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Patterns of variation in the benthic macroinvertebrate community of the Upper Columbia River, WA

A Thesis presented to

Eastern Washington University

Cheney, WA

In partial fulfillment of the requirements for the degree of

Master of Science in Biology

By

Sarah J. Hindle

Spring 2018

THESIS OF SARAH J HINDLE APPROVED BY

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Master's Thesis

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Table of Contents

Chapter 1: Spatial and temporal variation in the benthic macroinvertebrate community in the Upper Columbia River, WA and its potential suitability as a prey source for larval white sturgeon (*Acipenser transmontanus*)

Chapter 2: A comparison of three common benthic sampling methods and their efficacy for invertebrate community analyses in an impounded river system

List of Figures and Tables

All figures were created using R (version 3.2.2) and Microsoft Excel.

Chapter 1

Chapter 2

Chapter 1

Spatial and temporal variation in the benthic macroinvertebrate community in the Upper Columbia River, WA and its potential suitability as a prey source for larval white sturgeon (*Acipenser transmontanus*)

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Abstract

Benthic macroinvertebrate (BMI) assemblages are important in the field of aquatic ecology for assessing water quality and ecosystem health. However, knowledge of BMI community structure and seasonal patterns in large impounded rivers is relatively limited in scope. In the Upper Columbia River, WA, the BMI community is of particular concern due to hypothesized starvation of native fish larvae. The population of white sturgeon (*Acipenser transmontanus*) which inhabits the Upper Columbia and feeds primarily on BMIs has been experiencing high mortality rates at the onset of exogenous feeding, leading to chronic larval recruitment failure. To determine the potential suitability of the BMI community as a prey source in this crucial stage of development, I explored seasonal, annual and zonal differences in BMI density, biomass, diversity and community structure within the area of highest density for first-feeding sturgeon larvae. My results indicate that during periods of high discharge and reservoir drawdown, BMI density is high but diversity is low. This is due to high relative abundance of few taxa, primarily freshwater cnidarians (*Hydra* sp), and Chironomid larvae (Order Diptera). Community structure changed over the course of the season, with more taxa present later after the second reservoir drawdown, but in lower densities. Chironomids were the only taxon besides *Hydra* which were present in moderate abundance regardless of season. Along with evidence of consumption of Chironomids by sturgeon larvae in other populations, this suggests that they may be the most suitable prey source for Upper Columbia white sturgeon larvae at this time. Further study is needed to directly connect BMI community structure with prey selection behaviors in this population.

Introduction

Large rivers serve many functions in the regions to which they belong. They shape landscapes, transport sediment, nutrients and other resources downstream, and serve as habitat for organisms, including juvenile forms of terrestrial animals such as insects. Aquatic insect larvae and other benthic macroinvertebrates (BMIs) account for most of the secondary production in river systems (Beamesderfer and Farr, 1997), and are a crucial component of the food web. Local abundance and diversity of BMIs in lotic ecosystems is influenced by a variety of hydrological and geomorphological factors, including flow velocity and habitat morphology (Chi et al., 2017; Holzapfel et al., 2017; Szczerkowska-Majchrzak et al., 2014). When changes in flow are predictable, BMI assemblages exhibit a seasonal pattern of distribution and abundance based on the local hydrograph and the dispersal abilities of individual taxa (Wilson and McTammany, 2016). Periods of high discharge often result in a decrease in overall BMI density and diversity, favoring taxa which are resistant to flow or quick to recolonize (Jacobsen et al., 2013; Szczerkowska-Majchrzak et al., 2014; Zhou et al, 2014). Due to their relative inability to move in a current and their rapid response to changing conditions, BMI communities are commonly used as indicators of water quality and environmental change (Chi et al., 2017; Zhou et al., 2017). Several ubiquitous BMI taxa (i.e. Ephermeroptera, Trichoptera) are sensitive to changes in water quality parameters, and so communities can undergo shifts due to changes in environmental conditions (Chi et al., 2017; Santos et al., 2017). This often leaves behind more tolerant taxa, including Dipteran larvae (Chironomidae, Simuliidae) and Oligochaetes.

While BMI studies are pervasive in the fields of freshwater science and stream ecology, the community characteristics of large rivers remains relatively understudied. This is partially due to sampling difficulties; the depth and velocity of the river prohibiting certain common sampling procedures used on smaller, wadeable streams (DiSabatino et al., 2016), and the absence of a standardized theory for how river ecosystems function (Johnson et al., 1995). Generally, we can predict which functional groups of BMIs will be abundant in different sections of lotic ecosystems based on a series of hydrological parameters, including duration and frequency of high and low flow events (Kakouei et al., 2017), and sediment characteristics in the benthos (McCabe et al., 1997, McCabe and Hinton, 1998). However, assessments of taxonomic diversity and community structure of BMIs in large rivers and their reservoirs have been somewhat lacking. Given the utilization of these organisms in water quality analyses and as prey for fish species, it is important to understand the baseline structure of these assemblages in order to understand how they might be impacted by habitat alteration.

Though natural disturbances like flooding are common in riverine systems, the natural hydrographs of large rivers are often impacted by anthropogenic activities, particularly impoundment by dams. Impoundment of waterways is a common practice in the Western United States for the purposes of flood control, irrigation and hydroelectric power generation, and there are currently over 75,000 dams over 2m tall in the United States (Poff et al., 2006). Dams alter the natural flow regime of rivers, leading to decreased peak and increased minimum flows (Poff et al., 2006), and alter biotic and abiotic properties both above and below the dam. Behind a dam, slower flows create reservoirs which lead to thermal stratification and increased deposition of fine sediments

(McAdam 2015; Santos et al., 2017; Xie et al., 2017), while irregular pulses of water below a dam can lead to unpredictable benthic scour (Holzapfel et al., 2017). These changes in stream condition, coupled with fragmentation of an otherwise continuous riverine habitat, can have sizable impacts on biotic communities. BMI assemblages which inhabit impounded river reaches react to changes in flow and sedimentation, leading to temporal shifts in community structure and potential disappearance of sensitive taxa (Chi et al., 2017; Li et al., 2015; Santos et al., 2017, Szczerkowska-Majchrzak et al., 2014). Habitat fragmentation can also prevent migrations and gene flow in fish populations. Riverine fish tend to form metapopulations, which are more genetically diverse and generally healthier than discrete populations, since they have a wide availability of resources and high levels of gene flow (Jager et al., 2001). Fish populations in fragmented rivers, however, are unable to migrate and thus become limited in their resource availability and mate choice.

The Columbia River in the Pacific Northwest is an ideal system for studying effects of river impoundment. The main channel of the river alone is impeded by fourteen dams, along with dozens more throughout the watershed, which were implemented primarily for flood control and generation of hydroelectric power. The largest impounded reach on the main stem lies behind the Grand Coulee dam in central Washington, and includes a lentic zone known colloquially as Lake Roosevelt, and a riverine zone upstream near the US-Canada border. Dam operations in the spring and summer seasons lead to large fluctuations in water level over the course of a year, creating a unique and dynamic ecosystem. In early Spring, the reservoir is drawn down, causing the surface elevation to decrease by approximately 50-70 feet, depending on the year (U.S. Bureau of Reclamation), followed by a smaller, roughly 10 foot drawdown in August. Impoundment effects in this reach have been a topic of concern in recent years due to the rapid decline of the inhabitant population of white sturgeon, a native fish species. The white sturgeon (*Acipenser transmontanus*) is the largest, longest-lived freshwater fish in North America, reaching natural lifespans of up to 80 years (Drauch Schreier et al., 2013). Though its native range includes large rivers from Alaska to Central California (McCabe et al., 1993), the largest population resides in the Columbia River. An anadromous species, white sturgeon spend their adult lives in the ocean, then migrate into rivers each year to spawn and rear their young. Despite high fecundity, sturgeon populations are incredibly vulnerable due to exploitation, decline in habitat quality, and effects of impoundment (Beamesderfer and Farr, 1997; McAdam, 2015). Impoundment of rivers where sturgeon are native has had some notable negative effects on population health and year-class strength. More than half of landlocked white sturgeon populations are currently in severe decline (Beamesderfer and Farr, 1997; Drauch Schreier et al., 2013), including the Lake Roosevelt population in the Upper Columbia.

Prior studies have identified larval recruitment failure as the most likely cause of population decline in many landlocked sturgeon populations, including Lake Roosevelt (Howell and McLellan, 2005; McAdam, 2015). This includes high mortality at the onset of exogenous feeding, the stage when larvae deplete their yolk sacs and begin foraging in the benthos for prey (Boucher et al., 2014; Hunter, 1981). This is arguably the most risky phase in fish development for many reasons, most notably a combination of high predation risk and low feeding success (Hunter, 1981). These factors are often interrelated, as a lack of available prey may lead larvae to search for resources in less sheltered areas, leaving them more vulnerable to predation. Additionally, starvation conditions in young fishes have been shown to decrease responses of certain Heat Shock proteins, leading to decreased tolerance of thermal stress (Han et al., 2012), and can influence the communities of microbiota in the gut, potentially increasing susceptibility to disease (Xia et al., 2014).

The decline of most landlocked white sturgeon populations is likely related to the availability of prey during early life stages (Leggett and Deblois, 1994). Though adults may pursue large prey, sturgeon are primarily benthivorous, eating BMIs at most phases of their life (Beamesderfer and Farr, 1997; Braaten et al., 2007; Crossman et al., 2016; McCabe et al., 1993; Muir et al., 2000). Therefore, the density and stability of BMI communities is crucially important for feeding sturgeon, particularly at the earliest stages of their lives. According to Deng et al. (2003), the optimal feeding rate for white sturgeon during their first week of exogenous feeding is 26% of their body weight per day, decreasing to 6% after 4 weeks. Larvae are also most metabolically active during this early period of growth and development, limiting their ability to store nutrients acquired from their prey (Han et al., 2012; Hunter, 1981). This suggests that low prey availability at this crucial period could quickly lead to starvation and poor year-class strength (Leggett and Deblois, 1994; Muir et al., 2000).

A few studies of white sturgeon diet in the Columbia River have been conducted in the estuary and adjacent freshwater zone. This unimpounded reach supports the largest white sturgeon population in the watershed, shows no signs of larval starvation (Muir et al., 2000), and is open to recreational and some commercial fishing (McCabe et al., 1993). The impounded Upper Columbia, however, is notably understudied in terms of the BMI community and diets of first-feeding sturgeon. Analysis of juvenile sturgeon diets in the Lower Columbia and relative abundance of invertebrate taxa in the benthos have revealed that the BMI community consisted of 17-20 taxa, with the most abundant being *Corbicula fluminea*, an abundant bivalve, and Dipterans in the family Ceratopogonidae (McCabe et al., 1993, 1997). The tube-dwelling amphipod *Corophium salmonis* was found to be a particularly important prey item for young sturgeon, especially those in smaller size classes, despite being in relatively low abundance in the benthos (McCabe et al., 1993). Being an estuarine species, *C. salmonis* is not found in the Upper Columbia, thus eliminating it as a potential prey item for landlocked larvae. Cyclopoid copepods and Dipteran larvae and pupae (mostly Chironomidae) have also been identified as important prey taxa for Lower Columbia sturgeon (Muir et al., 2000). In Canadian populations, juvenile sturgeon have been shown to consume other small crustaceans, such as freshwater amphipods, isopods and mysids, as well as some Chironomids and other insect larvae (Crossman et al., 2016).

The issue of larval mortality in the Upper Columbia presents many questions about availability of potential prey during a narrow window of development. Since little is known about the nature of the BMI community in this reach and most larval sturgeon collected for diet study are captured with empty stomachs (Reihart, 2016), we have very little information about what is available and what specific taxa are being selected. While specific correlations between benthic abundance and prey suitability cannot be drawn without reliable diet data, understanding the spatial and temporal distribution of the BMI community in the upper Columbia River will provide some much needed elucidation on which taxa are available during important periods of white sturgeon development. My primary objectives were to characterize the BMI community in the Upper Columbia River, within the area of highest drift density and feeding habitat for first-feeding white sturgeon, and determine annual, seasonal and zonal patterns of BMI abundance and diversity. In addition, I aimed to determine how size distribution of abundant taxa changed across various zones and time periods, and whether the size of common BMI taxa might make them unsuitable prey for gape-limited sturgeon.

