dominant macrophyte distribution and lakes bathymetry were mapped in July 2016. The dominant macrophytes included *Equisetum fluviatile*, *Fontinalis antipyretica*, *Potamogeton natans*, *Potamogeton epihydrus*, *Ranunculus aquatilis*, and *Vallisneria americana*. Other non-dominant macrophytes included: *Sparganium angustifolium*, *Sphagnum* mosses, *Nuphar polysepala*, Characeae (stonewort), *Isoetes* (quillwort) and *Potamogeton pusillus*. Macroinvertebrate taxa were identified to the lowest practical taxonomic resolution; the dominant macrophytes were identified to species; other non-dominant macrophytes were identified to the lowest practical resolution.

Water quality samples were collected in June, July and August 2016 and were analyzed for temperature, dissolved oxygen, pH, conductivity, turbidity, alkalinity, chlorophyll, ammonium, nitrate+nitrite, total nitrogen, soluble reactive phosphorus, and total phosphorus. The summer water quality in Canyon Lake was characterized by warm water temperatures (11.4 - 20.2 °C) and high dissolved oxygen concentrations (8.6 - 9.9 mg/L) in the upper water column. The lake began to stratify in June, and by late August the dissolved oxygen was < 2 mg/L in the deepest part of the lake. The lake had soft, poorly buffered (< 60 mg-CaCO3 /L), and mildly acidic to near neutral water (pH = 6.6 - 7.2). The phosphorus and nitrogen concentrations were relatively low except in late summer near the bottom of the lake. The lake is stained brown from humic compounds and acids that leach from the extensive submerged and floating woody debris.

A total of 18,509 epiphytic macroinvertebrates were collected, in association with six different macrophyte taxa. Among these, 36 different macroinvertebrate taxa were identified. Basommatophora and Amphipoda constituted the most numerically abundant macroinvertebrate. Emergent and structurally simple macrophytes supported lower abundances of macroinvertebrates; more structurally complex macrophytes supported greater abundances of all macroinvertebrate groups. The simple, un-branched macrophyte *Equisetum fluviatile* consistently supported the lowest macroinvertebrate abundances; the highly branched and complex *Ranunculus aquatilis* supported the highest macroinvertebrate abundances. Odonata and Trichoptera, which were rare compared to most taxa, were most commonly found among *Fontinalis antipyretica*, *Ranunculus aquatilis*, and *Potamogeton epihydrus*, which were complexly structure macrophytes. Hierarchical clustering on principal components generated two clusters of samples that corresponded best to low and high macroinvertebrate abundances. The remaining data factors that were explored did not correspond well to the identified cluster groups.

Acknowledgments

This project would not have been possible without the help of many people and I am deeply grateful to them all. I cannot thank my thesis advisor Dr. Robin Matthews enough. Her never ending patience and constant positive attitude truly kept me moving the ball forward in the most stressful of times. She consistently allowed this thesis to be my own work, but kept me out of trouble at all the right junctures. To my committee members, Dr. Leo Bodensteiner and Dr. James Helfield, thank you both for your efforts and input into revising my work. My writing has improved greatly thanks to your perseverance.

I would like to thank everyone at the Institute for Watershed Studies with whom I've learned from and gained valuable experience working alongside. I expressly thank Mike Hilles and Joan Vandersypen for offering their skills and support when I needed them most.

A very special gratitude goes out to Raena Anderson for her tireless work with me in the field and lab. Thank you for your steadfast punctuality and for pouring great input and organization into my project. I hope the best for you in your future.

I am also grateful to several university staff, especially Dr. Brian Bingham, Dr. Andy Bunn, and Dr. Ruth Sofield. I've learned immensely from them and their excellence in teaching. Their challenging classes provided me with the graduate experience I'd always hoped for.

To my girlfriend Amie, my brother A.J, and my parents, thank you for the much needed moral and emotional support. Finally, last but by no means least, thank you to everyone at Western and the Huxley College who have been my friends, colleagues, and roommates. My grad experience wouldn't be the same without them.

Thanks for all your encouragement!

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1 Introduction

1.1 Objectives

I examined the associations between the macrophytes and epiphytic macroinvertebrates in Canyon Lake of the Canyon Lake Community Forest in Northwest Washington State. The primary goal of my research was to explore the relationships between macroinvertebrates, macrophytes, and habitat structure in Canyon Lake. My specific objectives were: to characterize the physical and chemical lake environment by collecting water quality data and depth profiles; to create bathymetry and macrophyte distribution maps for Canyon Lake; and to estimate the density of macroinvertebrates associated with macrophytes in Canyon Lake.

1.2 Macroinvertebrates and Macrophytes

The productivity of water bodies in the Pacific Northwest differs widely and to understand the drivers of their productivity is important for conservation, management and the preservation of biodiversity in aquatic ecosystems. Secondary productivity is a measure of production by heterotrophic organisms (Rigler and Downing, 1984). Secondary productivity reflects many properties and processes of populations such as reproduction, biomass, predation, survivorship, life span, emigration, immigration, trophic status, etc. (Benke and Huryn, 2010). One important component of secondary productivity in lake ecosystems is that which is generated by macroinvertebrates. Macroinvertebrates facilitate important functions in lakes by cycling nutrients and biomass through consumption of live macrophyte tissue and breaking down detritus. They also create important trophic connections in lake ecosystems by consuming primary producers and other secondary producers, and serving as a food resource for higher trophic level consumers in both aquatic and surrounding terrestrial communities (Merritt and Cummins, 1996). Measuring the

secondary productivity of lake macroinvertebrates has become widely used as a proxy of ecosystem function and integrity in many still and moving water applications (Dolbeth et al., 2012; Benke and Huryn, 2010).

Aquatic macroinvertebrates are resident to nearly all natural aquatic systems. Many macroinvertebrates spend the juvenile portion of their life cycle in aquatic habitats and emerge into the terrestrial environment as adults. These emergent macroinvertebrates generate a vector for the flow of nutrients between aquatic and terrestrial systems (Czarnecka, 2016). Terrestrial consumers such as birds (Epanchin et al., 2010), amphibians (Finlay and Vredenburg, 2007), lizards (Sabo and Power, 2002), and bats (Gruenstein, 2014) depend on emergent macroinvertebrates. Epanchin et al. (2010) demonstrated a significant decrease in the presence and reproductive success of the Grey-crowned Rosy-Finch surrounding high alpine lakes with introduced trout populations due to the reduced availability of emergent mayflies. Finlay and Vredenburg (2007) found a significantly higher abundance of the amphibian *Rana muscosa* in alpine lakes without introduced trout. The dependence of terrestrial consumers and amphibians upon aquatic macroinvertebrates has historically been poorly understood and overlooked but continues to become recognized in current management and conservation efforts in aquatic ecosystems.

To understand the health and function of macroinvertebrate communities in aquatic systems, the substrates and macrophytes that macroinvertebrates live upon must be considered. Each macroinvertebrate species has specific habitat preferences driven by the habitats in which they evolved. The behavioral and morphological characteristics of macroinvertebrates are also related to the habitats they occupy and evolved in. These attributes have led to the development of various macroinvertebrate functional groups based on feeding behaviors (functional feeding groups), or modes of attachment, mobility, defense, and shelter (functional habitat groups). The use of functional feeding groups has become common place stream ecology. The presence or absence of particular functional

groups or specific taxa in aquatic systems has been used as a tool to indicate water quality conditions and even applied in larger regional water quality assessments (Moulton et al., 2002).

The distribution patterns of macroinvertebrates in aquatic systems are reflected in the optimization of a species mode of attachment or clinging, mode of motility, and the abundance of substrates conducive to those modes. One type of substrate that is often abundant in nearshore areas is aquatic macrophytes. Aquatic macrophytes are plants that grow under water and may or may not grow to or above the waters surface. They can provide food, shelter from predation, and living space that best suits numerous macroinvertebrate behaviors and foraging styles (Merritt and Cummins, 1996). Aquatic macrophytes are a common feature of subalpine woodland lakes and ponds (Wetzel, 2001). Macrophytes provide refuge for macroinvertebrates from predation by fish and other larger predators. Macrophytes also provide vertical substrates that enable macroinvertebrates to subsist in the entire nearshore water column. Macrophytes can play a role in promoting water clarity by outcompeting phytoplankton for nutrients during summer months (Hilt et al., 2013). In lentic systems, nearshore submerged and floating-leaf macrophytes have been shown to support greater taxonomic diversity and density of macroinvertebrates than open waters, emergent macrophytes or non-vegetated benthic substrates alone (Beckett et al., 1992; Paice et al., 2016).

Many macroinvertebrates are substrate-dependent or facultativly associated with specific macrophytes. For example, many Trichoptera genera have been found to associate strongly with macrophytes bearing higher biomass of epiphytic filamentous algae (Tinsley et al., 2016; Cattaneo et al., 1998). Nymphs of species in suborders Anisoptera and Zygoptera (dragonfly and damselfly, respectively) rely on more complex plant structures such as *Sphagnum* mosses and *Potamogeton* (pondweed) for platforms from which to hunt prey (Henrikson, 1993; Remsburg and Turner, 2009). Anisoptera and Zygoptera nymphs are also considered climbers and they require the presence of emergent macrophytes or other emergent substrates to climb out of the water to molt into their adult

life stage (Merritt and Cummins, 1996). Macroinvertebrates that leave the water for part of their life cycle are considered emergent macroinvertebrates. The presence of a diverse and physically heterogeneous macrophyte population has been shown to support the secondary productivity and diversity of emergent macroinvertebrates (Kirby and Ringler, 2015; Heino, 2000). Together, macrophytes and their macroinvertebrate inhabitants contribute to the health and diversity of their aquatic environment and surrounding watershed (Rennie and Jackson, 2005). A well developed understanding of the relationships between macroinvertebrates and their host macrophytes is necessary to make management decisions that best support the linkage between the aquatic and terrestrial environment facilitated by macroinvertebrates. This is especially important in aquatic systems that are subjected to various environmental stressors.

1.3 Regional Description

The Cascade Range begins at the confluence of the Nicola and Thompson Rivers in southern British Columbia and stretches south through Washington and Oregon, terminating at Mt. Lassen in Northern California (Peattie, 1949). There are eighteen major volcanic peaks distributed throughout the Cascade Range, most of which are dormant. The Cascades are dominated by basalt and andesite, which is interspersed with granitic plutons, generating a diverse geologic topography. The elevation of the Cascade Range climbs from roughly 330 m to its highest point on Mt. Rainier at 4,392 m (Pelto, 2006). Weather systems commonly develop in the Pacific Ocean and move east to deliver as much as 350 cm of annual precipitation to the Cascades. As weather systems move east, orographic lift causes much of the moisture held in the air to precipitate out on the western slopes of the Cascades, resulting in a relatively arid climate on the eastern side of the Cascade Range (Siler et al., 2013). Canyon Lake sits in the North Cascades, which extend from the Skykomish River in Northwest Washington to the Fraser River in southwestern British Columbia.

1.4 Site Description

Canyon Lake is a 0.149 km^2 lake that is 19 m deep at its greatest depth. The lake is situated 32 km east of Bellingham and 20 km west by northwest of Mt. Baker. It sets at an elevation of 709 m above sea level on the western slopes of the North Cascades (Figure 1). Although currently inaccessible by vehicle to the public due to road closures from storm damage in 2009, some still hike there. Canyon Lake was a popular site for hiking, fishing, and bird watching. The lake was formed after a land slide off the southwest wall of the watershed was triggered by a magnitude 7.4 earthquake in 1873 (Agee and Vaugn, 1993). These types of slides are common in the region due to the steep underlying Chuckanut sandstone formations (Easterbrook, 1973). A portion of the river valley was flooded during this event. As a result, the lake contains woody debris in the form of remnant rooted snags and hundreds of free floating logs. The extensive woody debris in Canyon Lake is responsible for the presence of some unusual aquatic habitats and conditions, including aggregations of floating woody debris that supports fully established terrestrial and semi-aquatic plant communities, and many small communities of fully and semi-aquatic plants growing on portions of emerging and submerged static logs that are still partial rooted or restricted from free floating in some way.

The watershed of Canyon Lake is approximately 9.71 km^2 and contains two parcels of old growth forest (Summers et al., 1998). Logging had reached Canyon Lake by the 1930's and the lake's upper watershed was logged between 1970 and 1990. The discovery of a 1.3 km^2 old growth parcel prompted the acquisition and conservation of the watershed by the Whatcom County Land Trust, in partnership with Western Washington University and the Paul G. Allen Forest Foundation from Crown Pacific in 1993. The watershed drains from the southeast to the northwest and has a maximum elevation of 1450 m. The majority of the watershed's slopes are greater than 30° with the southern slopes being the most steep (Summers et al., 1998).

Two perennial streams (Toboagan and Canyon Creeks) and four unnamed ephemeral streams drain into the lake (Agee and Vaugn, 1993). These streams are prone to flash floods and were likely scoured to bedrock during three boulder-capacity floods recorded in 1961, 1976, and 1983 (Summers et al., 1998). Additional surface hydrological components of the Canyon Lake Creek watershed include a second small water body situated just beneath the northern drainage divide and a small ephemeral wetland near the upper parcel of old growth forest. These are not connected to Canyon Lake through surface flow except perhaps during very high flow events. The outlet of Canyon Lake lies at the western most portion of the lake where there is a foot bridge and small waterfall a short ways down stream (Figure 1).

The forests surrounding Canyon Lake are diverse in both age and species. The forest stands range in age from 30 to over 800 years old. They include "Mesic" and "Dry" forests dominated by Pacific silver fir (*Abies amabilis*) and mountain hemlock (*Tsuga mertensiana*). Other common species include the Douglas fir (*Pseudotsuga menziesii*) and Alaskan yellow-cedar (*Callitropsis nootkatensis*).

2 Materials and Methods

2.1 Macrophyte Mapping

To map the distribution of macrophytes in Canyon Lake, a Garmin GPS map 60[†] was used to collect GPS coordinates of the location and approximate extent of all the present macrophyte beds that could be found. This GPS unit is considered to be accurate to five meters when standing still, which was difficult to achieve on breezy days which are common in the Canyon Lake watershed. The extent of each macrophyte bed was estimated by circling the lake in a flatbed aluminum row boat and recording the beginning and end position along the shore line of each bed. The distance from the shore that each bed extended into the lake was also visually estimated at several points for each macrophyte bed. Macrophytes such as *Potamogeton natans* and *Potamogeton epihydrus* were found growing in sediments that had accumulated upon various submerged portions of the large dead wood in Canyon Lake. These small patches were also mapped and recorded. All macrophyte patches that were accessible and large enough to provide macroinvertebrate samples using a macrophyte rake were mapped. Macrophyte mapping was done 11 - 14 July 2016 with assistance from Huxley College undergraduate student Raena Anderson. Raena also contributed the initial identification of the macrophytes we collected and created the map seen in Figure 2. Her associated senior project is reported in Anderson (2016).

Macrophyte beds were commonly found in mixed and monoculture species compositions. In the instances where macrophyte beds were of mixed species compositions a visual estimation of the relative proportion of each present species was recorded to the nearest 10 percent. This estimation was used to determine what was a dominant macrophyte and what was a co-dominant macrophyte. The most prevalent macrophyte was considered the dominant macrophyte, and the next most prevalent macrophyte was con-

[†]Garmin International, Inc. 1200 E. 151st St. Olathe, KS 66062 USA

sidered the co-dominant macrophyte. Coarse woody debris were also regularly recorded as present or absent. The distribution and relative proportion of aquatic mosses were not recorded because they were present on nearly every piece of woody debris greater than 30 cm in diameter, and much of the woody debris was free floating and moved regularly with the wind.

2.2 Macrophyte and Macroinvertebrate Collection

From 22 to 26 August, 49 macrophyte samples were collected in Canyon Lake, along with associated epiphytic macroinvertebrates. This was done in order to examine what associations, if any, existed between macroinvertebrates and their host macrophytes. Macrophyte sample locations were chosen in attempt to gain equal representation of each of the previously established dominant macrophyte types. Each sample location was recorded using GPS (Figure 3). To collect the macrophytes, a macrophyte rake[†] was slowly lowered into a macrophyte bed and very slowly twisted until as much plant material as possible was retained by the rake. The captured macrophytes were quickly retrieved in an effort to minimize the loss of macroinvertebrates and placed into a large sorting pan. It is likely that some macroinvertebrates were lost in this overall process. Each sample depth was no deeper than what could be reached from the boat with the rake. Macrophytes in water more shallow than the maximum reach were collected from the deepest depth possible. The resulting range of macrophyte collection depths was 0.3 - 2.5 m.

Macrophytes were carefully examined and removed from the rake with trimming shears and placed into a sorting pan. If the retrieved sample contained a mixture of macrophytes a visual assessment of which macrophyte represented the greatest number of stems within the sample was done. The macrophyte with the greatest relative stem density was considered the dominant macrophyte in each sample. The second most dominant macrophyte

[†]Macrophyte rake was a double sided bow rake commonly sold at hardware stores with 16-tines, 40 cm wide, and a 150 cm wooden handle.

of each sample was considered the co-dominant species. The mixture and dominance of each sample is detailed in Table 1. On a few occasions, the dominant macrophyte in a retrieved sample differed slightly from the dominant macrophyte of the immediate surrounding habitat seen in Figure 2. In these instances, the dominant macrophyte was still determined based on the collected sample.

Each collected macrophyte sample was further examined to determine whether any macroinvertebrates were present. Sample material without macroinvertebrates was discarded. Samples containing macroinvertebrates were placed into heavy duty water-proof plastic bags and transported on ice to the laboratory for processing. In instances where no macroinvertebrates could be found after three or four attempts using a macrophyte rake, a D-net was used to look for the presence of macroinvertebrate taxa that were not found with a macrophyte rake. In total, six D-net samples were taken. D-net samples were taken at the same sample location and depth where no macroinvertebrates were found by swiftly passing the D-net through the same macrophytes with a vertically oscillating motion avoiding the underlying benthic substrate. This was done from the flatbed rowboat or from a standing position on a static log rather than wearing a pair of waders standing in the water. This helped avoid disturbing the benthic sediment prior to D-net sampling which could have led to an over-representation of benthic macroinvertebrates.

2.3 Bathymetric Mapping

On 18 August, depth measurements were collected along 12 transects across the lake in order to generate a bathymetric map (Figure 4). The path of each transect was chosen to best minimize gaps in the coverage of the lake. The transects that were followed are displayed in Figure 4. Depth measurements were collected approximately every 2 m across each transect using a continuously reading depth finder. The depth finders accuracy was verified using a weighted calibrated rope with comparisons made before, during, and after collecting the transect data. Its accuracy was found to be within $\pm$ 0.5

m. To create the bathymetric map, the depth data from each transect were aligned with the associated GPS track. Additional depth measurements that were gathered during macrophyte sampling were added to the dataset to increase the amount and spread of the data. This provided a three dimensional dataset of latitude, longitude and depth. A digital grid of latitudinal and longitudinal points with a one meter resolution was used to interpolate the surface of the lake bed creating the map of the lake bottom. The surface interpolation was done with R statistical software using a second degree polynomial smoothing spline with a smoothing span of 0.065[†] generated with the loess and predict functions found in the "geoR" package (Ribeiro and Diggle, 2001; R Core Team, 2018).

2.4 Macroinvertebrate and Macrophyte Processing

The number of macroinvertebrates collected from each sample was standardized relative to the wet, dry and ash-free dry weight of each cleaned macrophyte sample. All dry weight and ash-free dry weight procedures followed the methods listed in Table 4 which are outlined below. All macrophyte samples that were collected were cleaned of macroinvertebrates and processed for drying within 24 hrs of collection. Upon returning to the laboratory, the macrophyte samples were weighed to obtain total wet sample weight, which included macrophytes, macroinvertebrates, and retained sediment.

The macrophytes samples were gently washed in a bucket to dislodge the macroinvertebrates and any sediment. The rinse water containing the macroinvertebrates and sediment was then strained through a 500 μm mesh sieve. The filtrate was discarded, leaving macroinvertebrates larger than 500 μm for further identification and analysis. The collected macroinvertebrates were then stored in small jars containing 95% ethanol with a trace of glycerin. The macroinvertebrate samples were sorted and identified in a

[†]The smoothing span is the proportion of maximum distance across which the smoothing spline is applied.

randomized order to avoid any counting bias. Macroinvertebrates were sorted and identified to the lowest practical taxonomic resolution using the following identification guides: Morse (2009); Merritt and Cummins (1996); Thorp and Covich (2009). The process of identification and sorting occurred over the following year after all macrophyte weights were collected.

The wet macrophyte samples were allowed to dry for 30 minutes to remove excess water, and then weighed to obtain a initial wet weight for each macrophyte sample. Prior to drying, each macrophyte sample was photographed to document the species composition. Macrophytes were identified to the lowest practical taxonomic resolution using the following identification guides: Brayshaw (1985, 1989); McQueen and Andrus (2012); Hotchkiss (1967); Schofield (1969); Ecology (2001). Each macrophyte sample was placed in a paper bag until it could be further processed. The process of obtaining each samples dry weight and ash-free dry weight was started within one week of collecting the samples.

All the macrophyte material of each sample was homogenized by using garden sheers to cut the macrophytes into the smallest pieces possible. Three subsamples were taken from each homogenized sample such that each subsample was at least 10% of the total sample by weight. The subsamples were placed into pre-weighed crucibles, dried in a forced air oven at 103 °C for 48 hours, and weighed. The macrophyte subsamples were returned to the drier and were re-weighed at 4 hour intervals until two sequential weights were within ± 0.01 g, which was designated as the subsample dry weight. Ash-free dry weights were obtained by combusting the subsamples in a muffle furnace at 550 °C for six hours. The dry and ash-free dry macrophyte weights were estimated using the subsamples average wet to dry and dry to ash-dry weight ratios. The wet to dry weight and dry to ash-free dry weight ratios were averaged by dominant macrophyte and can be seen in Table 5. The coefficient of variation for each set of three subsamples was less than 10% with respect to weight both before and after the combustion process. This was checked to insure that the plant material of each subsample was combusted to a similar degree.

2.5 Macroinvertebrate Estimates

The mean of the subsample dry weights and of the ash-free dry weights from each macrophyte sample was used to estimate the abundance of macroinvertebrates per gram of dry and ash-free dry macrophyte weight. Estimates of relative abundance per gram dry weight and gram ash-free dry weight for each macroinvertebrate taxon were calculated using the following equations:

$$\frac{\text{Number of Macroinvertebrates}}{\text{g AFDW}} = \frac{\text{Number of Macroinvertebrates}}{\text{DW}_{\text{Total}}} \times \frac{\text{DW}_{\text{Subsample}}}{\text{AFDW}_{\text{Subsample}}} \quad (1)$$

$$\frac{\text{Number of Macroinvertebrates}}{\text{g DW}} = \frac{\text{Number of Macroinvertebrates}}{\text{WW}_{\text{Total}}} \times \frac{\text{WW}_{\text{Subsample}}}{\text{DW}_{\text{Subsample}}} \quad (2)$$

Where: WW_{Total} is the total wet weight of a macrophyte sample, and $\text{DW}_{\text{Subsample}}$ is the average dry weight of three subsamples of macrophyte material, and $\text{AFDW}_{\text{Subsample}}$ is the average ash-free dry weight used in the same three subsamples. Numbers of individuals in the higher level taxonomic groupings of all Insects, non-Insects, Anisoptera, Zygoptera, Trichoptera, Chironomidae, Diptera, Oligochaeta, and Amphipoda were generated by the summation of all individuals in each respective subordinate group.

Macroinvertebrates were also segregated into functional feeding groups using the information found in Hawking et al. (2013) and Merritt and Cummins (1996). The abundances of the functional feeding groups were determined by the summation of all individuals in each respective functional group. The functional feeding groups that macroinvertebrates were separated into were filter-feeders, collector-gatherers, predators, piercers, scrapers, and shredders. Filter-feeders either swim through the water or are benthic dwelling sessile filters, collecting suspended fine particulate organic matter. Some create and use net like structures to collect suspended fine particulate organic mater. Collector-gatherers wander submerged substrates in search of dead organisms, detritus, or other food particles. Some collectors such as Amphipoda are omnivorous herbivores, swimming freely in

search of any palatable organic material they can find. Predators are carnivores that are generally good swimmers. They attack and engulf whole animals or animal parts. Some pierce the tissue of other animals or eggs to suck fluids out. Piercers also pierce tissues and plant cells feeding on the living vascular tissues of macrophytes or macroscopic algal cell fluids. Scrapers are animals that specialize in feeding on biofilm layers. Some specialize in feeding on epiphytic algae that grows on the surface of macrophytes and others primarily consume epilithic algae that grows on rocky substrates. Shredders have mouth parts that are adapted to tearing, chewing up detritus. Others feed by mining live macrophyte tissue with specialized mouth parts. These are also know as plant mining macroinvertebrates.

2.6 Water Quality Data Collection

All water quality samples were collected from depth or at the surface (0.3 m) with a Van Dorn sampler, transfered to a clean 1 L Nalgene sample bottle with no head space and stored on ice to be brought back to Western Washington University and analyzed in the Institute for Watershed Studies laboratories. I measured temperature and dissolved oxygen in the field using a calibrated YSI Pro 20[†] field meter at the surface (0.3 m) and at 1.0 m depth intervals from the surface to the bottom. These measurements were collected in the early to mid afternoon on 16 June, 14 and 25 July 2016 at the deepest point of the lake. The water quality profile taken 16 June was taken very near the deepest portion of the lake. The exact location of the deepest point of the lake would not be identified until the following sampling date. A GPS unit that was accurate to 5 m was used to mark the deepest point of the lake, which was returned to during the later sampling dates. I also collected vertical profile samples of alkalinity, specific conductance, turbidity, and pH on 16 June (Figures 5 and 6 and Table 2). On 1 August, vertical profiles of temperature, dissolved oxygen, pH, specific conductance, alkalinity, soluble reactive phosphorus, total

[†]Yellow Science Instruments, 1725 Brannum Lane, Yellow Springs, OH 45387 USA

phosphorus, nitrate, ammonium, and total nitrogen were collected at 0.3, 5, 10, and 15 m and 17.5 m (Table 3) and analyzed by the Institute for Watershed Studies. Conductivity, pH, and turbidity were measured using a calibrated Orion Model 162 conductivity meter, Orion Dualstar pH meter, and a Hach 2100n turbidimeter and alkalinity was measured using the low level acid titration method. Nutrients (total phosphorous, soluble reactive phosphate, total nitrogen and nitrate + nitrite, ammonium) were analyzed on the Alpkem FS3100 autoanalyzer by the Institute for Watershed Studies. The measured parameters and methods used to analyze samples are presented in Table 4.

2.7 Statistical Analysis

All analyses were conducted using R-studio (R version 3.4.0). Modified boxplots and quantile-quantile plots demonstrated that the majority of my data were not normally distributed and non-parametric rank-based statistical evaluations were used when feasible. I used Kendal's τ rank-based correlation analysis to look for correlations in the abundances of individual macroinvertebrate taxon, functional groups of macroinvertebrates, and the physical complexity of macrophytes. The complexity of macrophytes was determined by assigning a rank to all of the macrophytes that were collected. Macrophytes with non-branching structure (*Equisetum fluviatile, Nuphar polysepala, Vallisneria americana*) were termed simple and given a rank of 1. Macrophytes with alternate or opposite branching stem structures (*Potamogeton epihydrus, Potamogeton natans*) were termed moderately dissected and given a rank of 2. Macrophytes with more complex branching structures (*Fontinalis antipyretica, Ranunculus aquatilis, Sphagnum* mosses) were termed complexly dissected and given a rank of 3. The total complexity of each sample was given by the summation of dominant macrophytes rank and 50% of the co-dominant macrophytes rank. Wilcoxon rank-sum tests were used to look for significant differences in the abundance of macroinvertebrates between macrophyte types and for differences between specific types of macroinvertebrates within macrophyte types. The

Wilcoxon rank-sum test is sensitive to low samples sizes. This is important to note because only three sample where *Ranunculus aquatilis* was the dominant macrophyte were taken. In these cases I used simple graphical observations to aid my interpretations.

Principal component analysis uses a linear model to reduce the number of variables in an n-dimensional multivariate dataset into smaller subsets of synthetic variables or dimensions that have meaning. Principal component analysis was used look for trends in the macroinvertebrate data that could not be easily identified through pair-wise comparisons or graphical representations. To do this, the dimensions of the data that contribute the most variance are identified in successive decreasing order such that each dimension is orthogonal to the last. The first few dimensions that define the most variance are considered the leading, or "principal components," in the overall multi-dimensional space. In order to create a simplified 2D visualization of a multivariate dataset, the data are commonly plotted on the first two principal components. Successive orthogonal dimensions are also useful for further analysis but generally not used for plotting purposes. Principal component analysis was performed on the partial and condensed macroinvertebrate data. PCA was conducted using R statistical software in addition to the 'FactoMineR' and "factoextra" package (Lê et al., 2008; Kassambara and Mundt, 2017; R Core Team, 2018).

Hierarchical clustering, based on Euclidean distance and Ward's minimum variance method (Ward Jr., 1963), was used with principal component scores from the macroinvertebrate data. This method takes the principal component scores derived from the raw data that are row centered and scaled and uses those compute a distance matrix of Euclidean distance using Ward's minimum variance. Hierarchical clustering with the computed distance matrix was done on the first three principal components. This was done to look for natural groups in the macroinvertebrate communities, and to see whether any such groups were associated with specific macrophytes. To look for data factors that best explained the resulting cluster groups, I applied labels of dominant macro-

phyte type, macrophyte sample complexity, sample macroinvertebrate abundance, sample depth, mixed or monoculture macrophyte sample, and collection location. Care was taken to insure that each data factor was applied to the resulting cluster diagrams in the order that matched each cluster result. Macrophyte sample complexity was assigned in as described in the first paragraph of this section. Sample macroinvertebrate abundance was applied as high, medium, or low macroinvertebrate abundance and simply high and low. Sample depth was the depth of water each sample was collected in. The collection location was applied based on whether the sample was collected from the north or south shore.

