5-2013

Effects of Inorganic Nutrients and Dissolved Organic Carbon on Oxygen Demand in Select Rivers in Northern Utah

Joseph L. Crawford

Utah State University

EFFECTS OF INORGANIC NUTRIENTS AND DISSOLVED ORGANIC CARBON

ON OXYGEN DEMAND IN SELECT RIVERS IN NORHTERN UTAH

by

Joseph L. Crawford

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Watershed Science

Approved:

Michelle A. Baker Major Professor	Wayne A. Wurtsbaugh Committee Member
David K. Stevens Committee Member	Mark R. McLellan Vice President for Research and Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2013

ABSTRACT

Effects of Inorganic Nutrients and Dissolved Organic Carbon on Oxygen Demand in Select Rivers in Northern Utah

by

Joseph L. Crawford, Master of Science

Utah State University, 2013

Major Professor: Dr. Michelle A. Baker
Department: Watershed Science

Sewage, agricultural runoff, and atmospheric deposition have greatly increased the amount of nutrients (largely nitrogen (N) and phosphorus (P)) in surface water nationwide. Excess nutrients are associated with algal blooms and dissolved oxygen depletion in many water bodies, but linkages between nutrients and dissolved oxygen have been largely correlative. Biochemical oxygen demand (BOD) is a regulated water quality parameter that is aimed at describing the amount of oxygen consumed during the decomposition of organic matter. Despite the awareness that excess nutrients are linked to dissolved oxygen in rivers, few studies in the nutrient criteria literature discuss BOD measurements or how nutrients may impact BOD. Accordingly, I used factorial experiments to test the effect of inorganic nutrients (as N, P and N+P) and dissolved organic carbon on BOD measurements in Utah streams. The study was carried out from January through summer baseflow in 2011, allowing me to evaluate the effects of spatial and temporal variation of ambient nutrient concentration on oxygen demand. The study design included measurements in streams above and below nutrient point-sources (publicly owned treatment works) and several reference sites. I used classification and regression trees to identify thresholds of TN and TP that separate BOD response to nutrients into statistically distinct groups. My results show that seasonal variation affected BOD levels. As temperatures rose and water levels increased during peak runoff, I observed the highest BOD response to nutrient additions. I also found a significant correlation between BOD and ambient nutrient concentrations during that time period. I identified potential nutrient-related thresholds that could be used to assign numeric criteria that would protect designated uses. The threshold values I found for TN and TP were 0.56 mg/L and 0.09 mg/L, respectively. My results suggest that BOD may be sensitive to nutrient inputs and my experimental approach could be used as one line of evidence to support nutrient criteria related to aquatic life uses.

(81 pages)

PUBLIC ABSTRACT

Effects of Inorganic Nutrients and Dissolved Organic Carbon on Oxygen Demands in Select Rivers in Northern Utah

by

Joseph Crawford

Our nation's waterways are a valuable resource whose quality is influenced by their surroundings as well the amount of nutrients (largely nitrogen and phosphorus) in the water. Nutrients play an important role in aquatic ecosystems; however, if nutrient levels become too high, it is detrimental to water quality. Excess fertilizer that runs off of agricultural land and into the rivers and streams is a common source of nutrients in our waterways. Other sources of nitrogen and phosphorus include effluent released from wastewater facilities.

There are many negative side effects of high nutrients in the water. They can create large algal and bacteria blooms that release toxins, such as those released by cyanobacteria. When the algae start to decompose it consumes large amounts of oxygen, which can create a stressful environment for aquatic animals such as fish.

Through the funding of the Utah Division of Water Quality I studied the effects that varying nutrient levels have on oxygen consumption in select rivers in Northern Utah. I began the study in January, and ended in September allowing insight on impact that seasonal changes have on nutrient levels and oxygen consumption. I found that during the cold months elevated nutrient concentrations had little impact on oxygen

consumption. However, as temperatures increased and during spring runoff elevated nutrient levels resulted in more oxygen consumption. I also identified potential nutrient-related thresholds that could be used to determine how much nitrogen and phosphorus can enter the waterways before it elevates oxygen consumption to unhealthy levels. Such information can be used by policymakers to protect aquatic life uses of water in the state of Utah.

ACKNOWLEDGMENTS

I would like to thank Dr. Michelle Baker for her insight, and input as well as for all of the time she spent editing and helping me write my thesis. I would also like to thank Dr. David Stevens for his help with the statistical analysis. I also want to thank Dr. Wayne Wurtsbaugh whose insight and direction were always helpful. Without the expertise and advice of my committee I would have never been able to finish this project. I would also like to thank Jeff Ostermiller and Mike Shupryt for establishing the study sites as well as their help and feedback during this project. I also want to thank the Utah Division of Water Quality who provided the funding.

I would also like to thank my family for their patience, support, and help they have given me as I have gone through this process.

Joseph L. Crawford

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Eutrophication

Anthropogenic activities, including runoff from agricultural lands as well as effluent from wastewater treatment plants, are increasing nutrient loads to freshwater ecosystems (Carpenter et al. 1998). The term 'eutrophication' was coined in the 1930s by Naumann (as cited in Hasler 1947), largely as an increase in the nutrients nitrogen (N) and phosphorus (P) in lakes.

There are many negative side effects caused by the overabundance of N and P in water. High levels of N and P lead to algal and bacteria blooms that release toxins, such as those from cyanobacteria. These toxins degrade water quality and can create human health problems (Paerl 1988). Other nutrients such as organic carbon (released from algae) combine with disinfecting agents released from wastewater facilities generating more water contamination (Gilinsky et al. 2009). Over the ensuing decades the ecological literature has abounded with examples of the effects of eutrophication on freshwater and marine ecosystems worldwide (Clarke et al. 2006; Dodds 2006; Smith et al. 2006; Wolowicz et al. 2006; Worm and Lotze 2006). A notable example includes the Gulf of Mexico, which receives large amounts of nutrients from the Mississippi River basin. Consequently, the Gulf contains one of the largest eutrophic and hypoxic zones in the world and continues to be a scientific and policy concern (Rabalais et al. 2007; Liu et al. 2010).

It is evident that eutrophication can be detrimental to the health of water bodies. However, it has been shown that the problems associated with eutrophication can be ameliorated by significantly reducing the amounts of nutrients entering the water. Edmonson (1970) conducted a study that showed if the amount of nutrients is significantly reduced a body of water can overcome the effects of eutrophication. When effluent from 11 POTWs that were emptying into Lake Washington (Seattle) was diverted, water quality improved in that there was a significant improvement in water transparency and a decrease in the concentration of phosphorus as well as algal biomass.

Although nutrients such as N and P at most concentrations are non-toxic in-and-of themselves, the example above highlights potential negative effects of nutrient pollution on water quality. The recent wadeable stream assessment by the U.S. Environmental Protection Agency showed that excess N and P are the most extensive stressors of stream ecosystems nationwide, and that together with excess sediment, are the most important stressors to stream biota (USEPA 2011). Furthermore, despite extensive documentation of the effects of excess nutrients on freshwater and marine ecosystems, levels of biologically available P and N in terrestrial ecosystems have continued to increase at a rate that parallels that of human population growth (Corvalan et al. 2005).

Water Quality Policy
Related to Nutrients

Before the establishment of the Clean Water Act (CWA) (1972) two thirds of the nations waterways were unsafe for fishing and swimming (Sachar and Currey 1999). To help improve water quality the National Pollutant Discharge Elimination System. (NPDES) was created as part of the CWA. Under the NPDES any facility that discharges pollutants (i.e. any type of agricultural, industrial, or municipal waste) into receiving waters of the United States are required to obtain a permit. The permits are issued through technology-based and water quality-based effluent limits that allow a facility to discharge a specified amount of waste into the receiving waters as long as certain conditions are met (Sachar and Currey 1999). Over the last few decades the NPDES has helped reduce the amount of excess nutrients and other pollutants that have entered our waterways, however many of our nations waterways still remain polluted.

Waters that are too polluted or degraded to be in compliance with the standards that have been established by the local or federal governments are considered to be impaired (EPA 2012b). Section 303 (d) of the Clean Water Act requires States to list water that is impaired and develop a total maximum daily load (TMDL) for those waterways (Heiskary and Markus 2001). A TMDL calculates the maximum amount of nutrients and other pollutants that can be allowed to enter the impaired waterways without causing degradation.

Since the creation of the TMDL program in 1972 until now, 40,283 TMDL documents have been implemented in waters nationwide (USEPA 2011). In 2006 the Utah Division of Water Quality (UDWQ) assessed 10,446 miles of streams and found that 2926 of them did not fully support their designated use, and 845 (8.2%) miles of the impairment were due to excess nutrients (Millier et al. 2006). In fulfillment of the CWA the state is currently implementing TMDLs on many of these impaired waterways.

To reduce the amount of pollution in our waterways both numeric and narrative standards have been established for different pollutants. Many toxic chemicals, for example mercury, have numeric standards that are grounded in toxicological studies.

Other pollutants, including nutrients, are regulated by most states using narrative standards. The narrative standards usually state that any pollutant that causes an undesirable condition is unacceptable. For example the state of Utah narrative standard states that any pollutant that "causes conditions which produce undesirable aquatic life or which produce objectionable tastes in edible aquatic organisms" is unlawful (DAS 2012a). However, due to the lack of specific information or requirements, and the subjective nature of the narrative standards, they are often difficult to enforce.

The U.S. Environmental Protection Agency (USEPA) has been advocating that States adopt numeric nutrient criteria to address the excess nutrient problem in our waterways (USEPA 2011). The USEPA's numeric criteria development process suggests that after degraded water bodies have been identified, the goals and needs concerning nutrient enrichment should be established. To reach these goals the USEPA suggest that States should first create a list of parameters that will and will not be used to establish numeric criteria. This is followed by deciding the approach that will be used to develop the numeric criteria. The States should then prioritize and classify the different water bodies. The criteria should then be applied to the different bodies of water within the State as well as those that share borders with other states. It is also imperative that States implement and follow a date specific schedule (http://n-steps.tetratech-ffx.com/nutrient-supportLiterature.cfm). Following this process the States are then encouraged to continue to monitor those waters to determine the effectiveness of the nutrient criteria (Buck et al. 2000).

Numeric criteria may be difficult to determine because the cause and effect relationship between nutrient stressors and the biological response can be difficult to interpret. For example, many factors such as bioavailability, riparian vegetation, and grazing by aquatic organisms can greatly influence the response of algae to nutrient stressors. If possible these influences should be understood and managed accordingly when creating and implementing numeric criteria (McLaughlin 2012). Because there are several factors that influence nutrient concentration the USEPA suggests that nutrient criteria be developed according to the needs of each State (Buck et al. 2000).

The first numeric nutrient criteria for the state of Florida were signed by EPA Administrator Lisa Jackson in November 2010 (Kaufman et al. 2011). However this resulted in dozens of lawsuits, trying to negate the criteria. Thus as managers formulate nutrient criteria for different waterways it is important that they are done with sound, transparent scientific assumptions and conclusions (McLaughlin 2012).

Challenge to Link Nutrients (Eutrophication) to Designated Uses

Regardless of the process by which nutrient-related water quality impairment is addressed, a central challenge lies in linking excessive nutrients to designated uses of impaired water bodies. Two of the main goals of the Clean Water Act (1972) are to eliminate the discharge of pollutants into United States waters and to make sure all waters are swimmable and fishable (Carson and Mitchell 1993; EPA 2012a). To achieve these goals each body of water has been assigned a designated use.

The majority of the water bodies in Utah have been designated for drinking, recreation, cold and warm water species of fish and other aquatic life, waterfowl, shore birds and other water-oriented wildlife, as well as agricultural uses (DAS 2012a). Controlling nutrient levels in all of these designated waterways is an important step towards achieving good water quality. Conversely, failing to eliminate excessive nutrients can be detrimental to these designated uses and possibly lead to human health hazards in our drinking water. For example, water designated for drinking that contains an excess of 10 ppm of N as nitrate (NO_3^-) has a human health concern because it can cause methemoglobinemia (Fan and Steinberg 1996). Relating excess N and P to other uses is more tenuous.

Waters designated with aquatic life uses may be most affected by dissolved oxygen concentrations. The amount of dissolved oxygen in rivers reflects the balance between processes that add oxygen, including photosynthesis, and processes that remove oxygen, including respiration, nitrification, and chemical oxidation (Sullivan et al. 2010). These biochemical processes are influenced by the amount of nutrients that are in the water. An increase in nutrients will lead to higher levels of microbial growth. As microbial growth increases more oxygen is produced via photosynthesis. However, this also leads to higher oxygen consumption during respiration, lowering the amount of oxygen available to other aquatic organisms in the water.

Dissolved oxygen in rivers is very important to fish and other aquatic organisms. When oxygen levels are reduced it places stress on animals which slows their activity and changes their breathing patterns (Cox 2003). If dissolved oxygen levels reach hypoxic or anoxic levels in rivers and streams the production and diversity of aquatic organisms will be reduced. An example of this was observed by Hamilton et al. (1997) on the Paraguay River. During the wet season water levels increased and inundated the floodplains. As the river came in contact with the flood plains excess amounts of decomposing labile organic matter, detritus and soil leached into the system. When this matter entered the river large amounts of oxygen were consumed resulting in anoxic conditions and massive fish kills.

The oxygen sag curve is a well-known phenomenon that is observed below wastewater treatment facilities (Streeter and Phelps 1925). As effluent is released from a publicly owned treatment work (POTW) oxidation of organic materials occurs, depleting oxygen levels. However, as natural reaeration processes occur downstream, the oxygen levels increase, resulting in what is known as the oxygen sag curve (Romalho 1977).

Biochemical oxygen demand (BOD) is a regulated water quality parameter (APHA 1998) that describes the amount of oxygen that is consumed during the decomposition of organic matter and oxidation of reduced compounds like ammonia (Udeigwe and Wang 2010). For regulatory purposes the State of Utah requires that BOD levels in waters designated for domestic, recreational, agricultural, or aquatic wildlife remain below 5 mg/L (DAS 2012b). Despite the importance of measuring BOD and the awareness that excess organic matter is linked to dissolved oxygen in rivers, few studies in the nutrient criteria literature discuss BOD measurements, as well as what leads to BOD.

Biochemical oxygen demand typically refers to the water column processes alone. It is typically defined by two components, nitrogenous biochemical oxygen (NBOD), and carbonaceous biochemical oxygen demand (CBOD) demand (Cooper 1986; Deai et al. 1991; Sullivan et al. 2010). A related process that consumes oxygen in rivers is sediment oxygen demand (SODst) – the demand for oxygen specifically by bottom sediments (APHA 1998).

Nitrogenous biochemical oxygen demand measures how much oxygen is consumed through nitrification of ammonia by bacteria. Nitrogenous matter in human waste is usually composed of organic compounds such as protein or urea. Eventually these organic compounds are broken down into smaller amino acids. During this process ammonia (NH_3) is released which can join with hydrogen ions to form an ammonium ion (NH_4^+). Different species of nitrifying bacteria are able to oxidize NH_4^+ to nitrite (NO_2^-) and nitrate (NO_3^-), thus reducing oxygen in the water (Cox 2003).

Carbonaceous biochemical oxygen demand measures how much oxygen is used in the water column by the decay of organic matter (Cooper 1986). CBOD is measured by adding a chemical (nitrapyrin) that inhibits nitrification and only allows oxygen to be consumed by microorganisms decomposing organic matter (Sullivan et al. 2010).

There are also two different processes occurring in the sediment that contribute to oxygen consumption. These processes include (1) decay of organic matter/respiration by the organisms living in the sediment and (2) chemical oxidation of reduced substances such as iron, sulfide, and manganese (Bowman and Delfino 1980). Studies have shown that SOD is more variable than BOD and in certain circumstances SOD consumes more oxygen than BOD (MacPherson et al. 2007).

Study Objectives

The CWA requires that all designated uses of surface waters are protected. In section 131.11 of the CWA it states "such criteria be based on sound scientific rationale" (Havens 2003). In a similar manner, Smith and Tran (2010) state that due to the economic consequences of controlling nutrients, as well as the negative impact the excess nutrients are having, it is critical that policies made to eradicate these problems are based on credible and defensible scientific data.

Even though BOD is a regulated water quality parameter there have been few studies that focus on the effect of nutrients on BOD. In this study I sought to use sound scientific data to create nutrient-related thresholds to assign numeric criteria for BOD that would equip water managers with better information to protect designated uses.

The objectives of this study were to 1) test experimentally whether or not biochemical oxygen demand measured over a short term (BOD_{st}) and sediment oxygen demand short term (SOD_{st}) respond to nutrient additions, and whether or not this response is affected by nutrient inputs from point sources; 2) evaluate whether or not spatial and temporal variation in ambient nutrients explain variation in rates of oxygen consumption; and 3) identify potential nutrient-related thresholds or breakpoints that could be used to assign numeric criteria that would protect designated uses.

METHODS

Study Sites

My study sites include four rivers that receive effluent from publicly owned treatment works (POTWs), including Brigham City (Box Elder Creek), Tremonton (Malad River), Oakley (Weber River), and Wellsville (Little Bear River), (Figure 1, Table 1). Each facility differs in how incoming wastes are treated, ranging from membrane bioreactors to lagoons (Table 1). I chose these sites because of anticipated differences in capacity to treat N and P and because these sites were in close proximity to Utah State University with adequate winter access so I could revisit them throughout the year. These "treatment" rivers were each paired with two reference sites that do not receive effluent: South Fork of the Little Bear River and Little Bear River (Brigham City), Logan River at 1000 West and Logan River by the Dugway in Logan Canyon (Tremonton), Blacksmith Fork River and Logan River below Twin Bridges in Logan Canyon (Wellsville), Weber River above Rockport Reservoir and Upper Provo River (Oakley), (Figure 1, Table 1).

All sample locations were established by the Utah Division of Water Quality (UDWQ) as part of a functional indicators study in support of the State's nutrient criteria development (http://www.nutrients.utah.gov/index.htm). The sites below POTW discharges were below the mixing zone, which is estimated to occur at a distance between 20-100 times the river depth (Vandenberg et al. 2005). Accordingly, all of the sampling sites located below the effluent discharge were 120-160 meters below the POTW outfall. Reference sites were identified using "best professional judgment" by

Table 1. List of publicly owned treatment works with associated reference sites. Permit limits for these sites as established by the Standards of Quality of the State of Utah (http://www.rules.utah.gov/publicat/code/r317/r317-002.htm) are Phosphorus as TP < 0.05 mg/L, Nitrogen as N < 4 mg/L, and BOD < 5 mg/L. (Numbers refer to sample site, see Figure 1).

POTW	Study Site	Reference Site	Reference Site	Treatment Type
Oakley	Weber River (1)	Upper Provo River (2)	Weber River Above Rockport (3)	Membrane Bioreactor
Wellsville	Little Bear River (4)	Logan River Below Twin Bridges (5)	Blacksmith Fork River (6)	Lagoon
Tremonton	Malad River (7)	Logan River Below Dugway (8)	Logan River at 1000 West (9)	Activated Sludge
Brigham City	Box Elder Creek (10)	South Fork Little Bear River (11)	Little Bear River, West of Avon (12)	Oxidation Ditch

DWQ (Whittier et al. 2007) and represent sites at similar elevations and watershed areas as at treatment sites, but without regulated point sources for nutrients.

Study Design

The sample season began January 2010 and continued through September 2010. I broke my sample period into three different seasons: January-May I considered being the spring season (before snowmelt); June-July was the summer season (snowmelt), and; August and September was the late summer season (baseflow). Samples were collected from the Brigham City, Tremonton, and Wellsville sites six different times: three times

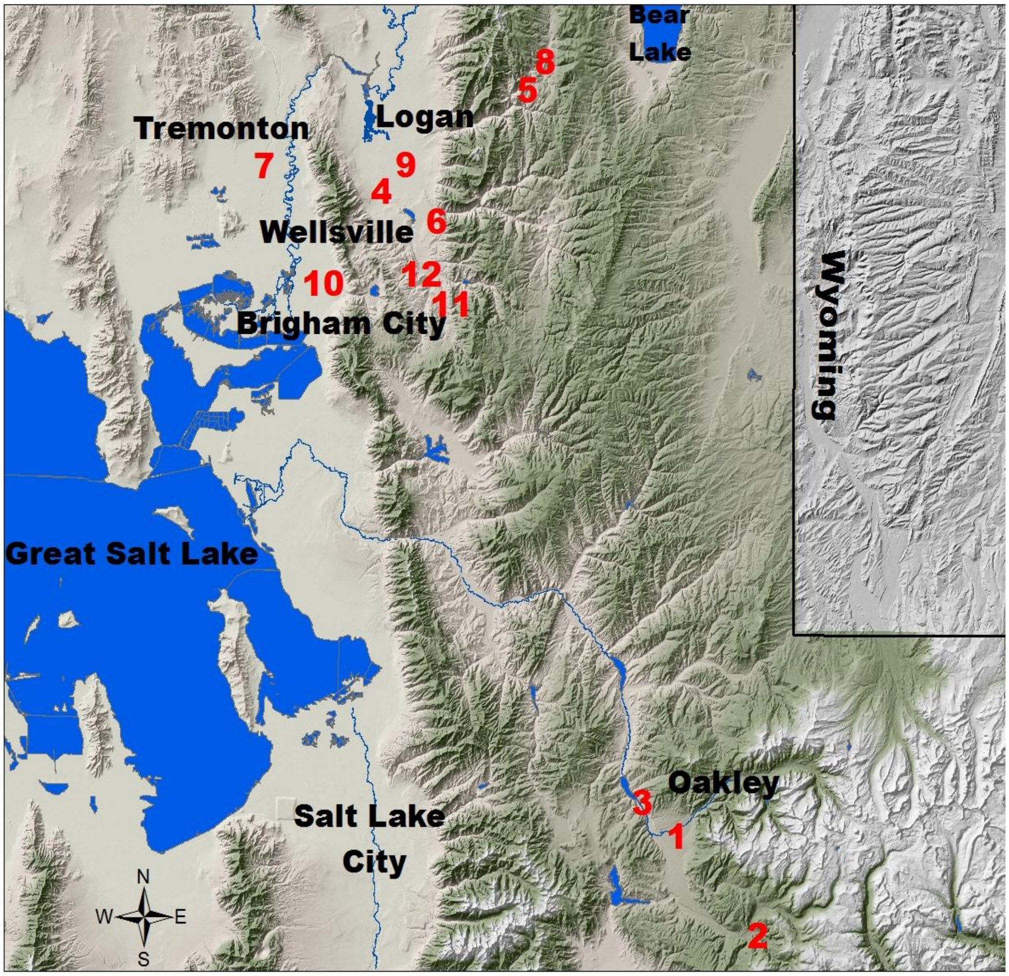


Figure 1. Map of sample sites. Numbers refer to specific study reaches defined in Table 1.

during the spring season, twice during the summer season, and once during the late summer season. Due to unusual amounts of snow, the Oakley sites were only accessible during four of the sample periods during 2010: twice during the spring season, once during the summer season and once during the late summer season. Samples were collected above and below the POTW on the same day and to ensure similar biological influences all of the reference sites were usually sampled within 48 hours of the POTW sample collection with the longest time between sample periods being seven days.

