

Analysis of Biochemical Oxygen Demand and Organic Matter after Stream Restoration

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Abstract

Regenerative stormwater conveyance (RSC) is a stream restoration method that is implemented to manage stormwater and reduce nitrogen concentrations downstream. Step-pools collect large amounts of stormwater until they overflow and travel through rocky riffles, where they move to the next pool. Hydrologic residence time is increased with the reduced flow velocity, which improves nitrogen retention through stream bed infiltration, uptake by vegetation, and denitrification. It is not currently understood how this stream restoration method affects other water quality measures, such as dissolved oxygen levels. Tradeoffs may exist with its effectiveness in nitrogen retention and the harmful impact on increased biochemical oxygen demand due to the pooling of water, accumulation of organic matter, and changes in redox conditions. Understanding the impacts of this form of stream restoration on oxygen levels can be studied through analysis of biochemical oxygen demand (BOD). Biochemical oxygen demand refers to the amount of oxygen consumed by microorganisms as they decompose organic matter; when BOD is higher, it lowers dissolved oxygen levels and can potentially create anoxic conditions.

Longitudinal stream synoptic monitoring was conducted monthly at 12 sites along Campus Creek, including water sampling sites that were RSC restored, unrestored, and sites that were recently constructed with channel stabilization techniques. Routine sampling of local unrestored sites such as Lower Campus Creek (LCC), Paint Branch (PB), and Northeast Branch Anacostia (NEA) was done to establish a baseline for comparison. BOD experiments were performed on the samples each week during the fall and winter; the samples were also analyzed using a HORIBA Aqualog to determine dissolved organic matter quality and investigate correlations between changes in BOD and organic matter quality across space and time. It was determined that RSCs significantly impacted ($p\text{-value} \leq 0.05$) dissolved oxygen, conductivity, pH, BOD, and organic matter quality; it was also determined that RSCs had a weak positive correlation with BOD, and a moderate positive correlation with organic matter quality. This suggests that tradeoffs exist with RSCs, specifically, with their impact of increasing BOD loads and organic matter presence. The results of this study can help us make informed decisions about stream restoration efforts by expanding our knowledge about the full impact RSCs have on stream water chemistry, specifically, unintended consequences on dissolved oxygen levels.

Plain Language Abstract

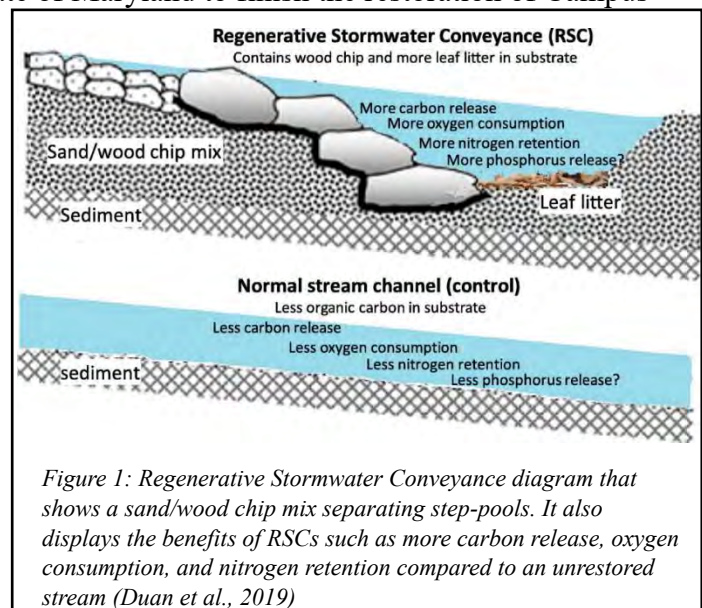
Urban watersheds experience increased amounts of runoff during storms due to the presence of parking lots, roadways, and sidewalks that prevent water from infiltrating into the ground. Flooding occurs when stormwater cannot be contained by stormwater drains and streams. Regenerative stormwater conveyance (RSC) is a stream restoration method that creates large pools for water to collect during storms. The pools remain filled with water even when it is not raining, and it slows down the flow of stream water. This can improve water quality by giving plants and bacteria more time to consume nitrogen. In this study, water samples were collected along Campus Creek, an urban stream on the University of Maryland campus, to

measure how much dissolved oxygen is present and how oxygen can become depleted by increased biochemical activity. Water samples were placed in an incubator for five days to allow the microorganisms in the water to consume oxygen as they break down organic matter. A final dissolved oxygen measurement was taken after five days and subtracted from the initial measurement to get the amount of dissolved oxygen consumed. Samples were also analyzed for the types and amounts of dissolved organic matter present, which allowed this study to evaluate possible connections between BOD and organic matter. This experiment was conducted once a month along Campus Creek, and once a week in the fall at three local unrestored streams. The results show that RSCs increased BOD levels and the amount of organic material in the stream. Understanding the impact RSCs have on the amount of dissolved oxygen in stream water will help us make more informed decisions on how we can protect stream ecosystems during restoration.

Introduction

Stream restoration is a billion-dollar industry in the United States. In 2023, the University of Maryland received \$1.7 million from the state of Maryland to finish the restoration of Campus Creek that began in 2019 (Haslam, 2023). The goal of this stream restoration project is to stabilize the channel downstream of regenerative stormwater conveyances (RSCs) that were previously constructed. RSCs reduce flooding during storm events by collecting large amounts of runoff in step-pools as the stormwater flows through the stream (as shown in Fig. 1). This is valuable because urban infrastructure is especially susceptible to flood damage during storm events due to widespread impervious surface land cover increasing runoff.

Studies have shown that RSCs improve certain water quality measures, such as nitrogen retention and carbon sequestration (Koryto et al., 2017; Duan et al., 2019). However, little is known about the effects they have on organic matter quality and biochemical oxygen demand. Organic matter quality is used to assess the ability of a stream to sustain freshwater ecosystems and cycle nutrients through the environment (Hill et al., 2022). Organic matter quality has multiple definitions, but in the context of this project, it refers to the type of organic matter and its origin. For example, organic matter quality can derive from terrestrial sources or microbial activity; it can also be protein-like or humic-like (Coble, 1996). Streams with higher organic matter quality can recycle nutrients more efficiently from natural sources such as leaf fall, decayed plants, or dissolved organic matter (DOM) (Duan et al., 2014). This causes nutrients to be readily available for consumption by plants and other organisms (Duan et al., 2014). On the contrary, streams with poor organic matter quality struggle to recycle nutrients



since they decompose organic material less efficiently (Duan et al., 2019). In addition to low nutrient availability, studies have shown that streams with poor organic matter quality can affect pCO₂ in connection with seasonal variations (Bodmer et al., 2016).

Biochemical oxygen demand (BOD) is a useful measure for quantifying the amount of dissolved oxygen (DO) consumed by microorganisms as they break down organic matter. For decades, BOD has also been a standard method used in the analysis of water quality, and it is widely used in routine monitoring and regulatory purposes for managing water and wastewater across the U.S. (Montgomery, 1967; U.S. EPA, 1974). In sections of streams with high discharge, there can be scouring of the streambed and decreased microbial activity, which can cause DO fluctuations (Blaszczak et al., 2019). In sections of streams with low discharge, such as RSC step-pools, there may be greater microbial activity (Duan et al., 2019). BOD quantifies the pollution level of a stream in relation to DO levels, which provides a potential mechanism for fish kill events to occur due to pollution or changes in redox conditions that mobilize Fe and Mn (Jouanneau et al., 2014; Kaushal et al., 2020).

This thesis investigates whether regenerative stormwater conveyance can influence BOD and organic matter quality in a restored stream across seasons. It explored this question along urban streams located near the University of Maryland. Routinely determining organic matter quality and BOD along the flow path of Campus Creek allowed me to track changes across space and time. Spatial data were collected at twelve longitudinal synoptic points along Campus Creek which included five RSC-restored sites, four unrestored sites, and three sites that recently underwent channel stabilization restoration (as shown in Fig. 2). The previously restored sites allowed me to track changes where RSCs are implemented, compared to sites that do not have RSCs. There was a unique opportunity to collect samples at newly restored sites shortly after restoration; this restoration project was completed in December of 2024. Data from newly restored sites was not necessary to determine the effects of RSCs on BOD and organic matter quality, but it provided rare and valuable data regarding changes in water quality shortly after restoration. Thus, Campus Creek provided the opportunity to monitor spatial changes in organic matter quality and BOD during three distinct phases of the restoration process.

In addition to Campus Creek, Paint Branch, and the Northeast Branch Anacostia were sampled routinely to provide a control for this experiment since they have not been restored using RSCs and are not currently undergoing stream restoration. Routine sampling occurred once a week at one location at Campus Creek, one at Paint Branch, and one at the Northeast Branch Anacostia (as shown in Fig. 3). The twelve synoptic sites were sampled once a month to provide data across monthly intervals during the fall and winter. Sampling during this period provided data during important seasonal events such as peak leaf fall. Peak leaf fall during autumn may be the time of year when there are large changes in organic matter quality and oxygen demand, but less work has explicitly studied changes in water quality during this time period. Spatial and temporal monitoring was necessary to accurately quantify changes in water quality. Without samples across multiple longitudinal points that are consistently collected, it would be difficult to characterize spatial heterogeneity in water quality along stream flow paths (Sivirichi et al., 2011; Kaushal et al., 2014; Newcomer-Johnson et al., 2014; Kaushal et al., 2023; Maas et al., 2023;

Malin et al., 2024; Shelton et al., 2024). Both spatial and temporal data allowed me to investigate how RSCs affect organic matter quality and BOD along Campus Creek. These results have implications for water quality tradeoffs associated with certain forms of stream and floodplain restoration.

Background

RSCs have been implemented in urban streams across the East Coast of the United States to reduce nutrient transport in runoff (Duan et al., 2019). The construction of RSCs involves the creation of step-pools, layering of sandy soil across the stream base, and the addition of vegetation along the stream banks (Flores, 2012). Step-pools create space for stormwater to collect before continuing downstream (Fanelli et al., 2017); this increases nutrient residence time and the likelihood of nutrient retention through vegetation and soil (Duan et al., 2019). The sandy soil substrate allows water to infiltrate and deposit nutrients with the sediments. The substrate is also an important organic matter source that can be altered in material to improve water quality measures such as the stream C:N ratio (Duan et al., 2019). Vegetation helps stabilize the banks of the channel through its roots, which can reduce erosion and absorb nutrients from stream water. Limiting the erosion of streambanks reduces suspended sediment flow downstream; suspended sediment carries phosphorus (P), which can increase P concentrations (Somura et al., 2012). Pairing this with increased denitrification rates in step-pools from increased microorganism activity, RSCs utilize multiple components to retain nutrients and major elements (Thomas et al., 1994; Malin et al., 2024).

The effectiveness of RSCs varies seasonally as a function of precipitation totals (Fanelli et al., 2017), water table elevation, and plant activity (Thompson et al., 2020). There is variability in RSCs ability to reduce stormwater volume across seasons (Fanelli et al., 2017). A factor in this is precipitation total since it influences the total volume of runoff entering the stream (Fanelli et al., 2017). Water table elevation may also impact the total stormwater volume; winter experiences the highest water table elevations, while summer has the lowest. Winter months may be the least effective time for RSCs due to increased infiltration of groundwater, which reduces the storage space for stormwater in structures like the streambed. Plant activity can strongly influence RSCs ability to control large amounts of stormwater (Fanelli et al., 2017). Significant variations in total streamflow volume have been found between growing and nongrowing seasons (Thompson et al., 2020). Differences are correlated with an increase in the ability of plants to uptake water and release it to the atmosphere during growing seasons through evapotranspiration (Thompson et al., 2020). Vegetation in streams also has an increased ability to reduce the flow of stormwater during growing seasons; this increases the residence time and allows for more infiltration through the streambed (Thompson et al., 2020).

BOD is an effective way to measure pollution levels in a stream (Rice et al., 2012). It is used in sewage treatment plants to gauge the effectiveness of their treatment methods (U.S. EPA, 1974; Hach et al., 1978). While BOD tests are performed in the wastewater industry, research on RSCs has focused on nutrient retention rather than DO consumption. The effects of organic matter input through leaves, along with fluctuating air temperatures, on BOD in streams were studied in the 1970s. It was found that BOD levels in streams that experienced temperature

Campus Creek Synoptic Sites

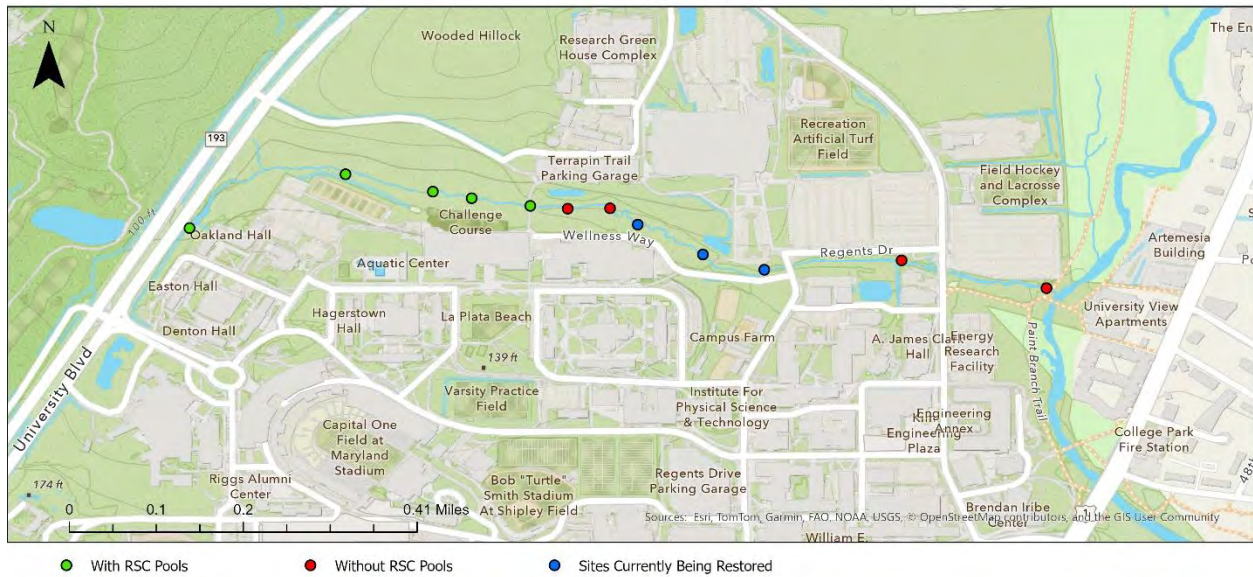


Figure 2: Map of Campus Creek at the University of Maryland displaying twelve longitudinal synoptic sites along Campus Creek. The green dots represent restored sites, the red dots represent unrestored sites, and the blue dots are sites currently undergoing restoration.

Routine Monitoring Sites

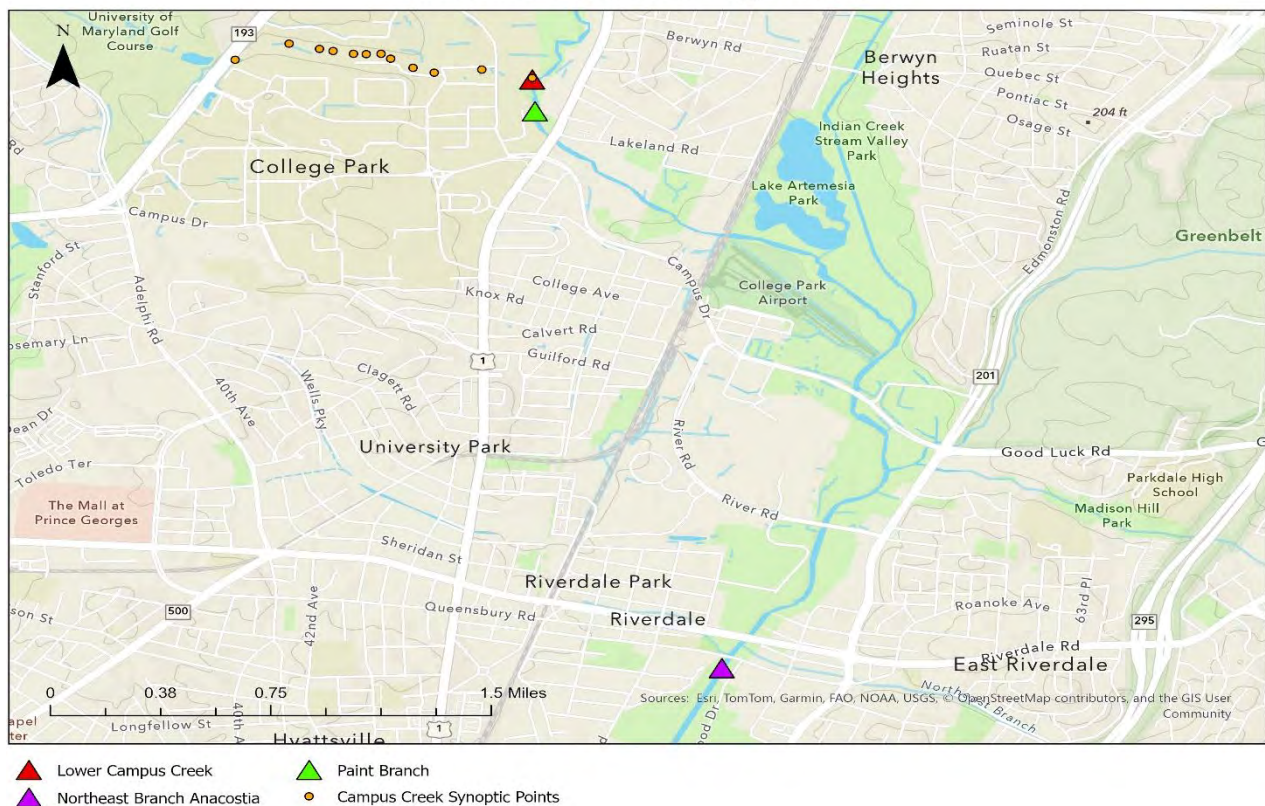


Figure 3: Map of three routine monitoring sites. The red triangle represents the Lower Campus Creek site, the green triangle shows the Paint Branch site, and the purple triangle shows the Northeast Branch Anacostia site. The orange dots represent the synoptic points along Campus Creek.

variations with large influxes of leaves and pine needles were between two and four times greater than the BOD of a controlled stream environment (Ponce, 1974). The findings of this study support the idea that increases in organic matter volume in streams during peak leaf and changing seasons may be correlated with increases in BOD. The presence of algae and high nutrient concentrations in relation to BOD was studied in rivers across Minnesota in the early 2000s (Heiskary and Markus, 2001). Less turbid rivers experienced larger algal blooms and higher nutrient concentrations, which caused higher BOD levels (Heiskary and Markus, 2001). It was also found that more turbid rivers experienced the opposite, where there were fewer algal blooms, nutrient concentrations, and lower BOD levels (Heiskary and Markus, 2001). This supports the idea that the step-pool features in RSCs with lower turbidity and discharge will be more likely to have higher nutrient concentrations and higher BOD. RSCs are designed to function as nutrient sinks that increase the residence time of the nutrients moving downstream. These studies support the idea that there could be unintended consequences with BOD levels.

Land use and development have led to changes in the quality of the organic matter that is entering streams through stormwater; these changes exist in both urban and rural settings (Kaushal and Belt 2012; Kaushal et al., 2014). In rural areas, the expansion of agricultural land cover has resulted in eutrophication of streams from fertilizers. In urban streams, impervious surfaces decrease infiltration through the ground, which increases inputs of polluted runoff. A study found that particulate organic matter (POM) was a significant predictor of BOD levels in streams (McCabe et al., 2021). POM requires large amounts of energy for microorganisms to break down due to their large mass; this requires more oxygen consumption by microorganisms as they attempt to break down the material. A positive correlation between BOD and POM was found through the R^2 test and suggests that POM has a more significant role in stream chemistry than previously believed (McCabe et al., 2021). Their stormwater management recommendation was to consider upstream inputs of POM through stormwater when planning restoration efforts to improve DO levels downstream. This study emphasizes the importance of measuring BOD and organic matter quality longitudinally along the stream's flow path because non-point sources upstream can have a significant effect on water quality downstream.

Objectives

RSCs are implemented for nutrient control, however, they may affect organic matter quality and biochemical oxygen demand (Duan et al., 2019). From this project, I aimed to gain valuable insight into potential tradeoffs with RSCs and further our understanding of the unintended consequences of restoration. Additionally, restoration strategies do not only involve the physical design of the restoration but also include the construction process. Data collected directly after the conclusion of construction may be especially useful in understanding both short-term and long-term effects RSCs can have. A study on the installation of culverts to create room for an airport runway in Ohio showed that the construction of best management practices can have minimal impacts on the water quality of a stream (Houser and Preuss, 2009). However, the construction of RSCs along Campus Creek undergoes a different construction process that may have a different influence on water quality. With the large investments being made in stream

restoration each year, we must continue to develop our understanding of how restoration strategies affect ecosystems.

