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Aquatic Ecosystems, while vital to everyday life, are threatened by anthropogenic activities and lack of management. Wetlands are a part of this, and recent research shows that they are of importance, but a large percentage has been lost over time. To mitigate this, research is being done in effectiveness of restoration/creation. Macroinvertebrates can help with identifying issues and assist in management. The piedmont region is lacking in research when it comes to Wetlands. To assist with this issue, I created prediction models for the EPT Orders utilizing historical data from the North Carolina Benthic Macroinvertebrate project and tested them for significance and correlation in R. Model testing showed that prediction modeling can be used to predict macroinvertebrate diversity in Wetlands though this predictability is moderate. Modeling also showed that Specific Conductivity having an effect on diversity.

WATER QUALITY MEASUREMENTS TO PREDICT MACROINVERTEBRATE DIVERSITY IN PIEDMONT AQUATIC HABITATS

by
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Approved by

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CHAPTER I: INTRODUCTION

Streams, rivers, ponds, and even wetlands are a vital resource to those who live near or around them. Many countries worldwide depend on these aquatic ecosystems for ecological and economic reasons(Khatri, Raval, and Jha 2021; Munyika, Kongo, and Kimwaga 2014; Zedler and Kercher 2005). Rivers across the African continent and India are vital for everyday functioning, such as fishing for food, drinking sources, and electricity (Onwona Kwakye et al. 2021; Munyika, Kongo, and Kimwaga 2014; Khatri, Raval, and Jha 2021). Wetlands are economically and ecologically beneficial by reducing costs incurred from flooding, increasing the fish population, providing water filtration (such as denitrification), and biodiversity support (Zedler and Kercher 2005; Verhoeven and Sorrell 2010; L. H. Moore and Best 2018).

However due to Anthropogenic activities such as farming, electricity generation, industrialization, urbanization, and mining, researchers are recognizing a decline (such as poor water health, inability to support pre-existing species, or becoming obsolete)in these aquatic ecosystem (Parker et al. 2019; Martin et al. 2012; Hutton et al. 2021; Onwona Kwakye et al. 2021; Munyika, Kongo, and Kimwaga 2014; Sweeney et al. 2020; Bytyçi et al. 2018; Arimoro et al. 2021; Hale et al. 2019). One study at a mining site in the Appalachian Mountain range showed the aquatic habitat was heavily polluted. This was determined based on the specific conductivity (measurement of ions) which can be used to estimate total dissolved solids (Hutton et al. 2021; Geological Survey (U.S.) 2019). A source of pollution for this study could have been stormwater runoff. Many studies have shown that stormwater runoff is a major pollution source as this water carries substances such as fuel, "compounds of arsenic, mercury, chromium, lead, cadmium, nickel, zinc, and many others" (Martin et al. 2012; Gołdyn et al. 2018). This could attribute to the increase stormwater wetlands and ponds, as these systems have shown to decrease pollutants from entering other aquatic habitats (T. L. Moore et al. 2011). Studies have reported a similar diversity for certain species such as bats upon reconstruction in relation to their natural or more established neighboring wetland (there are not many unaltered wetlands in the state of North Carolina left) (Li et al. 2021; Parker et al. 2019; Cashin, Dorney, and Richardson 1992; Dahl 2011; Fretwell 1996). However, it is not established if this is due to other factors such as food sources. In the case of macroinvertebrates, if a restored/constructed wetland is composed mostly of indigenous plants, then the community assemblage is similar to the natural wetland (Death and Collier 2010).

The issue is that certain wetlands can act as an ecological trap (Hale et al. 2019). A study focusing on stormwater wetlands found that certain amphibian species decreased fitness due to standing pollution—this pollution resulted from stormwater runoff from agricultural and urban areas. However, these polluted waters are often filtered by the wetland's vegetation absorption and surrounding soil before they contact nearby streams and rivers (T. L. Moore et al. 2011; Hale et al. 2019). The stormwater wetland does provide benefits as well as other wetland types (Hansen et al. 2018; Parker et al. 2019; Verhoeven et al. 2006; Zedler and Kercher 2005; Greenway, Jenkins, and Polson 2007; T. L. Moore et al. 2011).

This is why it is vital to ensure that these ecosystems have good water quality, to assist with this ecological management practices are implemented. These practices involve monitoring the habitat's water quality, how sustainable it is, and how it's resources are utilized (Arimoro et al. 2021). Water management, a branch of ecological management, requires factoring in water usage data, equipment maintenance, sewage use, staffing, adhering to federal law and requirements(in the case of the U.S.) and where to gain the funds(Federal Energy Management Program 2023). Aquatic ecosystems are encountering funding issues which is creating a reduction in efforts to properly manage these habitats (Arimoro et al. 2021; Onwona Kwakye et al. 2021). With increasing social concerns there is a necessity to constantly monitor all aquatic ecosystems, however, this is not economically plausible (Khatri, Raval, and Jha 2021; Munyika, Kongo, and Kimwaga 2014; Bytyçi et al. 2018; Salmaso et al. 2018; Arimoro et al. 2021; Orzechowski and Steinman 2022). There is a possible solution which is the use of bioindicators, specifically macroinvertebrates, as these species are have sensitivity to changes in their habitat (physical and chemical) (Etemi et al. 2020).

Macroinvertebrates are a easy to catch, observe, and analyze, making them an ideal resource for biomonitoring (Orzechowski and Steinman 2022; Heatherly et al. 2007; Onwona Kwakye et al. 2021, 2; Munyika, Kongo, and Kimwaga 2014; Khatri, Raval, and Jha 2021; Bytyçi et al. 2018; Arimoro et al. 2021; Gianopulos et al. 2021; Sweeney et al. 2020). Macroinvertebrates are sensitive to their environment (Bytyçi et al. 2018; Sweeney et al. 2020; Khatri, Raval, and Jha 2021; Onwona Kwakye et al. 2021), and changes in pollution directly alters the assemblage favoring pollutant tolerant invasive species (Gomes and Wai 2020). Studying macroinvertebrate assemblages and diversity, scientists and management personnel can determine what type of water quality is present in a waterbody (Munyika, Kongo, and Kimwaga 2014; Orzechowski and Steinman 2022; Khatri, Raval, and Jha 2021; Torres and Johnson 2001; Bytyçi et al. 2018; Arimoro et al. 2021; Gołdyn et al. 2018; Desrosiers et al. 2019). A study on the dead river study in Hong Kong found the presence of pollutant-tolerant(have a high tolerance to low water quality such as low dissolved oxygen) macroinvertebrates that were invasive (Gomes and Wai 2020). Other studies have found that when anthropogenic activities increase or occur, it causes a shift from pollutant intolerant native species to pollutant-tolerant invasive species (Torres and Johnson 2001; Bytyçi et al. 2018; Onyena, Nkwoji, and Chukwu 2021; Shen et al. 2020; Gołdyn et al. 2018). As this seems to be a reoccurring result, there has been a focus on using macroinvertebrates to determine the success and condition of wetlands, specifically those that are restored or created (Martin et al. 2012; Gołdyn et al. 2018; Chawaka et al. 2018; Stewart and Downing 2008; Gianopulos et al. 2021).