Field Sampling

The water temperature of each river was measured using a YSI 89 probe on each sample date. Water samples for BOD measurements were collected from the thalweg in a five gallon water cooler. I also collected grab samples for nutrient analysis at the same location. The general procedure was to collect a grab sample from the thalweg using either an acid washed 120-ml HDPE Nalgene bottle or 60-ml plastic syringe. Each was rinsed three times with stream water prior to sample collection. Unfiltered grab samples were used for analysis of total phosphorous (TP) and total nitrogen (TN). Samples collected via syringe were filtered using ashed 25-mm Pall A/E filters with 1.0 μM nominal pore size (Pall Corporation, Ann Arbor, Michigan) analyzed for nitrate (NO_3-N), ammonium (NH_4-N), soluble reactive phosphorous (SRP) and dissolved organic carbon (DOC). After collection, water samples were placed on ice then frozen until analysis. A large plastic scoop was used to collect sediment from the top layer (1-15 cm down from the top) of the river bed. All samples were kept cold in the field with icepacks until they were transported back to the lab. All water samples were processed for BOD_{st} and SOD_{st} in the lab within twenty four hours of being collected in the field. Samples for nutrient analyses were frozen upon return to the lab.

I also measured Volatile suspended sediments (VSS) by collecting water samples and bringing them back to the lab and filtering the sample onto ashed, pre-weighed GF/F glass fiber filters (GE Healthcare, Buckingamshire, UK). I did not collect VSS during spring.

Laboratory Analyses of
BODst and SODst

It is common practice to measure BOD for a period of 5 days or longer (APHA 1998). Prior to this study, tests were done to determine if performing the experiment for 24 hours, short-term BOD (BOD_{st}) would adequately measure BOD. These experiments showed that BOD_{st} would be sufficient to determine the effects that additional nutrients have on BOD levels. Thus in this study BOD_{st} acted as a proxy to BOD_5 (5-day BOD used commonly in water quality research and management, APHA 1998) and allowed us to perform the experiment six different times throughout the year for each POTW and their corresponding reference sites.

Water collected from each location was assigned one of six treatments and dispensed into a 5-gallon bucket. Treatments included a control (no chemical amendment), nitrate (NO_3-N), phosphate (PO4-3), nitrate plus phosphorus (N+P), carbon (C), or ammonium (NH_4-N). Nutrient treatments elevated the ambient concentration of N (1.12 mg N/L as KNO_3 or $(NH_4)_2SO_4$), P (2.48 mg P/L as KH_2PO_4), and C (0.163 mg C/L as C_2H_6O). Once the water was amended with the appropriate treatment, 300 ml was dispensed into glass BOD bottles, with four replicates per treatment. The background oxygen level of each replicate was then immediately measured using a YSI PrOBOD probe (Yellow Springs International, Yellow Springs, OH) that was calibrated in water-saturated air at ambient barometric pressure. Calibration was verified using Winkler titrations (APHA 1998).

All samples were then incubated at 22˚ C in the dark for 24 hours, after which dissolved oxygen was again measured and recorded. BOD_{st} was calculated by subtracting the amount of dissolved oxygen (mg/L) from the incubated samples from the background oxygen levels (Volkmar and Dahlgren 2006). I also used this data to determine if any of the BOD_{st} values violated (exceeded 5 mg/L of BOD) the Utah Division of Water Quality BOD standards (DAS 2012b).

Samples receiving a labile DOC amendment should exhibit increased BOD if the substrate is used for respiration. In some cases, addition of a labile organic substrate can stimulate additional respiration of ambient organic matter, in a process known as priming (Guenet et al. 2010). I calculated the amount of dissolved oxygen consumed by aerobic respiration of ethanol according to the stoichiometry:

$$C_2H_6O + 3O_2 = 2CO_2 + 3H_2O$$

Given that I added 0.163 mg/L of C_2H_6O3, 0.66 mg O_2/L should have been consumed by the additional carbon that was added.

Biochemical oxygen demand due to CBOD was determined by adding the nitrification inhibitor nitrapyrin (APHA 1998) to the samples that have water only. A separate water and NH_4-N treatment without nitrapyrin were also analyzed for BOD_{st}. By inhibiting nitrification I was able to calculate the amount of CBOD. Nitrogenous biochemical oxygen demand can then be calculated by subtracting CBOD from BOD (NBOD = BOD –CBOD).

Sediment oxygen demand was processed in separate BOD bottles by seeding each bottle with 1.5 ml of wet sediment. The SOD_{st} samples were subjected to the same experimental protocol as described above for BOD_{st} and NBOD. This was done to determine the effects of the sediments generally and was not used to extrapolate the data to the whole river. Due to the increase of nutrients below the POTWs I also expected to see an increase in the amount of organic matter in the sediment compared to the other sites. The amount of organic matter that was in the sediment was determined by mass loss on ignition (APHA 1998).

Volatile suspended sediment sample filters were dried, weighed, ashed at 450°C for 2.5 hours and weighed again to determine the amount of organic solids in suspension.

All samples were analyzed for nutrients in the Aquatic Biogeochemistry Lab at Utah State University using standard protocols summarized below. All analytical instruments were calibrated using standard reference materials (APHA 1998). Analytical quality control included use of reagent blanks, spikes, check standards and duplicate samples. Method detection limits were calculated as the product of the standard deviation of a minimum of seven replicates of a mid-range standard and the t-value from a one-sided t distribution (APHA 1998).

TN was quantified using a potassium persulfate digestion (Nydahl 1978) followed by cadmium reduction for measurement of NO_3+NO_2 (APHA 1998, EPA method 353.2). Measures of TP were made using a potassium persulfate digestion followed by an ascorbic acid molybdenum reaction for soluble reactive phosphorus (SRP), (Murphy and Riley 1962), EPA method 365.1. NH_4-N concentration was measured using an automated alkaline phenolhypochlorite reaction followed by spectrophotometric analysis (Solorzano 1969; SEMI 1993; APHA 1998). SRP and NO_3-N on filtered samples was also measured. All colorimetric analyses were done on an automated analytical system with FASPac II data acquisition software (Astoria Pacific International, Portland, OR).

Dissolved organic carbon (DOC) was quantified using oxidative combustion-infrared analysis on a Shimadzu TOC-Lcsh/TOC-Lcsn (Shimadzu Corporation, Kyoto Japan).

Statistical Analyses

All analyses were done using the statistical analysis program R (v 2.15 and v 2.15.2), with alpha = 0.05. I used several analyses to test the hypothesis that BOD_{st} and SOD_{st} can be limited by nutrients. First, I performed an analysis of variance (ANOVA) using all of the sampling sites (including reference sites) to assess whether control treatments had significantly different oxygen consumption rates than treatments with added nutrients. Because I was comparing a control with different treatments a Dunnett's test was then performed to determine which nutrients were significantly different from the control and when they were different. Second using BOD_{st} as the dependent variable, and treatment plants, month, and above and below the POTW as the predictor variables I used a three-way analysis of variance (ANOVA) to compare BOD_{st} responses at sites above and below POTWs. Because the main effects and the majority of their interactions were significant, I also performed pairwise t tests to identify which treatments were statistically different from each other ($p<0.05$). I also ran a two-way ANOVA to determine if the control SOD_{st} response to the treatments was greater than the control BOD_{st} response.

Frequency distributions were created to determine how often treatments from sites above POTWs were significantly different from the sites below. Frequency distributions were also done to determine how often the carbon treatment samples exceeded the UDWQ biochemical oxygen demand standard of 5 mg/L (DAS 2012b). Frequency distributions were also done to determine to determine how much BOD was due to carbonaceous biochemical oxygen demand (C-BOD) and nitrogenous oxygen demand (N-BOD). Since N-BOD may respond to ambient NH_4-N concentration and C-BOD may vary with ambient DOC, I evaluated relationships between these nutrients and BOD using linear regression on log-10 transformed data which is consistent with the EPA methodology (EPA 2012a). With BOD_{st} as the dependent variable and ambient nutrient concentration as the independent variable, I used analysis of covariance (ANCOVA) to evaluate whether there were significant differences among seasons in the relationship between ambient nutrient concentrations and N-BOD and C-BOD.

Once I established that oxygen consumption (as BOD_{st} and SOD_{st}) responded experimentally to nutrients, I wanted to evaluate whether or not measured BOD_{st} was related to ambient nutrient concentration. As per EPA guidance (EPA 2012a) I used simple linear regression on log-10 transformed data to analyze relationships between ambient BOD_{st} (measured in control treatments) and TN and TP. To evaluate how experimental nutrient additions might change regression patterns I conducted similar regression analyses for NO_3-N and SRP-amended treatments such that treatments with added NO_3-N were regressed against ambient concentrations of NO_3-N, NH_4-N and TN separately and treatments with added SRP-P were regressed against ambient SRP and TP concentrations. I also ran linear regression analysis for the SOD_{st} data and the different treatments. I expected that as the nutrient concentration increased there would be a stronger correlation with ambient BOD_{st} levels. Linear regressions were also run to evaluate relationships between ambient DOC and NH_4-N concentrations and C-BOD and the N-BOD levels, respectively. I also created a scatter plot matrix for TN, TP, DOC, VSS, and BODst during the spring, summer and late summer seasons. Scatter plot matrices were also created for NH_4, NO_3, SRP, TN, TP and temperature for each of the seasons. Scatter plot matrices were also created using the SOD_{st} data with the ambient nutrient concentrations.

With response of BOD to nutrients established using the statistical analyses above, I used classification and regression trees (CART, R v. 2.15) to identify thresholds in TN, TP, and carbon that allow separation of BOD_{st} and C-BOD into statistically distinct groups. CART does this by performing a complete search of all possible threshold values for each predictor and splits the data into two distinct homogenous groups. These groups are then split again. The splitting continues until an overlapping tree is made. Each group is then characterized by the categorical response or the numerical response of the response variable as well as other explanatory variables (De'ath and Fabricius 2000).

I used the party package in R to create my classification and regression trees. This package creates a nonparametric class of regression trees. This package enables for recursive portioning to build high and low tools for building regression trees and classification models. To avoid finding a threshold that was not statistically significant, a problem that occurs when models produce results that really do not exist (Babyak 2004), this package uses statistical stopping rules to determine threshold values (Hothorn et al. 2009).

RESULTS

Ambient Conditions

Ambient physicochemical conditions at each site varied seasonally. Northern Utah, the area of all of our sample sites, received an unusual amount of snow in winter 2010/11. During the early months of spring 2011, the region experienced cool temperatures and frequent rain showers. These conditions resulted in unusually high discharge levels; oftentimes with peak discharge three times the average peak (Table 2).

I also observed a large variation in temperature as the seasons began to change. The mean temperature from all of the sites during the spring season was 5.3°C (± 3.1), 13.6°C (± 4.7), in the summer season, and 15.2°C (± 3.6), in the late summer season (Table 3).

BOD was measured and recorded during each season for each site. The largest ambient (control treatment) BOD of 0.81 ± 0.20 mg/L was recorded during the summer

Table 2. Annual average peak runoff and peak runoff during 2011 for rivers in my study. No data was available for the Malad River or Box Elder Creek (all discharge data obtained from waterdata.usgs.gov).

River	Average Peak Runoff (CFS)	Peak Runoff 2011 (CFS)
Blacksmith Fork	300	1450
Logan River	800	1710
Weber River	1100	3180
Provo River	600	1890
Little Bear River	400	2300

Table 3. Seasonal average temperature of each sample site (standard deviation located in parenthesis). The temperatures without a standard deviation only had one sample collected for each season.

Site	Spring (°C)	Summer (°C)	Late Summer (°C)
Box Elder Creek-Above POTW	5.5 (±1.8)	18.8 (± 1.5)	17.6
Box Elder Creek-Below POTW	6.5 (± 1.5)	19.2 (± 0.1)	20.8
South Fork Little Bear River	6.1 (±1.1)	11.7 (± 1.1)	13.1
Little Bear River, West of Avon	6.0 (±1.5)	13.5 (± 0.1)	14.8
Little Bear River-Above POTW	5.7 (± 3.6)	15.6 (± 1)	14.2
Little Bear River-Below POTW	5.0 (± 2.6)	15.7 (± 1.8)	15
Blacksmith Fork River	4.7 (± 2.1)	11.7 (± 1.5)	11.2
Logan River Below Twin Bridges	3.7 (± 2.8)	9.0 (± 0.6)	10
Malad River-Above POTW	8.5 (± 5.0)	20.7 (± 0.1)	21.7
Malad River-Below POTW	8.6(± 4.4)	20.1 (± 0.7)	21.3
Logan River Below Dugway	5.8 (± 3.1)	8.2 (± 2.0)	no data
Logan River at 1000 West	5.9 (± 5.7)	no data	13.1
Weber River-Above POTW	1.8 (± 2.4)	8.2	14
Weber River-Below POTW	2.0 (± 2.3)	8.2	14.7
Upper Provo River	3.3	8.6	12.2
Weber River Above Rockport	6.5	10.9	13.9

season below the Tremonton POTW, while the lowest BOD was 0.07 ± 0.05 mg/L occurred during the late summer season at the Upper Provo River (Table 4). The BOD % of control for each experiment was also recorded (Appendix Table A 1).

Due to the effluent being released from the POTWs into the receiving waters, we expected higher ambient nutrient concentrations below the POTW's outflows and lower concentrations in the reference sites. The standard deviations are very large in some samples because the average was taken from the ambient nutrient concentration at different times during each season and not from replicate samples collected during each sample period. The highest ambient total nitrogen (TN) concentration of 3135 µg/L was found below the Tremonton POTW during the summer season. Conversely the lowest

Table 4. Mean Control BODst (mg/L) for all of the sites during each season (standard deviation located in parenthesis).

Site	Spring Control BODst	Summer Control BODst	Late Summer Control BODst
Wellsville-Above	0.35 (± 0.15)	0.31 (± 0.12)	0.16 (± 0.01)
Wellsville-Below	0.47 (± 0.09)	0.44 (± 0.22)	0.20 (± 0.03)
Blacksmith Fork River	0.36 (± 0.08)	0.16 (± 0.02)	0.15 (± 0.05)
Logan River Below Twin Bridges	0.33 (± 0.15)	0.15 (± 0.01)	0.14 (± 0.02)
Brigham City-Above	0.37 (± 0.14)	0.47 (± 0.13)	0.78 (± 0.05)
Brigham City-Below	0.38 (± 0.14)	0.44 (± 0.07)	0.31 (± 0.00)
Little Bear River, West of Avon	0.29 (± 0.11)	0.13 (± 0.02)	0.27 (± 0.05)
South Fork Little Bear River	0.25 (± 0.08)	0.18 (± 0.04)	0.13 (± 0.05)
Tremonton-Above	0.43 (± 0.09)	0.74 (± 0.15)	0.65 (± 0.07)
Tremonton-Below	0.43 (± 0.15)	0.81 (± 0.20)	0.51 (± 0.02)
Logan River at 1000 West	0.36 (± 0.12)	No Data	0.16 (± 0.03)
Logan River Below Dugway	0.28 (± 0.16)	0.23 (± 0.02)	0.26 (± 0.04)
Oakley-Above	0.26 (± 0.10)	0.20 (±0.05)	0.11 (± 0.03)
Oakley-Below	0.37 (± 0.24)	0.46 (± 0.11)	0.13 (± 0.06)
Upper Provo River	0.28 (± 0.09)	0.17 (± 0.01)	0.07 (± 0.05)
Weber River Above Rockport	0.17 (± 0.02)	0.15 (± 0.01)	0.11 (± 0.02)

ambient (TN) concentration was found at a reference site of 543.9 µg/L, this value was recorded at the Blacksmith Fork River during the late summer season (Appendix Table A 2). These results confirm what we expected to find in relation to nutrient concentrations among our different sampling sites.

Experimental Results

BODst

Having established that ambient nutrient concentrations varied widely in space and in time, I analyzed data from the bioassay experiments to test the hypothesis that BOD_{st} is limited by nutrient concentration. I expected BOD_{st} to increase in response tonutrient amendments if they were nutrient limited. The pairwise t-test results show that the sites were variably nutrient limited in some seasons. It is not surprising that the

Dunnett's tests showed that carbon was the most limiting nutrient during each season at all of the sites, with 75-100% of samples with significantly higher BOD_{st} in response to added ethanol (Figure 2 A-C, Appendix Table A 1). During the spring season the data suggests that inorganic N and P did not strongly limit BOD_{st} (Fig 2 A-C, Appendix Table A 1). As many as 35% of samples below POTWs responded to NO3-amendment in spring, with fewer samples responding to N and P at other sites. However, during the summer more samples significantly responded to nutrient amendment (Fig 2 A-C, Apendix Table A 1). Sites above and below POTWs were P-limited, with BOD_{st} in 84% of PO4-amended treatments higher than control treatments. Reference sites also showed P limitation, with 64% of BOD_{st} in SRP-amended treatments greater than controls. Some reference sites and sites below POTWs during summer were also N limited in that NO_3-N amended treatments from sites below POTWs were higher than control treatments 57% of the time and 42.9% of the time in reference sites. Sites below the POTWs were likely co-limited by N and P as 86% of N+P treatments were greater than controls. During the late summer season, P-limitation was maintained at sites below the POTWs with 75% of observations higher than controls.

It should be noted that during the spring season the N+P treatments from the Blacksmith Fork River were significantly lower than the control. This was also observed during the spring season for the NO_3-N and NH_4-N treatments above the Tremonton POTW and during the summer season for the NO3-N and NH_4-N treatments above the Brigham City POTW (Appendix Table A 1).

A

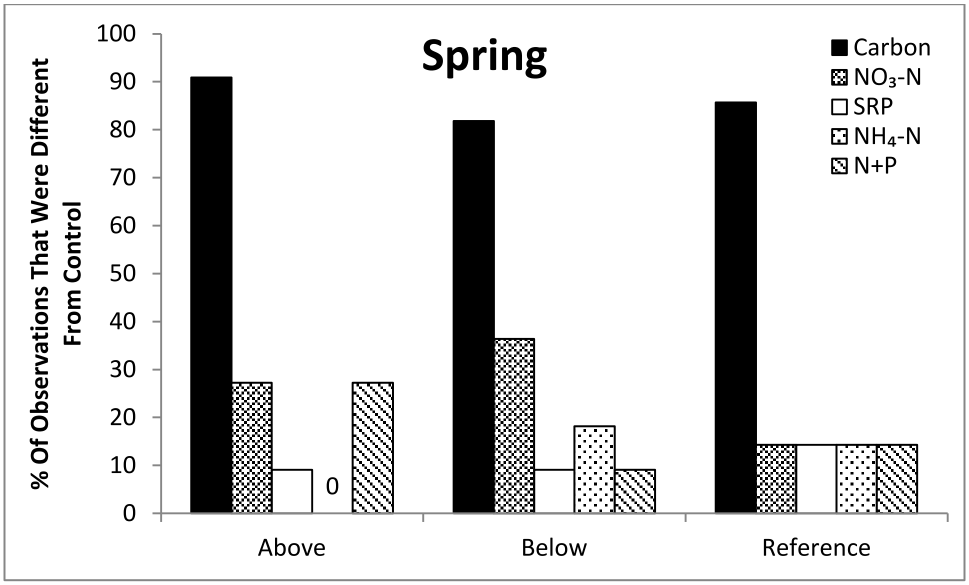
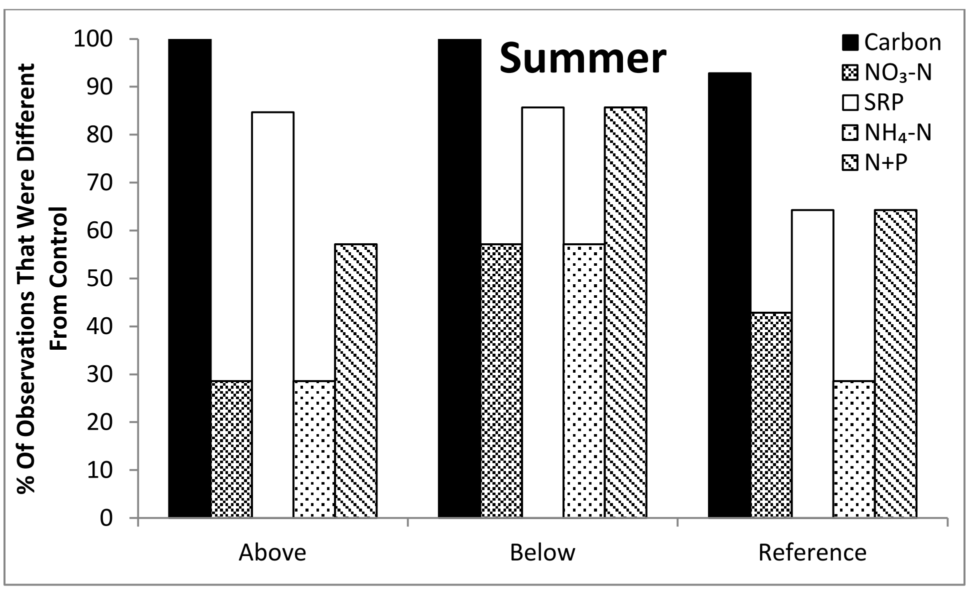
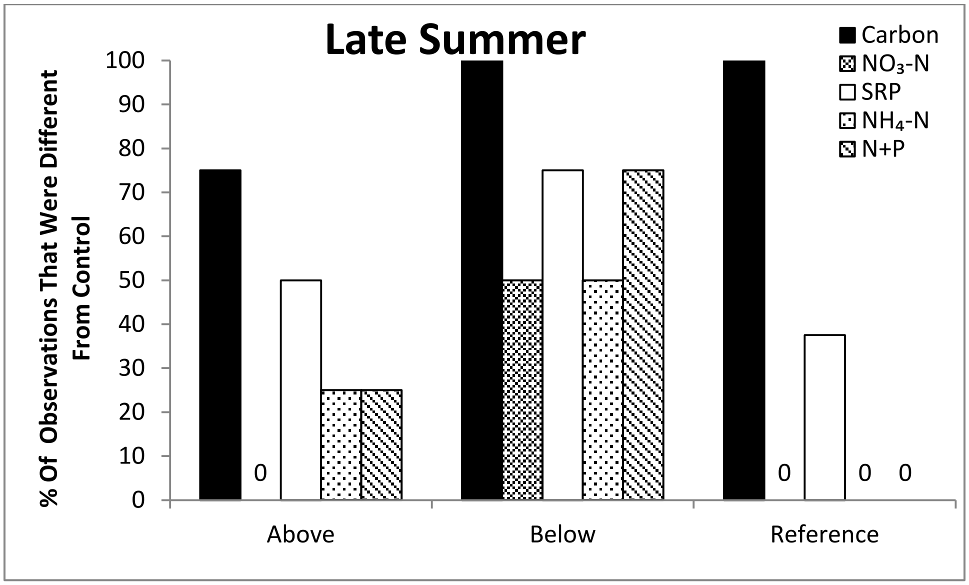


Figure 2 A-C Percent of observations indicating nutrient limitation, where mean BOD_{st} from nutrient amendments was significantly higher than from controls.