This clustering method was applied to the complete macroinvertebrate dataset for exploratory purposes, and reapplied to two revised datasets for additional analysis. The first dataset was revised by removing four samples that were identified as outliers because they were found to have a large component in a low variation direction (see section 3.9 Principal Component Analysis in Results and Discussion). This dataset was termed the partial dataset. Additionally, I aggregated the macroinvertebrate taxa at the level of order and removed any macroinvertebrate taxa that did not appear in at least 10% of the samples. This dataset was termed the condensed dataset. Principal component analysis was used on each dataset to gain better understanding of which macroinvertebrate factors had the greatest influence on the clustering groups.

3 Results and Discussion

3.1 Temperature and Dissolved Oxygen

The summer water quality conditions are presented in Tables 2 and 3. These measurements were taken across depth at the deepest point of the lake. Canyon Lake thermally stratified by mid-June and remained stratified throughout the sampling period. Temperature and dissolved oxygen concentrations exhibited a clinograde pattern, both decreasing with depth during the entire sampling period (Figures 5 and 6). By mid-July, oxygen concentrations were <0.5 mg/L O_2 at all depths from 15 m to the bottom. These oxygen conditions indicated the deeper benthic regions of the lake were not suitable for obligate aerobic organisms.

The Washington State Legislature has designated uses and criteria for fresh waters with the intent to protect all indigenous fish. This includes criteria for temperature and dissolved oxygen. Washington Administrative Code 173-201A-200(v) states that the 7-day average daily maximum temperature should not exceed 18°C and dissolved oxygen concentrations should not fall below 8.0 mg/L (WAC, 2016). Canyon Lake supports a self-sustaining population of cutthroat trout[†] and is therefore subject to protection under Washington State law. By August, the only depths where the waters temperature and oxygen concentrations were within this range was from 2 to 4 meters (Figures 5 and 6). These conditions were not measured for seven consecutive days but they did not likely change due to the stability of the stratified water column. This indicated that very limited ideal fish habitat is common in Canyon Lake.

[†]The exact subspecies of cutthroat trout present in Canyon Lake was not verified in this study. It is possible that westslope cutthroat (*Oncorhynchus clarki lewisi*) are present due to its known presence in the neighboring Racehorse Creek (Olis et al., 2018). Coastal cutthroat (*Oncorhynchus clarkii clarkii*) were also widely stocked in lakes of the Western Cascades (Behnke and Tomelleri, 2002) and could also be present.

3.2 Alkalinity, pH, Conductivity, and Turbidity

The vertical distribution of pH decreased from near neutral to slightly acidic (pH = 6.6 - 7.2) with depth during June and August (Tables 2 and 3). The production of CO_2 through decomposition in the lower water column can decrease pH generating slightly acidic conditions (Wetzel, 2001). In addition, acidic humic compounds leaching from submerged wood also contribute to the lowered pH. Turbidity was low in June (< 2 NTU) and secchi depth ranged between 3.9 and 5.0 m across the sampling period (Table 6). Canyon Lake's water was moderately brown stained which may help explain the shallow secchi depth readings amidst very low turbidity measurements.

The alkalinity and conductivity levels were very low, indicating that Canyon Lake is poorly buffered, with a low ionic content (Tables 2 and 3). In June and August, conductivity increased near the bottom of the lake. Ammonium was elevated near the bottom of the lake, so it may have contributed to the increased conductivity levels (Table 3). This is common in lakes where decomposition in the hypolimnion can consume enough oxygen to create anoxic conditions. Under anoxic conditions, in the absence of nitrate or nitrite to act as an electron acceptor, anaerobic microbes commonly shift to the production of ammonia as a metabolic endpoint (Wetzel, 2001; Dodson, 2005). This can cause the accumulation of ammonia and ammonium as the decomposition of organic matter continues.

3.3 Nitrogen and Phosphorus

Nutrient concentrations in Canyon Lake were low. The nitrate+nitrite were near or below analytical detection limits (<10 μg-N/L) at most depths (Table 3). Total phosphorus concentrations were also low, being at or below the analytical detection limit (<10 μg-P/L) at all depths. Soluble reactive phosphorus was also at or below analytical detection limit (<3 μg-PO_4^-/L) at all depths. Total nitrogen is all the nitrogen in a water sample,

or the sum of larger nitrogenous compounds (e.g. nitrogen bound up in organic material), nitrate, nitrite, ammonia, and ammonium. Total nitrogen concentrations were low in the upper water column and appeared to exist primarily as organic material. Total nitrogen increased sharply near the lake bottom and appeared to consist primarily of ammonium in the lower portion of the water column. These nutrient conditions are typical for mid-elevation mountain lakes in our region (Matthews, 2017).

The cause of the inverted distribution of nitrogen species is not fully understood. What is known is that woody debris can facilitate the conversion of nitrate (NO_3^-) to nitrogen gas (N_2) through microbial metabolism. Porter et al. (2015) and Rambags et al. (2016) found that nitrate was successfully removed from waste water in tests with bioreactors filled with chipped wood substrates. The extensive floating and submerged wood in Canyon Lake may contribute to the low nitrate and nitrite levels throughout the water column. Moreover, the combination of low oxygen concentrations, mild acidity, and continuous low temperatures slow the decomposition of organic matter in the deeper waters of the lake. These same conditions impede the nitrification of ammonia to nitrate or nitrite, all of which allow ammonia to accumulate.

3.4 Macroinvertebrate Estimations and Macrophyte Drying

Wet, dry, and ash-free dry macrophyte tissue weights were used to generate standardized estimates of macroinvertebrate abundances for each macrophyte sample. Analysis of macrophyte weights revealed that the best correlation between weights was between dry weight and ash-free dry weight ($\tau = 0.86$, Figure 7). Correlations between wet and dry, or wet and ash-free weights were slightly weaker ($\tau = 0.74$ and 0.65, respectively). This was important because the stronger correlation between dry and ash-free dry macrophyte subsample weights indicated that ash-free dry macrophyte weight would be the most accurate for macroinvertebrate estimates due to the lower variation across all the final subsample weights. Because of this better correlation, my remaining analyses were

based on macroinvertebrate densities normalized by ash-free dry weight. The weaker correlation between wet and dry weights was likely due to variations in the amount of water removed prior to collecting the wet measurements. Further, samples with greater amounts *Potamogeton natans* and *Nuphar polysepala* were more difficult to dry to a consistent weight, which introduced variability in final dry weight measurements. In general, samples with more rigid stems behaved hygroscopically, easily absorbing moisture from the air.

3.5 Macrophyte Composition, Distribution and Growth Form

A total of six dominant macrophyte taxa were collected in August 2016 (Table 1). These were the water horsetail *Equisetum fluviatile*, the antifever fontinalis moss *Fontinalis antipyretica*, the broad-leaved pondweed *Potamogeton natans*, the ribbonleaf pondweed *Potamogeton epihydrus*, the water buttercup *Ranunculus aquatilis*, and the watercelery *Vallisneria americana*. Other co-dominant macrophytes were also collected. They included the narrowleaf burr reed *Sparganium angustifolium* and the yellow pond lily *Nuphar polysepala*. The eight most prevalent macrophyte taxa do not represent the total number of macrophyte taxa in Canyon Lake. In addition, *Sphagnum* spp. (peat mosses) were present in many samples, but were not counted, as discussed above. At least one species of Characeae (stonewort), *Isoetes* (quillwort) and *Potamogeton pusillus* (sago pondweed) were observed during data collection, but were not abundant enough to collect macroinvertebrate samples from. *Potamogeton pusillus* was occasionally found in very small amounts when cleaning macrophyte samples in the lab. It is a short grass-like pond weed usually less than 70 cm in length. Its low abundance made it difficult to differentiate from other *Potamogeton* species during sampling and was never found in any aggregation dense enough to be sampled with a macrophyte rake. Other taxa were undoubtedly present, but were not collected using my sampling methods.

Macrophyte communities found along the northern shore of Canyon Lake occupied more littoral area than those on the southern shore. The largest patches of *Potamogeton natans*, *Potamogeton epihydrus*, and *Equisetum fluviatile* were found along the northern lake shore. This was not surprising because the southern watershed walls are much more steep and rocky. The lake bed quickly drops off into the lake leaving less littoral lake area with suitable depths for macrophyte growth along that portion of the lake shore. Furthermore, the high southern ridge line shades the southern lake shore, which reduces the available light for the growth of macrophyte beds. The input of Canyon Lake Creek into the upstream portion of the lake has deposited a notable delta and *Equisetum fluviatile* dominated the older deposits on this delta.

By August, *Equisetum fluviatile* became the most abundant macrophyte in the most shallow regions of the lake ($<$ 1.5 m). By the end of the sampling period it nearly encircled the entire lake, growing in the lake and the saturated soils surrounding the lake. No other macrophyte was as widespread. The next most abundant macrophytes were *Potamogeton natans* and *Potamogeton epihydrus*. *Potamogeton natans* was commonly found mixed with *Equisetum fluviatile* in the shallow littoral areas. Smaller patches of *Potamogeton natans* could also be found growing in sediments that had accumulated upon submerged down trees throughout the lake. The largest beds of *Potamogeton epihydrus* were found growing in isolated patches in slightly deeper regions (1.5 - 2.7 m) rooted in soft sediments. Other beds were adjacent to *Potamogeton natans* and *Vallisneria americana* but their footprints did not significantly overlap.

Small monoculture beds of *Ranunculus aquatilis* were often found in the northern littoral regions. Minor amounts of it were found among *Potamogeton epihydrus*, *Potamogeton natans*, and *Vallisneria americana*, but it was not abundant enough to be visible from the surface when these samples were collected. *Vallisneria americana* was found in small patches on both the northern and southern shores. *Fontinalis antipyretica* was only found on the remnant wood that perforates the lake and was consistently co-dominant

with *Sphagnum* and other mosses. *Nuphar polysepala* could be found among most littoral habitats but was not often collected due to constraints imposed by my sampling method. *Nuphar polysepala* develops large rhizomes and an extensive root system under the sediment making it extremely difficult to pull by its roots. Attempts to collect it with a macrophyte rake sheared off its thick stems from its rhizomes without becoming bound in the tines of the rake.

Aquatic macrophytes are generally categorized in four different growth forms. These forms are: fully submerged, floating-leaf, emergent macrophytes, or free floating. Each growth form is thought to contribute different ecological values in aquatic systems. In Canyon Lake, *Potamogeton epihydrus*, *Potamogeton natans*, and *Nuphar polysepala* were seen in floating-leaf stages, all with present, well developed reproductive structures. *Equisetum fluviatile* was also well developed with its stems and reproductive structures extending as much as a meter above the water's surface. *Sparganium angustifolium* and *Vallisneria americana* were seen only in floating-leaf stages. These macrophytes can produce reproductive structures that emerge above the water's surface. No emergent reproductive structures were found on either of these macrophytes, so they were considered to be floating-leaf macrophytes. When fully mature, *Ranunculus aquatilis* can develop floating leafs and produce small white flowers that extend above the water's surface for reproduction (Cook, 1969). At the time this macrophyte was collected, neither of these structures were present and no portion of it was at or near the water's surface, so I categorized *Ranunculus aquatilis* as a submergent macrophyte.

The growth forms of *Fontinalis antipyretica*, and *Sphagnum* mosses were somewhat indistinct with respect to those previously mentioned. These mosses were only sampled from remnant trees in the lake. They grew in expansive mats on the tops of logs which extended into and below the water. *Fontinalis antipyretica* naturally grows as a fully submerged macrophyte and *Sphagnum* mosses are tolerant to long periods of saturation but are not fully aquatic (Rydin and McDonald, 1985). When these mosses were sampled

from the many pieces of woody debris in Canyon Lake it was found that *Fontinalis antipyretica* grew on the undersides of large woody debris draping down into the water. *Sphagnum* mosses grew on the tops of the same large woody debris above the water's surface. The transition from one moss type to the other was continuous and they were impossible to separate for sampling purposes. These plants functioned together as an emergent substrate. Many molted exoskeletons of Anisoptera and Zygoptera odonates were found clung to the above water portions of these commingled mosses, as well as on emergent snags and woody debris.

3.6 Macroinvertebrate Abundance on Macrophytes

A total of 18,509 epiphytic macroinvertebrates representing 36 taxa in 15 orders and 20 families[†] were collected from the Canyon Lake macrophyte samples (Tables 7 and 8). The total number of taxa was a conservative number because many of the groups were only identified to family or order. Of this total, 26.7% were in the family Basommatophora, 20.0% were in the family Chironomidae, 18.5% were in the order Amphipoda, 13.8% were in the family Lumbricidae, and 7.7% were in the family Aphididae. All other taxa constituted less than 5% of all individuals collected. The total abundances and number of taxa collected per macrophyte type is shown in Figures 8 and 9. *Rhopalosiphum nymphaeae* (water lily aphid) was numerically abundant, but found exclusively among macrophytes with floating-leaf morphologies. Storey (2007) described the life history of *Rhopalosiphum nymphaeae* as heterogeneous, meaning they migrate between aquatic and terrestrial plants, such as fruit trees during their life cycle. Stroyan (1984) reported water lily aphids surviving underwater, perhaps by retaining an air film. Any foraging underwater by *Rhopalosiphum nymphaeae* appears to be rare. However, it does still qualify as an epiphytic invertebrate so it was not excluded from my analysis.

[†]One family of Coleoptera, five families of Diptera, two families of Hemiptera, and three families of Trichoptera were unidentified beyond order. Each of these taxa only contributed one to two individuals to the dataset or were damaged causing identification issues.

The macrophyte *Ranunculus aquatilis* had the highest median and maximum abundance of macroinvertebrates per gram ash-free dry macrophyte weight (Med = 356.0, Max = 2575.3 individuals /g ash-free dry weight). Only three samples of *Ranunculus aquatilis* were collected because its distribution was restricted to two small beds. The high macroinvertebrate abundance on *Ranunculus aquatilis* was the result of a single sample with an extremely high number of the gastropod *Gyraulus* (1314.2 individuals /g ash-free dry weight). The low total number of samples of *Ranunculus aquatilis* and the extreme outlier caused statistical issues that masked the relationships between other taxa and macrophytes. Because of this, I chose to omit *Ranunculus aquatilis* from the bulk of statistical analysis. Brown (1997) found in colonization experiments that the Gastropod *Gyraulus parvus* preferentially selected broad leaved macrophytes over thin or narrow leaved macrophytes. The macrophyte *Ranunculus aquatilis* is very narrow leaved, so the high density of *Gyraulus* was not expected. The narrow leaves of *Ranunculus aquatilis* give it a high surface are to volume ratio and its leaves were covered with a large amount of epiphytic algae, which could be a food resource for snails such as *Gyraulus*. This may explain the difference between these two findings.

Macroinvertebrate abundances found in association with the remaining dominant macrophytes varied. When *Fontinalis antipyretica* was dominant, macroinvertebrates were found in moderate to high abundances (Med = 196.0, Max = 468.0 individuals /g ash-free dry weight). Samples where *Equisetum fluviatile* was dominant macroinvertebrate abundances were the lowest (Med = 9.78, Max = 36.21 individuals /g ash-free dry weight). Statistical differences in the median macroinvertebrate abundance per gram of ash-free dry weight of each dominant macrophyte are discussed in the following section and are summarized Table 9 along with the minimum and maximum abundance of each. The median macroinvertebrate abundance per dry and wet gram of dominant macrophyte can be found in the Appendix in Tables 2 and 3.

3.7 Analysis of Macroinvertebrate Communities Among Dominant Macrophytes

Different Canyon Lake macrophytes appeared to have distinct communities of macroinvertebrates, at least with respect to abundance. *Equisetum fluviatile* supported a lower diversity of macroinvertebrate taxa (Figures 8 and 9) and consistently yielded lower median macroinvertebrate abundances than all other macrophytes as measured by any single taxonomic grouping other than Trichoptera (Table 9 and Figures 10 to 20). These low abundances were expected because the submerged stems of *Equisetum fluviatile* are simple and un-branched, and provide a homogeneous substrate that leaves macroinvertebrates exposed to predation. Verdonschot and Peeters (2012) and Heino (2000) found similar results where lower epiphytic macroinvertebrate abundance and numbers of taxa were found among macrophytes of lower structural complexity. Numerically, Odonates and Oligochaetes were the least abundant and Dipterans were the most abundant order of macroinvertebrate among *Equisetum fluviatile*, but no significant differences were detected compared to other taxa associated with this macrophyte (Figure 10).

Ranunculus aquatilis dominant samples supported an intermediate number of taxa (Figure 9) and high abundances of Basommatophora and Oligochaeta relative to other taxa (Figure Figure 25 and Table 9). However, no statistical differences could be determined among macroinvertebrates inhabiting *Ranunculus aquatilis* dominant habitats because of the small sample size. Only three plant samples were collected where *Ranunculus aquatilis* was the dominant macrophyte and the Wilcoxon rank-sum test is sensitive to small sample sizes.

Among *Fontinalis antipyretica* dominant samples, Amphipoda was the most abundant macroinvertebrate. Amphipoda were significantly more abundant than Odonata, Trichoptera, Diptera, Basommatophora, and Oligochaeta (Table 9 and Figure 21). The total macroinvertebrate abundances found on *Fontinalis antipyretica* dominant samples

were not significantly different from those on other dominant macrophyte types but were significantly greater than that of *Equisetum fluviatile* dominant samples (Figures 8, 10 and 21 and Table 9). The significantly greater numbers of Amphipods associated with *Fontinalis antipyretica*, *Sphagnum* and other mosses may be providing a food resource to the associated anisopterans. In Canyon Lake, Odonata, specifically individuals of the suborder Anisoptera, were prevalent on *Fontinalis antipyretica*. Odonata densities were not significantly more or less abundant on *Fontinalis antipyretica* dominant samples compared to other dominant macrophytes (Figure 18). However, *Fontinalis antipyretica* was the only dominant macrophyte where Anisoptera odonates were found among every sample. This was consistent with earlier work by Henrikson (1993) where Anisoptera, in particular *Leucorrhinia dubia*, were found to be more prevalent and more successful as predators among *Sphagnum* mosses in laboratory tests. Michiels and Dhondt (1990) also found that *Sphagnum* and another similar semi-aquatic moss was preferred by ovipositing female Libellulidae odonates over macrophytes with stemmed emergent structures.

Samples dominant with *Potamogeton epihydrus* and *Potamogeton natans* supported intermediate total abundances of macroinvertebrates relative to other macrophytes (Table 9). The overall macroinvertebrate abundances associated with *Potamogeton epihydrus* and *Potamogeton natans* was significantly greater than that associated with *Equisetum fluviatile* dominant samples (Figure 8). *Potamogeton natans* supported statistically lower abundances of Trichoptera than other orders of macroinvertebrates except Odonata (Figure 23). Within *Potamogeton epihydrus* I found significantly greater abundances of Diptera and Oligochaeta than Odonata, Trichoptera, Basommatophora, and Amphipoda (Figure 22). Oligochaeta were significantly more abundant on *Potamogeton epihydrus* dominant samples than all other dominant macrophyte types found (Figure 13). Diptera were significantly more abundant among *Potamogeton epihydrus* dominant samples than all others except *Vallisneria americana* (Figure 11). Chironomidae constituted 90.7% of all Diptera among *Potamogeton epihydrus* dominant samples. These larvae forage on periphyton, mine plant tissue, and some are predaceous (Merritt and Cummins, 1996).

The stems and leaves of *Potamogeton epihydrus* are less rigid than those of *Potamogeton natans*. This characteristic may offer a better substrate for plant-mining Chironomidae, which would explain the more common occurrence of Chironomidae among *Potamogeton epihydrus* dominant samples. *Potamogeton epihydrus* was observed to be associated with silt and fine sediments. When samples of *Potamogeton epihydrus* were collected, a cloud of what appeared to be silt could be seen in the water after the disturbance of collecting the sample. A silt-like substance was also noted on the leaves of *Potamogeton epihydrus* after collecting it. This was also later noted during cleaning *Potamogeton epihydrus* samples in the lab. Chironomidae and Oligochaeta were also associated with protective tubes of sediment and silk in the preserved macroinvertebrate samples, which were likely for refuge from predation. Together, these two observations may indicate that the observed siltation was contributing to the higher abundance of Chironomidae and Oligochaeta on *Potamogeton epihydrus*.

No statistical differences between the macroinvertebrate groups found on *Vallisneria americana* dominant samples could be detected. Significantly greater total macroinvertebrate abundance were found in association with *Vallisneria americana* than *Equisetum fluviatile* but no statistical differences could be detected between macroinvertebrate abundances associated with other dominant macrophyte types (Figure 8). The highest median and maximum abundances of Basommatophora (Med = 19.20, Max = 174.05, Figure 24) and Odonata (Med = 9.61, Max = 59.13, Figure 18) were found in association with *Vallisneria americana* but these abundances were not statistically different than other dominant macrophyte types. The relatively high numbers of Basommatophora and Odonata were surprising because *Vallisneria americana* has a simple un-branched structure, similar to *Equisetum fluviatile*. Such macrophytes often support lower macroinvertebrate abundances (Cattaneo et al., 1998; Heino, 2000; Rennie and Jackson, 2005). Phiri et al. (2012) compared *Vallisneria aethiopica*, a similar macrophyte with simple ribbon-leaved structure, and *Lagarosiphon ilicifolius*, a more complexly dissected macrophyte and consistently found similar macroinvertebrate taxa but in lower abundances

among *Vallisneria aethiopica*. In Canyon Lake, macroinvertebrate abundances among *Vallisneria americana* were not as low as might be expected given its simple structure.

In general, non-insect taxa ranged more widely with greater maximum sample abundances than did insect taxa among all dominant macrophytes, except for *Equisetum fluviatile* (Table 9). The greatest macroinvertebrate abundances found in any single sample, were in Basommatophora on *Ranunculus aquatilis* (Figure 25), Amphipoda on *Fontinalis antipyretica* (Figure 21), and Oligochaeta on *Potamogeton epihydrus* (Figure 22). The lowest macroinvertebrate abundances consistently occurred among *Equisetum fluviatile*. These differences in macroinvertebrate abundances may be related to macrophyte complexity.

In Canyon Lake, comparing macroinvertebrate abundances across simple, intermediate and complexly dissected macrophytes showed that total macroinvertebrate abundance significantly increased in macrophytes with greater structural complexity (Figure 26). Overall, macroinvertebrate abundance is known to increase in more heterogeneous habitats such as complexly structured macrophytes or macrophyte vegetation of mixed composition verses homogeneous monoculture macrophyte beds (Beckett et al., 1992; Rennie and Jackson, 2005). This is thought to be in part because macrophytes of greater complexity have higher surface area to volume ratios which encourages greater growth of periphyton and epiphytic algae which then supports more macroinvertebrates and other predators. Differences in macroinvertebrate assemblages and abundance among each macrophyte type is also related to macrophyte palatability (Suren and Lake, 1989). In Canyon Lake, the least palatable macrophyte was certainly *Equisetum fluviatile* because of its scaly rough stems. *Potamogeton natans* is also likely less palatable with its thick stems and a heavy cuticle. Danell and Sjöberg (1979) found that colonization of *Equisetum fluviatile* by macroinvertebrate biomass increased as decomposition of *Equisetum fluviatile* occurred over time. This suggest that the palatability of live mature *Equisetum fluviatile* is low and increases with decomposition. Reduced palatability and colonizable

surface area of macrophytes might help explain the lower abundances of Trichoptera and Odonata on *Equisetum fluviatile* and *Potamogeton natans*.

3.8 Taxonomic and Functional Macroinvertebrate Correlation Analysis

I used Kendall's τ correlation analyses to identify associations between individual macroinvertebrate taxa. (Table 1). Correlations were derived from data based upon the number macroinvertebrates per gram of ash-free dry macrophyte weight. These correlations were similar to those found with macroinvertebrates per gram of dry macrophyte weight, so only correlations based upon ash-free dry weight will be discussed here. *Gyraulus* was correlated with 10 other taxa, which was the greatest number of correlations with other macroinvertebrates. The strongest correlations (highest τ) between *Gyraulus* and other taxa were with the odonate *Zoniagrion exclamationis*, Chironomidae, and the trichopteran genus *Lepidostoma* (τ = 0.44, 0.37, and 0.35, respectively). *Gyraulus* was also the only taxa to have significant negative correlations with other taxa. It was negatively correlated with the odonate genus *Aeshna* and Hymenoptera (τ = -0.25 and -0.25, respectively). The correlations between *Gyraulus*, Chironomidae, and the most common Trichoptera, *Lepidostoma*, are consistent with the fact that many species of Chironomidae and *Lepidostoma* are known to use a scraping method for foraging (Merritt and Cummins, 1996; Thorp and Covich, 2009). Further, *Gyraulus* is equipped with a radula which is a minutely toothed, chitinous mouth part that functions as a rasp allowing it to scrape bio-films and soft plant epithelium. Their positive correlation and concurrent presence indicated that their macrophyte preference is overlapping, but these taxa may be utilizing different micro-habitats, food resources, or have varied temporal behaviors.

Amphipoda were correlated with five other taxa, which was the second greatest number of correlations among the most numerically abundant macroinvertebrate taxa. Amphipods were strongly correlated with the trichopteran Hydroptilidae, the megalopteran

Sialis, Pisidiidae (pea clams), Chironomidae, and the odonate *Aeshna* (τ = 0.29, 0.27, 0.26, 0.23, and 0.23, respectively). Amphipoda and *Aeshna* were found in the greatest densities together among *Fontinalis antipyretica*. This could be because both taxa are able swimmers and Amphipoda are known to seek fractured and high surface area substrates such as *Fontinalis antipyretica* (Kley et al., 2009). *Aeshna* are "sit and wait" predators and can easily cling on to mosses. They may be successfully preying upon Amphipoda in these types of habitats.

The associations of Amphipoda with Chironomidae and Hydroptilidae were most apparent on the two *Potamogeton* species. The more rigid stems of *Potamogeton natans* may provide a better attachment substrate for the anal claws of Chironomidae. The final instar of Hydroptilidae also require a rigid substrate to affix a small silk cocoon before they metamorphose into their adult stages (Back, 1983). The association of Amphipoda with *Sialis* and Pisidiidae is less clear. Amphipoda are good swimmers where as *Sialis* are poor swimmers, making a predator-prey relationship unlikely. Amphipoda, being opportunists, forage in both benthic and macrophytic habitats, which could contribute to their association.

Sialis and Pisidiidae are commonly found among benthic substrates. *Sialis* are known to climb and forage amidst macrophytes. Pisidiidae are only moderately mobile in benthic habitats and may be able to climb aquatic macrophytes with their long and strong foot. A different species of pea clam, *Sphaerium corneum*, was observed climbing aquatic plant substrates to aid in its filter feeding (Mackie et al., 1980). *Sialis* is known to feed on fingernail clams such as Pisidiidae (Merritt and Cummins, 1996). The correlation between the two (τ = 0.60) may reflect a predator-prey relationship between the two.

In order to reduce the noise in my Kendall's τ correlation analysis introduced by the least abundant taxa, I chose 17 different aggregations of macroinvertebrates that were taxonomic or functional in nature (Table 10). The functional groups I chose were collector-gatherers, piercers, predators, scrapers, shredders, and emergent macroinverte-

brates. The taxonomic groups I chose were: Insects, non-Insects, Odonata, Trichoptera, Diptera, Amphipoda, Basomatophora, and Oligochaeta. I also included total macroinvertebrate abundance by sample, macroinvertebrate taxa richness by sample, and macrophyte sample complexity in the correlation analysis. Macrophyte sample complexity was scored in the same fashion described in the section Statistical Analysis. Kendall's τ correlation analysis is a test of dependence between variables and it does not assume independence in the data being evaluated. Therefore, overlapping data categories were acceptable. All the Kendall's τ correlations were significant at least to the level of $\alpha < 0.05$. To highlight the best correlations, I only evaluated correlations with a τ value greater than 0.30. Restricting the τ correlation value to only those that were greater than 0.30 helped filter out weaker correlations which was necessary because the overall number of significant correlations was high and identifying the strongest relationships was important (Table 10).

The best correlation with macroinvertebrate abundance was with non-Insects (τ = 0.81). This was expected because the highest numerical abundances primarily occurred in Planorbidae, Amphipoda, and Oligochaeta. Collector-gatherers, predators, non-Insect taxa, and macroinvertebrate abundance was positively correlated with macrophyte sample complexity (τ = 0.40, 0.39, 0.38, and 0.35, respectively). Non-Insect taxa were numerically dominated by Amphipoda originating from *Fontinalis antipyretica* dominant samples and Oligochaeta originating from *Potamogeton epihydrus* dominant samples (Figures 14, 21 and 22). *Fontinalis antipyretica* was considered a complexly dissected macrophyte and the high abundance of Amphipoda and Oligochaeta found among it helps to explain this correlation. Kley et al. (2009) and others (Beckett et al., 1992; Brown, 1997; Heino, 2000) reported that taxa richness and diversity increased with measures of macrophyte substrate complexity. Taxa richness in Canyon Lake did not increase with complexity, at least at the correlation threshold I imposed. In my study, the measure of complexity was coarse. Physical measures of complexity at a finer scale, or perhaps accounting for stem densities of specific macrophytes may reveal more consistent trends.