Studies focused southeast US have found macroinvertebrates can be affected by other surrounding issues aside from water quality. Sedimentation toxicity can cause a change in the macroinvertebrate assemblage (Lenat, Penrose, and Eagleson 1981; Moran et al. 2020). Sediment type can be unsuitable for macroinvertebrates; specifically, sand substrates are found to be unsuitable habitats for several macroinvertebrates (Lenat, Penrose, and Eagleson 1981). Based on results from '*Variable effects of sediment addition on stream benthos*', it was discovered that there may be an inverse relationship between rainfall and macroinvertebrate density (Lenat, Penrose, and Eagleson 1981). This could explain why restored urban streams are failing due to a smaller watershed, as there are complications when it comes to restoring or installing an urban stream or wetland (Violin et al. 2011). This is unfortunate as wetlands in central Georgia are beneficial to predator macroinvertebrates that prey on crop pests in the agricultural area (Cardona-Rivera et al. 2021). This is important to changing farmers' minds about removing wetlands for agricultural land if it saves money on pesticides, which has been found to be one of the key problems of sediment toxicity (Lenat, Penrose, and Eagleson 1981; Moran et al. 2020; Violin et al. 2011; Miller, Paul, and Obenour 2019). From this information it would be beneficial to expand on studies involving the use of macroinvertebrates for ecological monitoring to assist with management in wetlands and other aquatic habitats.

While there is an increase in ecological management and wetlands research, there is a knowledge gap in what negatively or positively effects wetlands and how best to manage them.

Most research in the United States is generally focused on the coastal area. Such studies have found that urbanization affects streams' thermal and nutrient levels in the North Carolina Raleigh region, which is considered between the Piedmont and Coastal region (Hutton et al. 2021). A study on nitrogen levels before and after entering a stormwater wetland was located in the Piedmont region (T. L. Moore et al. 2011). Studies, like the salamander study, focused on the mountain region. Even with some studies having a location in the Piedmont region, most focus either on the mountain or coastal region. Which is why this study focused on aquatic habitats in the Piedmont region of North Carolina.

Aim 1. Create a statistical model for prediction using historical data from the NC Benthic Macroinvertebrates data. Specifically, I used publicly available raw data on macroinvertebrate diversity in different aquatic habitats that included basic water quality measurements recorded in the Piedmont region of North Carolina where there is limited research involving wetlands, water quality, and macroinvertebrates. Prediction models were previously used for determining trends in the healthcare field (Ying, Wei, and Lin 1992; Lee et al. 2015), ecological trends in habitats so as to better manage the habitats in question (Hamilton, McVinish, and Mengersen 2009; Sevinc, Kucuk, and Goltas 2020). The model will predict the macroinvertebrate diversity based on specific water quality levels. This will be tested by constructing a predictive model in R for three Orders Ephemoptera, Plecoptera, and Trichoptera. These three orders make up the EPT measurement index which is used frequently to indicate how good or bad the water quality is (Etemi et al. 2020). I predict Modeling of water quality measurements will predict macroinvertebrate diversity. I predict specific conductivity in particular will be significant.

Aim 2. Test the prediction model by assaying four selected wetlands and one Riverine in the region. Macroinvertebrate diversity will be impacted by water quality at four Piedmont wetlands. I predict data from four wetlands will be consistent with predictions from modeling. If I do not find support for these predictions, some interpretations of the pattern may include the difference between the aquatic environments in which macroinvertebrates were collected for the model, which are primarily riverine and swamps versus the field data I collected in restored and artificial. wetlands and the collection method. These differences may impact diversity of macroinvertebrate orders.

CHAPTER II: METHODS

Analysis

Using the statistical program R, historical data sent from the North Carolina Benthic Macroinvertebrate Project (Benthic Macroinvertebrate Assessment Data n.d.), a set of prediction models were created to determine if water quality could be used to predict macroinvertebrate diversity. The North Carolina Benthic Macroinvertebrate project was started in 1978 as a way to measure water health using macroinvertebrate abundance (Benthic Macroinvertebrate Assessment Data n.d.). The macroinvertebrate data contains the stream class, date, water body, location, latitude, longitude, county, basin, subbasin, ecoregion, sample type, drainage, scientific name, order, and abundance. The water quality data contains the same as macroinvertebrate excluding scientific name and order and instead having measurements of pH, specific conductivity, temperature in Celsius, and dissolved oxygen. Both data sets were filtered for the Piedmont region, NA (not applicable), and water quality that had a value of 0 were removed. A column of water class was added to the water quality data set. The water class was determined using the coordinates provided and the wetlands mapper on the U.S. Fish and Wildlife map, along with Google Earth (“Google Earth,” n.d.; “National Wetlands Inventory,” n.d.). The macroinvertebrate data set had three columns added, Class, Family, Genus. These were added by using databases such as animaldiversity.org to look up the species taxonomic tree (“Animal Diversity Web” n.d.). The two were than combined based on location and date. The new data set contains the date, county, abundance, temperature in Celsius, specific conductivity, pH, and dissolved oxygen.

Species with more than 10% of unknown at the Family and Genus Taxonomic level were removed from the data. Diversity was calculated by counting the amount of each genus present.

Each site was then assigned pre(Date ≤ 2001-01-01) or post (Date > 2001-01-01) Using the MuMIn package in R, a table was created to calculate the predictability of each possible model created from the pre period(Barton and Barton 2015). Only models that contained a ΔAIC ≤ 2.00 were considered for testing as these models are labeled as having substantial support in comparison to all other models(Anderson and Burnham 2004). These models were then tested to see if the two models were significantly correlated and by how much ($P < 0.05$) using Spearman's correlation test.