Generally, I expected to see a change in abundance and distribution of taxa in different years and seasons, as well as between the near-shore littoral zone and deep mid-channel thalweg. When comparing seasons, I expected density and diversity of BMIs to increase in the late summer season, once the water level in the reservoir had returned to maximum and peak flows from spring snowmelt had subsided (Figure 2). I also hypothesized that, when comparing years, those years with particularly high flow would have a different BMI community structure than other years. The years 2007 and 2010 were chosen as examples of two years with particularly wide discrepancies in flow (Figure 2). I also expected the littoral zone to have a higher BMI abundance and diversity than the thalweg, owing to a greater diversity of habitat and less hydraulic stress. When considering size distribution, I hypothesized that size of abundant taxa would be larger than the average gape size of first-feeding sturgeon, possibly contributing to low feeding success in early life stages of sturgeon larvae.

Methods

Study Site

The study was conducted in the section of the upper Columbia River between the Grand Coulee dam in Washington, USA and the Hugh Keenleyside dam in British Columbia, Canada. All samples for this study were collected within the riverine zone above the Lake Roosevelt reservoir, from the Kettle Falls boat launch (Rkm 1141) to Northport (Rkm 1175) (Figure 1).   This study area overlaps the zone of highest drift density for larval white sturgeon after hatching, and at the onset of exogenous feeding. At each site along the river channel, samples were taken from the littoral zone (nearshore), and from the thalweg (mid-channel). These represent two river zones with vastly different hydrological parameters and likely high variance in BMI abundance and community composition.

BMI collection

Benthic invertebrates were collected using artificial substrate deployment. Six concrete cones (surface area = 0.047 m^2 each) meant to replicate the large cobble substrate in the benthos were placed in metal baskets (0.04 m^2 substrate area covered) and deployed at each site on a line weighed down on one end by a large concrete pyramid and marked by a buoy on the other. Other methods (i.e. benthic sled) were attempted, but sampled primarily planktonic communities (See Chapter 2). This method was used in the years 2007 and 2010 by the Spokane Tribe of Indians (STOI) for BMI sample collections, which were analyzed to determine patterns in annual variation, and was subsequently used twice during 2017 for the collections to determine seasonal variation.

In previous years, the river channel was divided into nine sites, within each of which a random point had been chosen in each zone of the river for substrates to be deployed. Substrate baskets were incubated *in situ* for 6 weeks from May to July and then collected by STOI. Cones were scrubbed and rinsed to remove attached invertebrates, which were sieved through 0.5mm mesh, preserved in 70% ethanol and returned to the lab for analysis. During the seasonal analysis in 2017, substrates were deployed as above at ten littoral sites and seven thalweg sites. A different number of sites were used in 2017 due to an inability to access some upstream thalweg sites with particularly high flow velocity. The first seasonal incubation period lasted from mid-June to late July 2017, while the second lasted from early August to mid-September. Invertebrates were collected and processed in the manner above during both deployments, and returned to the lab where they were stained with Rose Bengal dye.

BMI analysis

Each benthic invertebrate sample was inspected under microscopy and individuals counted and identified to the lowest practical taxon, usually family or genus. The first 30 individuals of each taxon were also measured in length to the nearest 0.1mm. In samples which were particularly dense, a 500-individual subsample was taken. The sample was spread into square gridded petri dishes, each of which was divided into four equal quadrants. One quadrant was selected at random and its contents sorted in the manner described above. Additional quadrants were randomly selected until the total number of individuals counted reached five hundred.

BMI density (number of individuals per volume sampled) was calculated for each replicate. If density values were not normally distributed, a log transformation was performed. Shannon-Wiener Index values were calculated to determine taxon diversity, as well as an associated Equitability (a measure of evenness) value for each sample. Equations for Shannon-Wiener Diversity (H) and Equitability (E) are shown below. In these formulas, p_i represents the proportion of individuals sampled belonging to a particular taxon, and S represents taxa richness.

$$H = -\sum(p_i * \ln(p_i))$$

$$E = H / \ln(S)$$

Biomass calculations were conducted using the average lengths of individuals in each taxon and their relative abundance in the sample. The formula for biomass is as follows, where W and L represent biomass and length, respectively, and *a* and *b* are regression constants (Benke et al., 1999; Dumont et al., 1975; McCauley, 1984; Miserendino, 2001).

$$W = aL^b$$

All metrics were analyzed for effect of year/season and zone using two-way factorial Analysis of Variance (ANOVA) tests and Tukey's post-hoc in R (version 3.3.2).

Community structure was compared between years, seasons and zones by non-parametric multidimensional scaling (NMDS) and an associated PERMANOVA hypothesis test using the ADONIS function in R. Similarity Percentage analysis

(SIMPER) was used to explore contributions of specific taxa to overall community dissimilarity.

Sturgeon collection and analysis

Sampling of sturgeon larvae was conducted by STOI during the month of July in 2015 just upstream of China Bend within the study site. Double D-frame drift nets (0.8m wide x 0.6m high) with attached sieve buckets were deployed twice overnight for two to three hours per deployment, depending on debris loading. The contents of each collection bucket were sieved and larvae collected individually using small pipettes. The larvae were preserved in 10% neutral buffered formalin and returned for analysis. In the lab, fork length and gape size were measured to the nearest 0.1mm. Individuals had been previously dissected to determine diet (Reihart, 2016). A general linear regression (R, version 3.3.2) was used to explore relationships between fork length and gape size.

Results

Annual Variation: BMIs

Density of BMI samples was significantly higher in 2007 than in 2010 ($p<0.001$), though diversity and equitability showed a reverse trend, being significantly higher in 2010 ($p<0.001$) (Figure 3). Only diversity was affected by zone, with the littoral zone being more diverse than the thalweg ($p=0.037$). Sample biomass was significantly higher in 2007 than in 2010 ($p=0.009$), and higher in the littoral zone than the thalweg ($p=0.004$) (Figure 3). This was likely due to a few large organisms being present in the 2007 littoral samples. Community composition varied significantly between years and zones ($p<0.001$ and $p=0.008$, respectively) (Figure 4). Cnidarian polyps in the genus *Hydra* were the dominant taxon in 2007, making up 50-70% of the samples collected, depending on zone. In 2010, *Hydra,* Oligochaetes and Chironomids collectively made up a majority of individuals sampled (Figure 7).

Seasonal Variation: BMIs

Similar to the patterns of annual variation observed, BMI density in the early season of 2017 was significantly higher than in the late season of the same year ($p<0.001$) (Figure 5). Likewise, diversity and equitability followed the opposite trend and increased in the late season ($p=0.007$ and $p<0.001$, respectively). Biomass, however, was not significantly affected by either season or zone ($p=0.079$ and $p=0.219$, respectively) (Figure 5). Community composition varied by both season and zone ($p<0.001$), similarly to the way the annual samples differed (Figure 6). The early season collections contained mostly *Hydra* polyps, with some Chironomids and few other taxa. In the thalweg, up to 90% of individuals sampled were in the *Hydra* genus. In the late season, increased diversity and equitability meant a more even distribution of taxa. *Hydra* and Chironomids were still notably present, but samples also contained Caddisflies (Order Trichoptera), snails (Class Gastropoda) in the littoral zone, and assorted other taxa (Figure 7). SIMPER analysis revealed *Hydra* and Chironomidae as the largest contributing taxa to overall dissimilarity between seasons (63.3% total), as well as between years (85% total). Relative abundance of certain individual taxa was also significantly affected by season. Relative abundance of *Hydra* decreased in the late season ($p<0.001$), but persisted somewhat in the thalweg. Chironomid relative abundance did not vary by season (p=0.301), but was significantly higher in the littoral zone (p=0.002) (Figure 8). This family consistently accounted for up to 50% of individuals collected from littoral sites.

BMI size characteristics and sturgeon gape

Unfortunately, many of the taxa collected were found in such low abundance that conclusions cannot be confidently drawn about effects of year, season or zone on their size. However, *Hydra* showed a seasonal decrease in size, particularly in the thalweg (p<0.001). Average size of Chironomid larvae did not change by season (p=0.877) or zone (p=0.553) (Figure 9). A majority of Chironomid individuals sampled were between 1-3mm in length (Figure 11).

Gape size of drifting larval sturgeon increased linearly with fork length (p<0.001, N=561) (Figure 10). On average, the individuals sampled were 19mm in length, with a gape size of 1.88mm.

Discussion

The data revealed significant spatial and temporal shifts in the BMI community in the study reach. Over the course of one summer, density of BMIs peaked in the early season, from June to July. This goes contrary to the hypothesized trend, which predicted that BMI density would peak in the late season once the reservoir had reached full pool. This was not the case in this study, possibly due to a secondary drawdown of the reservoir in early August. While the water level only decreases by about ten feet, this can expose formerly inundated areas of the riparian and littoral zones, removing these areas as a potential source of nutrient input to support benthic production (Johnson et al., 1995; Junk et al., 1989). However, despite lower densities in the later season, Shannon-Wiener Diversity and Equitability values showed a marked increase. This may be due in part to colonization of newly inundated areas by adult insects, or by settlement of BMIs drifting down from upstream. Settlement is likely a particularly important mechanism of recolonization in the downstream-most sites where flow in the main channel slows entering the lentic pool of the reservoir (Howell and McLellan, 2005). Though the trend of decreasing density was clear during this period, biomass did not undergo significant change between seasons or zones. This suggests that *Hydra*, while numerically abundant, do not contribute much to the total sample biomass.

Interestingly, a similar trend was viewed when comparing BMI communities between years. Sampling years 2007 and 2010 were chosen due to a marked difference in flow despite samples being deployed and collected at the same time of year; in late spring when the reservoir had not yet reached full pool. During the May-June incubation period, the mean daily flow in 2007 was consistently almost double the mean daily flow in 2010, eventually converging in early July when the reservoir had re-filled (Figure 2). Despite flow values being similar when the samples were collected in July, the period of extremely high flow during the drawdown period may have resulted in forced drift of many epibenthic organisms (Holzapfel et al., 2017; Miller and Judson, 2014), likely contributing to low diversity in 2007. Similarly, 2017 was a high flow year due to heavy snowfall in winter, which may have led to trends of low diversity and high *Hydra* abundance in the early season when water levels were low and discharge was high.

Though many BMI taxa have morphological and behavioral adaptations to resist forced drift, periods of peak discharge have been connected to periods of peak drift biomass, as flow velocity supersedes the resistance threshold of several taxa (Gibbins et al., 2007; Miller and Judson, 2014; Schulting et al., 2016; Szczerkowska-Majchrzak et al., 2014; Zhou et al., 2017).

During the drawdown periods of 2007 and early 2017, when flow was highest, BMI communities were dominated by *Hydra* sp., with few other taxa present. *Hydra* are not generally considered prominent benthic organisms, yet routinely made up a majority of individuals sampled during these periods and was the most responsible taxon for driving dissimilarity in the benthic community. Even in 2010 and late 2017 when mean daily flows were lower and taxon diversity increased, *Hydra* still persisted in the thalweg where velocity remains naturally higher. Besides *Hydra*, the second most abundant taxon was the family Chironomidae, which is ubiquitous in freshwater habitats. Chironomids were relatively abundant compared to most other taxa regardless of season, though they were significantly more abundant in the littoral zone where flow is slower and fine sediment is deposited. Though Chironomids are prone to forced drift in high flow conditions like other BMI taxa, they are quick re-colonizers with high dispersal ability, leading to continued abundance in the benthos despite periods of high discharge (Szczerkowska-Majchrzak et al., 2014; Schulting et al., 2016; Wilson and McTammany, 2016; Zhou et al., 2017). Given the consistent presence of these insects in the Upper Columbia benthos and their utilization as a prey source for juvenile white sturgeon in other populations, it is possible that Chironomids are a suitable prey item for Lake Roosevelt sturgeon larvae. Additionally, a majority of sampled Chironomids were less than 3mm in length; within a suitable size range to potentially be consumed by first-feeding sturgeon larvae. *Hydra* were also within a reasonable size range for consumption, but their hostile morphology and low nutritional content make them a poor prey choice for larval sturgeon.