B



C



N-BOD & C-BOD

I found that over 50% all of my reference site samples experienced a higher percentage of C-BOD than N-BOD. My samples above and below the POTWs also experienced a higher percentage of C-BOD than N-BOD (Figure 3).

NH_4-N concentration explained about 41% of the variation in N-BOD across all sites and seasons, linear regression, p=0.017, r^2= 0.417 (Figure 4). Three points that exhibited a higher N-BOD when treated with nitrapyrin were excluded from the graph. Within seasons, NH4-N and N-BOD were positively related in summer and late summer seasons, p= 0.05, and 0.02, r^2=0.117 and 0.3638 respectively (Figure 4). ANCOVA revealed that the late summer season was significantly different from the spring and summer seasons such that the intercept was significantly greater in spring than summer. The spring season p= 0.152, r^2= 0.030 had a higher intercept than the summer season.

During the summer and late summer seasons I found NH_4-N to be positively related to N-BOD, p= 0.05, and 0.02, r^2=0.117 and 0.364 (Figure 5).

The relationship between ambient DOC concentration and C-BOD was weak across all sites and seasons, with DOC and C-BOD only being significantly related during the summer season explaining only about 29% of the variation in C-BOD p=0.006 (Figure 6). DOC and C-BOD were not significantly related in spring and late summer, p>0.05 (Figure 7). ANCOVA showed that the summer season was significantly different from spring and late summer seasons, p<0.05 (Figure 7).

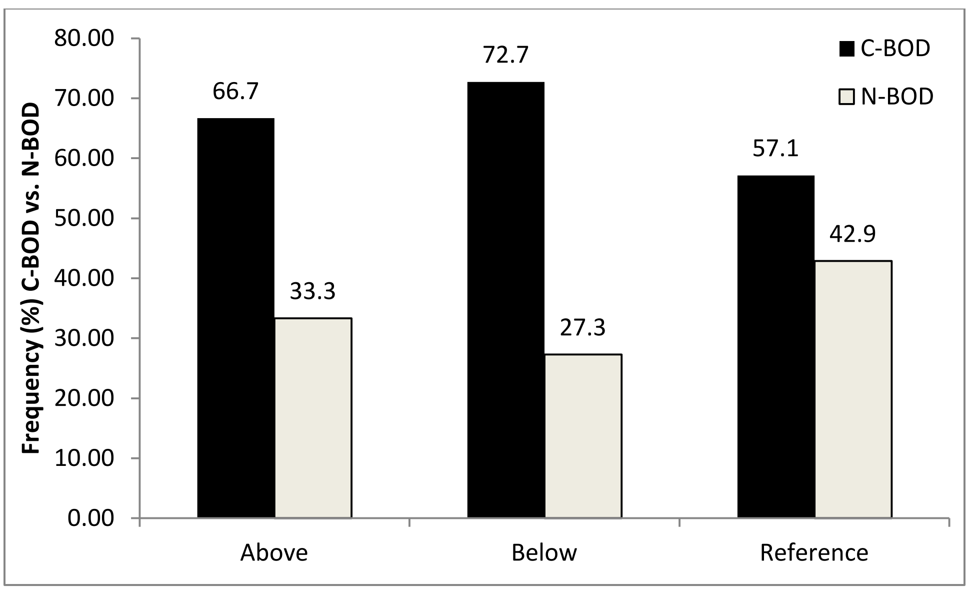


Figure 3: BOD results broken down into C-BOD and N-BOD. I found that all of my samples experienced a larger percentage of C-BOD than N-BOD.

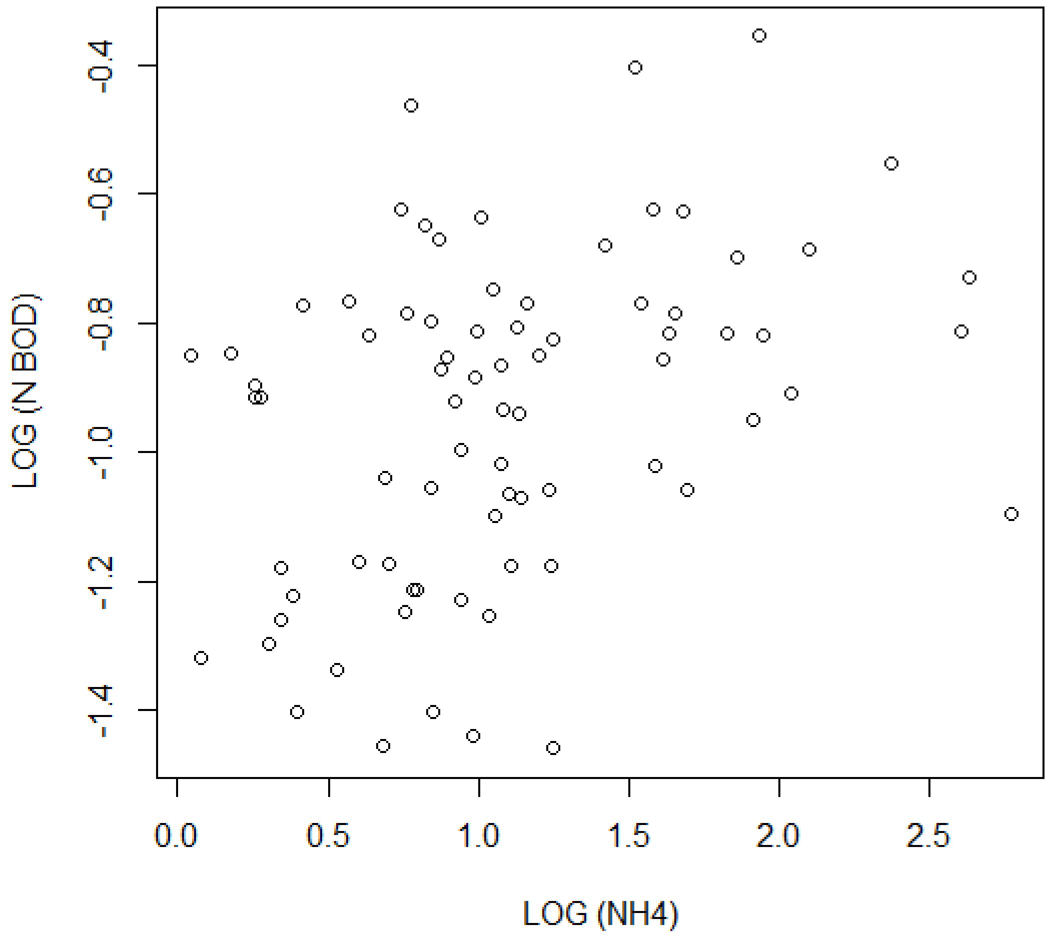


Figure 4: Relationship between ambient ammonium concentration and N-BOD (linear regression, p= 0.017, r2 = 0.417).

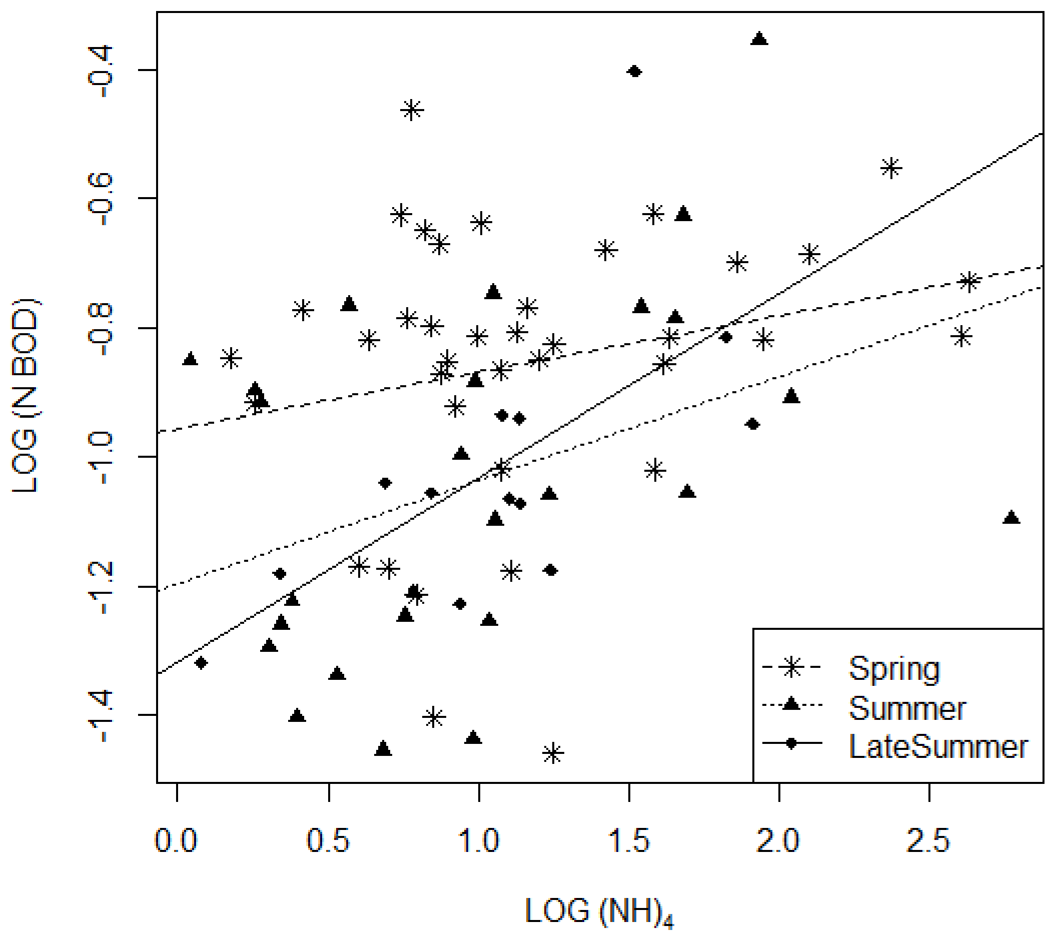


Figure 5: Ambient ammonium concentration vs. N-BOD. After running an ANCOVA I determined that the late summer season was different from the spring and summer seasons. The spring season p= 0.152, r^2= 0.02994 had a higher intercept than the summer season. The summer and late summer seasons found NH_4-N to be positively related to N-BOD , p= 0.05, and 0.02 , r^2=0.117 and 0.3638 respectively.

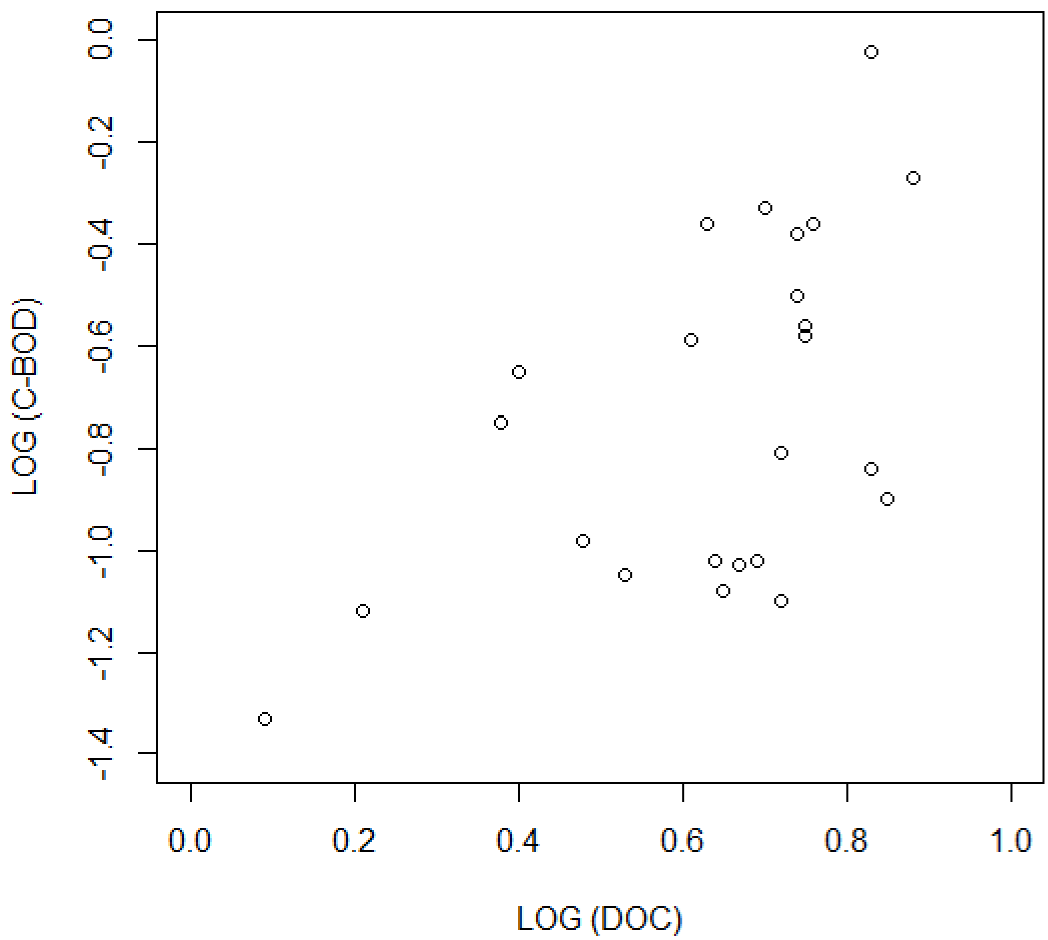


Figure 6: Relationship between ambient dissolved organic carbon and C-BOD during the summer season. Across all sites and the summer season was the only season that exhibited a significant relationship between ambient dissolved organic carbon concentration and C-BOD p-value = 0.006, $r^2 = 0.294$

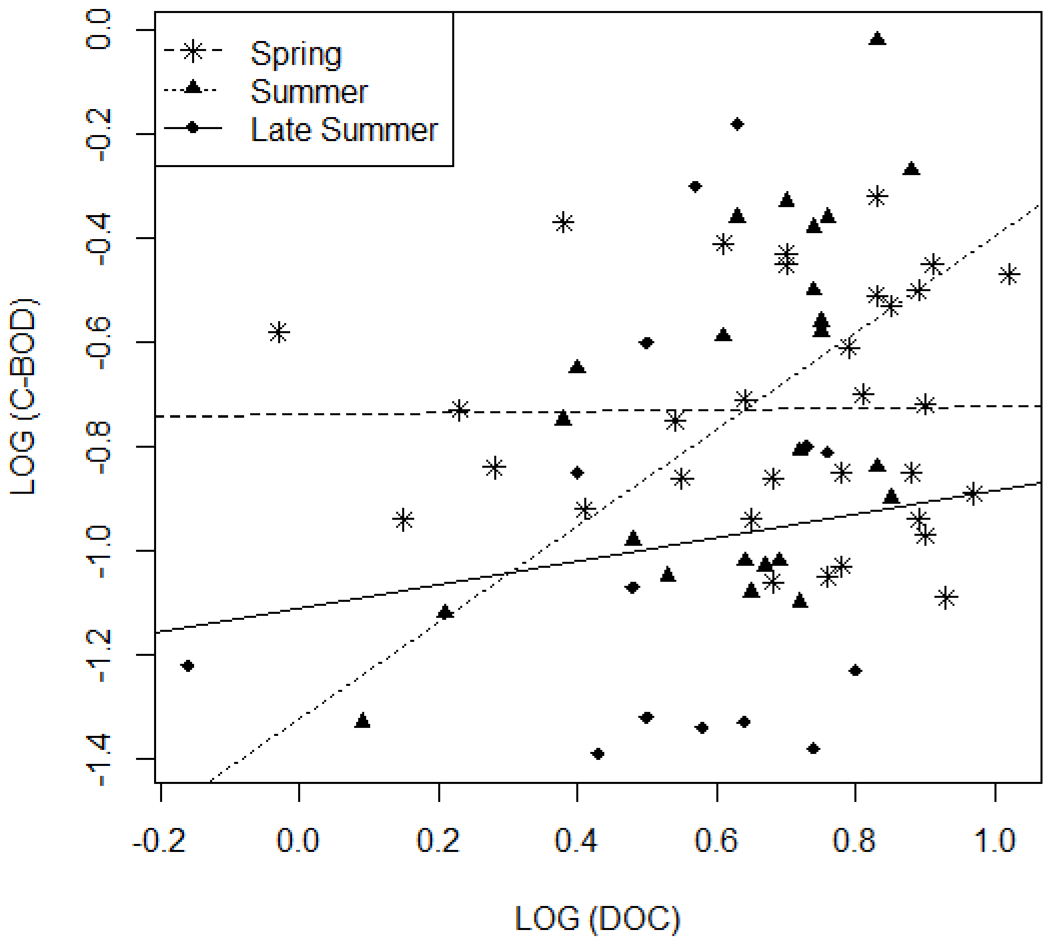


Figure 7: Seasonal ambient DOC concentration vs. C-BOD. ANCOVA showed the summer season to be significantly different from the spring and late summer season. It also showed that the spring season had a higher intercept than the late summer season. The summer season was found to have a significant relationship (p-value = 0.006, r^2 = 0.2939). C-BOD and DOC were not significantly related during the spring and late summer months (p= 0.928 and 0.649 respectively).

SOD_{st}

SOD_{st} should be different than the BOD in the water column. I found that for all of the inorganic nutrient treatments as well as for the carbon treatments the SOD_{st} samples were all significantly different from the BOD samples with the same treatments (Welch 2-sample t-test $p < 0.001$, Appendix Table A3).

I also analyzed data from the nutrient addition experiments to test the hypothesis that SOD_{st} was also limited by nutrient concentration. Due to the extreme runoff discharge levels I was not able to collect sediment samples during the summer season and analyze SOD_{st}. Similar to the BOD_{st} results I expected SOD_{st} to increase in response to nutrient amendments if they were nutrient limited. The pairwise t-test results show that sites were variably nutrient limited in some seasons. As was found with BOD carbon was the most limiting nutrient during the spring and summer seasons at all of the sites (Fig 8 A-B, Appendix Table A 4). During the spring season the data suggests that the sites located above the POTWs and the reference sites were possibly co-limited by N+P with 25% and 31.3% (respectively) of the samples experienced a significantly higher SOD_{st} than the control (Fig 8 A-B, Appendix Table A 4). As many as 25% the samples below POTWs responded to NO_3-amendment in the spring. However, during the late summer above the POTWs carbon appears to be the only limiting nutrient (Fig 8 A-B, Appendix Table A 4). I found 25% of the samples below POTWs experienced a significantly different SOD_{st} response from the control, possibly suggesting nutrient limitation. I also found that 13% of the reference site samples were different from the control in all of the treatments except the carbon treatment, suggesting that the sediments were slightly limited by the other nutrients than just carbon.

A

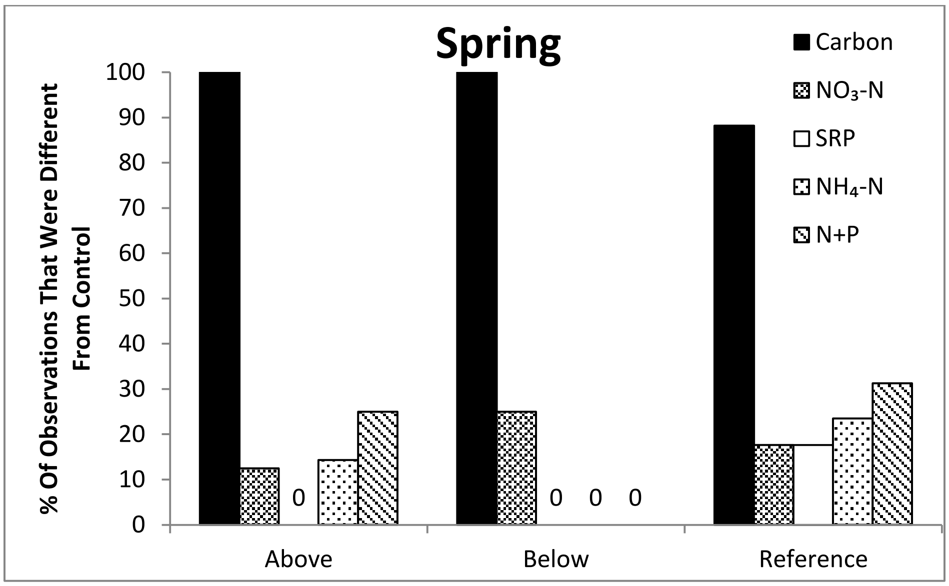
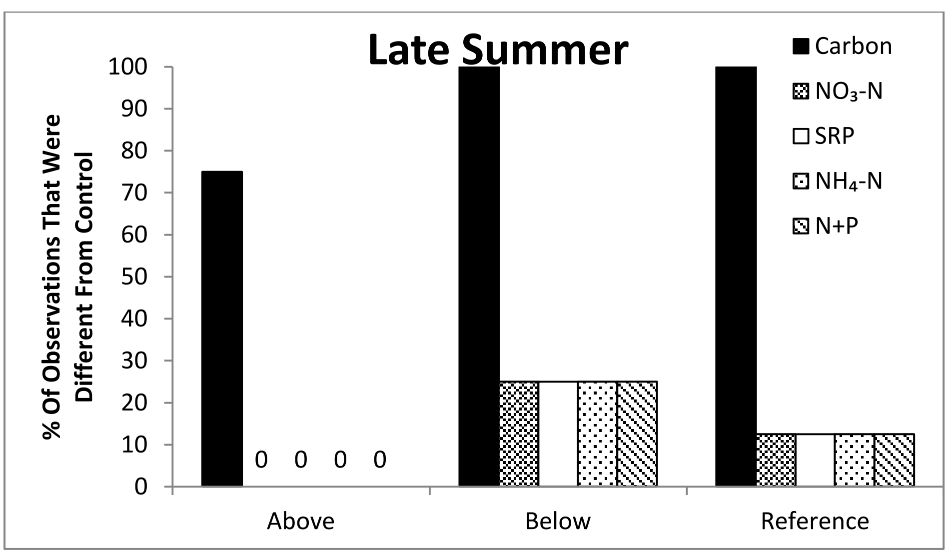


Figure 8 A-B. Percent of observations indicating nutrient limitation, where mean SOD_{st} from nutrient amendments was significantly higher than from controls as identified using 2-way ANOVA. See table Appendix A4 for the mean BODst in each experiment.

