Influence of *Cladophora sp.* on the Composition and Spatial Distribution of Macroinvertebrate Communities in Streams.

A
THESIS
SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
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BLOOMSBURG UNIVERSITY OF PENNSYLVANIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE

PROGRAM IN BIOLOGY
DEPARTMENT OF BIOLOGICAL AND ALLIED HEALTH SCIENCES

BY

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ABSTRACT:

Nitrogen and phosphorus from agricultural runoff cause blooms of the green alga, *Cladophora*. This alga provides surface area for epiphytes and invertebrates to colonize. However, *Cladophora* is not nutritious and hard to digest for many invertebrates, which could impact invertebrate communities around this alga. The purpose of this research was to determine how invertebrate communities differ between patches in streams where *Cladophora* is present and absent in the Susquehanna River watershed. To do this, 13 streams were sampled. Invertebrates were collected using a Surber sampler by collecting five composites and combining them into one sample per patch type. In streams where *Cladophora* was present, patches consisted of areas that either contained or did not contain *Cladophora*. In streams where *Cladophora* was not present, a single sample was collected. The samples were preserved in ethanol and macroinvertebrates were identified to the family level. The communities were compared between patch types from *Cladophora* streams using four metrics: percent Ephemeroptera, Plecoptera, and Trichoptera (%EPT), Hilsenhoff Biotic Index (HBI), Shannon-diversity index and percent Chironomidae (%Chironomidae). Densities were compared between patch types from *Cladophora* streams and non-*Cladophora* streams. Data loggers were left at sites to collect dissolved oxygen and temperature data every 15 minutes for a week. Percent Chironomidae was greater in *Cladophora* patches than non-*Cladophora* patches ($p<0.005$) which decreased Shannon diversity in *Cladophora* patches ($p<0.05$). Densities of invertebrates per m^2 were higher in *Cladophora* patches than non-*Cladophora* stream patches ($p<0.05$). Stream type and percent agriculture within the stream watershed both directly impacted invertebrate communities ($p<0.05$).

Logistic regression supported the prediction of *Cladophora* presence or absence in streams using alkalinity as a predictor (p=0.0516). This research supports that agriculture and the alga, *Cladophora* affect invertebrate communities at both patch and stream-level spatial scales in the Susquehanna River watershed by increasing invertebrate densities and decreasing diversity.

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Influence of *Cladophora sp.* on the Composition and Spatial Distribution of Macroinvertebrate Communities in Streams.

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INTRODUCTION:

Nutrients such as nitrogen and phosphorus often enter waterways from anthropogenic sources like agriculture fields, lawns, septic systems and construction sites (Cole and Weihe, 2016). Nitrogen concentrations in waterways are often positively associated with the amount of agricultural land within watersheds (Castillo et al., 2000; Carpenter et al., 1998). However, phosphorus concentrations are more strongly associated with suspended particles, like sediment (Jordan et al., 1997). This is likely due to phosphorus binding to these sediment particles which can be transported into waterways during rainfall events (McDowell et al., 2001). The addition and overapplication of phosphorus in the form of fertilizers and manures increases the content of phosphorus in soil and creates a surplus of phosphorus in many agricultural areas (Sharpley et al., 1994). The greater the concentration of phosphorus in the soil, the more phosphorus enters waterways through surface water runoff (Carpenter et al., 1998).

Stream periphyton communities are reactive to environmental variables including water chemistry, light availability and nutrients found within the watershed of the stream (Feminella and Hawkins, 1995). Stream periphyton communities are a mixture of autotrophic algae, heterotrophic microbes and detritus embedded in a matrix of exopolymeric substances (Cole and Weihe, 2016) covering boulders, cobble, gravel, sand, silt, and organic detritus (Lamberti and Steinman, 1997; Fairchild and Holomuzki, 2002). Periphytic algae in streams often includes diatoms, cyanobacteria, and filamentous green algae (Stevenson et al., 2006; Wellnitz and LeRoy Poff, 2012). Cultural eutrophication is the process of increasing primary productivity, like the abundance of periphytic algae, in waterways due to anthropogenic inputs of nitrogen and phosphorus

(Cole and Weihe, 2016). Whereas nitrogen is often associated with primary productivity, phosphorus is the limiting nutrient that regulates the biomass of periphytic algae in most streams (Francoeur, 2001). Therefore, when phosphorus is added to a lotic system, this can cause a shift in the periphytic algal community to contain predominantly filamentous, green algae which can grow to levels that can overtake streambeds and harm stream health (Stevenson et al., 2012).

The genus of filamentous, green alga, *Cladophora*, is one of the most widespread taxon of algae on the planet (Higgins et al., 2008) and is a prominent member of periphytic algal communities (Blum, 1956). The ability to out-compete other algae and reproduce in massive quantities in nutrient-rich waters are what allow this alga to be so widely distributed (Whitton, 1970). *Cladophora sp*., hereafter referred to as *Cladophora*, can withstand the high shear stresses that are common in streams, allowing for the colonization of lentic and lotic systems (Dodds and Gudder 1992). This alga is also capable of using inorganic carbon sources from the water to undergo photosynthesis and therefore does not rely solely on carbon dioxide from cellular respiration to photosynthesize, allowing for large growths of *Cladophora* to continue to grow without creating competition for resources between individual filaments (Choo et al., 2002).

Cladophora large biomasses through a variety of reproductive strategies can overtake nutrient-rich waterways (Dodds and Gudder, 1992; Van den Hoek, 1963; Zulkifly et al., 2013). In some *Cladophora* species there is no record of sexual reproduction (Dodds and Gudder, 1992). *Cladophora* can reproduce sexually, but the most common form of reproduction is through the asexual reproduction of biflagellated spores, called zoospores (Van den Hoek, 1963). The alga can also asexually reproduce through a resting-dormant cell called an akinete (Zulkifly et al., 2013). These methods of reproduction can cause differences in algal blooms within a year. *Cladophora* typically blooms twice annually (Whitton, 1970). In Northern temperate streams and rivers, this filamentous alga blooms in mid-summer and then dies off in the late-summer months. The alga then re-blooms in early fall (Higgins et al., 2008; Whitton, 1970).

There is some debate as to which nutrient limits the growth of *Cladophora.* Some studies provide evidence for nitrogen limitation (Penick et al., 2012; Lohman and Priscu, 1992), whereas others provide evidence for phosphorus limitation (Zulkifly et al., 2013; Dodds and Gudder, 1992). In many streams, the limiting nutrient is likely phosphorus, but it depends on the individual stream and which nutrient is in shorter supply (Francoeur, 2001).

Cladophora are rarely found in waters below a pH of 7 and are most commonly found in waters between a pH of 7 and 10 (Whitton, 1970). Research suggested that *Cladophora* can withstand a pH of 10.4 and still photosynthesize (Wood, 1975). This alga is likely to proliferate in waterways with higher alkalinity including areas with limestone bedrock, rich in $CaCO_3$ (Fairchild and Holomuzki, 2002). During a field experiment in Lake Erie, McMillan and Verduin (1953) documented *Cladophora glomerata* in waters with a total alkalinity of 90 mg $CaCO_3$/L. In a laboratory experiment in an artificial stream, water alkalinity was controlled at 134 mg $CaCO_3$/ L to study optimum growth rates of *C. glomerata* (Robinson and Hawkes, 1986). In Lake Michigan, *Cladophora* was collected in areas with total alkalinity between 152 and 179 mg/$CaCO_3$/L (Cheney and Hough, 1983).

Cladophora can grow to levels that can alter stream communities and harm overall stream health (Stevenson et al., 2012) and has been associated with reductions in the diversity of macroinvertebrate communities (Ellsworth, 2000). A fish kill in the Illinois River was attributed to large growths of filamentous green algae, including *Cladophora*, which caused hypoxic dissolved oxygen concentrations (0.9mg/L) (Stevenson et al., 2012). Hypoxia is the depletion of oxygen content in the water to about 3 mg/L (Berezina et al., 2007). Decaying *Cladophora* has also been shown to decrease dissolved oxygen to hypoxic levels in littoral areas of the Baltic Sea (Berezina et al., 2007, Berezina, 2008). In the absence of light, cellular respiration by both heterotrophic and autotrophic organisms removes oxygen from the water. Some macroinvertebrates can tolerate changes in oxygen better than others and thrive in low oxygen conditions, e.g. Chironomidae can absorb oxygen directly through their cuticle (Baranov et al., 2016) and pump water through their cases for irrigation in low flow conditions (Walshe, 1951).

Cladophora can be consumed by several species of macroinvertebrates, including the caddisfly *Gumaga nigricula*, *Helicopsyche borealis* (Feminella and Resh, 1991), *Dicosmoecus gilvipes* (Holomuzki et al., 2013), and the mayfly *Ephemerella subvaria* (Bird and Kaushik, 1984). However, this alga can be hard to digest for macroinvertebrates (Dodds and Gudder, 1992). The cell walls of *Cladophora* are thicker and the cellulose is more crystalline in structure than that of terrestrial plants (Zulkifly et al., 2013). There are few amino acids in *Cladophora*, which gives it a poor nutritional value (Dodds and Gudder, 1992).

Cladophora grow in filamentous tufts (Blum, 1956), which create a microhabitat including epiphytes, like diatoms, and the organisms that feed on these epiphytes

(Higgins et al., 2008; Ólafsson et al., 2013; Power, 1991). Many macroinvertebrates are likely ingesting *Cladophora* for the epiphytes that colonize the surface of this macroalga, essentially acquiring nutrition from the diatoms and particulate matter collected on the thalli of *Cladophora* rather than from the *Cladophora* itself (Dodds and Gudder, 1992). Therefore, even with a poor nutritional value, this alga has been associated with increased densities of macroinvertebrates from the genera *Baetis* and *Simulium* and the family Chironomidae (Ellsworth, 2000). Dudley et al. (1986) found that the genera *Baetis*, *Hydroptila*, *Ochrotrichia*, and *Euparyphus,* along with the Chironomidae family, were positively associated with *Cladophora* growths in a second order stream in Southern California. *Cladophora* has also been positively associated with *Hydropsyche*, *Rhyacophilla*, and *Microsema* due to the increased surface area this alga provides for colonization (Dudley et al., 1986). *Cladophora* has been recorded by Power (1991) to be used by Chironomidae to make cases or refuges. Therefore, *Cladophora* is not only a source of food, but also a source of shelter and building materials for some macroinvertebrates (Dudley et al., 1986; Power, 1991).

Despite all the research that has been conducted on *Cladophora* and macroinvertebrates, no studies have examined the differences in community composition of macroinvertebrates in relation to *Cladophora* distribution at varying spatial scales, both within streams at the patch level and between streams at the stream level. It is important to gain a better understanding of the interactions between this alga and its associated macroinvertebrate communities over varying spatial scales, both small and large. The objectives of this study are to firstly, see if *Cladophora* is influencing macroinvertebrate community composition in middle order streams at both a patch-level spatial scale within the same streams, and a between-stream spatial scale; secondly, to determine the direct and indirect effects of *Cladophora* on macroinvertebrates as bioindicators of water quality and to determine what these numbers actually mean for ecosystem managers; thirdly, to examine the possible mechanisms that could explain the response of macroinvertebrates to *Cladophora*; and fourthly, what factors influence the distribution of *Cladophora* in streams within central Pennsylvania.

Hypotheses:

1. *Cladophora* biomass will alter macroinvertebrate community membership at varying spatial scales both on a scale of patch distribution, within streams, and between streams.
2. Areas with *Cladophora* present will have different macroinvertebrate densities, percent Chironomidae (%Chironomidae), Shannon-diversity Index values, Hilsenhoff Biotic Index (HBI) values, and percent Ephemeroptera, Plecoptera, and Trichoptera taxa (%EPT taxa), when compared to areas without *Cladophora*.

MATERIALS AND METHODS:

Stream selection:

I collected data from thirteen middle order (4-6) streams in the Susquehanna River watershed, including: Briar Creek, Chillisquaque Creek, Fishing Creek, Green Creek, Hemlock Creek, Huntington Creek, Little Fishing Creek, Mahoning Creek, Mauses Creek, North Mahantango Creek, Penns Creek, Turtle Creek, and Warrior Run (Fig. 1). Streams were selected according to their stream order (4-6), proximity to Bloomsburg University, and the previous knowledge of *Cladophora* presence or absence in the streams from previous field experiments. Streams that contained patches of *Cladophora* large enough for five Surber samples with at least 50% *Cladophora* cover within each Surber sample were classified as *Cladophora* streams. If a stream did not contain patches of *Cladophora* large enough for five Surber samples with at least 50% *Cladophora* cover for each Surber sample, they were classified as non-*Cladophora* streams. Streams in this study had a pH of 7 or greater because *Cladophora* tends to be found in streams with higher pH (Whitton, 1970). A map of the collection sites was created using QGIS (Version 3.12) (QGIS Development Team, 2020) (see Fig. 1).

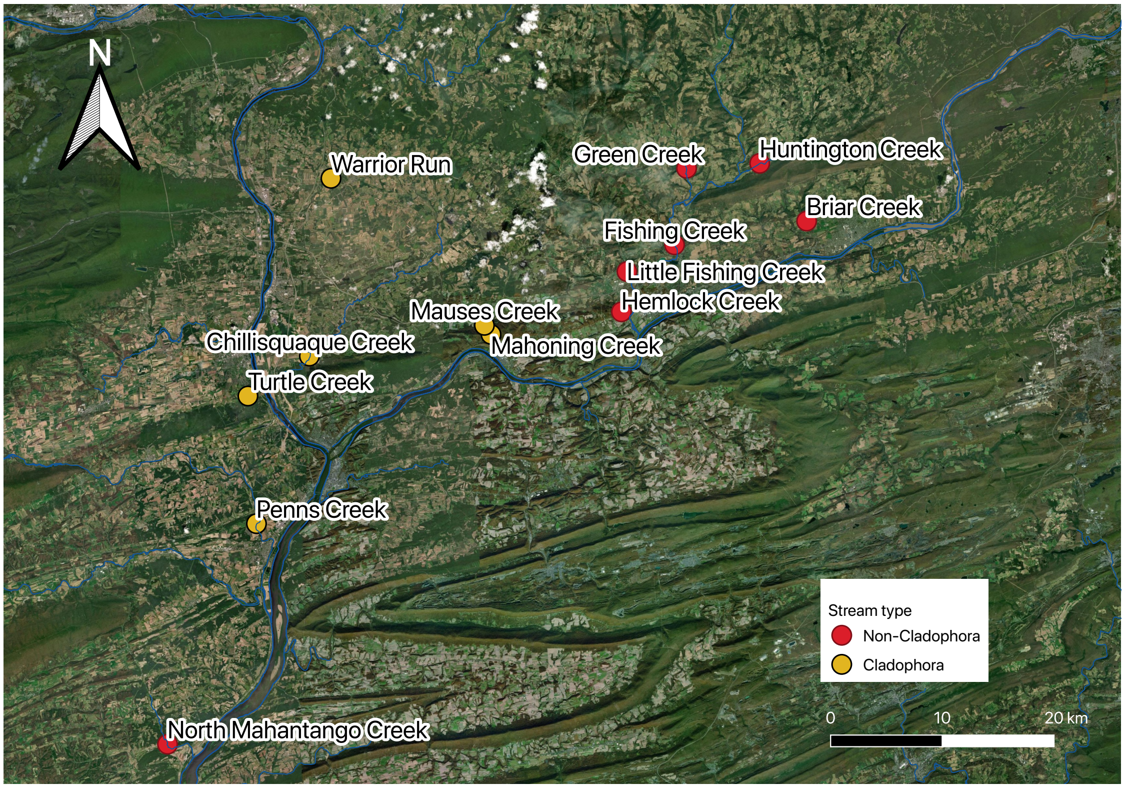

Figure 1. Map of all sites categorized as *Cladophora* (yellow) and non-*Cladophora* (red) streams within the Susquehanna River watershed. Layers data were obtained from Pennsylvania Spatial Data Access (PASDA).

Macroinvertebrates were collected when weather conditions allowed for safe access to streams and when *Cladophora* was present. Therefore, the sampling dates fell between mid-July and mid-August 2019. The summer months may exhibit decreased diversity values due to emergence of adult macroinvertebrates (Chalfont, 2015; PADEP, 2012). In streams where *Cladophora* was present (*Cladophora* streams), two macroinvertebrate collections were taken, one from *Cladophora* patches and another from non-*Cladophora* patches. For each patch type collection, five composites, or grabs, from the Surber sampler (0.096 m^2), were collected and compiled into one composite sample for a given patch type. Therefore, the total surface area of streambed disturbed per patch type was 0.48 m^2. This was be done by placing the Surber sampler in areas within riffles where at least 50% of the area within the Surber sampler was covered with *Cladophora* and disturbing the substrate for 30 seconds, making sure to fully disturb the total area within the Surber sampler. The same was done where *Cladophora* was totally absent, in the non-*Cladophora* patches. The macroinvertebrates were temporarily stored in Ziploc® bags along with a 95% ethanol solution and then transported to the laboratory to be identified under a dissecting microscope (Shull and Lookenbill, 2017; Johnson et al., 2013). The macroinvertebrates were permanently stored in 95% ethanol in scintillation vials in a repository at Bloomsburg University.

Macroinvertebrate Processing and Identification:

Macroinvertebrates were identified to the family level according to the protocol set forth by the Pennsylvania Department of Environmental Protection (PADEP, 2012). Macroinvertebrates were identified according to Merritt et al. (2008). Macroinvertebrates were identified to the family-level because a majority of individuals were early instar or immature and did not exhibit the necessary characteristics to confidently identify them to the genus-level.

A sub-sampling protocol was implemented to decrease the time for processing and identifying the collected macroinvertebrates. I developed this protocol by modifying the existing Pennsylvania Department of Environmental Protection Division of Water Quality's protocol for wadeable freestone riffle-run streams of Pennsylvania (PADEP, 2012) by taking 350 ± 70 individuals from each sample. I recorded the proportion of macroinvertebrates taken from the whole sample as a fraction, which was modified from the procedures used by Frost (2006).

