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Macroinvertebrate Community and Temperature Changes in a Michigan Stream

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Contents

Abstract

As the climate warms, global biodiversity is plummeting and extinction rates are rising (Jenkins, 2003). Freshwater ecosystems specifically are experiencing huge losses in diversity (Jenkins, 2003; Ormerod et al., 2010; Collier et al., 2016). Conservation of invertebrates is particularly urgent because they are foundational to the trophic systems in streams and lakes, comprising 95% of all species on earth (Titley et al., 2017), and over 60% of biodiversity in freshwater ecosystems (Collier et al., 2016). Through analysis of both my own field measurements and historical data I seek to gain a preliminary understanding of temperature and macroinvertebrate community changes that have taken place in a small stream in Michigan, in order to develop baseline data that will aid in identifying changes in the future. I utilize data before and after the installation of a unique stream cooling modification to explore: (1) How temperature has changed over time in Pine River? (2) How the macroinvertebrate community changed over time, as compared to a 1993 baseline? and (3) What do these changes mean for the community health of this stream? I compare my own 2020 aquatic macroinvertebrate samples to a 1993 macroinvertebrate dataset and look for statistically significant changes in order level percent abundance and taxa density. I analyze temperature data and find a significant cooling trend as a result of an anthropogenic temperature manipulation. Although Pine River has cooled since 1993 the macroinvertebrate community remains quite similar, with the exception of a significant increase in Trichoptera. My research suggests that this location could provide a refuge for species in the region that are negatively affected by warming water temperatures, and highlights the need for a better understanding of the factors that influence macroinvertebrate communities.

Acknowledgements

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I am grateful to Sam Desmet and the Huron Mountain Club for allowing me to access and study the unique and special ecosystem of Pine River.

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Lastly I would like to thank my fearless field assistant and father Jamie Campbell for braving near freezing water conditions, in the name of science, and my mother for her constant support through many long hours of work.

Keywords: *Aquatic, Biodiversity, Biology, Climate Change, Entomology, Environmental Monitoring, Environmental Sciences, Freshwater, Macroinvertebrates, Survey, Temperature Manipulation, Water Resource Management.*

Introduction

It is well understood that temperatures across the globe are rising due to anthropogenic greenhouse gas emissions, primarily from the burning of fossil fuels (Allen M.R. et al., 2018). Indeed, global temperatures have already risen by nearly 1˚C (Allen M.R. et al., 2018). Exactly how much temperatures will increase in the future is highly dependent on our actions in the coming decade, and will vary extensively by region. This widespread warming harms both aquatic and terrestrial ecosystems. Despite having the highest diversity relative to area of any aquatic ecosystems, freshwater aquatic ecosystems have seen the most dramatic decreases in biodiversity to date (Jenkins, 2003; Ormerod et al., 2010; Collier et al., 2016).

Since invertebrates make up 95% of all species on earth, biodiversity losses will have huge consequences for these small but important organisms (Titley et al., 2017). Invertebrates are systematically underrepresented in literature about biodiversity, which is why further research on these organisms is critical (Titley et al., 2017). Although less charismatic than larger organisms, aquatic macroinvertebrates such as insects, molluscs, gastropods, crustaceans, and worms comprise over 60% of the biodiversity found in freshwater aquatic ecosystems (Collier et al., 2016).

Climate change is already harming aquatic macroinvertebrates, with roughly 14% of freshwater species listed as vulnerable or near threatened and 11% endangered or critically endangered (Collier et al., 2016). Warming water is also changing the composition of macroinvertebrate communities across the globe, pushing many cold water species north (Daufresne et al., 2004; Chessman, 2009). Sadly, 0.7% of freshwater macroinvertebrates species are already extinct, and this is likely an underestimation due to the nearly 8,000 species (30%) currently classified by the IUCN red list as *Data Deficient* (Collier et al., 2016). Driving this loss is the fact that aquatic ecosystems are particularly vulnerable to compounding threats from multiple sources because water serves to efficiently vector changes occurring across a large area (Ormerod et al., 2010; Fausch et al., 2010).

These losses and changes are important because freshwater macroinvertebrates are important food sources for fish such as trout, as well as many other aquatic and terrestrial species (Fausch et al., 2010; Glaz et al., 2012). Stream ecosystems are closely linked to their surrounding forest ecosystems (Fausch et al., 2010). A lack of data at the macroinvertebrate level makes it difficult to predict how both communities will respond to changing climate stressors (Fausch et al., 2010). In the Northern Lakes and Forests ecoregion macroinvertebrates can be used as indicators to assess the health of entire ecosystems, data regarding macroinvertebrates is critical for conservation (Weigel et al., 2003).

Michigan is predicted to experience 6˚C of warming by 2100 under RCP 8.5, and 3.5˚C under RCP 6.0. (WMO Regional Climate Centre, Royal Netherlands Meteorological Institute, [KNMI], 2020). Rising temperatures pose a dire threat to the biodiversity of Michigan's 58,000 km of streams and well over 10,000 bodies of still water, (Michigan Department of Environment, Great lakes, and Energy, 2020). Understanding how the macroinvertebrate community responds to temperature in this ecoregion is key to preserving freshwater biodiversity and ecosystem health in the face of climate change. This is why I have chosen to examine the relationship between temperature and macroinvertebrates in this region.

I aim to gain a preliminary understanding of changes that have already taken place, and to develop baseline data that will aid in quantifying future changes. I use a combination of my own field samples and historical data to analyze changes in temperature and macroinvertebrate populations over time in Pine River, a lake feeder stream in the Upper Peninsula of Michigan.

In my research I seek to answer three main research questions: (1) How has temperature changed over time in Pine River as a result of anthropogenic temperature manipulation? (2) How has the macroinvertebrate community changed over time, as compared to a 1993 baseline? and (3) What do these changes mean for the community health of Pine River?

1.1 Historical Context of Pine River

Pine River is located on the private land of a hunting and fishing club, and has been remarkably well preserved from anthropogenic disturbances. I chose to study Pine River because previous research there provides macroinvertebrate and temperature data to which I could compare my own measurements. Pine River also has a unique history of anthropogenic temperature manipulations which make it a unique laboratory in which to study temperature changes.

The most notable disturbance on Pine River concerns the development of the impoundment at the outlet of Pine Lake. Prior to 2005 there was a small semi-permeable dam made of sticks, rocks, and mud, left from logging prior to 1900, over which water flowed when leaving Pine Lake into Pine River (Jamie Campbell, personal communication, May 2020). See Appendix A for a photograph of the old rock and stick dam.

In 2005 a concrete exotic species barrier was installed to replace the old rock and stick dam at the outlet of Pine Lake into Pine River to prevent Round goby, *(Neogobius melanostomus)* and Eurasian ruffe, *(Gymnocephalus cernua)* from swimming upstream and invading Pine Lake. See Appendix B for a photograph of the concrete invasives barrier. Flow over this barrier ranges from 4.2 cubic meters per second in the spring to 0.42 cubic meters per second in mid-summer (Jamie Campbell, CWT Project Engineer, personal communication, May 2020). Water flowing over both the old rock and stick dam and the invasives barrier came only from the topmost layer of Pine Lake, above the thermocline. In midsummer water temperatures in the upper reaches of Pine River routinely reached temperatures over 25˚C (Jamie Campbell, CWT Engineer, personal communication, May 2020; Temperature data provided by David Costello, personal communication, 2015). Anglers suspected that these high temperatures were killing trout fingerlings. To mitigate these warm temperatures a cold water manipulation was installed in 2015. Known as the *Cold Water Tap* (CWT) this system of pipes brings 0.17 cubic meters per second of 6.6 ˚C degree water from 12 meters down in Pine Lake, below the thermocline, out through pipes penetrating the bottom of the concrete barrier (Jamie Campbell, CWT Engineer, personal communication, May 2020). See Appendix C for more information on the CWT. The anglers anticipated that under low flow conditions the CWT would significantly reduce the stream temperature.

2.0 Methods

2.1 Study site

The study site is located in the Huron Mountain region of Michigan's Upper Peninsula. This region is characterized by rolling hills and numerous small lakes. Bedrock in this area is generally granite or sandstone. It has a boreal climate and forests are dominated by mixed conifer and deciduous hardwood forests (Yanoviak & McCafferty, 1996).

Pine River is located within Powell Township outside of Marquette, Michigan on land owned by a private organization, the Huron Mountain Club. Pine River is a 2.2 kilometer stream which connects inland Pine Lake to Lake Superior. Although it is small it has a relatively large watershed, draining 25 square kilometers of mostly undisturbed mixed conifer deciduous forest (Yanoviak & McCafferty, 1996). Pine River is virtually undisturbed along its length, with the exception of minor foot paths, two small road bridges, some shoreline cabins in the lower reaches, and an invasive species barrier at the outlet of Pine Lake. Riparian vegetation includes hemlock (*Tsuga canadensis*), northern white cedar (*Thuja occidentalis*), red and white pine (*Pinus resinosa* and *P. strobus*, respectively), as well as sugar maple (*Acer saccharum*), (*Quercus rubra*), yellow birch (*Betula alleghaniensis)*, and alder (*Betulaceae alnus*) in the lower reaches (Yanoviak & McCafferty, 1996). These species provide partial shading to the upper reaches of the stream where the channel is around 30 feet wide with vertical or gently sloping banks. The channel in the lower reaches becomes wider, less shaded, and is punctuated with sandbars

The streambed of Pine River is primarily sand, gravel, and cobbles in the upper reaches, where water flows swiftly creating riffles, runs, and slower pools around downed logs. Some submerged logs and branches are found along the sides of the channel in the upper reaches, with woody debris and submerged organic matter increasing downstream. Gravel and cobbles are generally not embedded, providing habitat for macroinvertebrates (United States Environmental Protection Agency [EPA], 2012). Water is generally clear and odorless. The lower reaches of Pine River slow significantly forming a braided flow punctuated by marshy areas and vegetated sandbars. In these lower reaches the substrate is primarily sand and silt.