Emergent taxa were correlated with non-Insects ($\tau = 0.41$). This was surprising because the life histories of these two groups of macroinvertebrates differ in many ways. Almost all emergent macroinvertebrates are Insects whose reproductive strategies rely on the terrestrial environment. Conversely, nearly all aquatic non-Insect invertebrates never leave the aquatic environment. This physical distinction between emergent Insects and non-Insects creates the expectation that their niche overlap would be minimal. This correlation can be explained in part by the strong relationship between emergent Insects and total abundance ($\tau = 0.59$). The increase in total macroinvertebrate abundance increases the likelihood of finding both emergent or non-Insect taxa. Further, both Oligochaeta and Diptera were abundant among both *Potamogeton* species and *Vallisneria americana*. The correlation between emergent taxa and non-Insects is likely driven by the abundance of Oligochaeta and Diptera on these macrophytes.

3.9 Principal Components Analysis

Prior to running the final PCA, I omitted four macrophyte samples from the clustering analysis. These samples were found to have extreme outliers or outliers that were approximately a whole order of magnitude greater than the samples inter-quartile range and maximum abundance. In the case of these four samples, the entire sample was removed prior to running PCA leaving only 45 total samples. The extreme difference within these samples drastically reduced the proportion of variability accounted for in the leading principal components. These same samples also appeared to cause instability in the cluster groups identified with hierarchical clustering as discussed next section. The resulting dataset will be referred to as the partial dataset in this and the next section.

Principal component analysis is used to help identify the most important variables or dimensions in a multivariate data space. In attempt to reduce the total number of variables or dimensionality of the macroinvertebrate data prior to analyzing it with PCA, I condensed it to the taxonomic level of order and then removed any taxa that did not

appear in at least 10% of samples. This dataset will be referred to as the condensed data in this and the next section.

Principal component analysis was performed on the partial and condensed macroinvertebrate datasets. Each dataset was row centered and scaled. In the partial data 10.0%, 8.1%, and 7.6% of the variance was explained by the first three principal components, respectively. In the condensed data 16.8%, 15.0%, and 13.6% of the variance was explained by the first three principal components. The top six PCA scores for the first three principal components of each dataset can be seen in (Table 11).

With the partial dataset, I created a loading plot showing which taxa had the best correlations to the first two principal components (Figure 31). *Ferrissia* and *Rhopalosiphum* had the greatest positive correlations with PC 1. *Lepidostoma* and *Gyraulus* had the greatest negative correlations with PC 2 (Table 11). I repeated this process with the condensed dataset where taxa were aggregated into taxonomic orders. The resulting loading plot (Figure 32) showed that the greatest correlations with PC 1 were with the orders Trichoptera and Basommatophora and were positive. The greatest correlations with PC 2 were with the orders Megaloptera and Pisidiidae and were negative (Table 11).

3.10 Hierarchical Clustering on Principal Components

Hierarchical clustering is an iterative tool with which to search for meaningful groups or associations based on a chosen measure of similarity (Kassambara, 2017). I used Hierarchical clustering to partition the macroinvertebrate data into groups based on Euclidean distances and Ward's minimum variance method of clustering. Principal components analysis was used with the partial and condensed datasets to help partition the majority of variability in the original macroinvertebrate variables into the first three leading principal components (Table 11). In their work detailing methods for detecting stable clusters with principal component analysis, Ben-Hur and Guyon (2003) found that clustering with

a few leading components can help stabilize clusters and improve structure.

When cluster analysis was performed on the first three principal components of the macroinvertebrate data, it generated two primary cluster groups (Figure 27). I examined the stability of the two cluster groups in two ways. First, I checked to see if they were retained when clustering on four, five, or six principal components. Generally, the two groups were retained in four, five, or six groups but destabilized when clustering on six or seven principal components. Second, I validated their structure by repeatedly removing a randomized 10% of the samples from the partial and condensed dataset when they were clustered on just three principal components. This demonstrated consistent stability in both datasets. Increasing the amount of randomly removed data began to diminish the stability of the cluster groups in each dataset.

I looked for associations of data factors with the cluster groups. The first factor I examined was macrophyte type (Figure 28). Neither of the two primary cluster groups were well explained by the macrophyte type in either dataset. I also examined macrophyte complexity as a data factor that might explain the two resulting cluster groups. I applied labels of high, medium, and low macrophyte complexity in addition to just high and low macrophyte complexity. Refer to the Statistical Analysis portion of the Methods section for the description of complexity determination. Macrophyte complexity also did not strongly associate with the two cluster groups. I hypothesized that total macroinvertebrate abundances were influencing the differentiation of clusters into two groups. To explore this, I first applied two different data factors as labels to the dendrograms. The first were high, medium, or low abundances based on the total abundance of samples split into equal thirds. The second were high or low based on the total macroinvertebrate abundance of samples split into equal halves. The two cluster groups were most closely associated with high and low total macroinvertebrate abundances in the case of both the partial and condensed datasets (Figure 29 lower panel). When applying the labels of high, medium, and low abundances the medium abundance group did not associate well

with either of the two primary cluster groups or subordinate group. This indicated that the macroinvertebrate abundances found among the collected macrophytes were bimodal. Macroinvertebrate abundances were either very high or low, which was difficult to predict based on the dominant macrophyte type, macrophyte sample complexity, sample depth, or collection location with hierarchical clustering. In general, macrophyte samples with higher macroinvertebrate abundance also supported more taxa (Figures 8 and 9), but this was not reflected in the hierarchical clustering results.

To look at how samples of high and low macroinvertebrate abundances were influencing the separation of the two cluster groups, I generated a factor map which plotted the samples on the first two principal components and labeled them as high or low by color (Figure 30). I found that the two categories were not perfectly disjoint but the cluster groups were clear. Samples with higher abundances had positive loadings on PC 1 and negative loadings on PC 2 (clustered toward the bottom right). Samples with lower abundances had just the opposite; negative loadings on PC 1 and positive loadings on PC 2 (clustered toward the top left). The direction of separation of the high and low abundance groups on PC 1 and 2 is similar to the abundance levels of the taxa loadings in Figures 31 and 32.

4 Conclusion

I investigated the associations between macroinvertebrates among the six different dominant macrophytes of Canyon Lake Washington. My objectives were to characterize the abundance of macroinvertebrates and number of macroinvertebrate taxa relative to the different macrophyte communities present in Canyon Lake. To do this, I determined the abundance of macroinvertebrates relative to the dry and ash-free dry weight of the macrophytes that were found among, and determined what physical macrophyte characteristics supported the greatest abundance of macroinvertebrates. I also collected summer water

quality data, mapped the approximate location and distribution of macrophytes, and generated a bathymetric map of the lake bottom.

I collected a total of 18,509 epiphytic macroinvertebrates in association with six different macrophyte taxa. Among these, I identified 36 different taxa to the lowest practical taxonomic resolution. All of my macrophyte samples supported varying communities of macroinvertebrates. The most notable differences were seen in total macroinvertebrate abundances which appeared to be driven primarily by the physical structure and complexity of the dominant macrophytes. I found that emergent and structurally homogeneous macrophytes communities supported lower abundances of macroinvertebrates. These macrophytes also supported a lower number of macroinvertebrate taxa. Conversely, submerged, floating leaf, and more structurally complex macrophytes communities supported greater abundances of all macroinvertebrate groups. These macrophytes also supported greater numbers of macroinvertebrate taxa. This was most readily apparent in the contrast between *Ranunculus aquatilis* and *Fontinalis antipyretica* with *Equisetum fluviatile*. Both *Ranunculus aquatilis* and *Fontinalis antipyretica* are structurally complex and dissected macrophytes. *Equisetum fluviatile* is a simple un-branched emergent macrophyte. *Equisetum fluviatile* dominant samples were consistently associated with low macroinvertebrate abundances indicating that it provided poor habitat for nearly all macroinvertebrate taxa. *Ranunculus aquatilis* dominant samples supported the most Odonata and Trichoptera, Planorbidae, Oligochaeta and Diptera and *Fontinalis antipyretica* supported the highest numerical abundances of number of Amphipoda and predatory taxa. *Ranunculus aquatilis* dominant samples also held the highest number of macroinvertebrate taxa.

All of the most common emergent and fully-aquatic macroinvertebrates increased in abundance with increasing structural complexity of their associated macrophytes. The highest numerical abundances occurred in Basomatophora, Amphipoda, and Oligochaeta. Basomatophora were most abundant among the complexly dissected macrophyte *Ranun-*

culus aquatilis. Amphipoda was statistically more abundant among the complexly dissected macrophyte *Fontinalis antipyretica* and Oligochaeta were statistically more abundant among the moderately dissected macrophyte *Potamogeton epihydrus* than other macrophytes. The less abundant taxa also increased with macrophyte complexity. Odonata and Trichoptera were the least abundant macroinvertebrates overall but were commonly found among the more complex macrophytes *Fontinalis antipyretica* and *Potamogeton epihydrus*. One exception to this was that the odonate *Zoniagrion exclamationis* and the trichopteran *Lepidostoma* were common among *Vallisneria americana*, which has a simple un-branched structure.

The macroinvertebrate data were further evaluated with principal component analysis and hierarchical clustering on principal components. Principal component analysis showed that greatest variability in the multivariate data space was introduced by Planorbidae, the trichopteran *Lepidostoma*, and the water lily aphid *Rhopalosiphum*. Hierarchical clustering on principal components revealed two distinct natural groupings in the data. These two cluster groups were best defined by high or low total abundance. Other factors including dominant macrophyte type, macrophyte sample complexity, sample depth, macrophyte co-dominance, and collection location were explored to attribute a more informative ecological meaning to the data separation but no additional associations in the data could be identified with the collected data factors.

This study provides a foundation for further studies in Canyon Lake and Canyon Lake Creek. Knowledge about the associations between macroinvertebrates and the macrophytes they associate with in Canyon Lake will help better predict changes in macroinvertebrate communities as changes in the macrophyte communities occur over time. It will also help to understand how the inhabitants of the Canyon Lake watershed benefit from the macroinvertebrate and macrophyte food resources in Canyon Lake.

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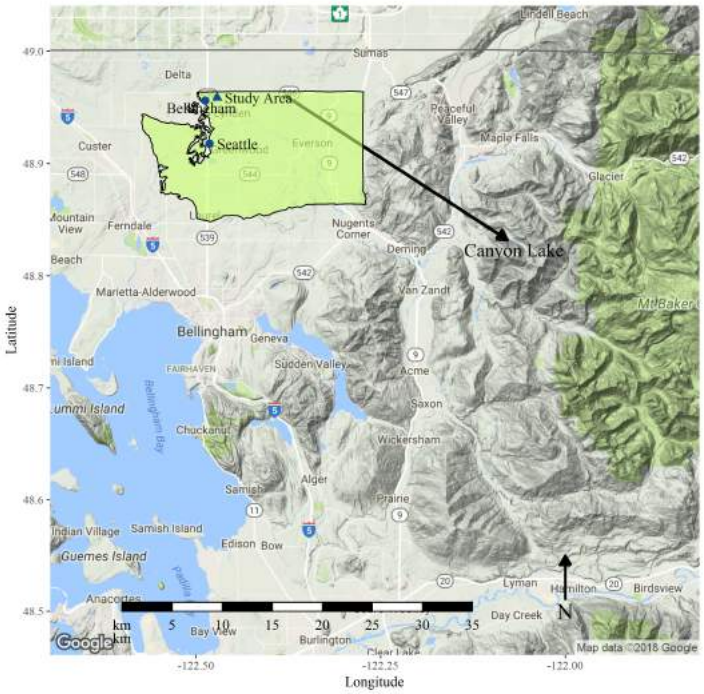
6 Figures

a)



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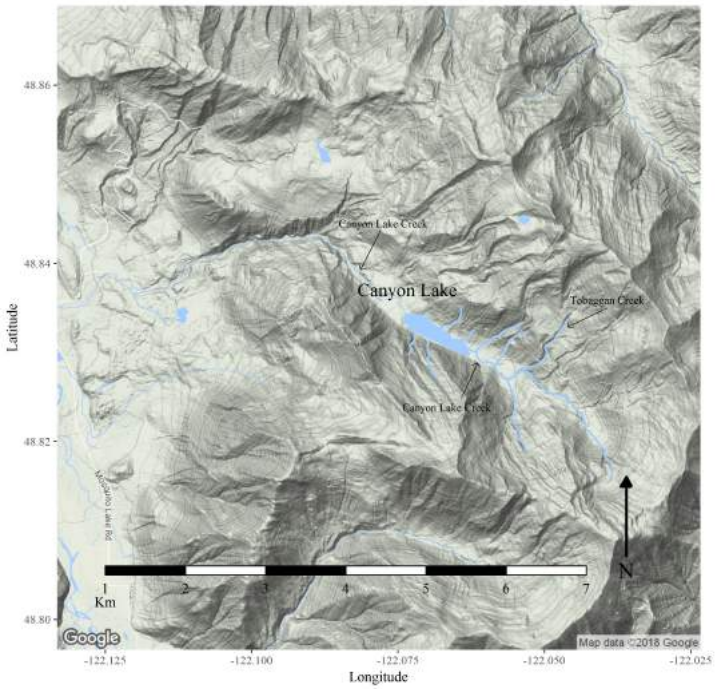


Figure 1: a) Shaded relief map of the Washington State region surrounding Canyon Lake. Canyon Lakes location is precisely at 48°49'55.04"N, 122° 4'5.84"W (WGS84). b) Shaded relief map of the Canyon Lake watershed. Maps created by Jesse Klinger using ggmap package in R (Kahle and Wickham, 2013). Canyon Lake Creek and Toboggan Creek are marked in the panel b and the only perennial tributaries of Canyon Lake.

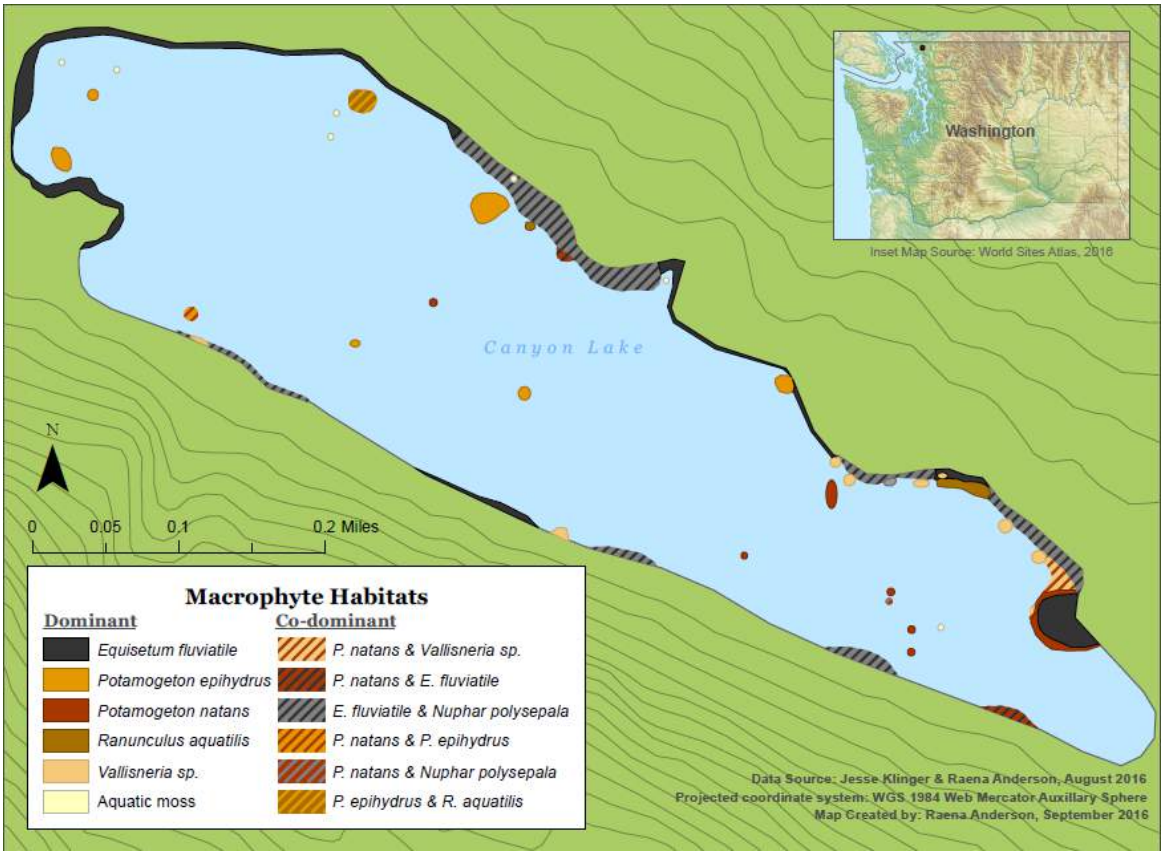


Figure 2: Macrophyte distribution in Canyon Lake, WA. Macrophytes were collected and mapped on 14 July, 2016. Solid colors represent mono-culture macrophyte beds and striped patterns represent co-dominant macrophyte beds. Distribution of aquatic mosses is incomplete and only represents sampled locations. Map created by Raena Anderson, Western Washington University; used with permission (Anderson, 2016).

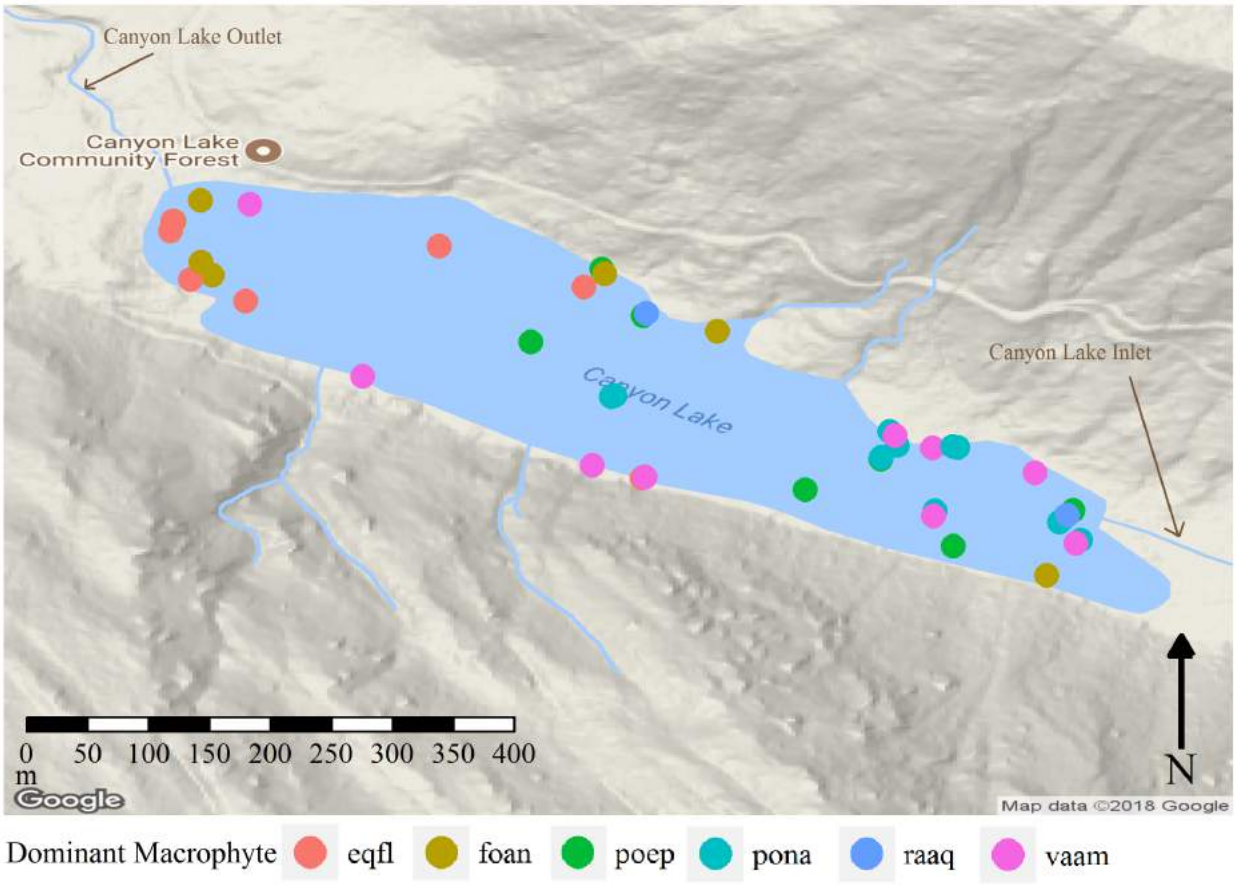


Figure 3: Macrophyte sample collection sites and the dominant plant type at each location in Canyon Lake, WA. Each tributary is also noted in blue.

Dominant macrophyte abbreviations are as follows:					
eqfl	-	*Equisetum fluviatile*	pona	-	*Potamogeton natans*
foan	-	*Fontinalis antipyretica*	raaq	-	*Ranunculus aquatilis*
poep	-	*Potamogeton epihydrus*	vaam	-	*Vallisneria americana*

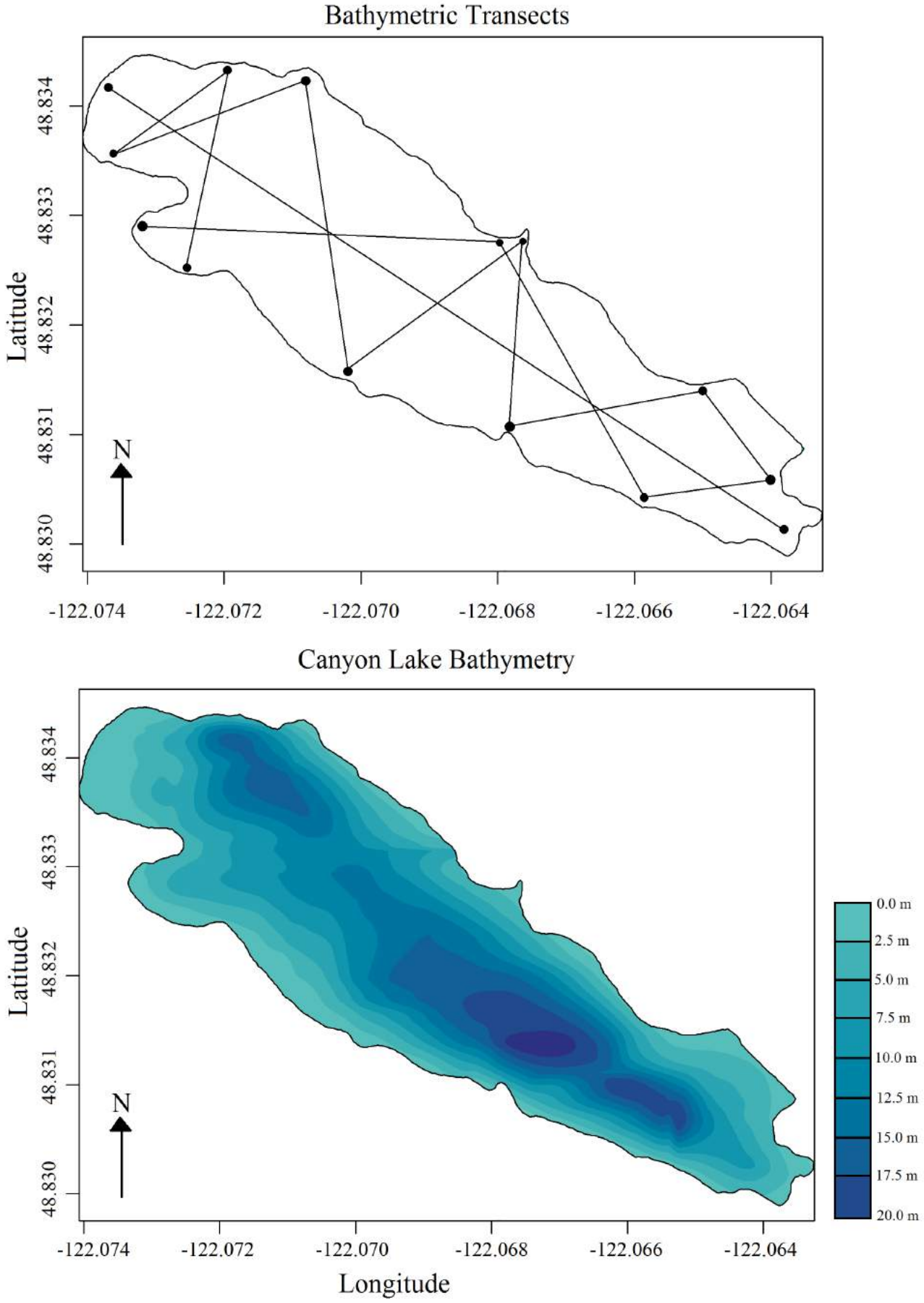


Figure 4: Above) The distribution of depth transects gathered to generate data used in bathymetric map. Below) Bathymetry of Canyon Lake. Map was generated by surface interpolation using a second degree polynomic loess smoothing spline with R statistical software (R Core Team, 2018)

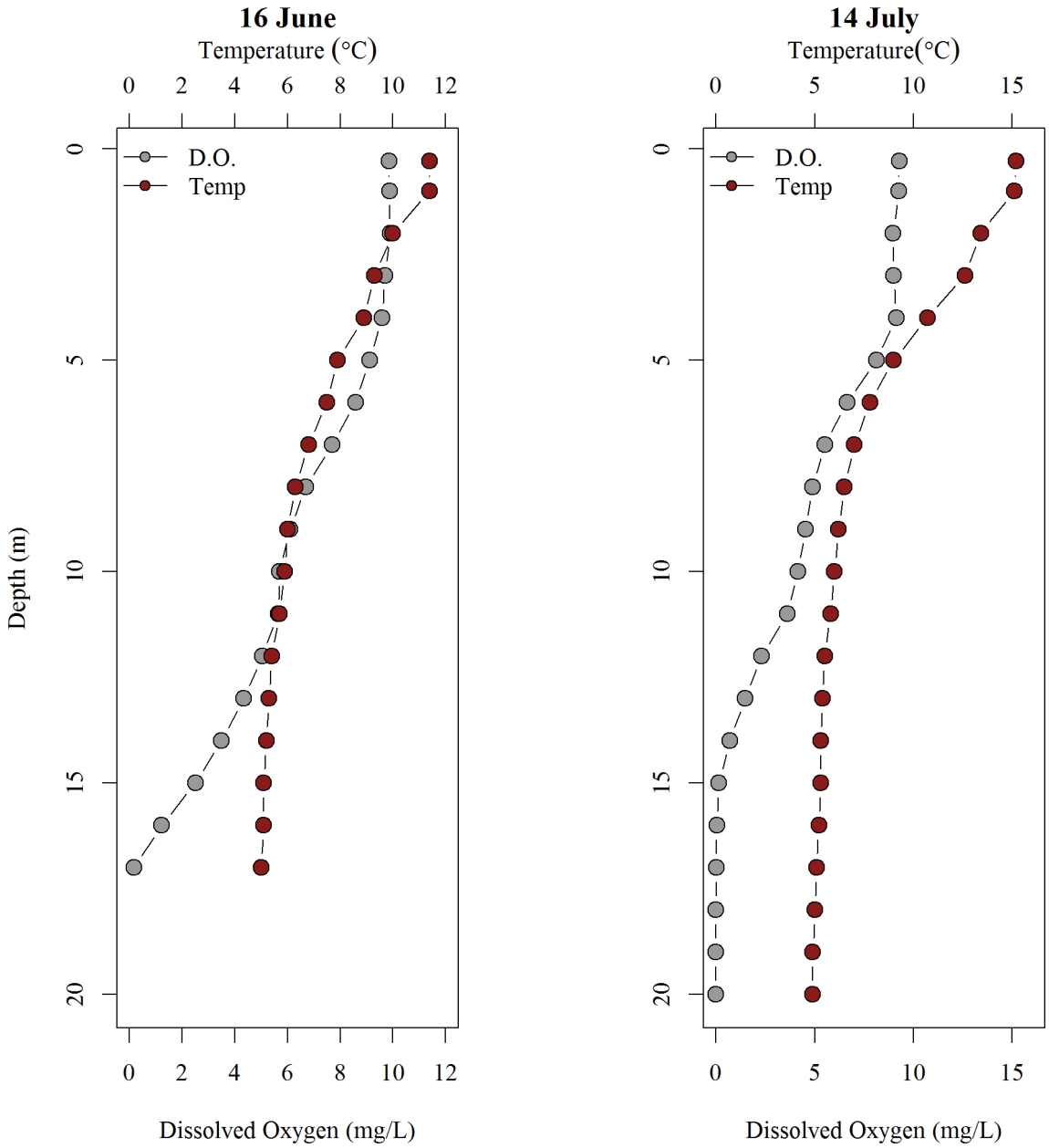


Figure 5: Depth profiles of temperature and dissolved oxygen taken with a YSI Pro20 in June and July, 2016 at Canyon Lake, WA. 16 June profile was taken prior to discovering the lake was slightly deeper at a near by point. The 16 June profile does not extend to 20 m because the deepest point of the lake, which it was within 10 m, would not be identified until the following sampling date.