Macroinvertebrate Metrics:

After macroinvertebrates were identified to the family level, I calculated the density of macroinvertebrates per m^2 (hereafter referred to as density). I also applied the taxonomic information to the following metrics:

<u>Percent EPT Taxa:</u>

Richness of Ephemeroptera, Plecoptera, and Trichoptera taxa, hereafter referred to as EPT taxa, was calculated by adding the total number of these taxa in a sample, modified from the method used by Chalfont (2015). Percent Ephemeroptera, Plecoptera, and Trichoptera taxa, hereafter referred to as %EPT taxa, was calculated from the EPT taxa richness scores (Equation 1). To calculate the %EPT taxa the total EPT richness is divided by the total number of individuals identified in the sample and multiplied by 100, to form a percent (Equation 2). The Ephemeroptera, Plecoptera, and Trichoptera are generally sensitive taxa that are not associated with polluted waterways (Barbour et al., 1992) and knowing the number of these taxa within a sample can provide insight into the level of pollution in a waterway. This is a common macroinvertebrate metric used by many agencies (Chalfont, 2015; PADEP, 2012).

Equation 1:

$$EPT\ Taxa\ Richness = \sum Ephemeroptera + Plecoptera + Trichoptera$$

Equation 2:

$$\%EPT\ Taxa = \frac{EPT\ Taxa\ Richness}{N} \times 100$$

N= Total number of identified organisms in the sample.

Shannon Diversity Index:

Shannon diversity (1948) scores are a metric that takes into account the family-level richness and evenness within the sample (Chalfont, 2015). Shannon diversity scores were calculated for each sample from the community data, excluding the unknown individuals (Equation 3). I excluded pupa, and damaged individuals that could not be identified from this calculation. Shannon diversity scores were calculated using the Vegan Package (R Core Team, 2019; Oksanen, 2019).

Equation 3:

$$Shannon\ Diversity\ Index\ (H') = -\sum_{i=1}^{S} p_i \ln p_i$$

S= the taxa richness, or total number of taxa identified

p_i= proportion of taxon i

Hilsenhoff Biotic Index (HBI):

Hilsenhoff Biotic index (1988), hereafter referred to as HBI, was calculated for each sample according to pollution tolerance values for each family of macroinvertebrate according to the table provided by the Pennsylvania Department of Environmental Protection (2012) (Equation 4). The families without pollution tolerance values were excluded from the calculation. The HBI is an index that depicts higher values for areas that are influenced by anthropogenic sources (Chalfont, 2015). The pollution tolerance values for each family were originally written to identify organic pollution of raw sewage and can provide insight into how hypoxic a waterway is due to this organic pollution (Hilsenhoff, 1988).

Equation 4:

$$Hilsenhoff\ Biotic\ Index = \sum_{i=0}^{10} [(i \times n_{indv\ PTV\ i})]/N$$

$n_{indv\ PTV\ i}$ = number of individuals identified in a sample with PTV i

N= total number of individuals identified in the sample

Percent Chironomidae:

The percent Chironomidae, hereafter referred to as %Chironomidae, is a metric that was calculated by taking the number of Chironomidae present in each sample and dividing by the total number of individuals identified in each sample and multiplying by 100 to get the percentage of individuals which belonged to the Chironomidae in each sample. The number of Chironomidae in samples can be useful to understand why diversity levels can be lower in some samples due to increased abundances of this taxon. The numbers of this tolerant taxon in a sample can provide researchers with information of the water quality and can help identify polluted areas (Pinder, 1986).

Water Chemistry:

Along with collecting macroinvertebrates, water chemistry measurements were also taken at each site. Data loggers (MiniDOT, PME) were deployed to measure diel fluctuations in dissolved oxygen and temperature. These data loggers were deployed for at least one week before or after macroinvertebrate samples were collected. Data loggers measured dissolved oxygen and water temperature every 15 minutes. The diel change in dissolved oxygen, was calculated by subtracting the minimum value of dissolved oxygen from the maximum value of dissolved oxygen for each day data were collected. The same calculation was used for the diel change in water temperature. The diel change in dissolved oxygen and diel change in water temperature were calculated for all full 24-hour days within the dataset of each stream. The average diel change in dissolved oxygen and average diel change in water temperature were calculated for each stream. A Eureka Manta Sonde was used to record turbidity, dissolved oxygen, pH, temperature, and conductivity at the time of macroinvertebrate collection. Total alkalinity was measured in the field using a Hach digital titrator. Total alkalinity is a measure of the buffer capacity of the water and is a measure of inorganic carbon sources (Cole and Weihe, 2016). Total phosphorus, hereafter referred to as TP, and total nitrogen, hereafter referred to as TN, were analyzed in the laboratory from whole water samples using an alkaline persulfate oxidation followed by colorimetric determination on a Seal AQ1 Discrete Analyzer. The TP and TN are the amount of all forms of phosphorus and nitrogen, both organic and inorganic, respectively, found within the unfiltered water sample collected from a body of water (Cole and Weihe, 2016).

Percent Agriculture Land Cover:

Percent agriculture (%Ag) for each stream watershed was calculated by delineating each watershed at the site of field data collection using modelmywatershed.org (Stroud Research Center, 2020). To calculate the total %Ag land cover the percent pasture/hay was added to the percent cultivated crops to get a total %Ag land cover within each stream watershed.

Statistical analyses:

All statistical analyses were performed using R statistical software (R Core Team, 2019). A logistic regression is a statistical analysis that can be used to test relationships between a dependent categorical variable and independent continuous predictor variables (Peng et al., 2002). A logistic regression was used to determine if environmental variables could predict the presence or absence of *Cladophora* in each stream sampled. The following environmental variables were analyzed in this model: %Ag, total alkalinity, and TP. These environmental variables were transformed using a log base 10 transformation to improve normality and homoscedasticity. An Akaike information criterion (AIC) is a way to compare multiple models to ensure the most parsimonious model is selected (Akaike, 1974 as cited by Akaike 1979). I performed a stepwise AIC test to select the most parsimonious categorical logistic regression. I carried out this categorical logistic regression using the generalized linear model (glm) function in base R (R Core Team, 2019). It is important to note, the non-*Cladophora* streams may have had *Cladophora* present in small amounts.

I used a series of paired t-tests to compare % EPT taxa, Shannon diversity scores, HBI scores, and %Chironomidae values between *Cladophora* patches and non-*Cladophora* patches from the same streams. These tests were performed using the t.test function in base R (R Core Team, 2019).

I used a Kruskal-Wallis rank sum test as a nonparametric equivalent to a one-way analysis of variance (ANOVA) to compare the densities of macroinvertebrates by patch type. This analysis compared densities from *Cladophora* patches, non-*Cladophora* patches and patches collected from non-*Cladophora* streams. When a Kruskal-Wallis rank sum test indicated a significant difference between one or more groups, I used a Dunn (1964) test as a nonparametric equivalent to a Tukey post hoc test. The Kruskal-Wallis rank sum test was performed using the kruskal.test function in base R (R Core Team, 2019). The Dunn test was performed using the dunnTest function in the FSA Package version 0.8.27 in R (Ogle et al., 2020).

Nonmetric multidimensional scaling (NMDS) ordination is a way to take multiple variables and arrange them in a multidimensional space according to the variable's similarity or dissimilarity to each other and then plot them in a specified number of dimensions (Kruskal, 1964). In this case, macroinvertebrate communities (samples from different patch types) were the variables arranged according to Bray-Curtis dissimilarity. Bray-Curtis dissimilarity was calculated from Bray-Curtis similarity (Bray and Curtis, 1957) by the metaMDS function in the Vegan Package version 2.5-6 in R (Oksanen et al., 2019). This function also plotted the communities in a two-dimensional space, a biplot (Oksanen et al., 2019; R Core Team, 2019). The data were Wisconsin Double standardized and square root transformed for normality by the Vegan Package version 2.5-6 (Oksanen et al., 2007).

Two NMDS biplots were created using the (ggplot) function in the ggplot2 package in R (Wickham, 2016). One biplot compared the macroinvertebrate communities from *Cladophora* patch samples to the non-*Cladophora* patch samples from the same streams. Another biplot compared the combined samples from *Cladophora* streams (*Cladophora* patch samples and non-*Cladophora* patch samples from the same streams) to the samples from streams where *Cladophora* was absent.

The permutational multivariate analysis of variance (PERMANOVA) is a nonparametric alternative to a multivariate analysis of variance, MANOVA (Anderson, 2001). PERMANOVA can be used to test the null hypothesis that the dependent variables, each taxon in the community, does not affect the community structure (Anderson, 2014). Two PERMANOVAs were performed using the macroinvertebrate community data. One PERMANOVA was used to test the hypothesis macroinvertebrate community structure was influenced by patch type, *Cladophora* patch or non-*Cladophora* patch within the same stream. Another PERMANOVA was used to test the hypothesis that macroinvertebrate community structure was influenced by stream type (*Cladophora* stream or non-*Cladophora* stream), %Ag, TP, average diel change in dissolved oxygen, or average diel change in water temperature. This statistical analysis was performed using the adonis function in the Vegan Package version 2.5-6 in R (Oksanen et al., 2007; R Core Team, 2019).

RESULTS:

Cladophora was generally found in streams with higher alkalinity values (see Fig. 2). The stepwise AIC test indicated the best model was one that used the log10 of total alkalinity values to predict the presence or absence of *Cladophora* in streams (AIC=14.73). The second-best model used the log10 of total alkalinity along with the log10 of TP (AIC=15.82). I used the log10 of total alkalinity values for each stream as the sole predictor variable for the categorical logistic regression to predict the presence or absence of *Cladophora* in streams (p=0.0516).

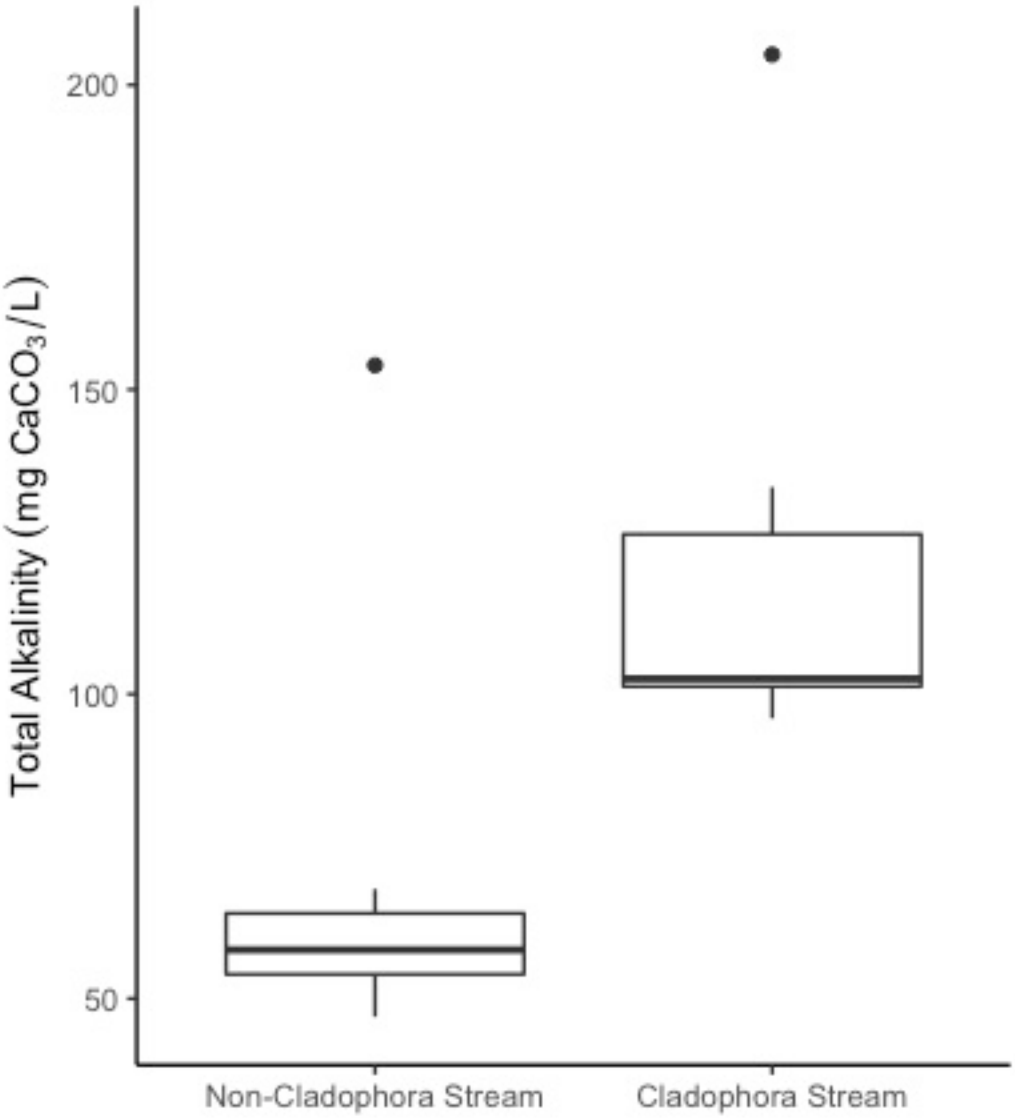

Figure 2. Total alkalinity in mg $CaCO_3$/L from *Cladophora* streams and non-*Cladophora* streams in central Pennsylvania. *Cladophora* streams contained patches of *Cladophora* that covered at least 50% of the area within a Surber sampler. Non-*Cladophora* streams had very little or no *Cladophora* present.

Samples collected from *Cladophora* patches contained greater numbers of macroinvertebrates compared to the samples collected from non-*Cladophora* patches. The lowest densities were found in samples collected from patches in non-*Cladophora* streams, then samples from non-*Cladophora* patches within *Cladophora* streams had higher densities, and the highest densities were found in samples collected from *Cladophora* patches (see Fig. 3). The Kruskal-Wallis rank sum test indicated a difference ($p<0.05$) when comparing densities between all patch types. The Dunn test indicated the *Cladophora* patch densities were higher than the non-*Cladophora* stream densities ($p<0.05$). The non-*Cladophora* patch densities and the non-*Cladophora* stream densities exhibited less distinction ($p=0.079$). The differences in the densities for the *Cladophora* patches and the non-*Cladophora* patches from the same streams were similar ($p=0.473$).

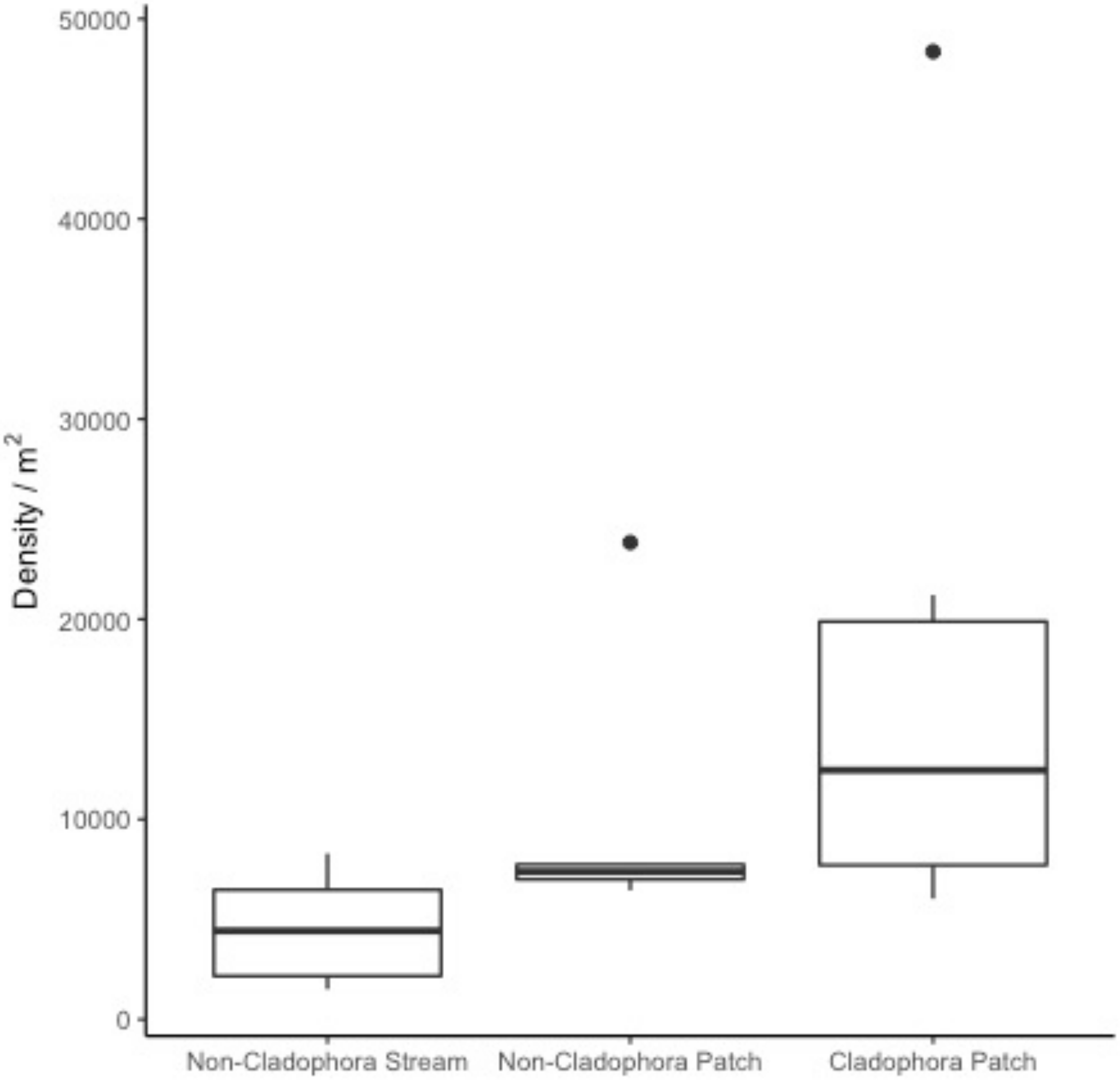

Figure 3. Macroinvertebrate densities in non-*Cladophora* streams and in non-*Cladophora* and *Cladophora* patches within *Cladophora* dominated streams in central Pennsylvania.

Samples collected from *Cladophora* patches contained greater numbers of Chironomidae and exhibited lower diversity than samples collected from non-*Cladophora* patches (see Fig. 4 and Fig. 5). The %Chironomidae was greater in *Cladophora* patches compared to non-*Cladophora* patches ($p<0.005$). Therefore, Shannon diversity scores were lower in *Cladophora* patches compared to non-*Cladophora* patches ($p<0.05$).