Macroinvertebrate Field Collection and Identification

Leaf litter bags were deployed in three stormwater wetlands and one 'natural' wetland. Natural in the structure was naturally occurring but has gone through anthropogenic reconstruction or add-ons, and one riverine area which is located near a 'natural' wetland. Bags were stuffed with dried magnolia leaves collected around UNCG's campus. Leaves were baked at 170 degrees Fahrenheit for 10 minutes to sterilize (zbrinks 2021). Four bags were made for each site-- two went into the water at different areas of the habitat and two went near the bank of each water location. They were secured with a twine rope. Deployment occurred in the first week of March, 2023 and were collected in the first week of April, 2023. The contents were emptied into a Berlese funnel made following Derek Hennen's method (hennen 2021). A container of 95% ethanol was placed under the funnels for about 3+ hours or until the wet samples were dry. Macroinvertebrates were ID'd using a collection of key guides, presentations, experts, and websites (Caterino 2022a; 2023; 2022b; Gibb and Oseto 2005; Krantz and Walter 2009; Marek 2022a; 2022b; Marek and Caterino 2022; Canada. Department of Agriculture. Research Branch. 1981; Mound and Kibby 1998; Scott 1986; Ubick et al. 2017; White 1983; "Animal Diversity

Web" n.d.; "BugGuide," n.d.; "iNaturalist" 2023; "Leeches (Glossiphoniidae)" 2023; "Mite ID Tool" 2023).

Site description

UNCG Wooded Wetland and Open Wetland

Peabody Park was originally a hot spot for student activity. The park was land that was both donated and bought from Pullen and Gray (E.A. Bowles 1967; A.W. Trelease and Noble 2004). The Park was given funds from George Peabody to have a walking trail with historic markers in the schools' early days. This was a way for students to learn about the state and campus history and the walks were mandatory (E.A. Bowles 1967). Later a sewer system was installed under Peabody Park which was later replaced when a leak was discovered during the typhoid epidemic (A.W. Trelease and Noble 2004). In 1897 McIver created a farm to supply the school with milk, pork, and produce and act as a horticultural lab. The park was used for other student activities such as putting on productions by different groups, a part of the lantern walks, and a place of study (E.A. Bowles 1967; A.W. Trelease and Noble 2004). Unfortunately, around the 1920s the park fell out of favor and practice with the loss of the "Walk and Park" night. Plans were made to establish a golf course and finish by 1934 but was not used as planned either (Allen W. Trelease and Noble 2004). Eventually, in 1941, new deal funds were used to reestablish Peabody and build an amphitheater and lake (both of which are no longer a part of campus). The farm was sold in 1945 due to loss of money and better prices for milk (Allen W. Trelease and Noble 2004). Peabody Park is now home to two reconstructed wetlands which are surrounded by vegetation, both native and invasive. The open wetland at the University of North Carolina's at Greensboro campus is located inside the golf course in Peabody Park. A part of the golf course was selected to build this wetland. The wetlands were restored in March of 2017

(“The Wetlands Project” 2022). Both wetlands are located near buffalo creek. The more wooded wetland is in the more forested and preserved area of Peabody Park located near the music building on campus and directly across from a section of the buffalo creek. This wetland is close to traffic and hosts a myriad of plants such as Horse Tail a native species and English ivy an invasive species. Each wetland are hot spots for different lab courses which was one of McIver’s original plans for the park (Elisabeth Ann Bowles, University of North Carolina Press, and Seeman Printery 1967; Allen W. Trelease and Noble 2004).

Bog Garden

The Bog Garden, located at Greensboro’s Bicentennial Gardens, was originally a declining lake. The lake was initially owned by Starmount Farms and purchased by Dr. Joe Christian after coming upon it in 1987(“Bog Garden at Benjamin Park History” 2022). Since then, the lake and the wetland have been restored, and a walkway through the wetlands has been built. The wetland is fed by a creek connected to Buffalo Creek and is flush with vegetation. This is a restored wetland with high anthropogenic activity. The sample area is a riverine located across the wetland and flows into the main body at the end of the walkway.

Cortland Park

Courtland Park is in Reidsville, North Carolina. The wetland is a constructed stormwater wetland that is 8+ years old and is designated as a bird sanctuary like the Bog Garden. This wetland is a part of the Little Troublesome Creek project to reduce the fecal coliform in the creek watershed. The Piedmont Council was given funds from the government in 2003 to construct these stormwater wetlands. Part of the project was planting Button Brush, Marsh Hibiscus, Soft Rush, Swamp Milkweed, Pickerel Weed, Lizard Tail, Cardinal Flower, and White Top Sedge.

All of which are native to North Carolina and have thick roots(“Little Troublesome Watershed” n.d.).

Hogan’s Creek

Hogan’s creek is categorized as a freshwater/emergent wetland according to the U.S. Fisheries and Wildlife wetland mapper (“National Wetlands Inventory,” n.d.). The creek flows into the Dan River and according to a soil survey, was found to help with drainage at surrounding or nearby farms (“National Register of Historic Places Multiple Property Documentation Form,” n.d.). This stream was part of a land grant awarded to Thomason Harris (“National Register of Historic Places Multiple Property Documentation Form,” n.d.), and was one of Rockingham’s earliest land grants. The creek was a host to one of the first operating mills by the 1750s in Rockingham County and the Searcy and Moore gun factory -- a supplier to the Florence armory in Guilford County from 1862-1863(Walker 2016). This wetland has quite the historic significance and runs behind roadways and houses. One area is seeing recent development and could be seeing some possible pollution in the future. The area I have selected is located behind my house where the previous owners left quite a bit of trash and we are working on removing this ourselves. Thankfully it hasn’t made it into the stream. The located area is surrounded by a small forest and is host to a myriad of species. At night you can hear owls, a few coyotes, and cows from the nearby farm.

CHAPTER III: RESULTS

The data from the North Carolina Benthic Macroinvertebrate Project (Benthic Macroinvertebrate Assessment Data n.d.) contained data on macroinvertebrates that were identified to a variety of taxonomic levels. Taxon level was identified based on the scientific name provided by the data, those whose taxa did not reach the a certain level were marked as unknown for that category. To reduce the amount of unknowns, R was used to identify those which held more than 10% unknown at the Family and Genus level and removed. Each location was assigned a site number based on geographical coordinates. Pre and Post was assigned based on the period before 2001 and after. Water quality measurements were calculated for the average for each site number and period. Any water quality measurements with a value of zero or N/A were removed from the data set. Genus richness was calculated for each site at the order and class level for that specific time period.

Prediction modeling focused on the three orders in the Piedmont region, Ephemoptera, Plecoptera, and Trichoptera, as these three orders are a standard for good water quality when present and absence of these can indicate poor health. Prediction model was created using data from the pre period. Dredge function from the MuMIn package in R was used to create all possible general linearized models for the Poisson family. The top model was selected to test calculate possible genus richness. The top candidate model was selected as this is the model with the highest predictability out of all other candidate models(Table 1). Models were tested for accuracy by inputting the water quality from the post period into the model and calculate predicted richness. Predicted richness was compared to observed using the Spearman's rank test. The results of the correlation showed a weak correlation for Order Ephemoptera, and a weak-moderate correlation for Plecoptera and Trichoptera (Table 2).