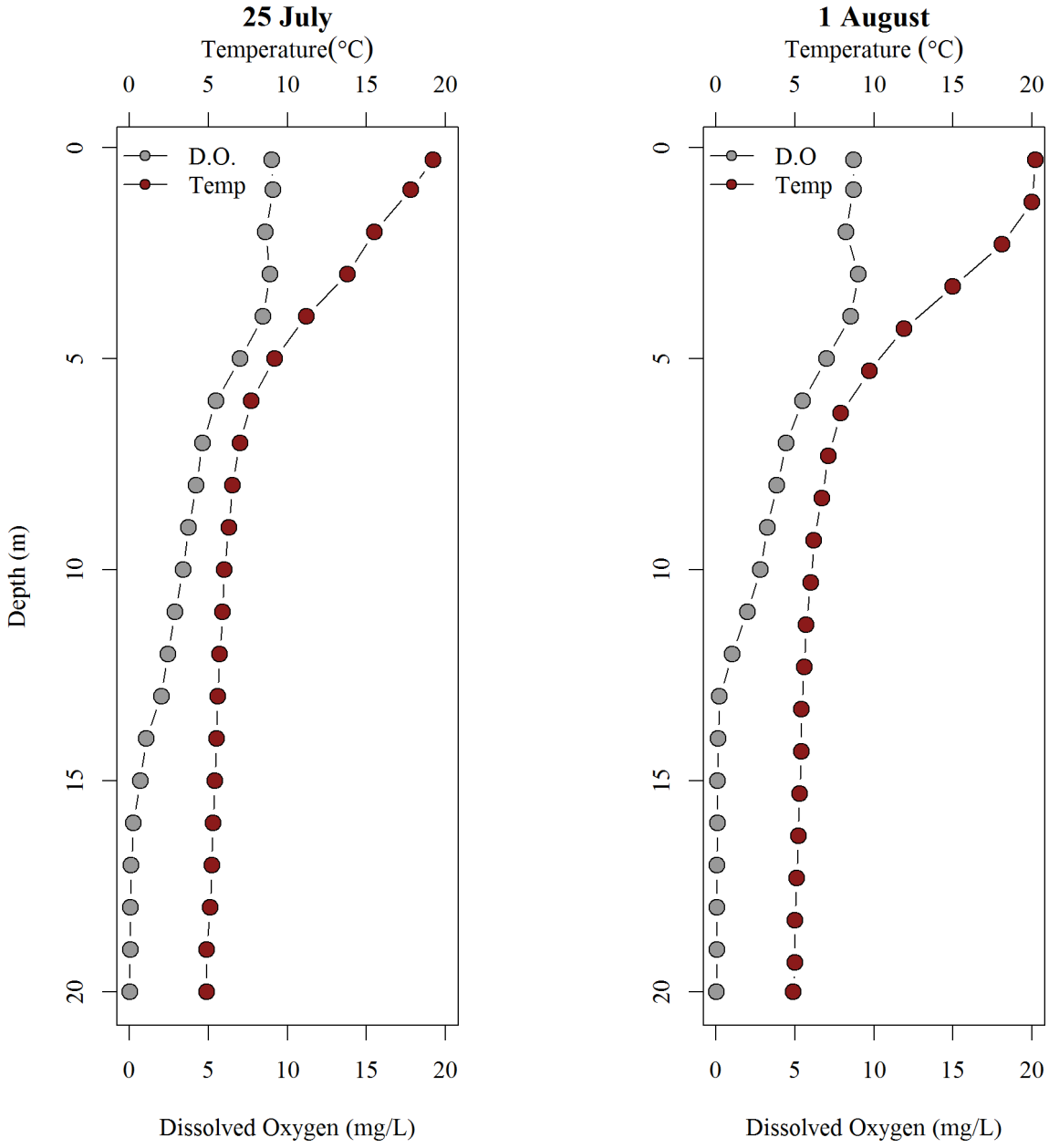
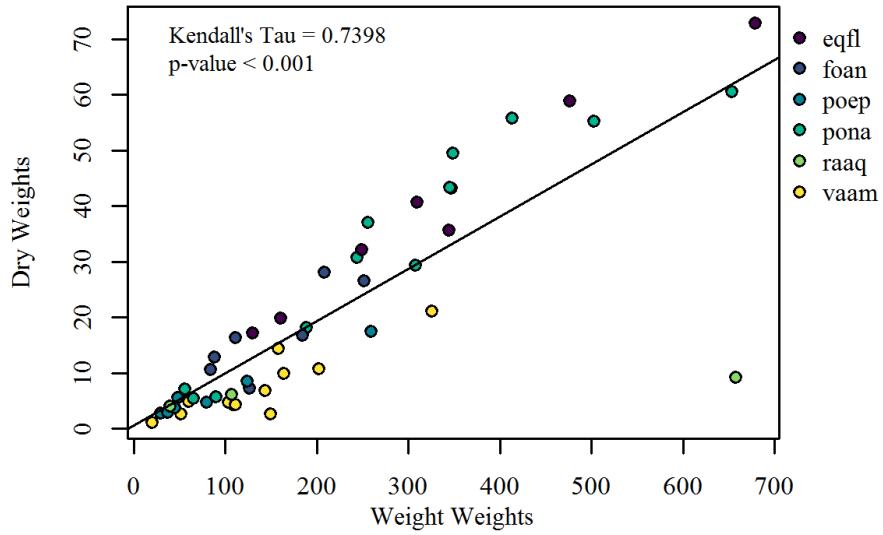
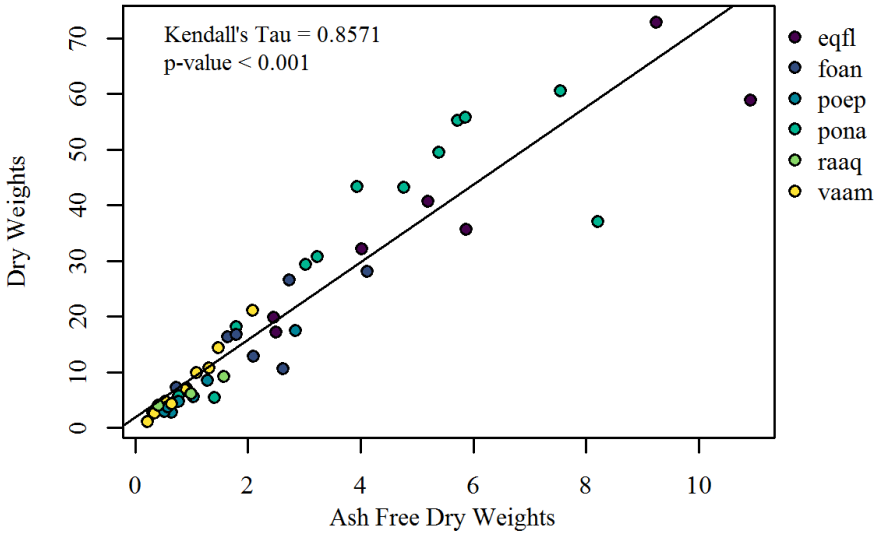


Figure 6: Depth profiles of temperature and dissolved oxygen taken with a YSI Pro20 in July and August, 2016 at Canyon Lake, WA. The profile collected 1 August was provided by the Institute for Watershed Studies.





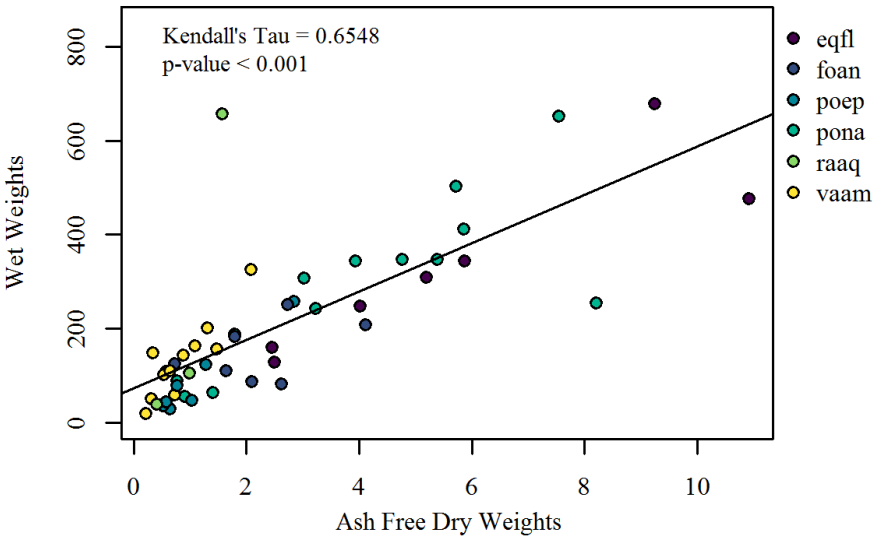


Figure 7: Correlations between macrophyte weight types colored by dominant macrophyte type. Each macrophyte sample consists of the dominant macrophyte and a lesser amount of other non-dominant macrophytes. Dominant macrophyte abbreviations are as follows: eqfl - *Equisetum fluviatile*, foan - *Fontinalis antipyretica*, poep - *Potamogeton epihydrus*, pona - *Potamogeton natans*, raaq - *Ranunculus aquatilis*, vaam - *Vallisneria americana*

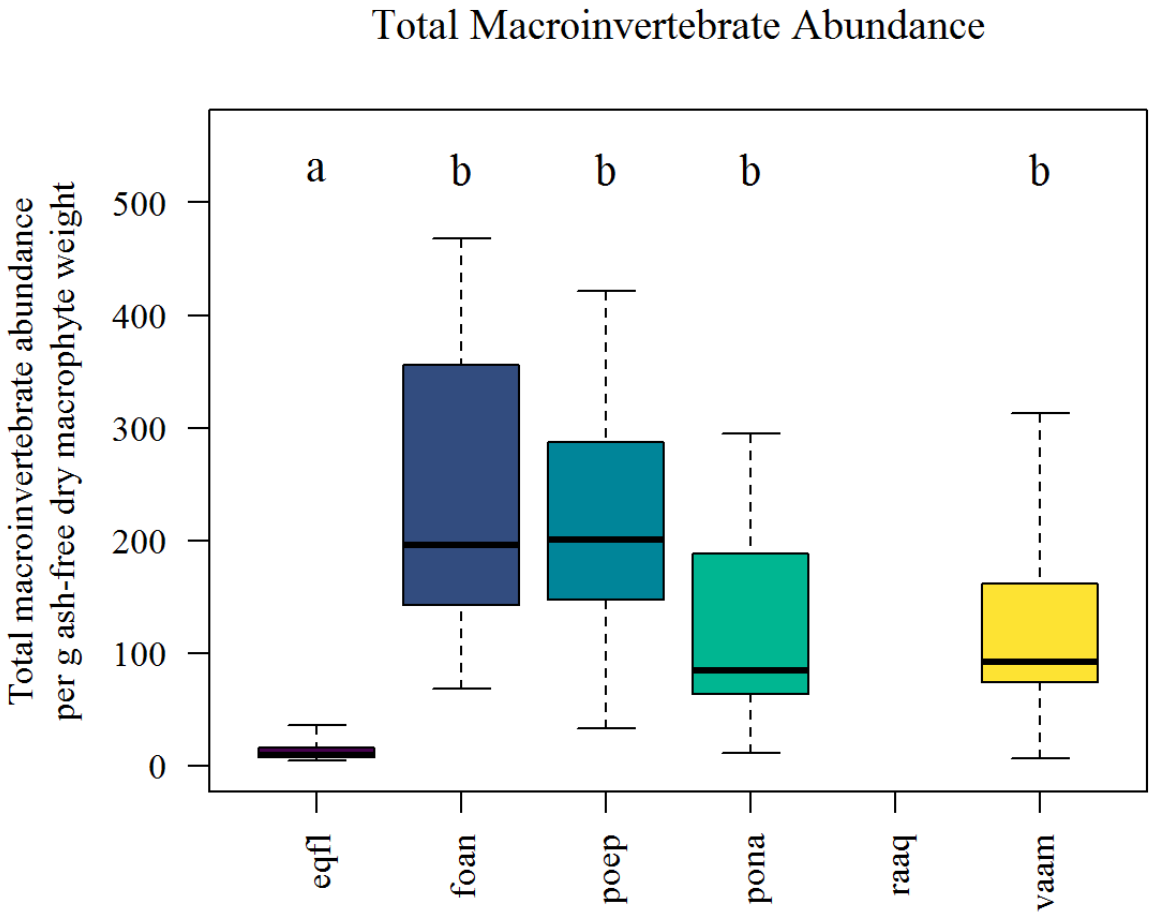


Figure 8: Total abundance of macroinvertebrates per gram of ash-free dry weight by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

Dominant macrophyte abbreviations are as follows:					
eqfl	-	*Equisetum fluviatile*	pona	-	*Potamogeton natans*
foan	-	*Fontinalis antipyretica*	raaq	-	*Ranunculus aquatilis*
poep	-	*Potamogeton epihydrus*	vaam	-	*Vallisneria americana*

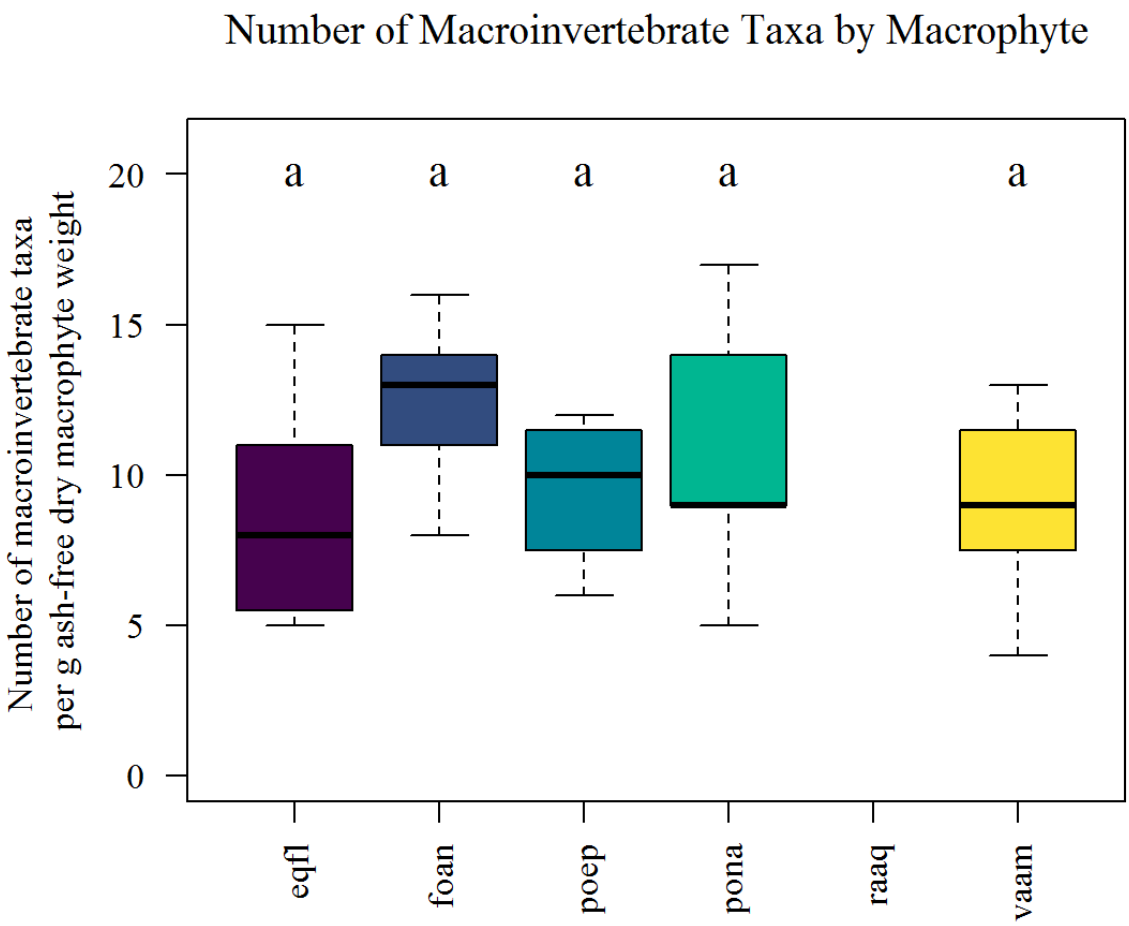


Figure 9: Boxplots of the number of macroinvertebrate taxa found per sample separated by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

Dominant macrophyte abbreviations are as follows:					
eqfl	-	*Equisetum fluviatile*	pona	-	*Potamogeton natans*
foan	-	*Fontinalis antipyretica*	raaq	-	*Ranunculus aquatilis*
poep	-	*Potamogeton epihydrus*	vaam	-	*Vallisneria americana*

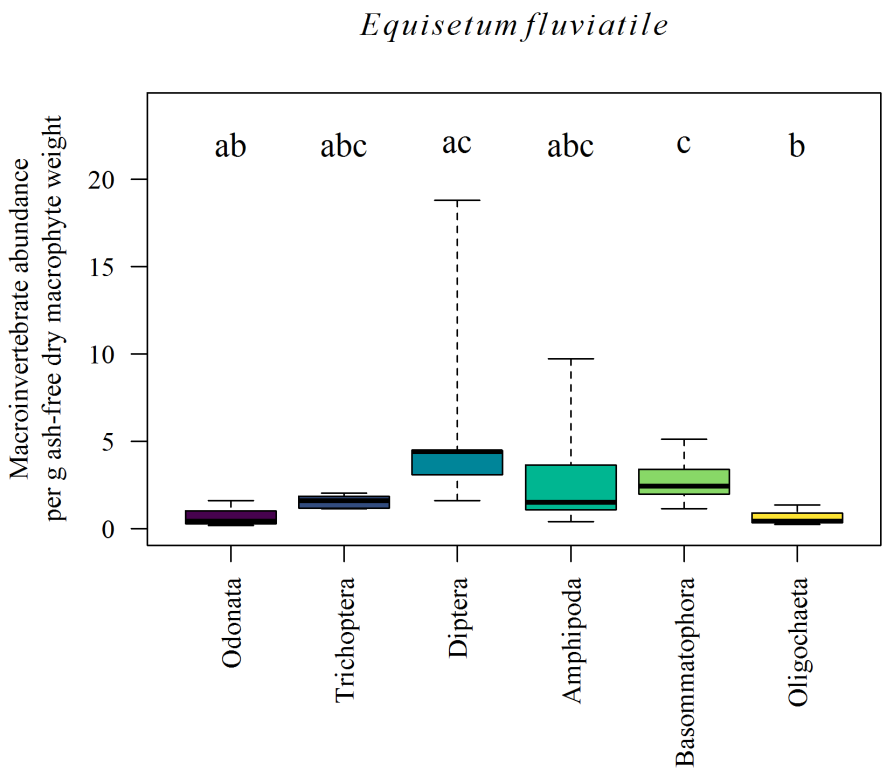


Figure 10: Number of macroinvertebrates per gram of ash-free dry weight of *Equisetum fluviatile*. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

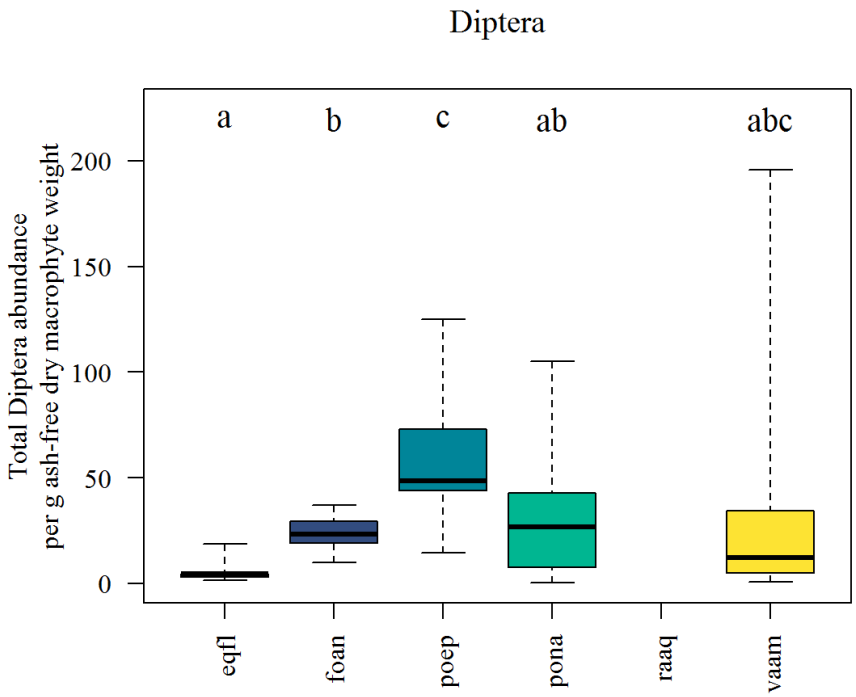


Figure 11: Boxplots comparing the number of Diptera per gram of ash-free dry weight by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

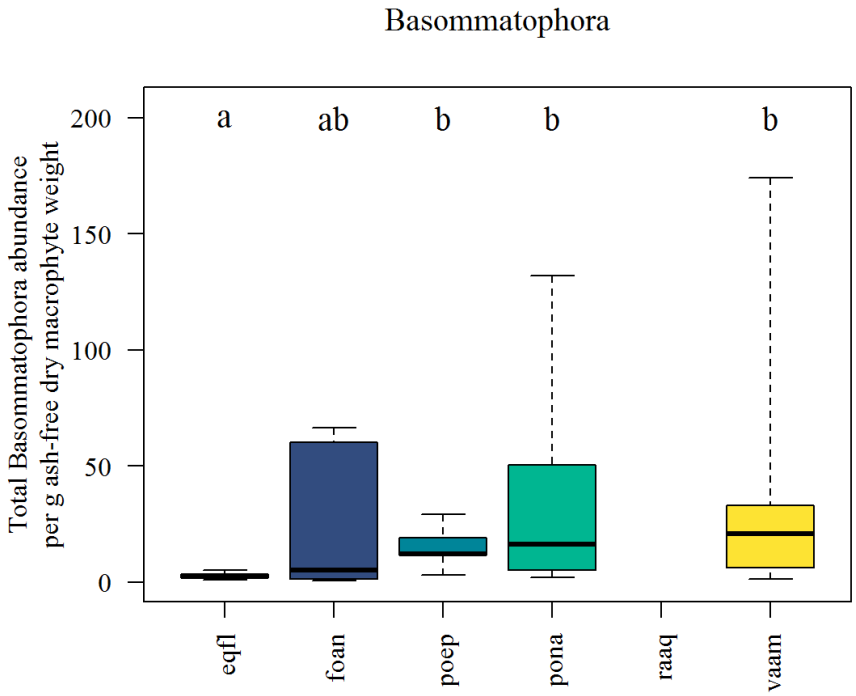


Figure 12: Boxplots comparing the number of Basommatophora per gram of ash-free dry weight by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

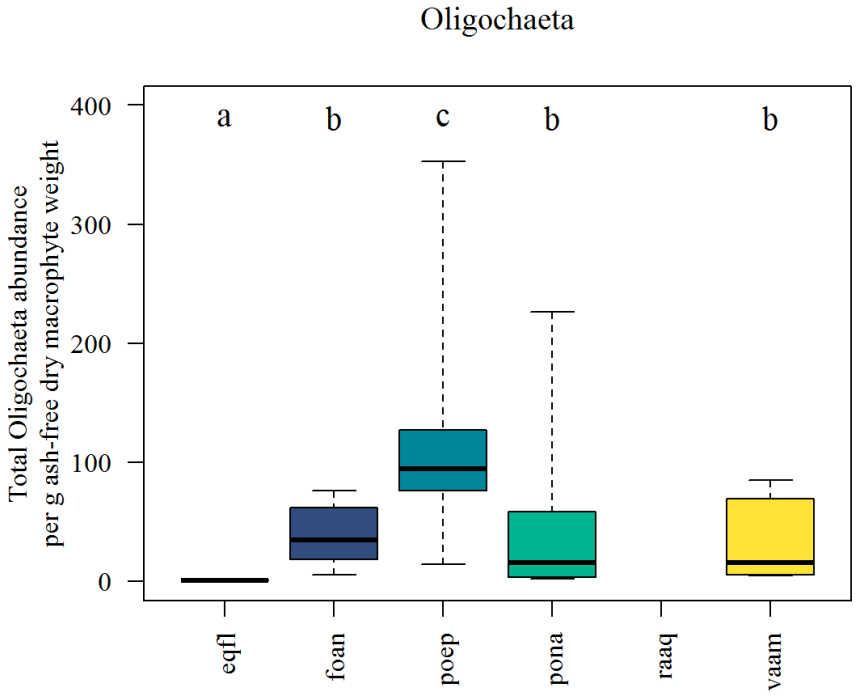


Figure 13: Boxplots comparing the number of Oligochaeta per gram of ash-free dry weight by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

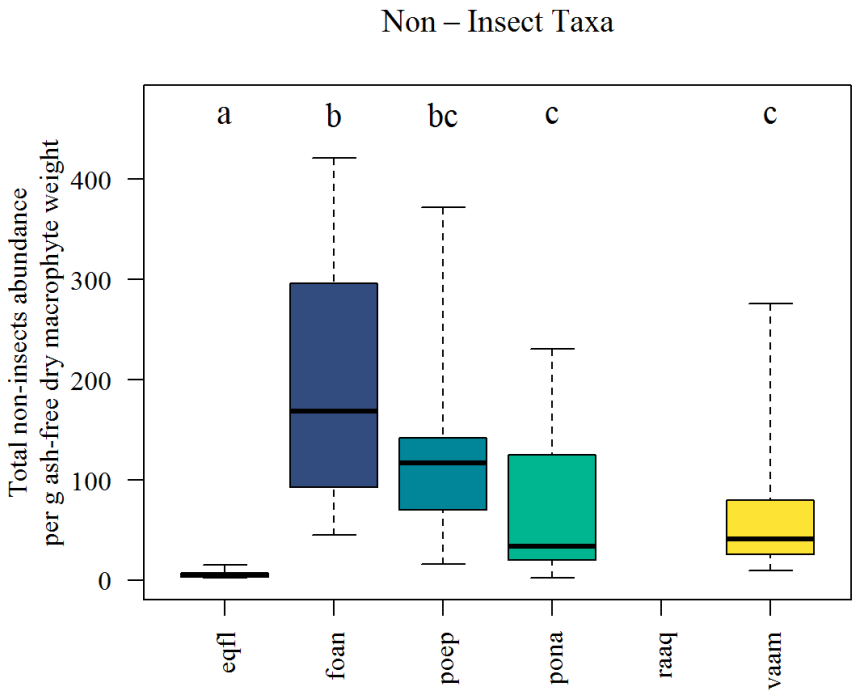


Figure 14: Boxplots comparing non-insect macroinvertebrates per gram of ash-free dry weight by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

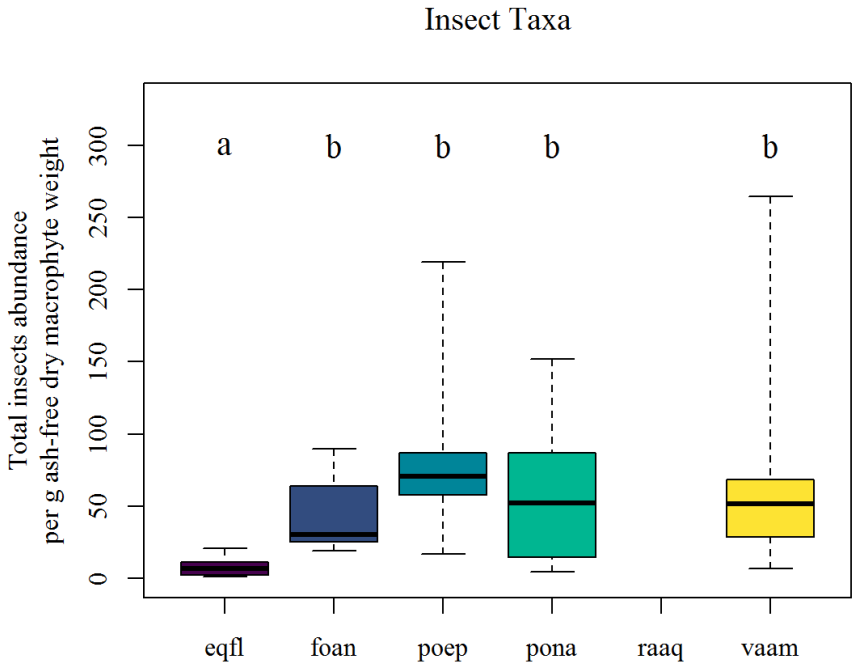


Figure 15: Boxplots comparing insect macroinvertebrates per gram of ash-free dry weight by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