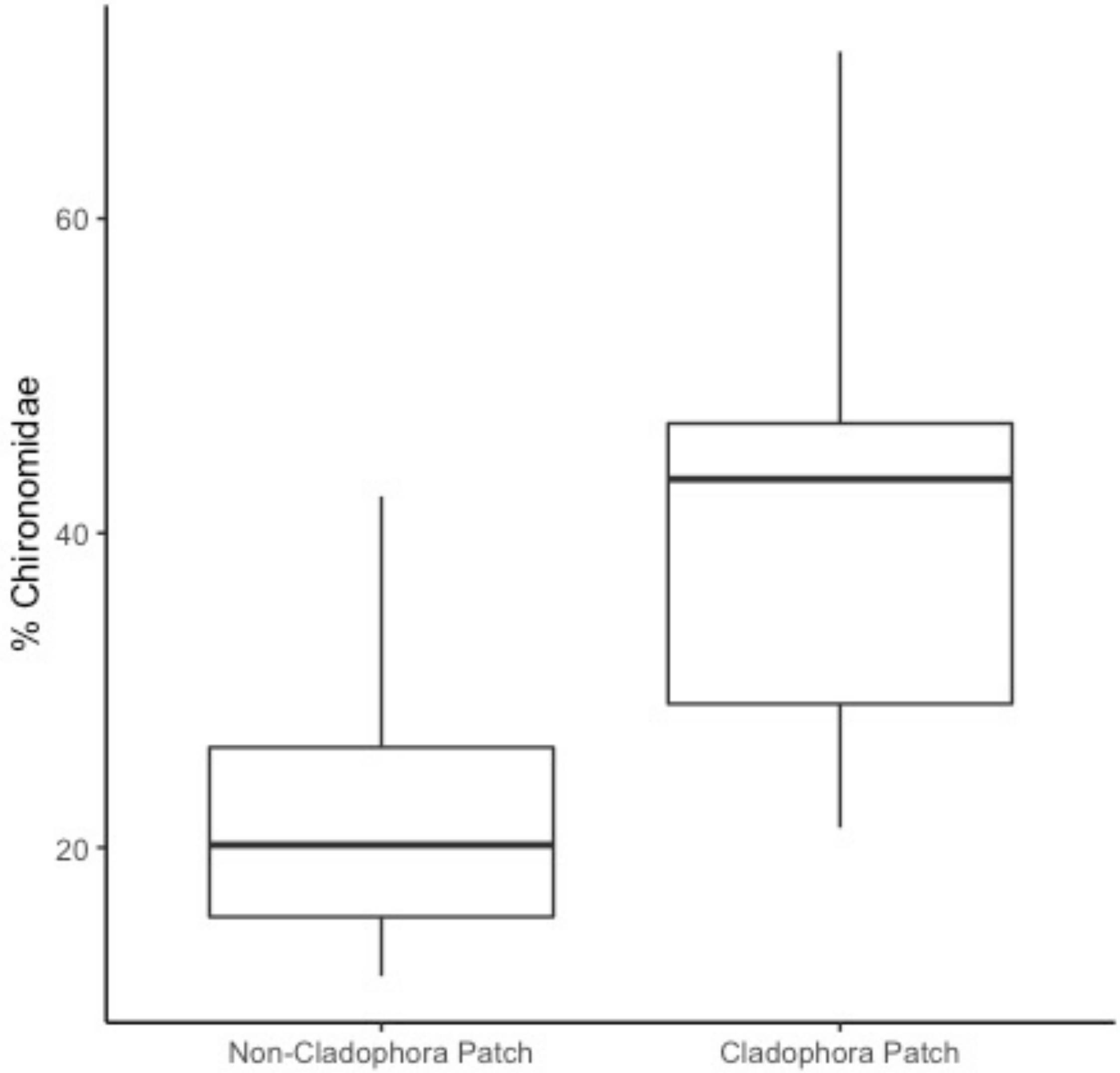

Figure 4. The %Chironomidae in *Cladophora* patches and non-*Cladophora* patches in *Cladophora* dominated streams in central Pennsylvania.

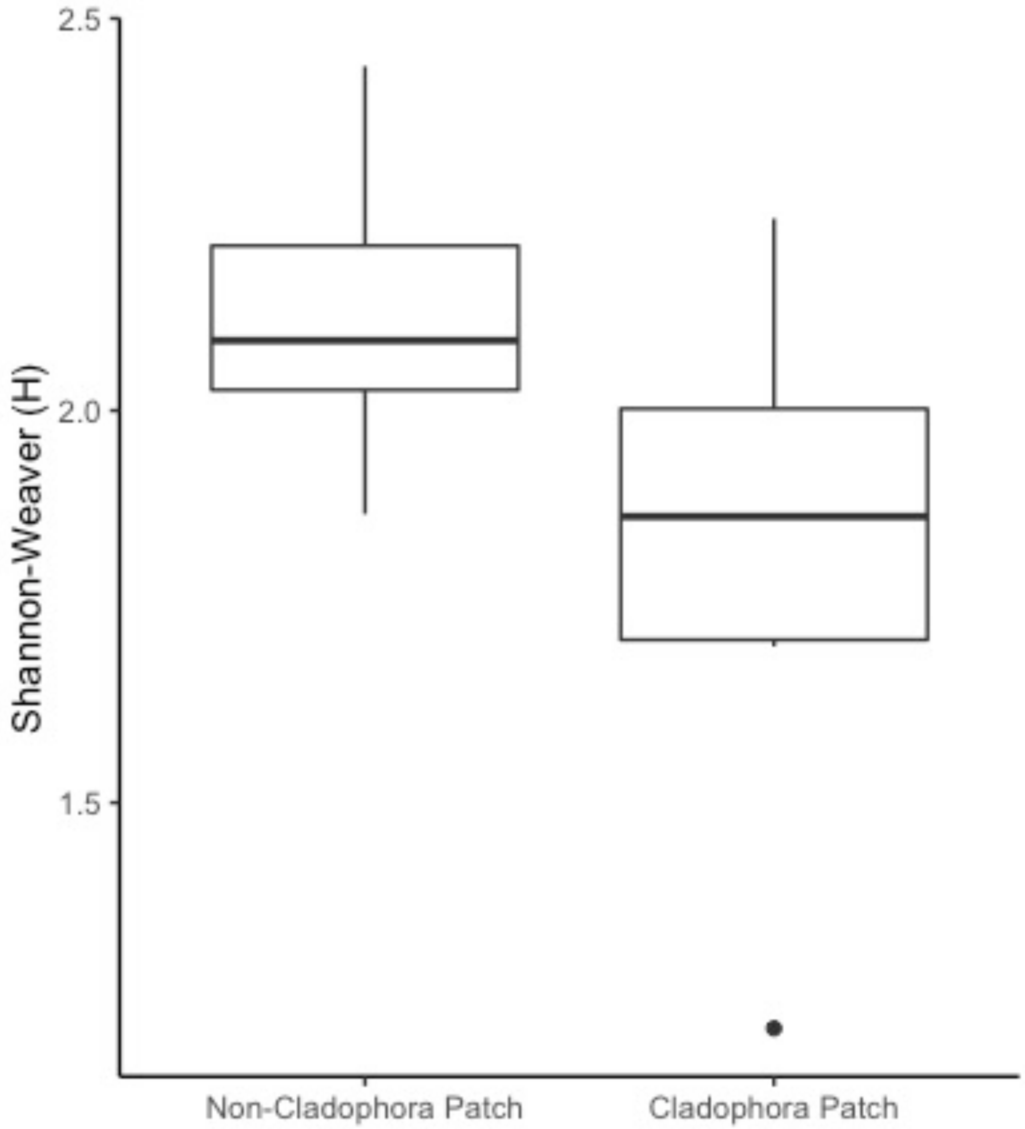

Figure 5. Shannon-Weaver diversity (H) scores for *Cladophora* patches and non-*Cladophora* patches in *Cladophora* dominated streams in central Pennsylvania.

Similar abundances of macroinvertebrate from the orders Ephemeroptera, Plecoptera, and Trichoptera were found in both *Cladophora* patches and non-*Cladophora* patches from the same streams. While the %EPT taxa for *Cladophora* patches were generally lower than that of the non-*Cladophora* patches (see Fig. 6) the differences were subtle (p=0.3508).

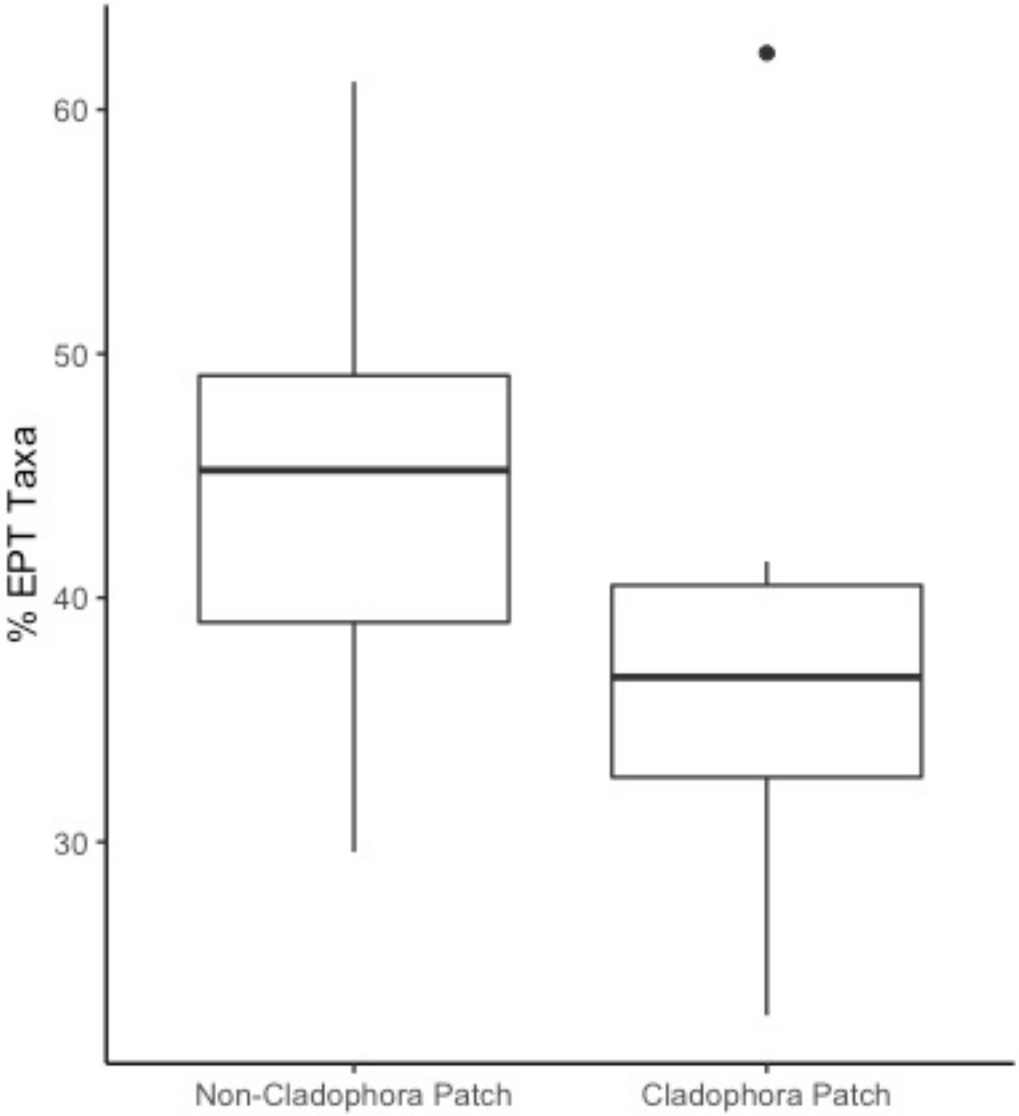

Figure 6. The %EPT taxa from non-*Cladophora* patches and *Cladophora* patches within *Cladophora* dominated streams in central Pennsylvania.

Many of the same families of macroinvertebrates were found in both *Cladophora* patches and non-*Cladophora* patches within the same streams. Therefore, HBI scores between *Cladophora* patches and non-*Cladophora* patches from the same streams were comparable (p=0.1463) (see Fig. 7). When comparing macroinvertebrate community structures by patch type using a PERMANOVA, I did not find a clear difference between the macroinvertebrate community structures of *Cladophora* patches and non-*Cladophora* patches within the same streams (p=0.296). It should be noted, in most cases, *Cladophora* patch samples (circles) were found to the left of the non-*Cladophora* patch samples (triangles) (see Fig. 8). This could indicate a trend that each *Cladophora* patch sample was separated from the non-*Cladophora* patch samples due to a difference in community structure in a similar direction, the negative direction on the NMDS1 axis. However, based on the results from the PERMANOVA, this trend is not strong enough to indicate clear differences in macroinvertebrate community structures between the two patch types.

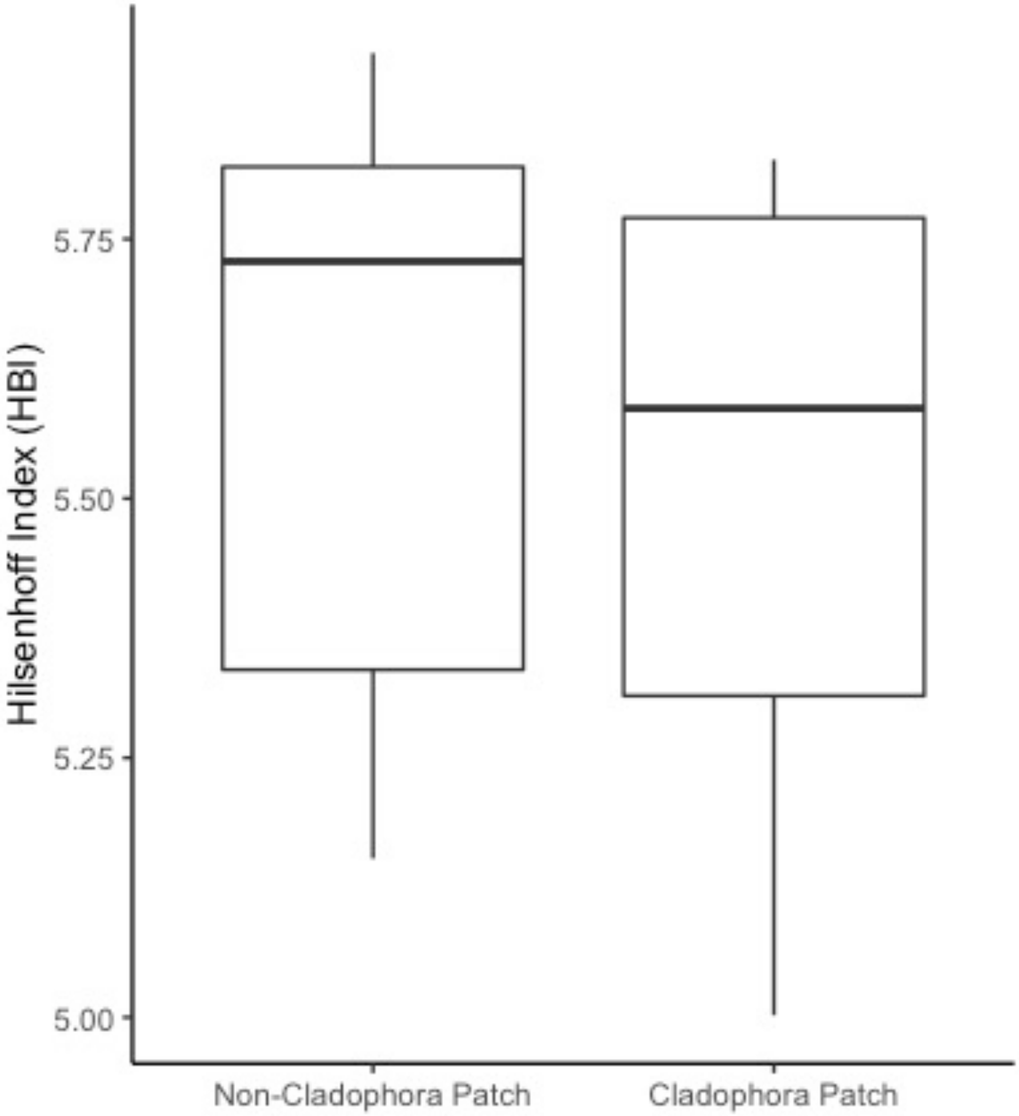

Figure 7. Hilsenhoff Biotic Index (HBI) scores of Cladophora patches and non-Cladophora patches within Cladophora dominated streams in central Pennsylvania.