My null hypothesis states that there is no trend in organic matter quality or biochemical oxygen demand along the flow path of Campus Creek because RSCs do not affect them. My first alternative hypothesis states that there is a trend in organic matter quality, but not biochemical oxygen demand, along the flow path of Campus Creek, because RSCs can improve rates of biogeochemical processes such as organic matter decomposition (Duan et al., 2019). My second alternative hypothesis states that there is no trend in organic matter quality, but a trend in biochemical oxygen demand along the flow path of Campus Creek because RSCs can create anaerobic conditions in their step-pools, which impact DO levels (Koryto et al., 2017). My third alternative hypothesis states that there is a trend in both organic matter quality and biochemical oxygen demand along the flow path of Campus Creek because RSCs can affect multiple water quality measures (Cizek et al., 2018).

Methodology

Field Methods

This study included both fieldwork and lab tests. I sampled the routine monitoring sites weekly as previously shown in Fig. 3. At these sites, I used a Yellow Springs Instrument ProQuatro Multiparameter Meter (YSI) to record data on standard water quality indicators including, temperature, dissolved oxygen (DO), specific conductance (SpC), conductivity, total dissolved solids (TDS), salinity, pH, and oxidation-reduction potential (ORP). A YSI is a device that uses multiple sensors to determine these water quality indicators in a stream (as shown in Fig. 4). I placed the YSI into the stream and waited for the readings to stabilize before recording them in a field notebook.

Routine Monitoring of Water Quality and Streamflow

During routine monitoring, I also collected water samples to take back to the lab for further testing; I collected a 1,000 mL sample at the Lower Campus Creek site, and 500 mL samples at Paint Branch and the Northeast Branch Anacostia. The size of the water sample was determined by the tests I conducted in the lab. Since I conducted a BOD experiment, I needed at least 300 mL of water to fill the BOD bottles. I collected 500 mL samples to ensure there is enough of each sample in case of spillage, and I collected a 1,000 mL sample at synoptic site CC1 so that I had enough of the sample to test a duplicate to evaluate accuracy. The water samples were immediately placed into a cooler with ice to preserve the sample and minimize interactions with light. The samples required minimal exposure to light to prevent photosynthesis from occurring because it could alter dissolved oxygen levels. For an experimental control, I sampled at the biogeochemistry lab's routine monitoring site for Paint Branch and the Northeast Branch Anacostia. These sites are experimental controls because they have not undergone restoration, which allowed me to make comparisons with the restored sites of Campus Creek.

This testing method gave me data on the water quality of the stream each week that I tracked during the fall and winter.

To determine the effects of restoration on biochemical oxygen demand, I conducted a longitudinal synoptic of Campus Creek once a month. During a synoptic, I collected water samples at each routine monitoring site as well as twelve points along Campus Creek that the biogeochemistry lab has designated as synoptic points (as shown in Fig. 2). At these points, I also measured water quality indicator data using YSI readings just as I did at the routine monitoring sites. Additionally, I measured the width of the stream at each synoptic point using an open reel measuring tape. To do this, I wore waders and walked a traverse across the stream from one stream bank to the opposite one while extending the tape measure until the total width was determined. At two-foot intervals along the transect, I measured the velocity and depth using a Flow Meter.

Laboratory methods

BOD Incubations

In the lab, the samples were immediately placed into a dark cabinet until they were at room temperature. Then, one sample was removed and shaken for one minute to saturate the sample. It was immediately poured into a 300 mL opaque bottle, and a DO meter was inserted. After one to two minutes, the dissolved oxygen reading became stable, and I recorded the value in a BOD datasheet. The DO meter was removed from the bottle and rinsed with de-ionized water three times to avoid contamination. This process was repeated until all of the samples' DO levels were recorded. The samples were moved to an incubator set to 20°C for five days. After five days, I carefully removed them from the incubator and placed the DO meter in the samples to get a final DO reading. To calculate the biochemical oxygen demand, I inserted the initial and final DO values in the following formula:

$$DO_{\text{initial}} - DO_{\text{final}} = \text{Biochemical Oxygen Demand}$$

Finally, I plotted the data and conducted statistical analyses to determine the changes in biochemical oxygen demand over the course of the project.

Horiba Aqualog

I also analyzed the samples I collected using a HORIBA Aqualog. The Aqualog uses fluorescent spectroscopy to evaluate the absorbance and fluorescence spectra of dissolved organic matter (Hansen et al., 2018). To use the Aqualog, I first checked the lamp by running a blank sample, a cuvette filled with de-ionized water; the absorbance should have a peak of 467 ± 1 nm. The emission spectra plot produced checks for “noise” or contamination on the cuvette; if there is no contamination, the plot will have no slope



Figure 4: YSI ProQuatro Multiparameter meter from YSI Company Website.

between 300 and 500 nm. These runs were saved as baselines for absorbance and emission analysis, which was then performed on water samples. Every three samples, another blank was analyzed to ensure that there was no contamination in the cuvette. Finally, the data were collected and analyzed to get valuable information on the changes in organic matter quality along Campus Creek.

Characterization of Dissolved Organic Matter Quality

The Aqualog quantifies fluorescence indices that indicate the origin and type of organic matter. These data provide information on the quality of DOM in water samples, which will allow me to track changes in DOM, which can influence BOD. In the study about DOM mentioned previously, it was found that humic substances and protein-like organic matter had a direct correlation with BOD downstream (McCabe et al., 2021). Analyzing the dissolved organic matter quality of Campus Creek and routine monitoring sites allowed me to relate BOD to changes in organic matter across space and time in restored and unrestored streams. This strengthened correlations between changes in BOD and the restoration of Campus Creek because it factors in the collection and decomposition of organic matter that occurs in RSC pools.

Protein-like and Humic-like

The protein-like index is a measure of the proportion of DOM that is typically produced from other organisms and is more easily degraded by microbes than humic-like DOM. Studies have determined that the intensities of this index are related to tryptophan concentrations in natural waters (Yamashita and Tanoue, 2003). Tryptophan is an aromatic amino acid with fluorescence properties that make it a useful fluorescence index (Teale and Weber, 1957). High protein-like values indicate more labile DOM is present for microbes to consume, potentially impacting BOD loads.

Humic-like is an Aqualog index that describes how much older and more degraded DOM is present in a water sample (Gabor et al., 2014; Pucher et al., 2019). This type of DOM is typically derived from plant material or soil, and studies have shown that it is less bioavailable, meaning microbes cannot break it down for energy as easily as protein-like DOM (Coble, 1996).

Coble M and Coble C

Coble M is an index that represents humic-like, complex DOM that is derived from microbial activity (Coble, 1996). In comparison, Coble C is an index that represents humic-like, complex DOM that originates from terrestrial sources (Coble, 1996). These indices allow us to further classify humic-like DOM and determine the relative contributions of microbes and terrestrial sources to DOM concentrations.

Decadic.a254

Decadic.a254 is a measure of the UV absorbance of a sample at a wavelength of 254nm, which is then normalized to the path length. This is used to determine the concentration of chromophoric DOM, which can be described as aromatic (Helms et al., 2008; Hansen et al., 2018; Pucher et al., 2019). Studies have shown that UV absorbance at a wavelength of 254nm

can effectively be used to estimate aromatic carbon content in aquatic environments (Weishaar et al., 2003). Aromatic carbon compounds can pose a threat to organisms in these environments due to their resistance to degradation, which can lead to their accumulation.

These fluorescence indices were used in this study to characterize changes in DOM across space and time. Determining these values allowed me to interpret the sources and types of DOM present and find correlations between DOM, BOD, and RSC restoration. These data were used as supporting evidence for determining whether to accept or reject my null hypothesis that there is no trend in BOD or organic matter quality along Campus Creek because RSCs do not affect them.

Calibrations and Errors

SpC, ORP, and pH Calibration

All devices used in this experiment were checked for accuracy before each sampling trip. There are three known samples that are used to check for the accuracy of the YSI readings: SpC, ORP, and pH standards. These standards are water-based solutions that come in a sealed container with a known value for the parameter listed on the bottle. Enough of each known standard was poured into a small cup so that the YSI sensors are completely submerged. The YSI sensors were placed into a sample until there was a stable reading. The reading was recorded in a field notebook if it was $\pm 5\%$ of the known value. However, if the YSI reading was off by more than this, it could be recalibrated to the known value of the solution. This was completed by selecting the recalibrate setting in the YSI and manually inputting the known value before it was removed from the solution. For example, the known value for ORP is 238 mV; if the YSI reading is 231 mV, this is within 5% and acceptable for sampling. However, if this value were 215 mV, it would require manual recalibration before use. This process was repeated until each of the known samples was used to check the accuracy of the YSI (as shown in Fig. 5). In between each sample, the sensor was rinsed with de-ionized water three times to avoid contamination. Before going into the field, the known samples were covered with an airtight lid and stored at room temperature on a shelf so they could be reused for accuracy checks for up to a month.

SpC ORP and pH Accuracy						
	SpC: 1413 $\mu\text{S/cm}$	% Error	ORP: 238 mV	% Error	pH: 7.00	% Error
09/25/2024	1380	2.34	238.5	0.21	7.02	0.29
10/30/2024	1415	0.14	239	0.42	7.36	5.14
11/13/2024	1381	2.26	231.8	2.61	7.31	4.43
01/10/2025	1435	1.56	235	1.26	7.38	5.43
02/13/2025	1322	6.44	238.4	0.17	7.15	2.14
03/14/2024	1440	1.91	237.2	0.34	7.12	1.71
Avg % Error		2.44		0.83		3.19

Figure 5. Table containing YSI measurements made at the beginning of sampling days. SpC, ORP, and pH average percentage errors were determined using the percent error of the YSI measurements compared to the known samples value.

The accuracy of the measurements I recorded in the field with the YSI can be determined using percentage error. The percentage error is calculated using the following formula:

$$\text{Percent Error} = (|\text{Experimental Value} - \text{Accepted Value}| / \text{Accepted Value}) \times 100\%.$$

Dissolved Oxygen Calibration

The last step for preparing the YSI for field data collection was to calibrate the DO sensor. This was done by putting one inch of de-ionized water into the solid cap of the YSI sensors. The cap was screwed onto the YSI sensor just enough so that the sensor would not fall off when flipped upside down; this allowed for air to flow into the cap. The cap was placed onto a flat surface so that the YSI sensor was upright, surrounded by water-saturated air. The YSI was then set to DO% calibration mode; DO was calibrated in % because this calibrates mg/L and ppm at the same time. The YSI was set to calibrate for up to ten minutes or once it reached a stable DO reading, whichever came first. When this process was complete, I accepted the calibration, and the YSI recalibrated the final DO measurement to 100%. This sets a water-saturated air sample to the maximum DO reading possible for the device.

Temperature, C, TDS, and Salinity Error

The errors for these parameters were used based on the errors given by the Yellow Springs Instrument company for the YSI Multiparameter meter. Temperature had an uncertainty of $\pm 0.1^\circ\text{C}$, and 1% for C and TDS, and ± 0.1 ppt for salinity (YSI, n.d.).

BOD Uncertainty

The uncertainty of the BOD experiments was measured by doing a duplicate of site CC1 during each experiment (as shown in Fig. 9). In three of the six experiments the percentage difference was less than 10%. The first experiment had a sizable percentage difference of 17%, which raised the average percentage difference. The average uncertainty for the BOD measurements through the first three synoptics is approximately 7%.

BOD Duplicates on CC1 (mg/L)			
Date	CC1	CC1 Dup	% Diff
09/25/2024	1.63	1.39	17.27
10/30/2024	1.17	1.16	0.86
11/13/2024	3.58	3.49	2.58
01/10/2025	1.23	3.49	7.89
02/13/2025	1.49	1.14	49.00
03/14/2025	0.88	1	37.50

Figure 6: BOD Duplicates performed on Campus Creek site 1.

Data Presentation and Results

Longitudinal and Temporal Changes in Field Parameters

Temperature

The temperature of Campus Creek represents the average kinetic energy of the water at each synoptic site measured in degrees Celsius. Changes in temperature along the flow path of Campus Creek are plotted in Appendix 2. The plots show a general trend of temperature

decreasing from September to January before increasing from January to March, this is likely due to changing air temperatures throughout changing seasons.

Dissolved Oxygen

The dissolved oxygen level of a stream is a measure of the amount of oxygen dissolved in the water per unit volume. Appendix 3 shows the dissolved oxygen level of Campus Creek along the flow path for each synoptic. There is a general trend of dissolved oxygen content increasing over time from September through February, before the DO levels were lower in March. This makes sense when considering the relationship between temperature and DO levels, as previously mentioned, but there is an outlier. November had a large decrease in dissolved oxygen content that did not follow the general trend; this can likely be attributed to peak leaf fall, where BOD levels were at their highest for multiple sites.

SpC

The SpC of a stream describes the ability of the water to conduct an electrical current (USGS, 2019). SpC along the flow path of Campus Creek is plotted over time in Appendix 4. There is no obvious general trend when analyzing the plots, however, it is clear the highest SpC values occur in the January and February synoptics.

Conductivity

The conductivity of stream water is similar to SpC since they both describe the water's ability to carry an electrical current, however, they differ in that SpC measures conductance to a standard temperature of 25°C. The conductance plots in Appendix 5 follow the same patterns seen in the SpC plots in Appendix 4. This is true for patterns across space from site to site, and across time, with the same trend of highest C values in January and February.

Total Dissolved Solids

Total dissolved solids are a measure of all substances that are dissolved in the stream water. This includes all organic substances, inorganic substances, metals, minerals, ions, salts, etc. Appendix 6 displays plots of the change in TDS along Campus Creek for each synoptic. There is a general trend of higher TDS measurements from the January and February synoptics before they begin to decrease again in March. This may be associated with the increased input of dissolved ions from road salt runoff throughout the winter, as previously discussed.

Salinity

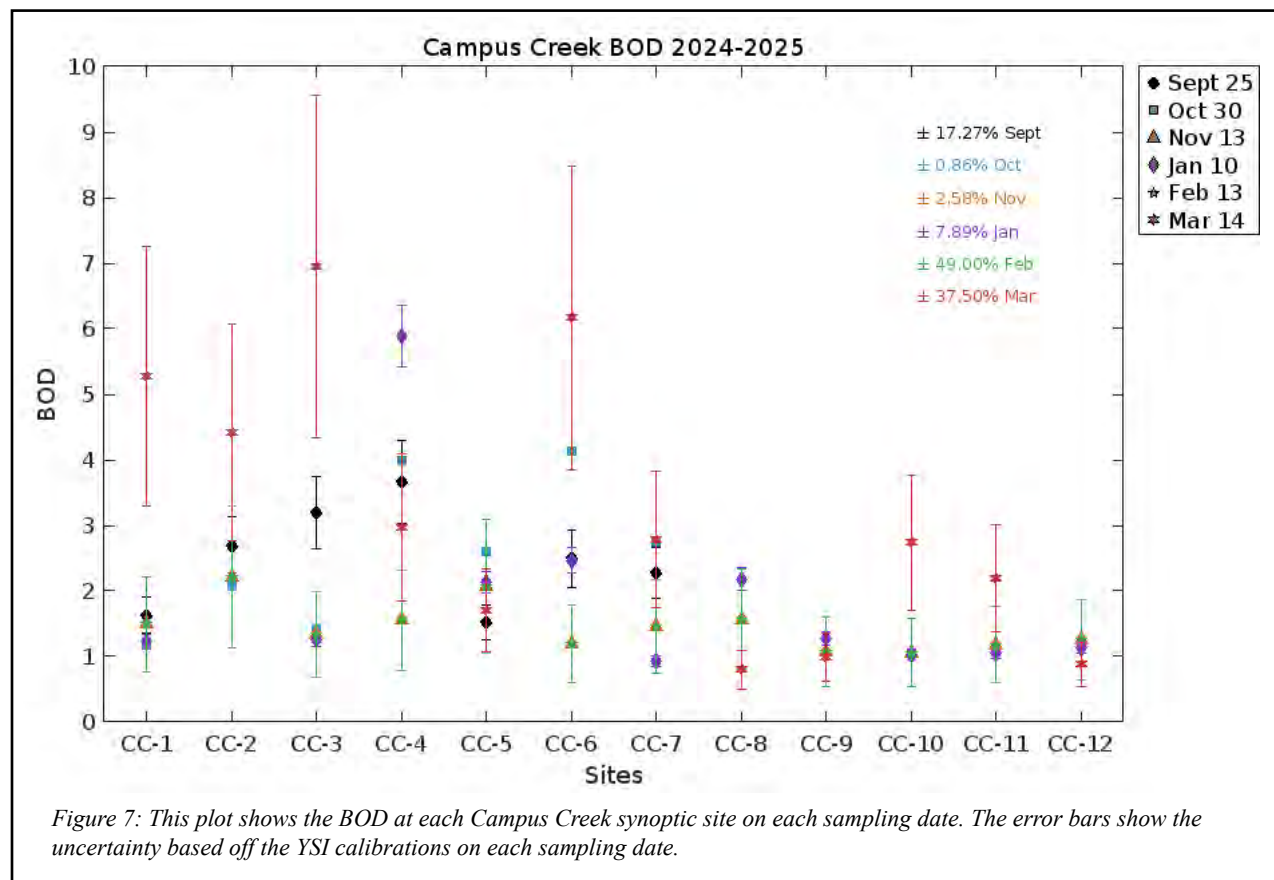
Salinity is a measure of the amount of salt dissolved in a body of water. Changes in salinity in Campus Creek over time are shown in Appendix 7. The same general trend that TDS experienced of increased values in January and February was observed for salinity. Specifically, there is a steady increase in salinity and TDS from CC8 to CC12 on January 10th that is not observed on any of the other synoptic dates.

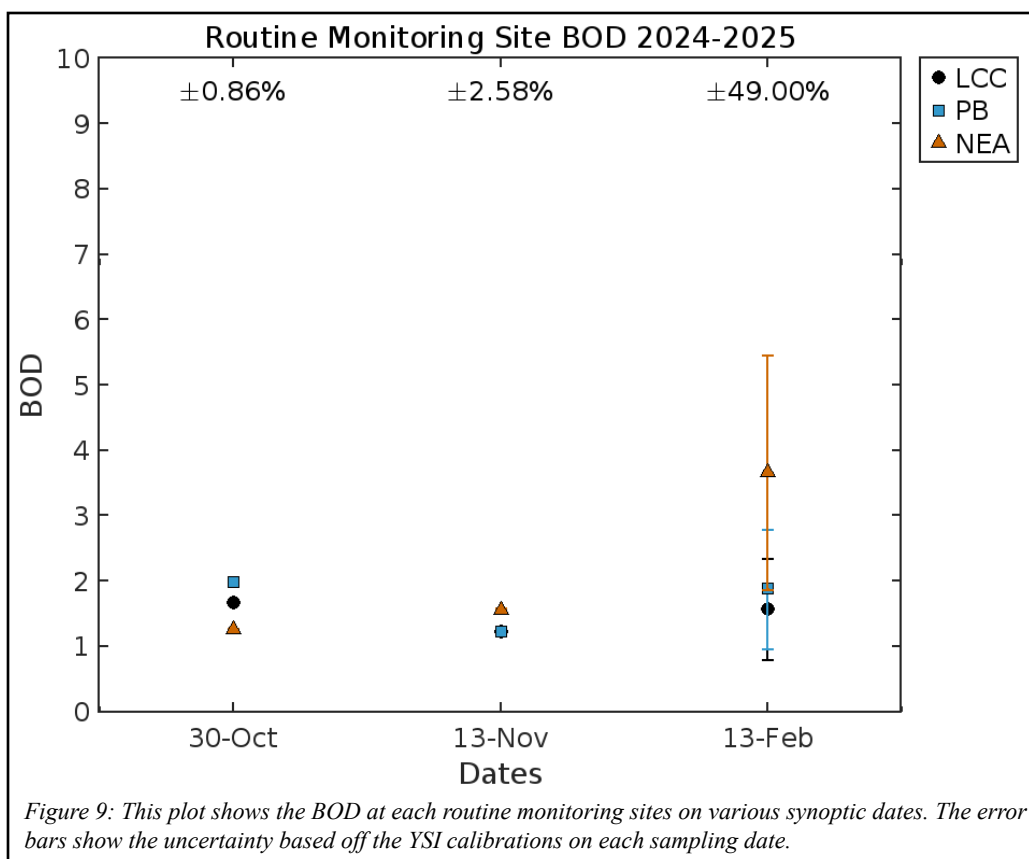
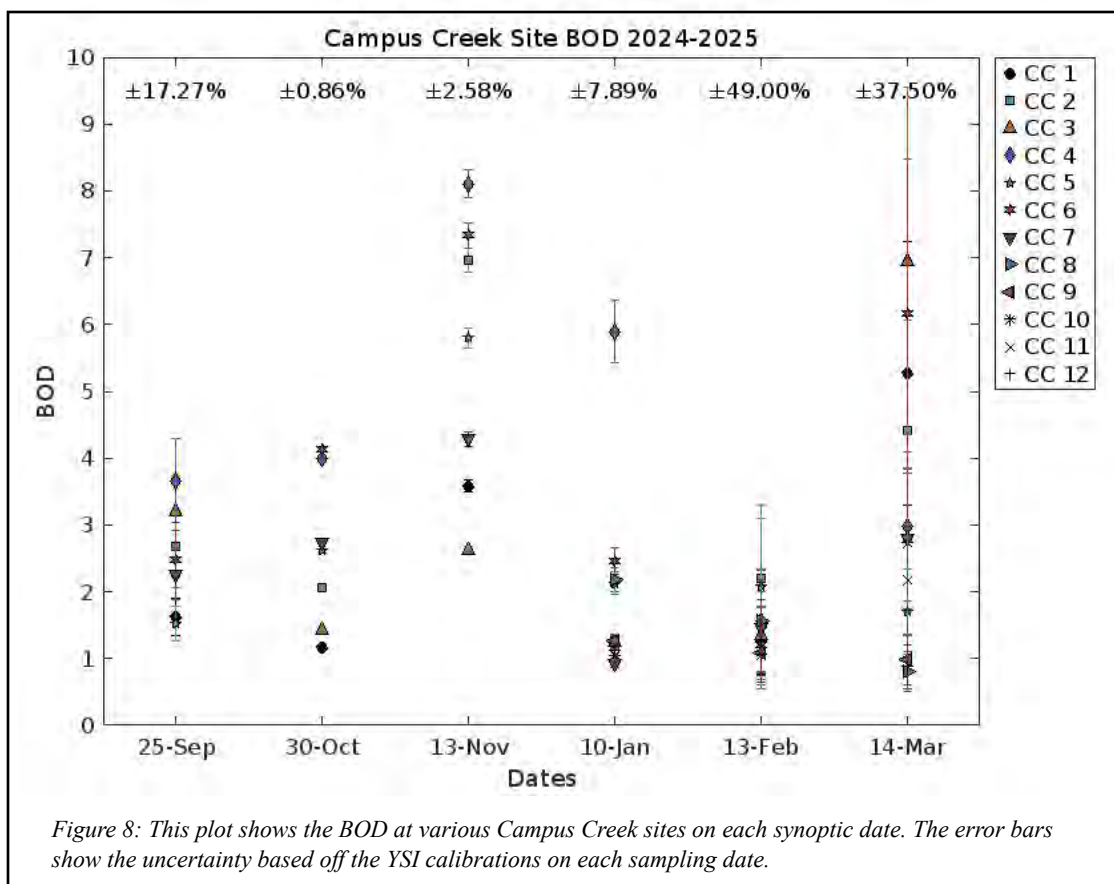
pH

Appendix 8 displays the pH changes along Campus Creek over time. There is no general spatial or temporal change apparent, and statistical analyses are necessary to determine any significance in the data.