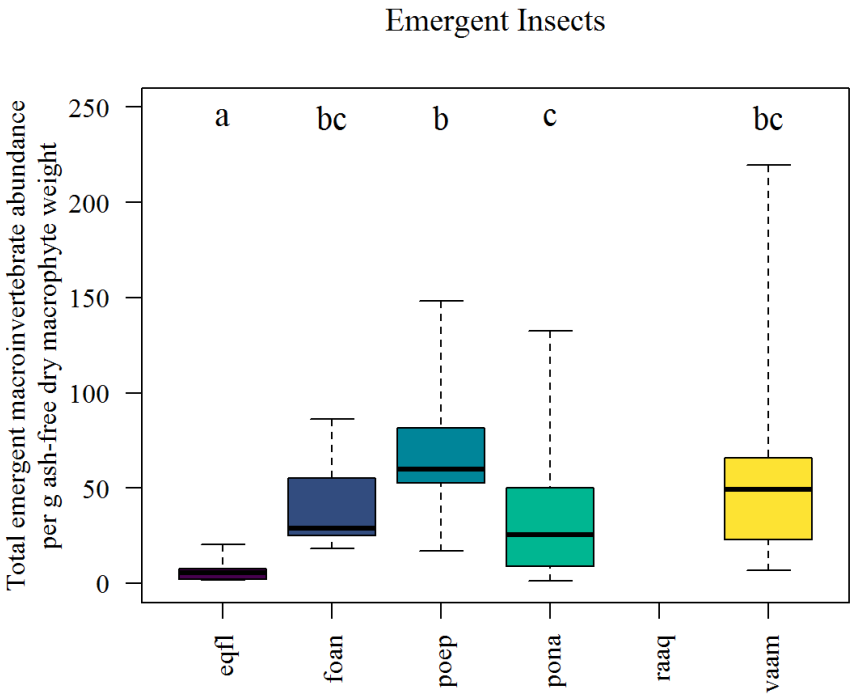


Figure 16: Boxplots comparing number emergent macroinvertebrates per gram of ash-free dry weight macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

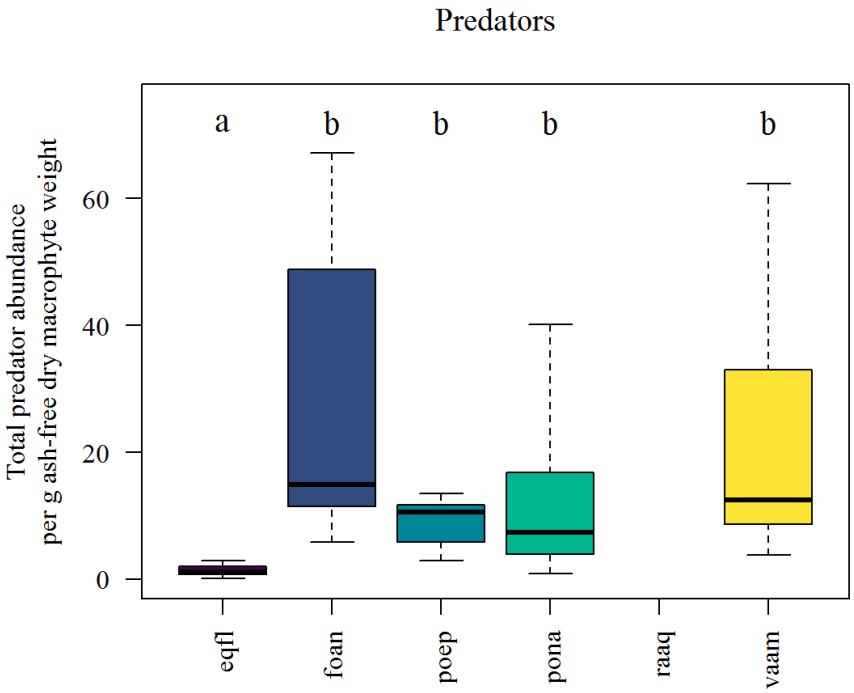


Figure 17: Boxplots comparing predators per gram of ash-free dry weight by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

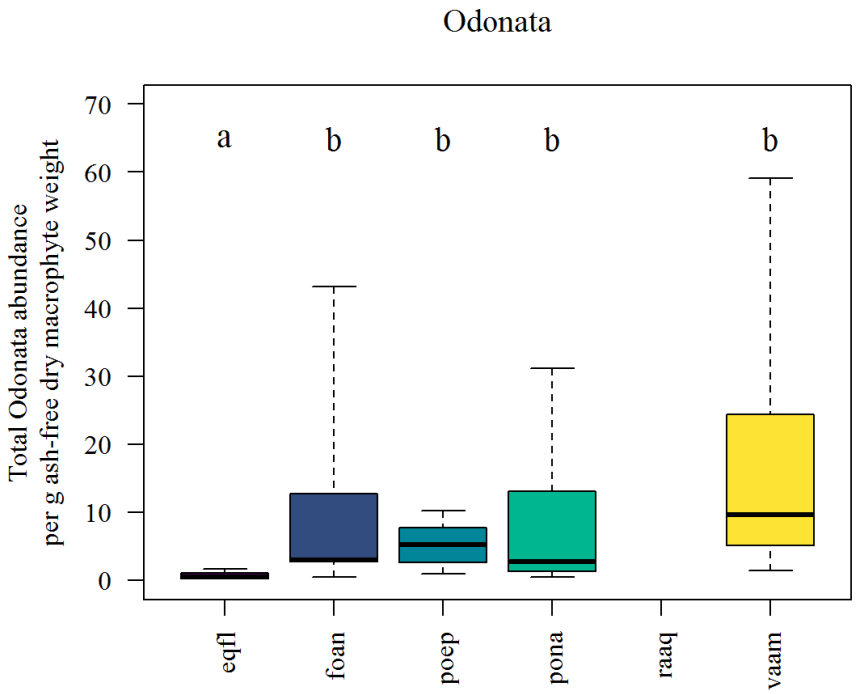


Figure 18: Boxplots comparing the number of Odonata per gram of ash-free dry weight by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

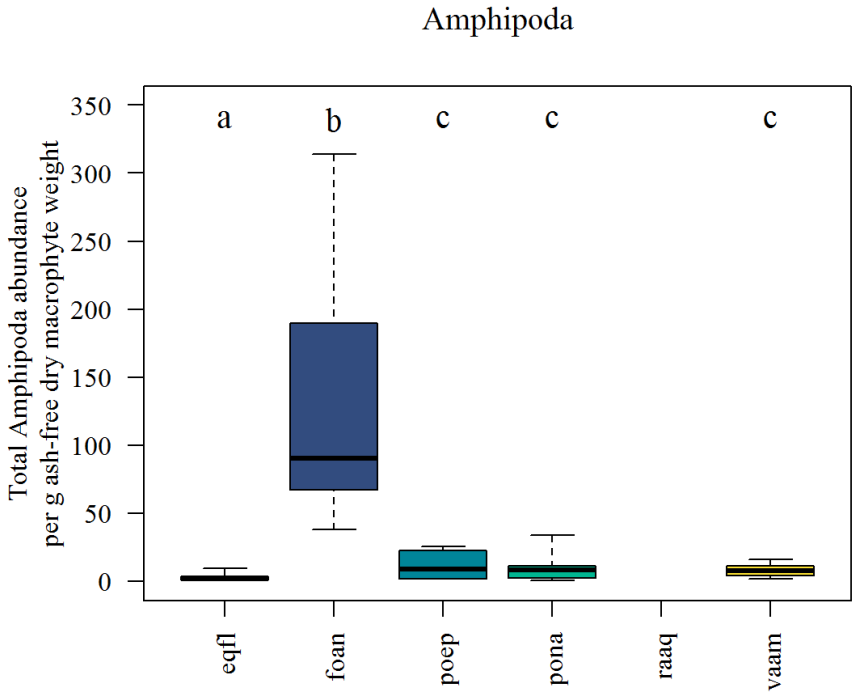


Figure 19: Boxplots comparing the number of Amphipoda per gram of ash-free dry weight by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

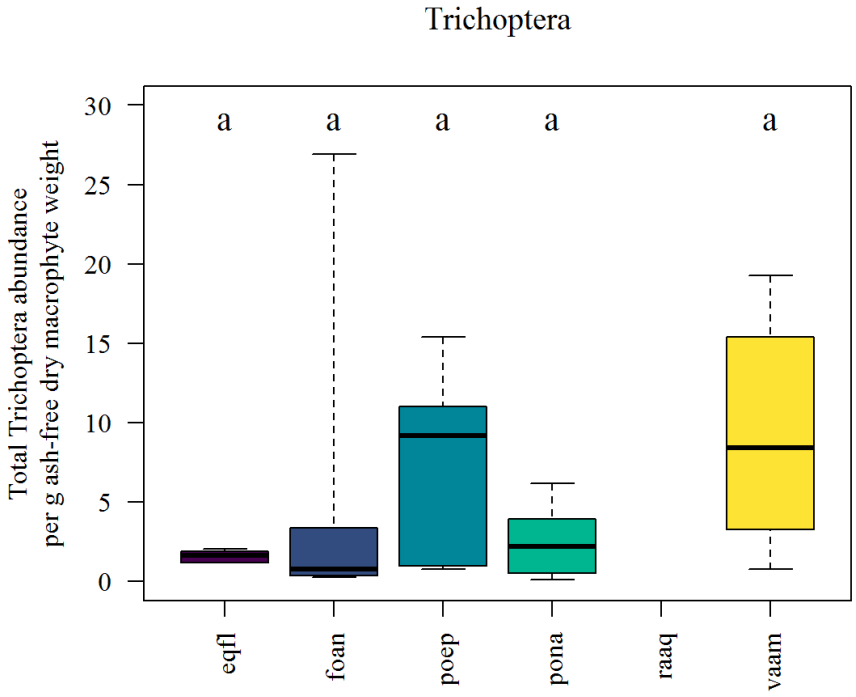


Figure 20: Boxplots comparing the number of Trichoptera per gram of ash-free dry weight by macrophyte type. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$). *Ranunculus aquatilis* was omitted due to small sample size.

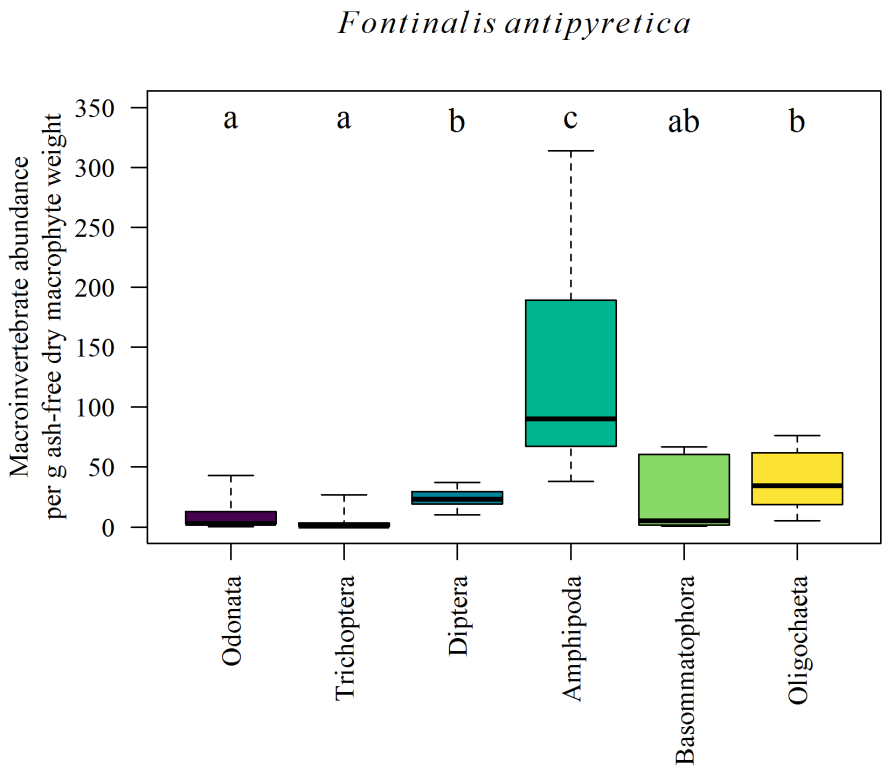


Figure 21: Number of macroinvertebrates per gram of ash-free dry weight of *Fontinalis antipyretica*. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$).

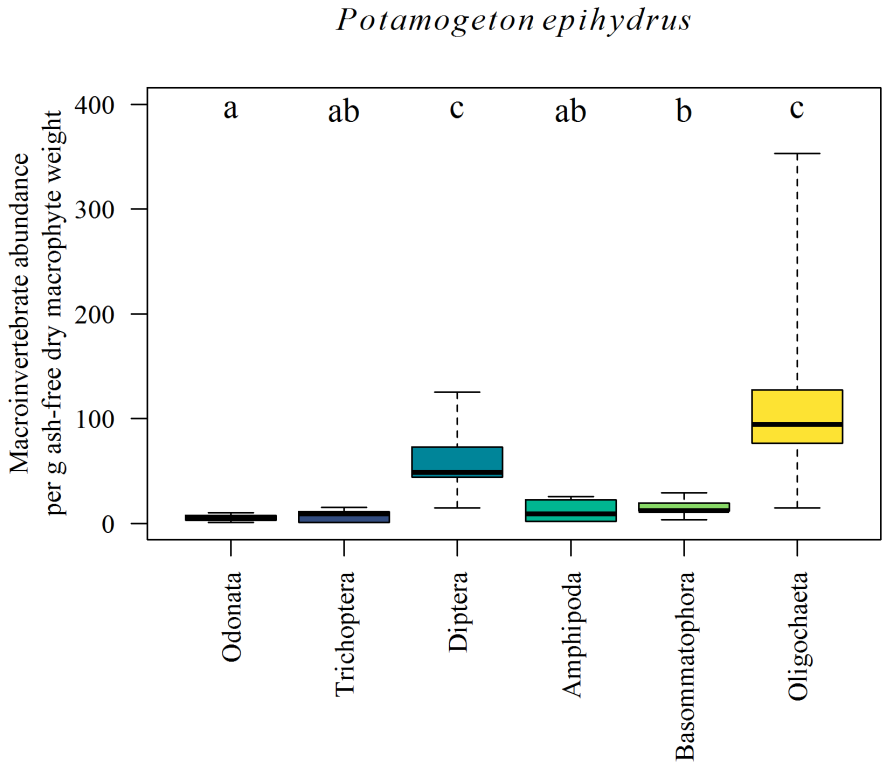


Figure 22: Number of macroinvertebrates per gram of ash-free dry weight of *Potamogeton epihydrus*. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$).

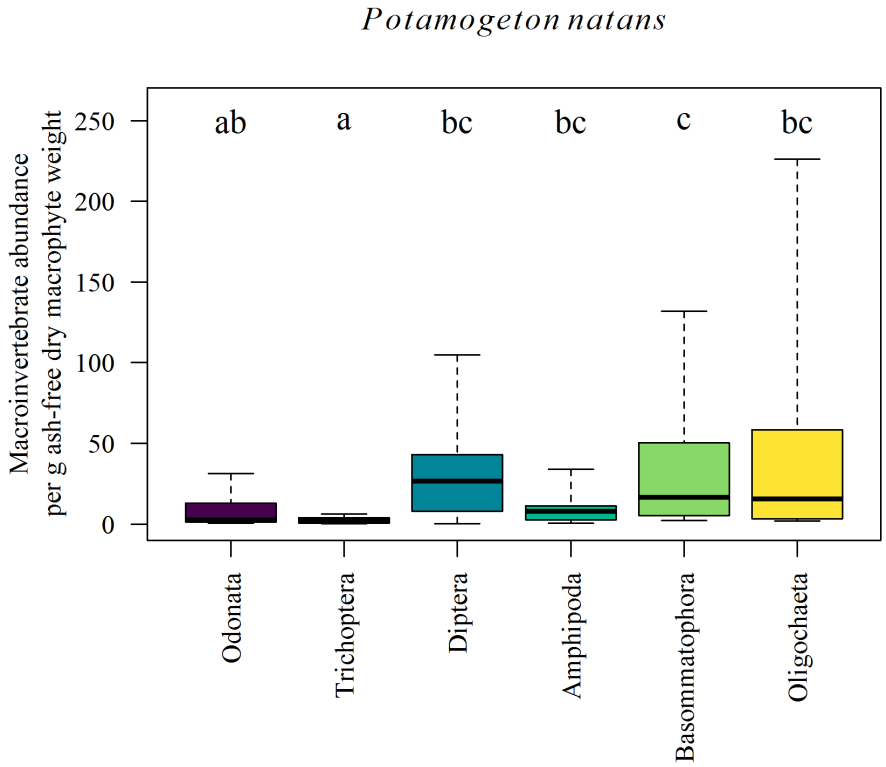


Figure 23: Number of macroinvertebrates per gram of ash-free dry weight of *Potamogeton natans*. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$).

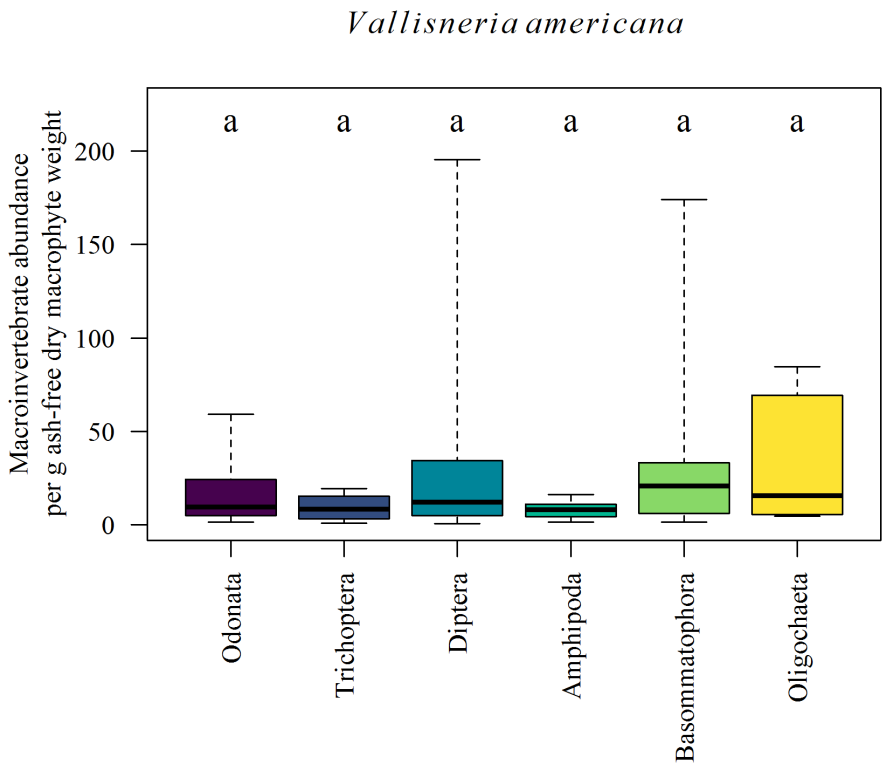


Figure 24: Number of macroinvertebrates per gram of ash-free dry weight of *Vallisneria americana*. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$).

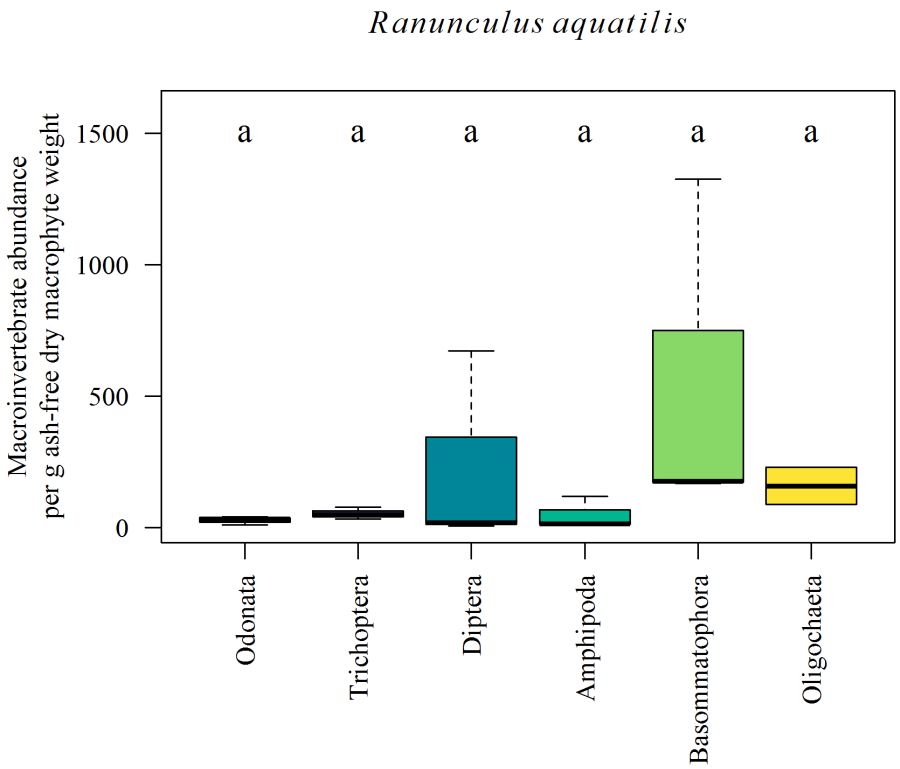


Figure 25: Number of macroinvertebrates per gram of ash-free dry weight of *Ranunculus aquatilis*. Only three total samples of *Ranunculus aquatilis* were available to compare. Pair-wise Wilcoxon rank-sum tests were used for comparisons which is sensitive to low sample numbers contributing to the lack of significant differences.

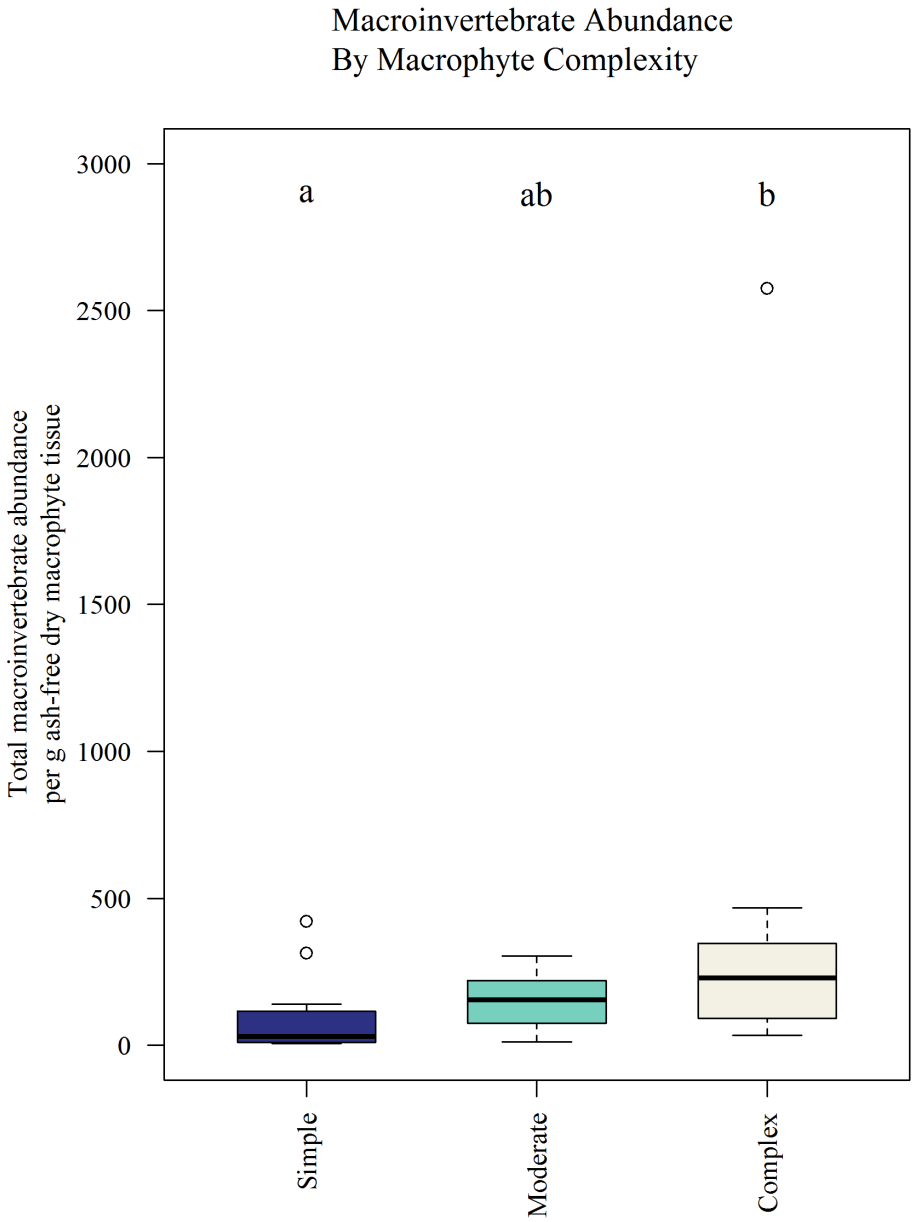


Figure 26: Total abundance of macroinvertebrates sorted by macrophyte complexity. Different letters at top indicate significantly different medians. Macrophyte complexity values are as follows: Simple = un-branched, Moderate = dichotomously dissected branching, Complex = complexly dissected branching. Significant differences detected with pair-wise Wilcoxon rank sum tests are indicated by different letters ($\alpha < 0.05$).

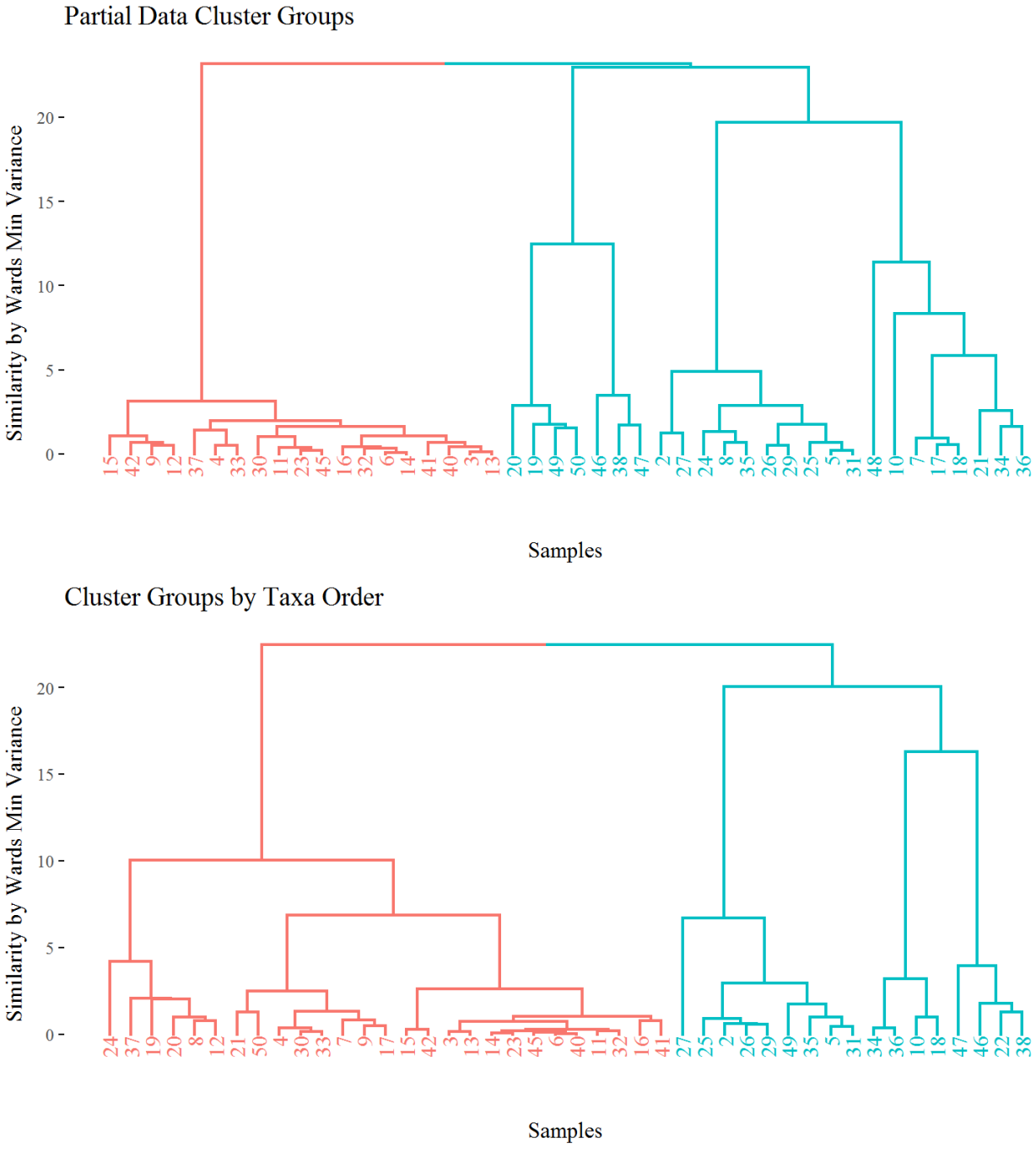


Figure 27: Hierarchical clustering analysis on the first three principal components derived from the partial and condensed macroinvertebrate datasets with leaf nodes labeled by sample number. The upper panel shows the clustering of the partial dataset, the lower panel the clustering of the condensed dataset.