I focused only on the upper reaches of Pine River because this was the site of the 1993 study which I used for baseline data, (Yanoviak & McCafferty, 1996).

2.2 Temperature data

Temperature data in Pine River was recorded using Onset® HOBO® Pendant Temperature Loggers. Data prior to the installation of the CWT was provided courtesy of David Costello at Kent State University and is used here with his permission. In 2011 data was recorded every 10 minutes July-August. In 2012 it was recorded hourly July-October.

Temperature data after the installation of the CWT, 2015-2019, came from the personal records of Jamie Campbell, angler and project engineer for the Pine CWT installation. During 2015-2019 data was recorded at 20 minute intervals during the summer months. The months recorded, however, vary from year to year due to timing of downloads, human error and technical difficulties.

To determine if the Pine CWT project had a significant effect on the temperature of Pine River I created two datasets, one with 2011 -2012 data representing pre-CWT conditions, and one with 2018-2019 data representing post-CWT conditions. I excluded 2015-2017 temperature data because during those years temperature was recorded in a different location.

I compared the mean, maximum, and minimum, river temperature for the months of July and August using the pre- and post-CWT datasets. I chose to analyze only data from July and August because these are the months when flow is lowest and the water temperature is generally highest. Before this period the water temperature is influenced by snowmelt runoff, and after this period cooling fall air temperatures begin to cool the river. This is also the time period of concern for anglers worried that high river temperatures will negatively impact the survival of trout fingerlings.

I used Welch's two sample t-tests to compare the mean, maximum, and minimum July and August temperatures between pre- and post-CWT years. All assumptions for this test were met. Since I could not be sure of equal variance between the two datasets (2011-2012 vs. 2018-2019) I used the effective degrees of freedom calculated using the Welch-Satterthwaite equation. Data loggers for 2011-2012 and 2018-2019 were placed in approximately the same location, and analysis was conducted on the same time periods each year, (July-August), ensuring that the pre- and post-CWT datasets are independent, random samples. I visually examined qq-plots and histograms for each dataset and determined that mean, maximum and minimum temperature follow normal distributions for both 2011-2012 and 2018-2019 datasets.

2.3 Site selection

I defined the sampling area as the area from the footbridge at the outlet of Pine Lake to a point 30 meters above the Road Bridge, a 270 meter stretch of fast flowing river. I chose this as the sampling area because it fell within the 500 meter stretch of river sampled by Yanoviak & McCafferty (1996), and including the locations of both Campbell's and Costello's temperature sensors. See Appendix D for a map of the study area and sampling locations. This section of Pine River is also the most promising spawning habitat for trout due to the gravel substrate. I chose six sampling sites within this sampling area. I chose four sampling sites, (sites 2-five) using randomly generated distances within the pre-defined 270 meter stretch of river. Two other sites were chosen non-randomly. Site 1 was chosen for convenience due to its accessible location and used to test the feasibility of our sampling methods in high water conditions. Site six was chosen to expand the diversity of our sampling sites and capture stream flow and substrate types that had not yet been sampled. At each distance I sampled as far out into the current as was permitted by the water and substrate conditions. This ranged from 0.6 to 5.0 meters offshore. Each sampling location was identified using a distance from the Road Bridge landmark, and a width measured from shore or a prominent tree landmark. I photographed all sites for future identification, as well as recording shore location GPS coordinates, and a satellite image with a pin. All measurements and satellite imagery were made using Google Maps (2020). Distances upstream from the road bridge were measured in the middle of Pine River using satellite imagery and the Google Maps measure function. See Appendix E for sample site locations and information.

At each site I recorded the water depth and estimated canopy cover and substrate type. Estimates were done by the same person at each site for consistency.

2.4 Biotic sampling

I used a 0.3 m square Surber sampler with ~500 micron mesh to sample the macroinvertebrate community. Water conditions were much higher than I anticipated, about 0.9 meters due to snowmelt and surface runoff in the watershed. I adapted the Surber sample procedures in Merritt & Cummins (1996), for high water conditions

After determining the location for sampling I approached the research site from downstream so as not to disturb the site. I plunged the Surber sampler to the bottom of the channel facing upstream. The sampler was completely submerged in the water. I opened the bottom part of the frame, thus defining a 0.6 meter square area of streambed. My assistant held the sampler open at the bottom of the stream while I removed all rocks from the area defined, depositing them into a bucket. I then used a small tool to disturb the substrate within the sampling area to a depth of 4 cm. I retrieved the sampler pulling it upstream to prevent samples in the net from washing out. Once on shore I added clean water, (water filtered through 500 micron mesh), to the bucket of rocks and scrubbed each rock, picking off visible macroinvertebrates with tweezers. Scrubbed rocks were set aside to be returned. I poured the rock-scrub water through the Surber sampler to catch all organisms that may have been clinging to rocks in the sampler.

I then emptied the contents of the sampler into a plastic container and rinsed any remaining contents in the sampler into the container with 90% ethanol, filling the container to cover the entire sample. All equipment was thoroughly rinsed with clean water. This process was repeated for each of six sampling sites.

2.5 Water Chemistry

I used LaMotte's *Earth Force® Low Cost Water Monitoring Kit* to test various abiotic water chemistry parameters. These included dissolved oxygen (ppm and % saturation), biochemical oxygen demand (BOD) (ppm and % saturation), nitrate level (ppm), phosphate level (ppm), PH, temperature (C), turbidity (JTU), and coliform bacteria level (presence/absence, threshold = 20 colonies/ 100 ml). I followed all recommended test procedures as outlined in the kit manual.All water in the stream was well mixed due to fast flow, aggressive riffles, and small waves.

I collected a single water sample approximately 0.3 meters offshore at sampling site 1. Water was collected in a sterile container following the directions provided in the monitoring kit. The temperature, turbidity, dissolved oxygen, and biochemical oxygen demand tests were performed in the field immediately upon sampling. The nitrate, phosphate and PH tests were performed three hours later after leaving the field. The coliform bacteria test was performed approximately 26 hours after the water sample was taken because moving/transporting the coliform bacteria test once the testing process had begun is not recommended.

2.6 Identification

I hand-picked and identified all macroinvertebrates visible to the naked eye in samples from each site. I identified organisms confidently to order, or when possible family level, using 10x magnification. Due to the small size of many early season organisms and the effects of preservation I was unable to separate organisms of the taxa Annelida, Nemetomorpha, and Turbellaria after preservation. These three taxa I combined into a single category called Annelida/Nemetomorpha/Turbellaria or abbreviated A/N/T.

I used the following identification resources: Merritt & Cummins, (1996), Tip of the Mitt Watershed Council, (2019); Mid-Michigan Environmental Action Council, (2016); Stroud Water Research Center, (2002); Michigan Department of Environmental Quality, Surface Water Quality Division, (1997).

2.7 Data Manipulation

To examine change in the macroinvertebrate community over time I compared my macroinvertebrate data to baseline data from Yanoviak & McCafferty, (1996). I used primary data from Yanoviak & McCafferty (1996), provided by S.P. Yanoviak, and used here with his permission. This primary data included counts of the number of individuals of each taxa, identified to species or family level, caught in each of five replicate samples taken at five sampling sites in Pine River in May 1993 (individual counts of taxa for 5x5= 25 total Surber samples).

I totaled Yanoviak & McCafferty's species level data at the order or family level to create categories which matched those that I was able to identify in my samples. Using these counts I calculated the density with standard deviation, total percent abundance, and average percent abundance with standard deviation for each taxa category. See Appendix F, Figure F2 for summary statistics of 1993 data. Total percent abundance was calculated by dividing the total number of individuals collected in each taxa at all sites by the total number of individuals collected at all sites. Average percent abundance was calculated by dividing the total number of individuals of each taxa at each sample site (sum of the five samples) by the total number of individuals at that site (sum of the five samples). This yielded a single percent abundance number for each sample site. I averaged the percent abundance at each site to get the mean percent abundance across all sites. When the numbers for *total percent abundance* and a*verage* *percent abundance* are similar it indicates that taxa were evenly distributed across sites. If total percent abundance is larger than average percent abundance it indicates that the majority of individuals in that taxa occurred in just one or two sampling sites.

I repeated these calculations to find the same metrics for the 2020 data. See Appendix F, Figure F1 for summary statistics of 2020 data.

2.8 Statistical Analysis

I used these summary statistics, (Appendix F, Figures F1 and F2), to look for significant changes in the density and average percent abundance of each taxa using Welch's two sample t-tests. All assumptions for this test were met.

2.8.1 Assumptions. Since I could not be sure of equal variance between the 1993 and 2020 data I used the effective degrees of freedom calculated using the Welch-Satterthwaite equation. I totaled 1993 data to the site level because data at the sample level was not independent. T-tests were conducted on site-level data ensuring that both 1993 and 2020 datasets are independent, random samples. By visually examining qq-plots of each year and taxa I determined that percent abundance and species density for both years follow normal distributions This makes logical sense because they are repeated measures of a population parameter.

2.8.2 Software. Data handling, graphics, and statistical analysis were done using R Core Team (2019). Packages used include tidyverse by Wickham et al., (2019); knitr by Yihui Xie (2019); reshape by H. Wickham (2007); extrafont by Winston Chang, (2014); and ggplot2 by H. Wickham, (2016).