Table 1 lists all possible models for each EPT order for each habitat. Plecoptera model had the lowest AIC score out of all three Orders for all three scenarios (no habitat selection, Wetland, Riverine) indicating this model for this order has the highest substantivity out of all three orders. Based on results shown in table 2 with a rho score of 0.36 as opposed to Ephemoptera (rho = 0.28) and Trichoptera (rho = 0.35) this corroborates that while the correlation is moderate (rho = 0.3-0.5) it is still the model with the best model when no habitat is selected out of all three Orders . This correlation does change when habit is selected in the case of Riverine Trichoptera was of a higher correlation than Plecoptera.

Table 1. Candidate models with ΔAIC < 2.00 indicating little statistical difference from the top model. Top model being the model with the highest predictability compared to all others. Order = Taxa, Habitat type of aquatic ecosystem, Models structure = water quality included as a predictor, df = degrees of freedom, AIC = AIC score, ΔAIC = calculated ΔAIC score, Weight = Akaike weight.

Order	Habitat	Model Structure	df	AIC	ΔAIC	Weight
Ephemoptera	N/A	Diss_Oxy + Sp_Cond + Temp_C	4	1309	0	0.681
Ephemoptera	N/A	Diss_Oxy + pH_SU + Sp_Cond + Temp_C	5	1310	1.52	0.319
Plecoptera	N/A	Diss_Oxy + pH_SU + Sp_Cond + Temp_C	5	825.7	0	0.44
Plecoptera	N/A	Diss_Oxy + pH_SU + Sp_Cond	4	826.8	1.09	0.255
Trichoptera	N/A	Diss_Oxy + Sp_Cond + Temp_C	4	1312	0	0.707
Trichoptera	N/A	Diss_Oxy + pH_SU + Sp_Cond + Temp_C	5	1314	1.76	0.293
Ephemoptera	Wetland	Sp_Cond + Temp_C	3	240.8	0	0.357
Ephemoptera	Wetland	Diss_Oxy + Sp_Cond + Temp_C	4	242.5	1.74	0.15
Ephemoptera	Wetland	pH_SU + Sp_Cond + Temp_C	4	242.6	1.79	0.146
Plecoptera	Wetland	Diss_Oxy + Sp_Cond + Temp_C	4	157.2	0	0.486
Plecoptera	Wetland	Diss_Oxy + pH_SU + Sp_Cond + Temp_C	5	158.9	1.72	0.205
Trichoptera	Wetland	Diss_Oxy + Sp_Cond + Temp_C	4	249	0	0.514
Trichoptera	Wetland	Diss_Oxy + pH_SU + Sp_Cond + Temp_C	5	250.9	1.94	0.195
Ephemoptera	Riverine	Diss_Oxy + Sp_Cond + Temp_C	4	1025	0	0.725
Ephemoptera	Riverine	Diss_Oxy + pH_SU + Sp_Cond + Temp_C	5	1027	1.94	0.275
Plecoptera	Riverine	Diss_Oxy + pH_SU + Sp_Cond	4	640.9	0	0.55
Plecoptera	Riverine	Diss_Oxy + pH_SU + Sp_Cond + Temp_C	5	642.4	1.53	0.256
Trichoptera	Riverine	Diss_Oxy + Sp_Cond + Temp_C	4	997.5	0	0.525
Trichoptera	Riverine	Diss_Oxy + pH_SU + Sp_Cond + Temp_C	5	997.7	0.2	0.475

Table 2. EPT Spearman correlation results, $p < 0.05$ indicates significant correlation, rho indicates how correlated the two richness are with +1 being the highest possible correlation. Order = Taxa, Habitat type of aquatic ecosystem, Models structure = water quality included as a predictor, S = is the test statistic result, p_value = the statistical significance, rho = the correlation result.

Order	Habitat	Model Structure	S	p_value	rho
Ephemoptera	N/A	Diss_Oxy + Sp_Cond + Temp_C	39866246	1.86E-13	0.2750244
Plecoptera	N/A	Diss_Oxy + pH_SU + Sp_Cond + Temp_C	14451792	< 2.2e-16	0.3577228
Trichoptera	N/A	Diss_Oxy + Sp_Cond + Temp_C	36633715	< 2.2e-16	0.3536514
Ephemoptera	Wetland	Sp_Cond + Temp_C	984956	0.08753	0.1246239
Plecoptera	Wetland	Diss_Oxy + Sp_Cond + Temp_C	281077	0.0002031	0.3145139
Trichoptera	Wetland	Diss_Oxy + Sp_Cond + Temp_C	731447	7.98E-07	0.3499293
Ephemoptera	Riverine	Diss_Oxy + Sp_Cond + Temp_C	12422985	2.95E-16	0.3586139
Plecoptera	Riverine	Diss_Oxy + pH_SU + Sp_Cond	4960409	2.26E-15	0.3978929
Trichoptera	Riverine	Diss_Oxy + Sp_Cond + Temp_C	11694692	< 2.2e-16	0.4214696

This is reflected when predicted richness is plotted against observed diversity. For the Plecoptera order very few observations land on the slope in the graph those that are intercepting on the slope are richness that was predicted accurately. As can be seen in figure 1 there are very few points on the slope line for all three habitats. Indicating the model works but not as well as desired.

Some of this can be explained when plotting the predictors from the top model with the genus richness from the pre period. Order Plecoptera showed a massive grouping at each predictor. Dissolved oxygen there was a group of observations of varying amounts between 5.0 and 7.5. A strange occurrence to note is that the genus richness was on the higher end at a DO of 2.5 which is indicative of pollution and so we should not be seeing a genus richness of 10 at this level. Though when looking at the specific conductivity graph in figure 2 for Plecoptera we see that richness above 8 has a specific conductance less than 200. A further look shows that those with a genus richness of 10 held a pH between ~6.9 and ~7.6. Temperature varies though most observations tend to be between 20 and 30 degrees Celsius. As Dissolved oxygen and temperature are positively related this could explain why the dissolved oxygen was so low at this observation. This is all to say that for the Order Plecoptera all four water quality measurements should be collected to gauge the health of the habitat.

When isolating for the habitat wetland the Order Plecoptera AIC score drops to 157.2 for the top model indicating a better fit model than before this is not reflected in the rho score however as this score dropped to 0.31 and the best model no longer includes the water quality measurement pH though the model does still contain a significant correlation at 0.0002031. The graph in figure 1 middle shows fewer observations landing on the slope, which shows less predictability success. With fewer observations though it is easier to see possible relationships in the graphs than before. The graphs for dissolved oxygen and specific conductivity both have data located mainly in the center, while temperature seems to skewed to the right. These plots make a bit more sense as Plecoptera do not tend to habitat wetlands and prefer cooler temperatures.