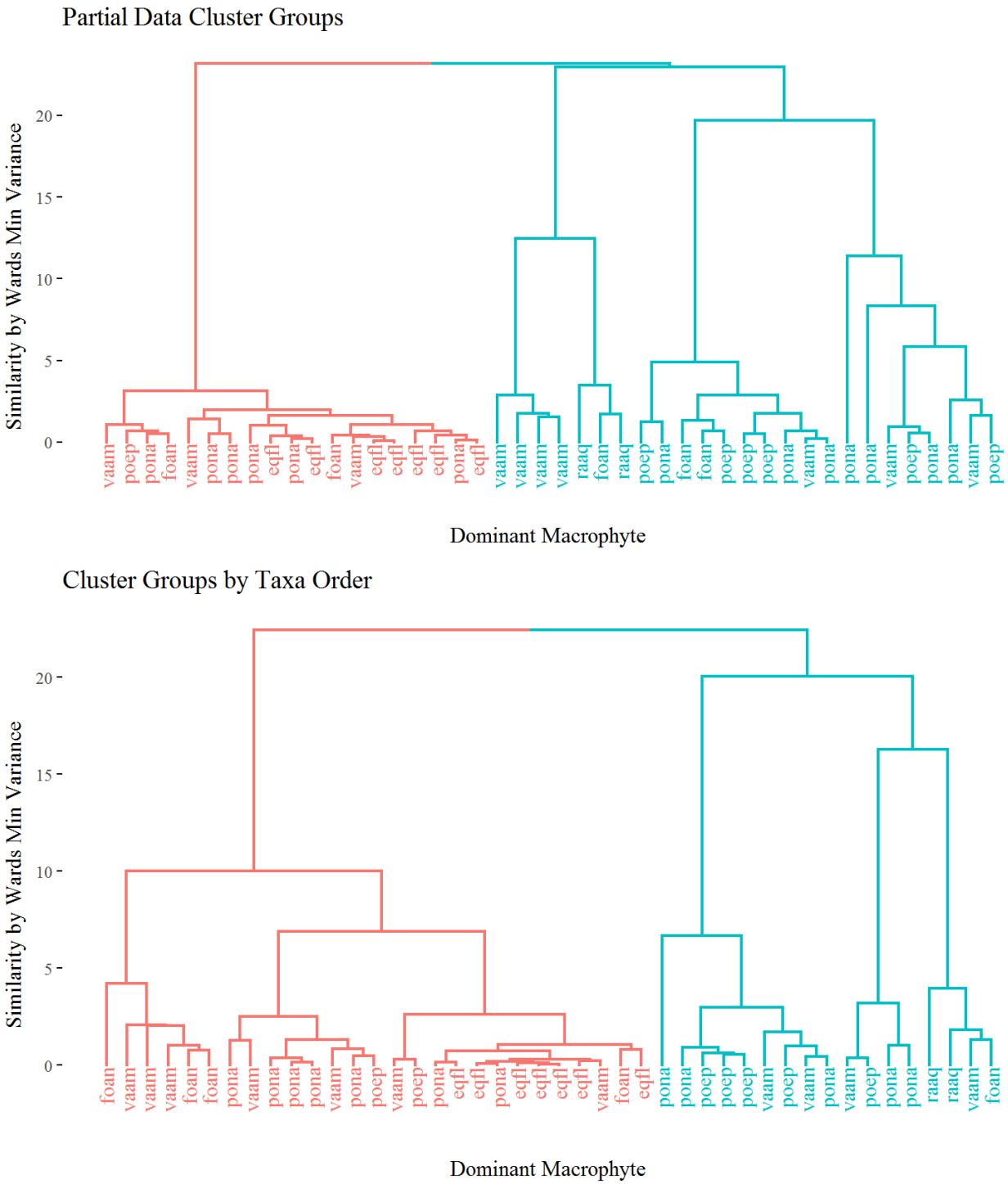


Figure 28: Hierarchical clustering analysis on the first three principal components derived from partial and condensed macroinvertebrate datasets with leaf nodes labeled by macrophyte type. The upper panel shows the clustering of the partial dataset, the lower panel the clustering of the condensed dataset.

Dominant macrophyte abbreviations are as follows:					
eqfl	-	*Equisetum fluviatile*	pona	-	*Potamogeton natans*
foan	-	*Fontinalis antipyretica*	raaq	-	*Ranunculus aquatilis*
poep	-	*Potamogeton epihydrus*	vaam	-	*Vallisneria americana*

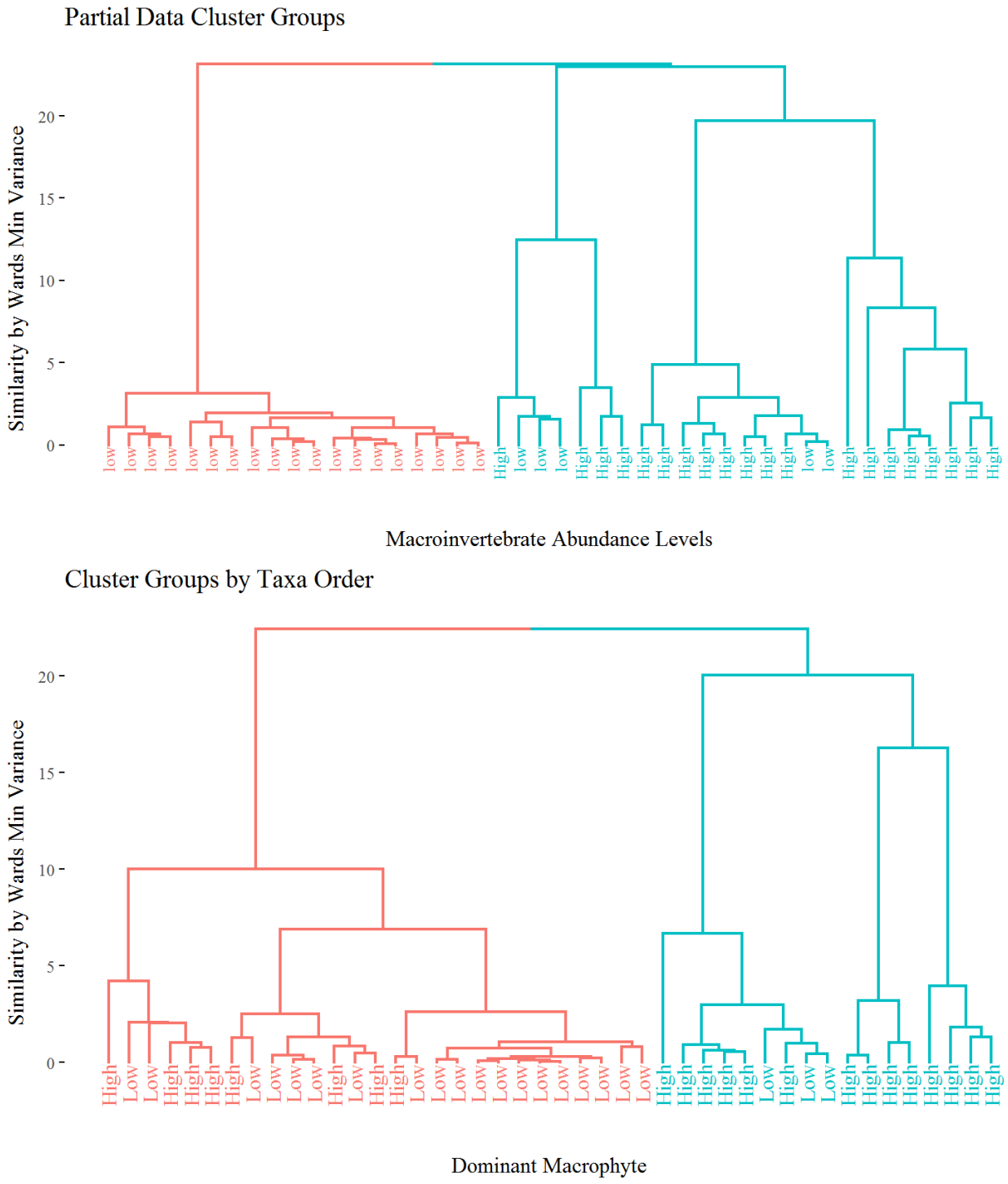


Figure 29: Hierarchical clustering analysis on the first three principal components derived from partial and condensed macroinvertebrate datasets with leaf nodes labeled by high or low sample abundance. The upper panel shows the clustering of the partial dataset, the lower panel the clustering of the condensed dataset.

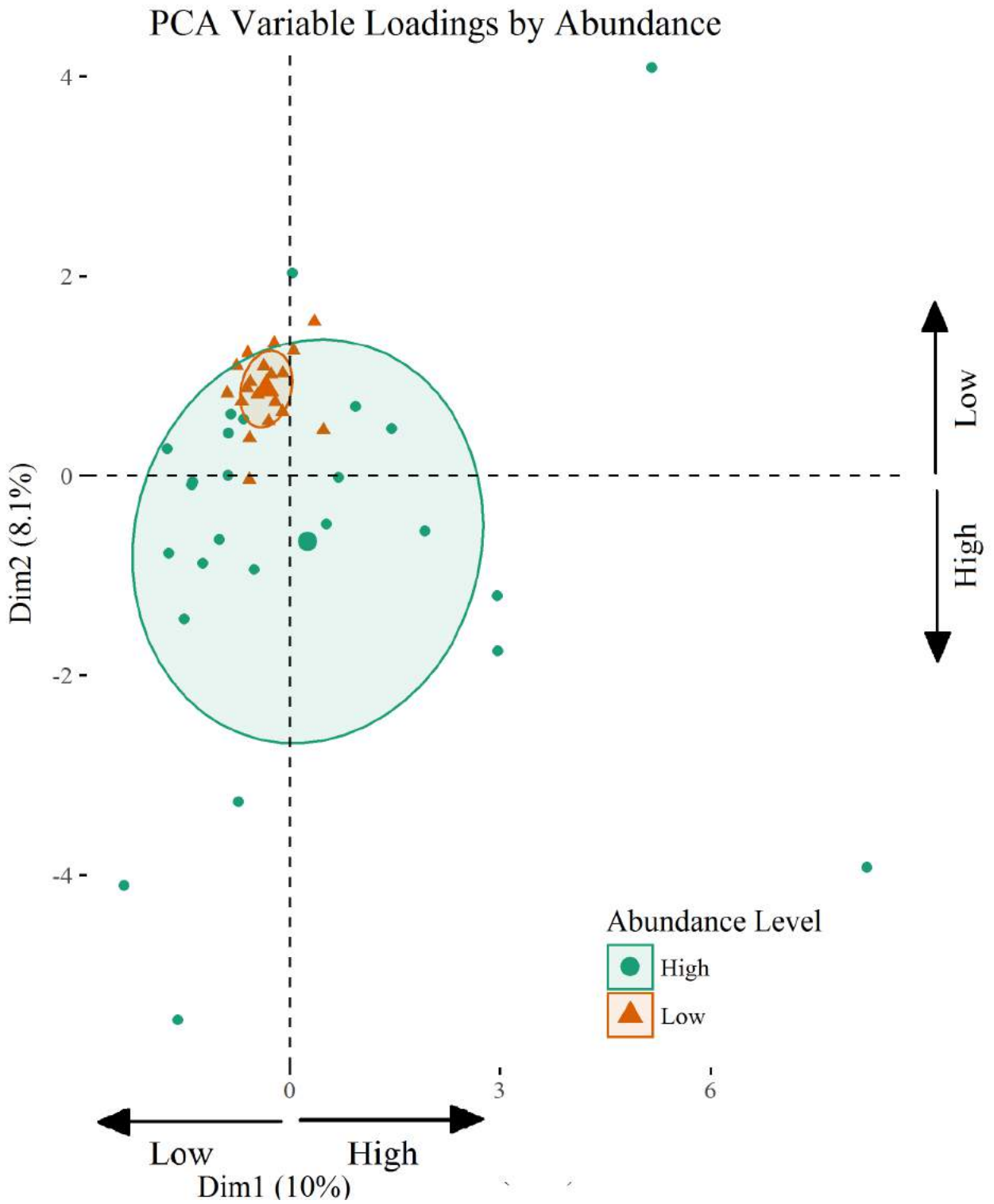


Figure 30: Principal component analysis macroinvertebrate abundance factor map based on condensed data. Macroinvertebrate abundance levels are clustered by group defined as high or low abundance on principal components one and two. The larger symbol of each group represents the centroid of each cluster group. The surrounding ellipse represents on third of each clusters distribution.

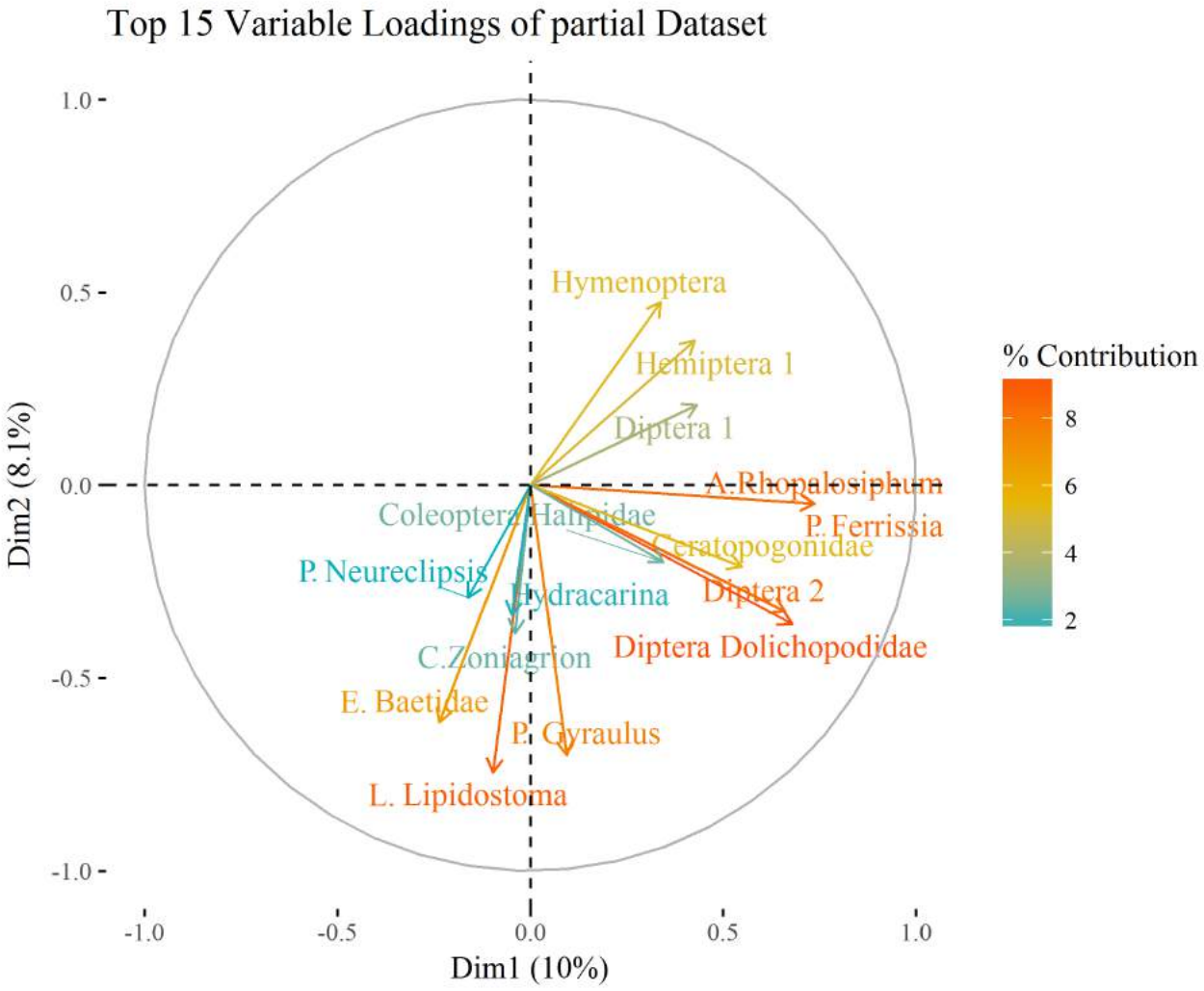


Figure 31: Principal component analysis variable loadings by individual taxa. Colors represent the relative proportion of variation that is accounted for in each dimension that is contributed by each taxon.

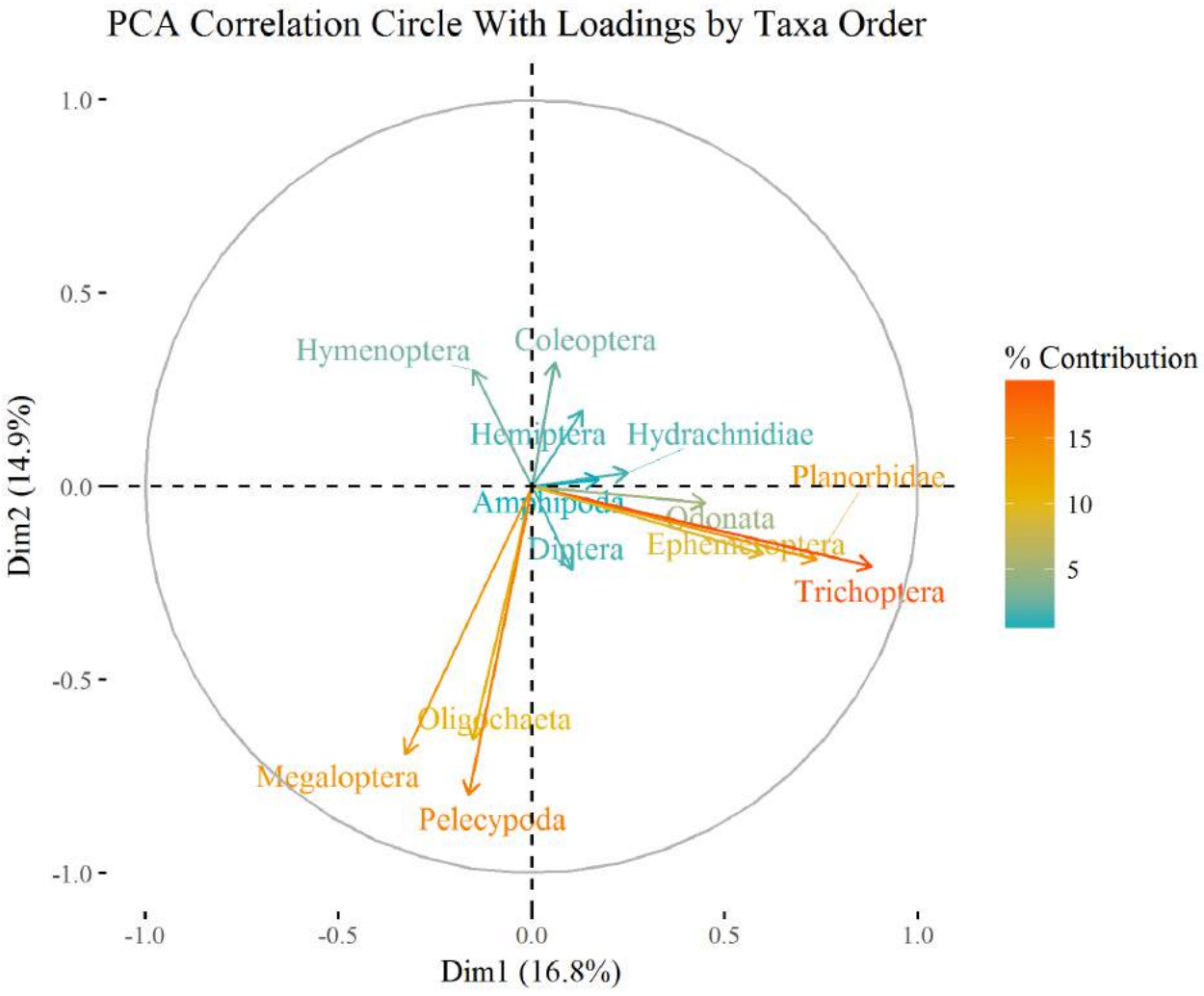


Figure 32: Principal component analysis variable loadings by condensed taxa order. Colors represent the relative proportion of variation that is accounted for in each dimension that is contributed by each taxon.

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7 Tables

Table 1: List of dominant macrophytes with the type and number of associated co-dominant macrophyte species found in the sample set. Samples taken from monoculture macrophyte communities are listed along with those without a co-dominant macrophyte. Co-dominant macrophytes represent the next most dominant macrophyte found in a sample. Sub-dominant macrophytes not included.

Dominant Macrophyte	Co-dominant Macrophyte	Number of Samples
Equisetum fluviatile	- - -	2
	Nuphar polysepala	4
	Potamogeton natans	1
Fontinalis antipyretica	- - -	2
	Sphagnum mosses	5
Potamogeton epihydrus	- - -	4
	Ranunculus aquatilis	3
Potamogeton natans	- - -	1
	Equisetum fluviatile	8
	Potamogeton epihydrus	2
	Ranunculus aquatilis	2
Ranunculus aquatilis	- - -	0
	Nuphar polysepala	2
	Vallisneria americana	1
Vallisneria americana	- - -	2
	Equisetum fluviatile	1
	Nuphar polysepala	1
	Potamogeton epihydrus	2
	Potamogeton natans	1
	Ranunculus aquatilis	4
Total		49

Table 2: Water quality depth profiles taken on 16 June, 2016 from the deepest point of Canyon Lake, WA

Depth (m)	**Temperature (°C)**	**Dissolved Oxygen (mg/L)**	**pH**	**Conductivity (μS/cm)**	**Alkalinity (mg-$CaCO_3$/L)**	**Turbidity (NTU)**
0	11.4	9.86	6.95	19.90	7.94	0.73
1	11.4	9.87	6.92	19.70	8.02	0.77
2	10.0	9.90	6.86	18.80	7.70	1.96
3	9.3	9.70	6.81	18.21	6.94	0.65
4	8.9	9.60	6.69	17.72	6.98	1.30
5	7.9	9.12	6.51	18.23	7.26	1.03
10	5.9	5.70	6.51	25.51	10.45	1.11
15	5.1	2.50	6.37	26.32	10.66	1.29

Table 3: Canyon Lake water quality data collected 1 August 2016 by the Institute for Watershed Studies.

Depth (m)	**Temperature (°C)**	**D.O.**[1] **(mg/L)**	**pH**	**Conductivity (µS/cm)**	**Total Phosphorus (µg-P/L)**
0.3	20.2	8.71	6.85	24.06	<5
5	9.7	5.43	6.21	24.04	6.5
10	6.0	2.80	6.17	31.91	12.7
15	5.3	0.11	6.04	34.11	10.4
17.5	5.0	0.12	6.19	75.64	14.6

Depth (m)	**Soluble Reactive Phosphate (µg-P/L)**	**Total Nitrogen (µg-N/L)**	**Nitrate+ Nitrite (µg-N/L)**	**Ammonium-N (µg-N/L)**	**Alkalinity (mg-$CaCO_3$/L)**
0.3	<3	63.1	<10	<10	9.6
5	<3	105.3	<10	15.3	9.2
10	<3	117.5	51.2	20.9	14.2
15	<3	198.3	<10	137.4	NA
17.5	<3	702.4	<10	629.3	15.2

[†]D.O. = Dissolved Oxygen

Table 4: Canyon Lake sample analysis summary and methods summary for water quality and macrophyte processing.

Location Analysis	**Parameter**	**Abbreviation**	**Method**	**Minimum Detection Limit**
Field	Temperature (°C)	Temp	YSI (2012)	
Field	Dissolved Oxygen (mg/L)	DO	YSI (2012)	
IWS	pH	pH	4500-H+	
IWS	Conductivity (µS/cm)	Cond	#2510	
IWS	Turbidity (NTU)	Turb	#2130	
IWS	Alkalinity (mg-$CaCO_3$/L)	Alk	#2320	
IWS	Chlorophyll (mg/L)	Chl	#10200-H	
IWS	Ammonium-N (µg-N/L)	NH_4	#4500-NH_4	>10 µg/L
IWS	Nitrate+Nitrite (µg-N/L)	NO_2+NO_3	#5400-NO_3-I	>10 µg/L
IWS	Total Nitrogen (µg-N/L)	TN	#4500-N-C	>30 µg/L
IWS	Soluble Reactive Phosphate (µg-P/L)	SRP	#4500-P-G	>3 µg/L
IWS	Total Phosphorus (µg-P/L)	TP	#4500-P-J	>5 µg/L

Analysis Location	**Macrophyte Processing**	**Abbreviation**	**Method**
IWS	Total Wet Macrophyte Weight (g)	WW	See Methods Section
IWS	Oven Dried Macrophyte Weight (g)	DW	#10300-C
IWS	Ash-free Dry Macrophyte Weight (g)	AFDW	#10300-D

IWS - Institute for Watershed Studies. All numbered methods can all be found in APHA (2012)

Table 5: Macrophyte drying ratios. The number of subsamples that were used to generate each mean is given by n.

Dominant Macrophyte	n	Mean WW to DW Coefficient	Mean DW to AFDW Coefficient
Equisetum fluviatile	21	0.1161	0.1373
Fontinalis antipyretica	24	0.0774	0.1655
Potamogeton epihydrus	33	0.1148	0.1292
Potamogeton natans	39	0.0801	0.1292
Ranunculus aquatilis	9	0.0527	0.1283
Vallisneria americana	21	0.1220	0.1421

Table 6: Secchi depth values collected from Canyon Lake in June and July, 2016

Secchi Depth
6 June - 4.0 m, 4.1 m
7 July - 4.6 m, 5.0 m, 4.0m
25 July - 5.0 m

Table 7: Macroinvertebrate species of class Insecta of Canyon Lake, WA 2016. Individuals/g ash-free dry weight are macroinvertebrate counts adjusted relative to ash-free dry macrophyte tissue.

Order	Family	Genus	Feeding Group	Individuals/g Ash-Free Dry Weight
Coleoptera	Chrysomelidae	*Donacia*	Shredders (herbivores)	35.74
	Noteridae	*Notomicrus*	Predators (engulfers)	1.37
	Lutrochidae†	*Lutrochus*	Shredders (detritivores)	
	Gyrinidae†		Predators (engulfers)	
	Haliplidae		Shredders (herbivores)	39.63
	Elmidae		Collectors/Gatherers	0.37
	1			0.82
Diptera	Chironomidae	1	Collector/Gatherers and Filterers	1835.19
	Chironomidae	2	Collector/Gatherers and Filterers	95.29
	Ceratopogonidae	-	Predators (engulfers)	46.35
	Dixidae	-	Collectors/Gatherers	6.49
	Dolichopodidae	-	Predator (piercers)	3.26
	1	-		0.37
	2	-		0.37
	3	-		1.89
Ephemeroptera	Baetidae	-	Collector/Gatherers	2.60
Ephemeroptera	Siphlonuridae	-	Collector/Gatherers	1.48
	(no appendix photo)	-		
Hemiptera	Aphididae	*Rhopalosiphum*	Piercers (herbivores)	418.81
Hemiptera	Gerridae	-	Predators (piercers)	0.21
Hemiptera	1 †	-		
Hemiptera	2	-		0.21
Hymenoptera	-	-	Parasites	7.48
Megaloptera	Sialidae	-	Predators (engulfers)	16.08
Megaloptera	1	-	Predators (engulfers)	3.11
Neuroptera	Sisyridae	-	Predators (piercers)	0.34
Odonata	Aeshnidae	*Aeshna*	Predators (engulfers)	34.22
Odonata	Corduliidae	*Cordulia*	Predators (engulfers)	45.56
Odonata	Coenagrionoidea	*Zoniagrion exclamationis*	Predators (engulfers)	433.70
Trichoptera	Lepidostomatidae	*Lepidostoma*	Shredders (detritivores)	177.91
	Polycentropodidae	*Polycentropus*	Predators (engulfers)	39.95
	Hydroptilidae	-	Piercers (herbivores)	71.78
	1	-		5.04
	2	-		0.93
	(no appendix photo)	-		

† indicates that taxa were found using a D-net but were not included in statistical analysis. Feeding groups were determined using Merritt and Cummins (1996) and Hawking et al. (2013).

Table 8: Macroinvertebrate species of class Arachnida, Crustacea, Gastropoda, Bivalvia, Oligochaeta and Hirudinea of Canyon Lake, WA 2016. Individuals/g ash-free dry weight are macroinvertebrate counts adjusted relative to ash-free dry macrophyte tissue.

Class	Order	Family	Genus	Feeding Group	Individuals/g Ash-Free Dry Weight
Malacostraca					
	Amphipoda	-	-	Collector/Gatherers and Filterers	1363.45
Gastropoda					
	Basommatophora	*Ancylidae*	*Ferrissia*	Scrapers	449.92
		Planorbidae	*Gyraulus*	Scrapers	2207.48
Bivalvia					
	Veneroida	Pisidiidae	*Pisidium*	Filter-Feeders	203.39
Clitellata					
	Opisthopora	Lumbricidae	-	Filter-Feeders	1988.70
	Hirudinida	Hirudinidae	*Hirudo*	Predators (piercers)	8.10
Arachnida					
	Trombidiformes	Hydrachnidae	-	Predators (piercers)	163.38

† indicates that taxa was found using a D-net and not included in statistical analysis.
Feeding groups were determined using Merritt and Cummins (1996) and Hawking et al. (2013).

Table 9: Median, minimum and maximum number of macroinvertebrates per gram ash free dry macrophyte weight. Data grouped by macroinvertebrate community metric and then by the six dominant macrophytes. *Ranunculus aquatilis* was omitted from statistical analysis due to small sample size (3) which caused issues with pair-wise Wilcoxon rank sum tests. Abundance refers to abundance of macroinvertebrates per gram of ash-free dry macrophyte weight. Median values within a row followed by mutually exclusive letters are significantly different as indicated by a pair-wise Wilcoxon rank sum test with Holmes error correction. EMG = Emergent Insects.