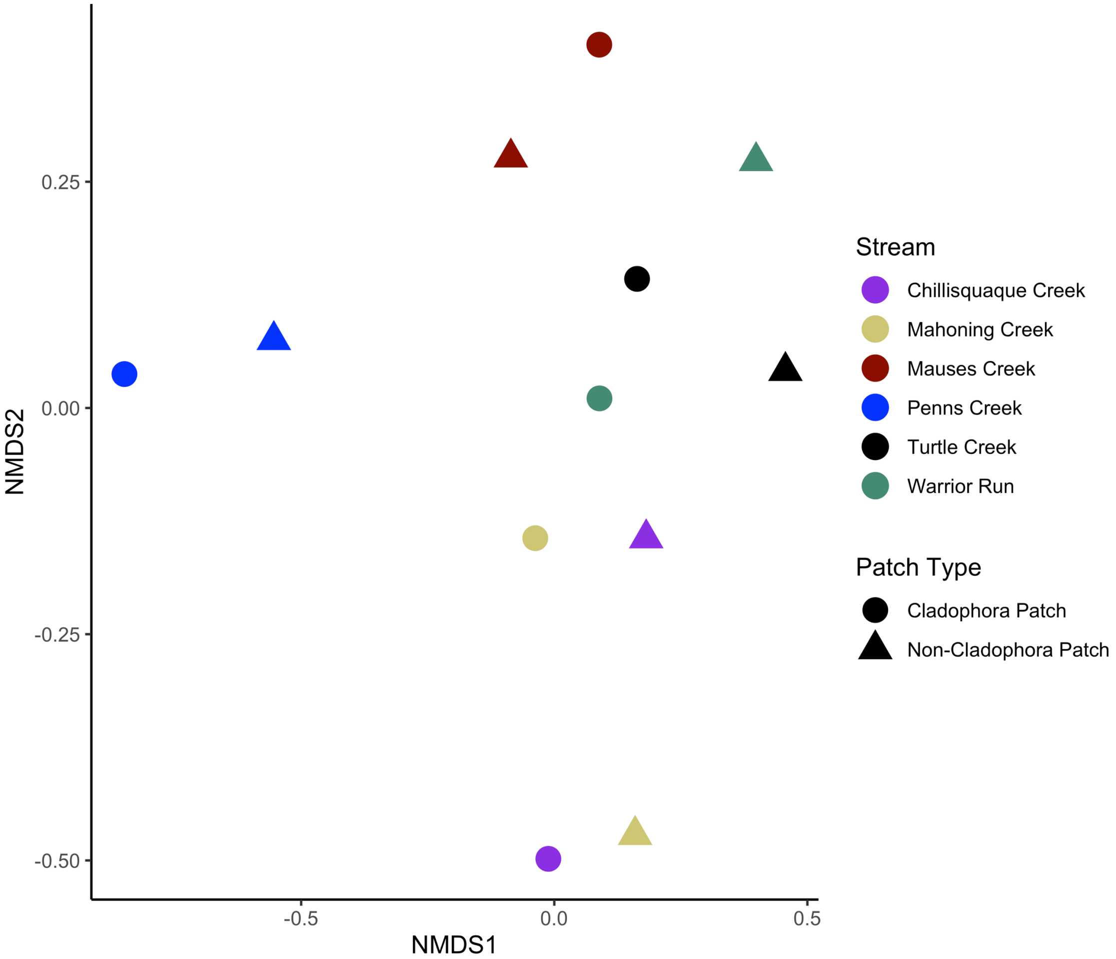

Figure 8. Nonmetric multidimensional scaling (NMDS) ordination comparing *Cladophora* patch communities (circles) to non-*Cladophora* patch communities (triangles) in six *Cladophora* dominated streams in central Pennsylvania (stress=0.10571).

Macroinvertebrate communities from *Cladophora* streams generally had greater numbers of macroinvertebrates and contained different families of macroinvertebrates when compared to non-*Cladophora* streams. *Cladophora* streams exhibited different community structure than non-*Cladophora* streams ($p<0.05$), indicated by closer grouping of *Cladophora* streams (circles) toward the middle of the biplot when compared to non-*Cladophora* streams (triangles; see Fig. 9). This indicates many of the same taxa are found at similar abundances in *Cladophora* streams. Essentially, the *Cladophora* streams are more similar in community structure to each other than they are to the non-*Cladophora* streams. The non-*Cladophora* streams show greater variation in their community structure and therefore do not group as tightly together as the *Cladophora* streams. It should be noted, when visually comparing *Cladophora* stream communities to non-*Cladophora* stream communities using NMDS ordination, an outlier was identified. This outlier was Briar Creek, which had far fewer individuals in the sample compared to all other samples. This caused a greater weight on the NMDS analysis and therefore, Briar Creek data were removed when making the NMDS biplot. Briar Creek data were not removed from the PERMANOVA test when comparing *Cladophora* stream and non-*Cladophora* stream macroinvertebrate communities.

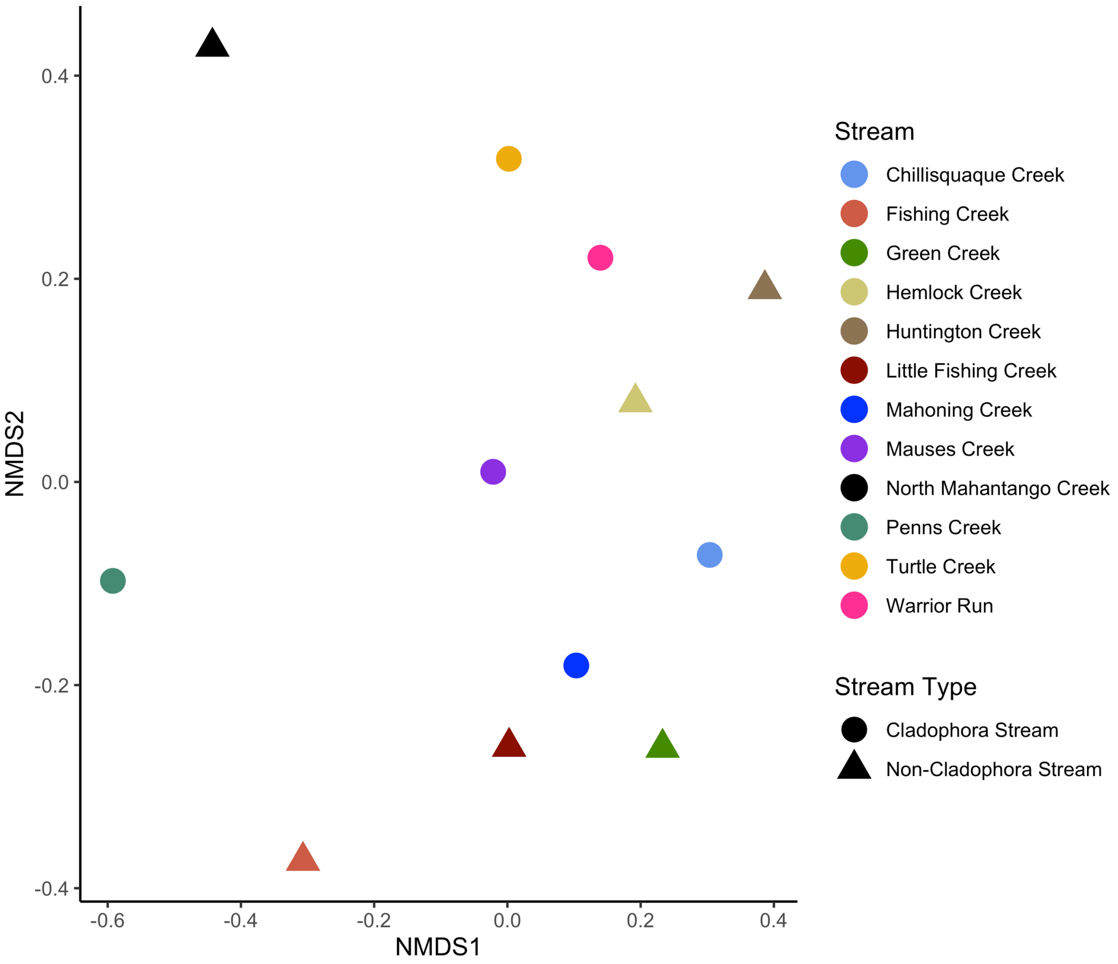

Figure 9. Nonmetric multidimensional scaling (NMDS) ordination comparing *Cladophora* stream communities (circles) to non-*Cladophora* stream communities (triangles) from 12 streams in central Pennsylvania. Briar Creek was omitted from this NMDS due to low numbers of macroinvertebrates heavily weighting NMDS analysis (stress=0.14163).

The percent agriculture within stream watersheds impacted the macroinvertebrate community composition ($p<0.05$). However, my findings suggest that average diel change in dissolved oxygen ($p=0.235$), average diel change in water temperature ($p=0.221$), total phosphorus ($p=0.094$), and total alkalinity ($p=0.191$) along with an interaction between stream type : average diel change in dissolved oxygen ($p=0.676$) did not seem to directly impact macroinvertebrate community composition between *Cladophora* streams and non-*Cladophora* streams.

DISCUSSION:

The findings of this study further support the large body of evidence that humans affect the communities of macroinvertebrates found in streams through agricultural land use and nutrient enrichment. The alga *Cladophora* is commonly associated with nutrient enrichment in streams (Stevenson et al., 2012; Whitton, 1970; Zulkifly et al., 2013). I found higher macroinvertebrate densities and decreased Shannon diversity in patches dominated by *Cladophora* due to higher Chironomidae abundances in these patches. These findings can help ecosystem managers by providing evidence for agricultural impact on macroinvertebrate communities, and potentially explain reasons for decreased diversity in nutrient-rich waters containing the filamentous, green alga, *Cladophora*.

My findings support the hypothesis that agriculture land use impacts macroinvertebrate communities in streams. This means %Ag within the upstream watershed from the site of macroinvertebrate collection impacted the community structure of macroinvertebrates found within the streams. Hanna et al. (2020) found agriculture land use was associated with decreased ecosystem services and lower diversity of macroinvertebrates in streams. In a review by Weijters et al. (2009) many studies found significant negative relationships between the percent agriculture and macroinvertebrate diversity scores. It is important to note that the likely reason for seeing the percent agriculture directly influence macroinvertebrate community structure was likely due to phosphorus playing a role in *Cladophora* growth. Agriculture increases phosphorus content in soil (Sharpley et al., 1994) and phosphorus allows for large growths of filamentous green algae, like *Cladophora* (Stevenson et al., 2012). Therefore, the percent agriculture indirectly influences *Cladophora* biomass which has a direct impact on macroinvertebrate community structure. I was unable to determine exactly how the percent agriculture specifically alters the community structure of macroinvertebrates. However, I suspect it is altering the community by increasing macroinvertebrate densities as well as the abundance of Chironomidae.

In previous studies, macroinvertebrate densities have been shown to increase with *Cladophora* presence (Dudley et al., 1986; Ellsworth, 2000; Fairchild and Holomuzki, 2002; Feminella and Resh, 1991). My findings further support this trend. The higher densities of macroinvertebrates in *Cladophora* patches compared to samples from non-*Cladophora* stream patches, suggested the *Cladophora* provided something to these macroinvertebrates, allowing for population sizes to increase. The macroinvertebrates could be benefitting from the increased surface area the *Cladophora* provides in streams, essentially providing a substrate for macroinvertebrate colonization (Dudley et al., 1986). Macroinvertebrates could also be feeding on epiphytic algae found on the *Cladophora* or potentially feeding on the *Cladophora* itself (Dudley et al., 1986; Feminella and Resh, 1991).

The likely reason for increased macroinvertebrate densities is due to much higher abundances of Chironomidae in samples from *Cladophora* patches. The association of this dipteran larvae with *Cladophora* has been well documented (Berezina, 2008; Dudley et al., 1986; Higgins et al., 2008; Whitton, 1970). Chironomidae have been found to make their cases from filaments of this alga (Power, 1991). Though this study did not focus on what this alga provides for the Chironomidae, a higher %Chironomidae was found in samples collected from patches containing *Cladophora* compared to patches without *Cladophora* from the same streams. Identification of Chironomidae past the family level would be necessary to determine the functional feeding groups present in this population. A potential future research project could be identifying the Chironomidae to the genus level to determine functional feeding groups present in each sample by taking a subsample of the Chironomidae and mounting head capsules for further identification. It is unknown exactly, what the *Cladophora* is providing the Chironomidae. However, research suggested the Chironomidae are likely feeding upon the diatoms and other epiphytes that typically colonize *Cladophora* and could also be using the *Cladophora* as a substrate to attach for filter feeding (Dudley et al., 1986; Furey et al., 2012; Pinder, 1986). Therefore, the *Cladophora* could be supplying the Chironomidae with both shelter and food, allowing for significant increases in Chironomidae populations in areas dominated by *Cladophora* compared to areas that lack this alga. The increase in Chironomidae in samples from *Cladophora* patches is likely the driving force for why Shannon diversity was lower for those samples. Essentially, the greater the abundance of one taxon in a sample, the lower the diversity for that sample. My research supports the findings of Ellsworth (2000), who found lower macroinvertebrate diversity associated with dense *Cladophora* growths in streams. However, Ellsworth (2000) attributed this decrease of diversity to increases of *Baetis*, *Simulium* and Chironomidae. My findings suggest Chironomidae are the dominant taxon in *Cladophora* patches and play a more significant role in the diversity of these patches than other taxa in central Pennsylvania.

My research indicated macroinvertebrate community composition is likely determined at the stream-level spatial scale rather than patch-level spatial scale. This claim is supported by finding distinct community composition separation between *Cladophora* streams and non-*Cladophora* streams, and not between *Cladophora* and non-*Cladophora* patches within the same streams. However, this should be interpreted with caution. The distinction between macroinvertebrate communities in *Cladophora* streams and non-*Cladophora* streams could be due to geographic separation of these two stream types. Many of the *Cladophora* streams are geographically closer together than the non-*Cladophora* streams, potentially causing similar families of macroinvertebrates to be found due to geographic proximity. The reason for a lack of distinction between communities at the patch level could be due to lower taxonomic resolution resulting from family-level identification. Though family level identification can tell us a great deal about macroinvertebrate communities (Osborne et al, 1980), most agencies recommend genus-level identification (PADEP, 2012). This is because genus-level identification would allow for further separation of the communities. It is possible that with greater taxonomic resolution (i.e. genus-level identification) a difference in community composition at the patch level could be found.

Total alkalinity did not have a direct impact on macroinvertebrate communities between streams. However, I found that alkalinity did impact the presence or absence of *Cladophora* in streams and that *Cladophora* can impact macroinvertebrate density and diversity. Therefore, alkalinity seems to indirectly impact the macroinvertebrate communities through the influence of *Cladophora*. *Cladophora* tends to be found in waters with alkalinity of at least 90 mg $CaCO_3$/L or higher (McMillan and Verduin 1953; Cheney and Hough, 1983; Robinson and Hawkes, 1986). Choo et al. (2002) were able to identify the uptake of inorganic species of carbon, directly related to alkalinity, in the

Baltic Sea. It has also been documented that *C. glomerata* is capable of utilizing HCO_3^- as a carbon source within streams (Raven et al., 1982; Raven et al., 1994). Therefore, it makes sense for this alga to be present in larger quantities in streams that have greater amounts of this inorganic carbon form, i.e. waters with higher alkalinity values.

The likely reason %Ag and TP were not identified as key predictors of *Cladophora* presence in the streams within this study is due to the limited number of stream sites examined. Variation within the %Ag and TP of these 13 streams was likely causing these variables to be excluded as predictors. It is well-known that *Cladophora* has a close association with higher levels of phosphorus (Whitton, 1970; Stevenson et al., 2012; Zulkifly et al., 2013; Dodds and Gudder, 1992). Agriculture is linked to phosphorus by the application of fertilizers and manures to agricultural lands increasing concentrations of phosphorus in soil (Sharpley et al., 1994), ultimately increasing the amount of phosphorus in streams (Carpenter et al., 1998). Therefore, if more sites, of both *Cladophora* and non-*Cladophora* streams, were included in this study then %Ag and TP would likely be good predictors of *Cladophora* presence in streams.

The reason for a lack of distinction between some macroinvertebrate metrics at the patch level could also be due to lower taxonomic resolution resulting from family-level identification. Many of the same families of macroinvertebrates were found in both *Cladophora* and non-*Cladophora* patches in the same streams, likely influencing the %EPT taxa and causing it to be similar for both patch types. However, *Cladophora* patches generally exhibited lower %EPT taxa, which would suggest the alga is creating slightly unfavorable conditions for these sensitive taxa. The orders Ephemeroptera, Plecoptera, and Trichoptera are generally sensitive to environmental variables, specifically lower dissolved oxygen (Connolly et al., 2004), and are often not found in abundance in waters with pollution (Barbour et al., 1992). *Cladophora* may be creating subtle changes in dissolved oxygen that these taxa do not prefer, causing them to be found less frequently in patches of *Cladophora*. A valuable future study could examine how subtle changes in dissolved oxygen could cause EPT taxa to avoid areas of *Cladophora*. To do this, a micro-sensor that collects dissolved oxygen data could be placed in patches of *Cladophora* before sampling for macroinvertebrates to see diel changes in dissolved oxygen at a smaller scale. Then the %EPT taxa could be calculated to determine how these small-scale diel changes in dissolved oxygen impact the EPT taxa found in *Cladophora* patches. If more sites were included in this study, a difference of %EPT taxa between *Cladophora* patches and non-*Cladophora* patches may be observed. The HBI metric was also calculated using family-level identified macroinvertebrates. Using family-level identified macroinvertebrates for HBI scores is a common practice (Hilsenhoff, 1988). However, HBI scores can also be calculated using the pollution tolerance values for macroinvertebrates identified to the genus level (Chalfont, 2015). Pollution tolerance values for macroinvertebrates identified to the genus level are more specific, whereas family level identified pollution tolerance values are more conservative and are typically higher than that of some of the genera within that particular family (PADEP, 2012). Therefore, if macroinvertebrates were identified to the genus level, a greater resolution could be found, further distinguishing any differences that may be found in the HBI values. Another explanation for the lack of difference between HBI values for *Cladophora* patches and non-*Cladophora* patches could be due to the limited number of sites to compare. In this comparison, six *Cladophora* patches were compared to six non-*Cladophora* patches from the same streams. If more sites containing *Cladophora* were found and separate HBI scores were calculated to compare *Cladophora* patches to non-*Cladophora* patches, it is possible a difference could be observed.

Large quantities of filamentous green algae, like *Cladophora*, can decrease dissolved oxygen to hypoxic levels in lotic systems, which harm fish and invertebrate communities (Stevenson et al., 2012). It was rare for any of the sites studied in this project to drop below dissolved oxygen concentrations of about 6 mg/L and so these sites were not in danger of becoming hypoxic. Hypoxia is when oxygen concentrations drop to around 3 mg/L (Berezina et al., 2007). Stevenson et al. (2012) reported a diel change in dissolved oxygen of around 7 mg/L that resulted in a fish kill. However, the reason for this fish kill was likely due to dissolved oxygen concentrations reaching as low as 0.9mg/L, not the actual diel change in dissolved oxygen. The highest average diel change in dissolved oxygen I found was from Penns Creek with an average diel change in dissolved oxygen of 5.45 mg/L. However, the lowest recorded dissolved oxygen for this site was 5.53 mg/L, well above hypoxic levels. Therefore, it is not surprising that the average diel change in dissolved oxygen did not have a direct impact on macroinvertebrate communities because these communities were likely receiving adequate dissolved oxygen, even at their lowest dissolved oxygen levels. I was unable to detect a change in the average diel change in dissolved oxygen due to the presence of *Cladophora* in streams. This is likely due to *Cladophora* growths not completely overtaking the streambeds. The site with the most *Cladophora* cover was Penns Creek (see Fig. 10). The *Cladophora* streams had *Cladophora* patches large enough to be considered *Cladophora* patches, greater than 50% *Cladophora* cover within a Surber sample, but I would not consider any of the sites in this study to be completely overrun with *Cladophora*. Therefore, it is unlikely the *Cladophora* would be significantly impacting the average diel change in dissolved oxygen within the streams at a detectable level. To see significant changes in average diel change in dissolved oxygen due to *Cladophora* with this number of sites, the sites in this study would need to have larger amounts of *Cladophora* present in the stream, greater than that of Penns Creek.