2.9 Ethics

This study used no human subjects. Animal subjects were all macroinvertebrates. Because my study is a macroinvertebrate survey aimed at obtaining baseline data about stream health and dynamics it was not possible to replace animal subjects with a model. After a thorough literature search I concluded that this study does not duplicate existing research and there is no other source to obtain current macroinvertebrate population data for Pine River. When sampling I used the minimum number of samples reasonable to develop an understanding of the community. I refined my sampling techniques so that when it was possible to identify macroinvertebrate subjects without harm, living subjects were identified and then returned to their natural habitat. The only subjects for which this was possible were the crayfish, *(Cambarida)*. Other macroinvertebrates were killed as humanely as possible by quickly submerging them in 90% ethyl alcohol. Throughout the study I followed Leave No Trace ethics while in the field. I returned rocks and organic matter that could be efficiently separated from the macroinvertebrate samples to the locations from which they were taken.Though the necessity of killing macroinvertebrate samples is regrettable, this research will be used to raise awareness about this ecosystem and advocate for protection and further monitoring for this stream, contributing to the development of a healthy stream ecosystem in the future.

During travel to and from the field site I maintained appropriate social distancing from all persons encountered.

3.0 Results

3.1 Site information

The weather on the day I sampled was 20 degrees ℃ with clear blue skies and no wind for the majority of the day. Around 4:30 pm cloud cover increased and wind was 4.8 kph from the north. The sky was totally cloudy by 5:00 pm. Only site six was sampled in cloudy conditions

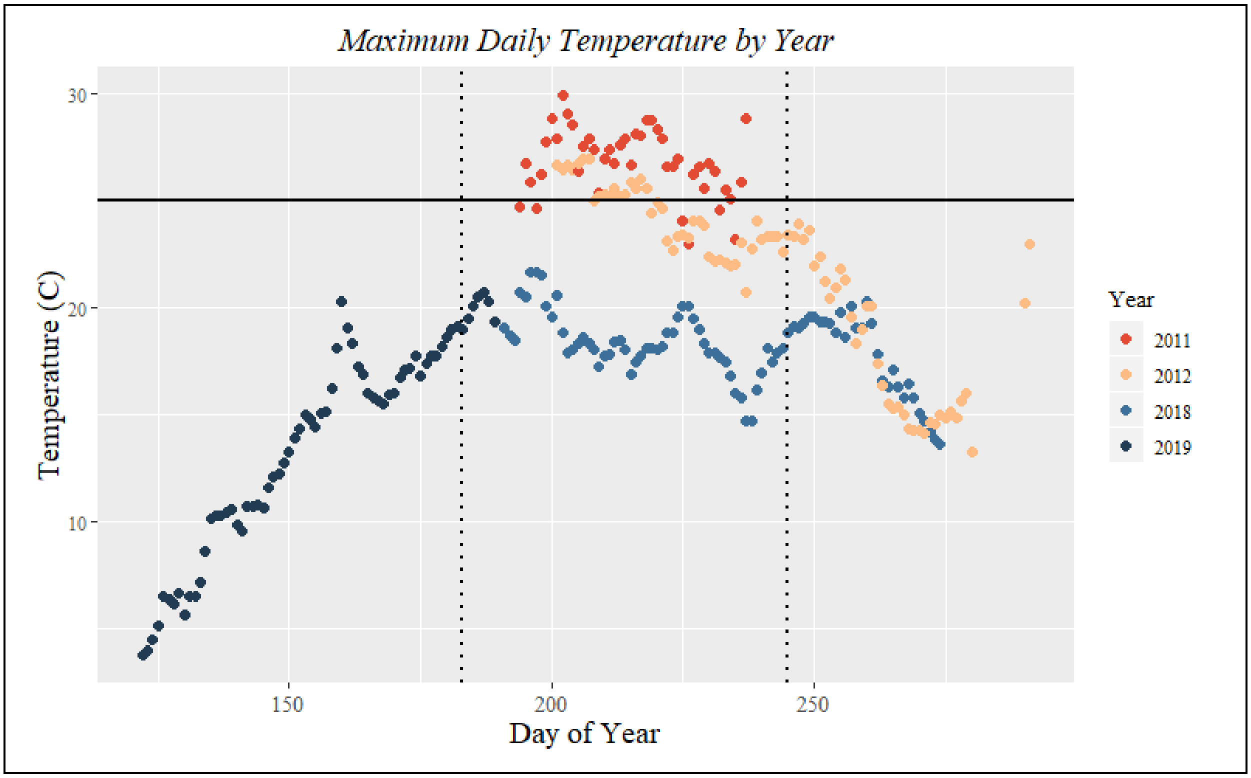
The average canopy cover across all sites was 36% cover. However this number is not an accurate representation of the Pine River as a whole, or even the upper reaches. Since most sample sites were close to shore where cover is thicker, the average canopy cover over Pine River would be distinctly lower than 36%.

Water conditions were categorized subjectively as fast riffles, slow riffles, or still eddies. Fast riffles had current strong enough to make walking upstream very difficult to impossible. Sites 1 and 2 were in fast riffles, sites 4 and 5 were in slow riffles, and sites 3 and 6 were pools. The substrate in sampling sites ranged from sand and fine organic litter to gravel and cobbles. Sand was more common in flows and pools while gravel and cobbles dominated both fast and slow riffles. See Appendix F for detailed sample site information.

3.2 Temperature

To determine if the Pine River CWT project had a significant effect on the temperature of Pine River I first examined the data graphically, Figure 1. It is apparent from this scatterplot (Figure 1) that the years before the installation of the Pine River CWT (shown in red) had noticeably higher maximum daily temperatures than during years after the installation of the Pine River CWT (shown in blue). This difference is particularly noticeable during July and August, the period indicated by the dotted lines. Mean daily temperature and minimum daily temperature showed the same trend. These graphs have been omitted for brevity. The black line indicates 25℃, the maximum survivable temperature for trout (Lessard & Hayes, 2003).

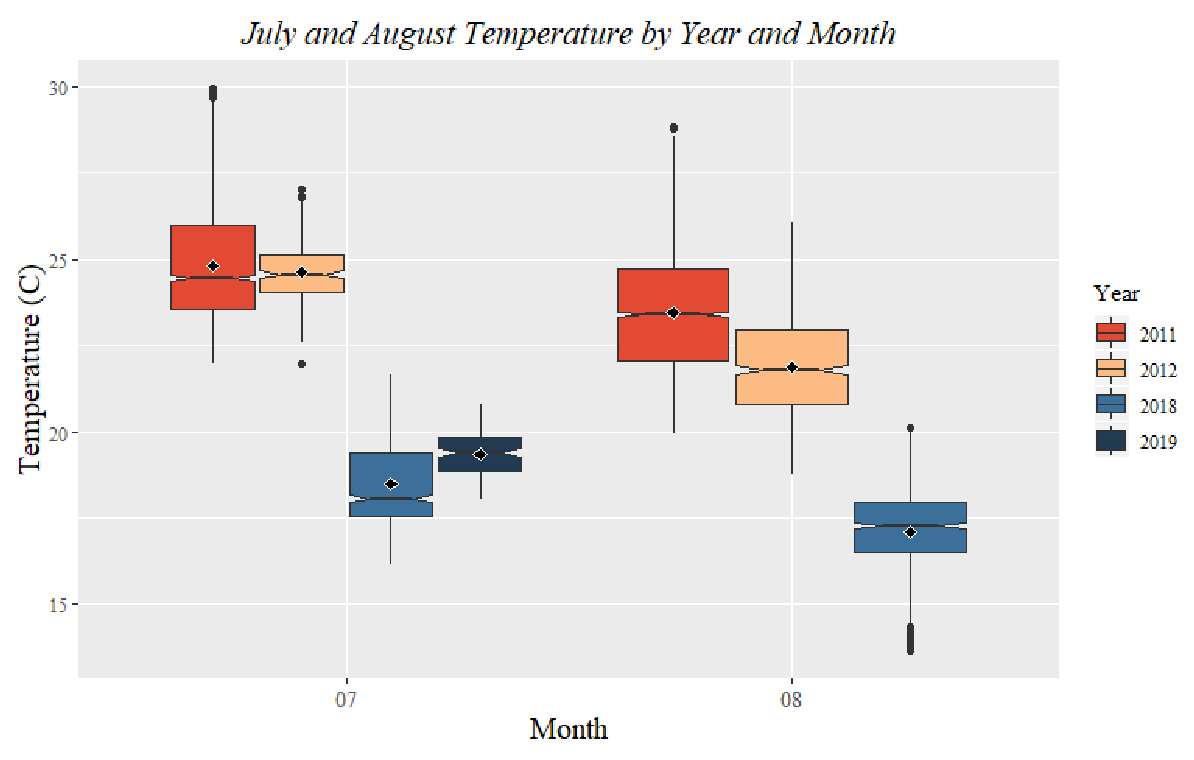
Figure 1



Note: Dotted vertical lines mark the beginning of June through the end of August. Black horizontal line indicates 25˚C.

This trend of warmer temperatures in 2011-2012 and colder temperatures in 2018-2019 can also be seen when examining July and August temperatures using box plots (Figure 2). The difference in temperature between pre-CWT and post-CWT years appears to be substantial as indicated by the minimal overlap of the box plots in Figure 2. Diamonds represent the mean temperature for each year and month.

Figure 2



Note: Compiled from un-averaged temperature data, multiple observations per hour. Diamonds represent the mean temperature for each year and month. Boxes represent 1st and 3rd quartiles. Vertical lines represent 1st and 4th quartiles. Notch represents the 95% confidence interval of the median value.

In order to determine whether the change in temperature is statistically significant I conducted a series of two tailed Welch's t-tests on the pre-CWT (2011-2012) and post-CWT (2018-2019) datasets. Significant results are detailed below and summarized in Table 1.

1. The average temperature of Pine River during July and August 2018 and 2019 (M=17.87, SD=1.52) was significantly lower than the average temperature of Pine River during July and August of 2011 and 2012, (M= 23.85, SD = 1.89); t(475.38)=69.57, p< 2.2e-16.