Understanding the structure of the BMI community in the Upper Columbia River is an important step in monitoring ecosystem health and determining potential prey sources for declining fish populations. However, it is important to remember that assessing abundance and diversity of BMIs doesn't necessarily predict survival rates of first-feeding larvae. Several studies of juvenile sturgeon diets have found a significant difference between relative abundances of taxa in benthic samples and taxa in gut samples, as well as seasonal patterns in diet that do not represent seasonal fluxes in BMI taxa (McCabe et al., 1993; Muir et al., 2000) In the Lower Columbia for instance, the bivalve *Corbicula fluminea* and Oligochaete worms were abundant in the benthos but were seldom selected as prey. Conversely, the amphipod *Corophium salmonis* was frequently selected as prey despite not being relatively abundant in the surrounding environment (McCabe et al., 1993). Chironomids, however, were a significant component of the BMI community and were also frequently chosen as prey at the onset of exogenous feeding (Muir et al., 2000), likely owing to their tolerance of a range of conditions and rapid turnover rate. Additionally, evidence suggests that survivorship of fish larvae is strongly related to feeding success, but not necessarily to benthic abundance. In other words, it matters little which taxa are abundant if the fish are not capturing and consuming prey items successfully (Leggett and Deblois, 1994). Feeding success could be influenced by a variety of factors other than benthic abundance, including prey size (Hunter, 1981) and even flow velocity. In the larval phase, sturgeon rely on currents to carry them to suitable feeding habitat before their nutritional yolk sac runs out. When velocity is low, there is a higher likelihood that the yolk will be depleted before reaching suitable habitat (Muir et al., 2000). In impounded reaches such as the Upper Columbia where flow is regulated by dams, incomplete downstream drifting patterns may impede feeding success in larval white sturgeon. Size of available benthic prey may also limit sturgeon feeding. Sturgeon are gape limited, so are limited in their prey choice by mouth size. I measured length as an indicator of size suitability, though other species of fish have been known to consume prey items larger than their gape. This suggests that the deciding factor for size suitability may be width of the organism rather than length (Hunter, 1981). If this is the case, further study may need to be done on the size structure of sturgeon prey.

Though larval recruitment failure and associated population decline of Upper Columbia white sturgeon is well documented, the specific causes remain unclear. Starvation at the onset of exogenous feeding remains a reasonable hypothesis to explain sustained larval recruitment failure in the Upper Columbia. However, it is necessary to obtain reliable diet data for first-feeding larvae within the study reach. This has proved to be a difficult endeavor, mainly owing to rapid mortality rates during the transition period and sampling difficulties. Monitoring of drift samples for free embryos were conducted in an attempt to time collections to coincide with the larval recruitment period (STOI, personal communication), yet many of the larvae collected during peak drift had not yet made the transition to exogenous feeding. Of the 590 individuals collected, all but nine had empty stomachs, and many still had yolks (Reihart, 2016). Due to the rapid mortality and declining density of drifting larvae during this period, cohesive diet studies from the Roosevelt reach have been effectively non-existent. In the few larvae collected with full stomachs, Reihart (2016) found that they had consumed mostly Chironomids and Calanoid copepods. This lends credibility to the idea that Chironomids are a suitable prey source, but sample sizes were not large enough to draw conclusions about prey selection patterns in Upper Columbia sturgeon larvae as a whole. Besides prey availability, there may be other potential limiting factors to successful larval recruitment in this population, including changes to habitat and substrate quality (McAdam, 2015), and heavy metal-contaminated slag from upstream sources, which collect in the benthos, presenting a danger to early stage white sturgeon (Little et al., 2014). Many metals are acutely toxic to young sturgeon, and slag contaminated sediments are also less readily colonized by BMIs, leading to a potential prey deficit and inhospitable habitat in those areas (Little et al., 2014).

Despite a lack of specific data on larval sturgeon diet in the study reach, the data collected help to paint a clearer picture of the BMI community in the impounded Upper Columbia and which taxa are most abundant at certain times and places. A pattern emerged connecting high flow events and drawdown periods with low-diversity benthic communities dominated by *Hydra*, followed by periods of higher diversity but lower density after the second reservoir drawdown. These data suggest that the BMI community in this reach experiences drastic changes based on seasonal and zonal hydrologic fluxes, similar to other stream BMI communities around the world (Chi et al., 2017; Li et al., 2015; Jacobsen et al., 2013; Miller and Judson, 2014; Santos et al., 2017; Schulting et al., 2016; Szczerkowska-Majchrzak et al., 2014; Villenueve et al., 2018;

Zhou et al., 2017). However, the dominance of freshwater Cnidarians during high flow events in large rivers is thus far undocumented. Further study is needed to understand how and why they persist in extreme hydrological conditions, and how their prevalence could affect prey choice in fishes.

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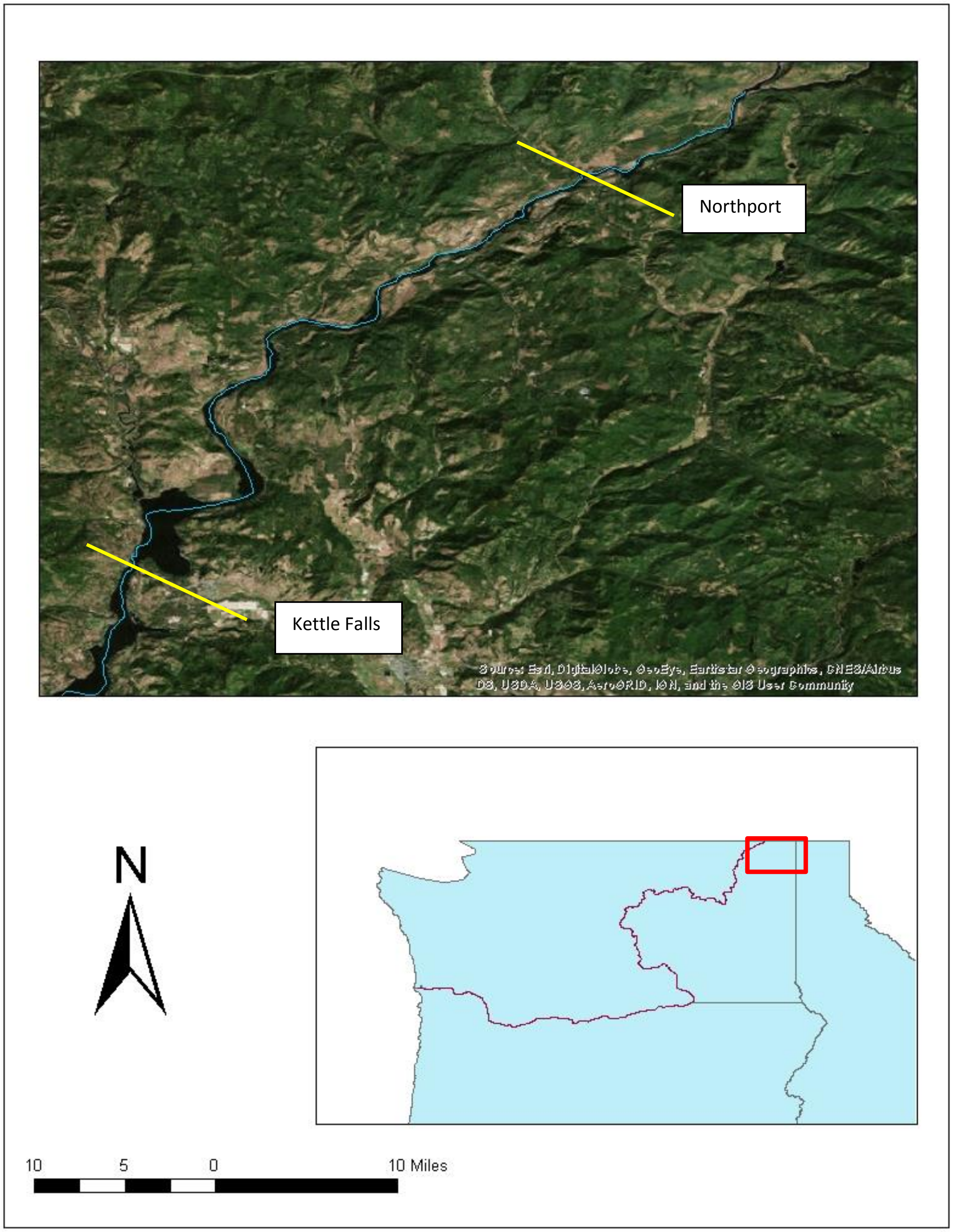
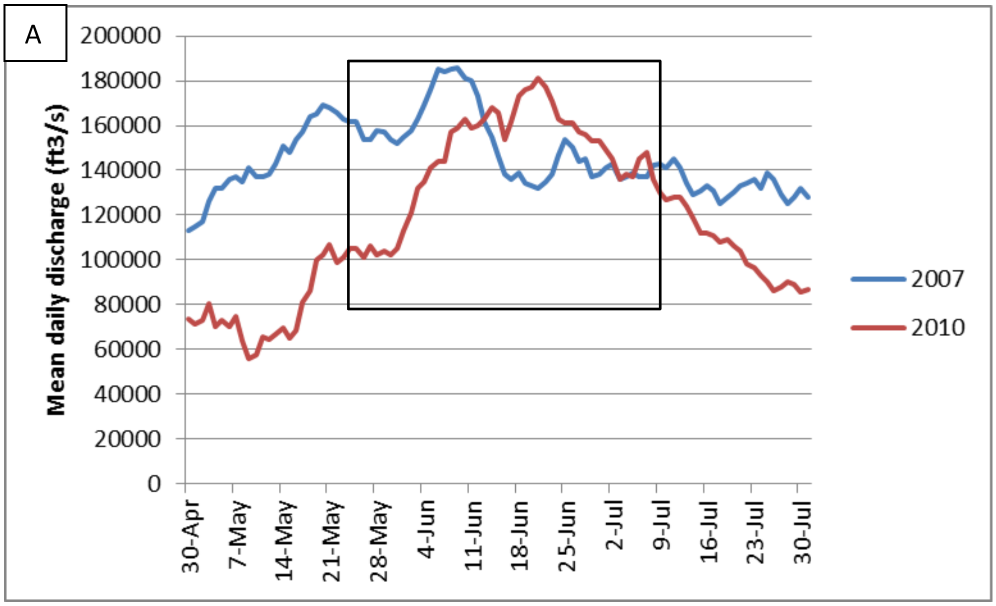


Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 1: Map of the study area, Upper Columbia River, WA, USA (Rkm 1141-1175).