ORP

ORP describes the ability of a stream to break down material such as organic matter and waste through oxidation and reduction. When ORP is positive, the stream has oxidizing conditions, and when it is negative, the stream has reducing conditions. Appendix 9 displays the changes in ORP along Campus Creek over time, where there is no general trend observed. However, there are noticeably lower ORP values from the November 13th synoptic compared to the others.





Longitudinal Changes in BOD Associated with RSC Restoration

Figure 7 plots all of the synoptic BOD data collected throughout this study. In this figure, the synoptic sites are in order on the x-axis from CC1 to CC12, and BOD is plotted on the y-axis. It is important to note that access to sites CC8 through CC12 did not become available until the January 10th synoptic due to the restoration project. The error of each measurement is shown by the error bars which were determined by analyzing a duplicate sample of CC1 sample during each synoptic (as shown in Fig. 6). This figure is most useful for observing changes in BOD over space; using this plot, we can see that BOD values were generally lower for sites CC7 through CC12 compared to CC1 through CC6. BOD values were measured to be in an approximate range of 1-7 mg/L for sites CC1 through CC6, while BOD values were measured to be in an approximate range of 1-3 mg/L. Higher BOD levels at the upstream sites may suggest that there is increased microbial activity and pollution levels compared to the downstream sites (Rice et al., 2012). More data is needed before any conclusions can be made, and since CC8 through CC12 were only sampled after January 10th, it is useful to analyze temporal changes of BOD.

Seasonal Variations in BOD During Fall and Summer

Figure 8 plots all of the synoptic BOD values, where the synoptic date is on the x-axis and the measured BOD value is on the y-axis. This figure plots each synoptic site on the x-axis with its correlating sampling date, displaying the range in BOD values measured on each date. The range of BOD values remained relatively unchanged from September to October but increased in November. This may be attributed to peak leaf fall, as previously mentioned, due to larger inputs of organic material that are subsequently decomposed by microorganisms. The BOD generally decreased in the winter, this is seen with the January and February synoptics having BOD ranges that are lower than the ranges observed in fall synoptics. Finally, the March synoptic saw a wider range of BOD levels compared to other synoptics; some sites increased while others decreased compared to the previous synoptic in February. This plot may suggest that BOD rises throughout the fall before decreasing again throughout the winter. To develop a better understanding of the temporal changes observed in Campus Creek, it is useful to compare them with data from the control sites that have not undergone any restoration.

Figure 9 is a plot of BOD data at routine monitoring sites on synoptic days when the control sites were able to be visited, it was not always possible due to transportation limitations. This plot is structured the same as Figure 7, with the synoptic date on the x-axis and the measured BOD value on the y-axis. The interesting result from this plot is that BOD levels did not increase from October to November, however, they did increase from November to February. This result is the opposite of what was observed in Campus Creek and suggests there may be a difference between Campus Creek and the control sites that affects BOD over space and time. Statistical analysis is necessary to determine if the observed differences in the plots have any significance.

Statistical Analysis

To determine statistical significance and correlate data collected across space and time, linear regression analysis was used. This analysis was conducted to test the significance of the relationships between RSC restoration and temperature, DO, SpC, conductivity, TDS, salinity, pH,

ORP, and BOD. Additionally, linear regression analysis was conducted to determine the strength of relationships between RSC restoration on the four fluorescence indices measured in this study, including Coble C, Coble M, humic-like, and decadic.a254; these analyses were necessary for making conclusions on the impact of RSCs on BOD, organic matter quality, and overall water quality. Linear regression analysis was also conducted on the same field data and fluorescence indices in relation to BOD to determine the significance of their relationships. These analyses produced p-values, r-values, and R^2 values for each parameter's relationship with restoration and BOD. For this study, a p-value less than or equal to 0.05 was used to determine significance (Fisher, 1925). For determining the strength of the Pearson coefficient (r), the following r-value ranges are used: 0-0.19 very weak, 0.20-0.39 weak, 0.40-0.59 moderate, 0.60-0.79 strong, 0.8-1 very strong (Rodgers and Nicewander, 1988). Conducting these statistical analyses was vital for determining the significance, strength, and variation of the relationships between variables, which allowed for quantitative conclusions to be made.

Discussion of Results

Temperature

Temperature did not show statistical significance with RSC restoration; however, it did have a p-value of 0.047 in relation to BOD, showing significance. In addition, temperature and BOD had an r-value was 0.27 and an R^2 of 0.07, indicating a weak positive correlation (as shown in Fig. 14). A conclusion can be made that rising temperatures may cause BOD levels to increase. This may not seem intuitive when considering the inverse relationship between temperature and dissolved oxygen content in streams, but it could potentially be explained through microbial processes (O'Connor and Dobbins, 1956). While higher water temperatures are related to less dissolved oxygen availability, they also cause increased microbial respiration (Kirschbaum, 1995, 2006). An increase in microbial respiration would correlate with increased BOD levels as oxygen must be consumed by microorganisms in this process.

Dissolved Oxygen

Statistical analyses determined that DO and RSC restoration had a p-value of 0.001, an r-value of -0.41, and an R^2 value of 0.16. This suggests that DO and RSCs have a significant relationship and have a moderate negative correlation. A conclusion can be made that RSCs may decrease DO levels in streams. In addition, analyses of the relationship between DO and BOD determined a p-value of 5.4e-5, an r-value of -0.51, and an R^2 value of 0.26 (as shown in Fig. 15). This indicates an inverse relationship between DO and BOD levels, which supports my second alternative hypothesis. This makes sense because as DO levels decrease due to oxygen consumption by microbes, BOD increases.

Specific Conductance

As previously mentioned, no general trend over space or time exists, however, January and February had the highest values. This is likely due to large road salt inputs during the winter to mitigate the effects of snow on urban infrastructure. When road salt enters the stream through runoff, it dissociates into ions that move freely throughout the stream and increase the SpC

(Moore et al., 2019). Statistical analyses showed no significant relationship between restoration and SpC, however, they did reveal a significant relationship between SpC and BOD. There was a p-value of 0.001, an r-value of -0.45, and an R^2 value of 0.20, indicating a moderate negative correlation (as shown in Fig. 16). The existence of a relationship between SpC and BOD is not currently understood, but this study suggests that they may be correlated. Future work is necessary to determine a possible mechanism for their relationship.

Conductivity

Conductivity produced similar values to SpC in statistical analyses. C and BOD have a p-value of 0.001, r-value of -0.43, and an R^2 value of 0.19 (as shown in Fig. 17). As SpC did, this indicates a significant relationship exists with BOD, and a moderate negative correlation. The main difference is that there was also a significant relationship with C and RSC restoration. There was a p-value of 0.05, an r-value of -0.29, and an R^2 of 0.05, indicating a weak negative correlation. It is worth noting that while C does show significance with RSCs and SpC did not, SpC and RSCs had a p-value of 0.06, which may potentially indicate a significant relationship if the dataset were expanded in a future study. The contrary is also possible that a larger dataset may not yield a significant relationship between C and RSCs.

Total Dissolved Solids

Statistical analyses produced nearly identical results as SpC in relation to both restoration and BOD; TDS had no significant relationship with restoration, a p-value of 0.001 in relation to BOD, an r-value of -0.45, and an R^2 value of 0.20. These results may indicate that TDS and SpC have a significant relationship, and further statistical analyses may support this. TDS includes organic materials in addition to salts and ions, so this significant relationship with BOD may support my third alternative hypothesis that organic matter quality has a trend with BOD; analysis of Aqualog indices is needed to determine this.

Salinity

Salinity has a direct connection to road salt usage, and a possible explanation for the increase from CC8 to CC12 may be due to increased impervious surface cover near these sites (Kaushal et al., 2005). Looking back at the map from Figure 2, sites CC8 through CC12 are closer to major parking lots and sidewalks, which are covered in road salt before storms, compared to the first seven sites. Greater impervious surface cover that is de-iced with road salt likely contributed to the increased salinity seen at sites eight through twelve. High-resolution sampling may be needed to explain why salinity increased at each site from CC8 to CC12 on January 10th, when this trend was not observed on any other synoptic date.

Statistical analyses determined that there was no significant relationship between salinity and RSCs, however, there was a significant relationship between salinity and BOD. They had a p-value of 0.002, an r-value of -0.41, and an R^2 value of 0.17, indicating a moderate negative correlation exists. Studies have shown that increasing salinity can decrease BOD due to impacts on the ability of microorganisms to break down organic matter, and this result is consistent with these findings (Dann et al., 2024). To further support this, linear regression analysis was also

conducted between salinity and the fluorescence indices measured to classify organic matter quality (as shown in Figs. 22 – 25).

Salinity had a significant, strong negative correlation with all four indices analyzed in this study including, Coble C, Coble M, humic-like, and decadic.a254. This suggests that as salinity increases, there is less terrestrial and microbial derived organic matter, as well as less humic-like and chromophoric DOM. This supports the negative relationship between salinity and BOD because as organic matter concentrations decrease, the BOD will also decrease since there's less organic matter to break down.

pH

It was found that there was no relationship between pH and BOD, however, there was a significant relationship found between pH and RSC restoration. pH and RSCs produced a p-value of 0.03, an r-value of -0.29, and an R^2 value of 0.07, indicating a weak negative correlation exists. RSCs collect larger amounts of leaf fall compared to narrow, faster-flowing sections of a stream, and the negative correlation between pH and RSCs is supported by previous experiments. Studies have shown that the breakdown of organic matter, such as leaves, is correlated with a decrease in stream water pH due to the release of organic acids in this process (David and Vance, 1991). While no relationship between pH and BOD was found, the results indicate that the installation of RSCs may cause a decrease in pH at those sites.

Oxidation-Reduction Potential

Studies have shown that ORP can decrease as organic matter content increases because organic matter can act as a reducing agent as it breaks down (Khanal and Huang, 2003). The November 13th synoptic occurred closest to peak leaf fall, and this may explain why Campus Creek had the strongest reducing conditions of any synoptic in this study. Based on this, it would be expected that ORP would have a negative correlation with BOD, where increased BOD would lead to more reducing conditions due to increased organic matter decomposition. Statistical analyses supported this hypothesis with ORP and BOD having a p-value of 0.032, an r-value of -0.29, and an R^2 of 0.08, indicating a weak negative correlation. In relation to RSC restoration, it was found that ORP and RSCs do not have a significant relationship. This may be consistent with the weak negative correlation that exists between ORP and BOD; if this was a strong relationship, it would be expected that RSCs and ORP would have a significant relationship due to greater amounts of organic matter being collected and decomposed in RSC pools as previous studies have shown (Duan et al., 2019). Since this is a weak relationship, it is reasonable that this relationship is not significant, and it may indicate that RSCs may not typically contain as much organic matter as previous studies have suggested compared to non-RSC sites. Collecting more data to analyze total organic carbon concentrations would be useful in determining the strengths of these relationships.

Coble C and Coble M

Appendix 10 displays box plots of Coble C values for RSC restored and unrestored sites. There is a general trend of values for restored sites compared to unrestored sites, which is shown

by the difference in means. Statistical analyses determined there was no significance between Coble C and BOD, however, there was significance between Coble C and RSC restoration. They had a p-value of 0.029, an r-value of 0.48, and an R^2 value of 0.18, indicating a moderate positive correlation. From this, we can conclude that RSC pools increase the presence of complex terrestrial-derived humic-like organic matter. This supports my first alternative hypothesis that there is a trend in organic matter along Campus Creek due to RSC restoration, but no trend in BOD.

Appendix 11 displays box plots of Coble M values for RSC sites and non-RSC restored sites. There is a general trend of greater Coble M values for restored sites compared to unrestored sites, like the results of Coble C. There was no significant relationship found between Coble M and BOD or RSC restoration. However, there was a moderate positive correlation found between Coble M and RSC restoration based on a calculated r-value of 0.44. This may be related to the moderate positive correlation Coble C and RSCs had, however, there is no statistical significance between the variables, and it does not support the first alternative hypothesis. These findings suggest that RSCs in Campus Creek increased the amount of terrestrial-derived DOM, but did not affect microbial-derived DOM. This is an important observation since it allows us to associate changes in organic matter quality in these specific RSC pools with terrestrial sources more so than microorganisms. This is consistent with previous studies that found RSCs can affect the type of organic matter present (Duan et al., 2019).

Decadic.a254

Appendix 12 displays box plots of Decadic.a254 values for RSC sites and non-RSC sites. There is a general trend of higher values at RSC sites compared to non-RSC sites, just as Coble C and Coble M had. There is statistical significance between decadic.a254 and RSC restoration as determined by a p-value of 0.009, an r-value of 0.55, and an R^2 value of 0.27, indicating a moderate positive correlation. In addition, there was no statistical significance found with decadic.a254, and BOD. This result supports my first alternative hypothesis that RSCs impact organic matter quality.

Humic-like

Appendix 13 displays box plots of humic-like values for RSC sites and non-RSC sites. These plots show a general trend of higher humic-like values in RSC sites compared to non-RSC sites, which is shown by the difference in means. Statistical analyses determined that a significant relationship exists between humic-like and RSC restoration, with a p-value of 0.05, an r-value of 0.43, and an R^2 value of 0.14, indicating a moderate positive correlation. Additionally, there was no significant relationship found between humic-like and BOD. This is another fluorescence index that supports my first alternative hypothesis and provides more evidence for RSCs impact on organic matter quality.

Biochemical Oxygen Demand

Changes in BOD along the flow path of Campus Creek are plotted in Appendix 1. Each plot shows the BOD measurement at each synoptic site on the corresponding synoptic date. The

statistical analyses mentioned previously determined that BOD had a p-value of 0.05, a Pearson coefficient (r) of 0.26, and an R^2 of 0.05 in relation to RSC restoration. This suggests that there is a significant relationship between BOD and RSC restoration, and they have a weak positive correlation. A conclusion can be made that RSCs may cause BOD levels to increase in streams, which supports my second alternative hypothesis. This supports the findings of previous studies where DO% decreased in RSC pools due to dissolved organic carbon release during decomposition by bacteria (Duan et al., 2018). While BOD had a relationship with RSC restoration, it did not have a significant relationship with any of the fluorescence indices, indicating that BOD and organic matter quality were not related in Campus Creek.

Suggestions For Future Work

Future research is necessary to fully understand the relationship between all water quality parameters, BOD, and RSC restoration. While this study provides insight into some of the relationships that exist, many questions remain in terms of the mechanisms behind them. It would be valuable to determine why SpC and C are related to BOD so that we can improve our methods of managing BOD loads in streams. It would also be useful for restoration designers to know why spatial patterns along the flow path exist so that future designs are more optimal for environmental health.

In terms of this study, improvements can be made to the design and datasets, which may allow for improved results. One change could be high-resolution sampling over a short period of time to collect data that accounts for more daily variations. For example, tracking water quality parameters continuously over 24 hours can collect data that includes diurnal fluctuations as well as storm events of various intensities. This study ensured data was collected at the same time of day each month, however, it was unable to account for the impact of episodic storm events that may alter the water chemistry from its usual conditions for a given month. This could increase the accuracy of temporal trends and relationships between parameters.

Increasing the number of sampling sites could also benefit this study. Only one stream with RSC restoration was sampled in this study, potentially providing biased results based on local environmental factors. This study included sites at multiple streams that were not restored with RSCs, but all of the data for RSC sites came from one section of Campus Creek. Increasing the variety of RSC sites could improve this dataset's accuracy and strengthen the relationships found with BOD, organic matter quality, and RSC restoration.

Finally, it would be interesting to determine the impact RSCs have on heavy metals and to determine if heavy metals could be impacting BOD loads. Studying heavy metal species longitudinally along Campus Creek would be valuable for determining if RSCs can be effective in retaining them and potentially locating point sources of pollutants that could be entering various portions of the stream. Correlating heavy metals with BOD, RSCs, and organic matter quality could improve our understanding of the mechanisms causing changes and help us develop a more complete understanding of RSCs impact on water quality.

Conclusions and Broader Implications

Stream restoration has benefits that can improve nutrient retention and stormwater management. This study found that RSC restoration can impact BOD levels, altering the amount of DO available for organisms in a stream's ecosystem. This supports my second alternative hypothesis that there is a trend in BOD along the flow path of Campus Creek due to RSC restoration. This study also found that RSCs had an impact on the amount and type of organic material present along the flow path of Campus Creek. These findings allow me to reject my null hypothesis and accept my third alternative hypothesis that both BOD and organic matter quality have a trend along the flow path of Campus Creek due to RSC restoration. It was also determined that RSC restoration impacted DO, C, and pH, which further supports my third alternative hypothesis by indicating RSCs affect multiple water quality parameters.

Additionally, other water quality parameters impact BOD. This study found that temperature, DO, SpC, C, TDS, salinity, and ORP all impact BOD levels along the flow path of Campus Creek. Monitoring and limiting all parameters that impact BOD is essential for ensuring stream restoration strategies are effective at improving water quality. With the state of Maryland investing millions of dollars to restore one section of Campus Creek, we must continue to improve our understanding of the potential impacts of RSCs on water quality. This information will become increasingly important as investments in urban stream restoration continue to be made across the United States.

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Code of Conduct

I pledge on my honor that I have not received any unauthorized assistance on this assignment.