B



Stressor-Response Analyses-BOD_{st}

After establishing that BOD_{st} can respond to experimental additions of nutrients, and that these responses vary seasonally, I evaluated potential relationships between this response indicator, and nutrients as a stressor. The ANOVA show that inorganic nutrients were related to total N and total P in all seasons (Figure 9 A-C, Appendix Tables A 5 and A 6). This is important because States are more likely to establish numeric criteria for total nutrients as opposed to inorganic nutrients (which were used in my experiments).

I also evaluated the potential relationships between BOD_{st} response and nutrient concentrations during the different seasons. Ambient BOD_{st} (from control treatments) was significantly related to TN and TP ($p<0.05$, Appendix Figures A 1 and A 2) during the summer and late summer seasons, but not during the spring (Figure 10 A-C). BOD_{st} was not significantly related to ambient DOC (Figure 10 A-C) and was only significantly related to VSS during the late summer season (R2=0.425, p=0.01, Figure 10 C). These results suggest that nutrient concentrations and seasonal changes influence BOD rates.

Stressor-Response Analyses – SOD_{st}

I also evaluated potential relationships between SOD_{st} and nutrients as a stressor. I found that during the spring season the only ambient nutrient level that had a significant positive relationship with the control SOD_{st} was NH_4-N, p-value = 0.03, $r^2 = 0.141$ (Figure 11). TN, TP, NO_3-N, were significantly related to the control SOD_{st} during the late summer season only (Figures 12 and 13). SRP was not significantly related to the control SOD_{st} during any season.

A

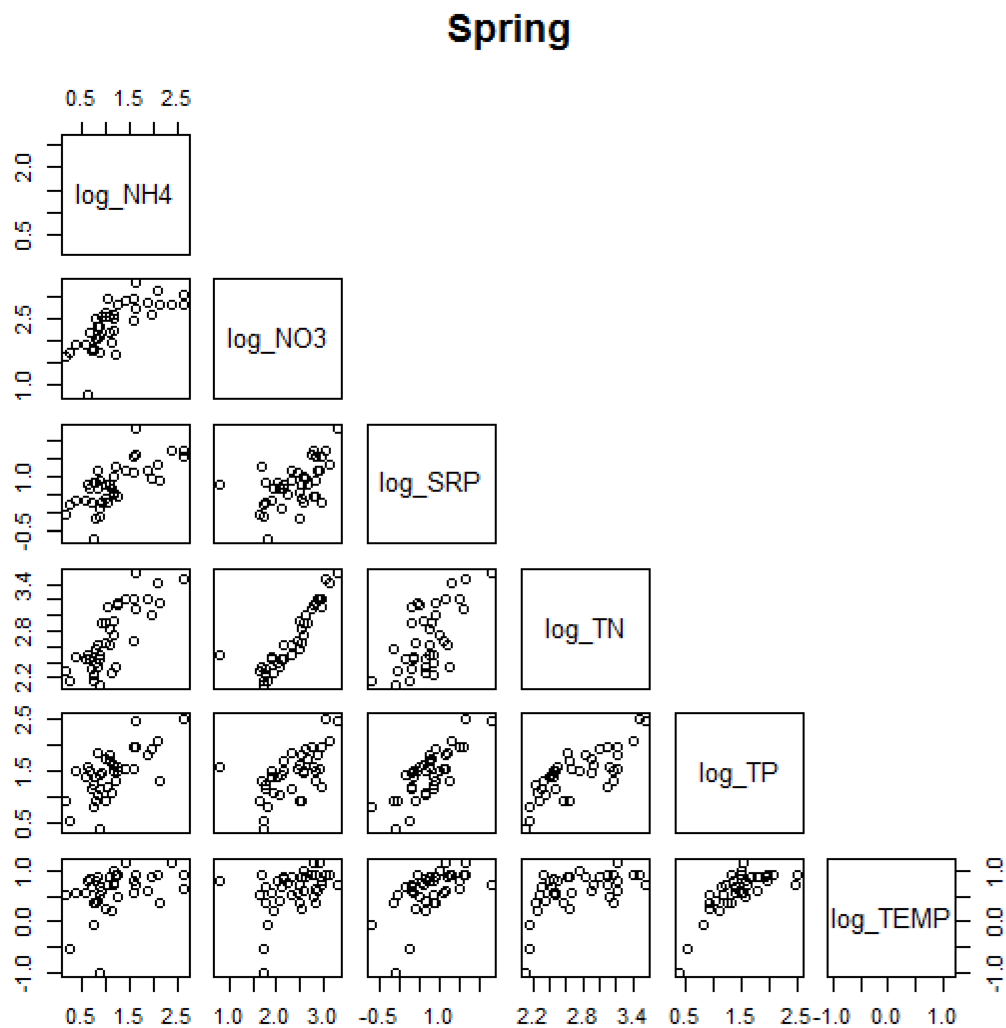
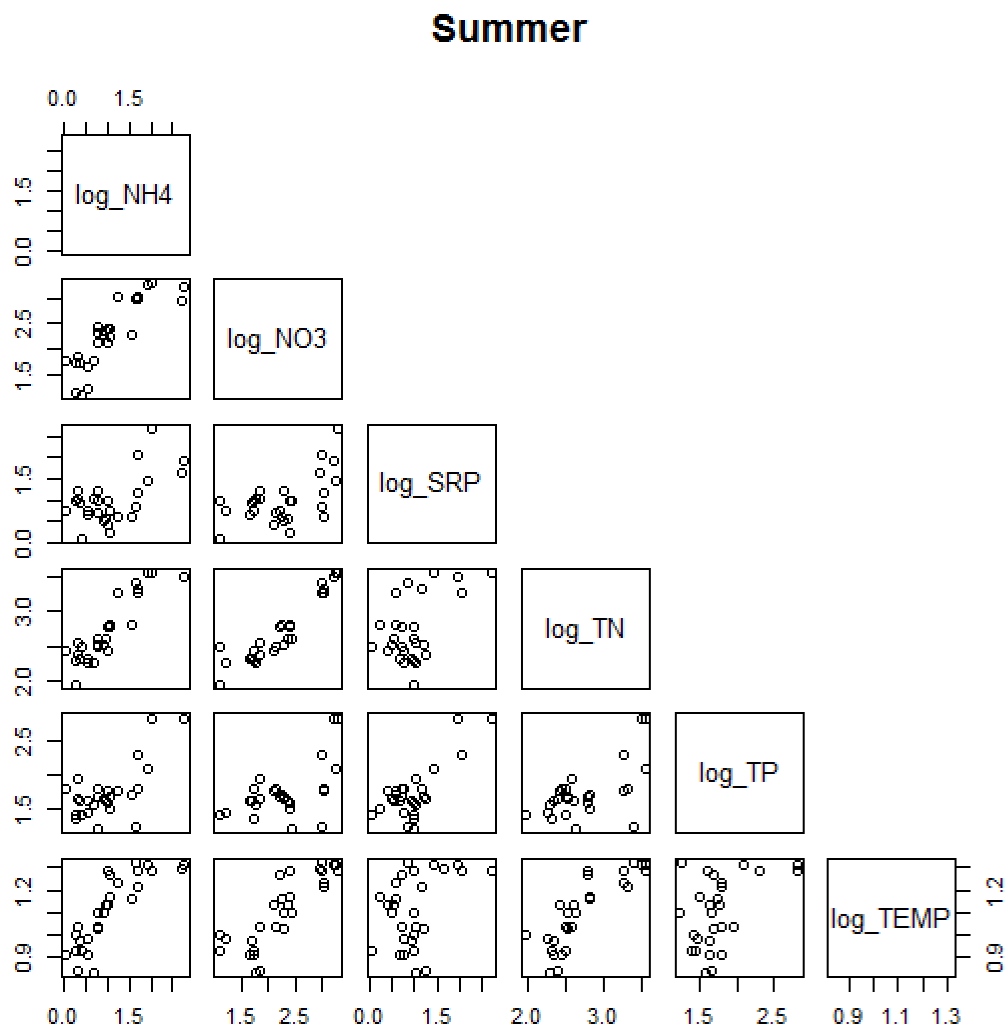
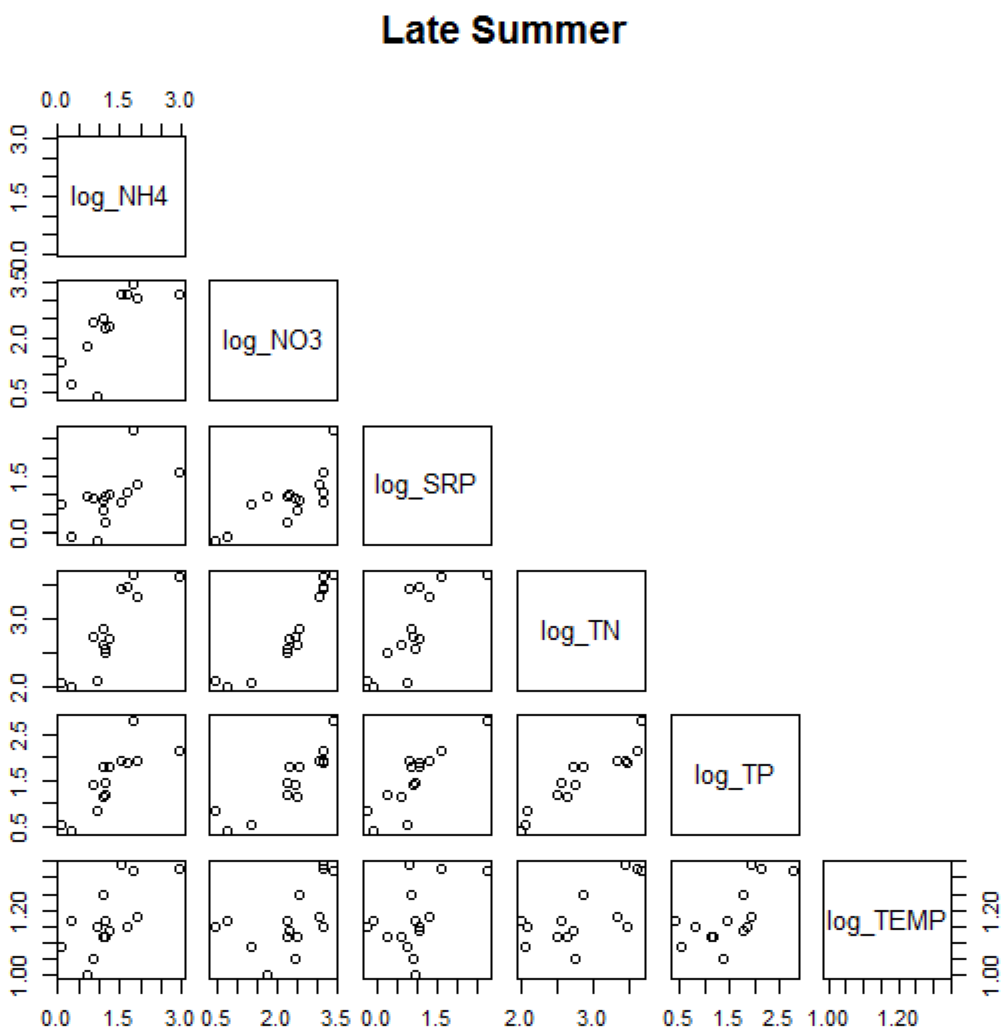


Figure 9 A-C: Scatter plot matrix for NH_4-N, NO_3-N, SRP, TN, TP and Temperature during spring (A), summer (B) and late summer (C). The inorganic nutrients were significantly related to TN and TP during all of the seasons. See Appendix Tables A5 and A6 for statistical results.

B


Summer
log_NH4
log_NO3
log_SRP
log_TN
log_TP
log_TEMP
0.0
1.5
2.5
2.0
3.0
0.9
1.1
1.3
1.2

C


Late Summer
log_NH4
log_NO3
log_SRP
log_TN
log_TP
log_TEMP
0.0
1.5
3.0
0.5
2.0
3.5
2.0
3.0
2.5
1.00
1.20

A

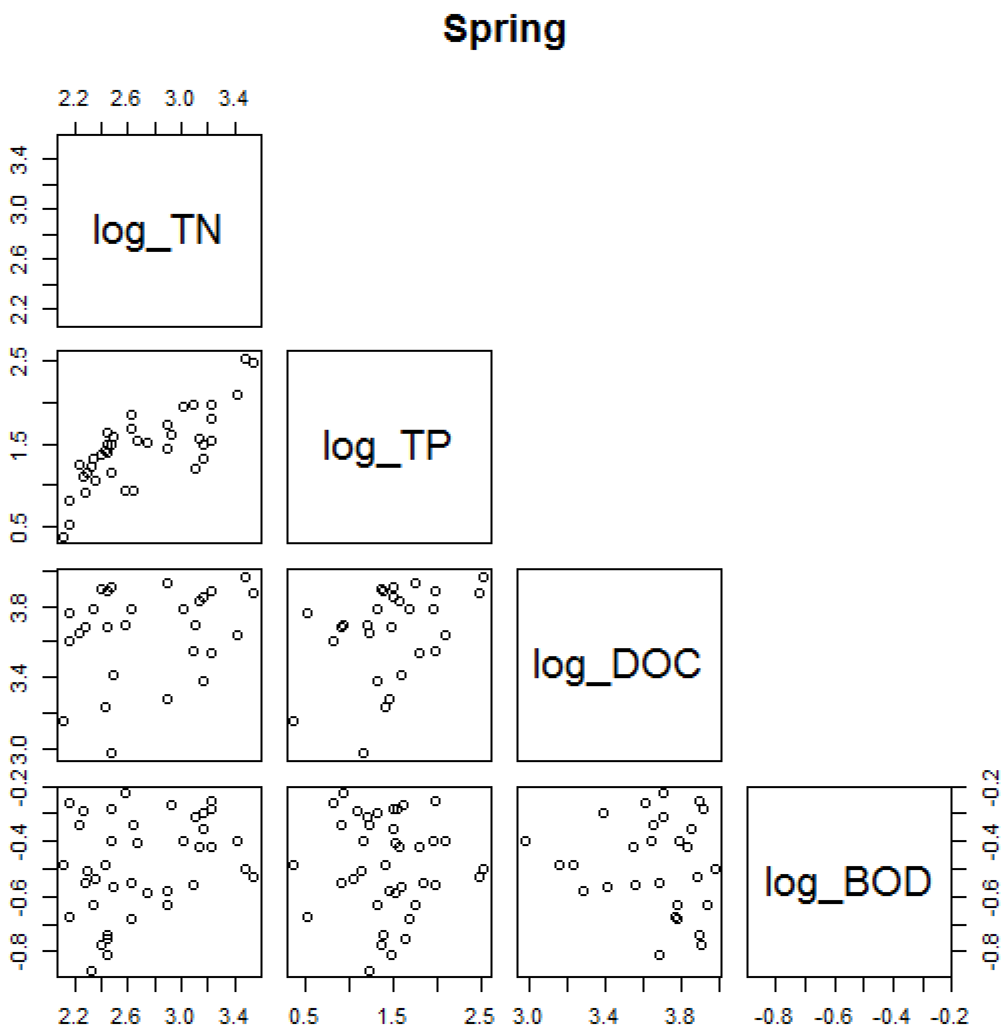
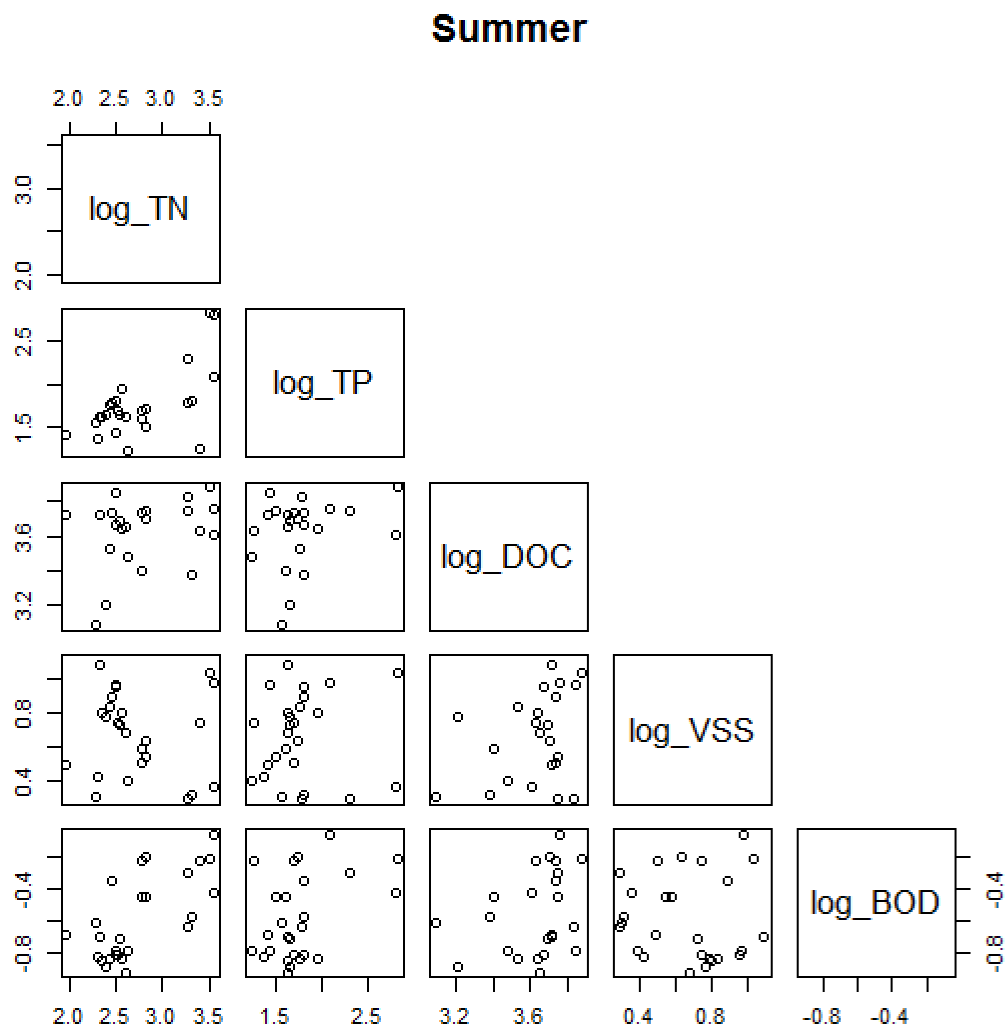
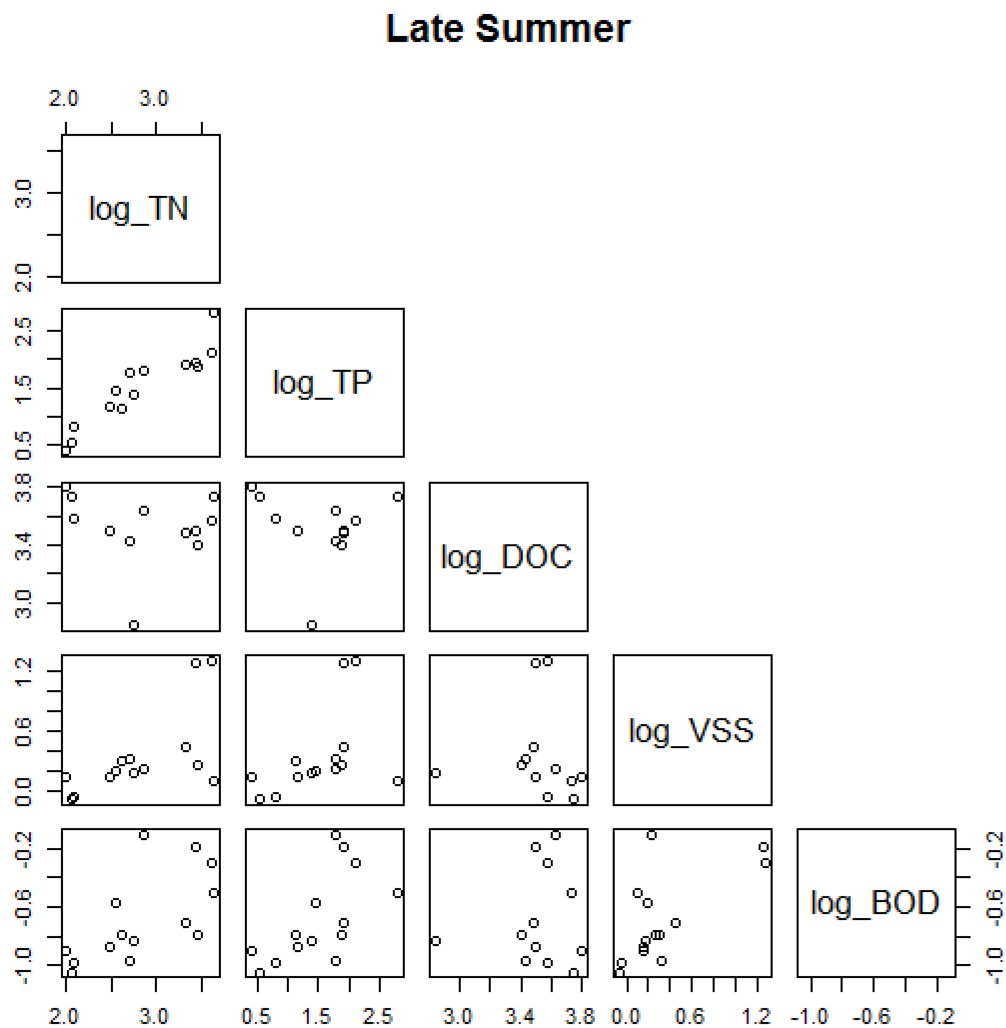


Figure 10 A-C: Scatter plot matrices for TN, TP, DOC, VSS, and BOD during spring (A), summer (B) and late summer (C). BOD_{st} was not significantly related to ambient DOC and was only significantly related to VSS during the late summer season (p=0.01, R2=0.425). See Appendix Figures A1 and A2 for statistical results.