When isolating the data for the riverine habitats AIC score increases compared to wetlands but is still lower than when models don't select for a habitat with the top model holding an AIC score of 640.9. The top model also includes pH but excludes temperature which is strange considering temperature and dissolved oxygen are inversely related (Table 1). The rho did increase to 0.4 and was significantly correlated (Table 2). This is reflected in the 3rd graph in figure 1 as it appears the observations group together more closely and appear to have more observations landing on the slope intercept. The scatter plot for riverine water quality shows dissolved oxygen and pH having a more centered distribution, specific conductivity is skewed more to the left(Figure 2). Specific conductance the genus richness drops to ~ 1 after 500 with maybe 1 or 2 outliers. This is reasonable as research has shown higher levels of specific conductance to affect species richness in a negative manner. The outliers could also be accounted for when inputting the other water quality measurements, as it could be a case where the DO is of a higher value.

Figure 1. Predicted genus richness against Observed of Order Plecoptera in all three habitats (1st = no selected habitat, 2nd = Wetland, 3rd = Riverine).

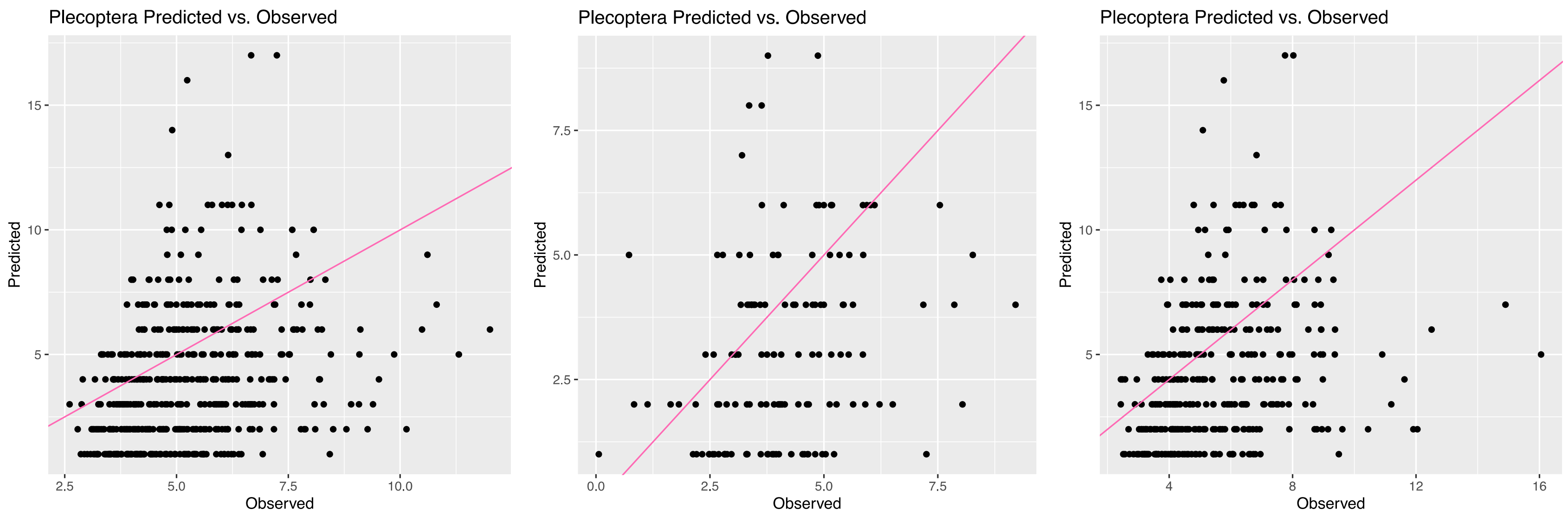
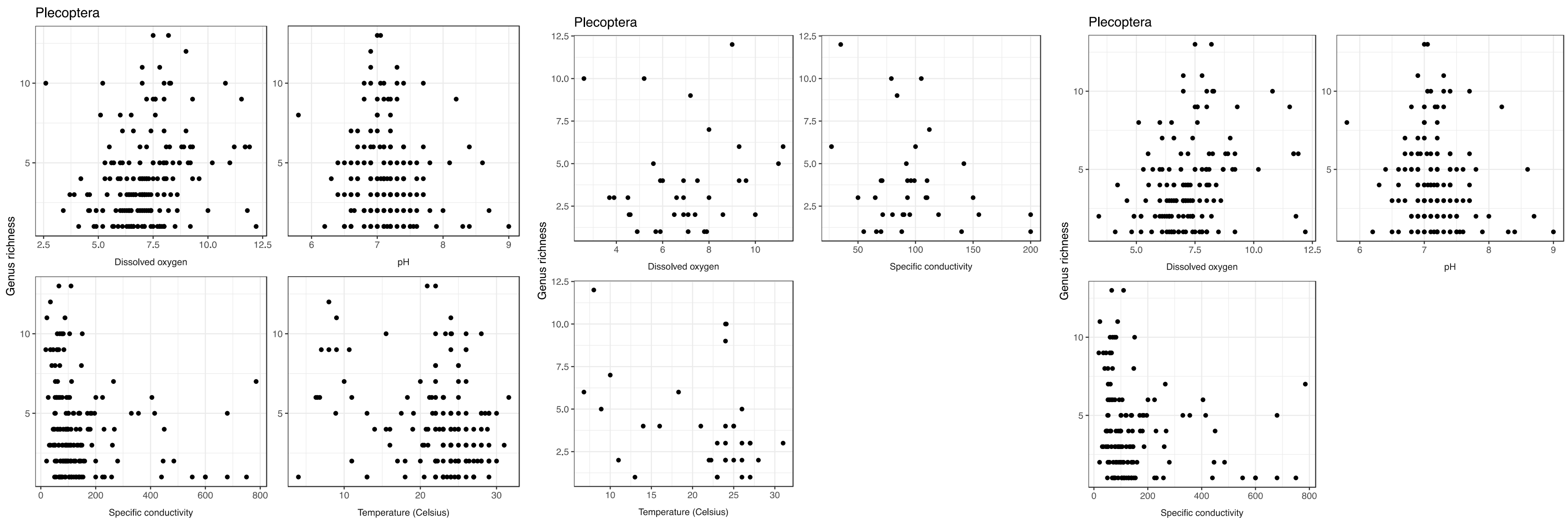


Figure 2. Genus Richness in comparison to Water Quality measurements for order Plecoptera in each habitat(1st = all habitats. 2nd = Wetland, 3rd= Riverine)



For Order Ephemoptera when no habitat is selected quite a few observations land on the slope in the first graph of figure 3. Those that are intercepting on the slope are richness that was predicted accurately. Order Ephemoptera showed a massive grouping at each predictor, though this grouping varies. Dissolved oxygen groups near the center, Specific conductivity groups around the left and Temperature groups around the right side. DO held an outlier of a richness of 20 at 2.5 which is indicative of pollution. In the case of specific conductance richness has negative relationship with specific conductance and very few outliers. All richness of 20 or higher is located between a specific conductance of < 200. Temperature does not seem to have a strong effect on richness but could explain a few outliers with Dissolved oxygen.