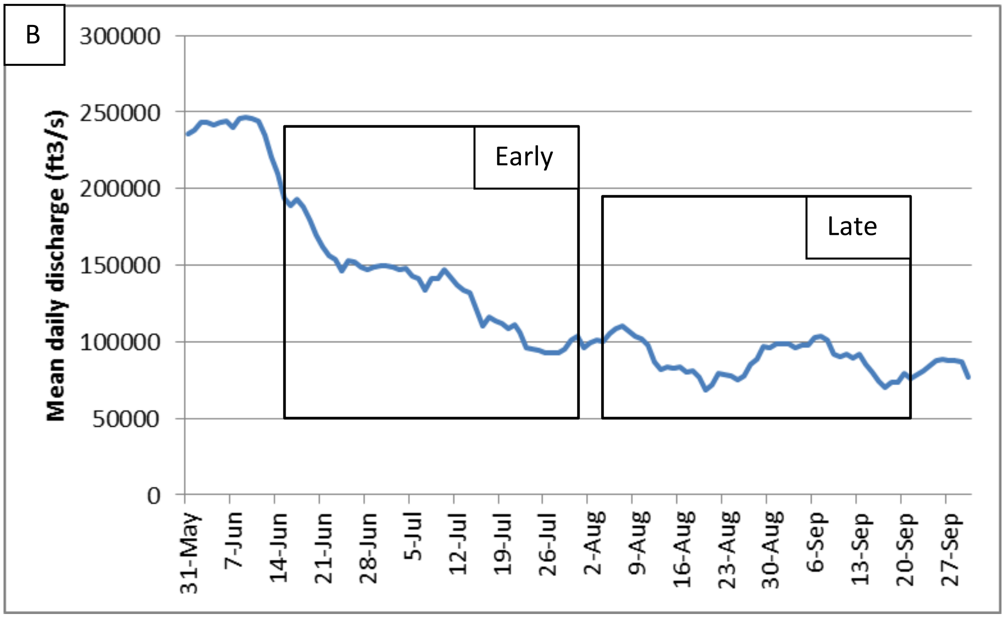


Figure 2: Changes in discharge over time during the sampling periods in 2007, 2010 (A), and 2017 (B). Black boxes indicate sampling periods. All data acquired from the US Geological Survey (2018).

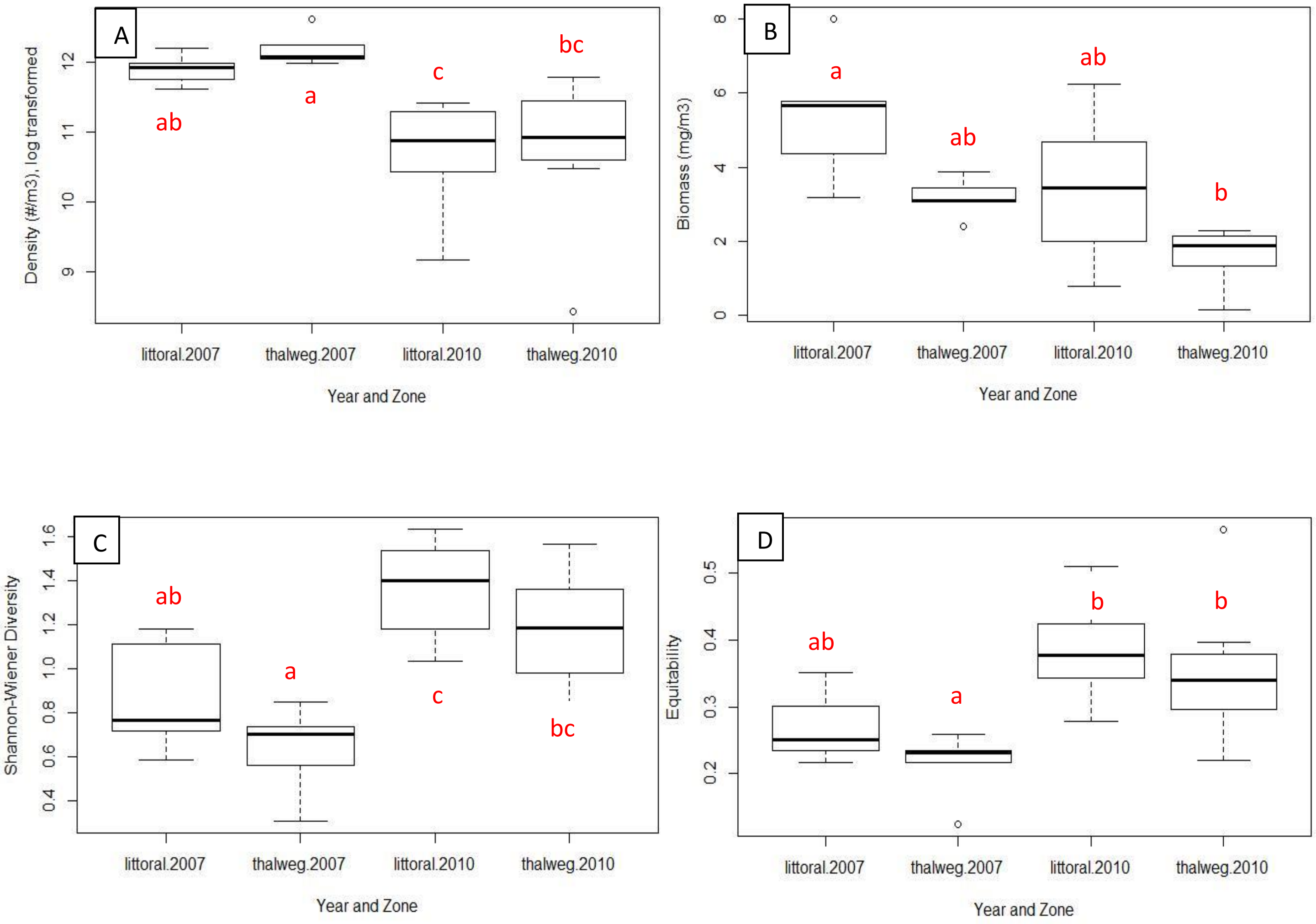


Figure 3: ANOVA boxplots displaying effects of year and zone on density (A, year $p<0.001$, zone $p=0.628$), biomass (B, year $p=0.009$, zone $p=0.004$), Shannon-Wiener Diversity (C, year $p<0.001$, zone $p=0.037$) and equitability (D, year $p<0.001$, zone $p=0.177$). Red letters represent Tukey's post-hoc results.

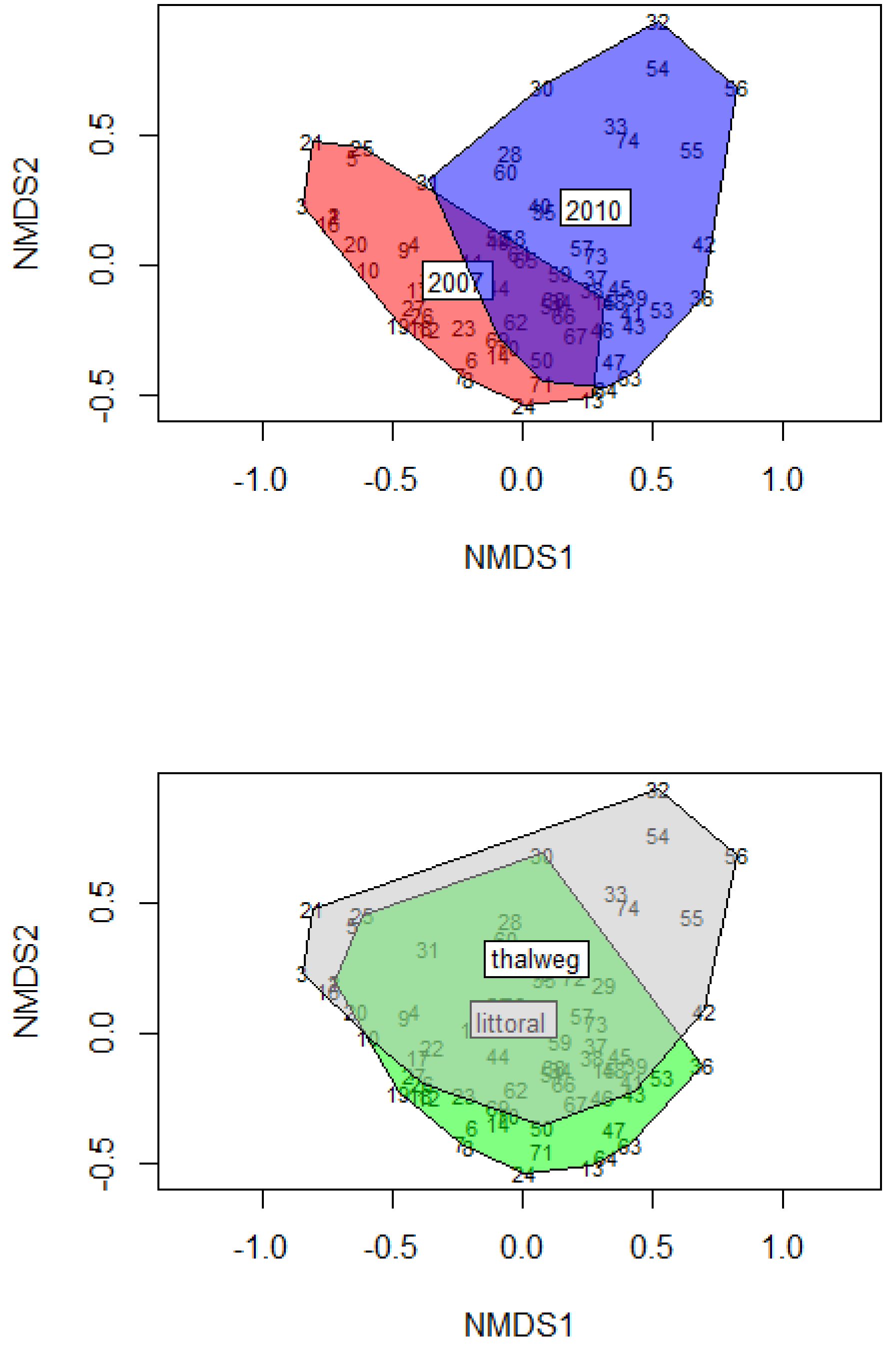


Figure 4: NMDS plots of the BMI community, grouped by year (top, p<0.001), and zone (bottom, p=0.008)

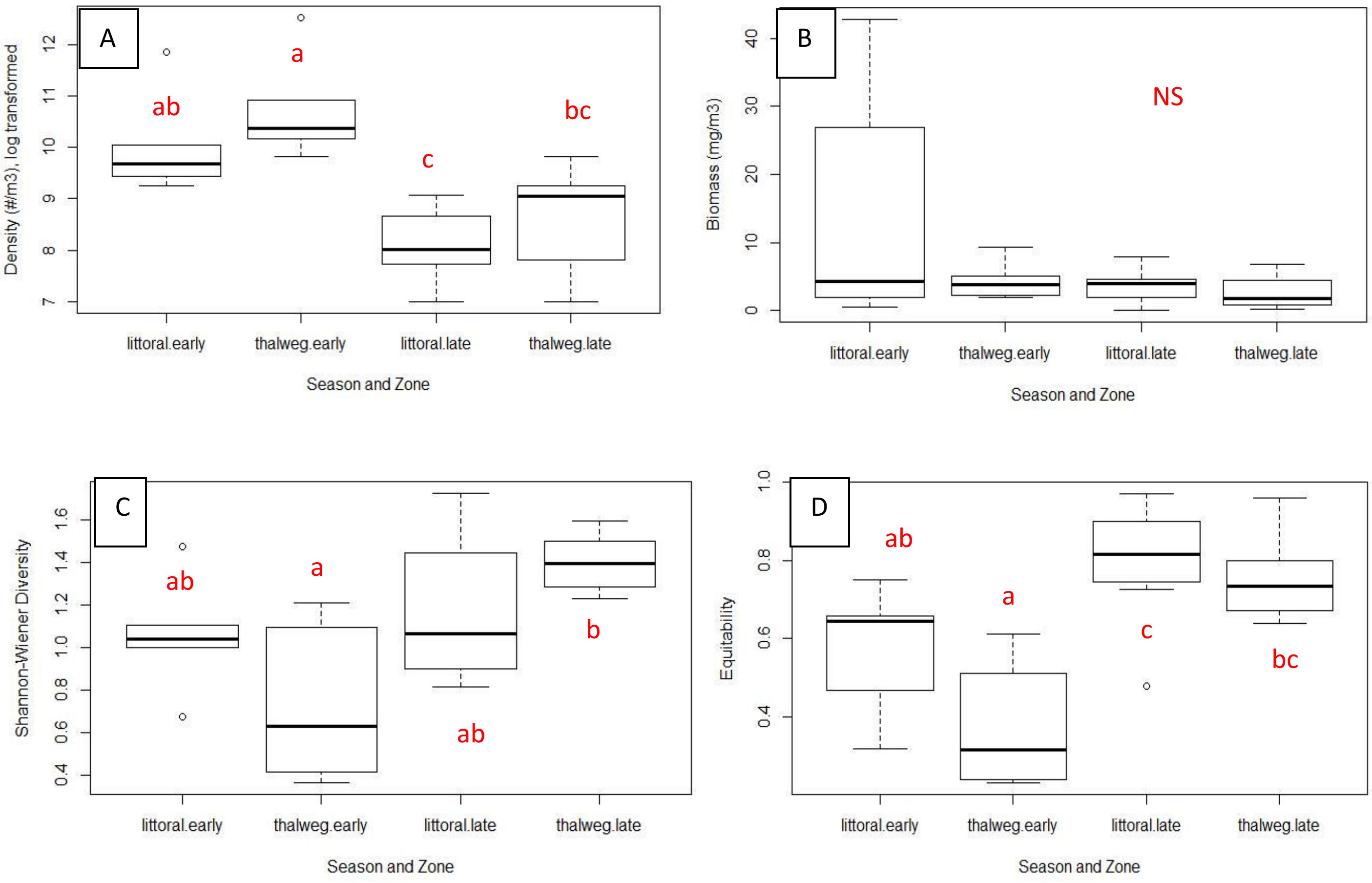


Figure 5: ANOVA boxplots displaying effects of season and zone of density (A, season $p<0.001$, zone $p=0.101$), biomass (B, season $p=0.079$, zone $p=0.219$) Shannon Wiener Diversity (C, season $p=0.007$, zone $p=0.904$) and equitability (D, season $p<0.001$, zone $p=0.072$). Red letters represent Tukey's post-hoc results.