Metric	*Equisetum fluviatile*	*Fontinalis antipyretica*	*Potamogeton epihydrus*	*Potamogeton natans*	*Ranunculus aquatilis*	*Vallisneria americana*
Abundance	9.78 A	196.00 B	187.00 B	84.70 B	356.00	91.80 B
(min-max)	(5.21-36.21)	(68.92-468.02)	(32.83-421.74)	(11.59-294.88)	(262.83-2575.3)	(6.78-312.61)
EMG	4.44 A	28.80 BC	57.30 B	25.20 C	77.60	47.60 BC
(min-max)	(0.25-20.44)	(19.11-84.01)	(15.76-148.16)	(1.1-132.16)	(76.74-782.76)	(4.75-219.61)
All Insect	6.52 A	30.20 B	68.30 B	52.80 B	82.80	51.30 B
(min-max)	(1-20.72)	(19.11-89.62)	(17.07-219.36)	(4.39-152.17)	(77.6-902.35)	(6.78-264.74)
Non-Insect	5.99 A	169.00 B	110.00 BC	34.10 C	274.00	39.10 C
(min-max)	(2.12-15.49)	(45.18-421.05)	(15.76-372.33)	(2.45-231.02)	(185.23-1672.96)	(0-275.96)
Predators	1.01 A	14.20 B	6.38 B	7.17 B	39.00	11.10 B
(min-max)	(0-2.92)	(5.84-66.09)	(0-11.78)	(0.85-39.37)	(12.12-42.55)	(3.84-59.13)
Odonata	0.19 A	5.49 B	3.30 B	3.72 B	32.60	9.61 B
(min-max)	(0-1.52)	(1.43-43.69)	(0-10.21)	(0-35.65)	(9.09-40.05)	(0.77-59.13)
Trichoptera	1.19 A	0.37 A	0.88 A	1.54 A	49.5	4.67 A
(min-max)	(0-2.04)	(0-26.89)	(0-15.39)	(0-6.16)	(32.54-78.02)	(0-19.26)
Diptera	3.09 A	23.30 B	46.40 C	12.50 AB	17.20 AB	6.42 ABC
(min-max)	(0-18.79)	(9.95-36.97)	(14.45-125.07)	(0-104.98)	(5.01-672.12)	(0-195.55)
Amphipoda	1.53 A	90.4 B	3.32 C	7.94 C	15.00	2.74 C
(min-max)	(0.41-9.72)	(38.29-313.72)	(0-25.37)	(0-34.14)	(8.08-117.67)	(0-16.14)
Oligochaeta	0.25 A	24.60 B	84.40 C	3.10 B	86.80	6.02 B
(min-max)	(0-1.36)	(0-76.11)	(0-352.92)	(0-226)	(0-229.58)	(0-84.74)
Basommatophora	2.44 A	2.56 AB	12.10 B	13.20 B	176.00	19.20 B
(min-max)	(1.16-5.13)	(0-66.65)	(0-29.28)	(0-131.98)	(167.71-1325.7)	(0-174.05)

Table 10: Non-parametric correlations between macroinvertebrates grouped by selected functional and taxonomic characteristics. EMG - emergent Insects, Complexity implies the structural complexity of macrophytes from which macroinvertebrates were collected: simple, simple dissected, or complexly dissected macrophyte. Kendall's τ values less than 0.30 were not included. p-values < 0.01 for all displayed correlations.

	Filter-feeders	Collector-Gatherers	Piercers	Predators	Scrapers	Shredders	Complexity	EMG	Abundance	Richness	All Insect	Non-Insect	Odonata	Trichoptera	Diptera	Amphipoda	Basommatophora	Oligochaeta
Collector-Gatherers	0.36																	
Piercers	∼	∼																
Predators	∼	0.31	∼															
Scrapers	∼	∼	∼	∼														
Shredders	∼	∼	0.33	∼	0.42													
Complexity	∼	0.40	∼	0.39	∼	∼												
EMG	0.30	0.58	∼	0.50	0.37	0.38	∼											
Abundance	0.56	0.59	∼	0.41	0.46	∼	0.35	0.58										
Richness	∼	0.43	0.35	∼	∼	∼	∼	0.32	∼									
Insects	0.31	0.55	∼	0.44	0.41	0.40	∼	0.84	0.61	0.40								
Non-Insects	0.60	0.52	∼	0.38	0.46	∼	0.38	0.41	0.81	∼	0.42							
Odonata	∼	∼	∼	0.74	∼	0.31	∼	0.45	∼	∼	0.39	∼						
Trichoptera	∼	∼	∼	0.32	0.37	0.71	∼	0.46	∼	∼	0.44	∼	0.36					
Diptera	0.37	0.68	∼	∼	∼	∼	∼	0.64	0.49	0.39	0.63	0.36	∼	∼				
Amphipoda	∼	0.56	∼	∼	∼	∼	0.38	∼	0.35	0.42	0.48	∼	0.35	∼	∼			
Basommatophora	∼	∼	∼	∼	0.97	0.42	∼	0.37	0.48	∼	0.42	0.48	∼	0.35	∼	∼		
Oligochaeta	0.94	0.33	∼	∼	∼	∼	∼	∼	0.55	∼	∼	0.58	∼	∼	0.34	∼	∼	

Table 11: The relative variable loadings of the top six predictor variables on each of the first three principal components (PC1, PC2, and PC3) of the partial dataset and condensed dataset followed by the proportion of variance accounted for by each principal component.

Partial Dataset		**Condensed Dataset**	
Variable Loadings	PC1	Variable Loadings	PC1
Ancylidae *Ferrissia*	0.394	Trichoptera	0.589
Aphididae *Rhopalosiphum*	0.394	Basomatophora	0.493
Diptera 6	0.363	Odonata	0.352
Ephemoroptera Baetidae	-0.127	Hemiptera	-0.063
Corduliidae *Cordulia*	-0.135	Megaloptera	-0.163
Pelecypoda	-0.156	Hymenoptera	-0.163
Proportion of Variance	10.0%	Proportion of Variance	16.8%
Variable Loadings	PC2	Variable Loadings	PC2
Hymenoptera	0.281	Hymenoptera	0.230
Hemiptera 1	0.223	Coleoptera	0.211
Ephemoroptera Siphlonuridae	0.135	Hemiptera	0.209
Ephemoroptera Baetidae	-0.366	Oligochaeta	-0.479
Planorbidae *Gyraulus*	-0.415	Megaloptera	-0.524
Lepidostomatidae *Lepidostoma*	-0.442	Pelecypoda	-0.577
Proportion of Variance	8.1%	Proportion of Variance	15.0%
Variable Loadings	PC3	Variable Loadings	PC3
Coenagrionidae *Zoniagrion*	0.355	Hemiptera	0.521
Trichoptera 2	0.293	Diptera	0.453
Chrysomelidae *Donacia*	0.285	Hymenoptera	0.240
Pisidiidae	-0.283	Amphipoda	-0.281
Oligochaeta Lumbricidae	-0.319	Coleoptera	-0.360
Chironomidae	-0.358	Hydracarina	-0.421
Proportion of Variance	7.6%	Proportion of Variance	13.6%

8 Appendix A - Data and Correlation Tables

Table 1: Correlation matrix of statistically significant Kendall's τ rank-based correlations between estimates of macroinvertebrate per gram ash-free dry weight based the complete dataset. The p-values associated with each significant Kendall's τ correlation statistic are indicated using asterisks (*<0.05, **<0.01, ***<0.001).

	Donacia	*Notomicrus*	Halipidae	Elmidae	Coleoptera 1
Donacia	∼				
Notomicrus		∼			
Halipidae			∼		
Elmidae				∼	
Coleoptera 1					∼
Chironomidae 1					
Chironomidae 2					
Ceratopogonidae					
Dixidae				0.465**	
Dolichopodidae				0.711***	
Diptera 1				1***	
Diptera 2				1***	
Diptera 3			0.294*		
Baetidae				0.534***	
Siphlonuridae					
Rhopalosiphum					
Gerridae					
Hemiptera 1					
Hymenoptera				0.336*	
Sialidae					
Megaloptera 1					
Sisyridae					
Aeshna					
Cordulia				0.274*	
Zoniagrion					
Lipidostoma					
Polycentropus	0.258*				
Hydroptilidae					
Trichoptera 1					
Trichoptera 2			0.319*		
Amphipoda					
Ancylidae *Ferrissia*	0.297*				
Gyraulus					
Pisidium					
Lumbricidae					
Hirudo	0.385**				
Hydrachnidia					

Table 1: Continued

	Chironomidae 1	Chironomidae 2	Ceratopogonidae	Dixidae	Dolichopodidae
Donacia					
Notomicrus					
Halipidae					
Elmidae					
Coleoptera 1					
Chironomidae 1	∼				
Chironomidae 2	0.349**	∼			
Ceratopogonidae			∼		
Dixidae				∼	
Dolichopodidae			0.322*	0.307*	∼
Diptera 1				0.465**	0.711***
Diptera 2				0.465**	0.711***
Diptera 3					0.38**
Baetidae					0.362*
Siphlonuridae					
Rhopalosiphum					
Gerridae					
Hemiptera 1					
Hymenoptera				0.298*	
Sialidae	0.284*		0.264*		
Megaloptera 1					
Sisyridae					
Aeshna					
Cordulia					
Zoniagrion		0.279**			
Lipidostoma					
Polycentropus	0.229*				
Hydroptilidae	0.377**				
Trichoptera 1			0.406**		
Trichoptera 2					
Amphipoda	0.231*				
Ancylidae *Ferrissia*					
Gyraulus	0.374***	0.23*			
Pisidium	0.29*				
Lumbricidae	0.304**				
Hirudo					
Hydrachnidia					

Table 1: Continued

	Diptera 1	Diptera 2	Diptera 3	Baetidae	Siphlonuridae	*Rhopalosiphum*
Donacia						
Notomicrus						
Halipidae						
Elmidae						
Coleoptera 1						
Chironomidae 1						
Chironomidae 2						
Ceratopogonidae						
Dixidae						
Dolichopodidae						
Diptera 1	∼					
Diptera 2	1***	∼				
Diptera 3			∼			
Baetidae	0.534***	0.534***		∼		
Siphlonuridae					∼	
Rhopalosiphum						∼
Gerridae					0.534***	
Hemiptera 1					0.534***	
Hymenoptera	0.336*	0.336*			0.342*	
Sialidae						
Megaloptera 1						
Sisyridae						
Aeshna						
Cordulia	0.274*	0.274*		0.312*		
Zoniagrion						
Lipidostoma						0.284*
Polycentropus						
Hydroptilidae						
Trichoptera 1						
Trichoptera 2						
Amphipoda						
Ancylidae *Ferrissia*						0.31**
Gyraulus						
Pisidium						
Lumbricidae						
Hirudo			0.465***			
Hydrachnidia						

Table 1: Continued

	Gerridae	Hemiptera 1	Hymenoptera	Sialidae	Megaloptera 1	Sisyridae
Donacia						
Notomicrus						
Halipidae						
Elmidae						
Coleoptera 1						
Chironomidae 1						
Chironomidae 2						
Ceratopogonidae						
Dixidae						
Dolichopodidae						
Diptera 1						
Diptera 2						
Diptera 3						
Baetidae						
Siphlonuridae						
Rhopalosiphum						
Gerridae	∼					
Hemiptera 1	1***	∼				
Hymenoptera	0.368**	0.368**	∼			
Sialidae				∼		
Megaloptera 1					∼	
Sisyridae						∼
Aeshna						
Cordulia						
Zoniagrion			-0.279*			
Lipidostoma						
Polycentropus						
Hydroptilidae						
Trichoptera 1						
Trichoptera 2						
Amphipoda				0.271*		
Ancylidae *Ferrissia*						
Gyraulus			-0.245*	0.237*		
Pisidium				0.603***		
Lumbricidae				0.241*		
Hirudo						
Hydrachnidia						

Table 1: Continued

	Aeshna	*Cordulia*	*Zoniagrion*	*Lipidostoma*	*Polycentropus*	Hydroptilidae
Donacia						
Notomicrus						
Halipidae						
Elmidae						
Coleoptera 1						
Chironomidae 1						
Chironomidae 2						
Ceratopogonidae						
Dixidae						
Dolichopodidae						
Diptera 1						
Diptera 2						
Diptera 3						
Baetidae						
Siphlonuridae						
Rhopalosiphum						
Gerridae						
Hemiptera 1						
Hymenoptera						
Sialidae						
Megaloptera 1						
Sisyridae						
Aeshna	~					
Cordulia		~				
Zoniagrion			~			
Lipidostoma			0.299**	~		
Polycentropus			0.339**	0.296*	~	
Hydroptilidae			0.267*	0.406***	0.288*	~
Trichoptera 1						
Trichoptera 2						
Amphipoda	0.229*					0.288*
Ancylidae *Ferrissia*				0.219*		
Gyraulus	-0.246*		0.44***	0.35**		
Pisidium						
Lumbricidae						
Hirudo				0.261*		
Hydrachnidia						

Table 1: Continued

	Trichoptera 1	Trichoptera 2	Amphipoda	*Ferrissia*	*Gyraulus*	*Pisidium*
Donacia						
Notomicrus						
Halipidae						
Elmidae						
Coleoptera 1						
Chironomidae 1						
Chironomidae 2						
Ceratopogonidae						
Dixidae						
Dolichopodidae						
Diptera 1						
Diptera 2						
Diptera 3						
Baetidae						
Siphlonuridae						
Rhopalosiphum						
Gerridae						
Hemiptera 1						
Hymenoptera						
Sialidae						
Megaloptera 1						
Sisyridae						
Aeshna						
Cordulia						
Zoniagrion						
Lipidostoma						
Polycentropus						
Hydroptilidae						
Trichoptera 1	∼					
Trichoptera 2		∼				
Amphipoda			∼			
Ancylidae *Ferrissia*				∼		
Gyraulus					∼	
Pisidium			0.262*		0.282*	∼
Lumbricidae					0.297**	0.401***
Hirudo		0.418**		0.249*		
Hydrachnidia					0.291	

No further correlations were found between Lumbricidae, *Hirudo*, or Hydrachnidia

Table 2: Mean number of macroinvertebrates per gram ash-free dry macrophyte tissue based on the complete dataset.

Macroinvertebrate Taxon	*Equisetum fluviatile*	*Fontinalis antipyretica*	*Potamogeton epihydrus*	*Potamogeton natans*	*Ranunculus aquatilis*	*Vallisneria americana*
Chrysomelidae *Donacia*	0.02	0.37	1.21	1	0.43	0.93
Noteridae *Notomicrus*	0	0.2	0	0	0	0
Coleoptera Elmidae	0	0.05	0	0	0	0
Coleoptera Halipidae	0.27	0	0.11	0.02	3.96	2.26
Coleoptera 1	0.12	0	0	0	0	0
Chironomidae Brachycera	0.19	1.46	3.64	1.32	5.76	2.16
Diptera Ceratopogonidae	0.51	1.98	0.55	1.4	0.64	0.46
Diptera Chironomidae	3.93	19.12	54.68	21.84	224.4	30.36
Diptera Dixidae	0	0.37	0.22	0	0	0.21
Diptera Dolichopodidae	0	0.42	0	0.03	0	0
Diptera 1	0	0.05	0	0	0	0
Diptera 2	0	0.05	0	0	0	0
Diptera 3	0.04	0	0	0.08	0.21	0
Ephemeroptera Baetidae	0	0.23	0	0	0.34	0
Ephemeroptera Siphlonuridae	0	0.14	0	0.04	0	0
Aphididae Rhopalosiphum	1.57	1.7	10.45	20.5	0	5.11
Hemiptera Gerridae	0	0	0	0.02	0	0
Hemiptera 1	0	0	0	0.02	0	0
Hymenoptera	0.04	0.21	0.19	0.22	0	0.14
Megaloptera Sialidae	0.01	0.53	0.25	0.51	0.21	0.29
Megaloptera 1	0	0	0	0	0	0.28
Neuroptera Sisyridae	0	0	0	0.03	0	0
Aeshnidae *Aeshna*	0.03	1.87	0	1.42	0	0.22
Corduliidae *Cordulia*	0.02	5.24	0.98	0.04	0	0.12
Coenagrionoidea *Zoniagrion*	0.32	5.6	3.42	7.24	27.25	17.5
Lepidostomatidae *Lepidostoma*	0.71	2.83	2.54	1.34	29.56	2.66
Polycentropodidae *Neureclipsis*	0.06	1.23	1.8	0.21	2.77	0.67
Hydroptilidae	0	0.09	0.6	0.25	17.27	1.08
Trichoptera 1	0.08	0.32	0.27	0.03	0	0
Trichoptera 2	0	0	0	0	0	0.08
Amphipoda	3.02	136.5	9.95	9.21	46.92	5.15
Planorbidae *Gyraulus*	0.26	19.72	11.69	10.13	552.53	17.87
Ancylidae *Ferrissia*	2.54	0.24	0.9	20.74	3.84	13
Pisidiidae *Pisidium*	0.01	1.21	4.2	2.79	41.46	0.44
Oligochaeta Lumbricidae	0.36	0.33	108.53	32.04	104.19	24.22
Hirudinidae *Hirudo*	0	0	0	0.05	1.28	0.33
Hydrachnidia	0.17	12.38	1.7	1.99	1.84	2.93

Table 3: Mean number of macroinvertebrates per gram dry macrophyte tissue based the complete dataset.

Macroinvertebrate Taxon	*Equisetum fluviatile*	*Fontinalis antipyretica*	*Potamogeton epihydrus*	*Potamogeton natans*	*Ranunculus aquatilis*	*Vallisneria americana*
Chrysomelidae *Donacia*	0	0.05	0.2	0.11	0.07	0.1
Noteridae *Notomicrus*	0	0.02	0	0	0	0
Coleoptera Elmidae	0	0.01	0	0	0	0
Coleoptera Halipidae	0.03	0	0.02	0	0.66	0.26
Coleoptera 1	0.01	0	0	0	0	0
Chironomidae Brachycera	0.03	0.16	0.59	0.16	0.96	0.26
Diptera Ceratopogonidae	0.07	0.23	0.09	0.17	0.11	0.05
Diptera Chironomidae	0.65	2.43	9.35	2.96	37.47	3.57
Diptera Dixidae	0	0.06	0.05	0	0	0.03
Diptera Dolichopodidae	0	0.04	0	0	0	0
Diptera 1	0	0.01	0	0	0	0
Diptera 2	0	0.01	0	0	0	0
Diptera 3	0	0	0	0.01	0.04	0
Ephemeroptera Baetidae	0	0.02	0	0	0.05	0
Ephemeroptera Siphlonuridae	0	0.02	0	0	0	0
Aphididae Rhopalosiphum	0.2	0.25	1.81	2.25	0	0.63
Hemiptera Gerridae	0	0	0	0	0	0
Hemiptera 1	0	0	0	0	0	0
Hymenoptera	0.01	0.02	0.03	0.03	0	0.02
Megaloptera Sialidae	0	0.08	0.04	0.11	0.04	0.04
Megaloptera 1	0	0	0	0	0	0.04
Neuroptera Sisyridae	0	0	0	0	0	0
Aeshnidae *Aeshna*	0	0.23	0	0.14	0	0.02
Corduliidae *Cordulia*	0	0.56	0.13	0	0	0.02
Coenagrionoidea *Zoniagrion*	0.05	0.81	0.55	0.86	3.61	2.23
Lepidostomatidae *Lepidostoma*	0.11	0.28	0.46	0.14	4.08	0.31
Polycentropodidae *Neureclipsis*	0.01	0.14	0.36	0.03	0.46	0.08
Hydroptilidae	0	0.01	0.1	0.03	2.89	0.13
Trichoptera 1	0.01	0.03	0.05	0	0	0
Trichoptera 2	0	0	0	0	0	0.01
Amphipoda	0.47	17.14	1.69	1.01	7.5	0.6
Planorbidae *Gyraulus*	0.03	2.44	1.94	1.16	88.32	2.2
Ancylidae *Ferrissia*	0.38	0.04	0.15	2.34	0.64	1.56
Pisidiidae *Pisidium*	0	0.13	0.72	0.47	6.94	0.06
Oligochaeta Lumbricidae	0.06	3.91	17.19	6.14	17.26	2.97
Hirudinidae *Hirudo*	0	0	0	0.01	0.21	0.04
Hydrachnidia	0.02	1.33	0.25	0.23	0.24	0.34

9 Appendix B - Macrophyte and Macroinvertebrate Photos

The following macrophyte and macroinvertebrate taxa were found in Canyon Lake in association with the macrophytes described in the Macrophyte Distribution and Community Composition section. The macroinvertebrate taxa were collected with a macrophyte rake and macroinvertebrate taxa were collected along with macrophytes with a macrophyte rake or with a D-net according to the methods described in the section Macroinvertebrate Collection.

c)

d)

e)

Figure 33: *Equisetum fluviatile* of Canyon Lake, WA, 2016.

a)

b)

c)

Figure 34: The top two panels are photos of *Fontinalis antipyretica* and the lower panel is an unknown genera of *Sphagnum* moss of Canyon Lake, WA, 2016.

a)

b)

c)

Figure 35: *Potamogeton epihydrus* of Canyon Lake, WA, 2016.

a)

b)

c)

Figure 36: *Potamogeton natans* of Canyon Lake, WA, 2016.

a)

b)

c)

Figure 37: *Ranunculus aquatilis* of Canyon Lake, WA, 2016.

a)

b)

c)

Figure 38: The top two panels are photos of *Vallisneria americana* and the lower panel is an less common of *Sparganium angustifolium* of Canyon Lake, WA, 2016.

a)

b)

Figure 39: Coleoptera (Chrysomelidae) *Donacia* of Canyon Lake, WA, 2016. Commonly known as aquatic leaf beetles. A - Adult, B - Larvae. Photos take from sample 45.

a)

b)

Figure 40: Coleoptera (Noteridae) *Notomicrus* of Canyon Lake, WA, 2016. Commonly known as burrowing water beetle. Photos take from sample 24.

a)

b)

Figure 41: Coleoptera (Lutrochidae) *Lutrochus* of Canyon Lake, WA, 2016. Photos taken from sample 12.

a)

b)

Figure 42: Coleoptera (Gyrinidae) of Canyon Lake, WA, 2016. Commonly known as whirligig beetle.

a)

b)

Figure 43: Coleoptera (Haliplidae) of Canyon Lake, WA, 2016.

a)

b)

Figure 44: Coleoptera (Elmidae) of Canyon Lake, WA, 2016.

a)

b)

Figure 45: Coleoptera # 1 of Canyon Lake, WA, 2016.

c)

d)

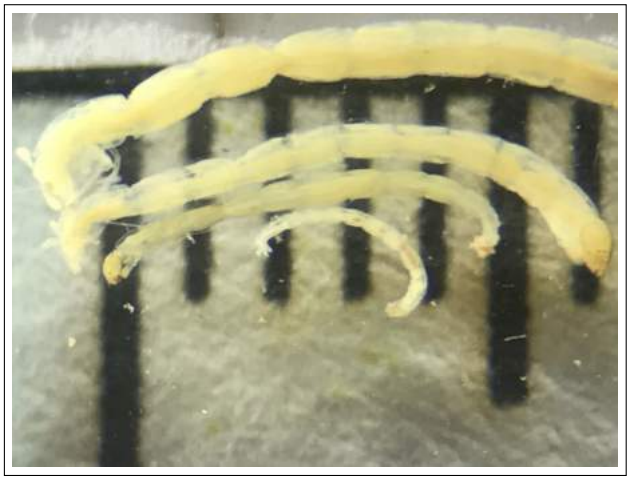
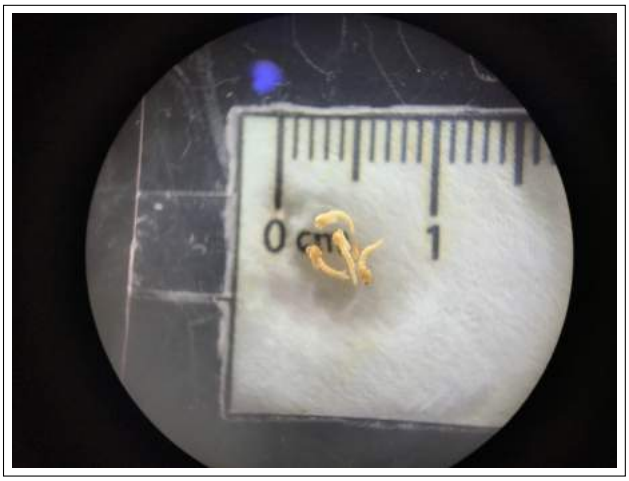


Figure 46: Diptera (Chironomidae) # 1 of Canyon Lake, WA, 2016. Photos taken from sample 51.

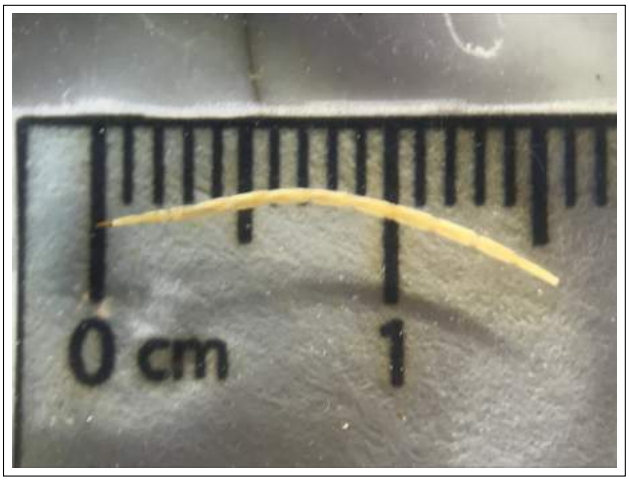
a)



b)

Figure 47: Diptera (Chironomidae) # 2 of Canyon Lake, WA, 2016. Photos taken from sample 51.

a)



b)

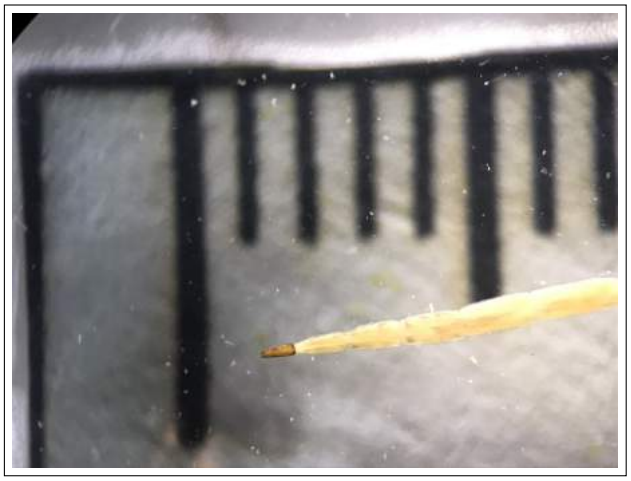


Figure 48: Diptera (Ceratopogonidae) of Canyon Lake, WA, 2016. Photos take from sample 51.

a)

b)

Figure 49: Diptera (Dixidae) of Canyon Lake, WA, 2016. Photos take from sample 15.

a)

b)

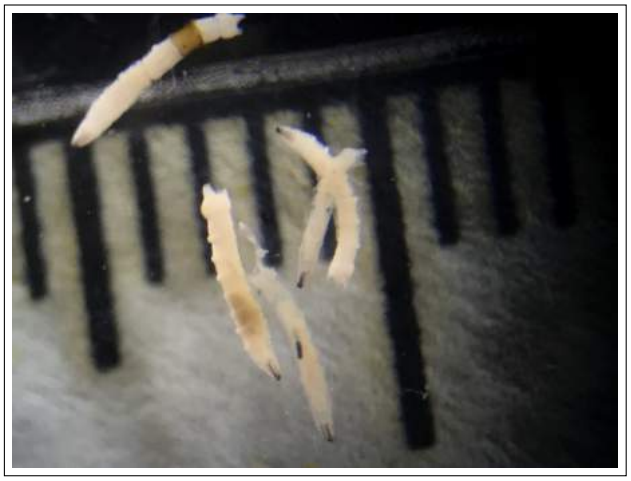


Figure 50: Diptera (Dolichopodidae) of Canyon Lake, WA, 2016. Photos take from sample 39.

a)

b)

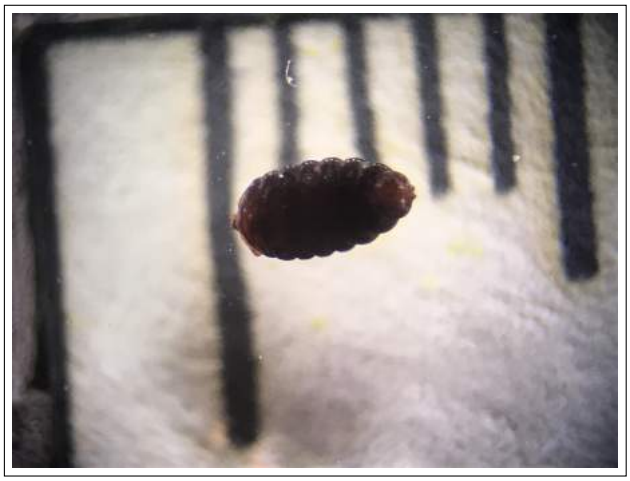
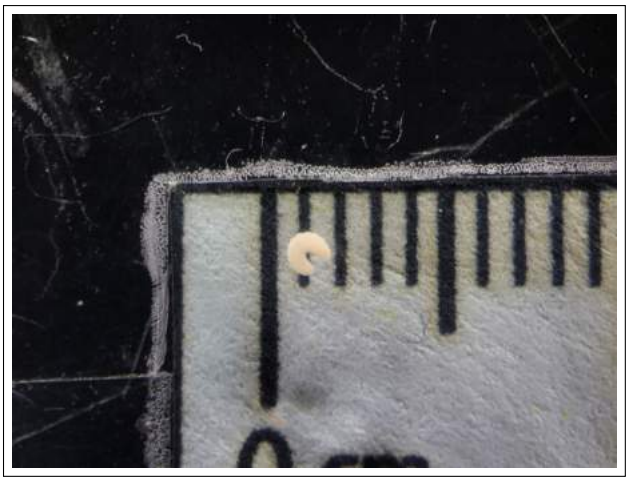