2. The maximum temperature during July and August 2018 and 2019 (M=18.55, SD=1.5) was significantly lower than the maximum temperature of during 2011 and 2012, (M= 25.54, SD = 2.05); t(147.23)=24.06, p< 2.2e-16.
3. The minimum temperature during July and August 2018 and 2019 (M=17.29, SD=1.47) was also significantly lower than the minimum temperature of during 2011 and 2012, (M= 22.27, SD = 1.72); t(141.02)=18.90, p< 2.2e-16.

Table 1

Results of T-tests on Temperature

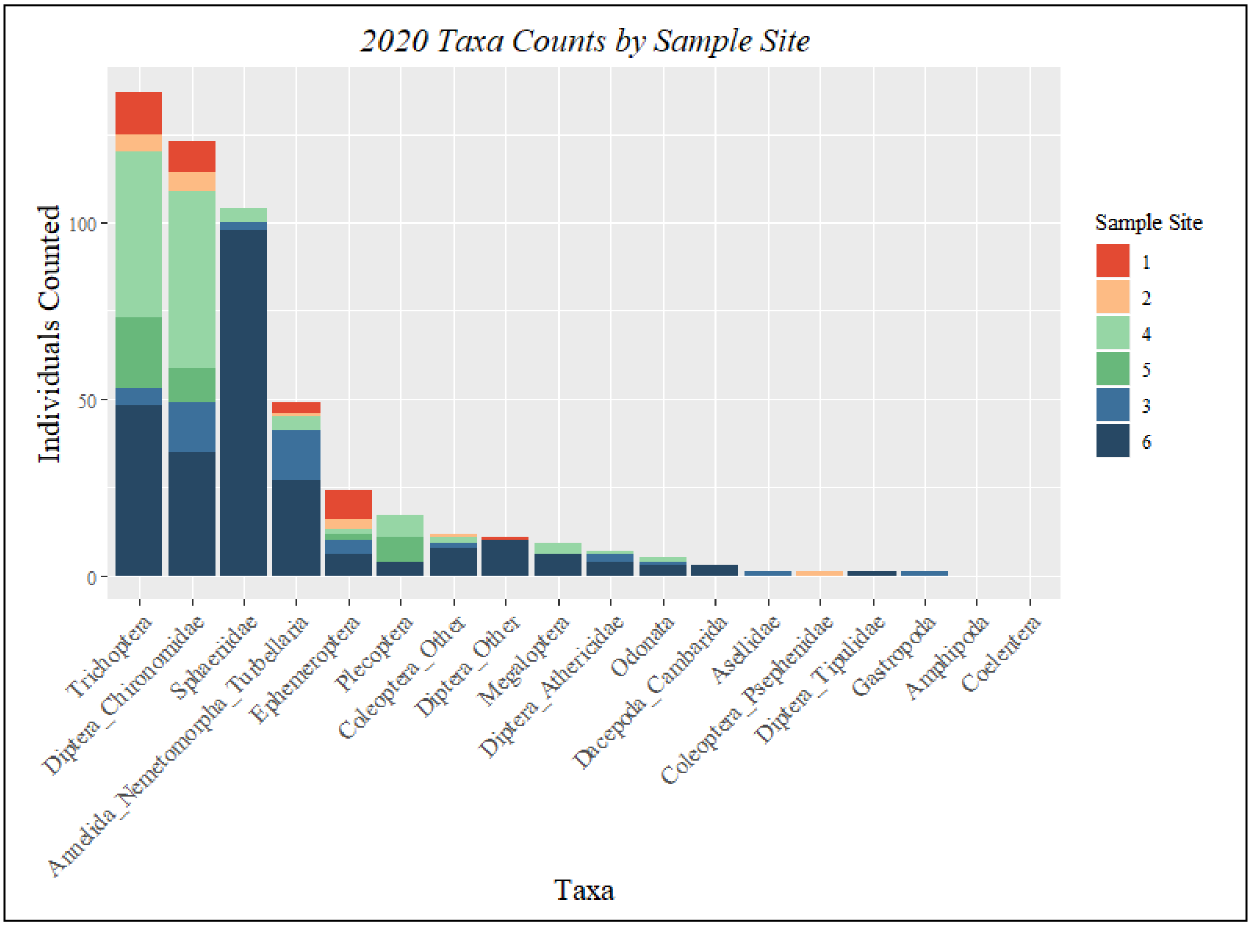
Temperature Metric	**2011-2012**		**2018-2019**		**T-value**	**Effective Degrees of Freedom**	**P- value**
	Mean	**SD**	**Mean**	**SD**			
Mean	23.85	1.89	17.87	1.52	69.57	475.38	<<< 0.001 ***
Maximum	25.54	2.05	18.55	1.5	24.06	147.23	<<< 0.001 ***
Minimum	22.27	1.72	17.29	1.47	18.9	141.02	<<< 0.001 ***

3.3 Macroinvertebrates

3.3.1 Results 2020. I collected 578 individual organisms comprising 18 taxa categories from six sample sites in Pine River. Counts of taxa by site are depicted in Figure 3. Sampling sites spanned a variety of water conditions. Only some taxa, such as Chironomids, Ephemeroptera, and Trichoptera were found in fast flowing water (depicted in red). However, Chironomids, Ephemeroptera, and Plecoptera were more common in medium flow riffles (depicted in green). Both fast and medium riffles tended to have cobble substrates. Slow water conditions (shown in blue) yielded the highest number of individuals including a large number of Sphaeriidae clams, Trichoptera, Chironomids, and Annelids/Nemetomorpha/Turbellaria. Overall the most abundant categories (average abundance) were Tricoptera (31.4 % abundance) Chironomids (28.5%), Annelida/Nemetomorpha/Turbellaria (10.1%), and Sphaeriidae (7.8%). The categories with the highest density were the same with 245.8 Trichoptera per m^2, 220.7

Chironomids, 186.6 Sphaeriidae, and 87.9 A/N/T. For more information on percent abundance and density see the summary statistics table in Appendix F, Figure F1.

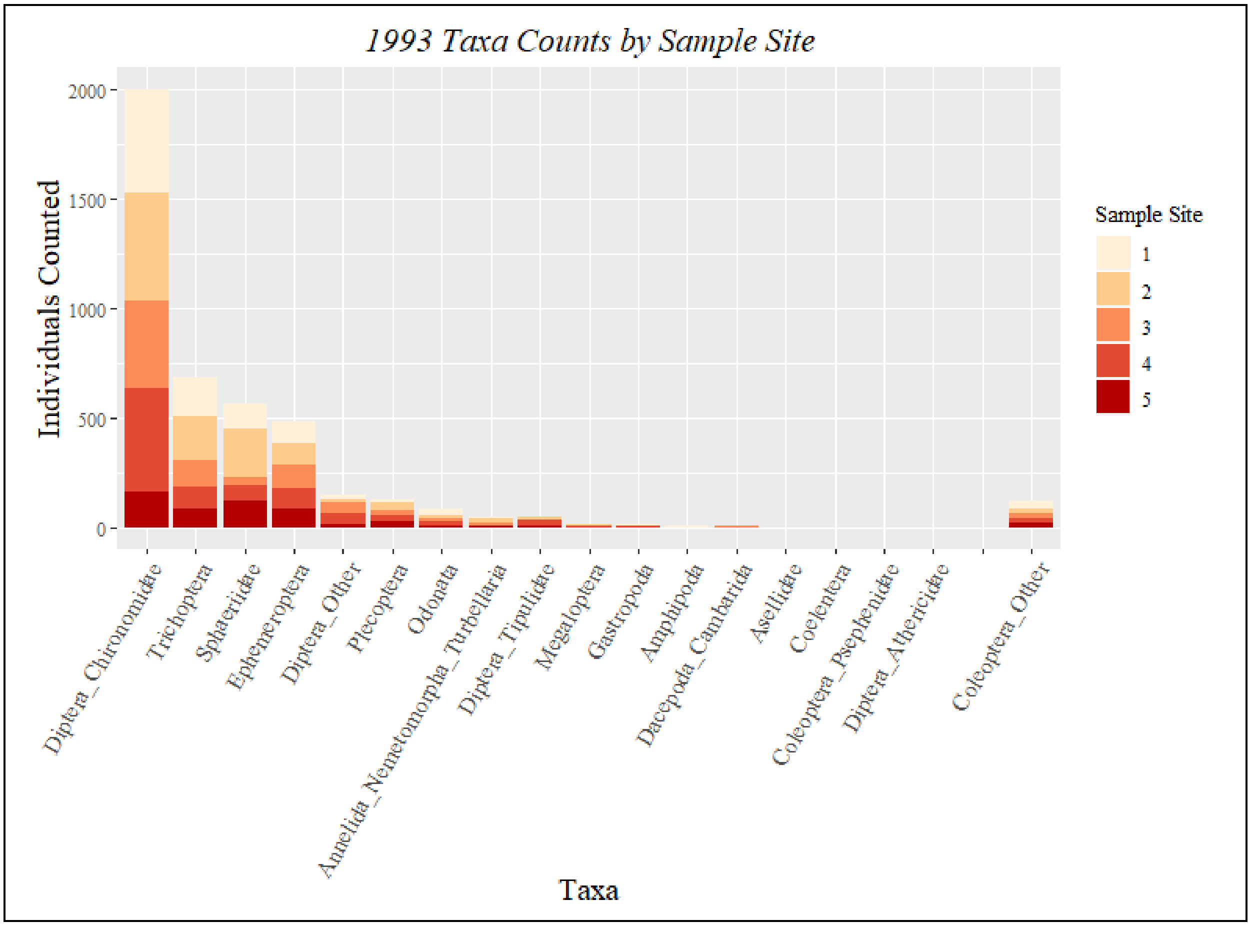
Figure 3



Note. Blue hues represent still water conditions, green hues represent medium flow riffles, and red hues represent fast riffles.