When isolating for the habitat wetland the Order Ephemoptera AIC score drops to 240.8 for the top model indicating a greater predictability than before this is not reflected in the rho score at 0.12 nor is significantly correlate, though it is close at a p-value of 0.08753. The graph in figure 2 middle shows fewer observations landing on the slope, which shows less predictability success. With fewer observations though it is easier to see possible relationships in the graphs than before. However even though there was no significant correlation between predicted and observed there does appear to be a relationship between specific conductivity and genus richness, though this seems minor.

When isolating the data for the riverine habitats AIC score increases compared to wetlands but is still lower than when models don't select for a habitat with the top model holding an AIC score of 1025 The top model is also the same when no habitats are selected as opposed to the top model in wetlands which excluded Dissolved oxygen. This could provide evidence that the reason the model did not have significant correlation because it was missing dissolve oxygen as a predictor. The rho did increase to 0.36 and was significantly correlated(Table 2). This is reflected in figure 3 as it appears the observations group together more closely and appear to have more observation landing on the slope intercept. The scatter plot for riverine water quality shows a vast distribution among all three(figure 4). Specific conductance appears to have a higher richness compared to Plecoptera, though the richness is still decreasing was specific conductance increase starting at ~250. Dissolved oxygen has a grouping of ≥20 at around 7.5 and a similar grouping at ~ 25 degrees temperature.

Figure 3. Predicted genus richness against Observed of Order Ephemoptera in all three habitats (1st = no selected habitat, 2nd = Wetland, 3rd = Riverine)

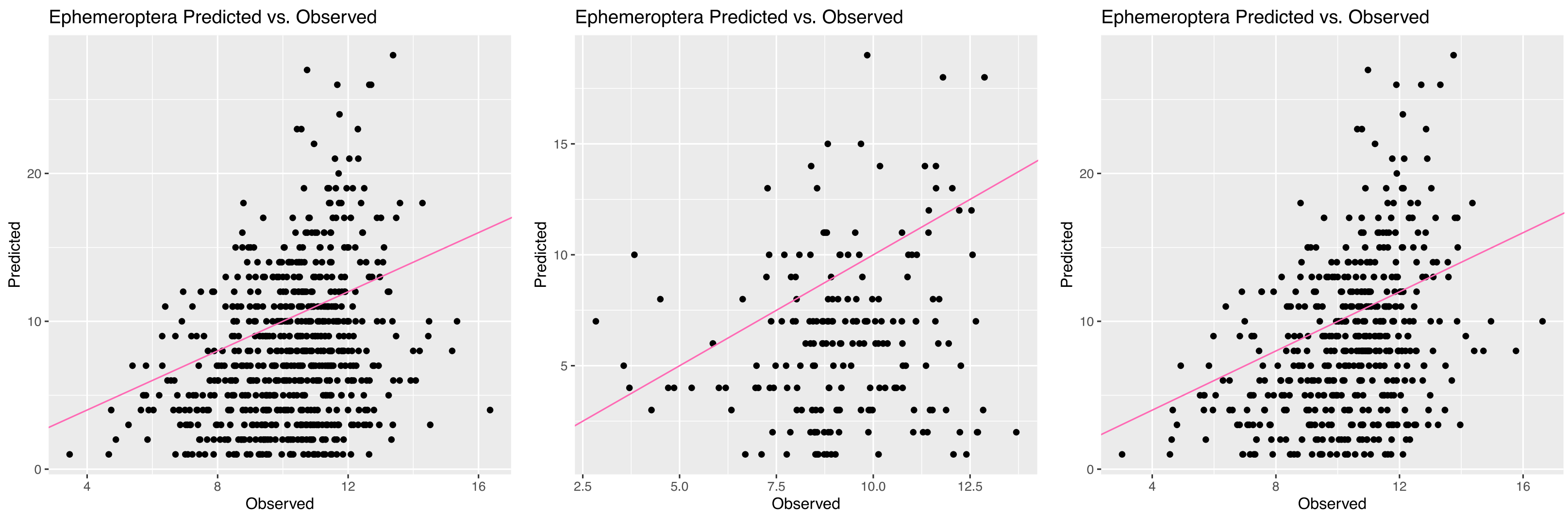
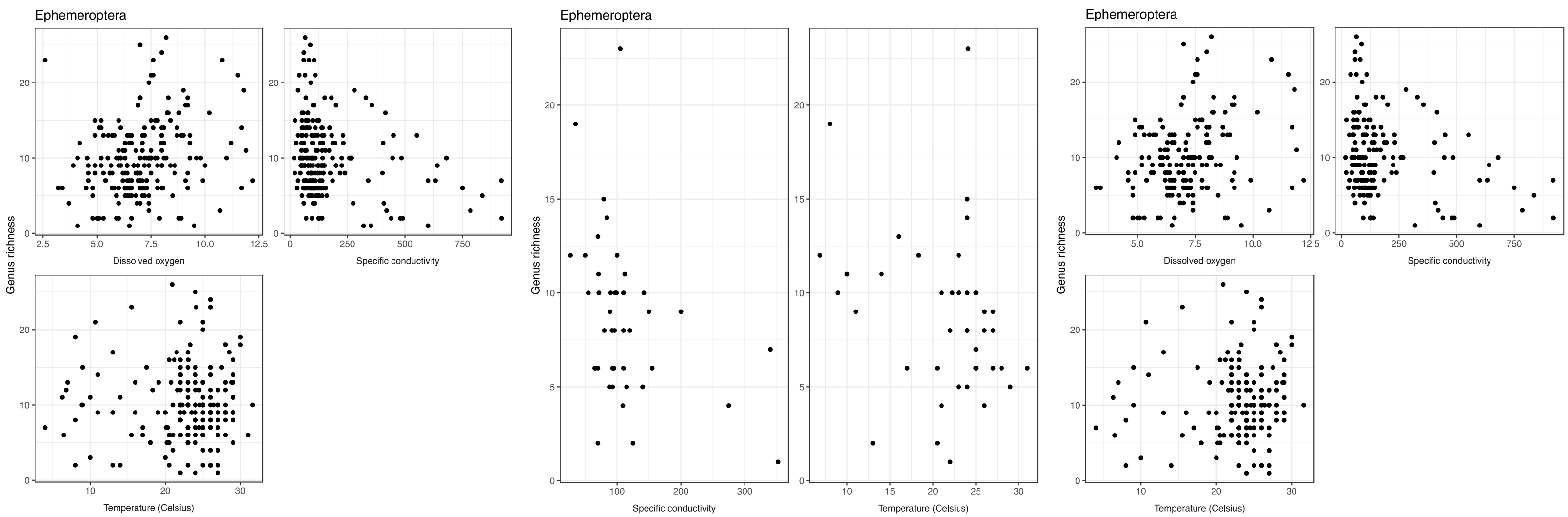