B


Summer
log_TN
log_TP
log_DOC
log_VSS
log_BOD
2.0 2.5 3.0 3.5
1.5 2.5
3.2 3.6
0.4 0.8
-0.8 -0.4

C


Late Summer
log_TN
log_TP
log_DOC
log_VSS
log_BOD
2.0
3.0
0.5
1.5
2.5
3.4
3.8
0.0
0.6
1.2
-1.0
-0.6
-0.2

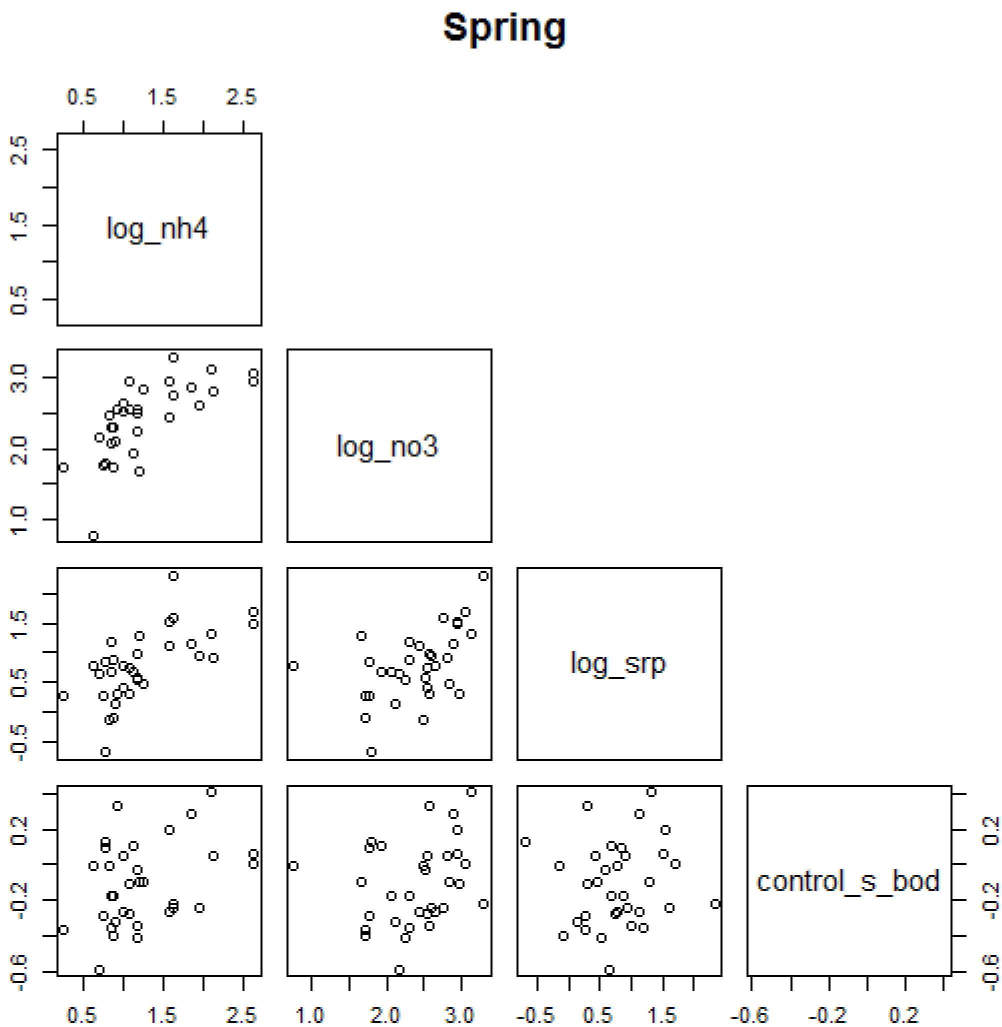


Figure 11: Scatterplot matrix during the spring season for control SOD_{st} and inorganic nutrients. NH_4 was the only nutrient significantly related to the control SOD_{st} during this season (p-value =0.03, r^2=0.141).

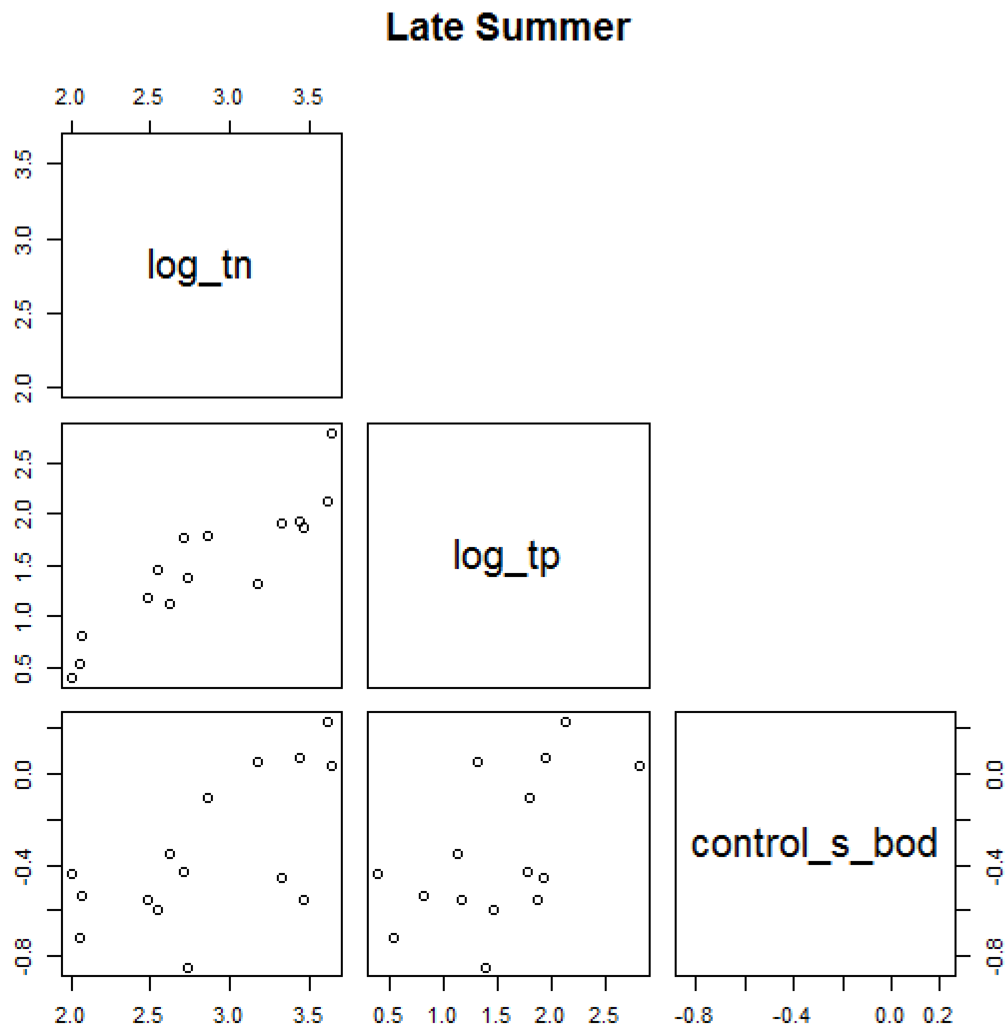


Figure 12: Scatterplot matrix during the late summer season for control SOD_{st} and organic nutrients. TN and TP were both significantly related to the control SOD_{st} during this season, p-value <0.01 and 0.03, $r^2= 0.425$ and 0.314, respectively.

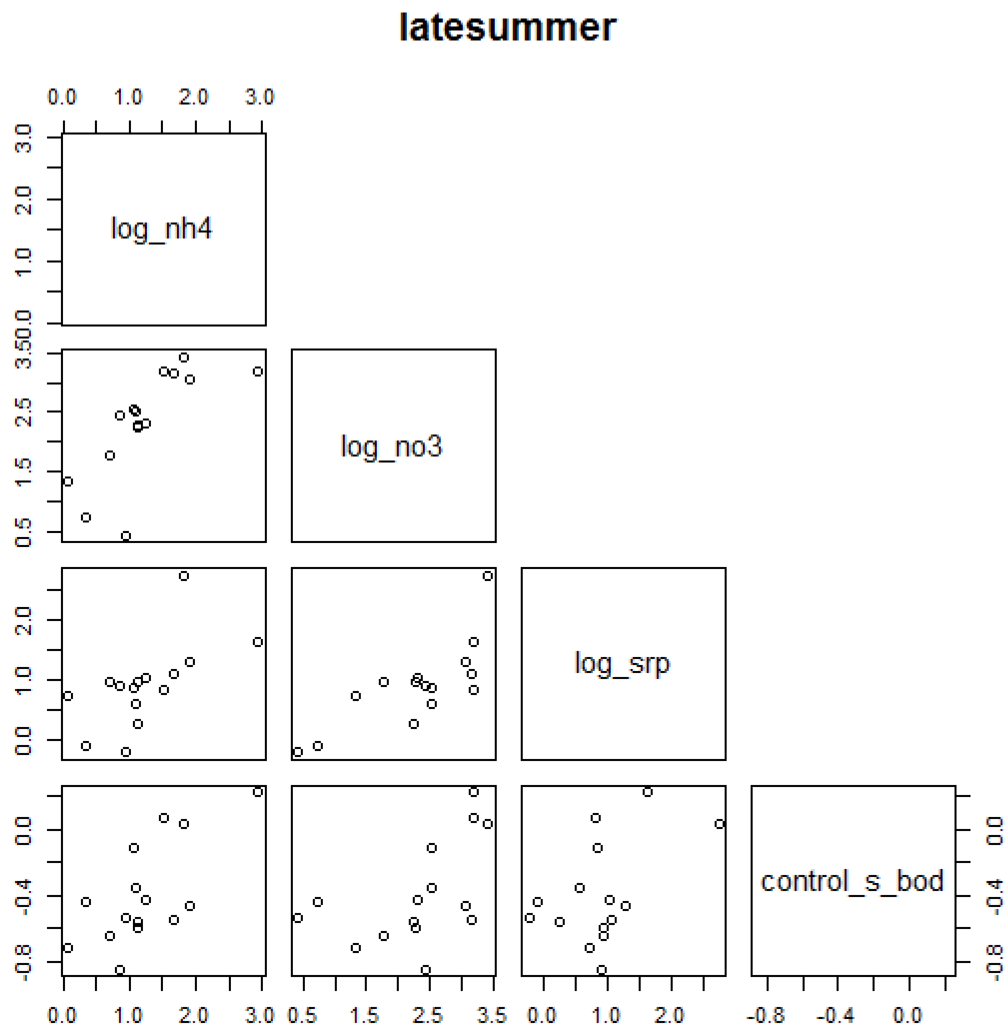


Figure 13: Scatterplot matrix during the late summer season for control SOD_{st} and inorganic nutrient levels. NH_4-N , NO_3-N were both significantly related to the control SOD_{st}, p-value <0.01, and 0.05, r^2= 0.474 and 0.27,1 respectively.

Comparison of Sites Above vs. Below POTWs

Given that BOD_{st} was positively related to TN and TP, I expected to see higher levels of BOD at sites below POTWs compared to those above the POTWs. While 2-way ANOVA (Appendix Table A 7) showed a significant result between the sites located above the POTW versus the sites below the POTW the results did not strongly support this prediction. During August, BOD_{st} rates in control treatments as well as those amended with inorganic nutrients were higher above the Brigham City POTW than below it (Figure 14 A). A pairwise t-test was performed to test significance (Appendix Table A 8). In contrast, BODst was generally higher below the Tremonton and Wellsville POTWs than above them (Figures 13 B and C).

A

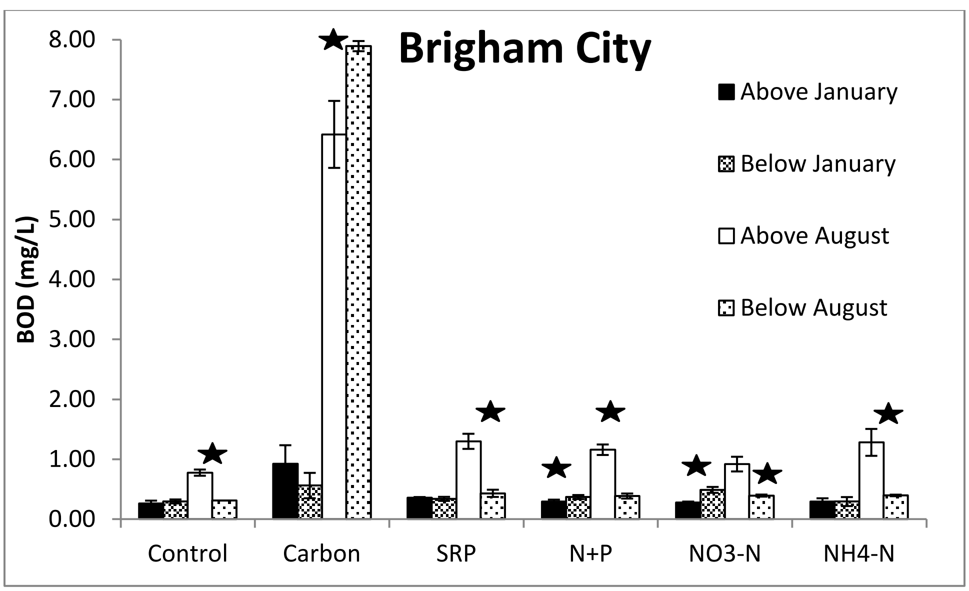
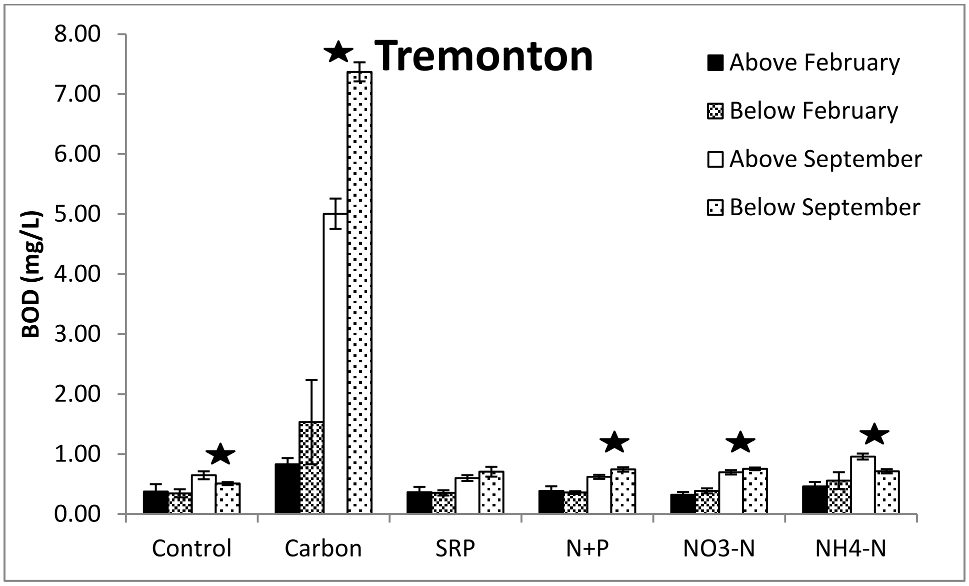
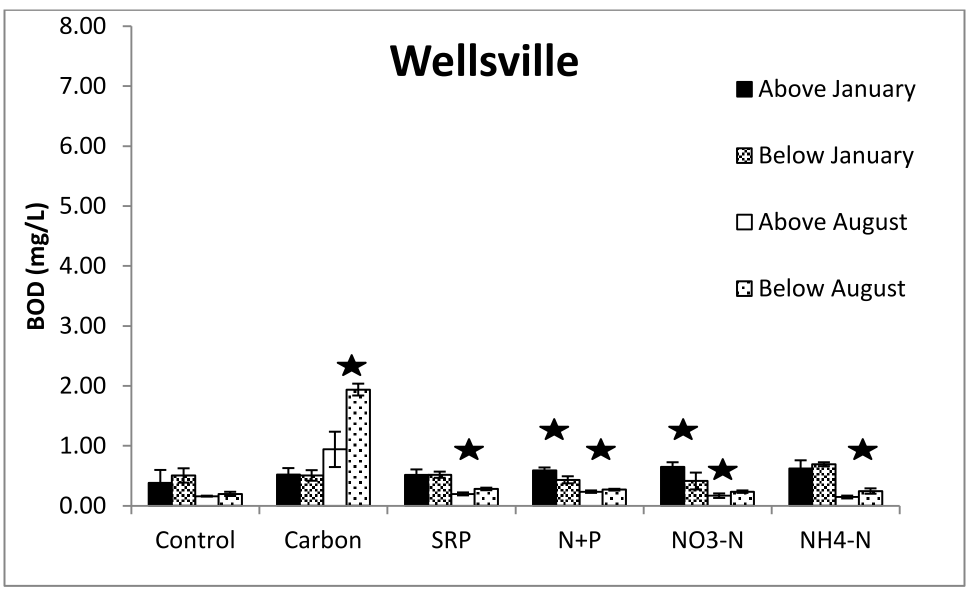


Figure 14 A-C: Bar charts comparing 24 hr. BOD levels of different treatments above and below the Brigham City and Wellsville POTWs for the months of January and August and Tremonton for the months of February and ★ = Significant difference above and below the POTW. A Pairwise t-test was performed to test significance (Appendix Table A8).

B


Tremonton
Above February
Below February
Above September
Below September
BOD (mg/L)
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Control
Carbon
SRP
N+P
NO3-N
NH4-N

C


Wellsville
Above January
Below January
Above August
Below August
BOD (mg/L)
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Control
Carbon
SRP
N+P
NO3-N
NH4-N

Due to the increase in nutrients released from the effluent below the POTWs, I also expected to see an increase in the SOD_{st} levels below the POTW compared to sites located above the POTW. After performing a two-way ANOVA (Appendix Table A 9) I found that the SOD_{st} levels were on average 23% significantly different ($p < 0.05$) below the POTW compared to the sites above. During spring the sites above the Brigham City and Tremonton POTW were significantly higher (except for the carbon and nitrate treatment in Tremonton). During the late summer season this trend was reversed and the sites below the POTW were significantly higher than above (except for the carbon and nitrate treatments in Brigham City) (Figure 15 A and C). A Pairwise t-test was performed to test significance (Appendix Table A 10). This trend suggests that during the spring the sites above the POTW were more nutrient limited and as the seasons progress the sites below the POTW became more nutrient limited. The Wellsville site also had samples that were significantly higher during the late summer season than during the spring season (Figure 15 B).