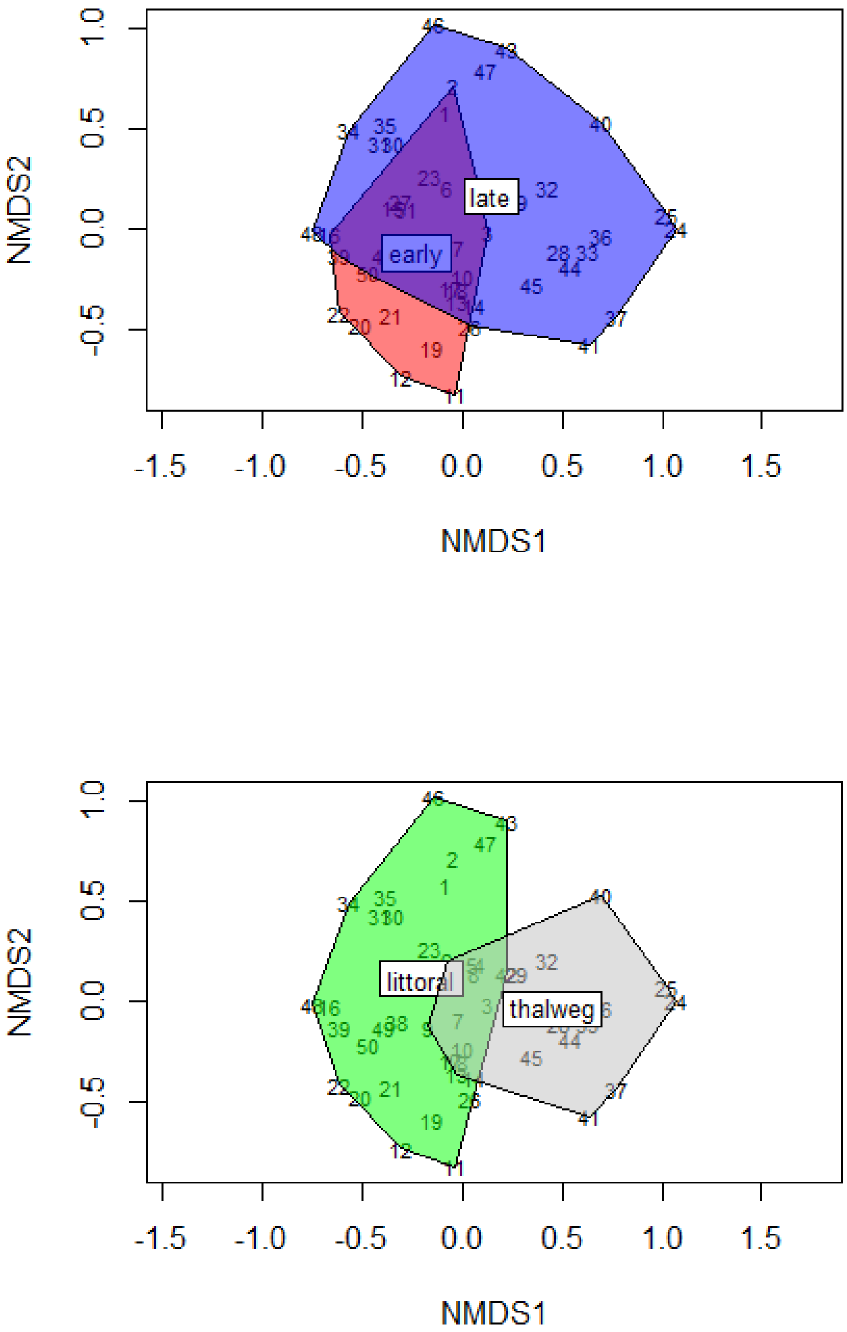
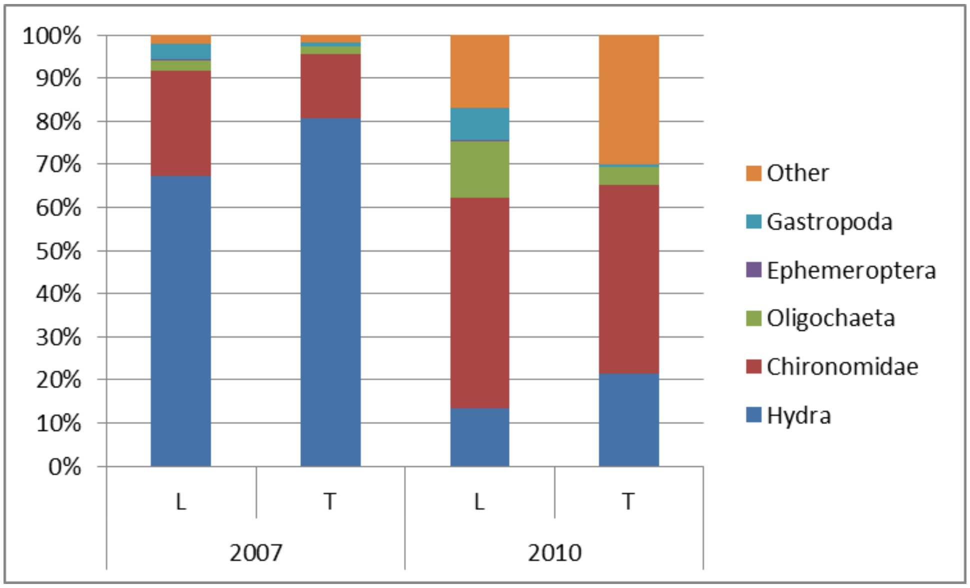


Figure 6: NMDS plots of the 2017 BMI community, grouped by season (top, p<0.001) and zone (bottom, p<0.001)



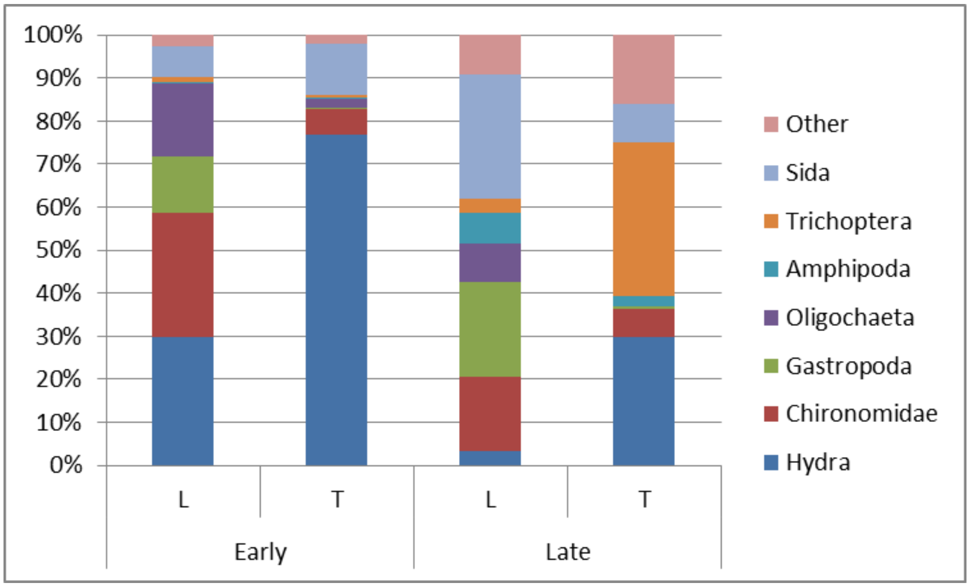
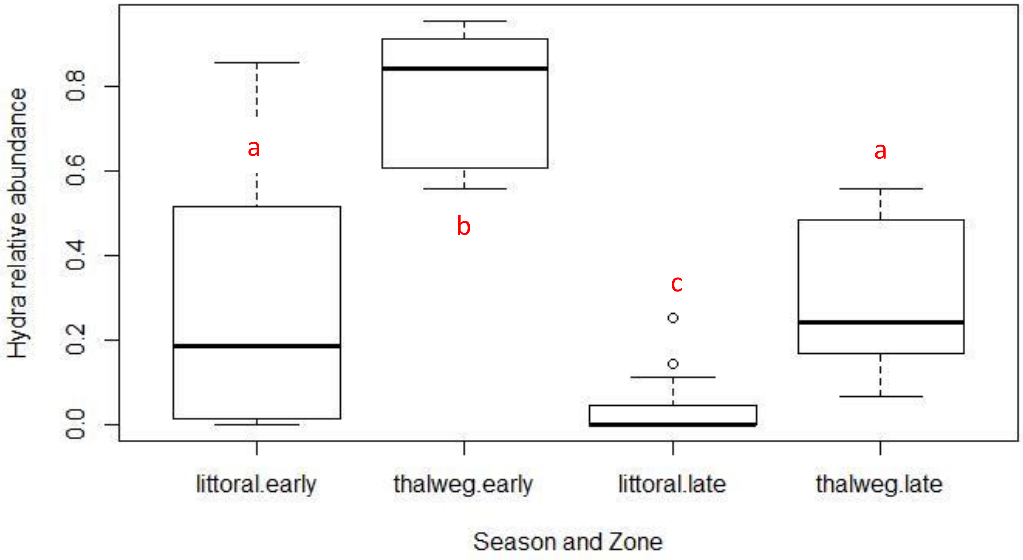


Figure 7: Comparisons of community structure between different years (top), seasons (bottom), and zones.



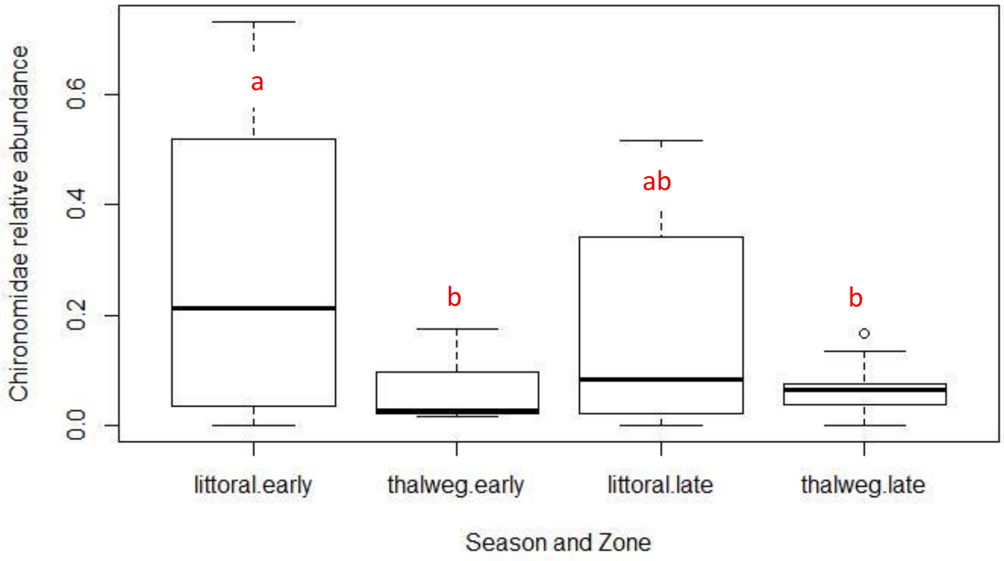


Figure 8: Relative abundance of prominent taxa *Hydra* (top, season and zone p<0.001) and Chironomidae (bottom, season p=0.301, zone p=0.002) in both seasons and zones. Red letters represent Tukey's post-hoc results.