Figure 4. Genus Richness in comparison to Water Quality measurements for order Ephemoptera in each habitat(1st = all habitats. 2nd = Wetland, 3rd= Riverine)



For the Trichoptera order quite a few observations land on the slope in the graph those that are intercepting on the slope are richness that was predicted accurately (Figure 5). Some of this can be explained when plotting the predictors from the top model with the genus richness from the pre period. Order Trichoptera showed a massive grouping at each predictor, though this grouping varies. Dissolved oxygen groups near the center, Specific conductivity is more left skewed, and Temperature is more the right skewed, this is similar to the Order Ephemoptera (Figure 6.). DO held an outlier of a richness of 20 at 2.5 which is indicative of pollution. In the case of specific conductance richness has negative relationship with specific conductance and very few outliers. All richness of 20 or higher is located between a specific conductance of < 200. Temperature does not seem to have a strong effect on richness but could explain a few outliers with Dissolved oxygen.

When isolating for the habitat wetland the Order Trichoptera AIC score drops to 249 for the top model indicating a greater predictability than before this is not reflected in the rho score at 0.35 as it does not change as much when no habitat is selected (Table 1). The graph in figure 5 middle shows fewer observations landing on the slope, which shows less predictability success. With fewer observations though it is easier to see possible relationships in the graphs than before. Dissolved oxygen has a similar outlier as the other two orders though once again this is more than likely do to the temperature as specific conductive showed genus richness declining as it increased (Figure 6).

When isolating the data for the riverine habitats AIC score increases compared to wetlands but is still lower than when models don't select for a habitat with the top model holding an AIC score of 997.5 (Table 1). The top model is also the same when no habitats are selected and as when wetland is selected. The rho did increase to 0.42 and was significantly correlated

(Table 2). This is reflected in the figure 5 as it appears the observations group together more closely and appear to have more observation landing on the slope intercept. The scatter plot for riverine water quality shows a vast distribution among all three. Specific conductance appears to have a higher richness compared to Plecoptera, though the richness is still decreasing was specific conductance increase starting at ~250. Dissolved oxygen has a grouping of ≥20 at around 7.5 and there seems to be a shift of the richness increasing with the DO, a similar grouping at ~ 25 degrees temperature.

Figure 5. Predicted genus richness against Observed of Order Trichoptera in all three habitats (1st = no selected habitat, 2nd = Wetland, 3rd = Riverine)

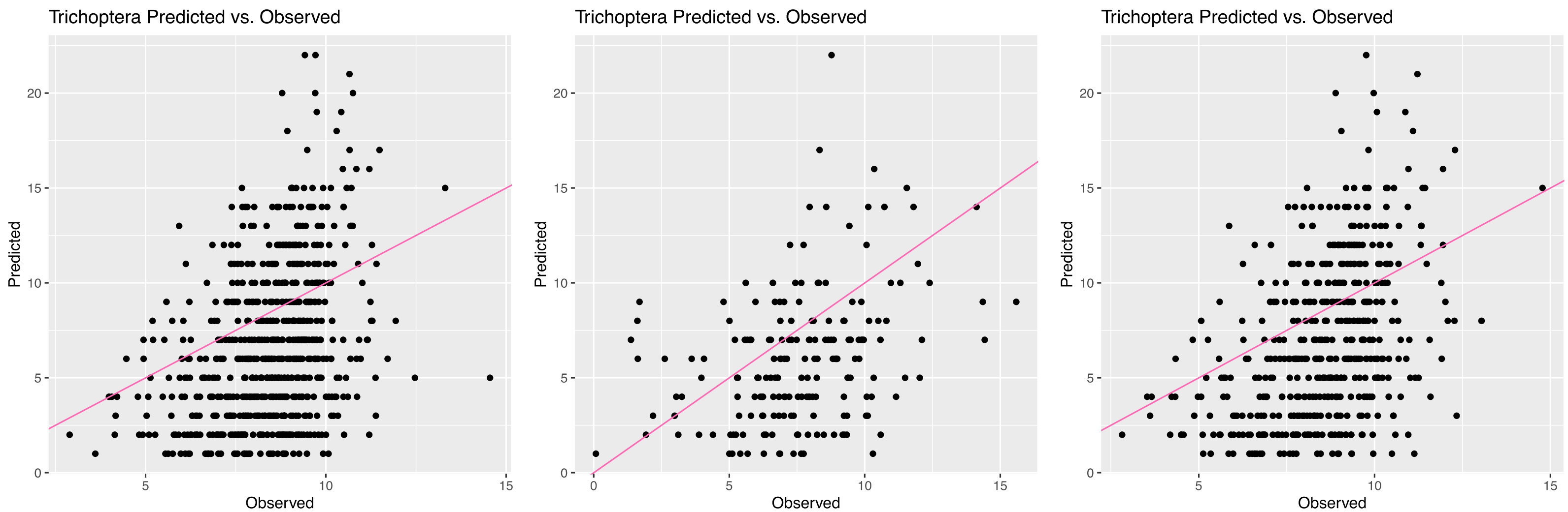
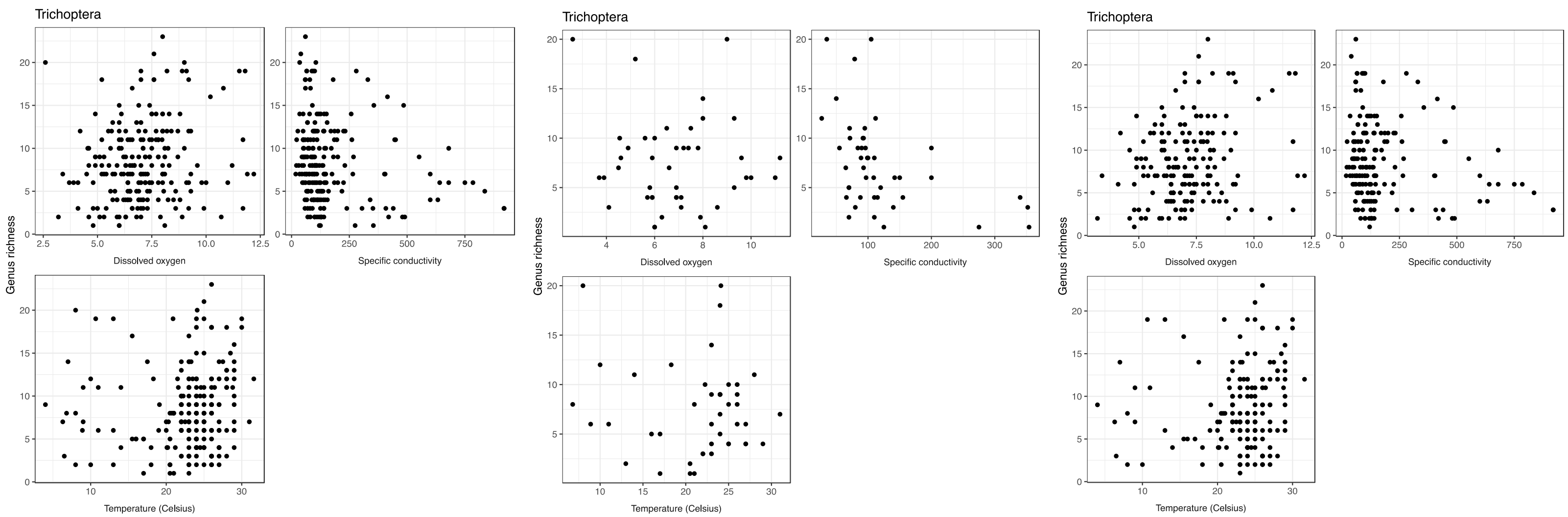