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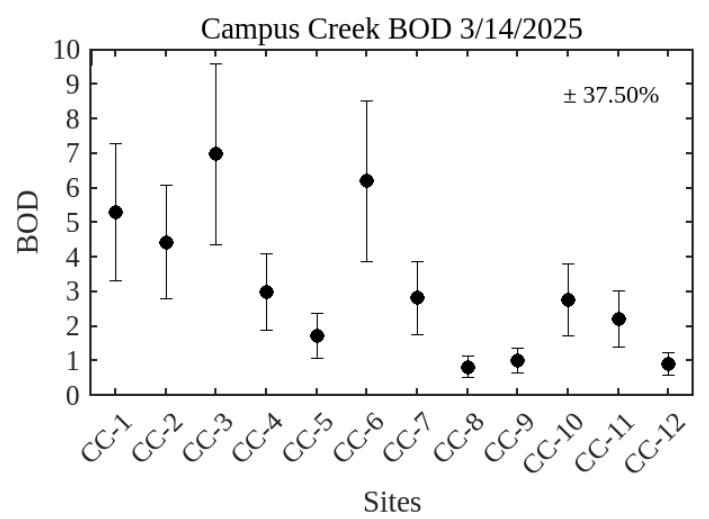
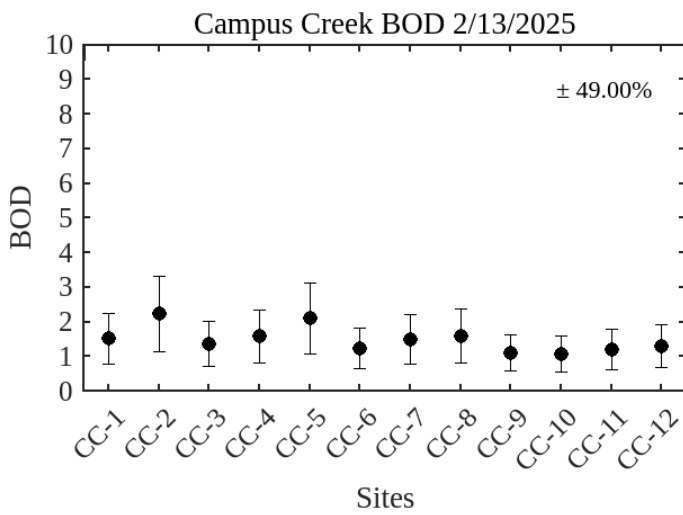
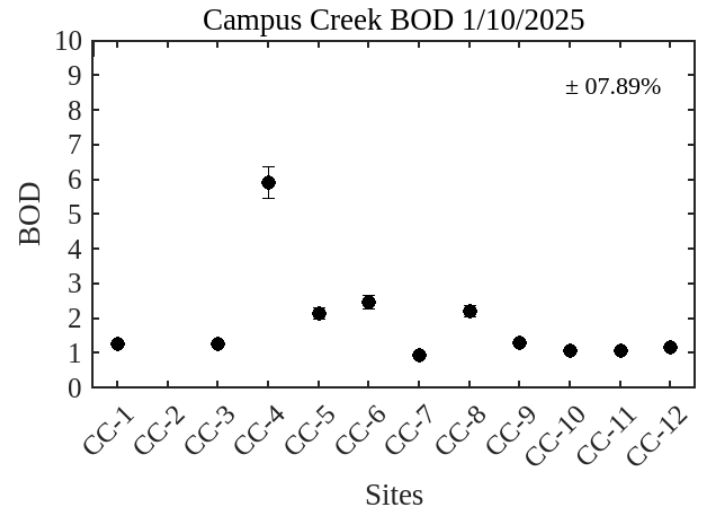
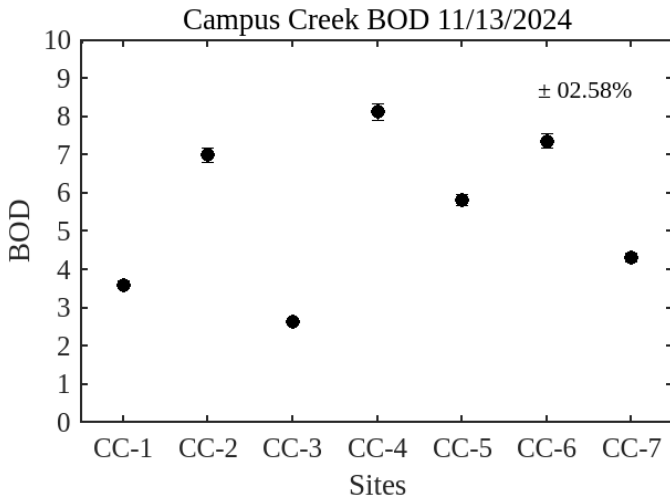
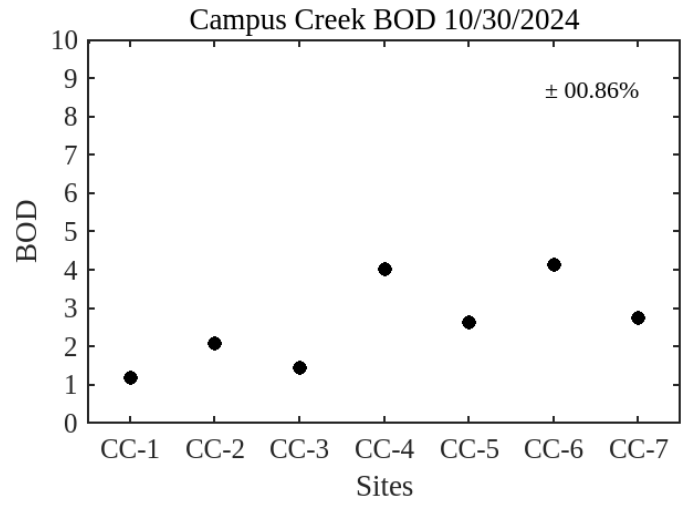
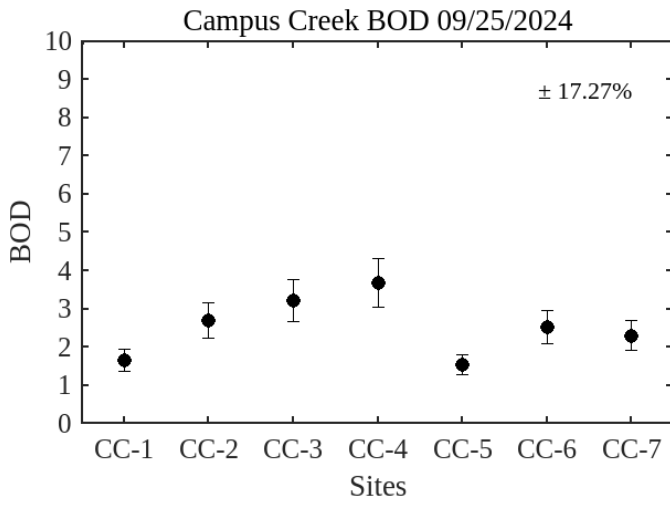
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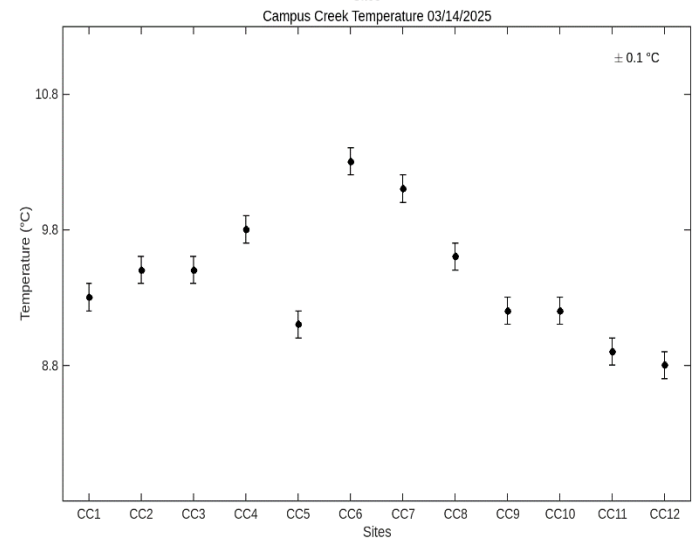
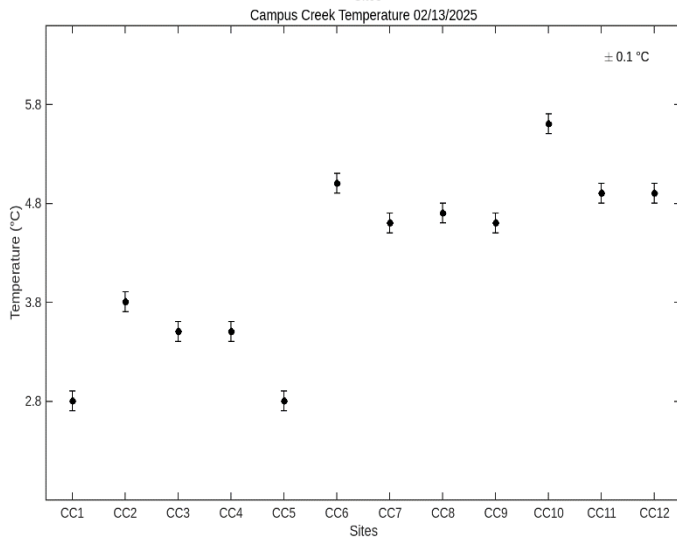
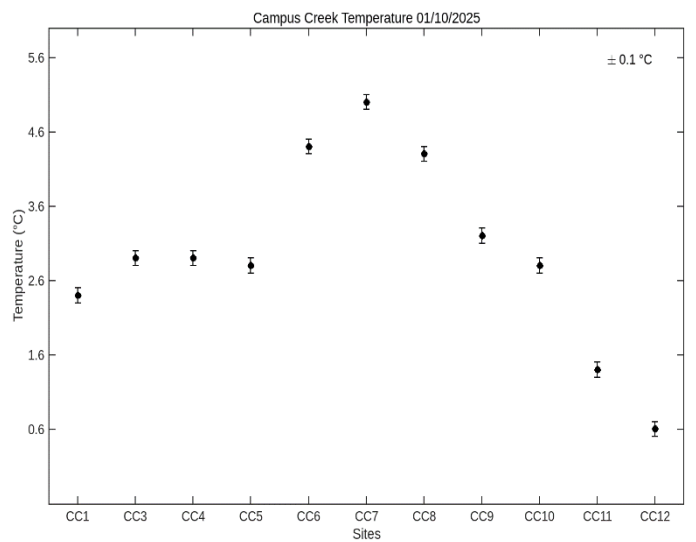
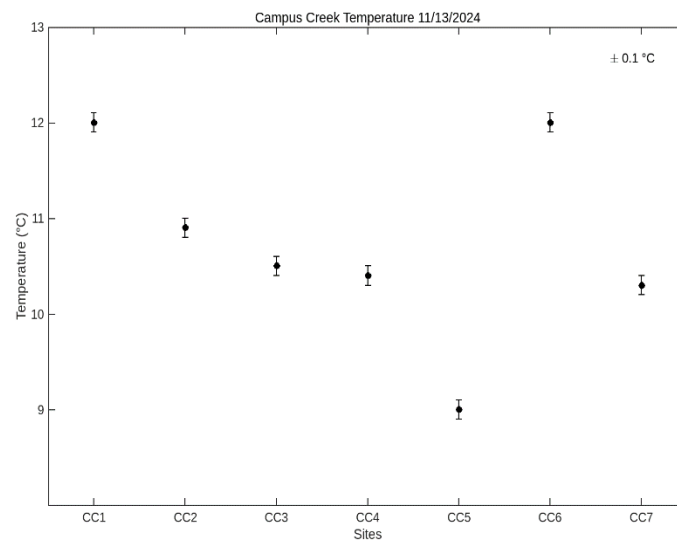
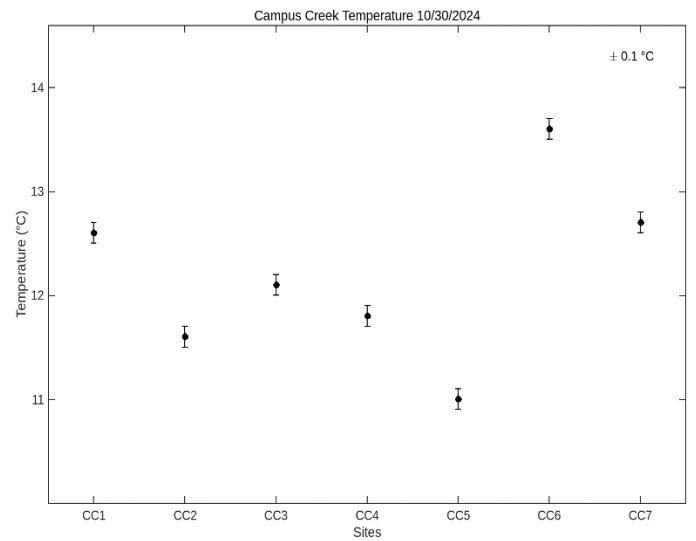
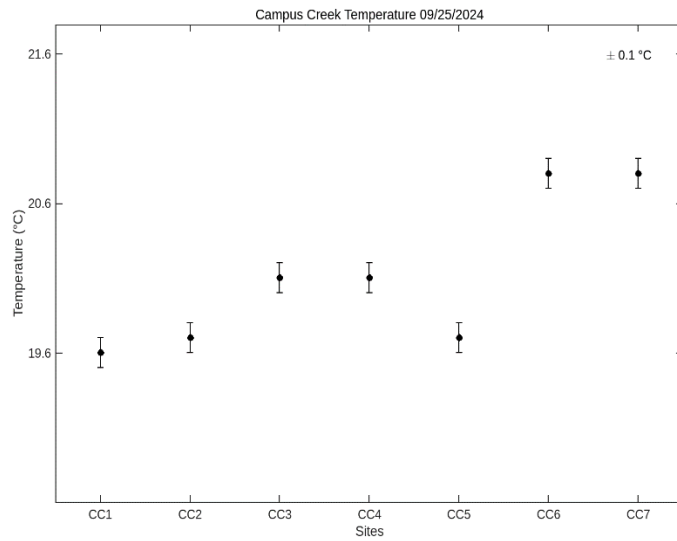
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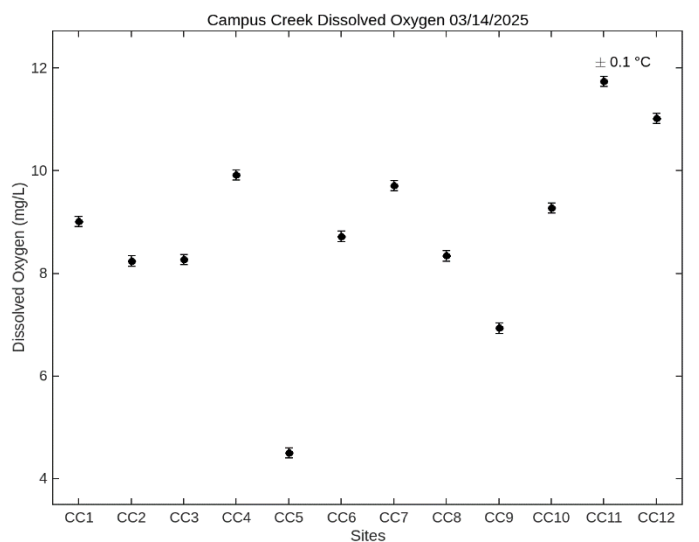
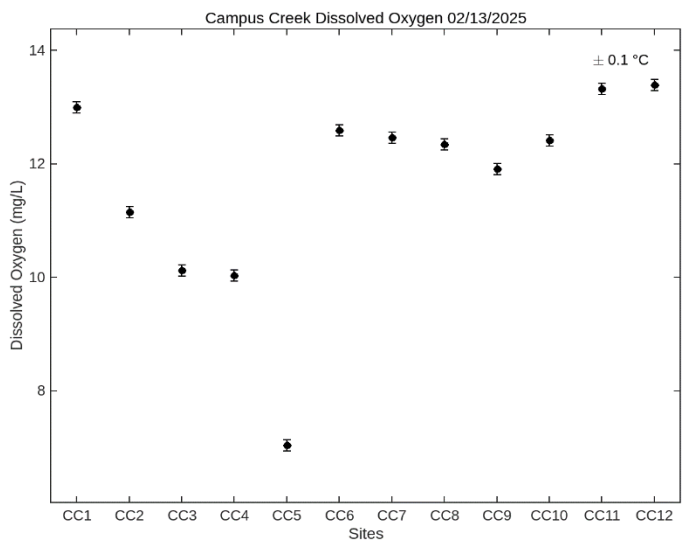
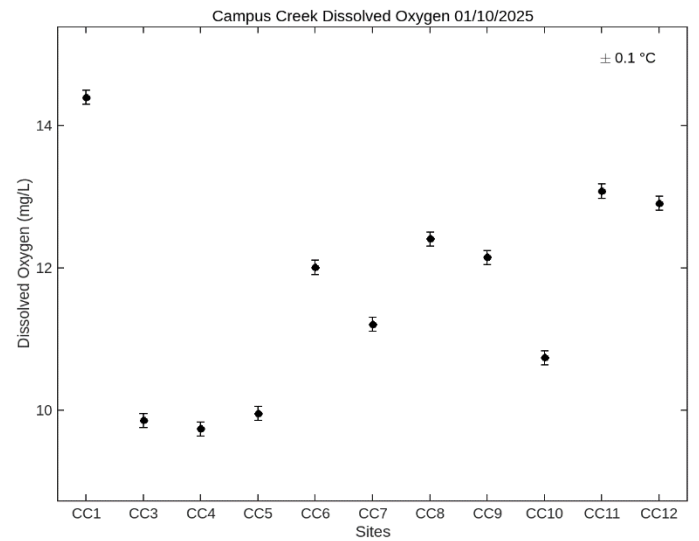
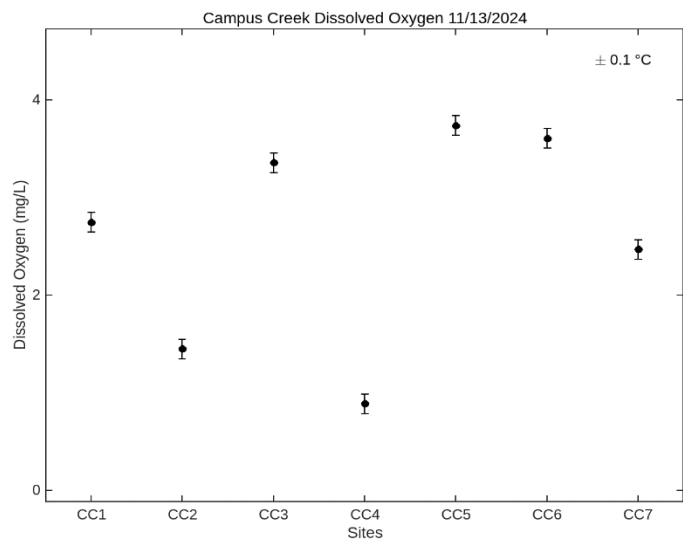
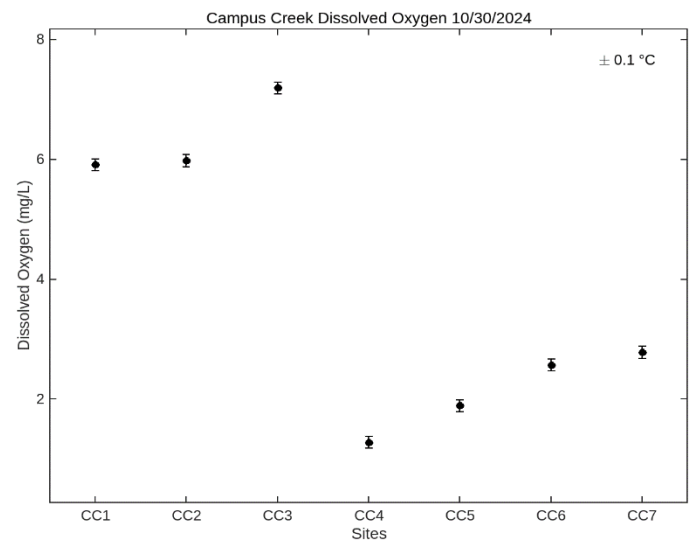
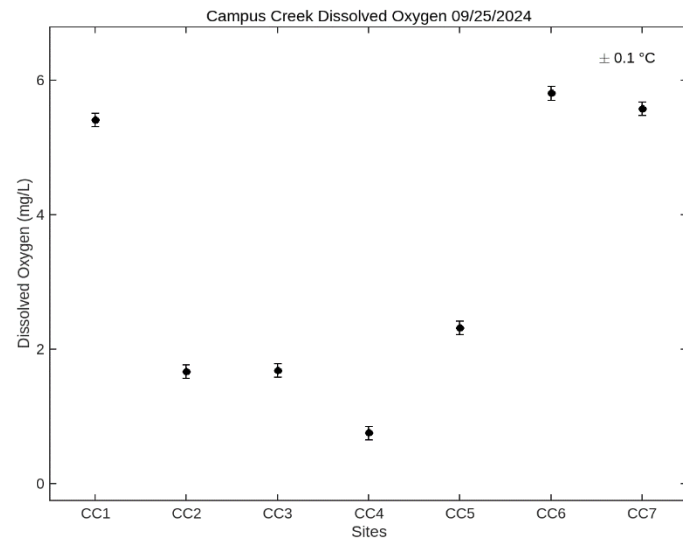
Appendix 1: Campus Creek Biochemical Oxygen Demand