3.3.2 Results 1993. Data from Yanoviak & McCafferty (1996), included 4329 individuals from 25 Surber samples covering five sample sites in Pine River in May. All sites were medium to fast riffle water conditions with gravel or cobble substrate. Overall the most abundant categories were Chironomidae (45.1% abundnace), Trichoptera (15.7%), Sphaeriidae (13.3%), and Ephemeroptera (11.8%). The highest density categories were the same with 859.8 Chironomidae per m^2, 294.9 Trihoptera, 241.5 Sphaeriidae, and 208.0 Ephemeroptera. For more information on percent abundance and density see the summary statistics table in Appendix F, Figure F2. These four taxa are clearly also the most common based on total counts of individuals as seen Figure 4.

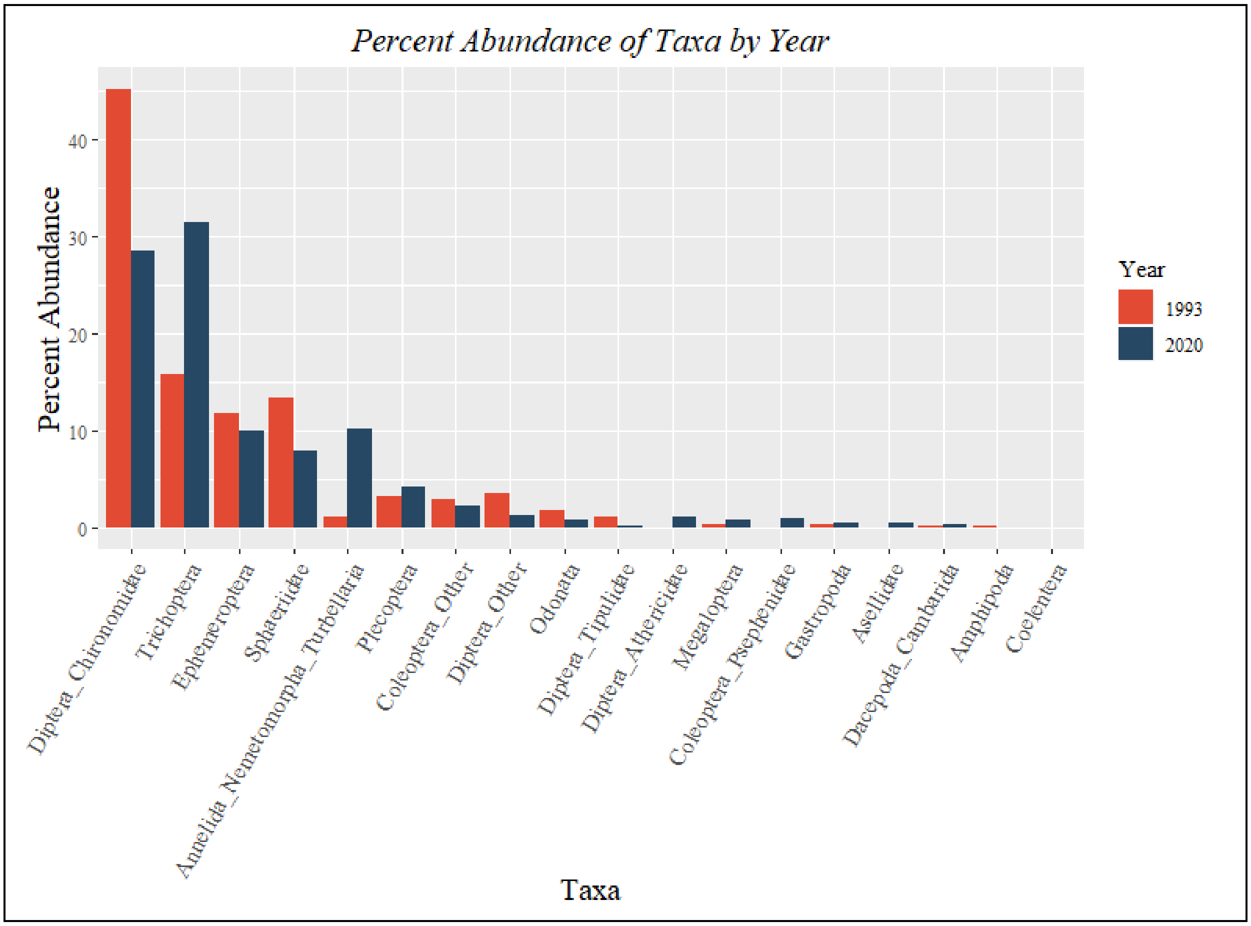
Figure 4



Note: Red hues represent fast riffles. All sites sampled in 1993 had fast riffle water conditions.

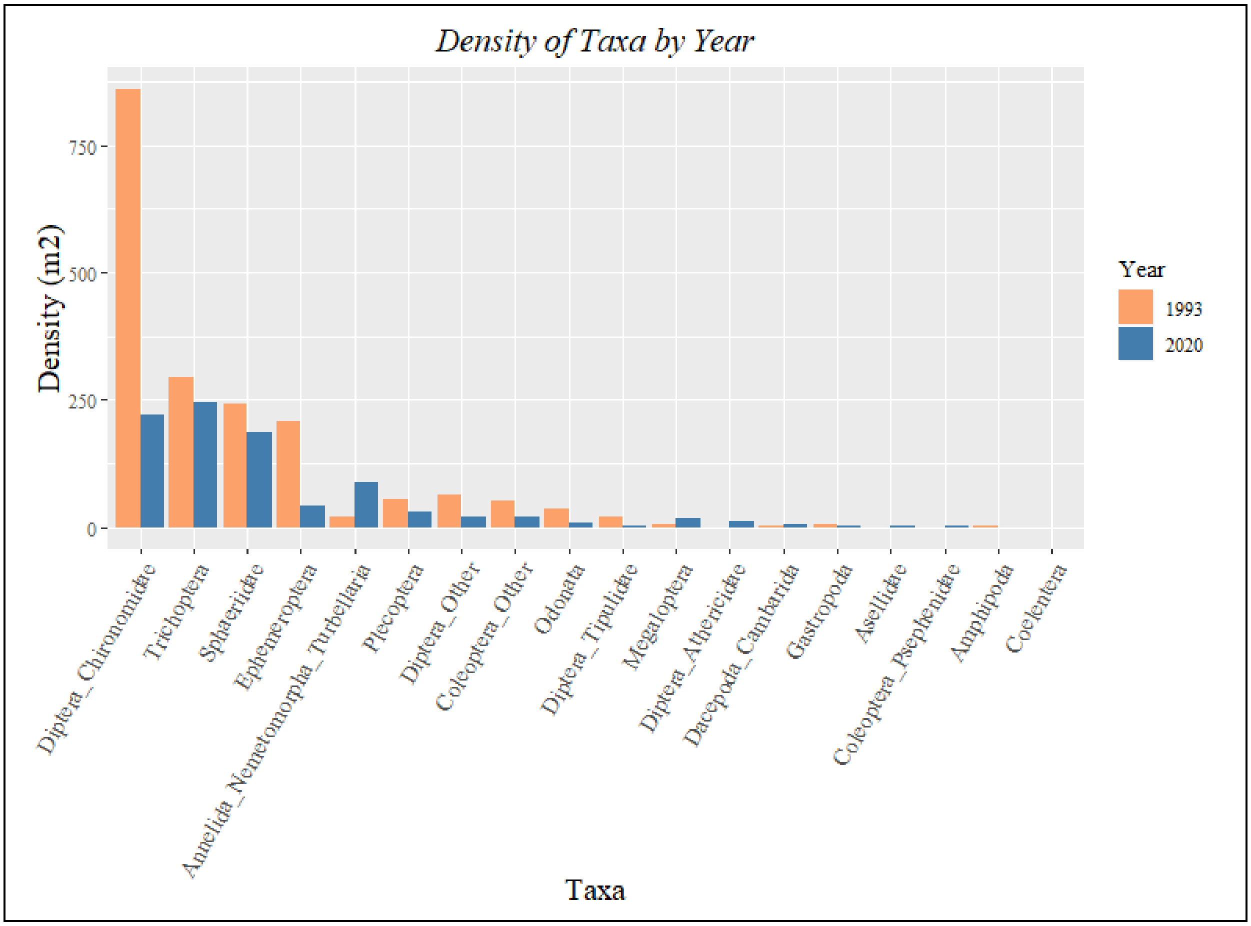
3.3.3 Comparison 1993 & 2020. Graphically, as shown in Figure 5, it is easy to see that the percent abundance of taxa in 2020 is remarkably similar to 1993. Possible exceptions are the Chironomids which appear to be more abundant in 1993 and the Trichoptera, as well as Annelida/Nemetomorpha/Turbellaria which appear to be more abundant in 2020. The bar graph comparing taxa density between years, Figure 6 shows the similar results, however Chironomids are much more dense in 1993, and Trichoptera are slightly more dense in 1993, despite being more abundant in 2020.

Figure 5



Note: Percent abundances are based on average percent abundance at each sample site.