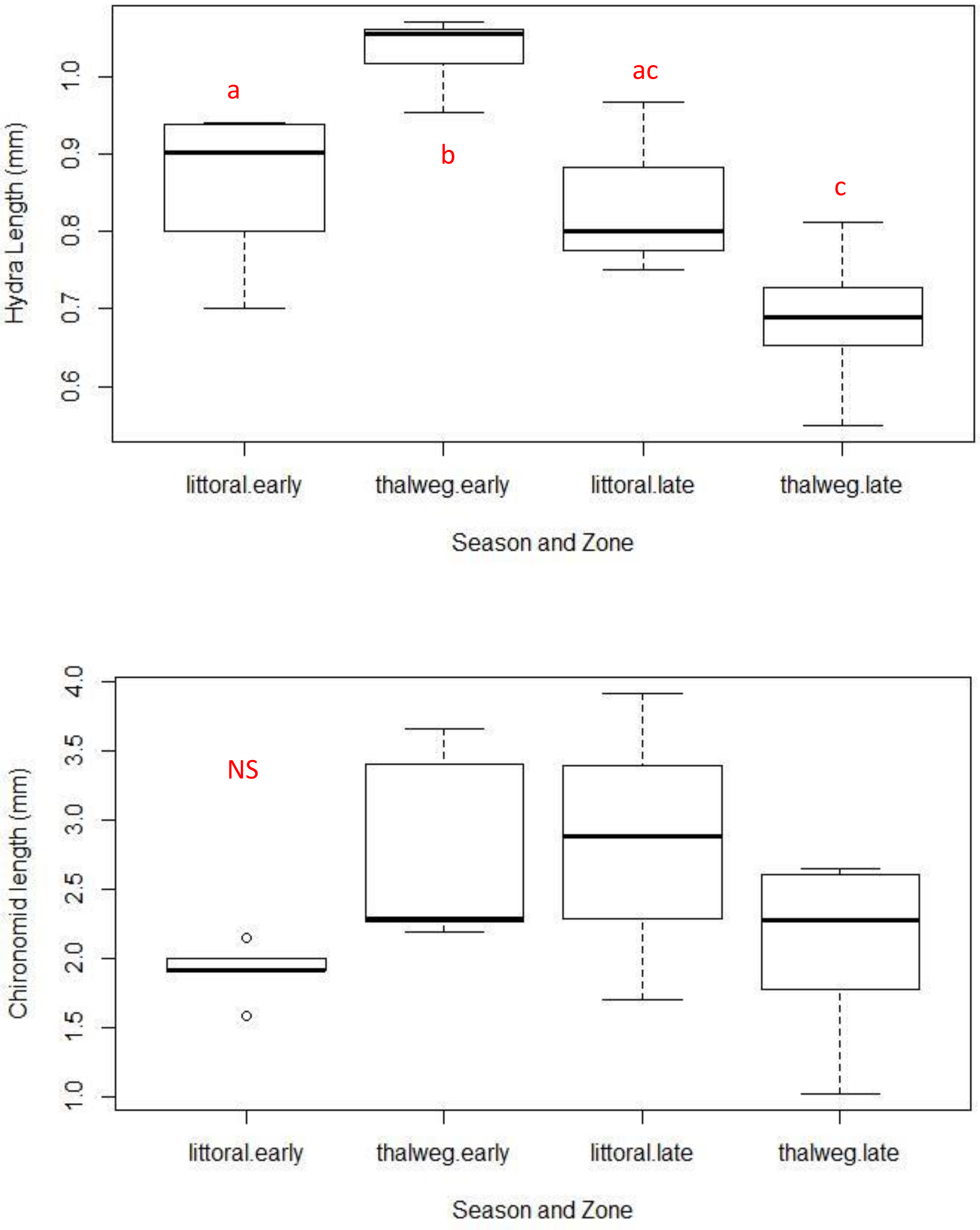


Figure 9: ANOVA boxplots displaying effects of season and zone on average size of *Hydra* (top, $p<0.001$ and $p=0.413$, respectively) and Chironomid (bottom, NS) individuals. Red letters represent Tukey's post-hoc results.

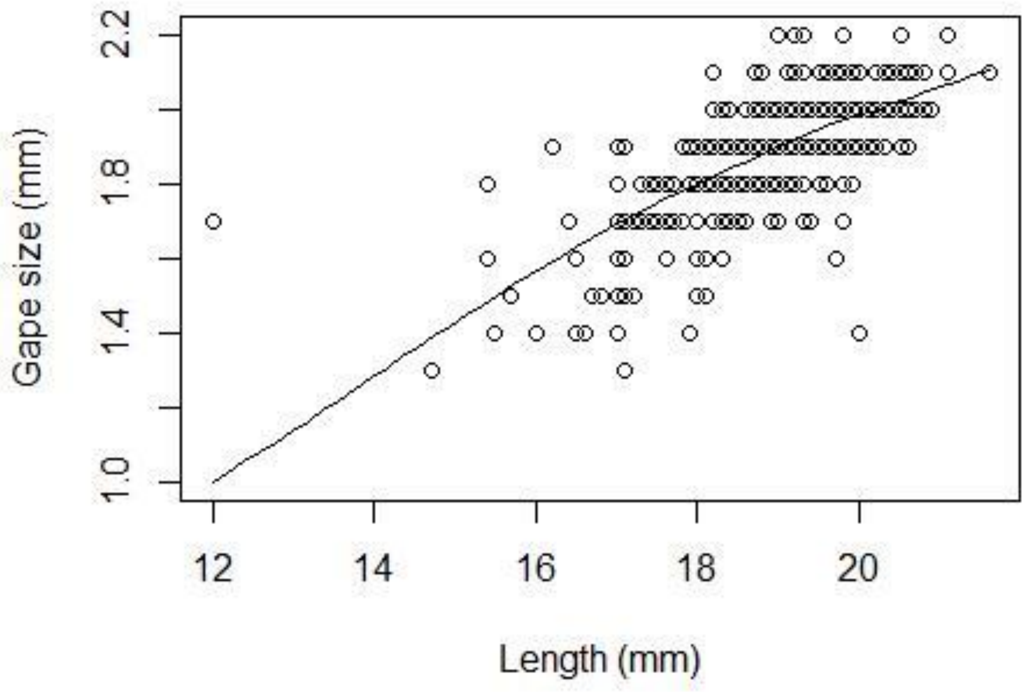


Figure 10: Linear regression between larval sturgeon fork length (mm) and gape size (mm) ($y=0.0962x + 0.0624$, $R^2= 0.4753$, $p<0.001$)

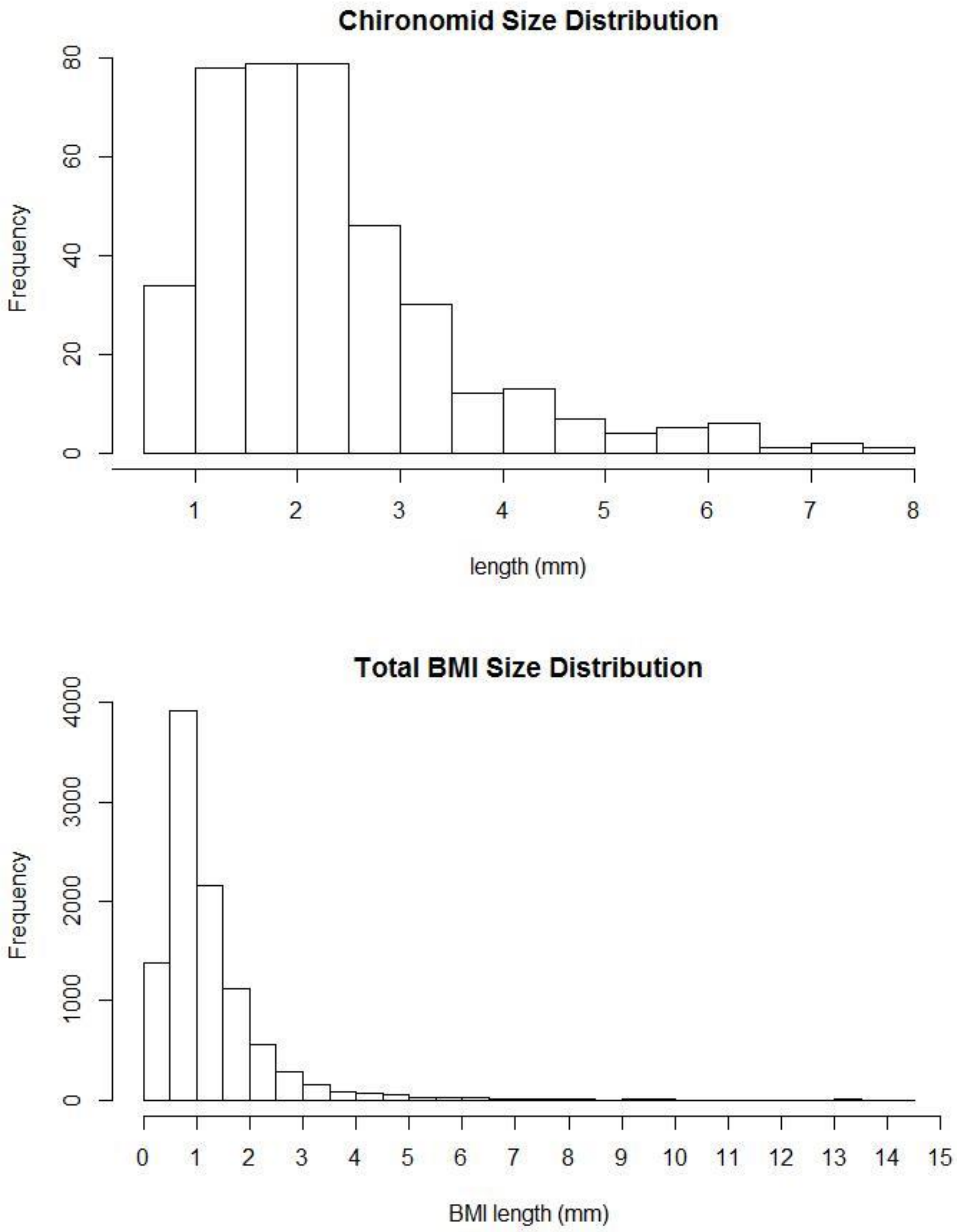


Figure 11: Distribution of length of Chironomids collected in 2017 (top), and total distribution in length of all BMI taxa throughout the study period (bottom)

Dependent Variable	Source of Variation	SS	df	MS	F
Density	Year	10.36	1	10.36	19.184**
	Zone	0.13	1	0.13	0.242
	Year x Zone	0.102	1	0.102	0.189
	Residual	11.882	22	0.54	
Biomass	Year	17.35	1	17.35	8.279
	Zone	21.91	1	21.91	10.453
	Year x Zone	0.62	1	0.617	0.294
	Residual	46.11	22	2.096	
Shannon-Wiener Diversity	Year	1.652	1	1.652	29.253**
	Zone	0.278	1	0.278	4.916*
	Year x Zone	0.005	1	0.005	0.092
	Residual	1.2423	22	0.0565	
Equitability	Year	0.0975	1	0.0975	16.505**
	Zone	0.0115	1	0.0115	1.948
	Year x Zone	0.0009	1	0.0009	0.157
	Residual	0.1299	22	0.0059	