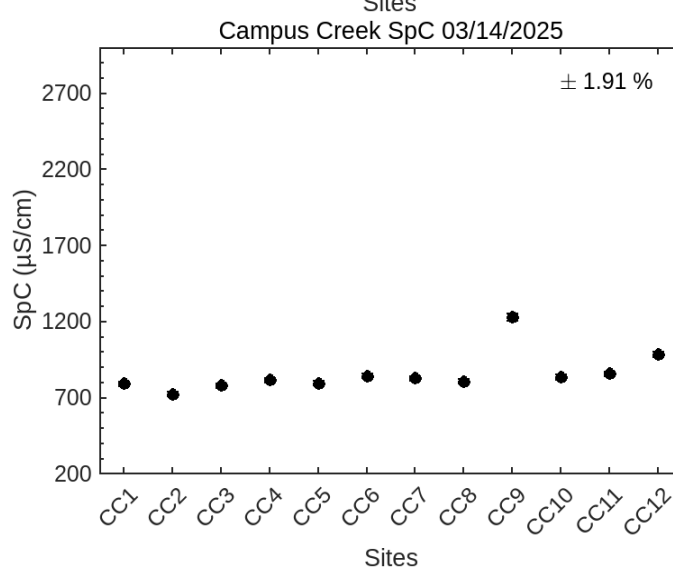
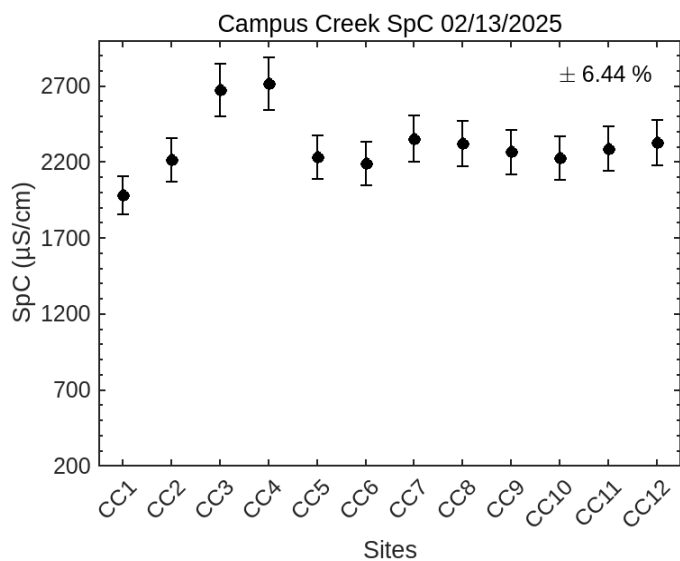
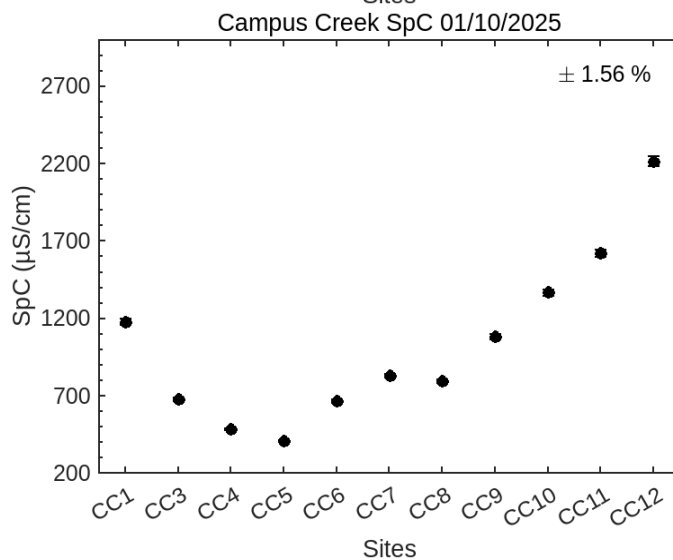
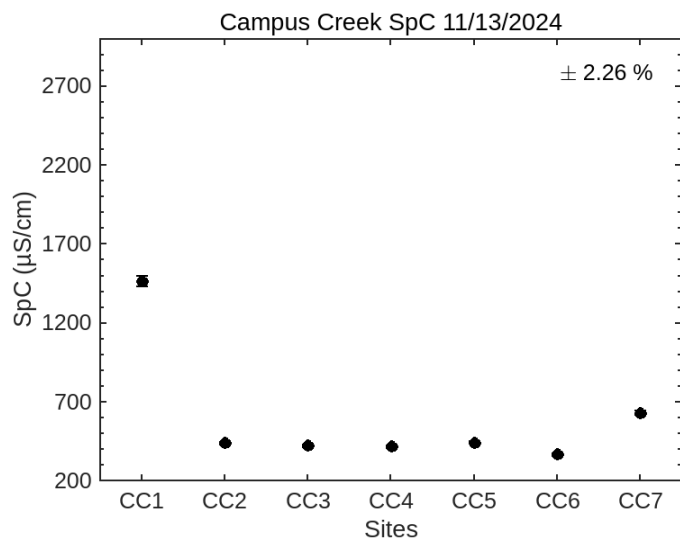
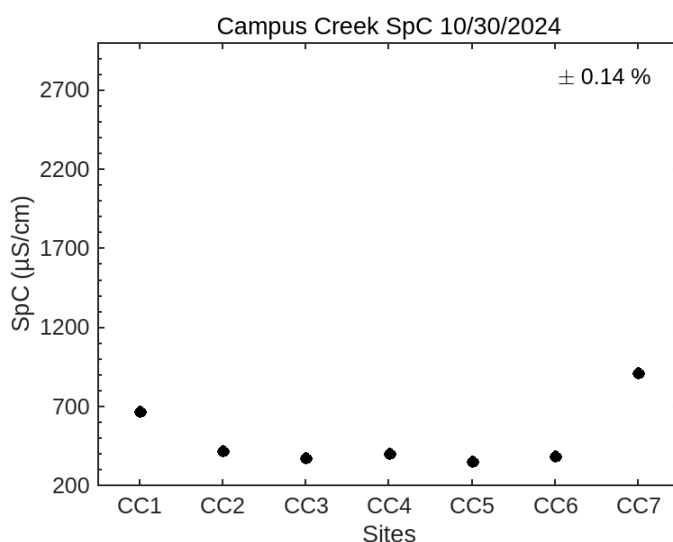
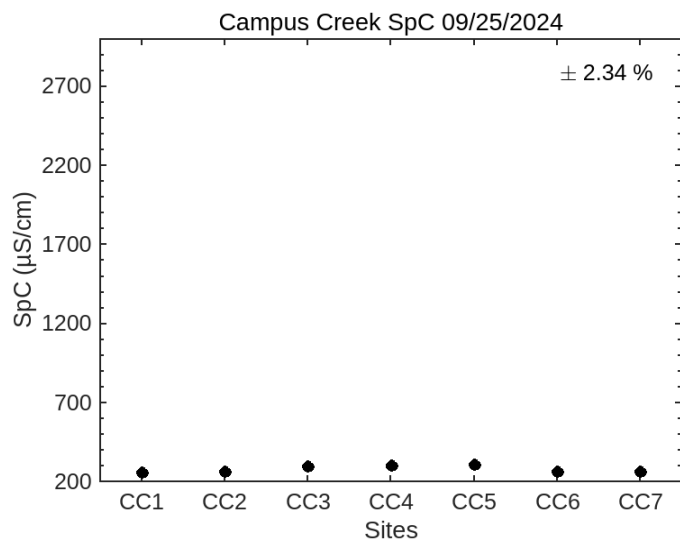
Appendix 2: Campus Creek Temperature



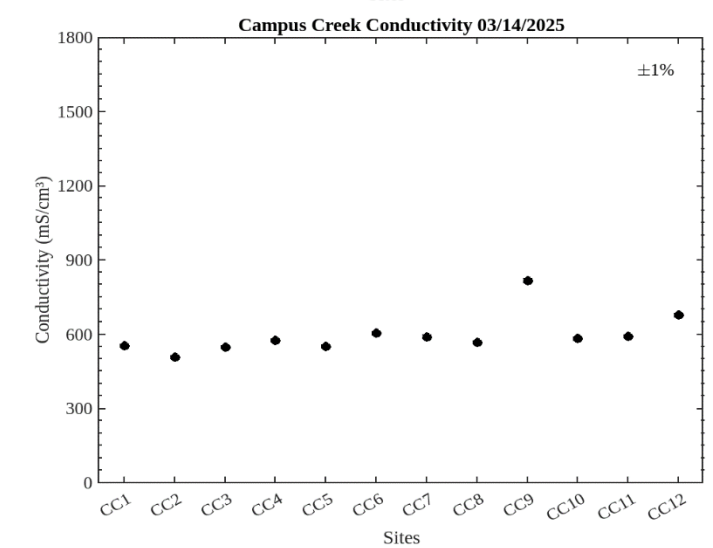
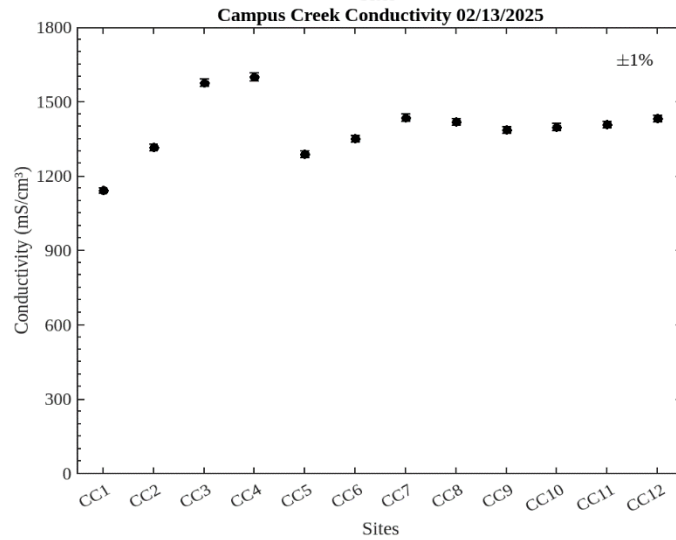
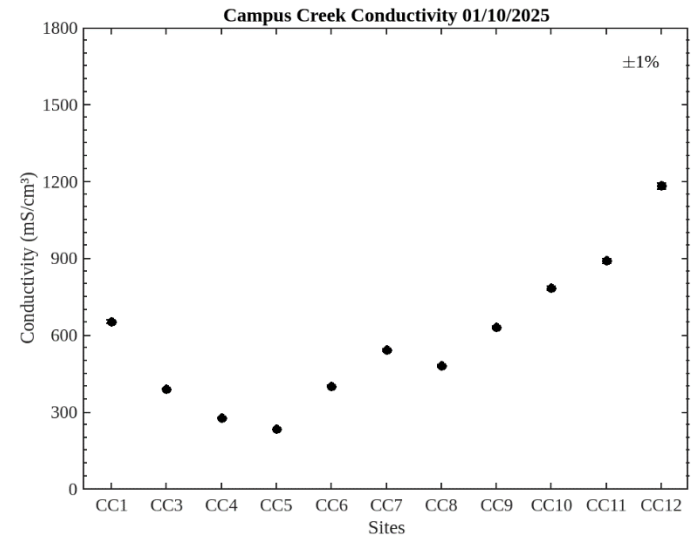
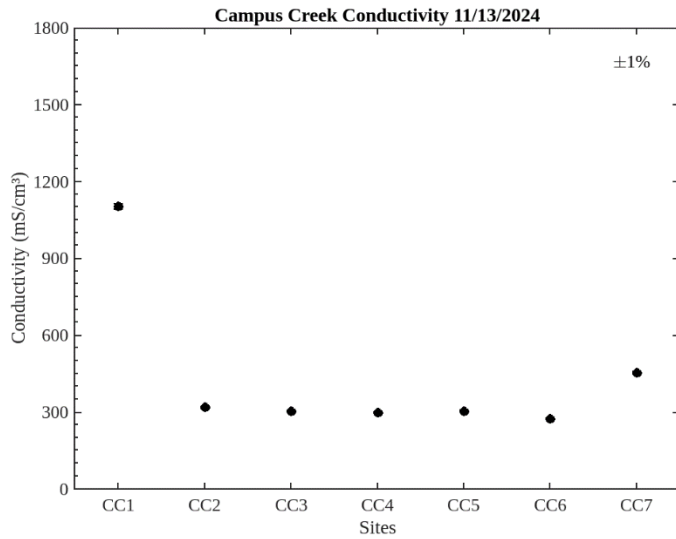
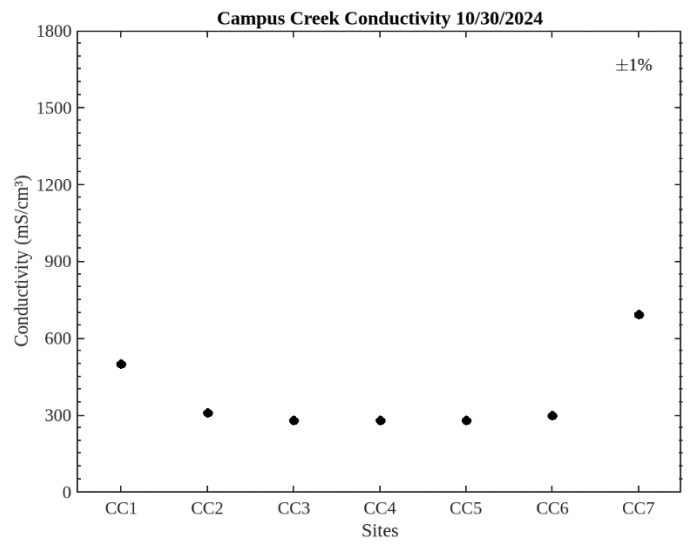
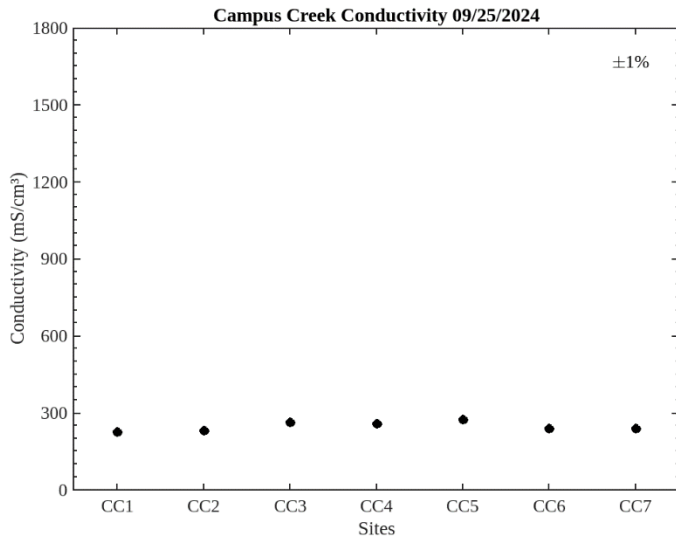
Appendix 3: Campus Creek Dissolved Oxygen



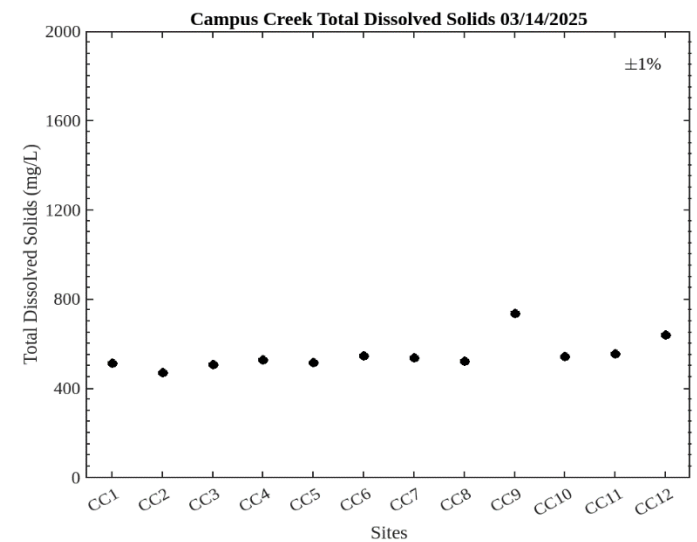
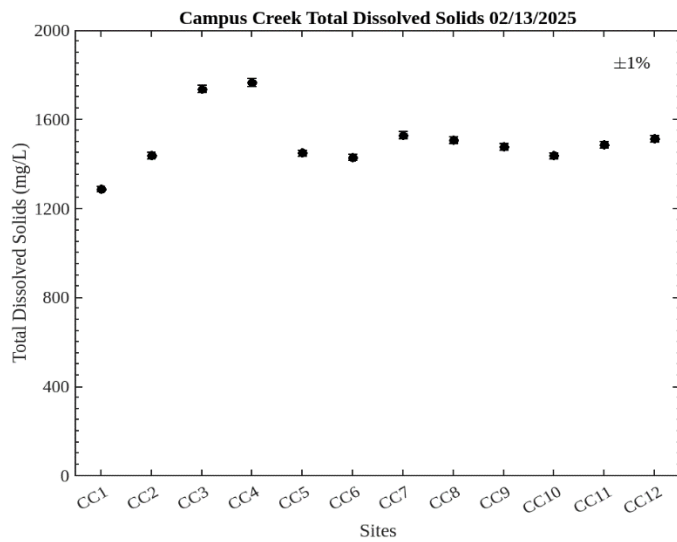
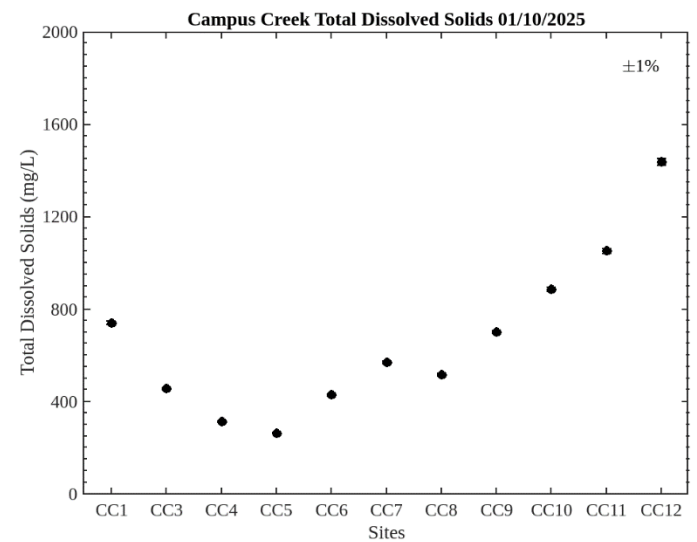
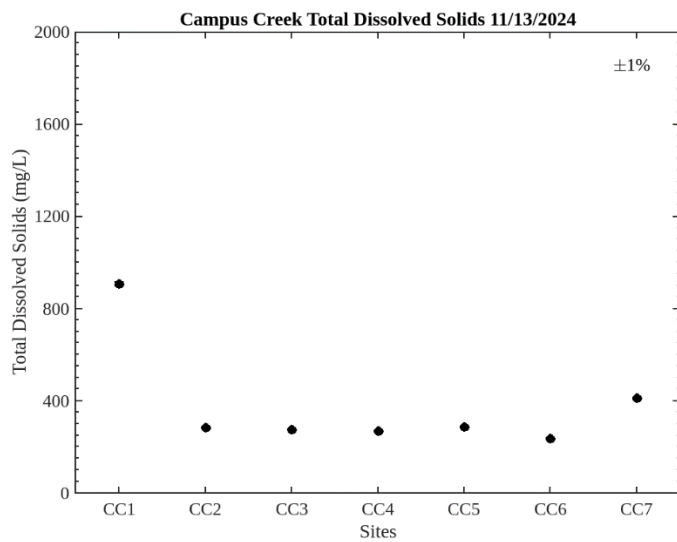
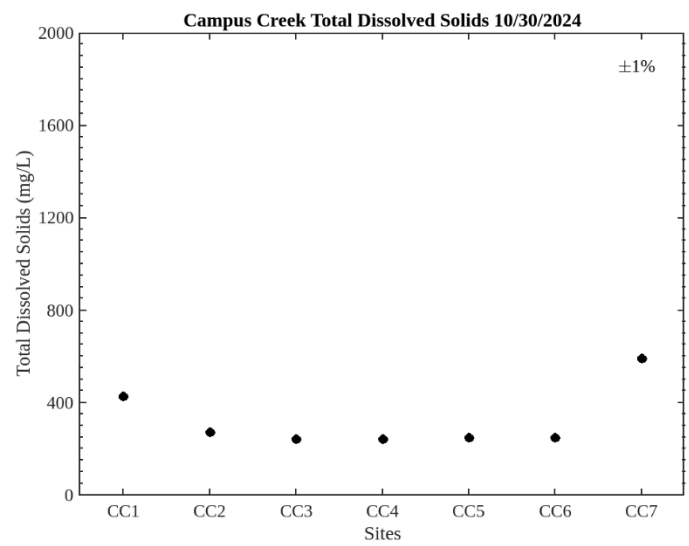
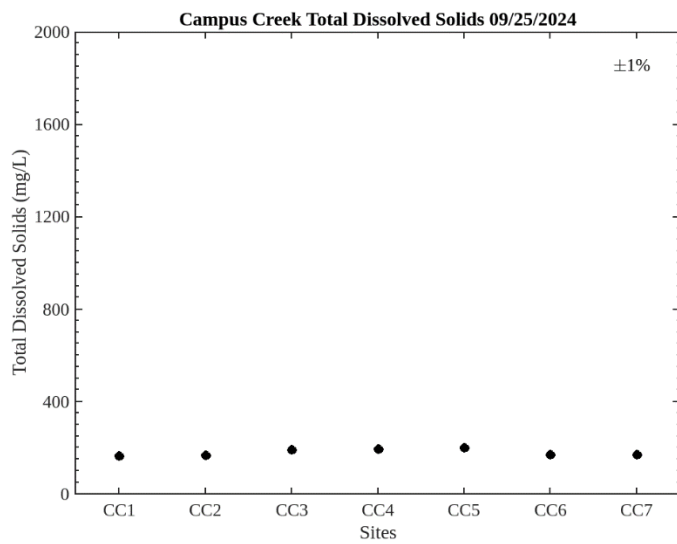
Appendix 4: Campus Creek Specific Conductance