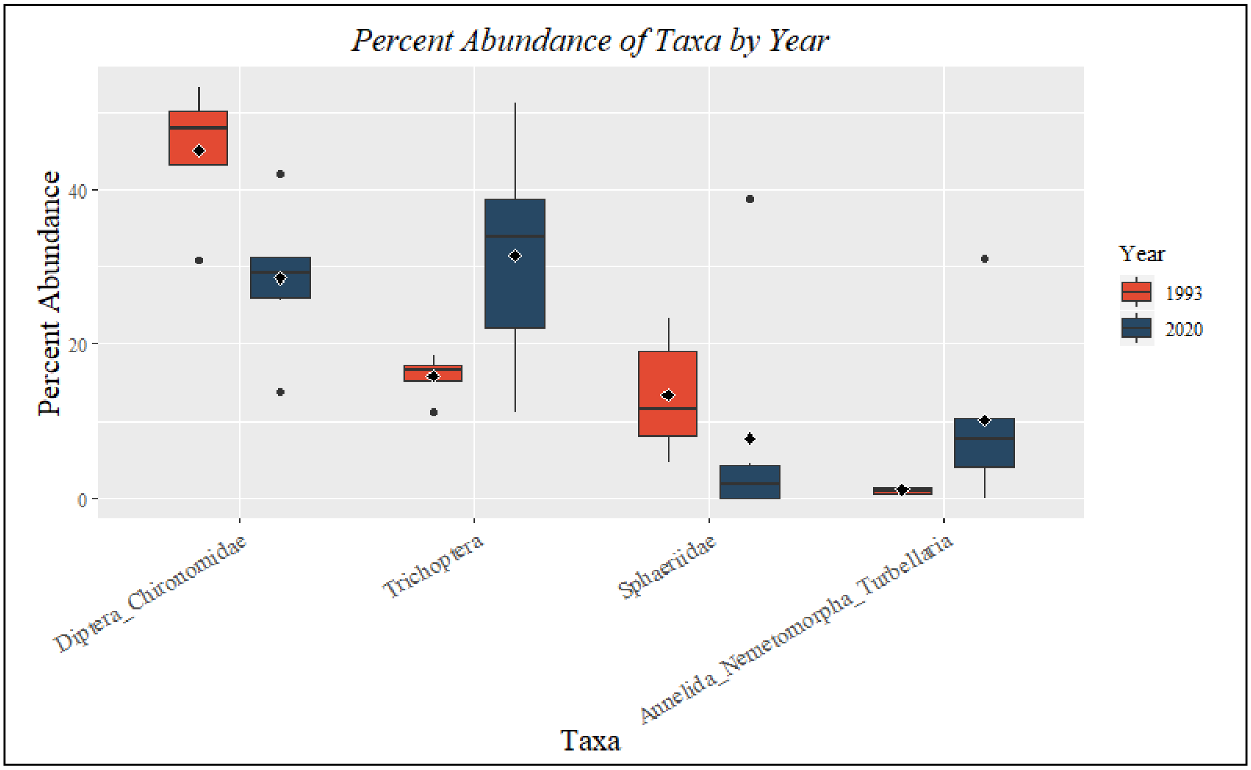
Figure 6



Note: Comparison of average density per m^2 *of taxa between years.*

Although these differences are distinguishable when looking at the means alone it is important to take into account measures of variance. Some sense of the large variance in these measures can be gained by examining the selected taxa in the box plot in Figure 7. While there are large differences in the percent abundances between year, there is also a lot of variance, and many outliers.

Figure 7



Note: Selected taxa represent the taxa with the most variation in mean average percent abundance between years. Diamonds represent the mean temperature for each year and month. Boxes represent 1st and 3rd quartiles. Vertical lines represent 1st and 4th quartiles.

What Figure 7 shows is that it is necessary to examine the standard deviations of these measurements and take into account sample size in order to determine whether any of these differences are significant. To do this I conducted a two tailed Welch's t-tests on average percent abundance and average density between 1993 and 2020.

The tests yielded no significant difference in the density of organisms for any taxa. There was, however, a significant increase in the percent abundance of Trichoptera from 1993 (M=15.7, SD=2.8) to 2020 (M=31.4 , SD=14.5); t(5.4)=2.59, p=0.04. There was also a slightly significant increase in the percent abundance of the combined category for Annelida, Nemetomorpha, Turbellaria from 1993 (M=1.1 , SD=0.7) to 2020 (M=10.1, SD=11.0);

t(5.1)=2.00, p=0.10. However, this is could be due to differences in the water conditions of sample sites. Most Annelida/Nemetomorpha/Turbellaria identified in 2020 came from still water sites. No still water sites were sampled in 1993. There were no other significant differences in percent abundance for any taxa between 1993 and 2020.

Table 2 depicts the results of Welch's two sample t-tests comparing the average percent abundance for each taxa between 1993 and 2020.

Table 2

Results of T-tests on Average Percent Abundance by Taxa

Taxa	SE	DF	T- value	P-value
Amphipoda	0.04	4	-2.24	1.91
Annelida_Nemetomorpha_Turbellaria	4.5	5.05	2	0.1 •
Asellidae	0.37	5	1.09	0.33
Coleoptera_Other	1.02	6.72	-0.59	1.42
Coleoptera_Psephenidae	1.06	5	0.94	0.39
Dacepoda_Cambarida	0.21	5.48	0.48	0.65
Diptera_Athericidae	0.69	5	1.58	0.17
Diptera_Chironomidae	5.44	8.78	-3.05	1.99
Diptera_Other	1.23	7.77	-1.87	1.9
Diptera_Tipulidae	0.5	4.22	-2.01	1.89
Ephemeroptera	4.08	6.26	-0.44	1.33
Gastropoda	0.37	5.15	0.54	0.61
Megaloptera	0.54	5.28	0.93	0.39
Odonata	0.45	8.67	-2.42	1.96
Plecoptera	2.95	5.34	0.31	0.77
Sphaeriidae	7.13	7.62	-0.77	1.54
Trichoptera	6.05	5.44	2.59	0.04 *

3.4 Water Chemistry

The results of water chemistry tests are displayed in Table 3. All water quality metrics measured in 2020 fell well within the ranges for healthy streams compared to similar streams in Michigan and other northern latitudes (Lessard & Hayes, 2003; Daufresne et al., 2004; Weigel et al., 2003).

Table 3

Water Chemistry Test Results

Test Parameter	Results
Dissolved Oxygen	>8ppm, >61% saturation
Biological Oxygen Demand	4ppm
Coliform Bacteria	Positive (> 20 colonies/ ml)
PH	7 (to nearest whole number)
Temperature	3.88 ˚C
Nitrate	< 5ppm
Phosphate	1ppm
Turbidity	0 JTU

4.0 Discussion

4.1 Changes in Temperature - Research Question 1

The Pine River CWT is a fascinating modification, essentially changing the invasives barrier from a *surface level release dam*, which draws water only from the topmost level of the lake, to a partial *lower level release dam*, which releases cooler, deeper lake water. This classification is important to note because various studies exist about the differing ecological effects of these two types of dams (Lessard & Hayes, 2003, Ward & Stanford, 1979).

Based on the results of my statistical analysis the Pine River CWT was successful in lowering the water temperature of the upper reaches of Pine River during July and August. This is expected since 0.16 cubic meters per second of 6.6 ˚C water is a large contribution to a total flow of roughly 0.42 cubic meters per second at this time of year. Thanks to this addition of cold water the river now stays about 6 ˚C cooler in July and August than it did before the installation of the CWT, when only the concrete invasives barrier was in place. It is also quite likely that the river is cooler now than it was even before the installation of the concrete invasives barrier, (during the days of the rock and stick dam, prior to 2005).

The concrete invasives barrier would likely only have raised the temperature of Pine River a few degrees above what it was prior to 2005. This is because the old rock and stick dam drew water from, at deepest, only one meter below the surface, and well above the thermocline. Measurements by anglers Jamie Campbell and Paul Rice of water temperature ~one meter below the surface near the outlet of Pine Lake found only a ~one degree difference in temperature with surface water (Jamie Campbell, Paul Rice, personal communication, May 2020). These measurements are in agreement with a temperature profile survey conducted by Peter Dykema and John Lehman in 2014 (Peter Dykema, personal communication, May 2020). Both the rock and stick dam and the concrete invasives barrier (pre-CWT) likely acted in similar capacities as surface level release dams, which are known to increase downstream water temperature (Lessard & Hayes, 2003; Ward & Stanford, 1979). This means that Pine River now, after the installation of the CWT, is likely cooler in July and August than it has been in the last 100 years.

4.2 Changes in the Macroinvertebrate Community - Research Question 2

Overall the macroinvertebrate community remained remarkably unchanged from 1993 to 2020. This is consistent with multiple studies indicating that changes in temperature cause macroinvertebrate taxa replacement at the species level, but little overall change in order level abundance (Lessard & Hayes, 2003; Chessman, 2009). The only taxa that saw significant change were the Trichoptera, which increased in abundance by around 15%. The combined category for Annelida, Nemetomorpha, and Turbellaria showed a marginally significant increase in abundance of 10%. This increase, however, is likely not reflective of true change in the macroinvertebrate community, but rather a difference in sampling techniques. I sampled multiple types of substrates and water flow conditions including sandy substrates and slow water conditions, while Yanoviak & McCafferty (1996), only examined areas with rocky substrate and fast flow conditions. In my samples 84% of Annelida, Nemetomorpha, and Turbellaria were found in still waters with sandy substrate. This could explain the difference between years. It is likely that Annelida, Nemetomorpha, and Turbellaria were present in greater numbers in 1993 in river conditions that were not sampled.

The reason for the change in Trichoptera abundance is unknown and could be due to a wide variety of factors. A survey of literature regarding macroinvertebrate communities below dams Ward & Stanford (1979), found that Trichoptera, more so than other taxa, did not show any predictable response to summer cooling caused by lower level release dams. This unpredictability could be due in part to the large degree to which macroinvertebrate communities are determined by their surroundings and other non-temperature variables.