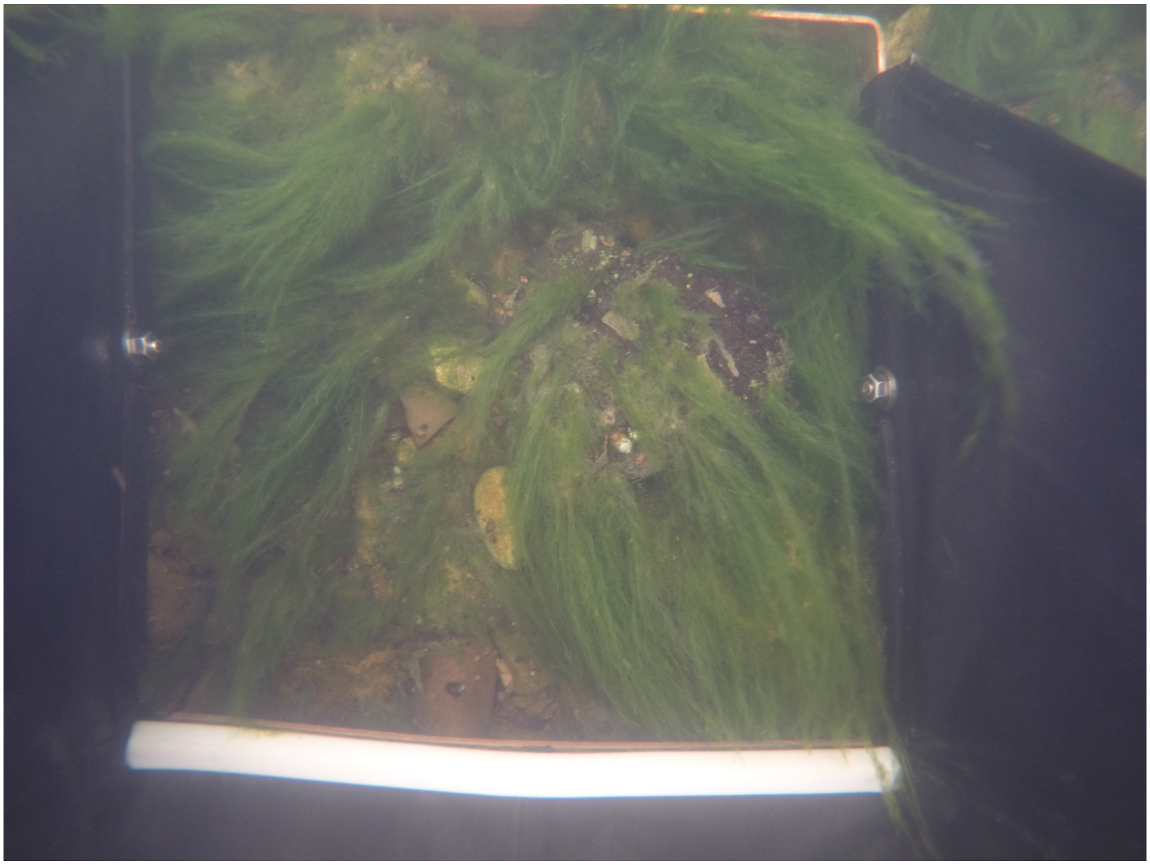

Figure 10. Image serving as an example of a *Cladophora* patch sample taken within Penns Creek before a Surber sample from a patch containing *Cladophora* was collected.

Low macroinvertebrate diversity in streams has been attributed to large (13°C) diel changes in water temperature (Jacobsen and Marín, 2008). However, the highest average diel change in water temperature I found was 5.25°C in Briar Creek. Most of the sites fell within a diel change in water temperature of around 3-4°C. Therefore, it is not surprising the diel change in water temperature did not have a direct impact on macroinvertebrate community structure at the stream.

Nutrient enrichment, like phosphorus, can cause shifts in macroinvertebrate communities, and more specifically, indirectly increase the Chironomidae populations within macroinvertebrate stream communities (Gafner and Robinson, 2007; Friberg et al., 2010). My results indicate total phosphorus did not directly impact macroinvertebrate communities between streams. However, it has been well documented that TP influences the growth of *Cladophora* in streams (Whitton, 1970; Stevenson et al., 2012; Zulkifly et al., 2013; Dodds and Gudder, 1992). Therefore, TP is indirectly influencing macroinvertebrate communities, and more specifically Chironomidae populations, through the growth of *Cladophora*.

My hypotheses were: (1) *Cladophora* biomass will alter macroinvertebrate community membership at varying spatial scales both on a scale of patch distribution, within streams, and between streams, and (2) areas with *Cladophora* present will have different macroinvertebrate densities, percent Chironomidae (%Chironomidae), Shannon-diversity Index values, Hilsenhoff Biotic Index (HBI) values, and percent Ephemeroptera, Plecoptera, and Trichoptera taxa (%EPT taxa), when compared to areas without *Cladophora*. My first hypothesis is supported by my findings. Though the community composition may not clearly differ between patches where *Cladophora* was present and where it was absent from the same streams, the % Chironomidae was significantly higher in the patches containing *Cladophora*. Therefore, at the patch scale, a greater number of Chironomidae can be found in areas containing *Cladophora* when compared to areas the alga is absent. Macroinvertebrate communities differed more at the stream level, than the patch level. The basis for this statement is founded on the difference between macroinvertebrate community structure when comparing the macroinvertebrate communities by stream type. My second hypothesis was also supported by my findings. While not all the macroinvertebrate metrics would support this claim, both %Chironomidae and Shannon diversity differed between patch types within the same streams. The Shannon diversity scores were lower in samples from *Cladophora* patches, due to the %Chironomidae being greater in these patches. I found supporting evidence that alkalinity of streams can determine the distribution of *Cladophora* in streams of central Pennsylvania. My findings suggest that *Cladophora* growth in the streams I examined has not reached the nuisance levels reported by Stevenson et al. (2012) and is likely not strongly influencing diel changes in dissolved oxygen at a scale large enough for me perceive with the equipment I used. *Cladophora* seems to be influencing the macroinvertebrate community structure to be skewed with a dominant presence of Chironomidae.

Future research could focus on the impacts of *Cladophora* on higher trophic levels. Beneficial questions which could be addressed include the following: What does the increased densities of macroinvertebrates mean for the organisms that feed on these macroinvertebrates? How does the presence or absence of *Cladophora* influence fish species and aquatic predators feeding on the aquatic life stages of macroinvertebrates? The results provided in this study lay the groundwork for studying the questions above and provide insight into how humans are impacting streams and, more specifically, the macroinvertebrates within streams.

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APPENDICES:

Appendix A

Raw data including the name of each stream site, the patch type, proportion of the total sample that was identified, date the macroinvertebrate samples were collected, total phosphorus (TP) in (ug/L), total nitrogen (TN) in (ug/L), total alkalinity in (mg $CaCO_3$/L), Latitude and Longitude coordinates for each site, percent agriculture (%Ag) in each stream watershed, average diel change in dissolved oxygen (Average ΔDO) (mg/L), average diel change in water temperature in °C (Average ΔTemperature), and the identified macroinvertebrate data for each stream.

Table 1. Raw data collected from each site including the stream site, patch type, proportion of the total macroinvertebrate sample that was identified, date the samples were collected, TP (ug/L), TN (ug/L), total alkalinity (mg $CaCO_3$/L), coordinates for each site, %Ag in each stream watershed, average diel change in dissolved oxygen (Average ΔDO) (mg/L), average diel change in water temperature in °C (Average ΔTemperature), and identified macroinvertebrate data for each stream.

Stream	Patch Type	Proportion subsampled	Date	TP	TN	Total Alkalinity
Briar Creek	Non-Cladophora Stream	1 (N/A)	7/10/19	61.9779566	1815.336	60
Penns Creek	Cladophora Patch	1/14	8/14/19	66.6792756	1174.874	96
Penns Creek	Non-Cladophora Patch	3/28	8/14/19	66.6792756	1174.874	96
Fishing Creek	Non-Cladophora Stream	3/28	7/16/19	30.0589682	1011.955	58
Green Creek	Non-Cladophora Stream	9/28	7/15/19	15.5895266	2491.359	50
Mahoning Creek	Cladophora Patch	1/28	8/20/19	62.3403174	1619.008	103
Mahoning Creek	Non-Cladophora Patch	3/28	8/20/19	62.3403174	1619.008	103
Chillisquaque Creek	Cladophora Patch	1/7	8/15/19	98.1796748	1982.938	101
Chillisquaque Creek	Non-Cladophora Patch	3/28	8/15/19	98.1796748	1982.938	101
Mauses Creek	Non-Cladophora Patch	3/28	8/20/19	43.3913466	1805.275	134
Mauses Creek	Cladophora Patch	3/28	8/20/19	43.3913466	1805.275	134
Hemlock Creek	Non-Cladophora Stream	9/28	7/15/19	22.580591	2650.922	68
Warrior Run	Cladophora Patch	3/28	8/22/19	29.0968378	731.019	102
Warrior Run	Non-Cladophora Patch	3/28	8/22/19	29.0968378	731.019	102

Table 1continued

Stream	Patch Type	Proportion subsampled	Date	TP	TN	Total Alkalinity
Little Fishing Creek	Non-Cladophora Stream	3/28	7/17/19	33.3045964	1591.275	58
Huntington Creek	Non-Cladophora Stream	1/7	7/16/19	26.8352066	720.604	47
Turtle Creek	Cladophora Patch	1/56	8/18/19	46.0340814	5128.933	205
Turtle Creek	Non-Cladophora Patch	1/28	8/18/19	46.0340814	5128.933	205
North Mahantango Creek	Non-Cladophora Stream	1/7	8/14/19	51.553836	1422.952	154

Table 1 continued

Stream	Patch Type	Latitude	Longitude	%Ag	Average ΔDO	Average ΔTemperature
Briar Creek	Non-Cladophora Stream	41.0679	-76.29013	34.39	1.702929	5.252929
Penns Creek	Cladophora Patch	40.826213	-76.87228	27	5.45675	3.921875
Penns Creek	Non-Cladophora Patch	40.826213	-76.87228	27	5.45675	3.921875
Fishing Creek	Non-Cladophora Stream	41.048969	-76.42997	28.08	2.199591	3.14
Green Creek	Non-Cladophora Stream	41.109922	-76.41719	52.15	1.261667	2.960667
Mahoning Creek	Cladophora Patch	40.977506	-76.62412	46.1	3.1626	2.908
Mahoning Creek	Non-Cladophora Patch	40.977506	-76.62412	46.1	3.1626	2.908
Chillisquaque Creek	Cladophora Patch	40.960146	-76.81623	65.71	1.433111	1.629444
Chillisquaque Creek	Non-Cladophora Patch	40.960146	-76.81623	65.71	1.433111	1.629444
Mauses Creek	Non-Cladophora Patch	40.984725	-76.63109	45.28	1.7227	2.4646
Mauses Creek	Cladophora Patch	40.984725	-76.63109	45.28	1.7227	2.4646
Hemlock Creek	Non-Cladophora Stream	40.995499	-76.4861	54.39	1.263313	4.056313
Warrior Run	Cladophora Patch	41.101962	-76.79345	39.97	1.41225	1.78
Warrior Run	Non-Cladophora Patch	41.101962	-76.79345	39.97	1.41225	1.78
Little Fishing Creek	Non-Cladophora Stream	41.027598	-76.48027	39.1	1.599444	3.647833
Huntington Creek	Non-Cladophora Stream	41.113928	-76.33979	26.61	1.394375	3.0385
Turtle Creek	Cladophora Patch	40.928275	-76.88129	46.72	4.377	4.170333
Turtle Creek	Non-Cladophora Patch	40.928275	-76.88129	46.72	4.377	4.170333
North Mahantango Creek	Non-Cladophora Stream	40.6495	-76.96636	35.31	2.715944	3.301778

Table 1 continued

Stream	Patch Type	Chironomidae	Psephenidae	Elmidae	Oligochaeta	Bivalvia
Briar Creek	Non-Cladophora Stream	665	2	12	20	4
Penns Creek	Cladophora Patch	138	2	107	2	2
Penns Creek	Non-Cladophora Patch	43	5	70	6	0
Fishing Creek	Non-Cladophora Stream	151	0	2	3	0
Green Creek	Non-Cladophora Stream	94	8	38	9	0
Mahoning Creek	Cladophora Patch	172	2	15	3	0
Mahoning Creek	Non-Cladophora Patch	96	6	47	21	0
Chillisquaque Creek	Cladophora Patch	88	1	26	2	0
Chillisquaque Creek	Non-Cladophora Patch	61	6	115	4	0
Mauses Creek	Non-Cladophora Patch	54	2	13	45	0
Mauses Creek	Cladophora Patch	173	3	18	19	0
Hemlock Creek	Non-Cladophora Stream	32	2	12	5	0
Warrior Run	Cladophora Patch	327	2	5	1	0
Warrior Run	Non-Cladophora Patch	152	2	19	6	0
Little Fishing Creek	Non-Cladophora Stream	48	2	37	65	0
Huntington Creek	Non-Cladophora Stream	117	3	22	18	1
Turtle Creek	Cladophora Patch	169	20	45	4	4
Turtle Creek	Non-Cladophora Patch	111	9	82	5	2
North Mahantango Creek	Non-Cladophora Stream	160	10	106	5	5

Table 1 continued

Stream	Patch Type	Chloroperlidae	Heptageniidae	Limoniidae	Tabanidae	Julida
Briar Creek	Non-Cladophora Stream	1	1	5	1	1
Penns Creek	Cladophora Patch	0	9	0	0	0
Penns Creek	Non-Cladophora Patch	0	7	0	0	0
Fishing Creek	Non-Cladophora Stream	0	8	0	0	0
Green Creek	Non-Cladophora Stream	3	34	2	0	0
Mahoning Creek	Cladophora Patch	0	15	0	0	0
Mahoning Creek	Non-Cladophora Patch	0	17	0	0	0
Chillisquaque Creek	Cladophora Patch	0	7	0	0	0
Chillisquaque Creek	Non-Cladophora Patch	0	21	0	0	0
Mauses Creek	Non-Cladophora Patch	0	11	7	0	0
Mauses Creek	Cladophora Patch	0	10	12	0	0
Hemlock Creek	Non-Cladophora Stream	0	11	2	0	0
Warrior Run	Cladophora Patch	1	4	5	0	0
Warrior Run	Non-Cladophora Patch	0	2	6	0	0
Little Fishing Creek	Non-Cladophora Stream	1	35	1	0	0
Huntington Creek	Non-Cladophora Stream	0	8	12	0	0
Turtle Creek	Cladophora Patch	0	0	7	0	0
Turtle Creek	Non-Cladophora Patch	0	0	8	0	0
North Mahantango Creek	Non-Cladophora Stream	0	2	3	0	0

Table 1 continued

Stream	Patch Type	Nematoda	Isopoda	Baetidae	Hydropsychidae	Arachnida	Viviparidae
Briar Creek	Non-Cladophora Stream	2	1	3	1	1	0
Penns Creek	Cladophora Patch	1	0	32	24	0	80
Penns Creek	Non-Cladophora Patch	0	0	48	45	0	39
Fishing Creek	Non-Cladophora Stream	5	0	70	57	0	0
Green Creek	Non-Cladophora Stream	1	0	86	72	0	0
Mahoning Creek	Cladophora Patch	1	0	31	82	0	0
Mahoning Creek	Non-Cladophora Patch	10	0	32	103	0	0
Chillisquaque Creek	Cladophora Patch	0	0	28	131	1	0
Chillisquaque Creek	Non-Cladophora Patch	5	0	25	79	0	0
Mauses Creek	Non-Cladophora Patch	4	0	84	76	0	0
Mauses Creek	Cladophora Patch	0	0	66	55	0	0
Hemlock Creek	Non-Cladophora Stream	6	0	80	103	0	0
Warrior Run	Cladophora Patch	2	0	33	49	0	0
Warrior Run	Non-Cladophora Patch	28	0	51	55	0	0
Little Fishing Creek	Non-Cladophora Stream	16	0	124	67	0	0
Huntington Creek	Non-Cladophora Stream	1	0	25	61	0	0
Turtle Creek	Cladophora Patch	1	3	26	65	0	0
Turtle Creek	Non-Cladophora Patch	7	0	26	46	0	0
North Mahantango Creek	Non-Cladophora Stream	8	0	7	62	0	0

Table 1 continued

Stream	Patch Type	Lepidostomatidae	Perlidae	Glossosomatidae	Philopotamidae
Briar Creek	Non-Cladophora Stream	0	0	0	0
Penns Creek	Cladophora Patch	1	5	32	1
Penns Creek	Non-Cladophora Patch	0	4	13	2
Fishing Creek	Non-Cladophora Stream	4	7	0	7
Green Creek	Non-Cladophora Stream	0	2	0	3
Mahoning Creek	Cladophora Patch	0	0	0	7
Mahoning Creek	Non-Cladophora Patch	0	0	0	5
Chillisquaque Creek	Cladophora Patch	0	0	0	9
Chillisquaque Creek	Non-Cladophora Patch	0	0	0	19
Mauses Creek	Non-Cladophora Patch	0	0	23	8
Mauses Creek	Cladophora Patch	0	0	1	4
Hemlock Creek	Non-Cladophora Stream	0	0	4	22
Warrior Run	Cladophora Patch	0	0	2	7
Warrior Run	Non-Cladophora Patch	0	0	1	20
Little Fishing Creek	Non-Cladophora Stream	0	2	0	5
Huntington Creek	Non-Cladophora Stream	0	3	0	0
Turtle Creek	Cladophora Patch	0	0	0	17
Turtle Creek	Non-Cladophora Patch	0	0	0	44
North Mahantango Creek	Non-Cladophora Stream	0	0	0	1