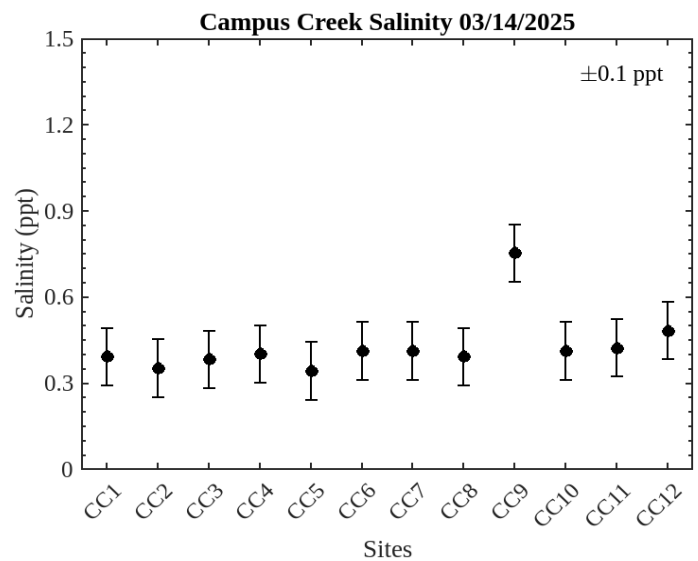
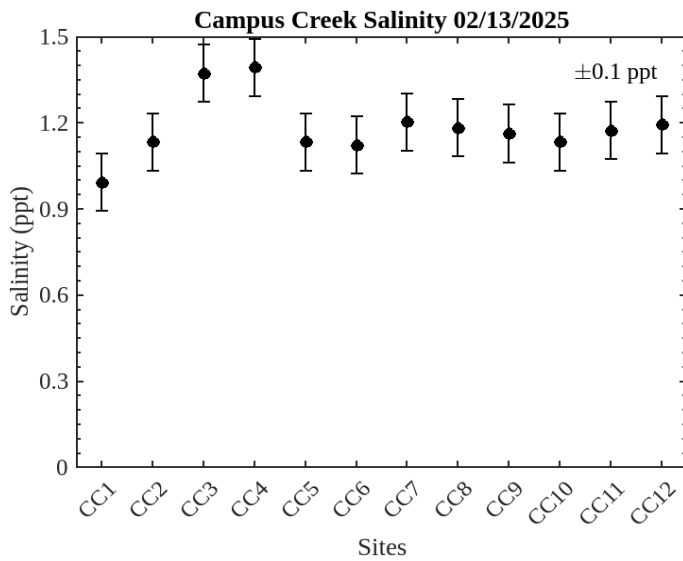
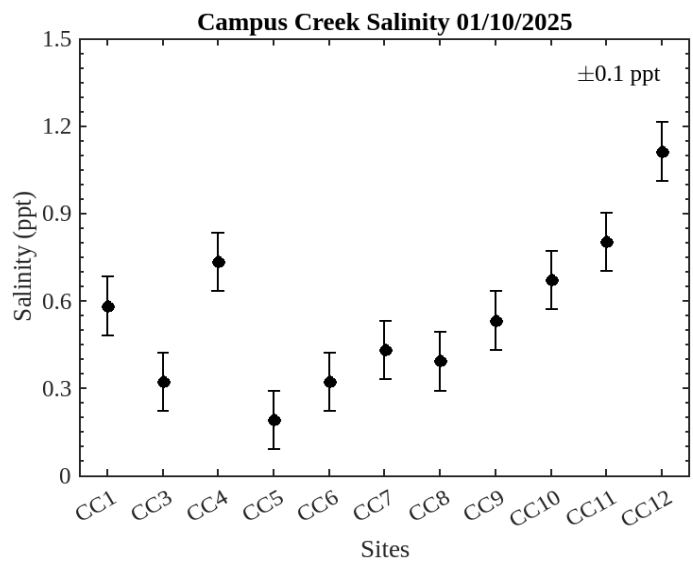
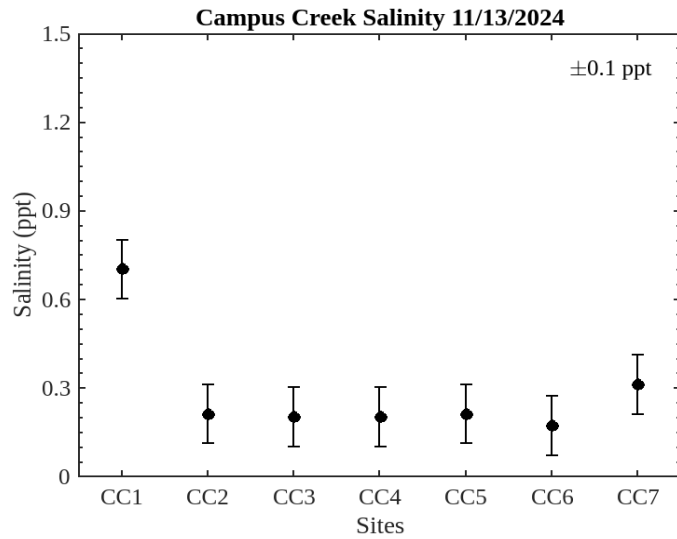
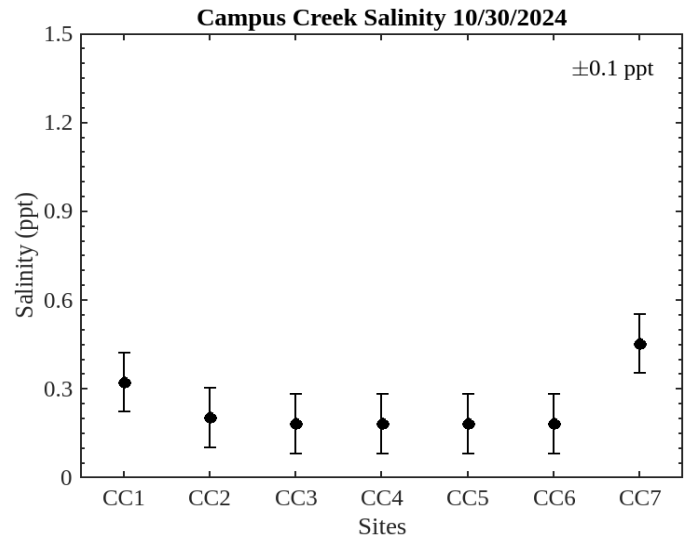
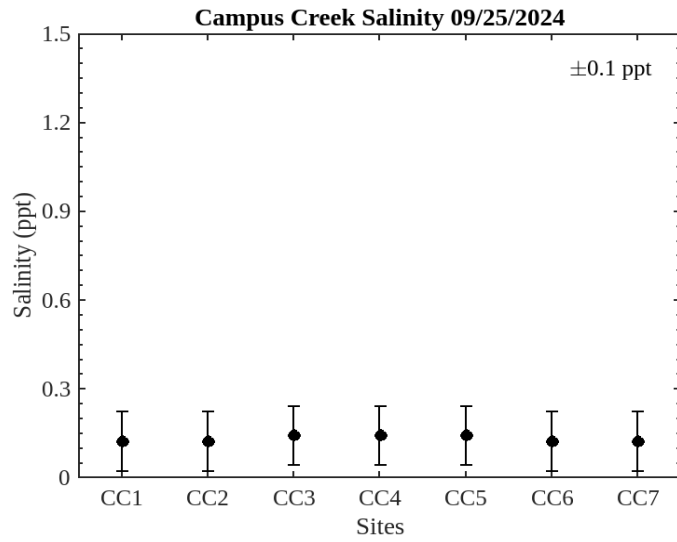
Appendix 5: Campus Creek Conductivity



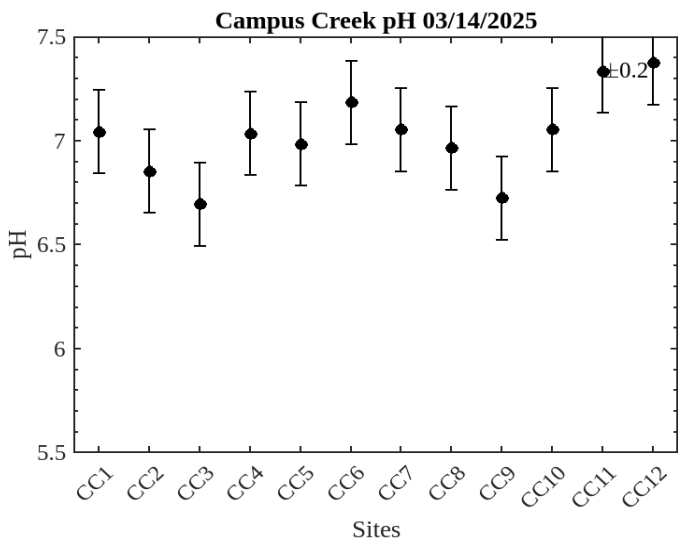
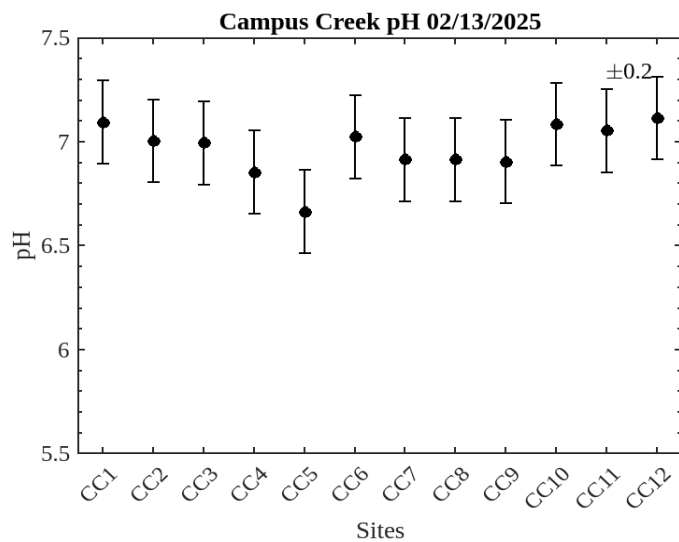
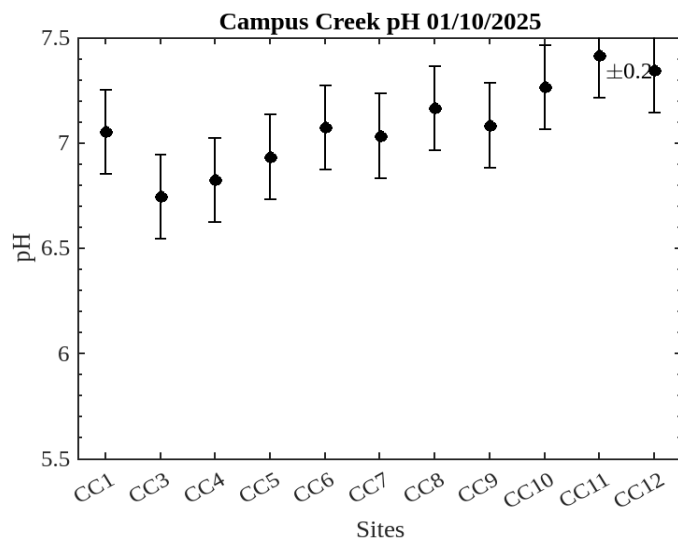
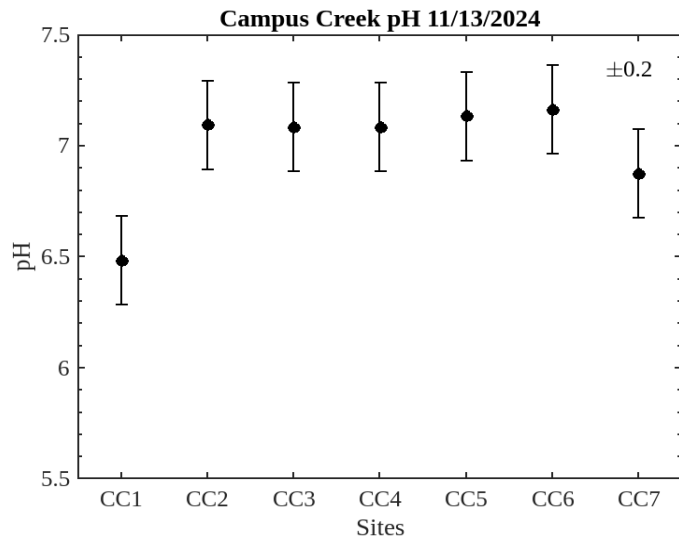
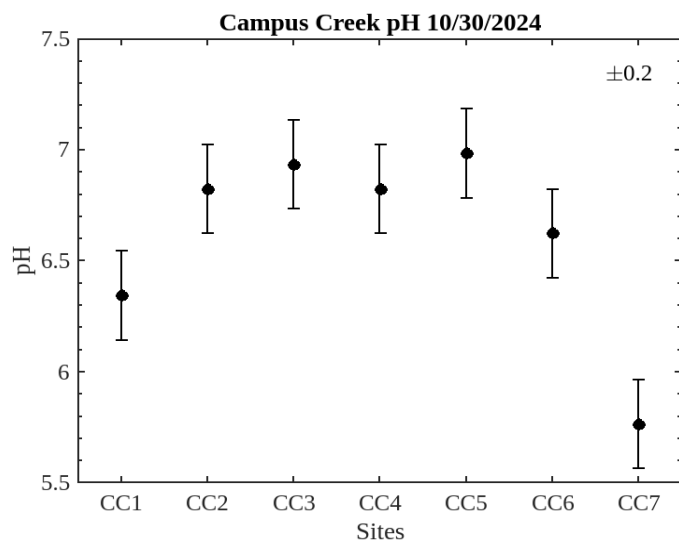
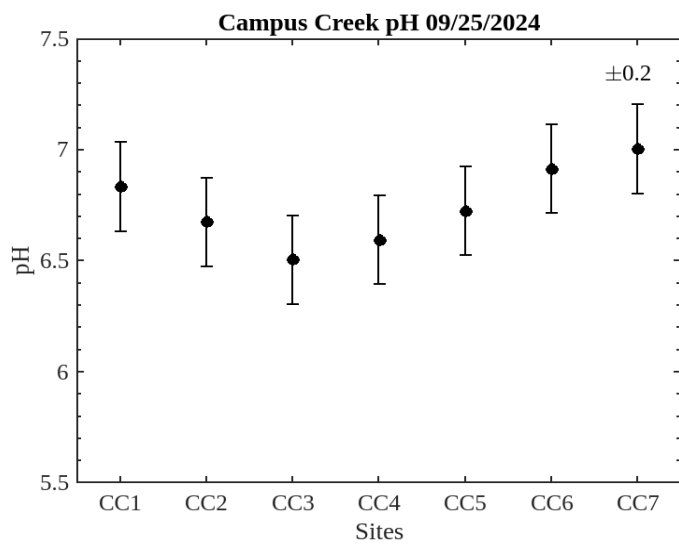
Appendix 6: Campus Creek Total Dissolved Solids



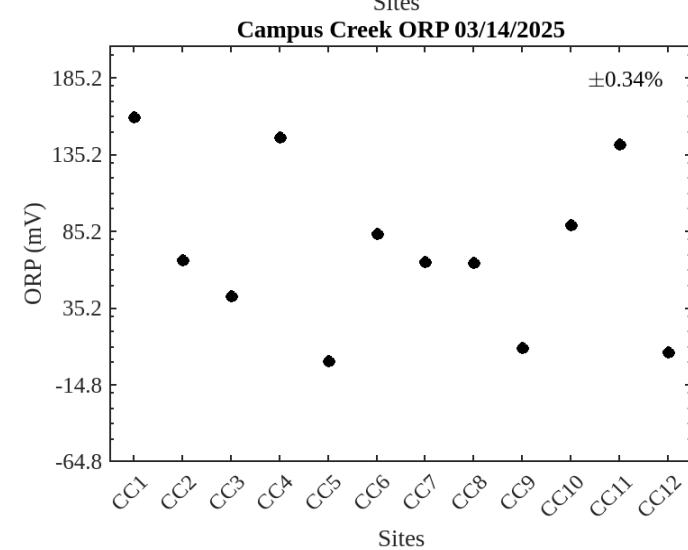
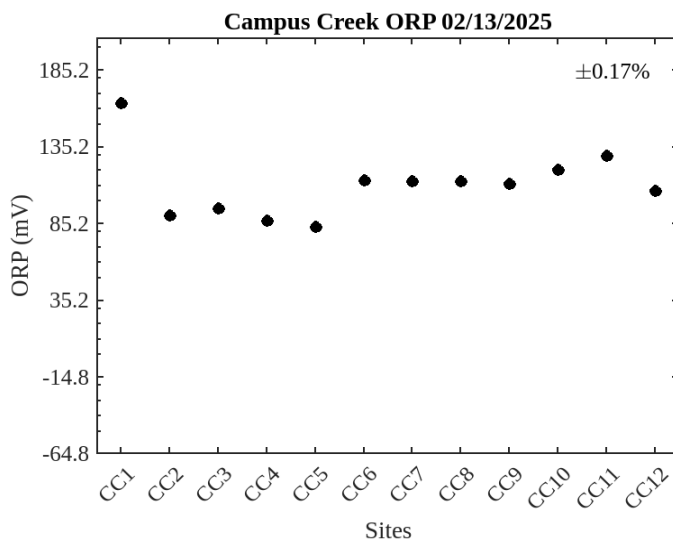
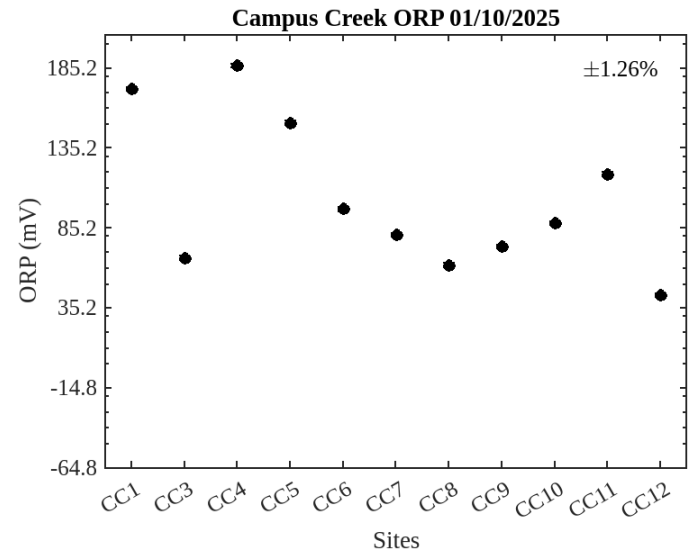
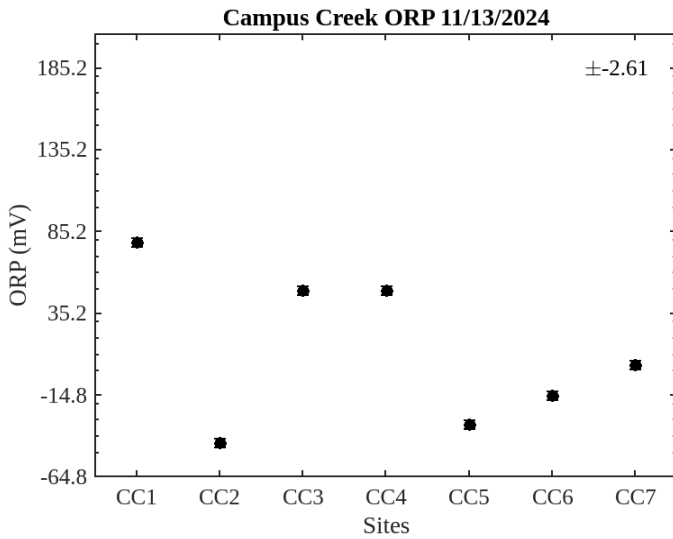
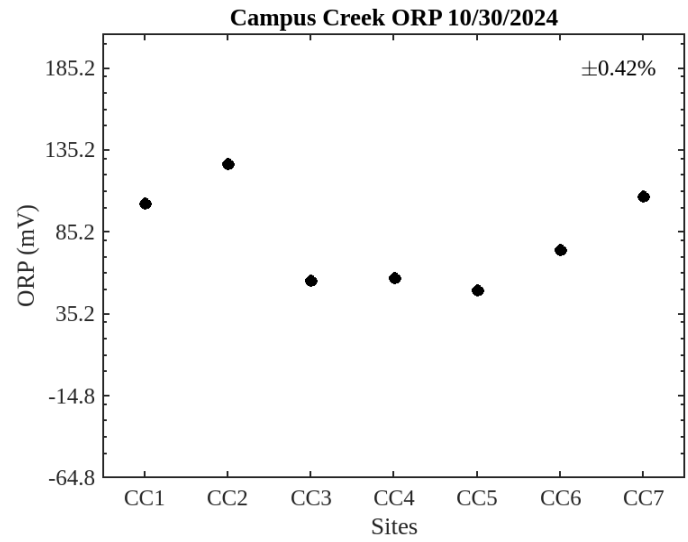
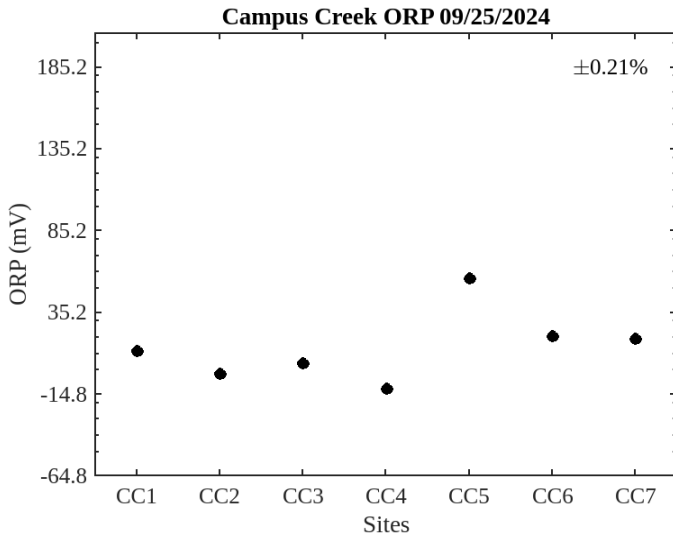
Appendix 7: Campus Creek Salinity