Table 1: Analysis of Variance results for effects of year and river zone on log-transformed BMI density, biomass, Shannon-Wiener diversity and equitability. * = $p < 0.05$, ** = $p < 0.001$

Dependent Variable	Source of Variation	SS	df	MS	F
Density (log transformed)	Season	24.9	1	24.9	29.754**
	Zone	2.45	1	2.45	2.922
	Season x Zone	0.113	1	0.113	0.135
	Residual	18.426	22	0.838	
Biomass	Season	250.7	1	250.7	3.387
	Zone	118.7	1	118.7	1.604
	Season x Zone	102.7	1	102.7	1.387
	Residual	1628.8	22	74.03	
Shannon-Wiener Diversity	Season	0.82	1	0.82	8.815*
	Zone	0.0014	1	0.0014	0.015
	Season x Zone	0.423	1	0.423	4.544*
	Residual	2.05	22	0.093	
Equitability	Season	0.548	1	0.548	24.894**
	Zone	0.079	1	0.079	3.574
	Season x Zone	0.035	1	0.035	1.601
	Residual	0.485	22	0.022	
Hydra abundance	Season	1.697	1	1.697	44.08**
	Zone	1.551	1	1.551	40.27**
	Season x Zone	0.133	1	0.133	3.45
	Residual	1.8098	47	0.039	
Chironomid abundance	Season	0.035	1	0.035	1.094
	Zone	0.332	1	0.332	10.433*
	Season x Zone	0.041	1	0.041	1.283
	Residual	1.494	47	0.032	
Hydra length (mm)	Season	0.203	1	0.203	27.685**
	Zone	0.005	1	0.005	0.708
	Season x Zone	0.119	1	0.119	16.169**
	Residual	0.118	16	0.0073	
Chironomid length	Season	0.01	1	0.01	0.025
	Zone	0.152	1	0.152	0.366
	Season x Zone	2.875	1	2.875	6.943*
	Residual	6.626	16	0.4141	

Table 2: Analysis of Variance results for effect of season and river zone on log-transformed density, biomass, Shannon-Wiener diversity and equitability of BMIs, as well as relative abundance of *Hydra* and Chironomidae, and length of individuals in both taxa. * = $p < 0.05$, ** = $p < 0.001$

Dependent Variable	Source of Variation	SS	df	MS	F
Community Composition 2007/2010	Year	4.1213	1	4.1213	31.2512**
	Zone	0.4735	1	0.4735	3.5908*
	Year x Zone	0.0768	1	0.0768	0.5824
	Residual	9.2313	70	0.1319	
Community Composition 2017	Season	2.4905	1	2.4905	10.2748**
	Zone	2.0083	1	2.0083	8.2855**
	Season x Zone	1.067	1	1.067	4.4019**
	Residual	11.3924	47	0.24239	

Table 3: Permutational Analysis of Variance results for effect of year, season and river zone on community composition of BMIs in the study reach. * = $p < 0.05$, ** = $p < 0.001$

Chapter 2

A comparison of three common benthic sampling methods and their efficacy for invertebrate community analyses in an impounded river system

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Abstract

In the summers of 2007-2010, the Spokane Tribe of Indians collected benthic macroinvertebrates (BMIs) from the Upper Columbia River using three techniques: benthic sled, benthic drift, and artificial substrates. An analysis of the samples collected by these methods revealed significant discrepancies in the types of organisms caught. Benthic sled and drift methods collected mainly planktonic taxa, primarily small crustaceans. This varies significantly from the community structure of the artificial substrate samples ($p<0.001$), which contained primarily Cnidarian polyps (Hydra sp.), and Chironomids. Collectively, these two taxa made up an average of 93.6% of the individuals in artificial substrates in 2007, and 63.7% in 2010. Though artificial substrate samples were less diverse than samples collected by other methods ($p<0.001$), the samples contained more benthic oriented taxa. These data suggest that, of the benthic sampling methods presented, artificial substrates are most applicable to the study area, as they provided a more accurate representation of the BMI community with little or no overlap with the zooplankton community.

Introduction

Benthic macroinvertebrates (BMIs) are important to the study of freshwater ecosystems as indicators of water quality, ecosystem health, and environmental change. BMIs include primarily insect larvae, as well as several non-insect invertebrate taxa including crustaceans, worms and other phyla. These organisms are ubiquitous in fresh waters around the world, and account for a majority of the secondary production in lotic ecosystems (Beamesderfer and Farr, 1997). Since several common taxa are sensitive to changes in physicochemical parameters, BMI communities can undergo drastic shifts based on environmental conditions and local hydrological fluxes (Chi et al., 2017; Santos et al., 2017). In stream ecosystems, BMI studies are relatively easy to conduct, and provide valuable information about the health and trophic status of the stream, as well as prey availability for larger animals.

Though pervasive in stream ecology, BMI studies on large impounded rivers are relatively limited, partially due to sampling difficulties caused by high depth and water velocity. This lack of reliable and standardized information about BMI assemblages leaves severe gaps in our understanding of riverine food webs and responses to natural and anthropogenic disturbance (DiSabatino et al., 2016). Such harsh conditions prohibit the use of many standard quantitative methods used in wadeable streams, and may lead researchers to choose an alternative study design, including using small-stream methods such as kick netting in nearshore areas or sampling tributaries instead of main channel habitats (Santos et al., 2017; Zhou et al., 2017). Several methods of collection on large rivers have been employed in prior studies, which are diverse in form and function. These include active methods like benthic grabs, as well as passive methods like benthic drift nets and several forms of artificial substrates which become colonized by BMIs over a designated time period. In order to obtain reliable BMI community data from large river and reservoir systems, it is necessary to determine which collection method will be most applicable to the study site.

In the summers of 2007 and 2010, the Spokane Tribe of Indians collected BMIs from the Upper Columbia River, WA as part of an ongoing study investigating potential causes for recruitment failure in larval white sturgeon. Since mortality occurred primarily during the first-feeding phase, it has been hypothesized that low availability of suitable prey (BMIs) is the limiting recruitment in this population. In order to address this question, it is first necessary to accumulate baseline data about the structure of the BMI community within the study reach and how it changes spatially and temporally in response to hydrological fluxes. Three techniques were used to collect BMIs from this system: artificial substrates, benthic drift, and benthic sled tows. Though theoretically meant to accomplish the same goal, different methods can lead to significant discrepancies in the types of organisms collected. The purpose of this analysis was to quantitatively compare the efficacy of these three methods for collecting benthic community data in a large impounded river like the Upper Columbia where accurate BMI studies are needed and large rocky substrate prohibits use of other techniques.

Methods

Study Site

This study was conducted in the Upper Columbia River, WA, upstream of the Lake Roosevelt reservoir (Rkm 1141-Rkm 1175). Samples were collected in both the littoral zone (nearshore) and thalweg (mid-channel) at nine sites along the study reach. Substrate consists primarily of large cobbles and boulders, with some deposition of finer sediments and organic matter in the shallow littoral zones.

Artificial Substrates

Artificial substrates consisted of cylindrical metal baskets filled with concrete cones meant to imitate large cobble substrate (Figure 1). Weighted lines with three baskets each were deployed on the river bottom at each site; one line in the littoral zone and one in the thalweg. After a 6-week incubation period from late May to July, lines were collected and each basket emptied. Concrete cones were scrubbed and rinsed to remove attached invertebrates, and the contents rinsed into collection jars and stained with Rose Bengal dye for analysis.

Benthic Sled

The benthic sled apparatus consists of an iron frame (112 x 35.5 cm), with two nets attached (each measuring 56 x 35.5 cm) (Figure 1). Benthic sled collections were taken at the same nine sites by dragging the apparatus along the substrate. The two nets represent two replications for each location. A flow meter (General Oceanics, model

2030 R) attached to each net was used to determine distance dragged at each location. Each replicate sample was rinsed into collection jars and stained with Rose Bengal.

Benthic Drift

Rectangular drift nets (15 x 30 cm) were deployed twice daily for 15 minutes at each location (Figure 1). Collections occurred at dawn and dusk, when drift densities of invertebrates tend to be highest. Following the collection period, BMIs were rinsed into sample jars and stained with Rose Bengal.

BMI analysis

Samples collected by all three techniques were analyzed in the same manner. Sample material was divided into square gridded petri dishes, each of which were divided into four quadrants. A quadrant was selected at random, and all BMIs within that quadrant were identified to the lowest practical taxon. Additional quadrants were chosen until the total number of BMIs counted reached 500. If the sample contained less than 500, all individuals were counted and identified.

Taxon diversity was calculated using the Shannon- Wiener Index, and compared between methods using Analysis of Variance (ANOVA) and Tukey’s post-hoc test. Differences in community structure between the three sampling methods in each year were explored using Non-metric multidimensional scaling (NMDS) and an associated PERMANOVA hypothesis test (R, version 3.3.2).

Results

In both years, artificial substrate samples contained significantly different BMI taxa than either benthic sled or drift samples ($p<0.001$)(Figure 2), though the latter two were similar. Both benthic sled and drift samples contained primarily small crustaceans, including copepods and Cladocerans. By contrast, freshwater Cnidarian polyps (*Hydra* sp.) were the dominant taxon in artificial substrates, followed closely by Dipteran larvae (family Chironomidae) (Figure 4). Despite this difference, taxa diversity was also lower on average in artificial substrate samples ($p<0.001$, see Figure 3).

Discussion

Analysis of BMI samples revealed significant differences in assemblage structure between samples collected by each method. Drift and sled samples contained a similar collection of taxa, but the taxa which dominated artificial substrate samples were significantly different. In these samples, we found mostly freshwater Cnidarians in the genus Hydra, along with Chironomid larvae and some Oligochaetes. Interestingly, the drift and sled samples contained mostly Cladocerans and Copepods, which are primarily planktonic taxa. This indicates that these two methods are failing to accurately sample the benthic community and instead are collecting mostly zooplankton from the water column. One potential reason for this discrepancy could be the nature of the substrate in this reach of the Upper Columbia. Substrate in the riverine zone of the reservoir consists of mostly large cobbles and boulders, which may not be suitable for methods which rely on the stirring of sediments to collect invertebrates. When using substrates, invertebrates are allowed to colonize the cones naturally, likely leading to increased representation of benthic taxa, even though taxon diversity was relatively low compared to the wide variety of zooplankton genera collected by the other two methods.

The artificial substrate method also more accurately displays the natural shifts in community structure which accompany local hydrological fluxes. The years 2007 and 2010 were chosen for this analysis due to a particularly wide difference in mean daily discharge during the study period. Discharge values in 2007 were consistently almost double those of 2010 throughout May and June, leading to notable shifts in the BMI community. However, in samples which contained mostly zooplankton, the effects of year on the BMI community are not as prominent. This suggests that using these methods in similar systems may lead to a less complete understanding of the natural patterns of BMI abundance and diversity within the study reach. Given the results of this study, artificial substrates appear to be the most effective benthic sampling method in the upper Columbia River. Various types of artificial substrates such as unglazed tiles and leaf nets have been used successfully in the past (DiSabatino et al., 2016), and such methods may be the most accurate and informative procedure to sample BMIs in similar large, non-wadeable rivers.