Table 1 continued

Stream	Patch Type	Potamanthidae	Simuliidae	Hydroptilidae	Helicopsyche
Briar Creek	Non-Cladophora Stream	0	0	0	0
Penns Creek	Cladophora Patch	22	10	9	1
Penns Creek	Non-Cladophora Patch	24	12	15	0
Fishing Creek	Non-Cladophora Stream	0	0	0	0
Green Creek	Non-Cladophora Stream	0	0	0	0
Mahoning Creek	Cladophora Patch	0	2	10	0
Mahoning Creek	Non-Cladophora Patch	0	0	13	0
Chillisquaque Creek	Cladophora Patch	0	4	75	0
Chillisquaque Creek	Non-Cladophora Patch	0	0	30	0
Mauses Creek	Non-Cladophora Patch	0	1	1	0
Mauses Creek	Cladophora Patch	0	0	0	0
Hemlock Creek	Non-Cladophora Stream	0	4	0	0
Warrior Run	Cladophora Patch	0	4	6	0
Warrior Run	Non-Cladophora Patch	0	0	0	0
Little Fishing Creek	Non-Cladophora Stream	0	2	0	0
Huntington Creek	Non-Cladophora Stream	0	0	3	0
Turtle Creek	Cladophora Patch	0	4	22	1
Turtle Creek	Non-Cladophora Patch	0	0	1	0
North Mahantango Creek	Non-Cladophora Stream	8	0	4	3

Table 1 continued

Stream	Patch Type	Turbellaria	Ephemerellidae	Caenidae	Brachycentridae
Briar Creek	Non-Cladophora Stream	0	0	0	0
Penns Creek	Cladophora Patch	7	57	1	1
Penns Creek	Non-Cladophora Patch	5	23	1	0
Fishing Creek	Non-Cladophora Stream	1	13	1	1
Green Creek	Non-Cladophora Stream	0	2	0	0
Mahoning Creek	Cladophora Patch	1	0	0	0
Mahoning Creek	Non-Cladophora Patch	7	0	3	0
Chillisquaque Creek	Cladophora Patch	8	2	0	0
Chillisquaque Creek	Non-Cladophora Patch	9	0	0	0
Mauses Creek	Non-Cladophora Patch	2	0	0	0
Mauses Creek	Cladophora Patch	0	3	0	0
Hemlock Creek	Non-Cladophora Stream	1	0	0	0
Warrior Run	Cladophora Patch	9	0	0	0
Warrior Run	Non-Cladophora Patch	8	0	0	0
Little Fishing Creek	Non-Cladophora Stream	0	0	1	0
Huntington Creek	Non-Cladophora Stream	0	3	0	0
Turtle Creek	Cladophora Patch	22	0	0	0
Turtle Creek	Non-Cladophora Patch	58	0	0	0
North Mahantango Creek	Non-Cladophora Stream	2	14	0	9

Table 1 continued

Stream	Patch Type	Athericidae	Isonychiidae	Ancylidae	Hydrachnidia
Briar Creek	Non-Cladophora Stream	0	0	0	0
Penns Creek	Cladophora Patch	1	0	0	0
Penns Creek	Non-Cladophora Patch	0	1	1	0
Fishing Creek	Non-Cladophora Stream	4	13	0	1
Green Creek	Non-Cladophora Stream	15	1	0	2
Mahoning Creek	Cladophora Patch	16	3	0	0
Mahoning Creek	Non-Cladophora Patch	21	4	0	3
Chillisquaque Creek	Cladophora Patch	20	3	0	4
Chillisquaque Creek	Non-Cladophora Patch	13	2	0	6
Mauses Creek	Non-Cladophora Patch	1	0	0	0
Mauses Creek	Cladophora Patch	1	1	4	4
Hemlock Creek	Non-Cladophora Stream	1	0	0	1
Warrior Run	Cladophora Patch	1	0	0	1
Warrior Run	Non-Cladophora Patch	0	0	1	1
Little Fishing Creek	Non-Cladophora Stream	3	12	0	3
Huntington Creek	Non-Cladophora Stream	0	0	1	2
Turtle Creek	Cladophora Patch	2	0	1	1
Turtle Creek	Non-Cladophora Patch	0	0	0	3
North Mahantango Creek	Non-Cladophora Stream	0	2	0	2

Table 1 continued

Stream	Patch Type	Capniidae	Aeshnidae	Ceratopogonidae	Polycentropodidae	Sialis
Briar Creek	Non-Cladophora Stream	6	0	0	0	0
Penns Creek	Cladophora Patch	0	0	0	0	0
Penns Creek	Non-Cladophora Patch	0	0	0	0	0
Fishing Creek	Non-Cladophora Stream	2	0	0	0	0
Green Creek	Non-Cladophora Stream	0	1	1	2	1
Mahoning Creek	Cladophora Patch	0	0	1	3	0
Mahoning Creek	Non-Cladophora Patch	0	0	0	4	1
Chillisquaque Creek	Cladophora Patch	0	0	0	3	1
Chillisquaque Creek	Non-Cladophora Patch	0	0	0	2	0
Mauses Creek	Non-Cladophora Patch	0	0	0	0	0
Mauses Creek	Cladophora Patch	0	0	0	0	0
Hemlock Creek	Non-Cladophora Stream	0	0	0	2	0
Warrior Run	Cladophora Patch	0	0	0	4	0
Warrior Run	Non-Cladophora Patch	0	0	0	4	0
Little Fishing Creek	Non-Cladophora Stream	1	0	1	1	0
Huntington Creek	Non-Cladophora Stream	0	0	0	7	2
Turtle Creek	Cladophora Patch	0	0	0	0	0
Turtle Creek	Non-Cladophora Patch	0	0	0	4	0
North Mahantango Creek	Non-Cladophora Stream	0	0	0	0	0

Table 1 continued

Stream	Patch Type	Amphipoda	Perlodidae	Gerridae	Formicidae
Briar Creek	Non-Cladophora Stream	0	0	0	0
Penns Creek	Cladophora Patch	0	0	0	0
Penns Creek	Non-Cladophora Patch	0	0	0	0
Fishing Creek	Non-Cladophora Stream	0	0	0	0
Green Creek	Non-Cladophora Stream	0	0	0	0
Mahoning Creek	Cladophora Patch	0	0	0	0
Mahoning Creek	Non-Cladophora Patch	4	1	1	0
Chillisquaque Creek	Cladophora Patch	0	0	0	1
Chillisquaque Creek	Non-Cladophora Patch	0	0	0	0
Mauses Creek	Non-Cladophora Patch	0	0	0	0
Mauses Creek	Cladophora Patch	0	0	0	0
Hemlock Creek	Non-Cladophora Stream	0	0	0	0
Warrior Run	Cladophora Patch	0	0	0	0
Warrior Run	Non-Cladophora Patch	0	0	0	0
Little Fishing Creek	Non-Cladophora Stream	0	0	0	0
Huntington Creek	Non-Cladophora Stream	0	0	0	0
Turtle Creek	Cladophora Patch	1	0	0	0
Turtle Creek	Non-Cladophora Patch	2	0	0	0
North Mahantango Creek	Non-Cladophora Stream	0	0	0	0

Table 1 continued

Stream	Patch Type	Leptophlebiidae	Leuctridae	Limnephilidae	Tipulidae
Briar Creek	Non-Cladophora Stream	0	0	0	0
Penns Creek	Cladophora Patch	0	0	0	0
Penns Creek	Non-Cladophora Patch	0	0	0	0
Fishing Creek	Non-Cladophora Stream	0	0	0	0
Green Creek	Non-Cladophora Stream	0	0	0	0
Mahoning Creek	Cladophora Patch	0	0	0	0
Mahoning Creek	Non-Cladophora Patch	0	0	0	0
Chillisquaque Creek	Cladophora Patch	0	0	0	0
Chillisquaque Creek	Non-Cladophora Patch	0	0	0	0
Mauses Creek	Non-Cladophora Patch	0	0	0	0
Mauses Creek	Cladophora Patch	1	0	0	0
Hemlock Creek	Non-Cladophora Stream	0	4	1	0
Warrior Run	Cladophora Patch	0	0	0	0
Warrior Run	Non-Cladophora Patch	0	0	0	1
Little Fishing Creek	Non-Cladophora Stream	0	0	0	0
Huntington Creek	Non-Cladophora Stream	0	0	5	0
Turtle Creek	Cladophora Patch	0	0	0	0
Turtle Creek	Non-Cladophora Patch	0	0	0	0
North Mahantango Creek	Non-Cladophora Stream	0	0	0	0

Table 1 continued

Stream	Patch Type	Corydalidae	Psychomyiidae	Pediciidae	Gomphidae
Briar Creek	Non-Cladophora Stream	0	0	0	0
Penns Creek	Cladophora Patch	0	0	0	0
Penns Creek	Non-Cladophora Patch	0	0	0	0
Fishing Creek	Non-Cladophora Stream	0	0	0	0
Green Creek	Non-Cladophora Stream	0	0	0	0
Mahoning Creek	Cladophora Patch	0	0	0	0
Mahoning Creek	Non-Cladophora Patch	0	0	0	0
Chillisquaque Creek	Cladophora Patch	0	0	0	0
Chillisquaque Creek	Non-Cladophora Patch	0	0	0	0
Mauses Creek	Non-Cladophora Patch	0	0	0	0
Mauses Creek	Cladophora Patch	0	0	0	0
Hemlock Creek	Non-Cladophora Stream	0	0	0	0
Warrior Run	Cladophora Patch	0	0	0	0
Warrior Run	Non-Cladophora Patch	2	0	0	0
Little Fishing Creek	Non-Cladophora Stream	0	0	0	0
Huntington Creek	Non-Cladophora Stream	0	10	0	0
Turtle Creek	Cladophora Patch	0	0	0	0
Turtle Creek	Non-Cladophora Patch	0	0	1	0
North Mahantango Creek	Non-Cladophora Stream	1	0	6	2

Table 1 continued

Stream	Patch Type	Polymitarcyidae	Unknowns	Totals
Briar Creek	Non-Cladophora Stream	0	4	730
Penns Creek	Cladophora Patch	1	22	568
Penns Creek	Non-Cladophora Patch	0	10	374
Fishing Creek	Non-Cladophora Stream	0	34	384
Green Creek	Non-Cladophora Stream	0	27	404
Mahoning Creek	Cladophora Patch	0	9	373
Mahoning Creek	Non-Cladophora Patch	0	14	413
Chillisquaque Creek	Cladophora Patch	0	15	429
Chillisquaque Creek	Non-Cladophora Patch	0	19	416
Mauses Creek	Non-Cladophora Patch	0	25	357
Mauses Creek	Cladophora Patch	0	30	405
Hemlock Creek	Non-Cladophora Stream	0	15	308
Warrior Run	Cladophora Patch	0	15	478
Warrior Run	Non-Cladophora Patch	0	28	387
Little Fishing Creek	Non-Cladophora Stream	0	17	444
Huntington Creek	Non-Cladophora Stream	0	15	319
Turtle Creek	Cladophora Patch	0	19	434
Turtle Creek	Non-Cladophora Patch	0	15	424
North Mahantango Creek	Non-Cladophora Stream	0	12	434

Appendix B

Dissolved oxygen and water temperature data for each site including the following variables: dissolved oxygen (a), water temperature (b), diel change in dissolved oxygen, labeled ΔDO (c), diel change in water temperature, labeled ΔTemperature (d). The figures for each stream are presented in the following order: Briar Creek (Fig. B1), Chillisquaque Creek (Fig. B2), Fishing Creek (Fig. B3), Green Creek (Fig. B4), Hemlock Creek (Fig. B5), Huntington Creek (Fig. B6), Little Fishing Creek (Fig. B7), Mahoning Creek (Fig. B8), Mauses Creek (Fig. B9), North Mahantango Creek (Fig. B10), Penns Creek (Fig. B11), Turtle Creek (Fig. B12), and Warrior Run (Fig. B13). All figures were created by the author using R statistical software (R Core Team, 2019).