Nutrient Criteria

I used Classification and Regression Trees (CART) with conditional inference tree significance tests to identify statistically significant TN, TP, and DOC thresholds that separated BOD_{st} into different groups. I ran separate CART analyses for each the seasons. I took the log values of the control BODst as well as the log values of TN, TP and DOC during the summer and found the following threshold values: Low < -0.38 (0.42 mg/L)> High, Low <-1.35 (0.04 mg/L)> High and Low <0.72 (5.25 mg/L)> High respectively. (Figure 16 A, B, and C). CART did not identify significant thresholds

A

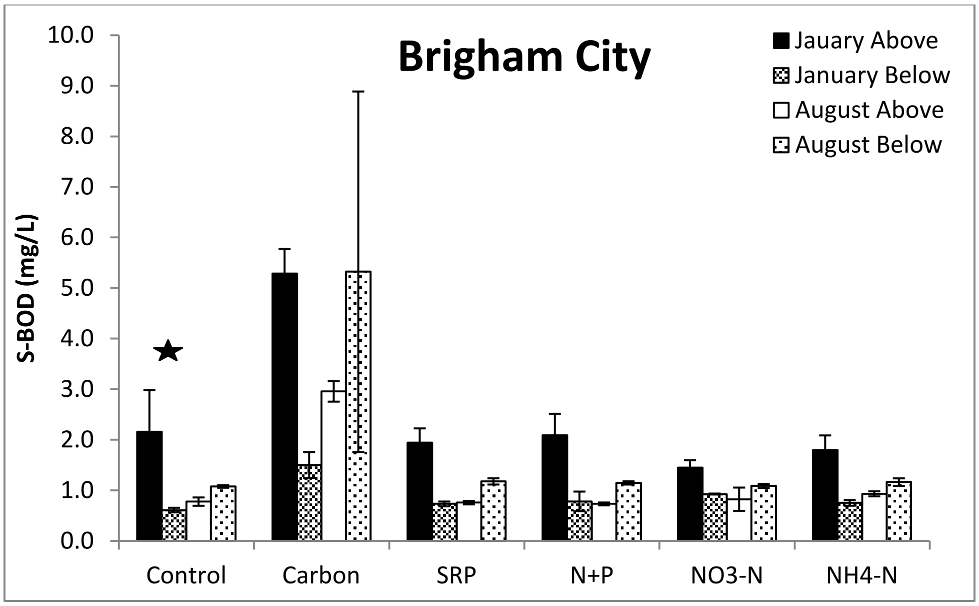
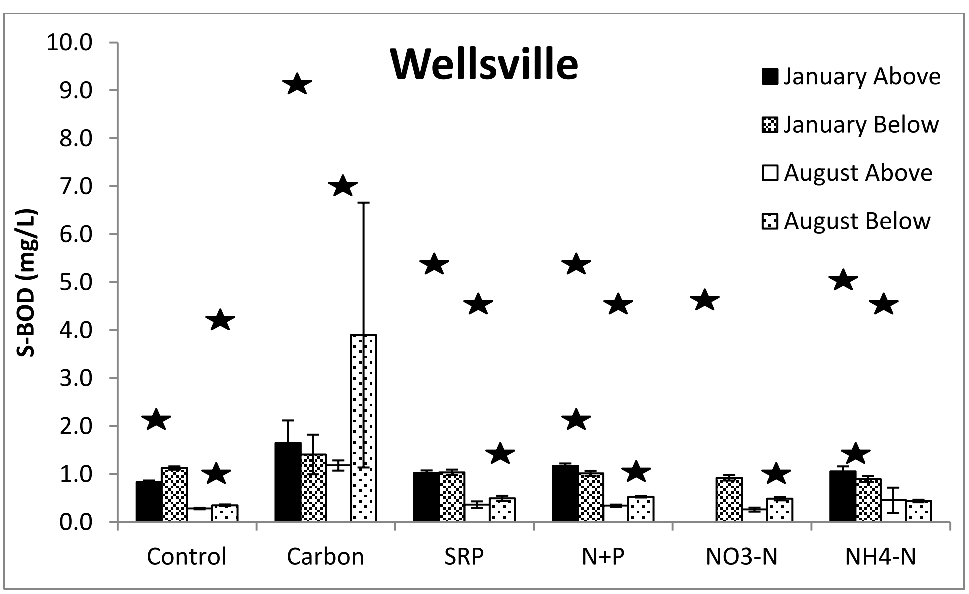
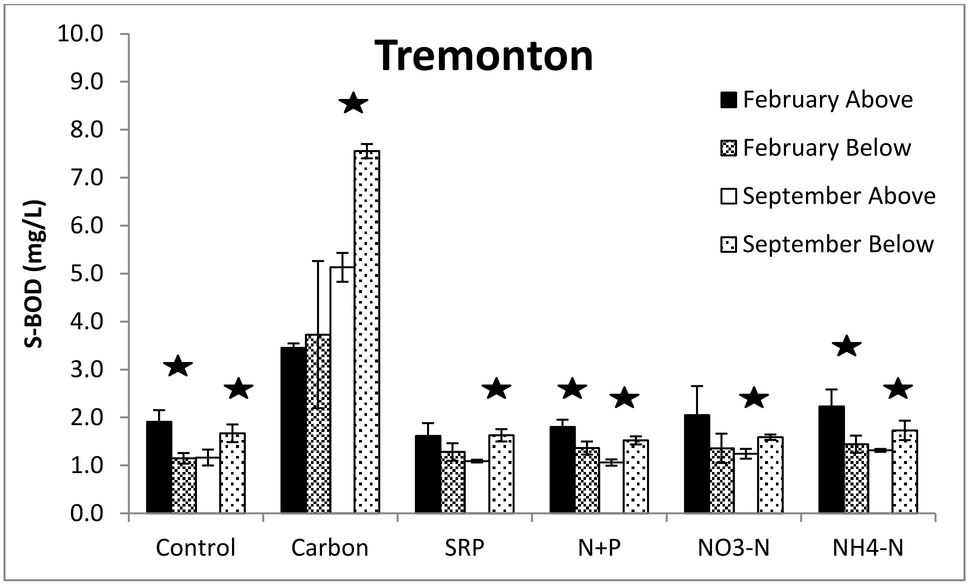


Figure 15 A-C: A (Brigham City), B (Tremonton), C (Wellsville). Bar charts comparing 24 hr. SOD_{st} levels of different treatments above and below the Brigham City, Tremonton and Wellsville POTWs for the months of January and August. ★= Significant difference. A pairwise t-test was performed to test significance (Appendix Table A10).

B



C



during the spring and late summer season for TP or DOD, or for the late summer TN (data not shown).

A

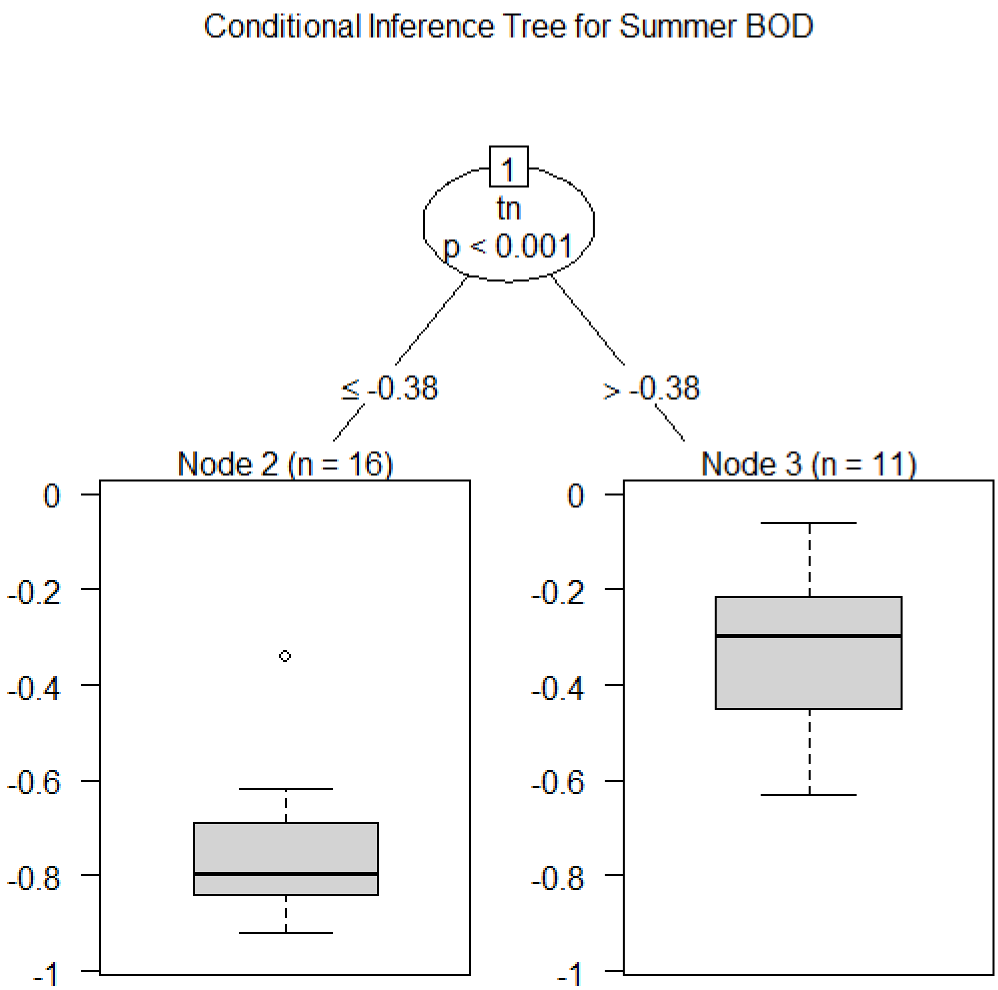
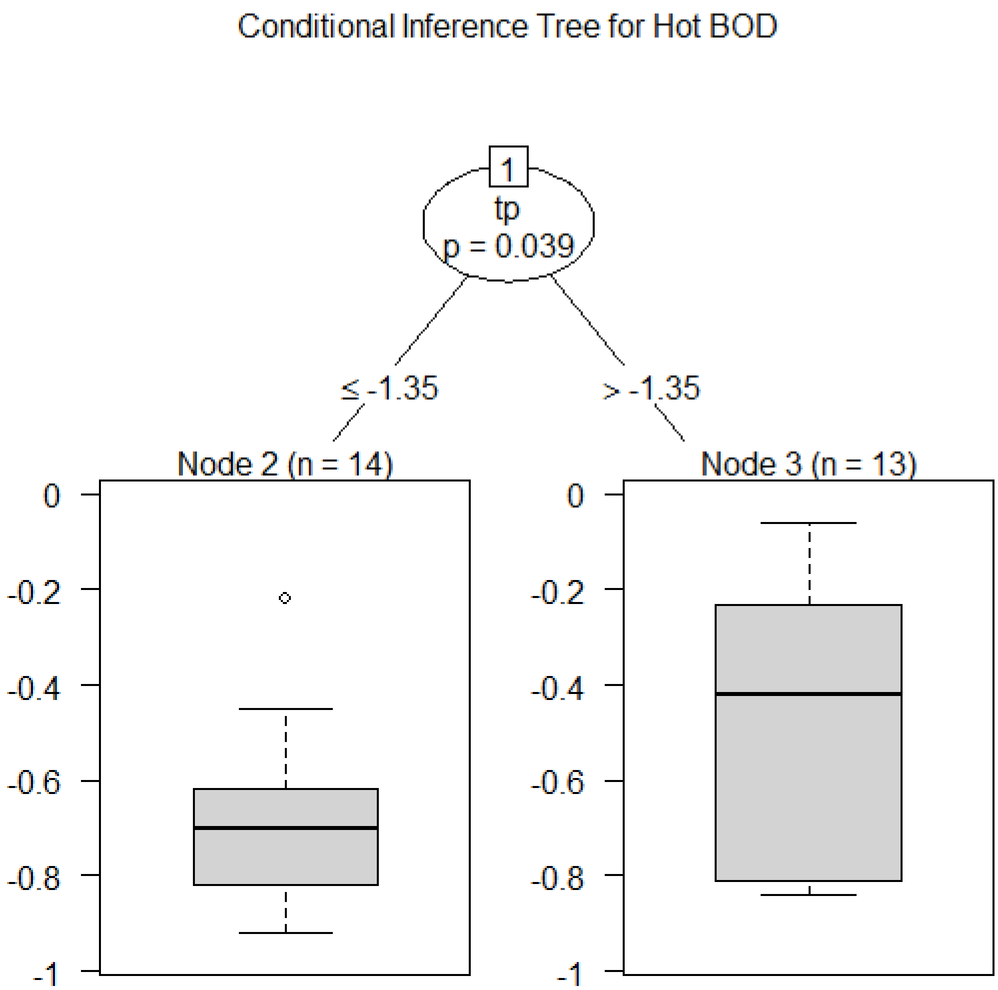
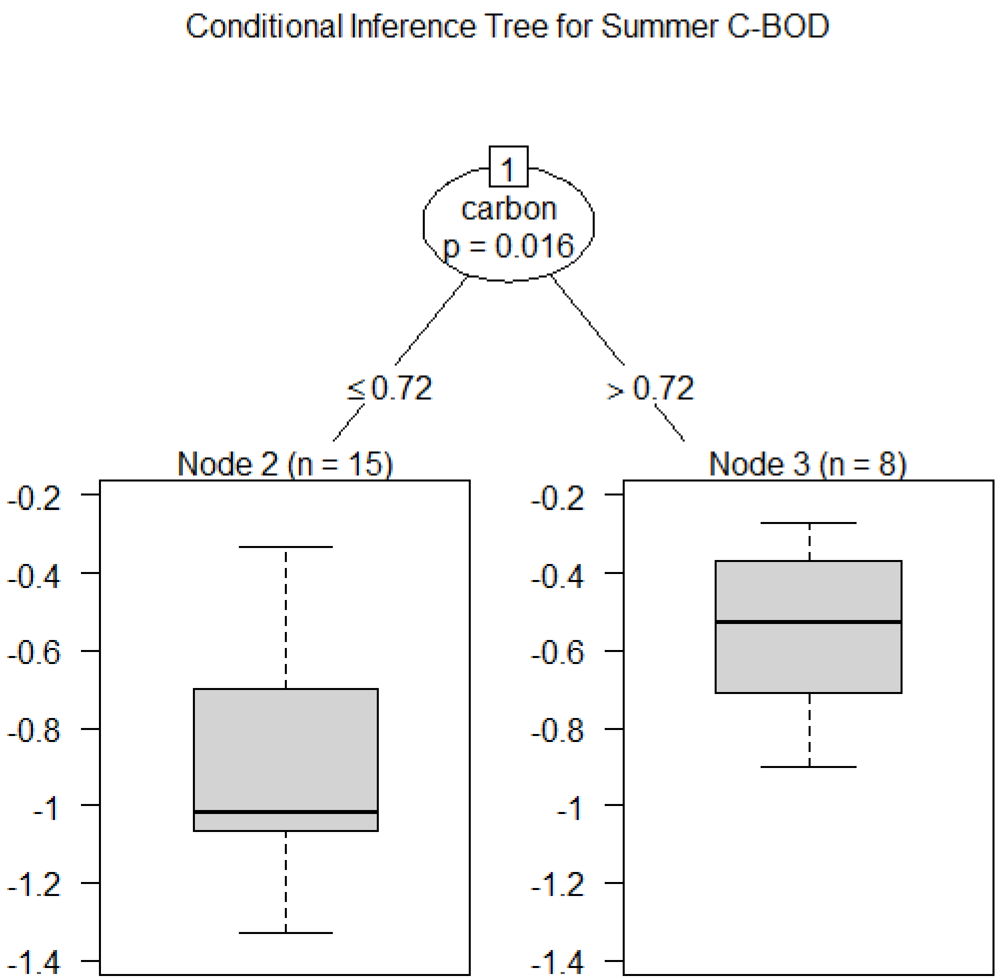


Figure 16 A-C: A (TP), B (TN), C (Carbon): Conditional inference tree for the summer season BOD. The boxplots show BODst of control treatments for the TP and TN data, and log of the C-BOD in for DOC. The values located beneath the p value are the log nutrient threshold values in micrograms/L. The summer season thresholds were TN (p-value < 0.001) and TP (p- value = 0.039) values of Low < 0.42 mg/L > High, Low < 0.04 mg/L > High, Low< 5.25 mg/L>High respectively.

B


Conditional Inference Tree for Hot BOD
1
tp
p = 0.039
≤ -1.35
> -1.35
Node 2 (n = 14)
Node 3 (n = 13)
0
-0.2
-0.4
-0.6
-0.8
-1
0
-0.2
-0.4
-0.6
-0.8
-1

C


Conditional Inference Tree for Summer C-BOD
1
carbon
p = 0.016
≤ 0.72
> 0.72
Node 2 (n = 15)
Node 3 (n = 8)
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4

DISCUSSION

Spatial and Temporal Variation in Physicochemical Conditions that Influence BOD

My study captured a wide range of physicochemical conditions in space and time. Temperature varied seasonally. Spring 2011 was unseasonably cool and sites experienced at least a twofold increase in temperature from the spring to summer seasons as well as a further increase in all but one of the sites during the late summer season (Table 1). BOD rates were low during spring season and were not related to ambient nutrients. As temperatures warmed in summer we saw significant effects of nutrients experimentally and along the ambient nutrient gradient. This is similar to the results found by Rosemond (1994) who found that ecosystem was most correlated to inorganic nitrogen during the summer.

Many mountainous rivers and streams receive their highest levels of discharge from snowmelt during spring runoff (Goldman et al. 1983), as was the case for my study sites. Snowmelt often carries with it large amounts of sediment and nutrients which are transported to streams (e.g. Pellerin et al. 2012). However, ambient nutrient concentrations at my study sites were often lowest in spring. I suspect that due to the atypical spring runoff with numerous rainstorms, which resulted in some of my sites experiencing more than three times the amount of discharge than regularly occurs during peak seasonal runoff, the ambient nutrient concentrations were diluted to lower levels than normal.

Nutrient Limitation

Newbold (1992) suggests that there are times when organic elements, such as carbon, and inorganic elements such as nitrogen and phosphorus may be in relatively short supply, in relation to the organisms that require them for growth, reproduction, and survival as well as other biological processes, as they travel downstream. It is accepted that over short periods nitrogen limits primary production in streams as often as phosphorus does and that this might vary temporally (Scott et al. 2008). Less research has focused on the effect of nutrients on community respiration or BOD.

My experiments established that BOD can be limited by inorganic nutrients, and also that BOD responds to labile carbon as expected. The nutrient concentration gradient between the reference sites and the sites located below the POTWs allowed me evaluate if nutrient limitations changed as a result of increases in ambient concentrations. I found that sites located above POTWs as well as reference sites were most likely P-limited because 37.5% of the observations made above POTWs were significantly different than the control BOD samples, and 33% of the reference site samples were different. This pattern changed below POTWs which were more often N-limited, with 50% of NO_3-N-amended observations higher than controls. The sites below the treatment facilities also had 41.8% of the N+P samples that were different from the control samples,

Because of the high nutrient concentration in effluent from the wastewater facilities I did not expect to observe nutrient limitation, much less N limitation, in the samples collected below POTWs. However, nitrogen limitation has been shown in many different bodies of water that receive wastewater effluents including the highly degraded

Mississippi River and the Danube River in Central Europe (Rabalais 2002; Pehlivanoglu and Sedlak 2004).

The observation of N limitation below POTWs might be a result of different factors. First, the N:P ratio in effluent may have shifted to favor N limitation. For example the average N:P ratio during spring in samples taken below the Brigham City POTW is 3.1 (±0.7) compared to the average in the samples taken above the Brigham City POTW is 44.1 (±19.6). During the late summer season the N:P ratio below the POTW is 1.5 and above is 25.7 Second, The bioavailability of dissolved organic matter (DOM) is highly variable (Wiegner et al. 2006). Thus the bioavailablity of N above and below POTWs may differ. Studies have shown that effluent TN contains anywhere from less than 10% of dissolved organic nitrogen (DON) to as much as 80% DON, the rest being in the form of inorganic nitrogen (Pehlivanoglu and Sedlak 2004). The average portion of DON below the Brigham City POTW was 47%, while below the Oakley POTW it was 78%, and it was at 58% below Tremonton and 51% below Wellsville.

BOD_{st} was most strongly DOC-limited compared to the inorganic nutrient treatments. The maximum BOD level that was obtained with carbon amendment was 9.18 mg/L. Compared to the other maximum levels of 1.71 mg/L for N+P, 1.65 mg/L for NO_3-N and 1.53 mg/L for SRP-P this was very high, and at least 79% of DOC-amended treatments were significantly different from the control treatments across all times and sample locations.

Despite the significant DOC limitation, there were not strong relationships between ambient BOD_{st} and DOC or between C-BOD and DOC. However, during the summer when DOC concentrations declined at all sites, I found a highly significant (p-value = 0.006) positive relationship between C-BOD and the ambient carbon concentration.

High rates of BOD_{st} in DOC-amended treatments may have been due to the priming effect, a hypothesis from soil science first introduced by Löhnis (1926). The priming effect is the stimulation of microbial activity by the introduction of labile organic matter. The priming effect enables large amounts of carbon or nitrogen and other nutrients to be released or immobilized for a short period of time (Kuzyakov et al. 2000). The mechanisms that control this rate are not completely known. It is generally accepted that it is controlled by energy, nutrient availability and stoichiometric constraints (Guenet et al. 2010). While there have been copious numbers of studies (Woods et al. 1987; Fontaine et al. 2003; Fontaine et al. 2004; Hamer and Marschner 2005; Kuzyakov 2010) done to show that the priming effect occurs in the terrestrial environment, very little research has been done to prove that the priming effect exists in aquatic ecosystems. However, Guenet et al. (2010) suggest that the priming effect is not unique to the terrestrial environment.

Treatments amended with DOC as ethanol (0.16 mg C/L) experienced the highest levels of BOD compared to the other nutrient treatments. In some cases these treatments were nearly anoxic after a 24-hour period. This occurrence of high levels of BOD, as a response of the carbon treatment, was not limited to waters below the wastewater facilities as hypoxic conditions existed in some of the reference sites as well. Based on stoichiometry, only 0.66 mg/L of oxygen should have been consumed as microbes respired the ethanol. However, 69% of my DOC-treatments exceeded that limit with the highest level being almost 14 times higher. This strongly suggests that the labile carbon added as ethanol stimulated more oxygen consumption (as measured by BOD) than was expected. These findings give support to the hypothesis of Guenet et al. (2010) that the priming effect occurs in the aquatic environment. Future studies should be done to obtain more evidence of this phenomenon occurring in rivers and streams.

N-BOD & C-BOD

I measured N-BOD directly by measuring BOD and C-BOD through inhibiting nitrification with nitrapyrin and then taking the difference between them (APHA 1998) instead of using a stoichiometric relationship (Deai et al. 1991). While some studies have shown that N-BOD is the dominant process in rivers (Deai et al. 1991) The mean C-BOD for my study sites was 0.26 mg/L and the mean N-BOD was 0.16 mg/L. These results suggest that C-BOD is playing a large role in these rivers..

Even though N-BOD happened, it was less important than C-BOD during this study. Had I performed the experiment for a longer period of time and incubated the samples at a higher temperature, the amount of nitrification would have increased and N-BOD would have possibly been the more dominant process. Gerardi (2002) noted that temperature and the inhibition of soluble forms of C-BOD have large impacts on nitrifying bacteria which slows down the N-BOD rate. Gerardi states that in activated sludge, as the temperature decreases there is a significant reduction in the nitrification rate and a significant increase with rising temperatures. It was noted that when temperatures increase above 45° C or decrease below 5° C nitrification ceases to occur

(Gerardi 2002). Gerardi (2002) also noted that in sludge activated waste the optimal temperature for nitrification is between 28°- 32° C and when nitrifying bacteria remain in the sludge for 7 days in that temperature range the bacteria have sufficient time to reproduce resulting in the rapid removal of ammonium ions. It is possible that since I incubated my samples at 22° C for 24 hours the nitrifying bacteria were not at their optimal temperature range. Moreover the population sizes could have been too low to significantly nitrify ambient NH_4. (Gerardi 2002). Further studies would have to be concluded at different temperatures for longer periods of time to determine the long term results of N-BOD.

However, even though my incubation time was for 24 hours nitrification was still occurring and was having an impact in BOD. It should be noted that in my study I found the ambient ammonium concentrations to be positively related to the N-BOD, p-value = < 0.001. Giving further evidence that even during the short time period of 24 hours nitrification is still playing a role in the river systems that I studied.

SOD_{st}

Sediment often contains a lot of organic matter and it has been shown to significantly influence the amount of dissolved oxygen in rivers and streams (Wang 1980). Boynton and Kemp (1985) looked at oxygen consumption budgets in the Chesapeake Bay in the spring and summer and found SOD_{st} to be an important term for water column O_2 budgets at all of their sites. They also found SOD_{st} occurred at higher levels in the summer season than in the spring. In my study, SOD_{st} rates were higher than BOD rates, and SOD_{st} rates were related to water column nutrient concentrations.