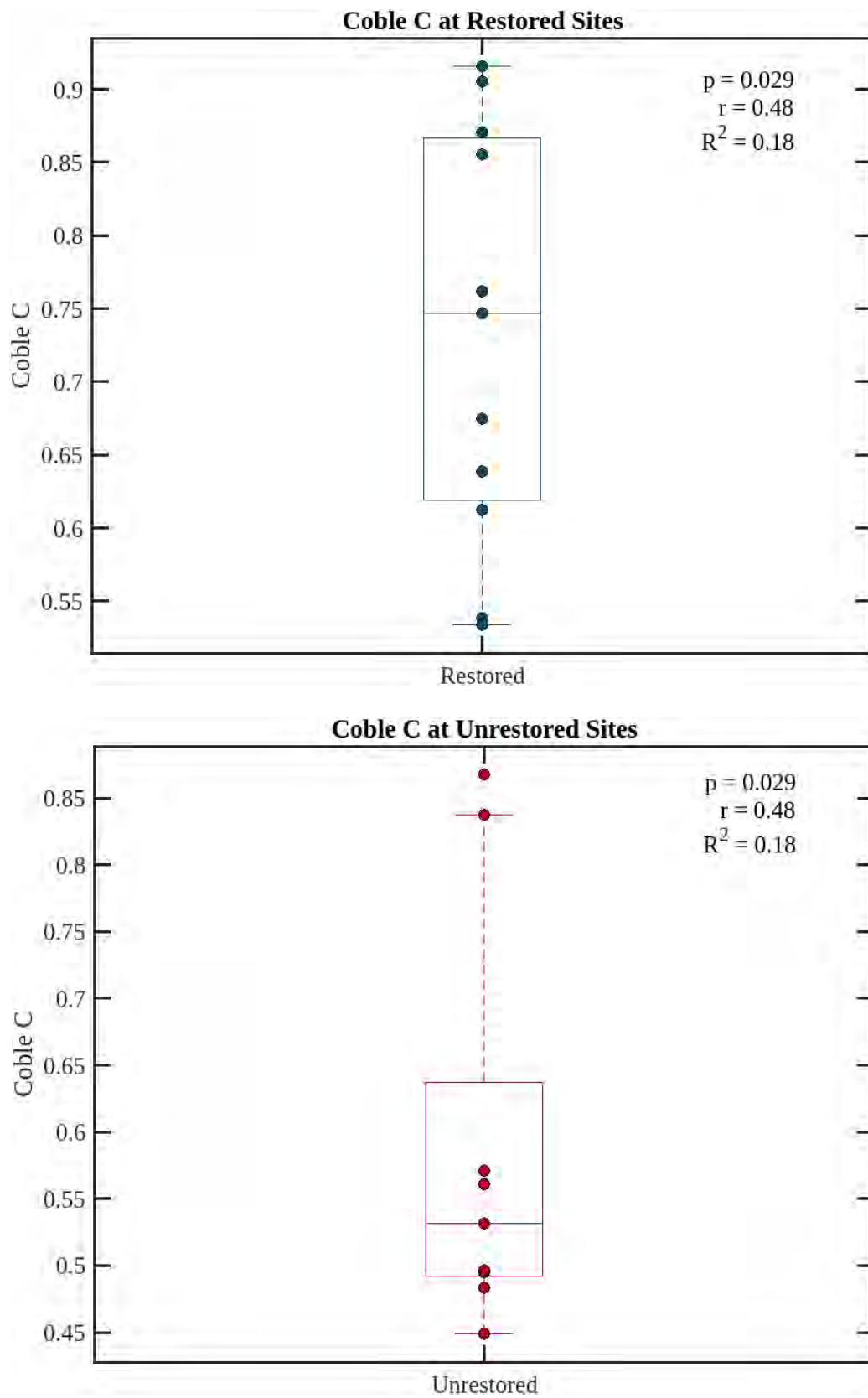
Appendix 8: Campus Creek pH



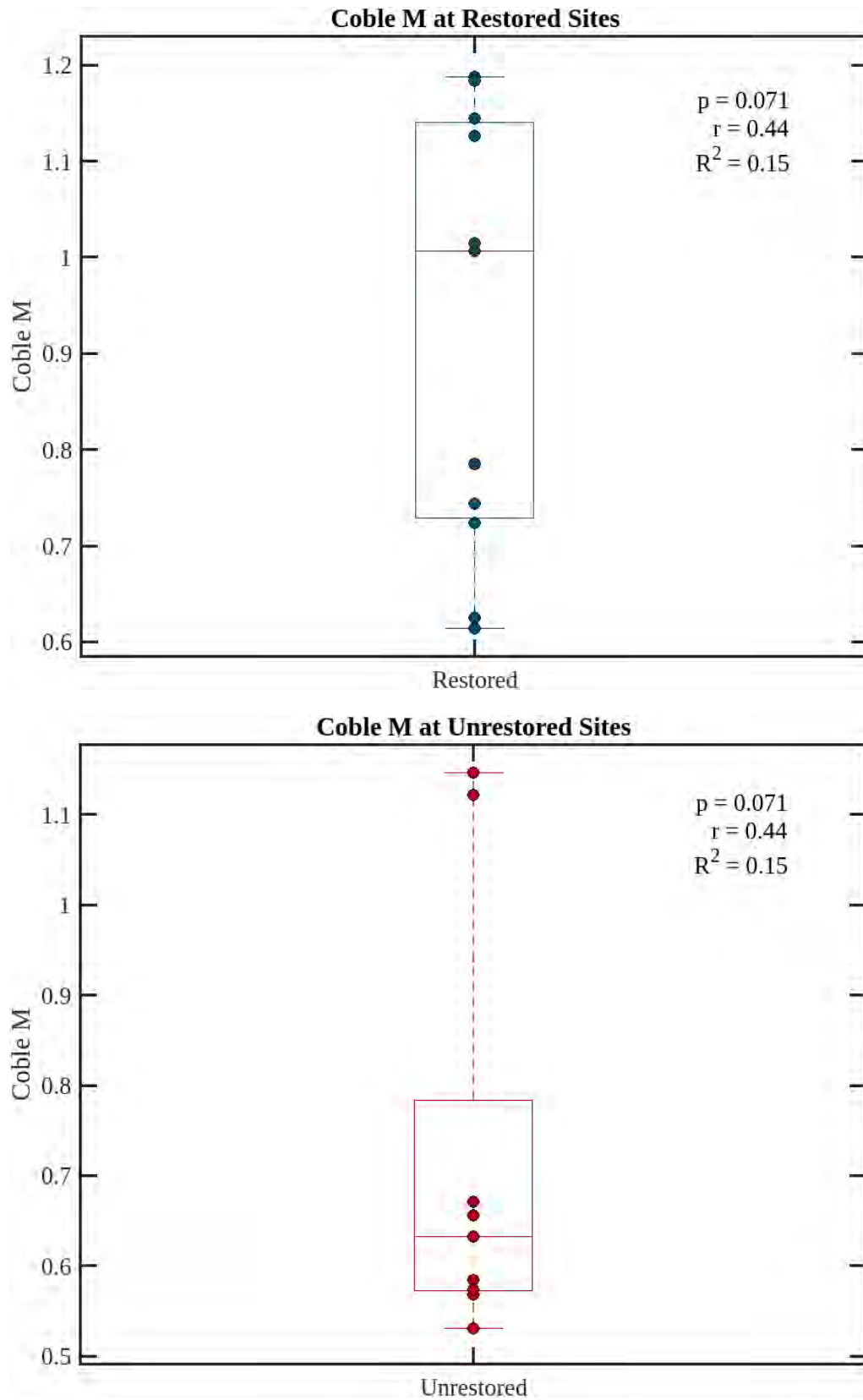
Appendix 9: Campus Creek Oxidation-Reduction Potential



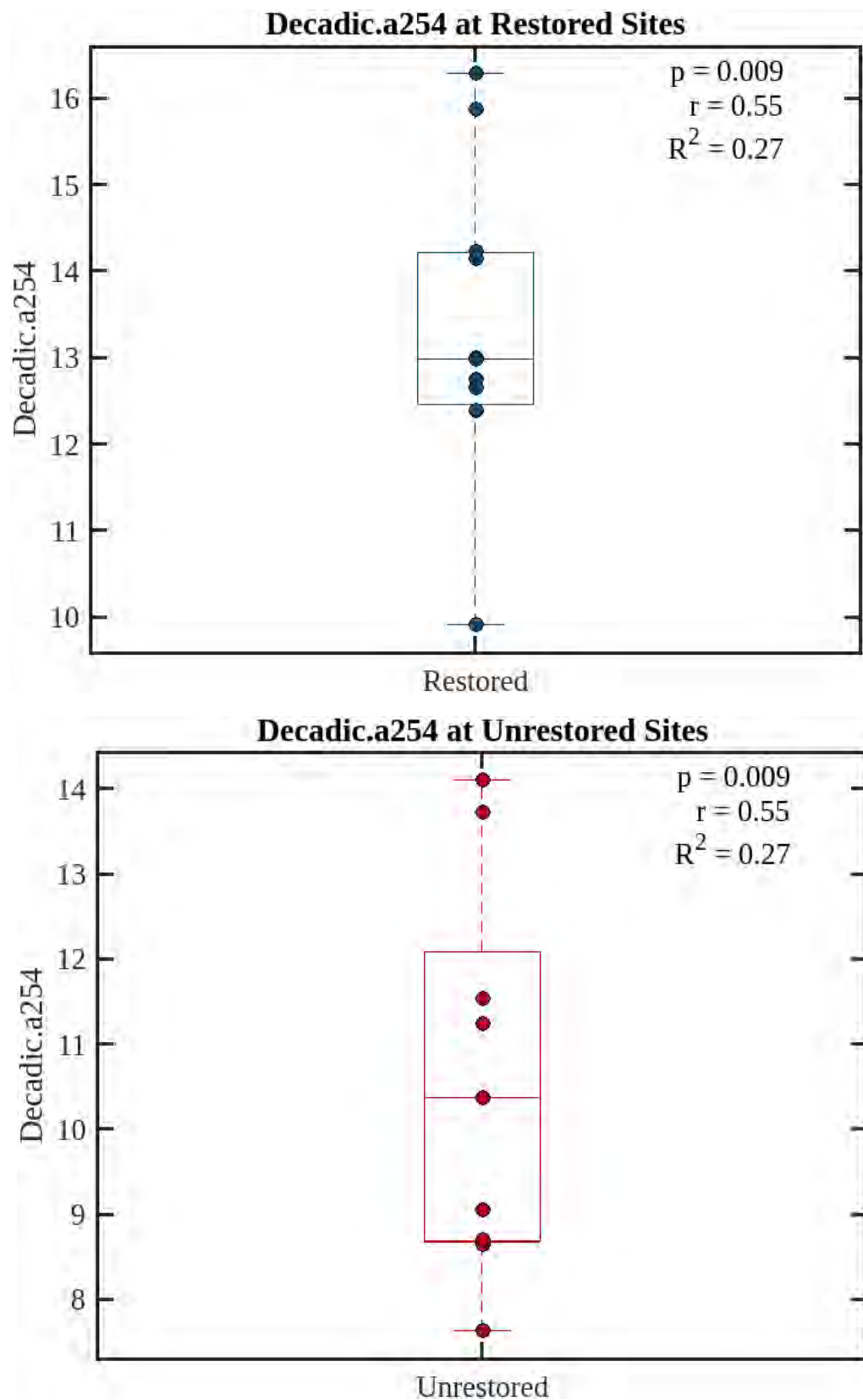
Appendix 10: Coble C Boxplot at Restored and Unrestored Campus Creek Sites



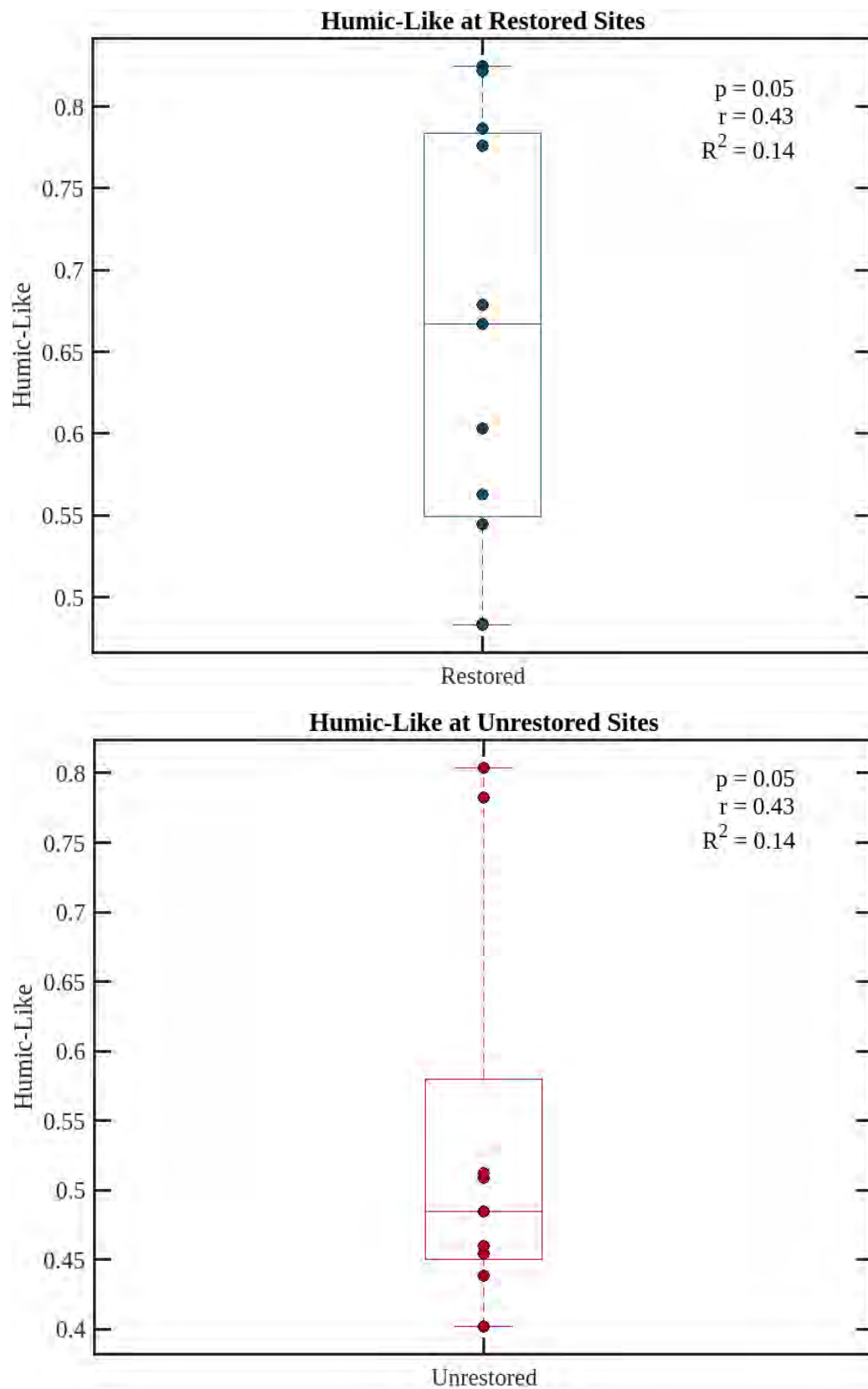
Appendix 11: Coble M Boxplot at RSC Restored and Unrestored Campus Creek Sites



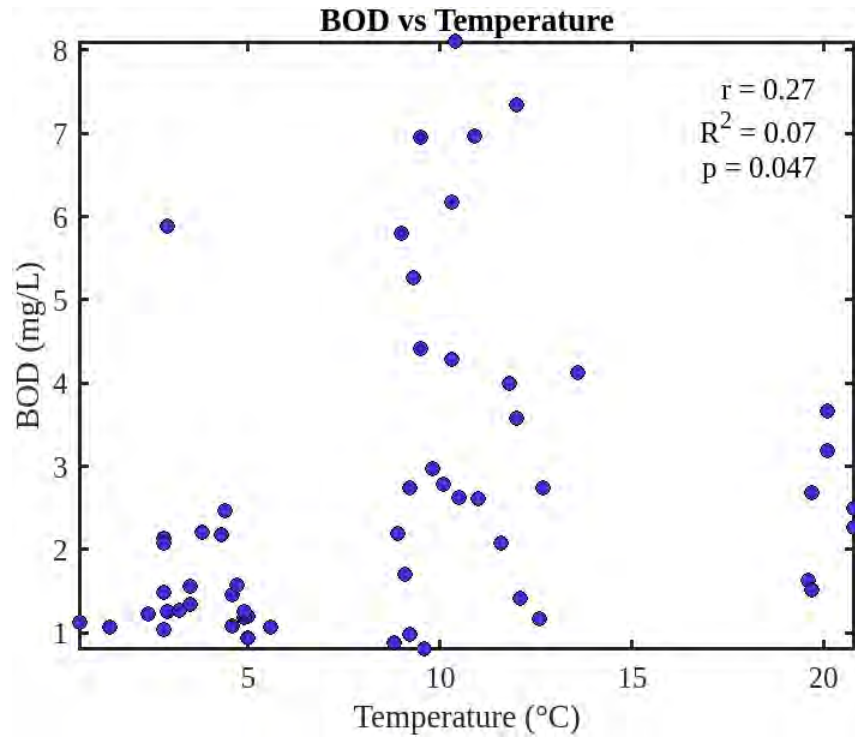
Appendix 12: Decadic.a254 Boxplot at RSC Restored and Unrestored Campus Creek Sites