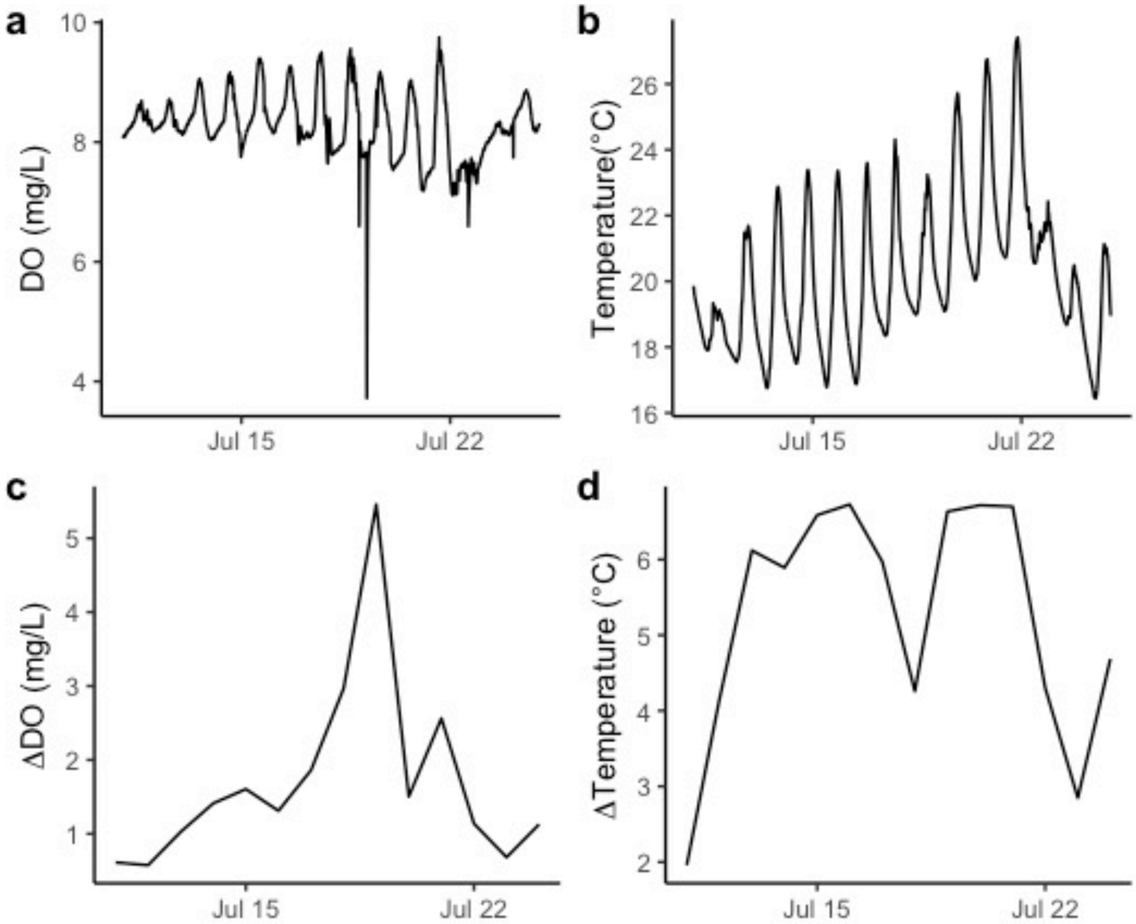

Figure B1. Briar Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

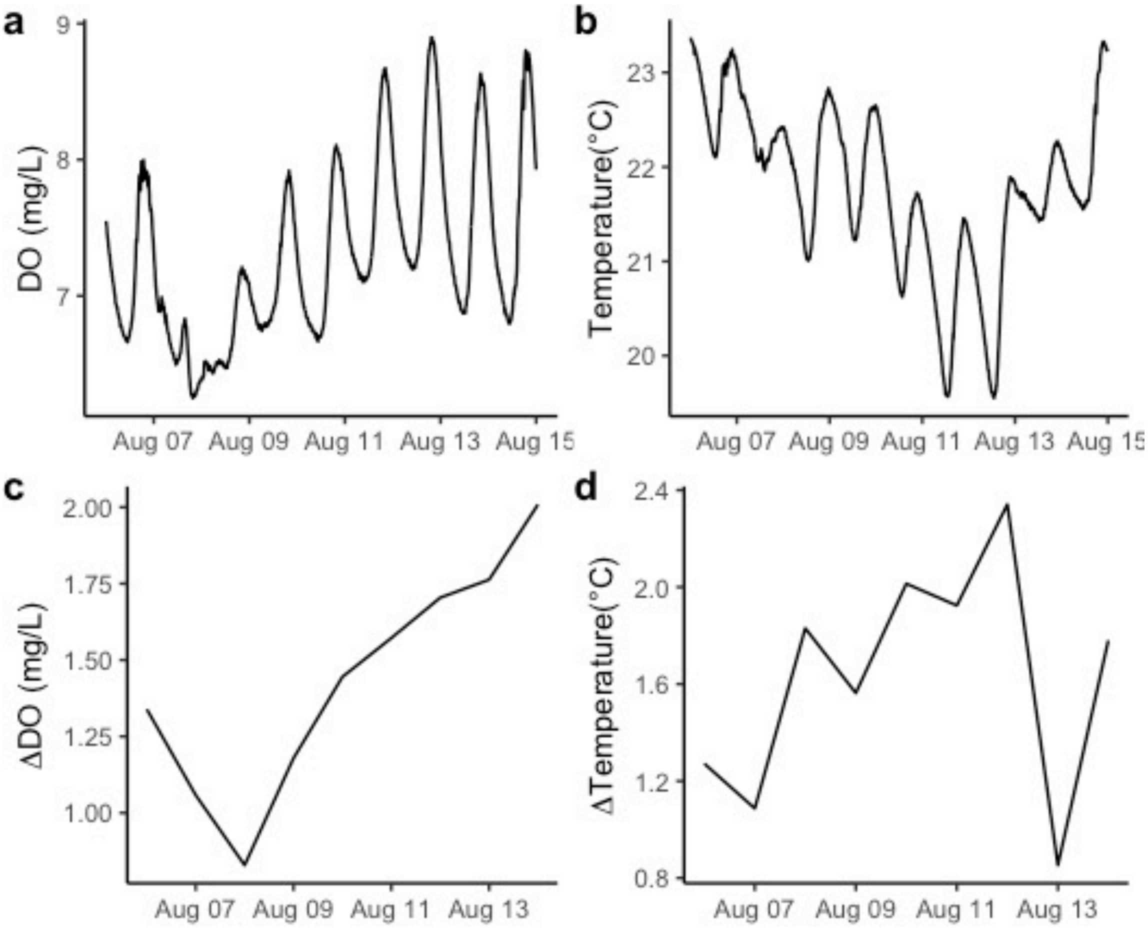

Figure B2. Chillisquaque Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

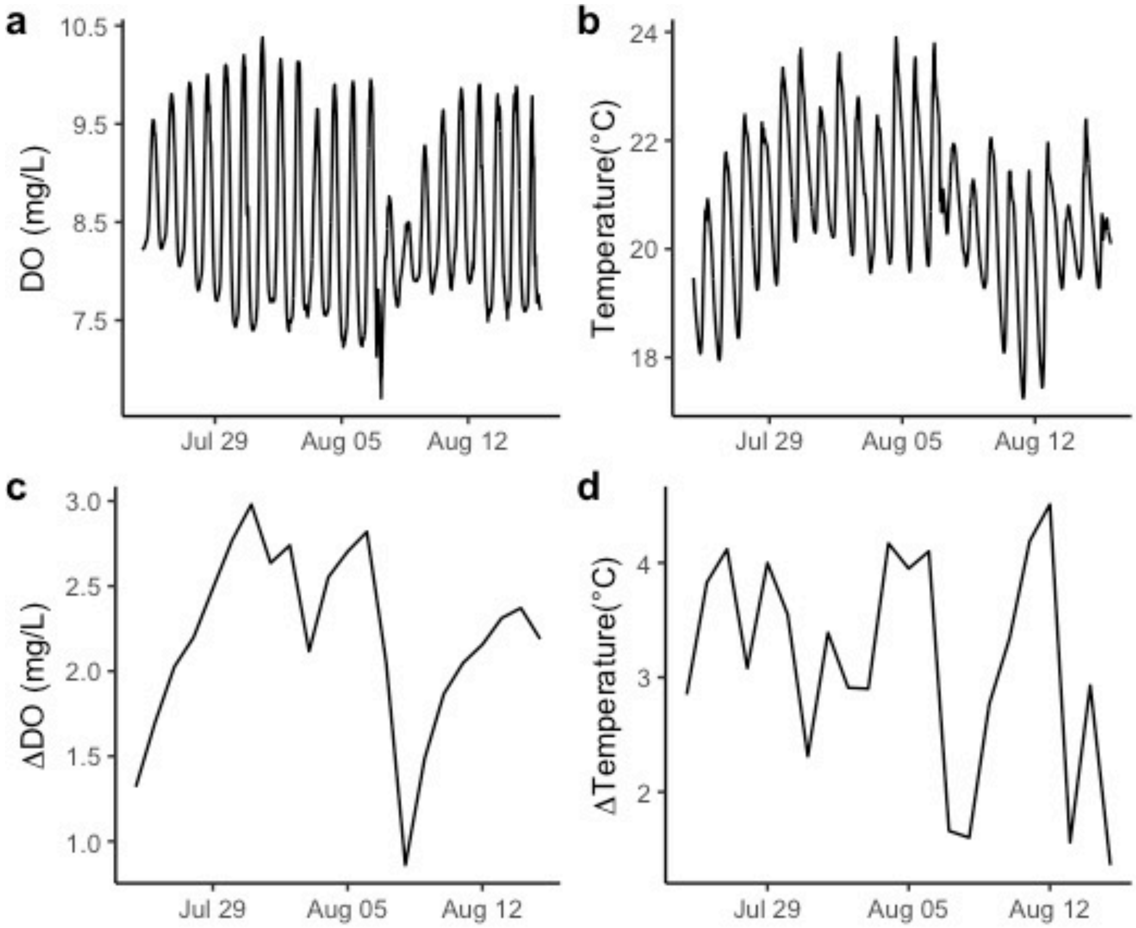

Figure B3. Fishing Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

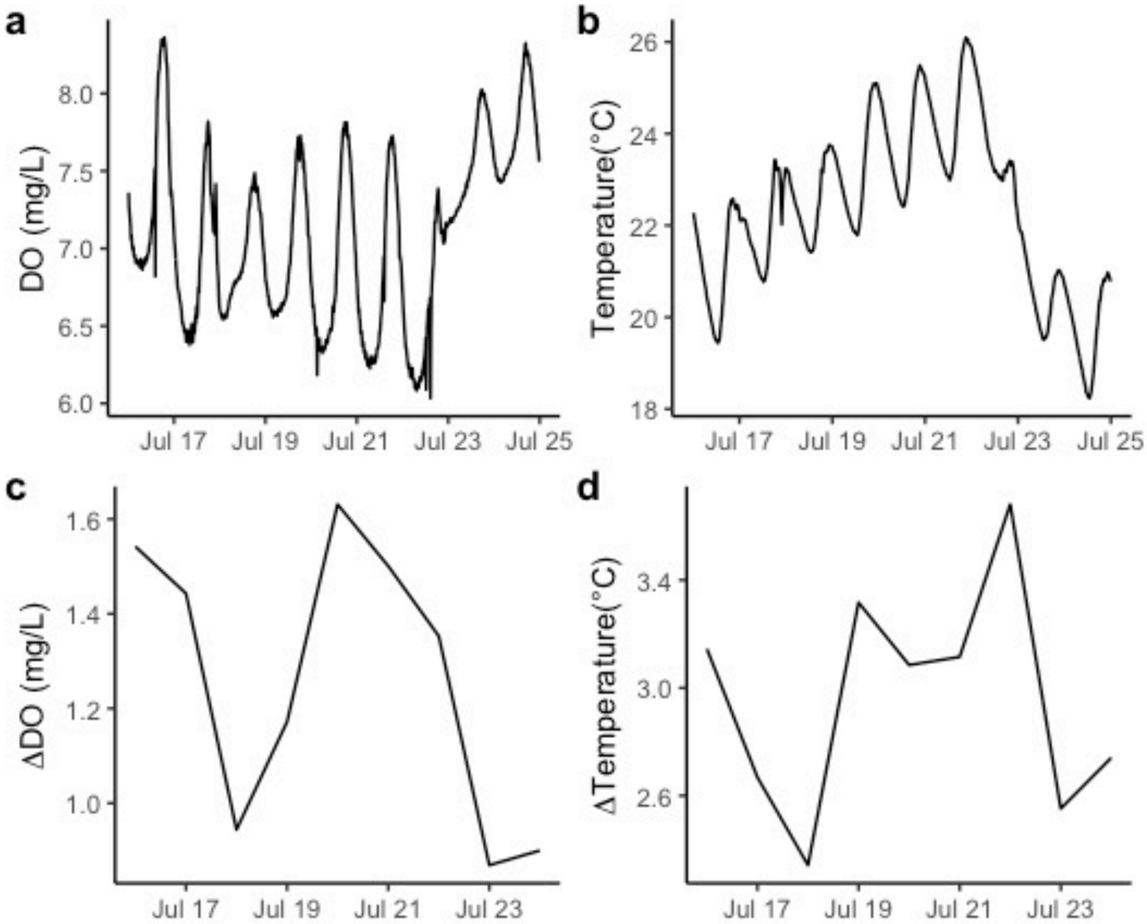

Figure B4. Green Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

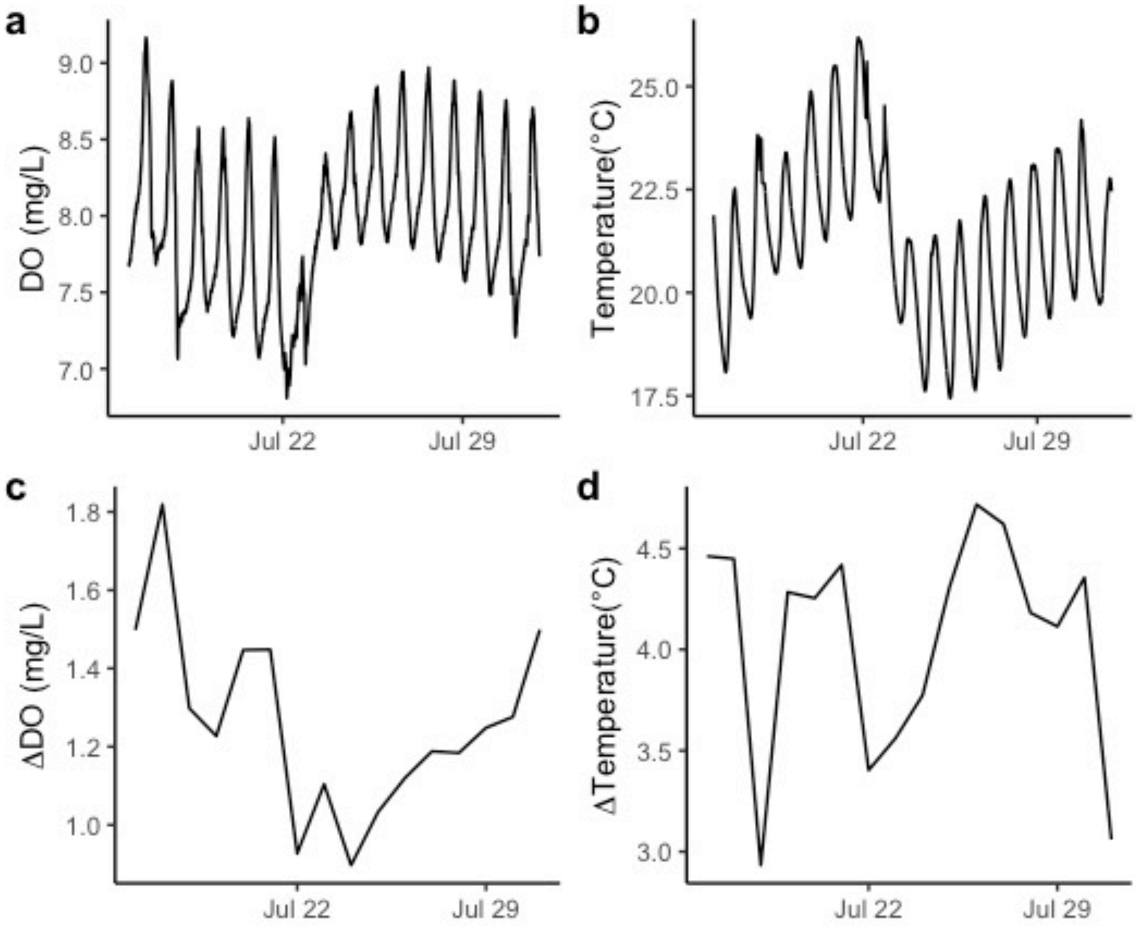

Figure B5. Hemlock Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

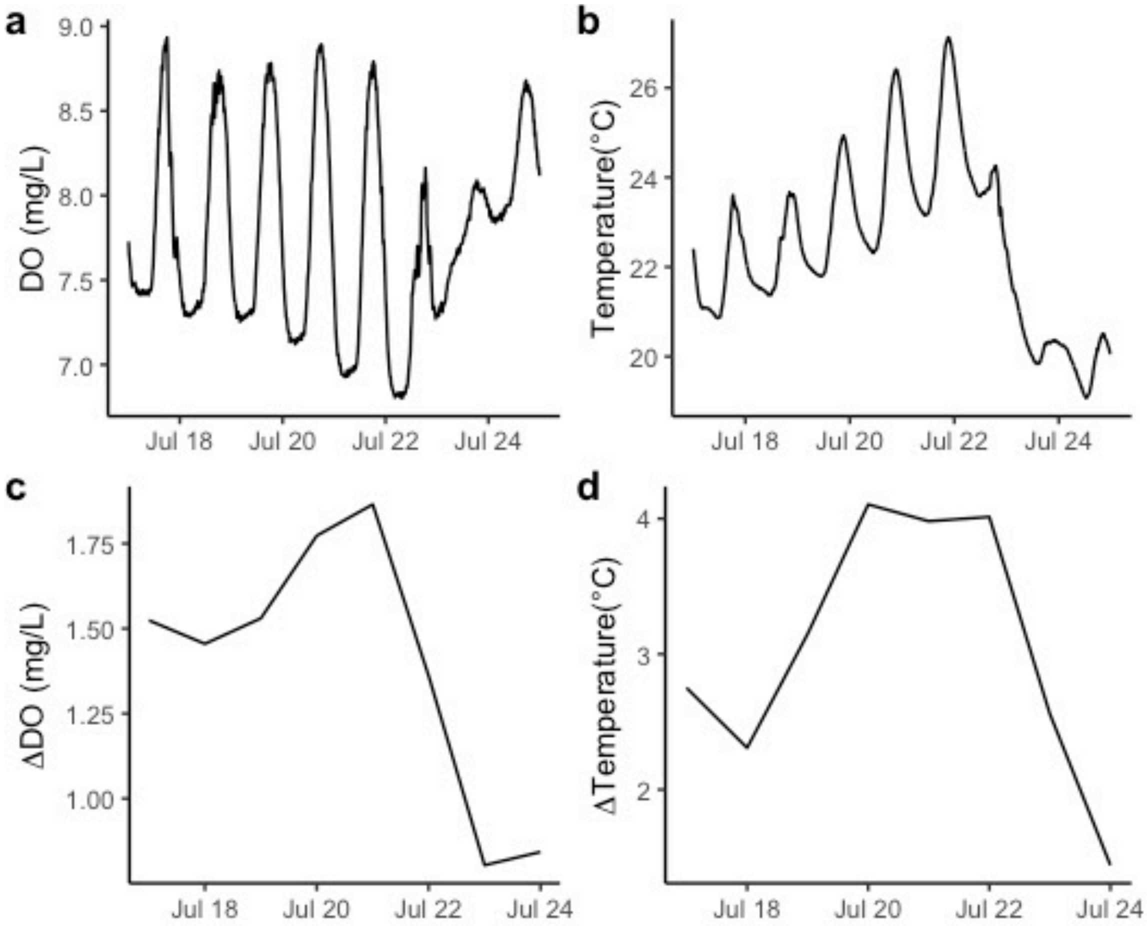

Figure B6. Huntington Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

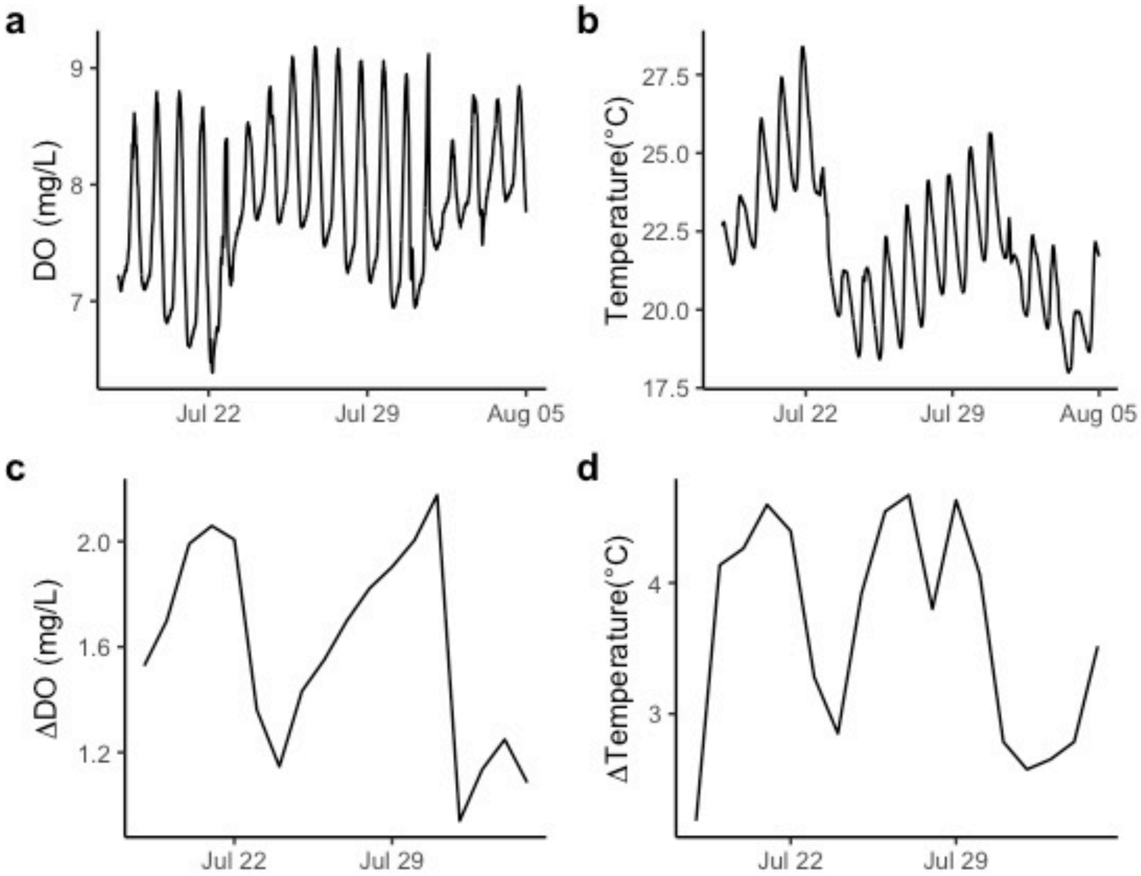

Figure B7. Little Fishing Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

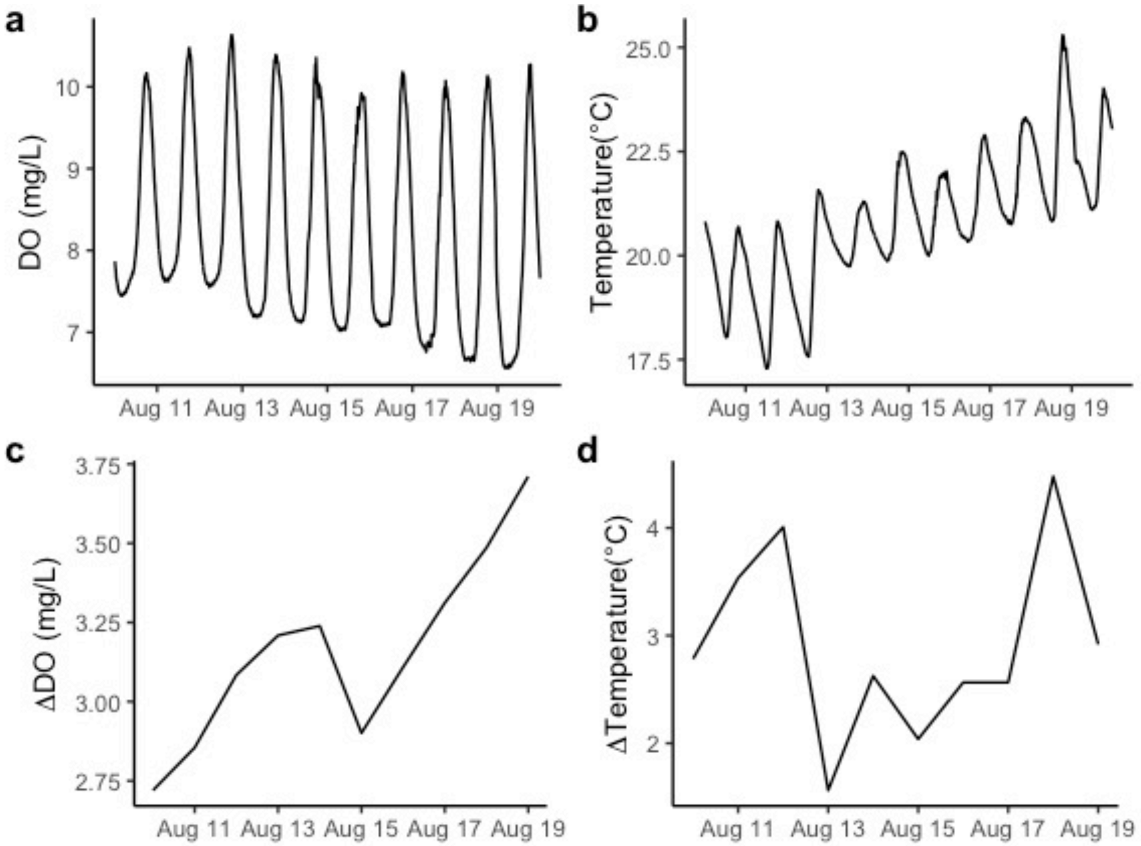