Figure 51: Diptera # 1 of Canyon Lake, WA, 2016. Photos take from sample 39.

a)



b)

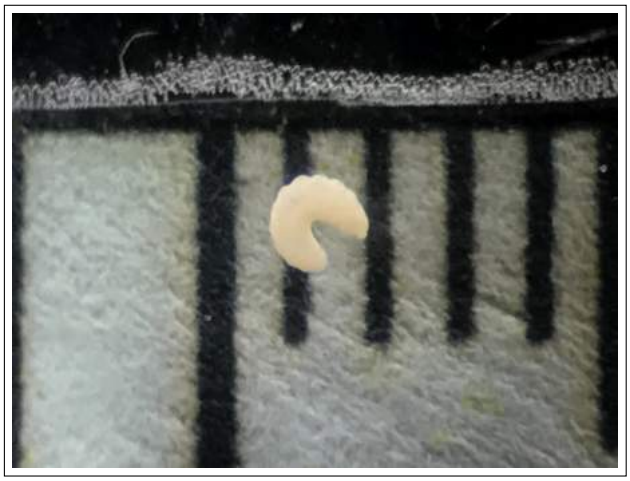


Figure 52: Diptera # 2 of Canyon Lake, WA, 2016. Photos take from sample 39.

a)

b)

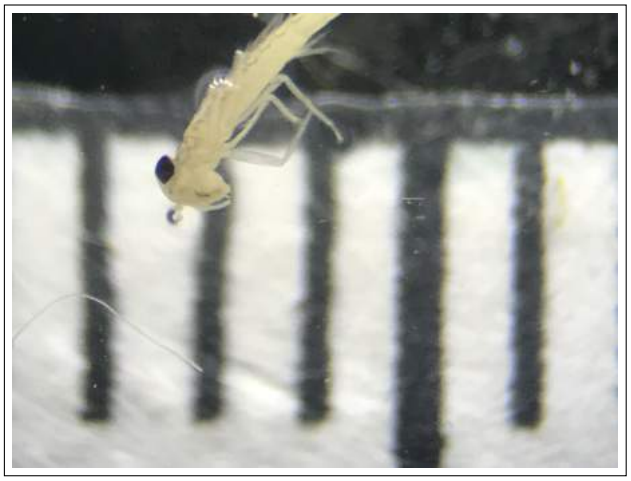


Figure 53: Ephemoroptera (Baetidae) of Canyon Lake, WA, 2016. Photos take from sample 15.

a)

b)

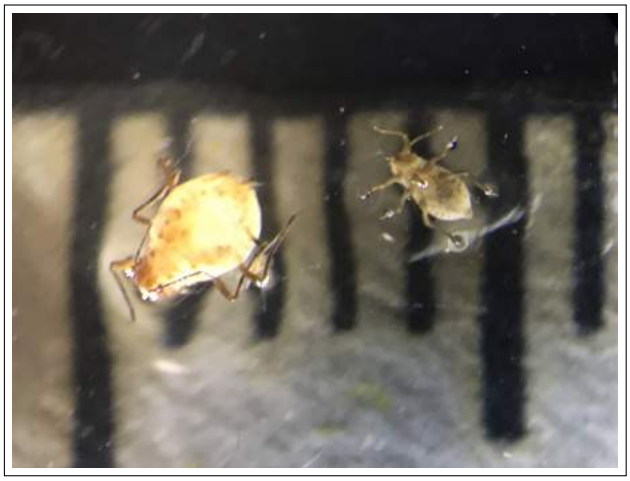


Figure 54: Hemiptera (Aphididae) *Rhopalosiphum* of Canyon Lake, WA, 2016. Photos take from sample 13.

a)

b)

Figure 55: Hemiptera (Gerridae) of Canyon Lake, WA, 2016. Commonly known as water strider. Photos taken from sample 10.

a)

b)

Figure 56: Hemiptera # 1 of Canyon Lake, WA, 2016. Commonly known as water strider. Photos taken from sample 10.

a)

b)

Figure 57: Hemiptera # 2 of Canyon Lake, WA, 2016. Photos taken from sample 10.

a)

b)

Figure 58: Hymenoptera of Canyon Lake, WA, 2016. Photos taken from sample 10.

a)

b)

Figure 59: Megaloptera (Sialidae) Sialis of Canyon Lake, WA, 2016. Photos take from sample 50.

a)

b)

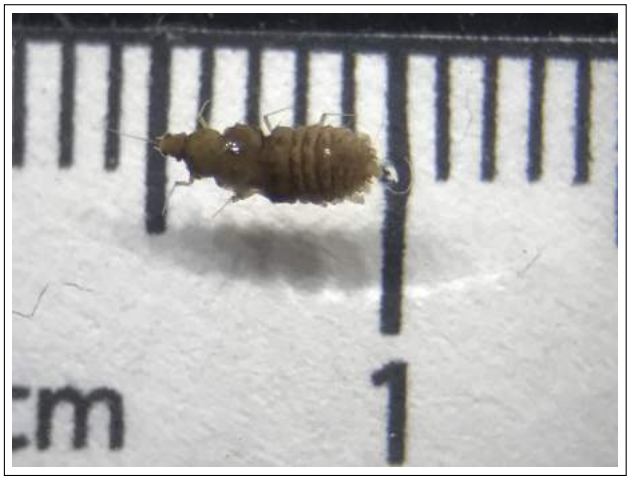


Figure 60: Neuroptera (Sisyridae) of Canyon Lake, WA, 2016. Commonly known as spongeflies. Photos taken from sample 31.

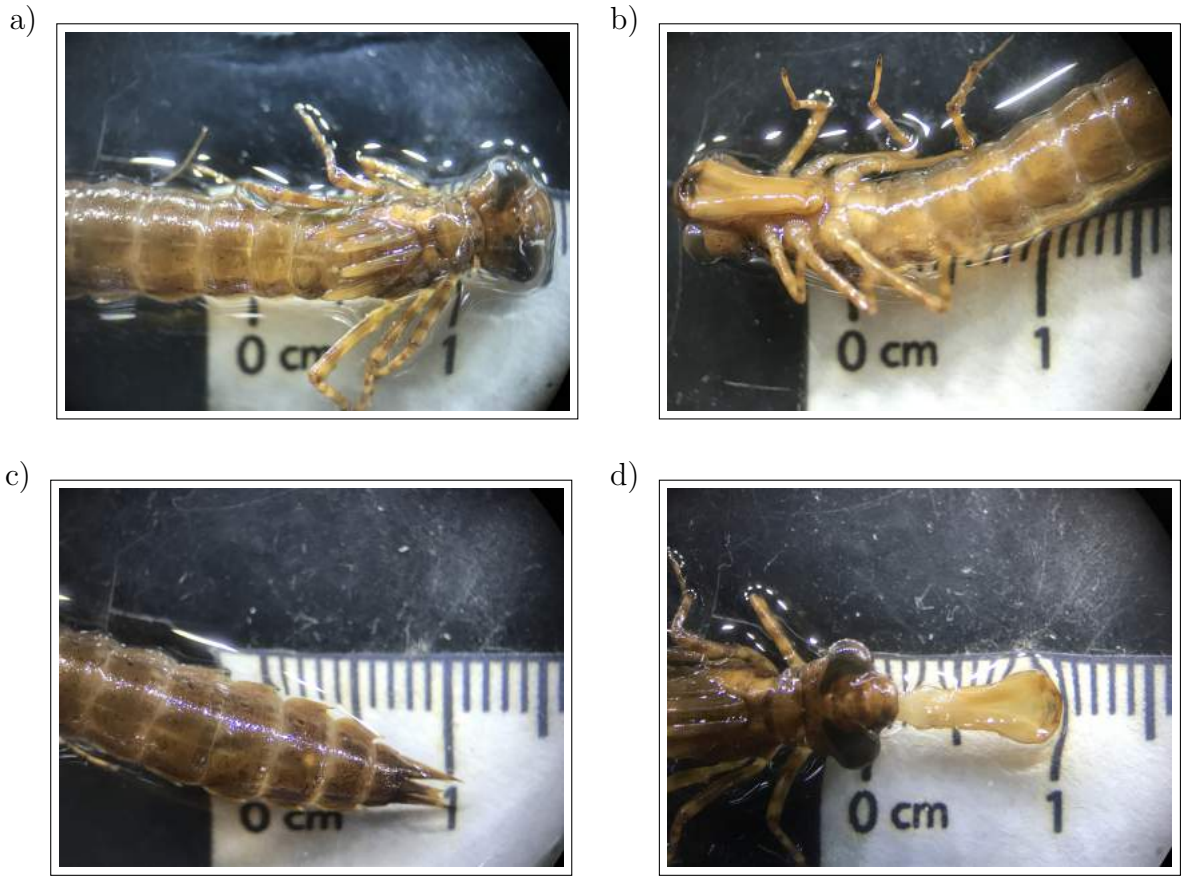


Figure 61: Odonata (Aeshnidae) *Aeshna* of Canyon Lake, WA, 2016. Commonly known as hawker dragonflies. Photos take from sample 11.

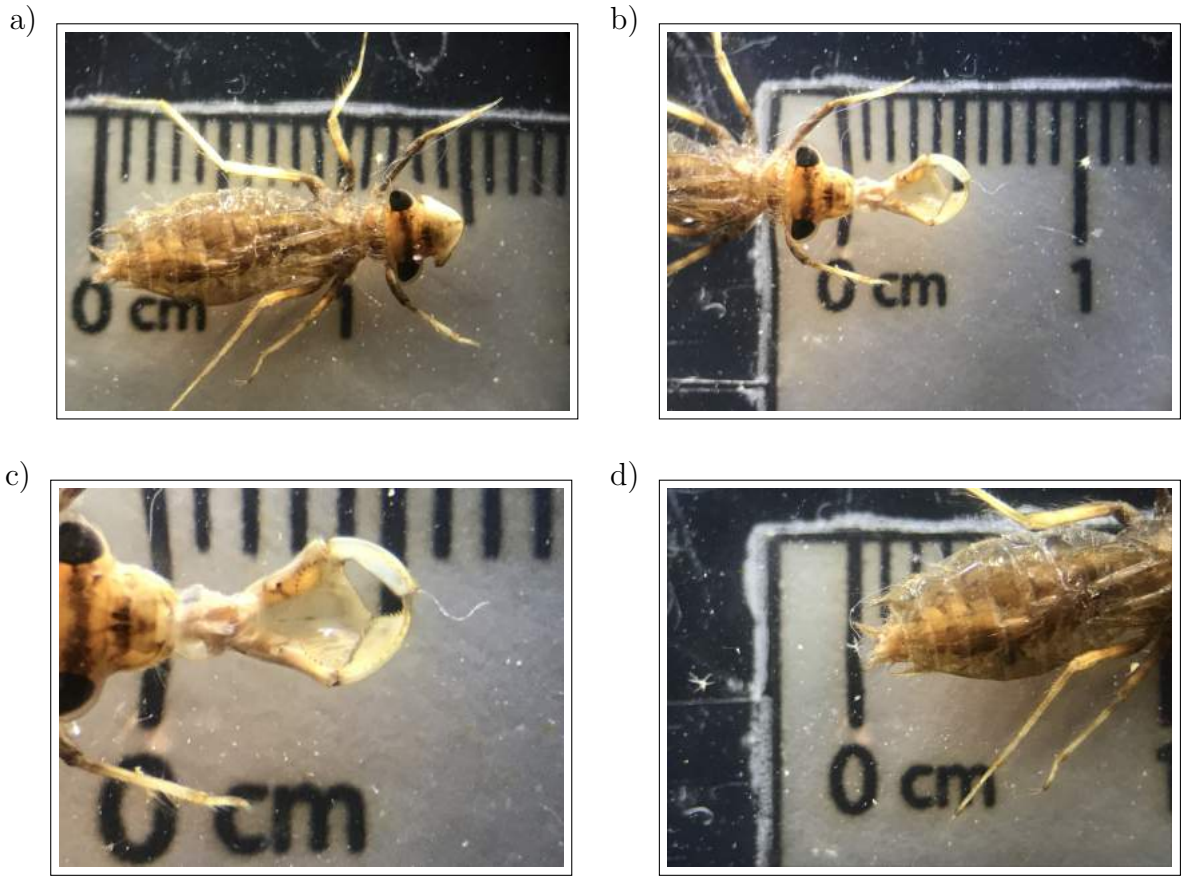


Figure 62: Odonata (Corduliidae) *Cordulia* of Canyon Lake, WA, 2016. Commonly known as hawker dragonflies. Photos take from sample 11.

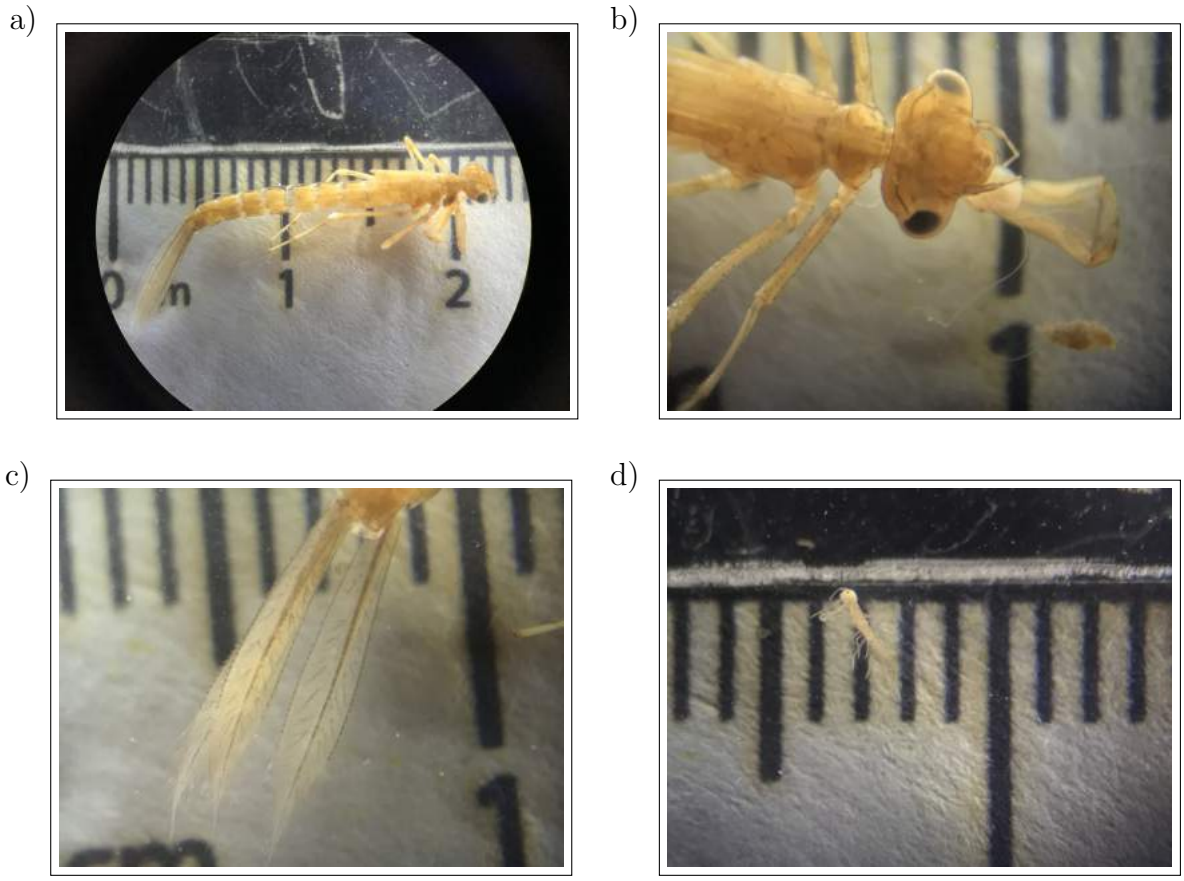
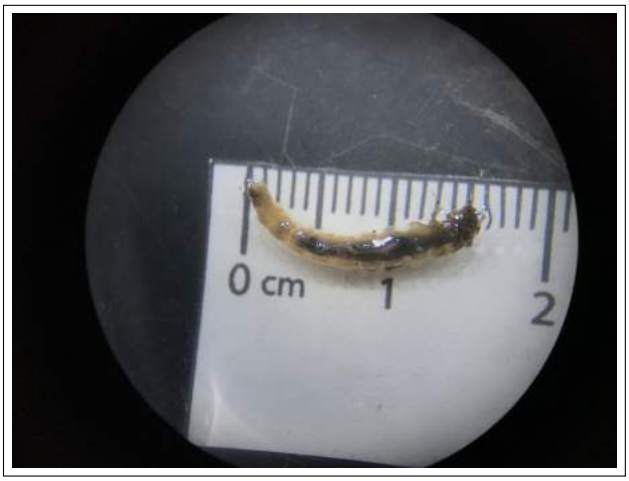


Figure 63: Odonata (Coenagrionidae) *Zoniagrion* of Canyon Lake, WA, 2016. Commonly known as the American Emerald. Photos take from sample 45.

a)



b)

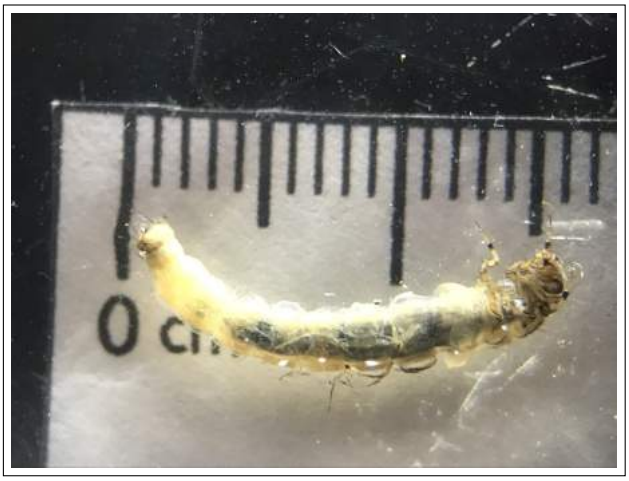


Figure 64: Trichoptera (Lepidostomatidae) *Lepidostoma* of Canyon Lake, WA, 2016. Commonly known as Bizarre Caddisflies. Photos take from sample 29.

a)

b)

Figure 65: Trichoptera (Polycentropodidae) *Neureclipsis* of Canyon Lake, WA, 2016. Commonly known as Tube Maker Caddisflies. Photos take from sample 17.

a)

b)

Figure 66: Trichoptera (Hydroptilidae) of Canyon Lake, WA, 2016. Photos take from sample 10.

a)

b)

Figure 67: Trichoptera (Hydroptilidae) in metamorphosing silk bottle cas, Canyon Lake, WA, 2016. Photos take from sample 51.

a)

b)

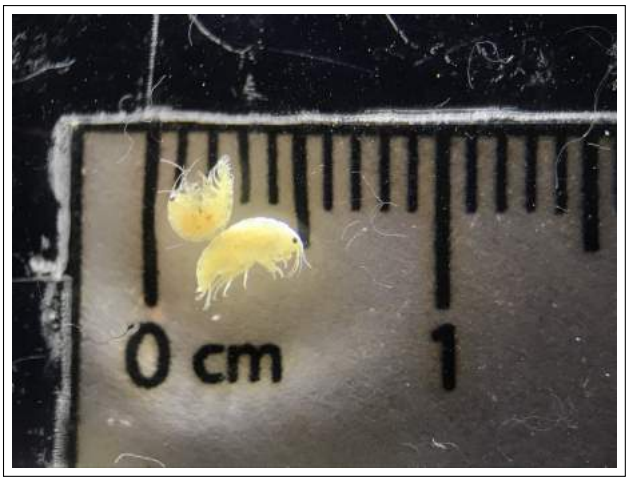
Figure 68: Trichoptera # 1 of Canyon Lake, WA, 2016. Photos take from sample 21.

a)

b)

Figure 69: Trichoptera # 2 of Canyon Lake, WA, 2016. Photos take from sample 50.

a)



b)

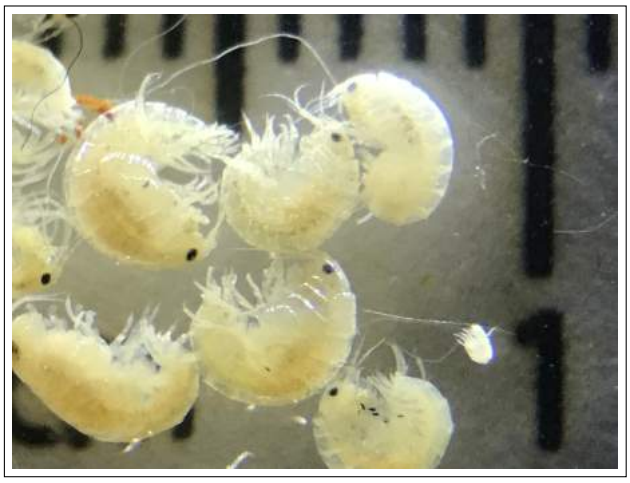
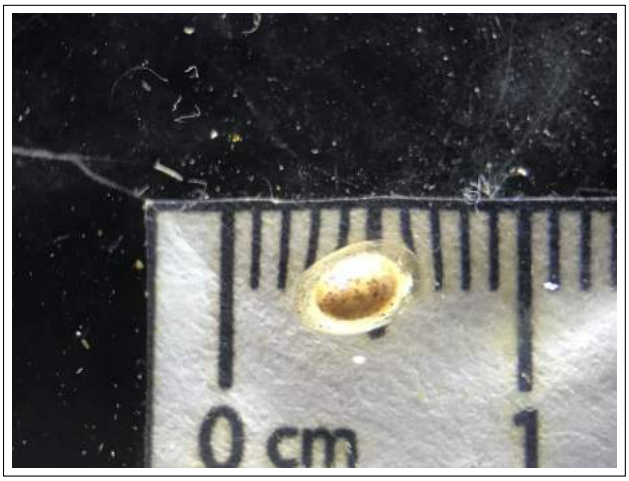


Figure 70: Amphipoda of Canyon Lake, WA, 2016. Photos take from sample 51.

a)



b)

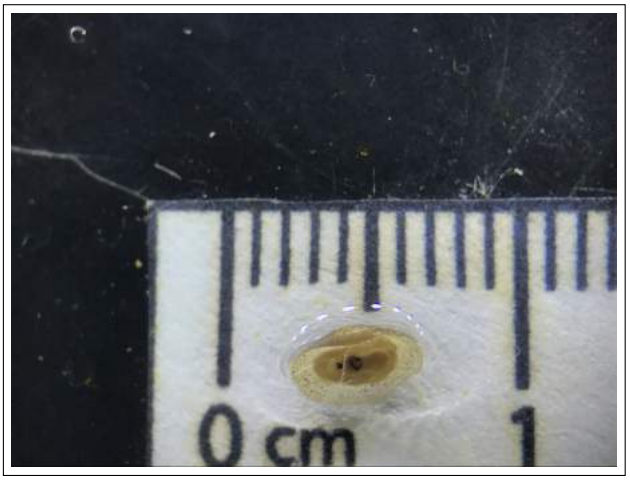
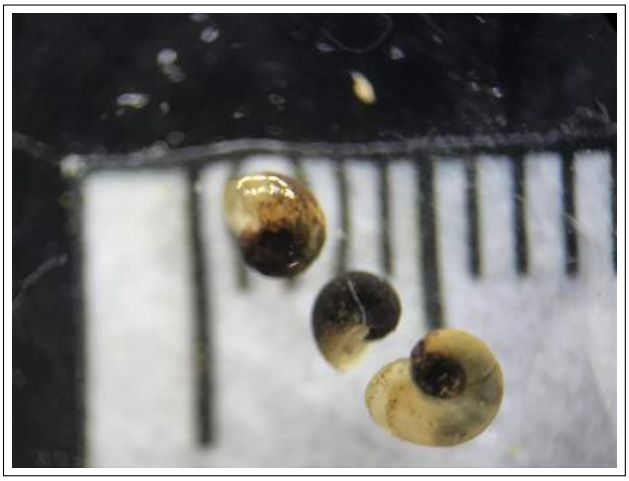


Figure 71: Basommatophora (Ancylidae) *Ferrissia* of Canyon Lake, WA, 2016. Photos taken from sample 12.

a)



b)

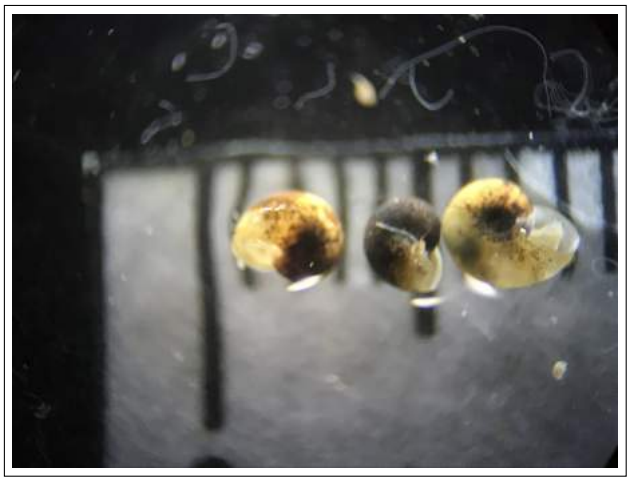


Figure 72: Basommatophora (Planorbidae) *Gyraulus* of Canyon Lake, WA, 2016. a) Largest individual with protoconch upward. b) Largest individual with umbilicus upward. Photos taken from sample 10.

a)

b)

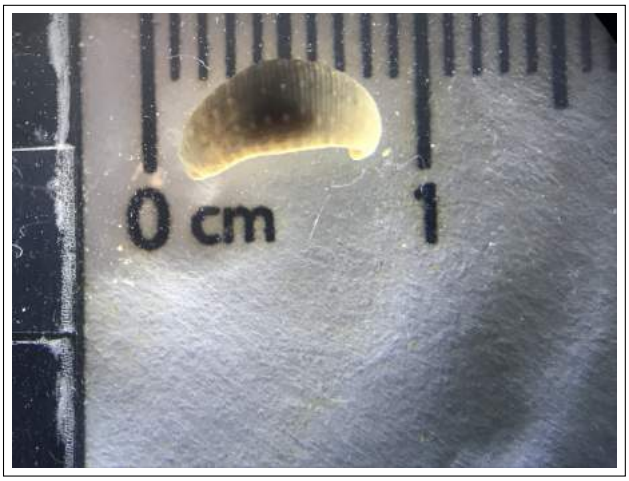
Figure 73: Veneroida (Pisidiidae) *Pisidium* of Canyon Lake, WA, 2016. Commonly known as fingernail clam or pea clam. Photos taken from sample 51.

a)

b)

Figure 74: Opisthopora (Lumbricidae) of Canyon Lake, WA, 2016. Photos taken from sample 51.

a)



b)

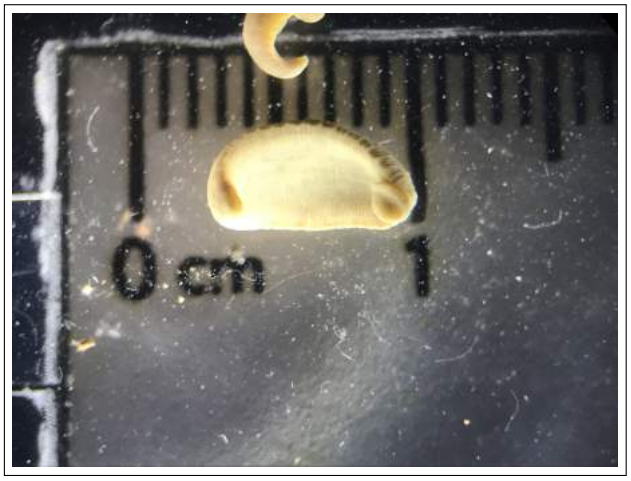
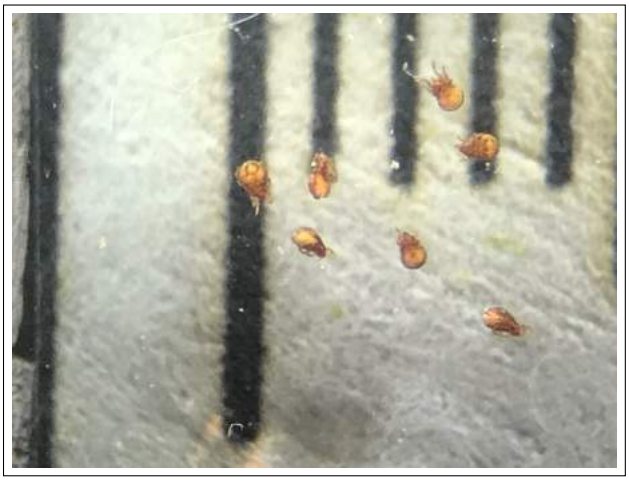


Figure 75: Hirudinida (Hirudinidae) *Hirudo* of Canyon Lake, WA, 2016. Commonly known as a leech. Photos taken from sample 51.

a)



b)

Figure 76: Trombidiformes (Hydrachnidae) *Hydrachna* of Canyon Lake, WA, 2016. Commonly known as water mites. Photos take from sample 12 and 21.