Figure 6. Genus Richness in comparison to Water Quality measurements for order Trichoptera in each habitat(1st = all habitats. 2nd = Wetland, 3rd= Riverine)



Field collection results

Macroinvertebrates collected in the field were classified as far down to the taxonomic Family level as possible. Three species were completely Unknown, 20 species could only by identified at the Phylum level (these were all of the Annelid phylum) There were no collections identified for the Ephemoptera, Plecoptera, and Trichoptera order, so there was no testing done involving the predicted model. However, based on the water quality measurements for some sites this could be indicative that the habitat was unsuitable as they held a DO < 2 and a specific conductance > 250 similar to the lack of observations in figures 2,4, and 6 (Table 3).

Table 3. Water quality results of field collection

Site	Sample	Temperature	Dissolved Oxygen	Specific Conductivity	pH
Bog	1	13.429277	0.8466256	225.4007884	7.12
Bog	2	13.204394	1.206752	237.9399851	6.42
Court	1	12.436543	1.6769404	114.78679	6.062605
Court	2	10.210372	1.7345979	63.871374	5.411819
Hogans Creek	1	14.667306	9.1059193	2.1874395	7.43
Hogans Creek	2	14.588273	3.9065187	52.7905044	6.05
Open	1	14.642833	10.2247783	25.1004075	7.45
Open	2	22.882342	9.9289024	0.3792475	6.94
Wood	1	11.23314	3.2161201	86.7074736	7.39
Wood	2	9.676055	10.2762675	83.1498754	7.18

CHAPTER IV: DISCUSSION

During the field collection, Ephemoptera, Plecoptera, and Trichoptera were not captured upon sampling. According to research, this could be caused by low dissolved oxygen, higher temperature or not enough stream flow (Thorp and Rogers 2015; Newman, DeWalt, and Grubbs 2021; Bytyçi et al. 2018). The lack of these species could also be due to my capturing methods as the leaf litter traps, I used in my field studies are not listed (Thorp and Rogers 2015). It is possible that because I used leaf litter traps, I did not catch the other two orders in the field. In the case of Trichoptera, pollution has been shown to influence their population which may explain why specific conductivity was a water quality predictor in all tested models and was skewed left indicating a drop in richness as specific conductivity increases, this provides support that specific conductivity has some effect on EPT(figures 2,4,6) (Thorp and Rogers 2015; Tszydel et al. 2015). Lack of Dissolved oxygen could also explain a lack of captured EPT as these species prefer good water quality and some field locations held a dissolved oxygen < 4.0 which is indicative of pollution (Table 3) (Bytyçi et al. 2018). Order Plecoptera did not change too drastically across the different habitats, though it is interesting that it held a moderate correlation in wetlands as they do not thrive in wetlands (Newman, DeWalt, and Grubbs 2021). Evidence has shown that this species tends to prefer streams at cooler temperatures, such as mountain temperate which would explain why one of the predictors was temperature (DeWalt, Kondratieff, and Sandberg 2015; Newman, DeWalt, and Grubbs 2021). Though from the graphs in figure 2 it appears it looks as though Plecoptera were frequent in warmer temperatures (Figure 2). This could be indicative that a new branch of Plecoptera species is adapting to warmer temperatures and possibly wetlands in general.

Based on the statistical analysis and that many macroinvertebrates, not just EPT, have been used as bioindicators, this study provides support that predictive analysis for wetlands in the piedmont region based on historical data can work, though not as well as desired (Fochetti and Tierno De Figueroa 2008; Thorp and Rogers 2015; Bytyçi et al. 2018; Newman, DeWalt, and Grubbs 2021; Gezie et al. 2020; Munyika, Kongo, and Kimwaga 2014). Even if my field results did not produce support that the models work in the field. It is my belief that the sampling method, sampling size, and possibly the historical data were the issue. The historical data does not have consistent replicates nor does it's abundance column have statistically likely results, though this could be a key for the actual species amount (Benthic Macroinvertebrate Assessment Data n.d.). Studies which performed predictive analysis on both Wetland and Riverine habitats with a larger sample size showed success in its predictive capabilities (Geipel, Jung, and Kalko 2013; Jung et al. 1999; Bytyçi et al. 2018). Such studies included plant diversity, land uses, anthropogenic uses at or near the aquatic habitat and found such inclusion to hold an impact on diversity. It is Recommended that further studies would benefit not only from a thorough collection method but increase the replications and analyze all data from the North Carolina benthic macroinvertebrate project (Gezie et al. 2020; Hutton et al. 2021; Wen et al. 2015; Thorp and Rogers 2015)(Gezie et al. 2020; Hutton et al. 2021; Wen et al. 2015; Thorp and Rogers 2015).

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