Figure B8. Mahoning Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

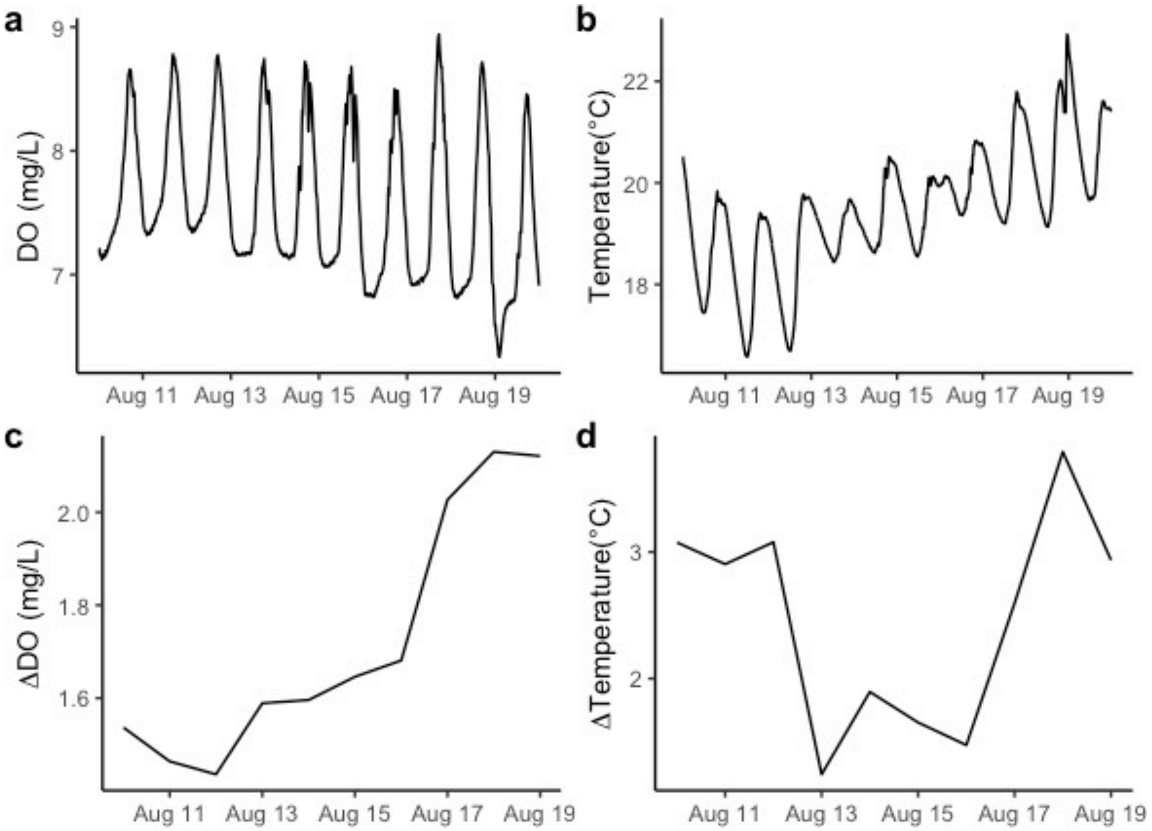

Figure B9. Mauses Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

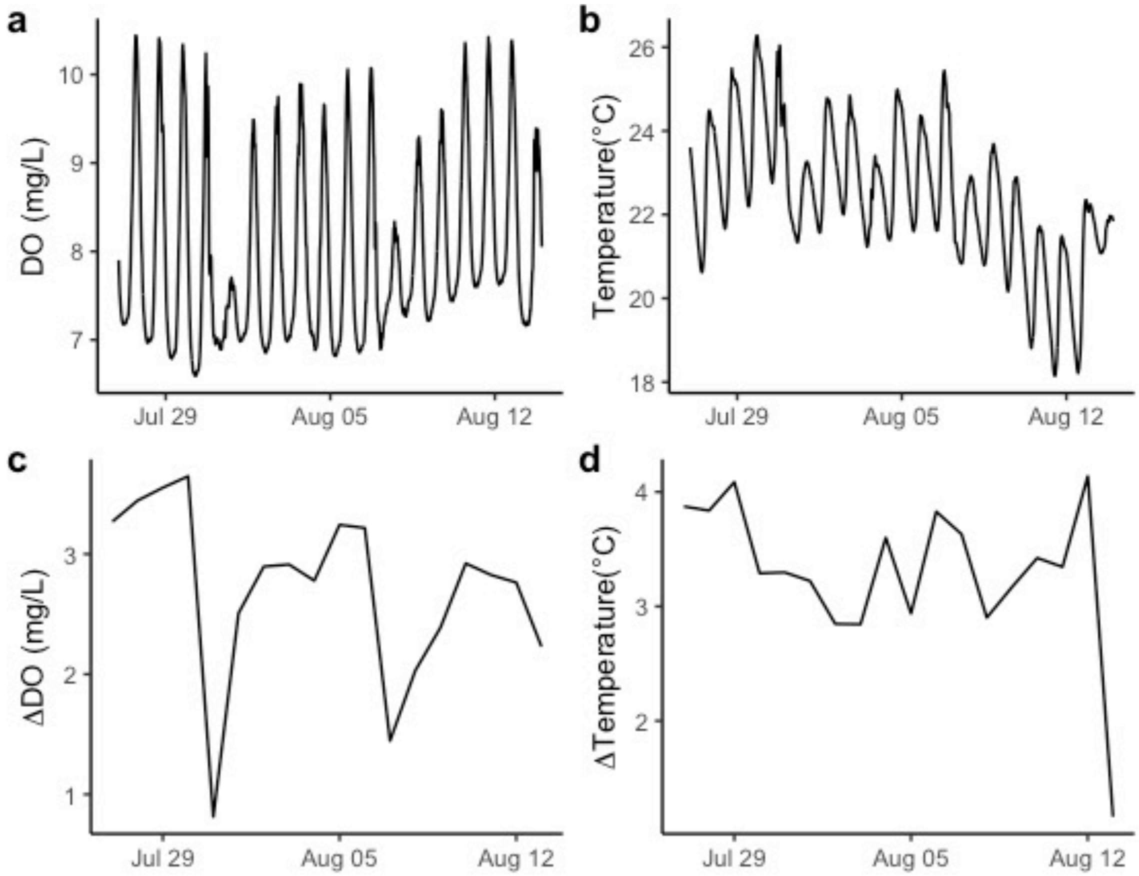

Figure B10. North Mahantango Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

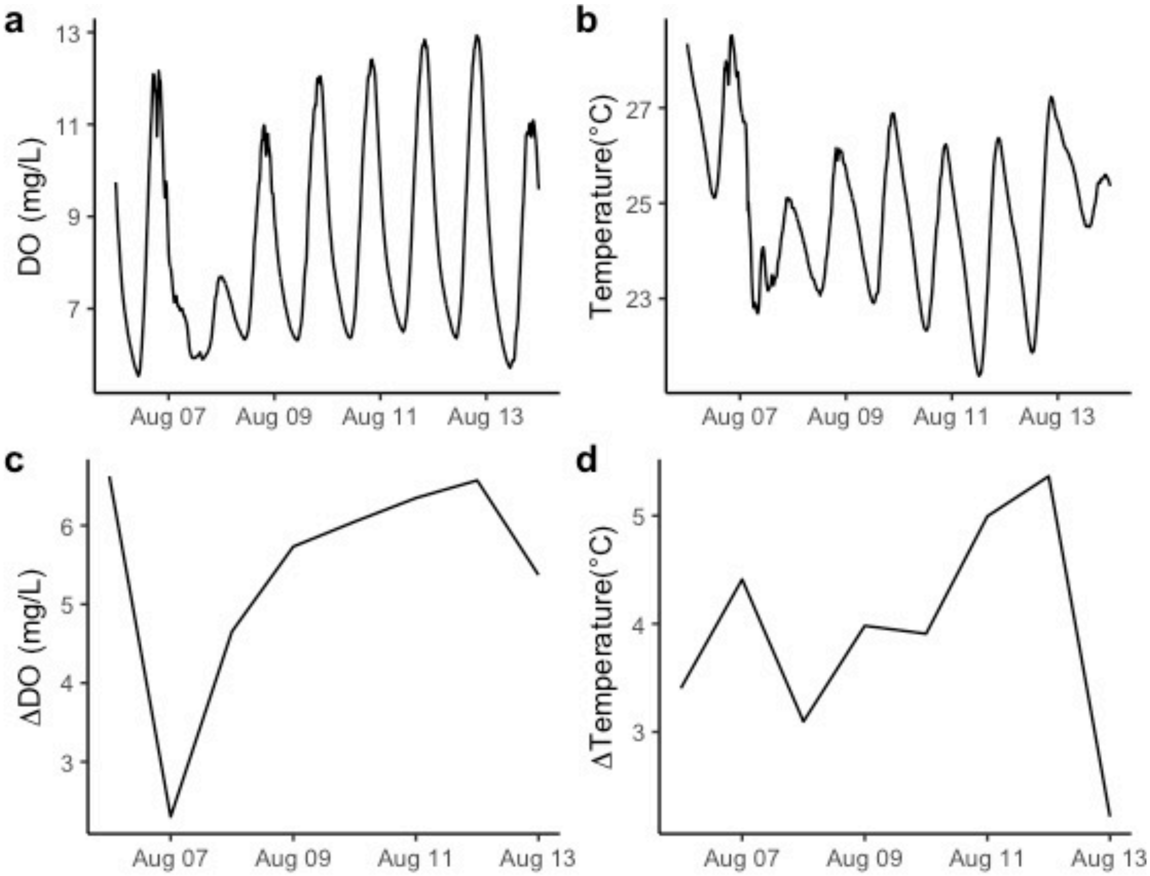

Figure B11. Penns Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

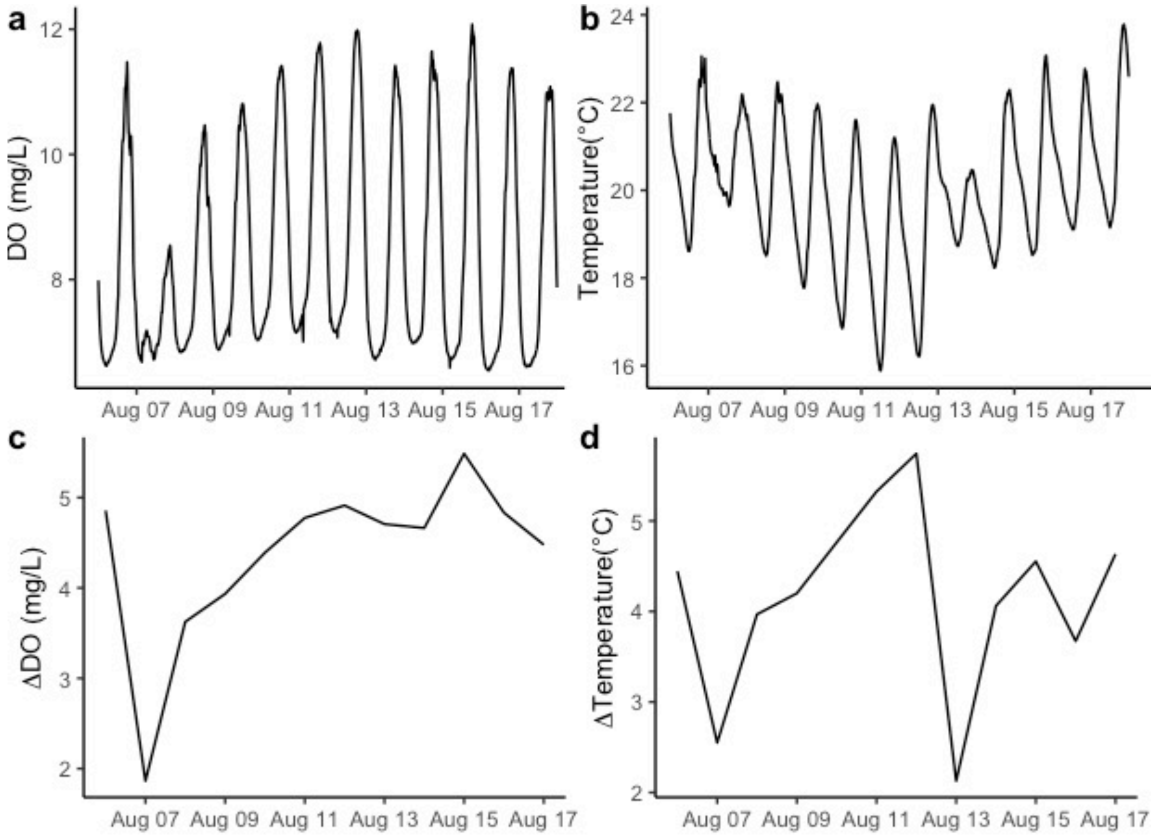

Figure B12. Turtle Creek data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.

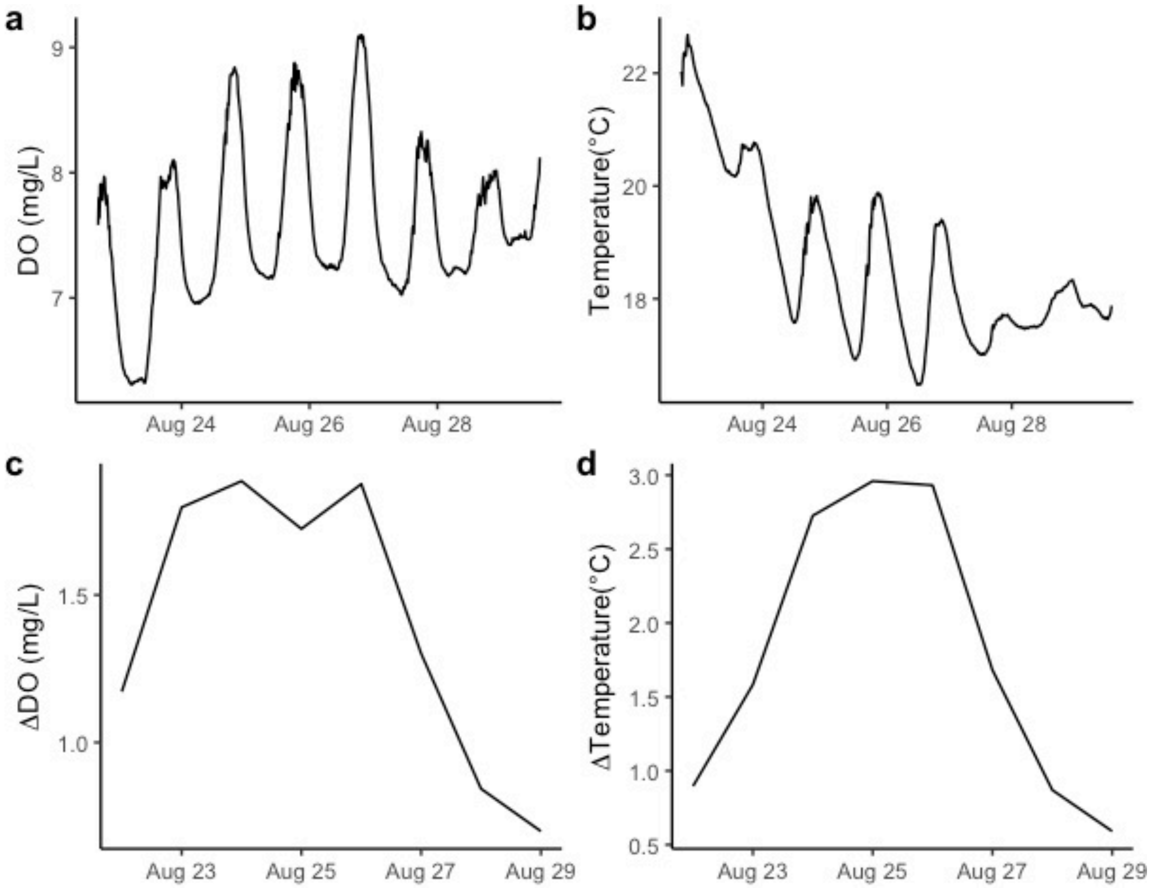

Figure B13. Warrior Run data including dissolved oxygen data (a) and water temperature data (b) collected every 15 minutes for the total amount of time the data loggers were deployed. The diel change in dissolved oxygen (c) and diel change in water temperature (d) for each day while data loggers were deployed is also displayed.