Weigel et al. (2003), analyzed stream macroinvertebrates in the Northern Lakes ecoregion and found that 35% of the relative abundance of macroinvertebrates could be explained by stream morphology and riparian forest traits. The increase in Trichoptera I noted could be due to changes in Pine River's morphology or watershed. Weigel et al. (2003), found that the abundance of Trichoptera taxa was significantly correlated with catchment area, stream depth, and dissolved oxygen. Around Pine River the catchment area and stream depth have not changed. Although the dissolved oxygen content is consistent throughout the water column of Pine Lake, even below the thermocline (Peter Dykema, John Lehman, personal communication, May 2020), the installation of the CWT could have changed the dissolved oxygen levels since 1993. Changes in dissolved oxygen could either be because of the change in temperature, (cold water can hold more dissolved gasses), or because the concrete barrier aerates water differently than the old rock and stick dam. Unfortunately, I was unable to find any historical dissolved oxygen measurements for Pine River to which I could compare current levels. Although Pine

River’s current dissolved oxygen level of >61% saturation falls well within the ranges found in other similar Michigan streams (Weigel et al., 2003), a change in dissolved oxygen levels since 1993 could be responsible for the observed change in the abundance of Trichoptera.

Another possible cause for the increase in Trichoptera could be changes in the forest surrounding Pine River. The degree to which stream catchment is forested affects Trichoptera abundance according to Weige et al., (2003). Since 1993 some logging has taken place on the banks of Pine River and within the watershed, and this change could have influenced the abundance of Trichoptera. Interactions between riparian forests and streams, such as the degree of shading, number of downed logs, and turbidity also influence the presence or absence of Trichoptera in Northern Forest streams (Weige et al., 2003). These factors could also be responsible for the change I observed, although it is more likely to be changes in shading than turbidity since turbidity has changed relatively little since 1993 (S.P Yannoviac, personal communication, May 2020).

Yet another reason for the change in Trichoptera abundance could be an influence suggested by Ward & Stanford (1979). Trichoptera have been known to increase below surface level release dams because an increase in the amount of lake plankton reaching the river provides additional food sources for them (Ward & Stanford, 1979). Pine River now has the unique trait of acting as both surface level release dam and a lower level release dam. However, the addition of the CWT in 2015 would have decreased the total flow of lake surface water into the river, thereby decreasing the amount of lake plankton reaching the river over the 1993-2020 period. Since the change in lake plankton has likely been negative over time, this does not explain a positive trend in Trichoptera abundance.

4.3 Implications for Pine River - Research Question 3

The decrease in temperature in Pine River is likely to be beneficial for coldwater fish communities. Lessard & Hayes (2003), found decreases in trout populations below small surface level release dams due to increases in water temperature. When water temperatures rise above 25 degrees the young fingerlings for species such as brook trout and brown trout cannot survive (Lessard & Hayes, 2003). Before the installation of the Pine River CWT both the old rock and stick dam and the invasives barrier drew water only from the warm surface of Pine Lake. In July and August water temperatures rose above 25℃ at least weekly (note the horizontal black line in Figure 1). Now temperatures stay well below 25 ℃ during these months. It is possible that lower temperatures in Pine River will allow the reestablishment of resident brook and rainbow trout populations. According to angler Paul Rice, Pine River is already home to small populations of smallmouth bass and pike, and some migratory populations of brook trout (coasters) and rainbow trout (steelhead) (Paul Rice, personal communication, May 2020). With more favorable temperature conditions brook and rainbow trout may now begin to live permanently in Pine River. Globally, warming temperatures are pushing cold water fish species north (Daufresne et al., 2004). It would be beneficial in preserving freshwater biodiversity if cooling in Pine River could provide a refuge for coldwater fish species, such as the endangered coaster brook trout, that are threatened by warming temperatures throughout the midwest, (KNMI, 2020).

The effects of cooling in Pine River on the macroinvertebrate community are less clear.. Macroinvertebrate composition is influenced not just by abiotic traits like temperature but also by top down influences such as predation by fish (Fausch et al., 2010). Benthic macroinvertebrates such as leeches, dragonflies and chironomids are the primary food for trout

(Weigel et al., 2003), so if colder water changes the fish community it could influence the macroinvertebrate community indirectly. So far the macroinvertebrate community showed only slight changes since 1993, and it is not possible to know the cause of this change, or to tie it to temperature. Other studies in other parts of the world have found that increases in temperature cause a range shift in the macroinvertebrate community as southern/warm tolerant species replace cold-adapted ones (Chessman, 2009; Daufresne et al., 2004; Fausch et al., 2010). A study of stream macroinvertebrates in Australia found most families showed a detectable trend tied to temperature changes over a 13 year period, but that approximately the same number of families show an increase in detection with temperature as those that showed a decrease (Chessman, 2009). This, however, does not take into account habitat loss and species replacement within families. Both Chessman, (2009) and Daufresne et al. (2004) highlight the need for species level identification in macroinvertebrate studies in order to develop an accurate picture of changes taking place.

4.4 Conclusions

Macroinvertebrate species are highly diverse and vary widely in their environmental tolerances and trophic roles, even within order and family categories. Any major changes in the Pine River macroinvertebrate community are likely to be observable first at a species scale. I identified my data only at order level, therefore I am not able to determine whether species level changes in community composition have taken place. In the future I would recommend identifying species in more detail, perhaps incorporating the classifications of functional feeding groups, in order to analyze possible changes in Pine River trophic schemes. What I can conclude from my analyses is that Trichoptera have increased significantly since 1993, and this change has many possible causes. Based on my examination of temperature data Pine River has cooled significantly since 2011, and this is almost certainly due to the addition of the Pine River CWT. Aquatic ecosystems are incredibly complex and there are many reasons why I may have observed a change of abundance of some taxa but not others. It will take more detailed studies in the future to unravel how macroinvertebrates, temperatures, fish populations, and other abiotic influences interact in the Pine River Ecosystem.

4.5 Directions for Future Research

In a review of other studies of macroinvertebrate communities below dams causing summer cooling (Ward & Stanford, 1979), Diptera and Amphipods consistently showed increases in abundance while Ephemeroptera, Plecoptera, Annelids, and Turbellaria showed decreases. Determining whether Pine River follows these trends would also be a valuable direction for future research.

In the upper Rhone river in France Daufresne et al. (2004), found an overall change in the macroinvertebrate community correlated with warming water temperatures. Cold water species, and those preferring fast water especially some Plecoptera, decreased in abundance while warm water and still water species, including several kinds of molluscs increased in abundance. Although the differences were statistically insignificant, I found that Plecoptera were more abundant in 2020, when Pine River was cooler, than in 1993 when it was likely warmer. Spheriidae, a type of mollusc, were more abundant in 1993 than 2020, although again the differences were statistically insignificant. If these trends noted in 2020 continue they would be in agreement with the responses to temperature change noted by Daufresne et al., (2004), although in the opposite direction since Pine River has been cooled over time. Examining these taxa in more detail would be a valuable direction for future research.

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Appendix A

Figure A1

Old Rock and Stick Dam on Pine River

Note: Photograph circa ~ 1896, provided courtesy of Gina Adamini.

Appendix B

Figure B1

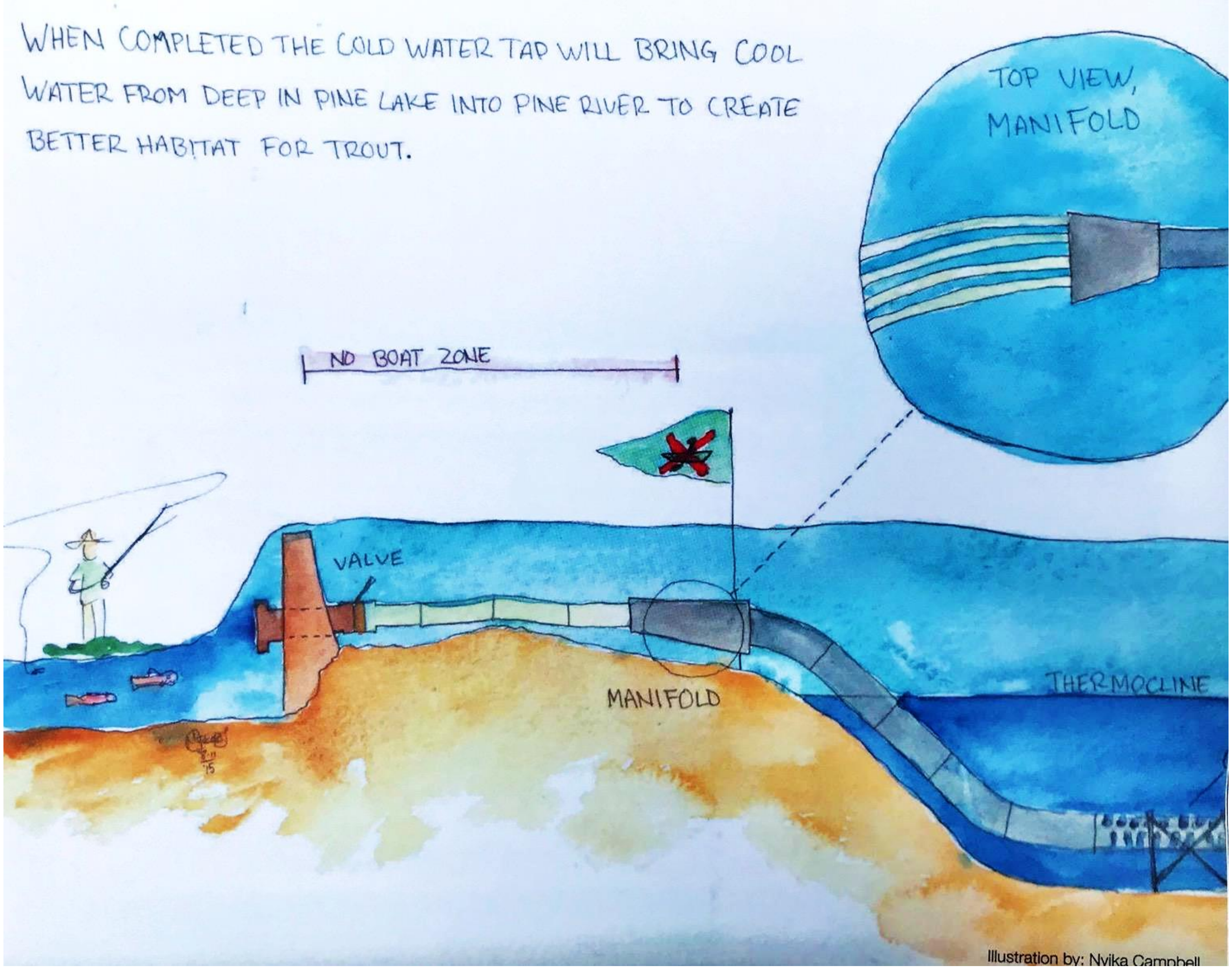
Concrete Invasives Barrier on Pine River

Note: Photograph taken in May 2020, unusually high spring water levels.