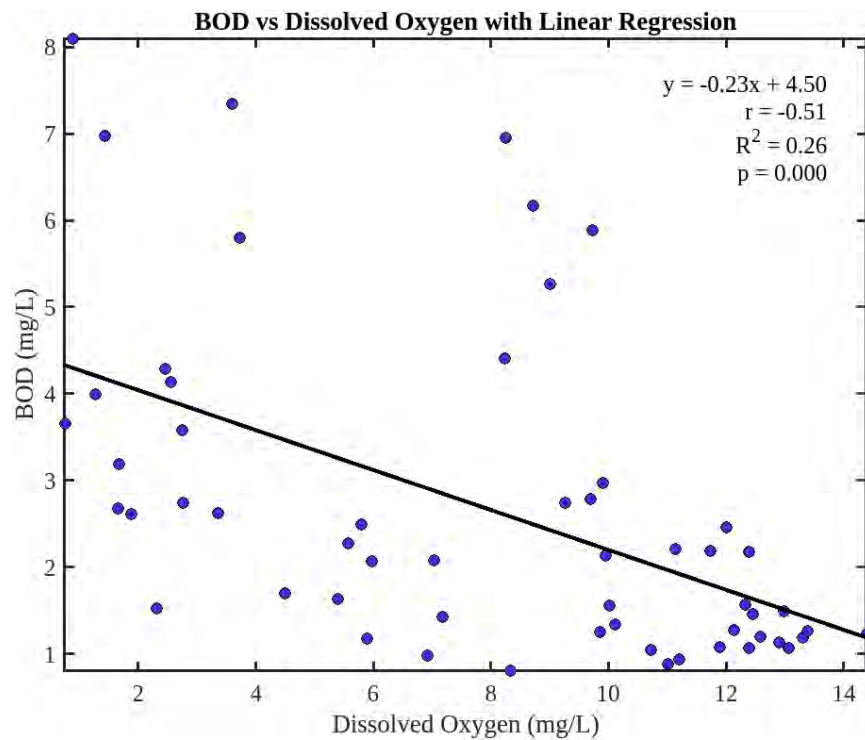
Appendix 13: Humic-like Boxplot at RSC Restored and Unrestored Campus Creek Sites



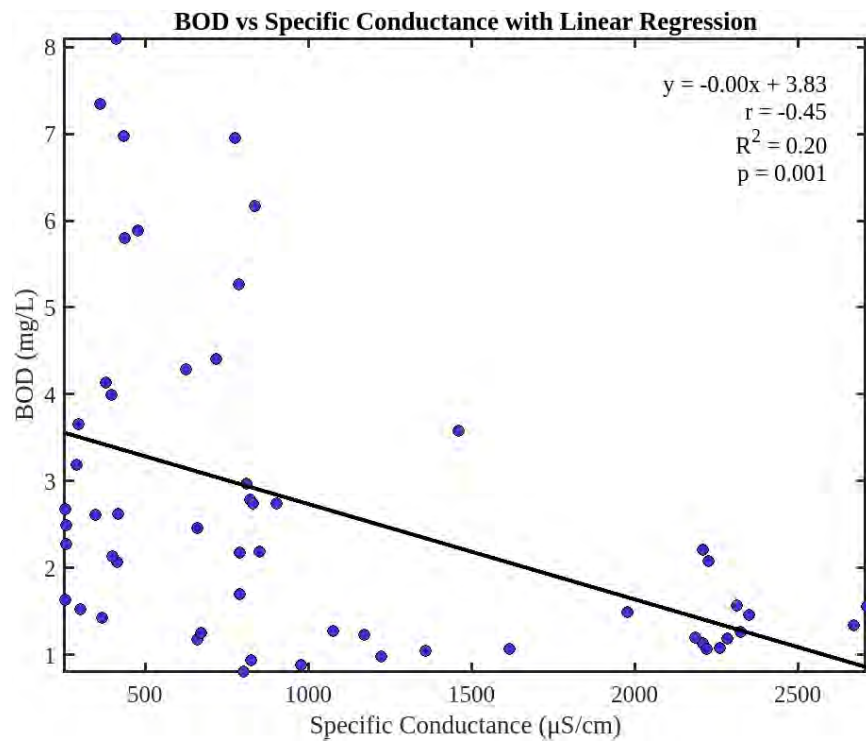
Appendix 14: Temperature vs BOD Linear Regression



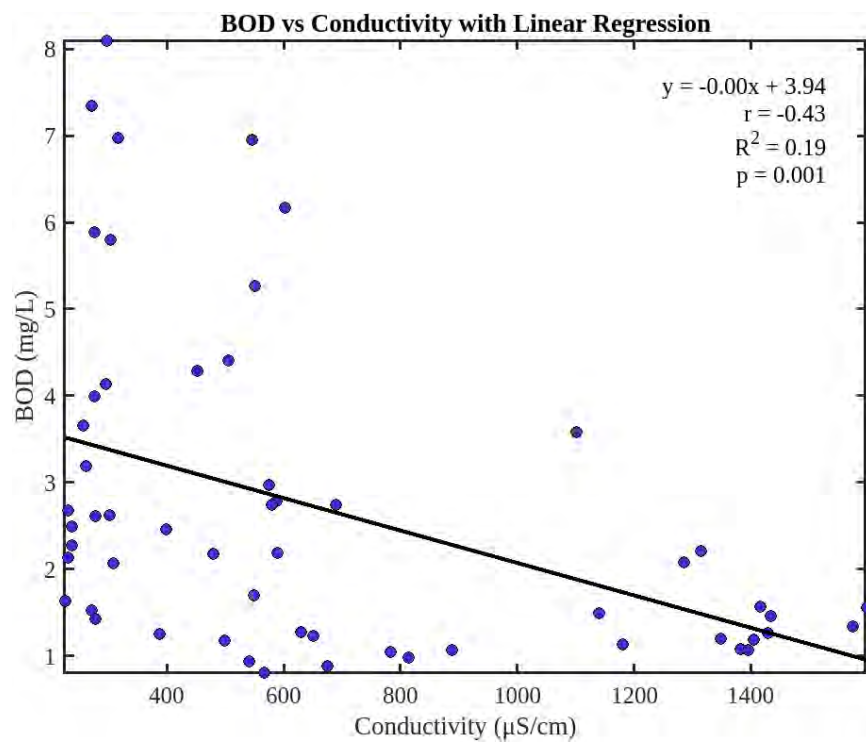
Appendix 15: Dissolved Oxygen vs BOD Linear Regression



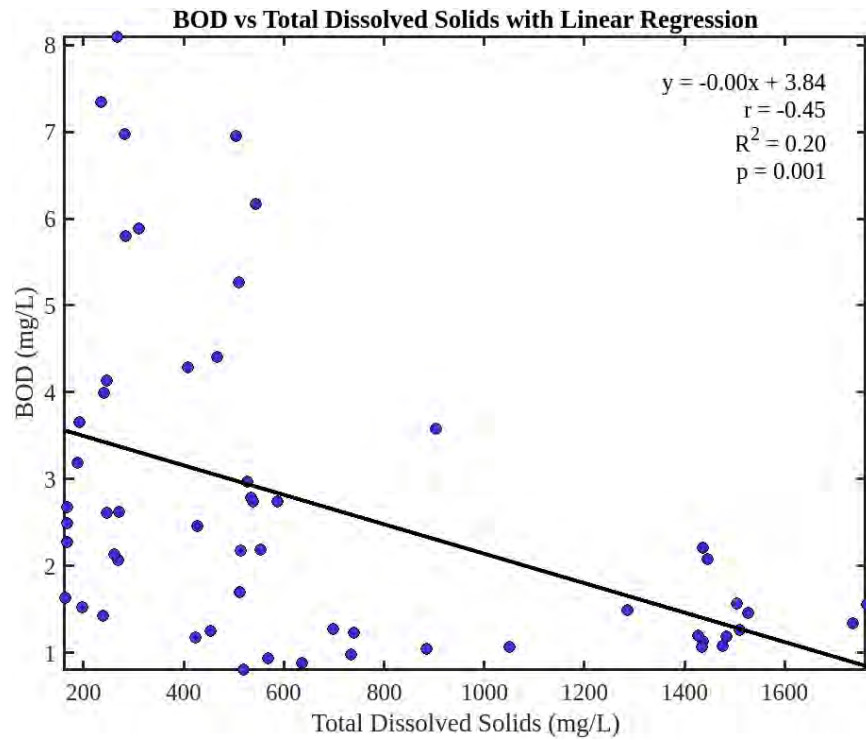
Appendix 16: Specific Conductance vs BOD Linear Regression



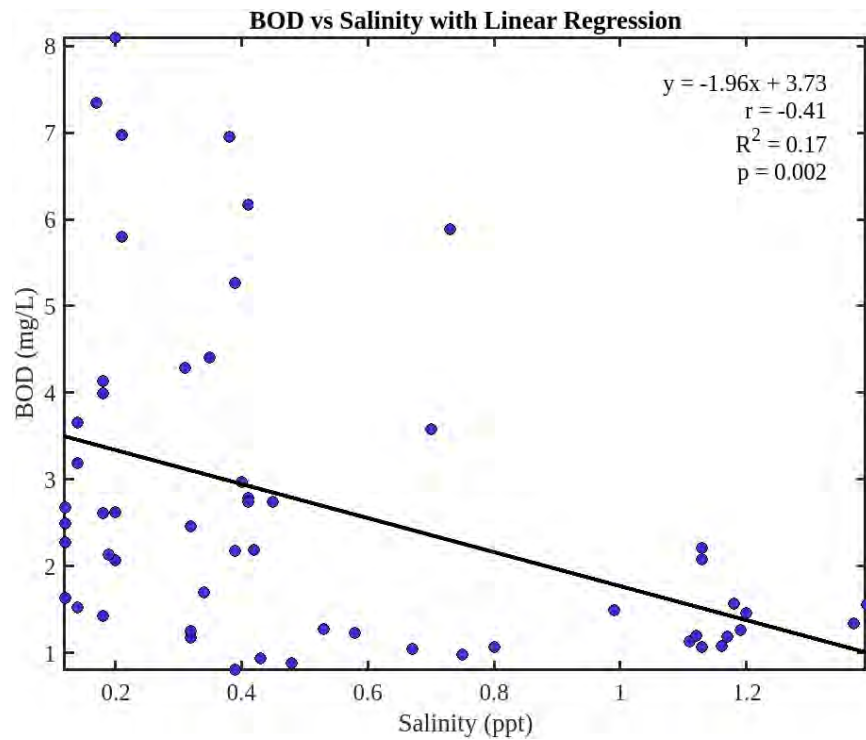
Appendix 17: Conductivity vs BOD Linear Regression



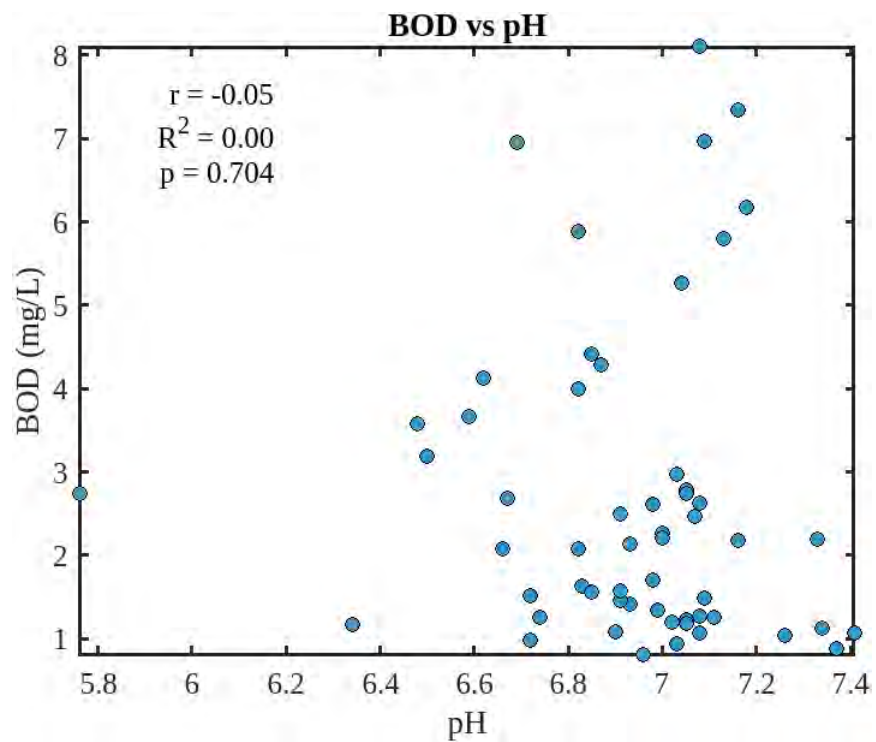
Appendix 18: Total Dissolved Solids vs BOD Linear Regression



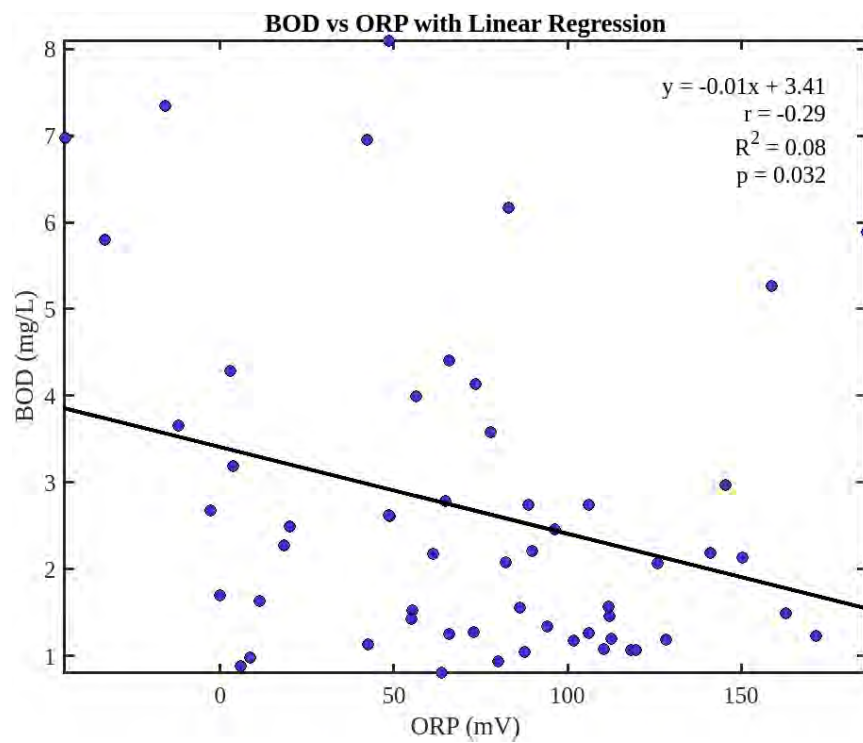
Appendix 19: Salinity vs BOD Linear Regression



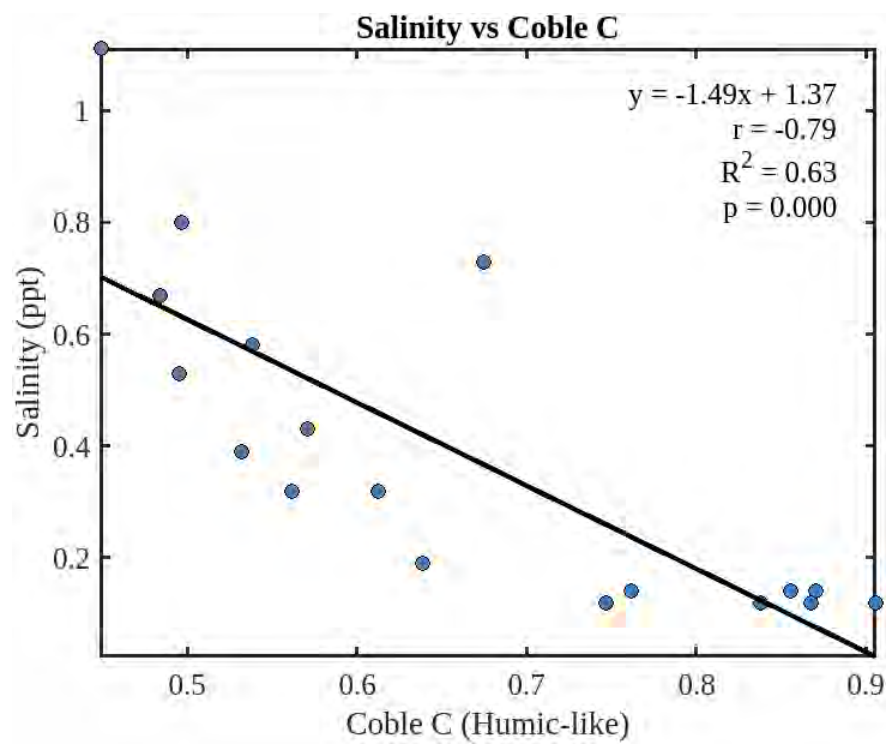
Appendix 20: pH vs BOD Linear Regression



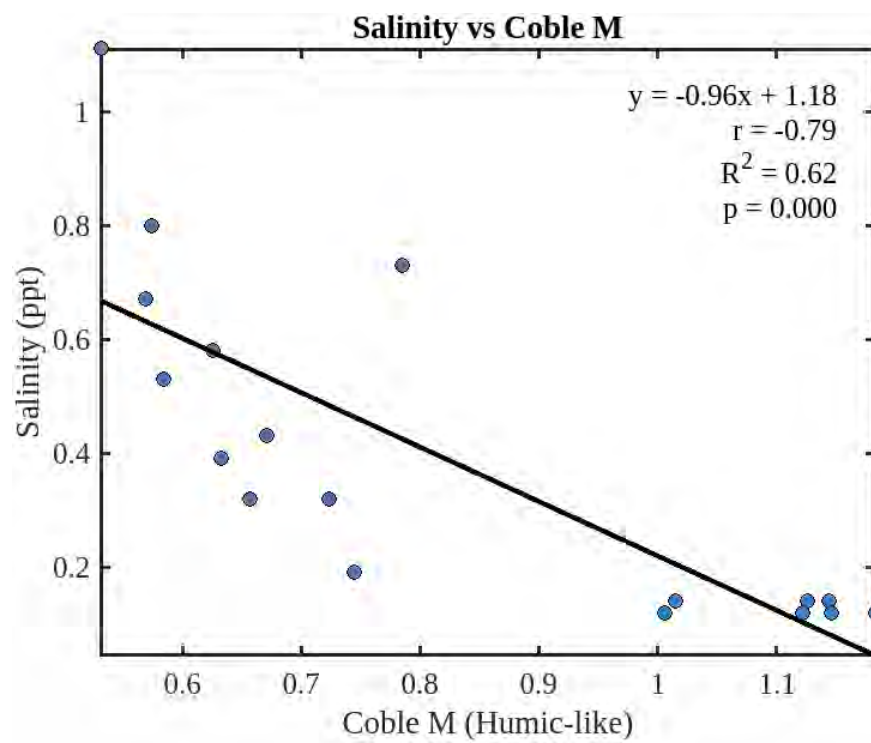
Appendix 21: Oxidation-Reduction Potential vs BOD Linear Regression



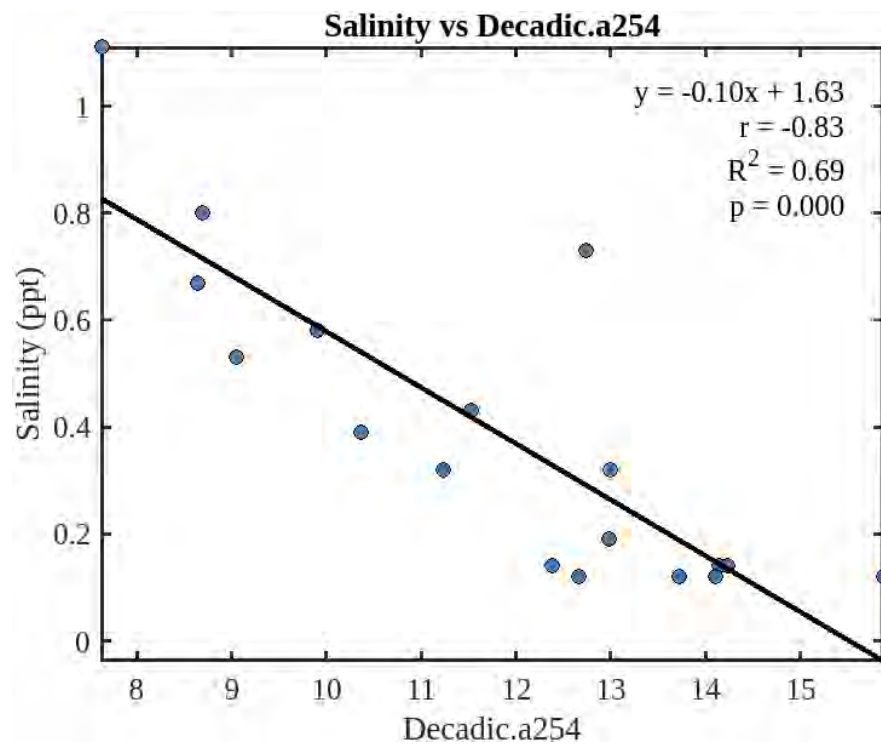
Appendix 22: Coble C vs Salinity Linear Regression



Appendix 23: Coble M vs Salinity Linear Regression



Appendix 24: Decadic.a254 vs Salinity Linear Regression



Appendix 25: Humic-like vs Salinity Linear Regression