Volatile Suspended Solids

Because VSS is organic material it is an important aspect of many ecosystem processes (Hauer and Lamberti 2006). Consequently I thought that VSS would impact BOD levels. Since I did not find a significant relationship between BOD and VSS during the summer season but I did during the late summer season this suggests that VSS does play a role in BOD however the impact of it was possibly diminished by the high water level during runoff.

Nutrient Thresholds

The Utah Division of Water Quality (UDWQ) is currently in the process of creating functional indicators of nutrient enrichment in the form of numeric standards for select rivers in northern Utah (UDWQ 2012). They recently studied the impacts of varying nutrient levels on stream metabolism. They determined threshold values that allow them to know how primary production and respiration will react when the nutrients in those rivers fall below or exceed certain concentrations. They found the following threshold values: TN (mg/L) values of low < 0.24 > medium <1.28 > high. And TP (mg/L) values of low <0.02 > medium < 0.09> high (UDWQ 2012).

Using the BOD$_{st}$ information from all of my sites, BOD levels were positively correlated with, making it possible to create a nutrient threshold. Using CART (R version 2.15) I found high and low nutrient threshold values during the summer season for TN and TP TN value of 0.42 mg/L and TP value of 0.04 mg/L. I also found a threshold value of 5.25 mg/L for DOC.

The TN and TP thresholds identified in my study are similar to the lower and upper threshold values that were found by the UDWQ (TN values of 0.24 mg/L and 1.28 mg/L and TP values of 0.02 mg/L and 0.09 mg/L) . These values are also supported by the results found by (Miltner et al. 1998). In their study they found that in low order streams fish populations began to decrease after background nutrient levels exceeded 0.61 mg/L of total inorganic nitrogen and 0.06 mg/L of phosphorus. These results also support the idea that functional indicators for nutrient enrichment are achievable.

Hopefully these nutrient thresholds that have been established can act as a stepping stone to help establish stronger water quality standards that will help Utah state agencies increase water quality. To fully achieve this goal further studies should be carried out with nutrients being added to water samples at different nutrient concentrations during the different seasons of the year to determine if there are further nutrient concentration thresholds that need to be established for those seasons.

CONCLUSION

As the world population continues to grow, the amount of water pollution will most definitely follow that trend. The need for more wastewater treatment facilities will also be necessary to accommodate this population increase. Obtaining information on how these increasing nutrients are affecting our water resources is critical to the health of our nation's waterways. It is also important that I continue to look for ways to ensure that the nutrients that are entering our waterways are managed in a manner that will ensure that I have enough water to continue to maintain healthy aquatic ecosystems, irrigate crops and land, recreate and enough clean water to drink in the future.

Creating functional indicators of nutrient enrichment will provide water managers with more knowledge of the impacts that varying levels of nutrients will have on our water sources. This knowledge will allow them to formulate and implement better plans of action when deciding how much nutrients should be allowed to enter our waterways.

Since each watershed is unique and receives varying levels of influence from anthropogenic activities I suggest that water quality managers across the nation work on implementing nutrient thresholds in their regions to ensure the long term health for our nation's waterways. Ultimately, being proactive and continuing to establish good functional indicators of nutrient enrichment in rivers will ensure that I do not end up with a crisis on our hands and end up not having enough clean water to support a healthy aquatic ecosystem and all of our aquatic needs.

LITERATURE CITED

APHA. 1998. Standard methods for the examination of water and wastewater, 20th edition. American Public Health Association, Washington DC.

Babyak, M. A. 2004. What you see may not be what you get: A brief, nontechnical introduction to overfitting in regression-type models. Psychosomatic Medicine 66(3):411-421.

Bowman, G. T., and J. J. Delfino. 1980. Sediment oxygen demand techniques: A review and comparison of laboratory and in situ systems. Water Research 14(5):491-499.

Boynton, W. R., and W. M. Kemp. 1985. Nutrient regeneration and oxygen consumption by sediments along an estuarine salinity gradient. Marine Ecology - Progress Series 23:45-55.

Buck, S., G. Denton, W. Dodds, J. Fisher, D. Flemer, D. Hart, A. Parker, S. Porter, S. Rector, A. Steinman, J. Stevenson, J. Stoner, D. Tillman, S. Wang, V. Watson, and E. Welch. 2000. Nutrient criteria technical guidance manual, rivers and streams

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications 8(3):559-568.

Carson, R. T., and R. C. Mitchell. 1993. The value of clean water: the public's willingness to pay for boatable, fishable, and swimmable quality water Water Resouces Research 29(7):2445-2454.

Clarke, A. L., K. Weckström, D. J. Conley, N. J. Anderson, F. Adser, E. Andrén, V. N. de Jonge, M. Ellegaard, S. Juggins, P. Kauppila, A. Korhola, N. Reuss, R. J. Telford, and S. Vaalgamaa. 2006. Long-term trends in eutrophication and nutrients in the coastal zone. Limnology and Oceanography 51(1):385-397.

Cooper, A. B. 1986. Developing management guidelines for river nitrogenous oxygen demand. Journal (Water Pollution Control Federation) 58(8):845-852.

Corvalan, C., S. Hales, A. McMichael, and C. Butler. 2005. Ecosystems and human well-being : Health synthesis. Millennium Ecosystem Assessment.

Cox, B. A. 2003. A review of dissolved oxygen modelling techniques for lowland rivers. The Science of The Total Environment 314-316:303-334.

DAS. 2012a. Division of administrative rules. Salt Lake City.

DAS. 2012b. Utah administrative code r317-2-14. Salt Lake City.

De'ath, G., and K. E. Fabricius. 2000. Classification and regression trees: A powerful yet simple technique for ecological data analysis. Ecology 81(11):3178-3192.

Deai, J., T. Yida, Y. Gong, Z. Jianrong, and S. Yicheng. 1991. Factors affecting the relationship between the nbod values and the amounts of nitrogenous pollutants: A field study on the lee river. Water Research 25(4):485-489.

Dodds, W. K. 2006. Eutrophication and trophic state in rivers and streams. Limnology and Oceanography 51(1):671-680.

Edmondson, W. T. 1970. Phosphorus, nitrogen, and algae in lake washington after diversion of sewage. Science (New York, N.Y.) 169(3946):690-691.

EPA. 2012a. Regression analysis. http://www.Epa.Gov/caddis/da_basic_2.Html.

EPA. 2012b. Water is worth it. http://water.epa.gov.

Fan, A. M., and V. E. Steinberg. 1996. Health implications of nitrate and nitrite in drinking water: An update on methemoglobinemia occurrence and reproductive and developmental toxicity. Regulatory Toxicology and Pharmacology 23(1):35-43.

Fontaine, S., G. Bardoux, D. Benest, B. Verdier, a. Mariotti, and L. Abbadie. 2004. Mechanisms of the priming effect in a savannah soil amended with cellulose. Soil Science Society of America Journal 68(1):125-131.

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: A question of microbial competition? Soil Biology and Biochemistry 35(6):837-843.

Gerardi, M. H. 2002. Nitrification in the activated sludge process. Water encyclopedia. John Wiley & Sons, Inc.

Gilinsky, E., J. M. Capacasa, M. G. Baker, and E. S. King. 2009. An urgent call to action – report of the state-epa nutrient innovations task group. Nutrient Innovations Task Group Report:1-34.

Goldman, C. R., Alexander J. Horne. 1983. Limnology. McGraw-Hill, New York.

Guenet, B., M. Danger, L. Abbadie, and G. Lacroix. 2010. Priming effect: Bridging the gap between terrestrial and aquatic ecology. Ecology 91(10):2850-2861.

Hamer, U., and B. Marschner. 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biology and Biochemistry 37(3):445-454.

Hamilton, S. K., S. J. Sippel, D. F. Calheiros, and J. M. Melack. 1997. An anoxic event and other biogeochemical effects of the pantanal wetland on the paraguay river. Limnology and Oceanography 42(2):257-272.

Hasler, A. D. 1947. Eutrophication of lakes by domestic drainage. Ecology 28(4):383-395.

Hauer, F. R., and G. A. Lamberti. 2006. Methods in stream ecology. Academic Press, San Diego, California.

Havens, K. E. 2003. Phosphorus–algal bloom relationships in large lakes of south florida: Implications for establishing nutrient criteria. Lake and Reservoir Management 19(3):222-228.

Heiskary, S., and H. Markus. 2001. Establishing relationships among nutrient concentrations, phytoplankton abundance, and biochemical oxygen demand in minnesota, USA, rivers. Lake and Reservoir Management 17(4):251-262.

Hothorn, T., K. Hornik, and A. Zeileis. 2009. Party: a laboratory for recursive partytioning. R package version 2.13.

Kuzyakov, Y. 2010. Priming effects: interactions between living and dead organic matter. Soil Biology and Biochemistry 42(9):1363-1371.

Kuzyakov, Y., J. K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32(11–12):1485-1498.

Liu, Y., M. A. Evans, and D. Scavia. 2010. Gulf of mexico hypoxia: exploring increasing sensitivity to nitrogen loads. Environmental Science & Technology 44(15):5836-5841.

Löhnis, F. 1926. Nitrogen availability of green manures. Soil Science 22(4):253-290.

MacPherson, T. A., L. B. Cahoon, and M. A. Mallin. 2007. Water column oxygen demand and sediment oxygen flux: patterns of oxygen depletion in tidal creeks. Hydrobiologia 586(1):235-248.

Millier, T., T. Toole, R. L. Denton, A. Hultquist, JamesHarris, C. Adams, A. Dickey, M. Allred, D. Wham, D. Griffin, M. Garn, L. Shull, J. Robinson, K. Lundeen, M. Herkimer, F. Reynolds, S. Jensen, S. Gerner, and M. Stanger. 2006. Utah 2006 integrated report volume 1 - 305(b) assessment. U. D. o. E. Quality, editor, Salt Lake CIty.

Miltner, R. J., Rankin, and T. Edward. 1998. Primary nutrients and the biotic integrity of rivers and streams. Freshwater Biology 40(1):145-158.

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27(0):31-36.

Newbold, J. D. 1992. Cycles and spirals of nutrients. Pages 379-408 *in* Rivers handbook, volume 1. Blackwell Scientific, Oxford, UK.

Paerl, H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnology and Oceanography 33(4):823-847.

Pehlivanoglu, E., and D. L. Sedlak. 2004. Bioavailability of wastewater-derived organic nitrogen to the alga selenastrum capricornutum. Water Research 38(14–15):3189-3196.

Pellerin, B., J. Saraceno, J. Shanley, S. Sebestyen, G. Aiken, W. Wollheim, and B. Bergamaschi. 2012. Taking the pulse of snowmelt: In situ sensors reveal seasonal, event and diurnal patterns of nitrate and dissolved organic matter variability in an upland forest stream. Biogeochemistry 108(1-3):183-198.

Rabalais, N. N. 2002. Nitrogen in aquatic ecosystems. AMBIO: A Journal of the Human Environment 31(2):102-112.

Rabalais, N. N., R. E. Turner, B. K. Sen Gupta, E. Platon, and M. L. Parsons. 2007. Sediments tell the history of eutrophication and hypoxia in the northern gulf of mexico. Ecological Applications 17(5):S129-S143.

Romalho, R. S. 1977. Introduction to wastewater treatement processes. Academic Press, New York.

Rosemond, A. D. 1994. Multiple factors limit seasonal variation in periphyton in a forest stream. Journal of the North American Benthological Society 13(3):333-344.

Sachar, J. H., and G. Currey. 1999. Water permiting 101. U. E. P. Agency, Washington D.C.

Scott, J. T., D. A. Lang, R. S. King, and R. D. Doyle. 2008. Nitrogen fixation and phosphatase activity in periphyton growing on nutrient diffusing substrata: evidence for differential nutrient limitation in stream periphyton. Journal of the North American Benthological Society 28(1):57-68.

SEMI, D. O. A. N. B. 1993. Method 350.1 determination of ammonia nitrogen by semi-automated colorimetry.

Smith, A. J., and C. P. Tran. 2010. A weight-of-evidence approach to define nutrient criteria protective of aquatic life in large rivers. Journal of the North American Benthological Society 29(3):875-891.

Smith, V. H., S. B. Joye, and R. W. Howarth. 2006. Eutrophication of freshwater and marine ecosystems. Limnology and Oceanography 51(1):351-355.

Streeter, W. H., and E. B. Phelps. 1925. A study of the pollution and natural purification of the ohio river. Public Health Bulletin 146, U.S. Public Health Service, Washington D.C.

Sullivan, A. B., D. M. Snyder, and S. A. Rounds. 2010. Controls on biochemical oxygen demand in the upper klamath river, oregon. Chemical Geology 269(1-2):12-21.

Udeigwe, T. K., and J. J. Wang. 2010. Biochemical oxygen demand relationships in typical agricultural effluents. Water Air and Soil Pollution 213(1-4):237-249.

UDWQ. 2012. Whole stream metabolism, unpublished. Page 6 *in* Utah Division of Water Quality, Salt Lake City.

USEPA. 2011. National summary of impaired waters and tmdl information.

Vandenberg, J. A., M. C. Ryan, D. D. Nuell, and A. Chu. 2005. Field evaluation of mixing length and attenuation of nutrients and fecal coliform in a wastewater effluent plume. Environmental Monitoring and Assessment 107(1):45-57.

Volkmar, E. C., and R. A. Dahlgren. 2006. Biological oxygen demand dynamics in the lower san joaquin river, california. Environmental Science and Technology 48(18):5653-5660.

Wang, W. 1980. Fractionation of sediment oxygen demand. Water Research 14(6):603-612.

Whittier, T. R., R. M. Hughes, J. L. Stoddard, G. A. Lomnicky, D. V. Peck, and A. T. Herlihy. 2007. A structured approach for developing indices of biotic integrity: Three examples from streams and rivers in the western USA. Transactions of the American Fisheries Society 136(3):718-735.

Wiegner, T. N., S. P. Seitzinger, P. M. Glibert, and D. A. Bronk. 2006. Bioavailability of dissolved organic nitrogen and carbon from nine rivers in the eastern united states. Aquatic Microbial Ecology 43(3):277-287.

Wolowicz, M., A. Sokolowski, A. S. Bawazir, and R. Lasota. 2006. Effect of eutrophication on the distribution and ecophysiology of the mussel mytilus trossulus (bivalvia) in southern baltic sea (the gulf of gdañsk). Limnology and Oceanography 51(1):580-590.

Woods, L. E., C. V. Cole, L. K. Porter, and D. C. Coleman. 1987. Transformations of added and indigenous nitrogen in gnotobiotic soil: a comment on the priming effect. Soil Biology and Biochemistry 19(6):673-678.

Worm, B., and H. K. Lotze. 2006. Effects of eutrophication, grazing, and algal blooms on rocky shores. Limnology and Oceanography 51(1):569-579.

APPENDIX

Table A 1. Mean BOD % of Control for each treatment during the different seasons (standard deviations in parentheses). In some cases, no replicates were run so the calculation of a standard deviation was not possible.

Site	Season	Carbon Treatment (BOD % of Control)		NO_3-N Treatment (BOD % of Control)		NH_4-N Treatment (BOD % of Control)		N+P Treatment (BOD % of Control)		SRP Treatment (BOD % of Control)	
Above Wellsville	Spring	387	±220	137	±30	136	±30	129	±23	134	±9
Above Wellsville	Summer	239	±8	95	±15	89	±14	128	±5	140	±16
Above Wellsville	Late Summer	588		105		91		147		123	
Below Wellsville	Spring	260	±161	94	±13	110	±25	93	±9	104	±9
Below Wellsville	Summer	379	±223	110	±3	113	±10	209	±82	190	±67
Below Wellsville	Late Summer	981		119		124		135		142	
Blacksmith Fork	Spring	526	±405	82	±28	82	±14	73	±37	104	±36
Blacksmith Fork	Summer	245	±110	145	±48	119	±20	182	±2	163	±8
Blacksmith Fork	Late Summer	393		110		115		119		124	
Bl. Twin Bridges	Spring	621	±550	109	±3	112	±13	106	±8	103	±9
Bl. Twin Bridges	Summer	207	±86	160	±26	173	±21	175	±2	187	±2
Bl. Twin Bridges	Late Summer	415		137		115		140		153	
Above Brigham City	Spring	593	±322	114	±9	112	±1	109	±7	125	±12
Above Brigham City	Summer	730	±559	95	±19	85	±7	129	±2	123	±7
Above Brigham City	Late Summer	828		118		165		149		167	
Below Brigham City	Spring	923	±700	133	±32	135	±78	115	±19	118	±11
Below Brigham City	Summer	1040	±909	205	±83	220	±107	152	±4	148	±12
Below Brigham City	Late Summer	2545		127		127		124		139	
Ltl. Bear @ R-Xing	Spring	567	±423	109	±30	116	±5	118	±18	122	±1
Ltl. Bear @ R-Xing	Summer	371	±232	126	±13	112	±12	160	±20	166	±7
Ltl. Bear @ R-Xing	Late Summer	2213		99		107		118		131	
Avon	Spring	1055	±1335	160	±20	123	±31	121	22	124	±31
Avon	Summer	281	±81	103	±9	76	±26	145	±49	133	±33
Avon	Late Summer	4187		143		126		145		159	

Above Tremonton	Spring	265	±44	127	±84	103	±18	100	±23	107	±10
Above Tremonton	Summer	284	±168	118	±37	125	±40	135	±6	131	±17
Above Tremonton	Late Summer	776		108		148		96		93	
Below Tremonton	Spring	407	±61	147	±34	120	±36	117	±17	108	±6
Below Tremonton	Summer	506	±173	167	±2	145	±58	134	±22	119	±13
Below Tremonton	Late Summer	1452		148		140		146		139	
Logan R@1000 W.	Spring	400	±167	104	±20	113	±14	102	±12	103	±15
Logan R@1000 W.	Summer					Site Flooded					
Logan R@1000 W.	Late Summer	5736		119		116		133		165	
Logan R. Dugway	Spring	397	±134	102	±7	131	±46	84	±25	103	±22
Logan R. Dugway	Summer	292	±292	139	±51	146	±30	121	±17	116	±6
Logan R. Dugway	Late Summer	1379		86		99		95		109	
Above Oakley	Spring	246	±24	102	±7	96	±9	108	±35	120	±28
Above Oakley	Summer	224		96		104		115		141	
Above Oakley	Late Summer	457		141		155		150		176	
Below Oakley	Spring	265	±238	136	±55	124	±55	111	±46	124	±54
Below Oakley	Summer	185		69		76		123		110	
Below Oakley	Late Summer	352		134		130		124		136	
Above Rockport	Spring	643		118		265		122		102	
Above Rockport	Summer	593		133		153		160		164	
Above Rockport	Late Summer	1191		119		147		149		161	
Provo River	Spring	127		94		127		71		108	
Provo River	Summer	177		138		155		158		144	
Provo River	Late Summer	167		53		117		100		131	

Table A 2. Seasonal average ambient nutrient concentration for all of the sites (standard deviation located in parenthesis). The data that do not have standard deviations are for data that only has one value because I only visited that site once during the season.

Site	Nutrient	Spring	Summer	Late Summer
Wellsville-Above	NH_4-N	15.10 (± 4.5)	30.35 (± 26.9)	47.8
	NO_3-N	588.9 (± 124.5)	671.9 (± 613.9)	1473
	SRP	4.03 (± 1.9)	7.85 (± 8.7)	11.8
	TN	1210 (± 371.8)	1373.90 (± 1028.3)	2870.
	TP	41.2 (± 12.1)	47.40 (± 22.8)	74.3
Wellsville-Below	NH_4-N	77.6 (± 61.1)	25.85 (± 12.2)	81.7
	NO_3-N	650.7 (± 256.7)	628.50 (± 630)	1167
	SRP	6.23 (± 3.7)	4.10 (± 0)	19.1
	TN	1251 (± 228.1)	1260.50 (± 862)	2121
	TP	42.2 (± 41.2)	56.90 (± 5.5)	82.3
Blacksmith Fork River	NH_4-N	17.6 (± 18)	5.85 (± 0.2)	7
	NO_3-N	226.7 (±43.3)	231.90 (± 47.9)	277.8
	SRP	12.0 (± 4)	12.60 (± 4.4)	7.9
	TN	395.7 (± 84.8)	377.7 (± 61.9)	543.9
	TP	39.2 (± 27.9)	33 (± 23.0)	24.1
Logan River Below Twin	NH_4-N	10.3 (± 5.5)	2.1 (± 0.3)	4.9
	NO_3-N	101.6 (± 32.1)	47.7 (± 24.1)	58.2
	SRP	4.7 (± 2.4)	11.35 (± 4.1)	8.9
	TN	202.9 (± 23.4)	189.0 (± 67.3)	No Data
	TP	11.8 (± 17)	33.6 (± 10)	No Data
Brigham City-Above	NH_4-N	11.7 (± 3.3)	10.45 (± 1.1)	12
	NO_3-N	342.7 (± 22.2)	210.10 (± 55)	347
	SRP	3.8 (± 1.9)	7.40 (± 2.7)	7.2
	TN	773.1 (± 84.9)	596.9 (± 0.1)	721.5
	TP	44.1 (± 18.3)	43.35 (± 5.7)	62.1
Brigham City-Below	NH_4-N	40.9 (± 2.5)	78.35 (± 43.4)	66.6
	NO_3-N	1125 (± 727.2)	1419 (± 666)	2648

	SRP	94.9 (± 100.2)	303.8 (± 270.3)	539.3
	TN	2120 (± 1191)	2747 (± 1173)	4390
	TP	162.1 (± 116.7)	411.7 (± 308.6)	633.7
Little Bear River, West of	NH_4-N	12.0 (± 3.7)	10.2 (± 0.9)	13.7
	NO_3-N	150.8 (± 23.8)	177.6 (± 71.6)	191.0
	SRP	3.5 (± 2.2)	3.1 (± 0.7)	8.8
	TN	346.3 (±111)	331.7 (± 88.1)	350.7
	TP	36.9 (± 15.9)	49.4 (± 11.5)	28.4
South Fork Little Bear River	NH_4-N	4.9 (± 0.6)	7.4 (± 1.8)	13.8
	NO_3-N	70.8 (± 70.7)	164.4 (± 39.8)	172.1
	SRP	4.1 (± 2.0)	4.1 (± 1.3)	1.8
	TN	262.8 (± 58.3)	324.7 (± 16.3)	303.2
	TP	27.8 (±12.9)	52.8 (± 13.6)	14.9
Tremonton-Above	NH_4-N	74.8 (± 49.7)	65.6 (± 28.9)	33.1
	NO_3-N	968 (± 315.6)	1369 (± 591.1)	1529
	SRP	16.3(± 4.1)	17.4 (± 14.4)	6.61
	TN	1965.3 (± 509.8)	3052 (± 738.2)	2780
	TP	73.5 (± 45.1)	69.6 (± 73.4)	85.5
Tremonton-Below	NH_4-N	354 (± 104.5)	556.1 (± 44.1)	874.9
	NO_3-N	813.3 (± 273.7)	1266.5 (± 491.4)	1510
	SRP	43.5 (± 8.9)	66.6 (± 29.9)	40.8
	TN	3031	3135	4111
	TP	337.9	656.5	132.3
Logan River at 1000 West	NH_4-N	8.4 (± 2.6)	No Data	12.7
	NO_3-N	320.5 (± 29.3)	No Data	329.4
	SRP	1.7 (± 1.3)	No Data	3.8
	TN	407.5 (± 41.4)	No Data	419.8
	TP	8.5 (± 0.2)	No Data	13.5

Logan River Below	NH_4-N	11 (± 7)	4.3 (± 0.8)	No Data
	NO_3-N	53.9 (± 10	38 (± 30.2)	No Data
	SRP	13.1 (± 8.6)	8 (± 3.3)	No Data
	TN	195.7 (± 30.4)	189.2 (± 1.34)	No Data
	TP	19.2 (± 2.8)	32.4 (± 6.2)	No Data
Oakley-Above	NH_4-N	5.7 (± 2.4)	3.4	8.7
	NO_3-N	65.7 (± 17.7)	45	2.7
	SRP	1.5 (±1)	4.6	0.6
	TN	206.3 (± 104.9)	215.1	11.5
	TP	13.3 (± 15.4)	43.1	6.5
Oakley-Below	NH_4-N	2.2 (± 0.6)	1.1	2.2
	NO_3-N	65.4 (± 16.7)	56.4	5.5
	SRP	2.1 (± 0.4)	5.7	0.8
	TN	220.5 (± 108.1)	282	99.9
	TP	17.5 (± 20.0)	61.1	2.5
Upper Provo River	NH_4-N	1.5	2.5	1.2
	NO_3-N	45.1	12.5	22.2
	SRP	0.9	1.2	5.4
	TN	189.1	312.4	112.7
	TP	8.2	26.9	3.4
Weber River Above	NH_4-N	6.2	2	17.4
	NO_3-N	108.8	68.1	207.9
	SRP	7	11	10.7
	TN	251.9	371	515.5
	TP	23.6	86.3	59.9

Table A 3. Welch two sample t-test comparing control BOD vs. control S-BOD. S-BOD was significantly different from BOD, p-value < 0.05.