Appendix C

Figure C1

Diagram of the Pine River CWT



WHEN COMPLETED THE COLD WATER TAP WILL BRING COOL WATER FROM DEEP IN PINE LAKE INTO PINE RIVER TO CREATE BETTER HABITAT FOR TROUT.

Note: Illustration by Nyika Campbell, 2014

Appendix C

Figure C2

Piping Installation for the Pine River CWT

Note: Photograph taken in 2015. Sandbags allow access to valves that regulate flow through pipes penetrating the invasives barrier.

Appendix C

Figure C3

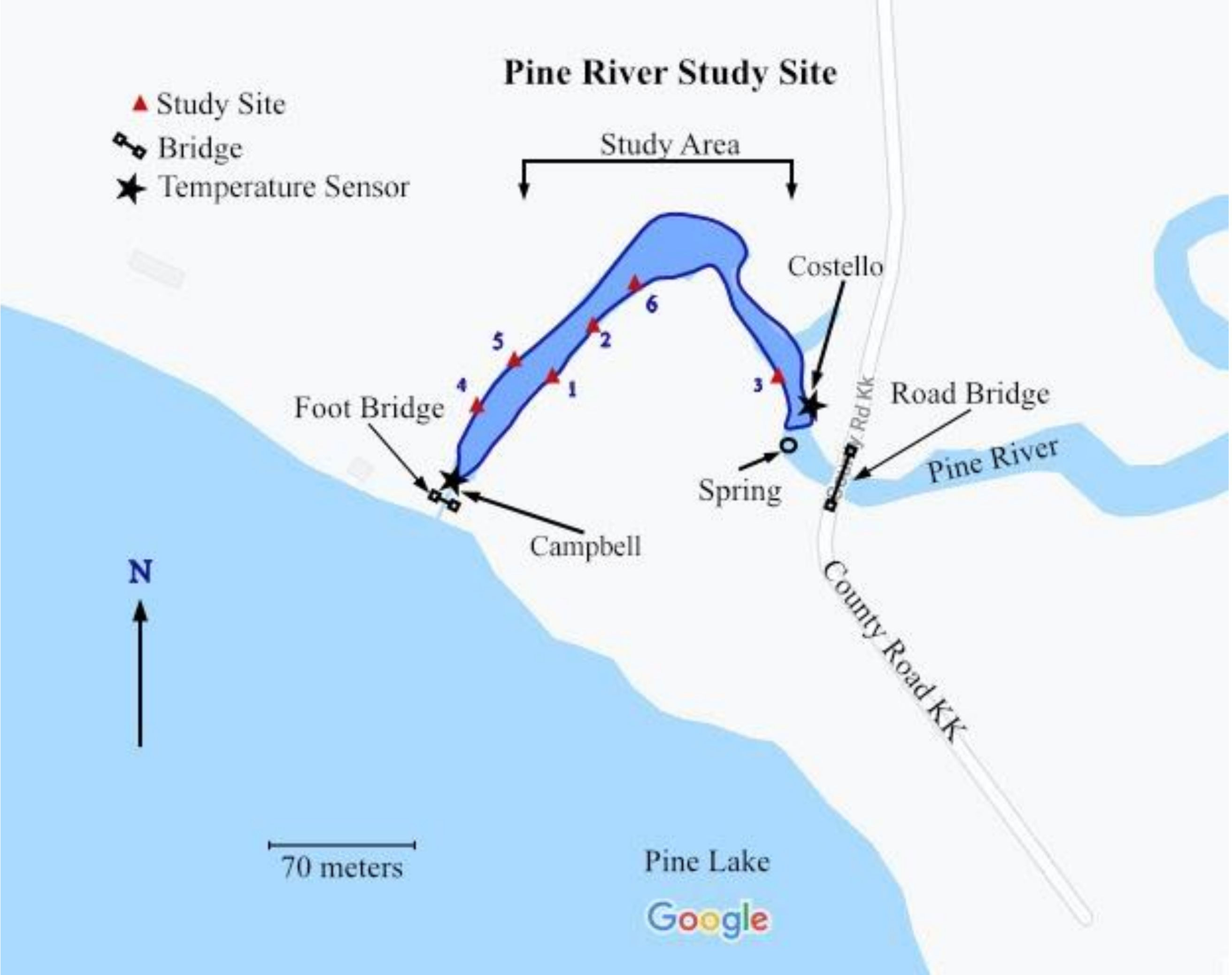
Checking Flow of Cold Water through the Invasives Barrier

Note: Photograph taken in 2015. Sandbags allow access to valves that regulate flow through pipes penetrating the invasives barrier. Jamie Campbell checks flow. Close up of pipes to the left.

Appendix E

Figure E1

Study Site Map



Note: This map depicts the study area, six study sites, and relevant landmarks.

Appendix F

Figure F1

Table of Sampling Site Information

Site	GPS Location on Bank	Width from Bank (m)	*Bank	Depth (May)	Cover	Substrate	Water Condition
1	46.883320, -87.870207	4.57	left	0.48	30%	gravel	fast riffles
2	46.883531, -87.869895	5.18	left	0.48	20%	gravel	fast riffles
3	46.883244, -87.868969	1.22	left	0.94	90%	sand	still eddy
4	46.883039, -87.870590	0.61	right	0.48	50%	gravel	slow riffles
5	46.883248, -87.870471	1.22	right	0.56	0%	cobbles	slow riffles
6	46.883586, -87.869755	4.88	left	0.57	30%	sand	still eddy

*Note: *Bank indicates which bank (facing upstream) the width measurement was taken from.*

Appendix G

Figure G1

2020 Summary Statistics

Taxa	Total Individuals Counted	Densi ty (m2)	SD of Density	Summed Percent Abundance	Average Percent Abundance	SD of Avg Percent Abundance
Trichoptera	137	245.8	214.1	27.1	31.4	14.5
Diptera_Chironomidae	123	220.7	193	24.4	28.5	9.2
Sphaeriidae	104	186.6	425.7	20.6	7.8	15.3
*A_N_T	49	87.9	113	9.7	10.1	11
Ephemeroptera	24	43.1	28.1	4.8	10	9.4
Plecoptera	17	30.5	35	3.4	4.1	7.1
Coleoptera_Other	12	21.5	32.6	2.4	2.2	2.3
Diptera_Other	11	19.7	43.3	2.2	1.2	1.8
Megaloptera	9	16.1	27	1.8	0.8	1.3
Diptera_Athericidae	7	12.6	17.2	1.4	1.1	1.7
Odonata	5	9	12.6	1	0.7	0.9
Dacepoda_Cambarida	3	5.4	13.2	0.6	0.2	0.5
Asellidae	1	1.8	4.4	0.2	0.4	0.9
Coleoptera_Psephenidae	1	1.8	4.4	0.2	1	2.6
Diptera_Tipulidae	1	1.8	4.4	0.2	0.1	0.2
Gastropoda	1	1.8	4.4	0.2	0.4	0.9
Amphipoda	0	0	-	0	0	-
Coelentera	0	0	-	0	0	-

*Note: * Abbreviation for category Annelida_Nemetomorpha_Turbellaria*

Appendix G

Figure G2

1993 Summary Statistics

Taxa	Total Individuals Counted	Density (m2)	SD of Density	Summed Percent Abundance	Average Percent Abundance	SD of Avg Percent Abundance
Diptera_Chironomidae	1997	859.8	476.2	46.1	45.1	8.8
Trichoptera	685	294.9	210	15.8	15.7	2.8
Sphaeriidae	561	241.5	232.2	13	13.3	7.7
Ephemeroptera	483	208	98.4	11.2	11.8	3.1
Diptera_Other	145	62.4	82.5	3.3	3.5	2.2
Plecoptera	130	56	48.4	3	3.2	1.2
Coleoptera_Other	118	50.8	45.9	2.7	2.8	0.9
Odonata	81	34.9	40.8	1.9	1.8	0.6
A_N_T *	51	22	26.1	1.2	1.1	0.7
Diptera_Tipulidae	50	21.5	47.4	1.2	1.1	1.1
Megaloptera	14	6	9.9	0.3	0.3	0.2
Gastropoda	9	3.9	6.1	0.2	0.2	0.1
Amphipoda	3	1.3	3.6	0.1	0.1	0.1
Dacepoda_Cambarida	2	0.9	3	0	0.1	0.1
Asellidae	0	0	-	0	0	-
Coelentera	0	0	-	0	0	-
Coleoptera_Psephenidae	0	0	-	0	0	-
Diptera_Athericidae	0	0	-	0	0	-

*Note: * Abbreviation for category Annelida_Nemetomorpha_Turbellaria*