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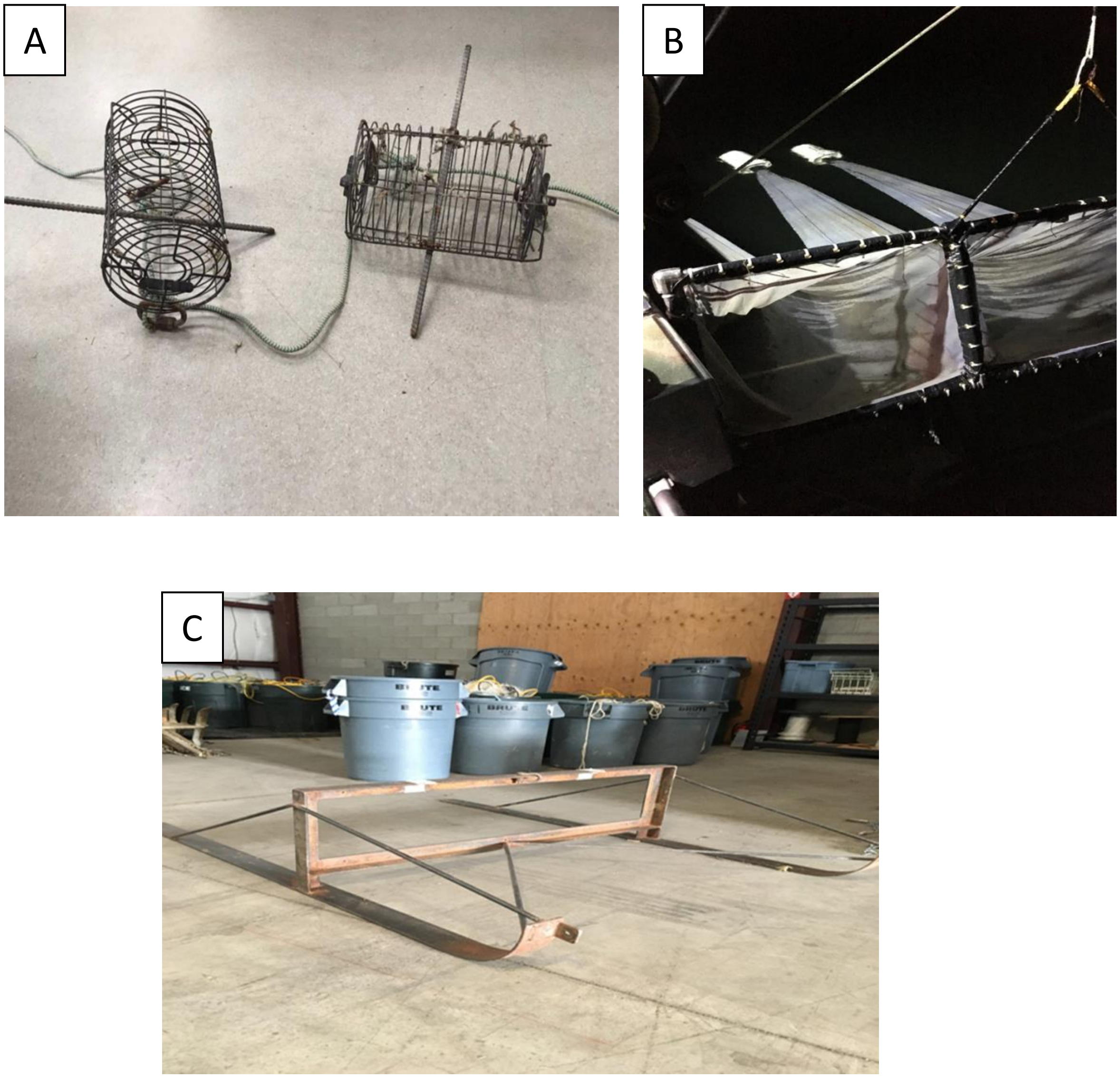

Figure 1: Three apparatuses used to sample the benthic macroinvertebrate community within the study site: artificial substrates (A), benthic drift (B) and benthic sled (C).

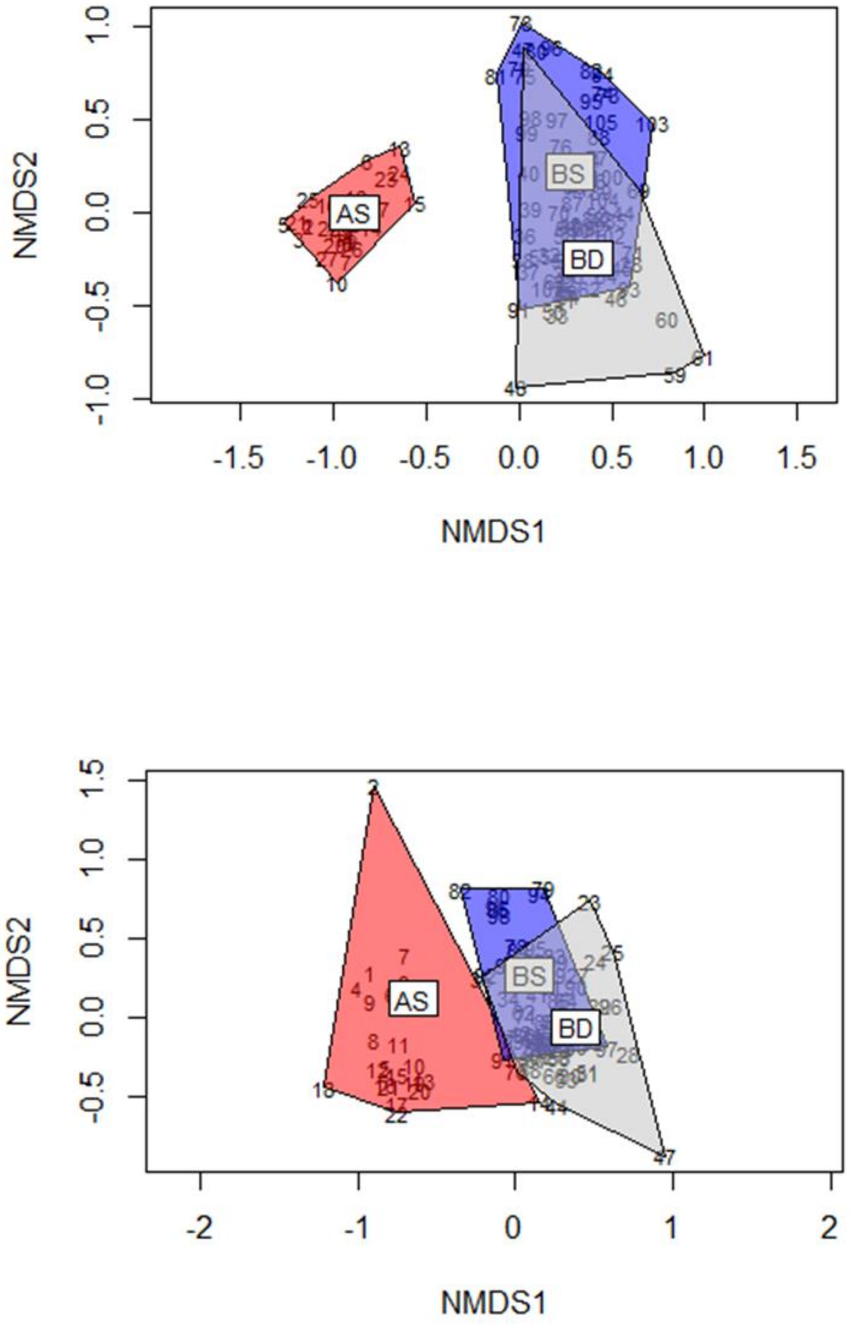

Figure 2: NMDS plots showing differences in BMI communities sampled by 3 techniques (AS = artificial substrate, BD = benthic drift, BS = benthic sled) in 2007 (top), and in 2010 (bottom) (PERMANOVA, p<0.001)

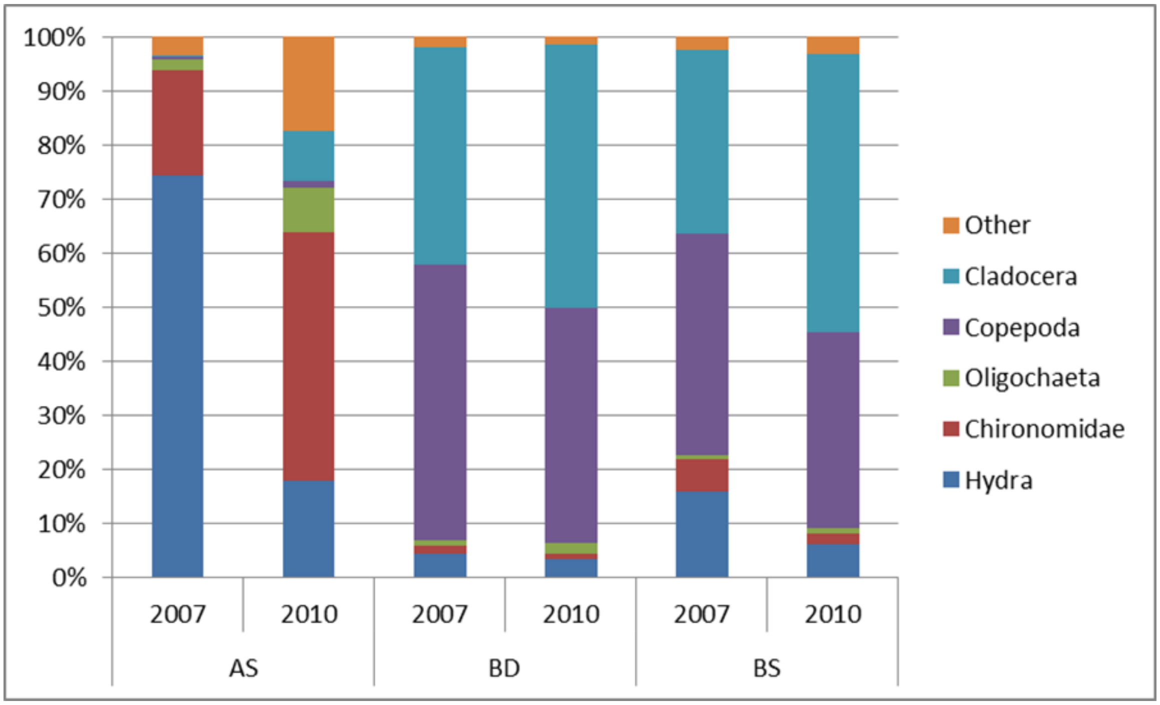

Figure 3: Taxa composition of benthic samples collected by three techniques (AS = artificial substrate, BD = benthic drift, BS = benthic sled).

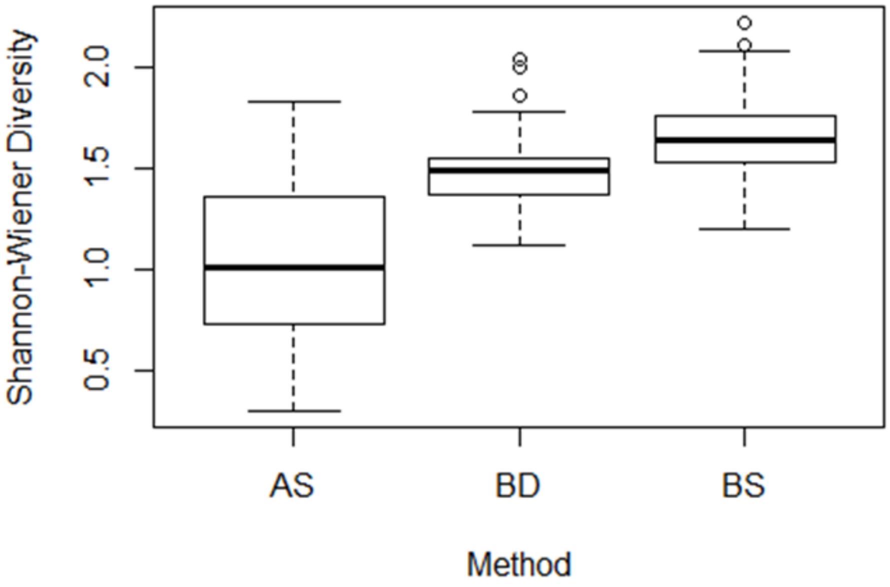

Figure 4: Shannon-Wiener Diversity of samples collected by three techniques (AS = artificial substrate, BD = benthic drift, BS = benthic sled; ANOVA, $p<0.001$)

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