Welch two sample t-test Control BOD vs Control S-BOD

```
> t.test(controlbodtest$log_wbod,controlbodtest$log_sbod)
        Welch Two Sample t-test
data:  controlbodtest$log_wbod and controlbodtest$log_sbod
t = -6.9109, df = 84.618, p-value = 8.45e-10
alternative hypothesis: true difference in means is not equal to
0
95 percent confidence interval:
 -0.4406987 -0.2437656
sample estimates:
 mean of x  mean of y
-0.5386905 -0.1964583
```

Table A 4. Mean S-BOD % of Control for each treatment during the different seasons (standard deviation located in parenthesis).

Site	Season	Carbon Treatment (BOD % of Control)		NO_3-N Treatment (BOD % of Control)		NH_4-N Treatment (BOD % of Control)		N+P Treatment (BOD % of Control)		SRP Treatment (BOD % of Control)	
Above Wellsville	Spring	327.3	±30.2	109.8	±9.9	125.3	±3.6	109.6	±7.4	115.4	±6.4
Above Wellsville	Late Summer	417.7		92.0		160.2		121.2		129.2	
Below Wellsville	Spring	353.1	±226.4	103.1	±19.0	104.8	±22.8	112.1	±21.7	110.5	±20.0
Below Wellsville	Late Summer	588.6		151.4		128.6		154.3		162.9	
Blacksmith Fork	Spring	600.2	±445.0	108.4	±3.9	110.3	±22.2	99.7	±22.7	100.8	±16.9
Blacksmith Fork	Late Summer	542.1		131.6		159.6		182.5		157.9	
Bl. Twin Bridges	Spring	282.3	±139.9	104.4	±10.3	128.9	±31.0	106.8	±13.0	101.0	±11.0
Bl. Twin Bridges	Late Summer	214.1		113.0		123.9		128.3		137.0	
Above Brigham City	Spring	387.3	±133.4	76.4	±17.4	91.0	±13.9	95.1	±1.3	100.1	±9.2
Above Brigham City	Late Summer	380.4		106.1		119.9		94.2		97.4	
Below Brigham City	Spring	610.3	±507.1	113.2	±34.1	109.3	±17.2	118.1	±14.8	108.9	±10.9
Below Brigham City	Late Summer	494.0		100.9		107.9		106.5		109.3	
Ltl. Bear @ R-Xing	Spring	453.9	±390.1	116.0	±13.8	133.6	±5.5	115.5	NA	127.1	±11.8

Ltl. Bear @ R-Xing	Late Summer	2170.3		110.9		113.9		131.7		139.6	
Avon	Spring	279.9	±135.4	117.6	±21.2	120.1	±42.2	134.6	±37.3	118.7	±23.8
Avon	Late Summer	1565.2		90.2		103.6		115.2		110.7	
Above Tremonton	Spring	168.9	±16.5	98.4	±12.4	102.2	±20.5	80.4	±13.6	88.7	±5.7
Above Tremonton	Late Summer	330.3		106.7		255.8		-79.6		93.6	
Below Tremonton	Spring	314.7	±14.3	94.7	±8.5	123.9	±2.6	110.6	±11.5	110.3	±1.8
Below Tremonton	Late Summer	452.2		95.1		103.6		91.2		97.5	
Logan R @1000 W.	Spring	323.4	±17.4	99.7	±0.5	100.7	±23.6	100.2	±4.1	100.3	±9.0
Logan R @1000 W.	Late Summer	696.1		83.7		87.1		100.6		96.1	
Logan R Dugway	Spring	306.6	±92.0	104.1	±7.1	139.4	±18.7	101.5	±2.2	102.5	±3.5
Logan R Dugway	Late Summer	3645.5		110.2		146.6		144.3		159.1	
Above Oakley	Spring	292.5		112.6		144.0		139.6		120.1	
Above Oakley	Late Summer	282.1		99.1		100.9		90.7		119.7	
Below Oakley	Spring	310.9		106.3		110.3		97.7		86.3	
Below Oakley	Late Summer	200.7		119.0		74.1		140.1		119.7	
Above Rockport	Spring	NA		NA		NA		NA		NA	
Above Rockport	Late Summer	437.3		114.0		99.3		123.3		130.7	
Provo River	Spring	NA		NA		NA		NA		NA	
Provo River	Late Summer	346.8		124.7		129.9		175.3		174.0	

Table A 5. TN vs NO_3 ANOVA. TN was significantly related to NO_3, p-value <0.05.
TN vs NO_3 ANOVA

```
                      Df Sum Sq Mean Sq F value   Pr(>F)    
matrix.spring$log_NO3  1  4.826   4.826   128.9 1.88e-13 ***
Residuals             36  1.347   0.037                     
---
```

Table A 6. TP vs SRP ANOVA. TP was significantly related to SRP, p-value <0.05.
TP vs SRP ANOVA

```
                      Df Sum Sq Mean Sq F value   Pr(>F)    
matrix.spring$log_SRP  1  5.009   5.009   62.02 2.42e-09 ***
Residuals             36  2.907   0.081                     
```

Figure A1

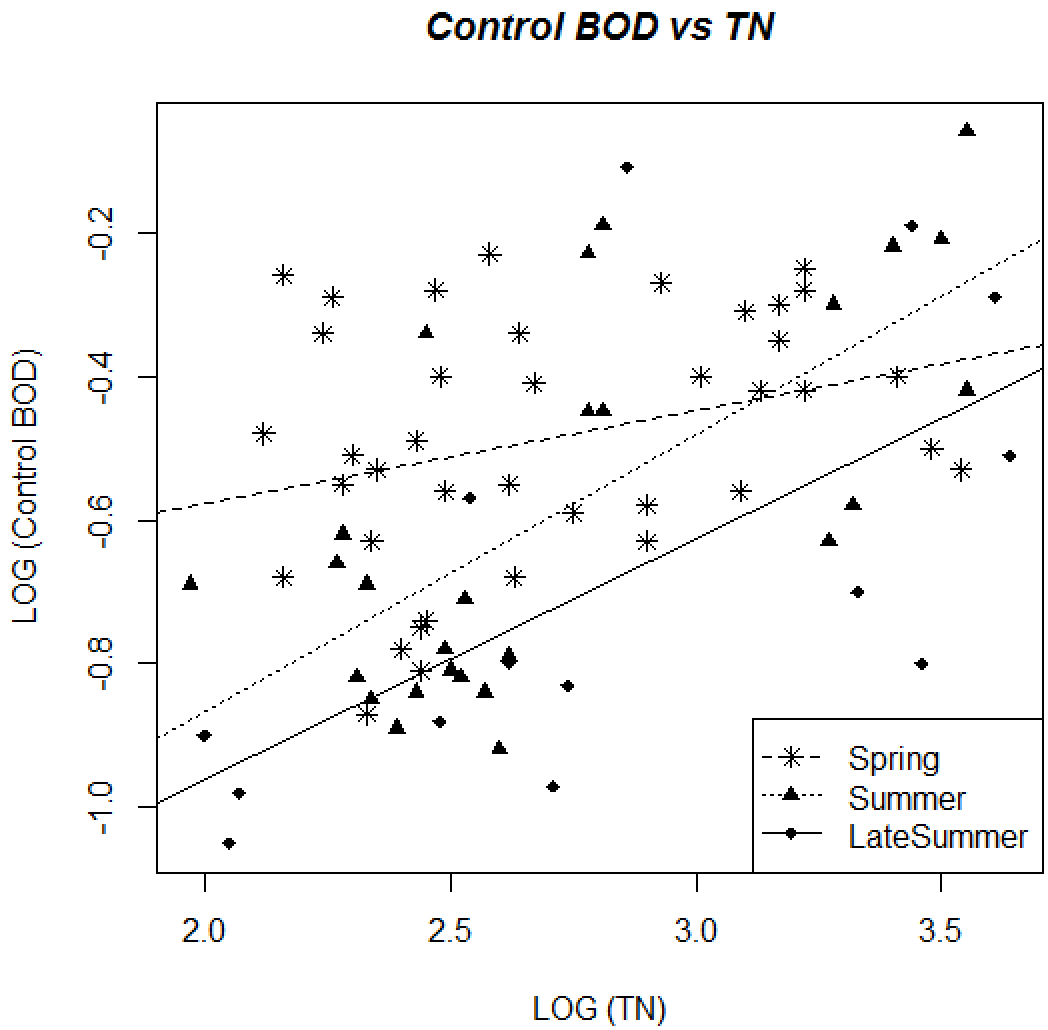


Figure A 1. Seasonal ambient total nitrogen concentration vs. control BOD. Linear regression was also run for total nitrogen (TN) to determine if ambient TN was significantly related to control BODst as well as NO_3 BODst during the different seasons. During the spring season no significant relationship was determined when comparing control BOD and ambient nutrient concentrations (p-value = 0.058, $r^2 = 0.0937$). Both the summer and late summer seasons were significantly related (p-value < 0.001, $r^2 = 0.4608$, and p-value = 0.014, $r^2 = 0.4103$) respectively.

Figure A2

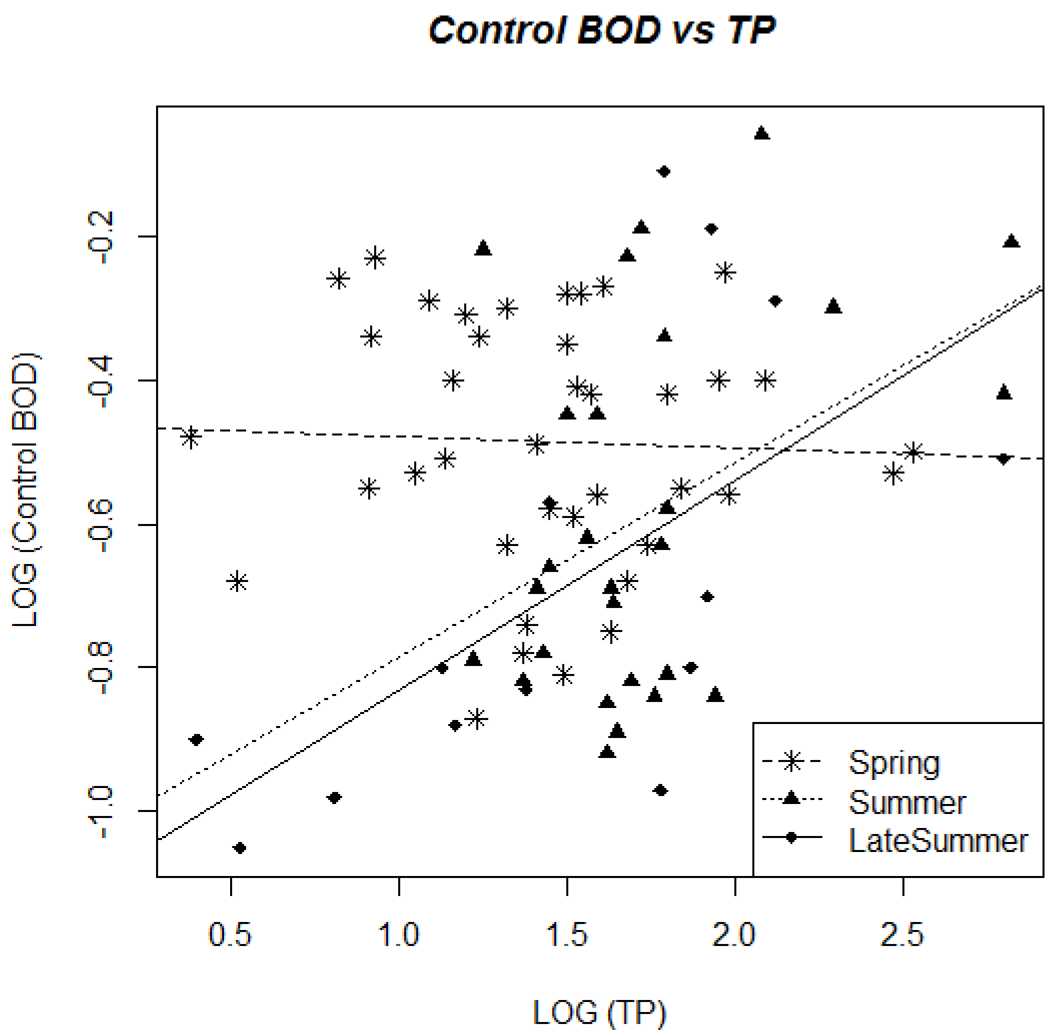


Figure A 2. Seasonal ambient total phosphorus concentration vs. control BOD. Linear regression for total phosphorus (TP) to determine if ambient TP was significantly related to control BODst as well as SRP BODst during the different seasons. During the spring season no significant relationship was determined when comparing control BOD and ambient nutrient concentrations (p-value = 0.794, r^2 = 0.0019). Both the summer and late summer seasons were significantly related (p-value = 0.036, r^2 = 0.1638, and p-value = 0.016, r^2 = 0.3936) respectively.

Table A 7. Two-way ANOVA comparing the sample sites BOD response above and below the POTWs. POTW = Publicly Owned Treatment Works Facility, M = Month, AB = Above/Below, Trt = Treatment. There was a significant difference found in all of the factors as well as all of the interactions.

Above vs. Below BOD Two Way ANOVA Limiting nutrients

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
POTW	3	24.12	8.041	949.223	< 2e-16	***
M	18	22.83	1.268	149.731	< 2e-16	***
AB	1	1.59	1.591	187.754	< 2e-16	***
Trt	5	44.92	8.985	1060.574	< 2e-16	***
POTW:AB	3	0.65	0.218	25.746	5.62e-16	***
POTW:Trt	15	3.94	0.263	31.032	< 2e-16	***
M:AB	18	4.54	0.252	29.748	< 2e-16	***
M:Trt	90	9.80	0.109	12.847	< 2e-16	***
AB:Trt	5	0.32	0.063	7.494	6.68e-07	***
Residuals	890	7.54	0.008			

Table A 8. Above vs. Below BOD pairwise t-test. These are the statistical results for Brigham City and Wellsville during January and August and for Tremonton during February and September.

POTW	Month	Treatment	p-value
Brigham City	26-Jan-11	Carbon	0.069
Brigham City	26-Jan-11	NO_3-N	<0.001
Brigham City	26-Jan-11	NH_4-N	0.848
Brigham City	26-Jan-11	Control	0.285
Brigham City	26-Jan-11	N+P	0.018
Brigham City	26-Jan-11	SRP	0.231
Brigham City	23-Aug-11	Carbon	0.003
Brigham City	23-Aug-11	NO_3-N	<0.001
Brigham City	23-Aug-11	NH_4-N	<0.001
Brigham City	23-Aug-11	Control	<0.001
Brigham City	23-Aug-11	N+P	<0.001
Brigham City	23-Aug-11	SRP	<0.001
Tremonton	8-Feb-11	Carbon	0.055
Tremonton	8-Feb-11	NO_3-N	0.089
Tremonton	8-Feb-11	NH_4-N	0.475
Tremonton	8-Feb-11	Control	0.752
Tremonton	8-Feb-11	N+P	0.552
Tremonton	8-Feb-11	SRP	0.923
Tremonton	1-Sep-11	Carbon	<0.001
Tremonton	1-Sep-11	NO_3-N	0.041
Tremonton	1-Sep-11	NH_4-N	<0.001
Tremonton	1-Sep-11	Control	0.005
Tremonton	1-Sep-11	N+P	0.003
Tremonton	1-Sep-11	SRP	0.072
Wellsville	12-Jan-11	Carbon	0.886
Wellsville	12-Jan-11	NO_3-N	0.028
Wellsville	12-Jan-11	NH_4-N	0.325
Wellsville	12-Jan-11	Control	0.331
Wellsville	12-Jan-11	N+P	0.008
Wellsville	12-Jan-11	SRP	0.947
Wellsville	3-Aug-11	Carbon	0.035
Wellsville	3-Aug-11	NO_3-N	0.004
Wellsville	3-Aug-11	NH_4-N	0.006
Wellsville	3-Aug-11	Control	0.058
Wellsville	3-Aug-11	N+P	0.046
Wellsville	3-Aug-11	SRP	0.004

Table A 9. Two-way ANOVA comparing the sample sites S-BOD response above and below the POTWs. POTW = Publicly Owned Treatment Works Facility, M = Month, AB = Above/Below, Trt = Treatment. There was a significant difference found in all of the factors as well as all of the interactions.

Above vs. Below S-BOD Two Way ANOVA Limiting nutrients

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
POTW	4	21.615	5.404	838.026	< 2e-16	***
M	8	6.410	0.801	124.267	< 2e-16	***
AB	1	0.044	0.044	6.806	0.00935	**
Trt	5	21.915	4.383	679.729	< 2e-16	***
POTW:AB	4	0.689	0.172	26.730	< 2e-16	***
POTW:Trt	20	0.873	0.044	6.769	< 2e-16	***
M:AB	8	4.489	0.561	87.027	< 2e-16	***
M:Trt	40	2.450	0.061	9.497	< 2e-16	***
AB:Trt	5	0.219	0.044	6.779	3.91e-06	***
Residuals	514	3.314	0.006			

Table A 10. Above vs. Below S-BOD pairwise t-test. These are the statistical results for Brigham City and Wellsville during January and August, and for Tremonton during February and September.

POTW	Month	Treatment	p-value
Brigham City	26-Jan-11	Carbon	< 0.001
Brigham City	26-Jan-11	NO_3-N	< 0.001
Brigham City	26-Jan-11	NH_4-N	< 0.001
Brigham City	26-Jan-11	Control	0.004
Brigham City	26-Jan-11	N+P	0.001
Brigham City	26-Jan-11	SRP	< 0.001
Brigham City	23-Aug-11	Carbon	0.462
Brigham City	23-Aug-11	NO_3-N	0.055
Brigham City	23-Aug-11	NH_4-N	0.002
Brigham City	23-Aug-11	Control	0.001
Brigham City	23-Aug-11	N+P	< 0.001
Brigham City	23-Aug-11	SRP	< 0.001
Tremonton	8-Feb-11	Carbon	0.909
Tremonton	8-Feb-11	NO_3-N	0.096
Tremonton	8-Feb-11	NH_4-N	0.005
Tremonton	8-Feb-11	Control	0.001
Tremonton	8-Feb-11	N+P	0.010
Tremonton	8-Feb-11	SRP	0.079
Tremonton	1-Sep-11	Carbon	< 0.001
Tremonton	1-Sep-11	NO_3-N	0.001
Tremonton	1-Sep-11	NH_4-N	0.698
Tremonton	1-Sep-11	Control	0.006
Tremonton	1-Sep-11	N+P	< 0.001
Tremonton	1-Sep-11	SRP	< 0.001
Wellsville	12-Jan-11	Carbon	0.515
Wellsville	12-Jan-11	NO_3-N	No Sample
Wellsville	12-Jan-11	NH_4-N	0.027
Wellsville	12-Jan-11	Control	< 0.001
Wellsville	12-Jan-11	N+P	0.012
Wellsville	12-Jan-11	SRP	0.601
Wellsville	3-Aug-11	Carbon	0.018
Wellsville	3-Aug-11	NO_3-N	< 0.001
Wellsville	3-Aug-11	NH_4-N	0.792
Wellsville	3-Aug-11	Control	0.002
Wellsville	3-Aug-11	N+P	< 0.001
Wellsville	3-Aug-11	SRP	0.021