Whole-stream Nitrogen and Phosphorus Addition Study to Identify Eutrophication Effects in a Wadeable Prairie Stream

In Cooperation with the Carter County Conservation District

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ABSTRACT

A whole-stream nitrogen and phosphorus (nutrient) addition study was carried out in a perennial prairie stream in eastern Montana. The study site (on Box Elder Creek) has been identified by DEQ since the 1990s as a reference-quality stream. The study's purposes were to (1) better understand the effects of nutrient enrichment in prairie streams, and (2) collect data which lent themselves best to the interpretation of harm to prairie stream beneficial uses. DEQ is constantly striving to better understand the linkages between pollutants and the effects they manifest on legally-defined stream beneficial uses and their associated water-quality standards; observing controlled eutrophication effects on a reference stream was an ideal way to study these linkages. The study design was a Before After Control Impact Paired (BACIP) study in which there was an upstream Control Reach followed by a Low Dose Reach and then a High Dose Reach. Work was carried out in summer and early fall over three years beginning in 2009. In 2009, 'Before' data were collected from all three reaches. Ambient soluble nutrient concentrations in the stream were found to be low (3 µg NO_3-N/L and 4 µg SRP/L). In 2010, soluble nutrients (nitrate and phosphate) were dripped into the stream in a controlled manner using gravity-fed supply tanks. Over the nearly two-month period during which nutrients were added, concentrations in the Low Dose Reach were increased to 38.6 µg NO_3-N/L and 4.4 SRP/L, and in the High Dose Reach to 118.7 µg NO_3-N/L and 15.6 SRP/L. In 2011 no nutrients were added, when follow-up/stream recovery data were collected. In 2010 and as a result of dosing, impacts were documented in the High Dose Reach, and included seasonal DO concentrations below state standards, pH increases bordering on exceeding standards, development of nuisance attached algae levels, and a decline in macroinvertebrate metric scores to the threshold DEQ has used to define impairment of that biotic community. The DO impact in the High Dose Reach was seasonal, occurring in the early fall when large volumes of algae senesced and these decaying algae exerted a strong DO demand on the stream. In the Low Dose Reach ecological changes were documented, but definitive harm to beneficial uses was largely absent. These findings were in alignment with our pre-study predictions. Both the Low and High Dose reaches returned to their original (i.e., 2009) biological and chemical status in 2011, less than a year after nutrient dosing ended. During the study there was rotational grazing by cattle occurring at the study site, but no discernable impact on water quality from their presence was documented. The report concludes with a number of recommendations which can be used to inform future updates to DEQ's stream assessment methodologies.

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ACRONYMS

Acronym	Definition
AFDM	Ash Free Dry Mass
ARM	Administrative Rules of Montana
AUM	Animal Unit Month
BACIP	Before After Control Impact Paired (study design)
BOD	Biochemical Oxygen Demand
Chl*a*	Chlorophyll-*a*
DEQ	Department of Environmental Quality (Montana)
DO	Dissolved Oxygen
NRCS	Natural Resource Conservation Service
QAPP	Quality Assurance Project Plan
SOP	Standard Operating Procedure
SRP	Soluble Reactive Phosphate
TN	Total Nitrogen
TP	Total Phosphorus
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
USGS	United States Geological Survey
WWTP	Wastewater Treatment Plant

1.0 INTRODUCTION

Development of numeric nitrogen (N) and phosphorus (P) criteria for surface waters for all regions of the state is one of the Department of Environmental Quality's many activities supporting its statewide water quality management goals and responsibilities. The Department of Environmental Quality (DEQ) used ecoregions (Omernik 1987) to establish zones in which specific N and P (nutrient) standards[2] apply (Montana Department of Environmental Quality 2014). These standards are intended to be protective of the regional streams' water quality and beneficial uses (e.g., fisheries, aquatic life, recreation). Scientific studies showing linkages between nutrient concentrations and effects on streams are critical to the criteria derivation process, and DEQ has been able to identify quite a number of these studies in the scientific literature which pertain to Montana's ecoregions (Suplee and Watson 2013). However, the Northwestern Great Plains ecoregion in southeastern Montana was not well studied, and for this reason DEQ decided to carry out a scientific study there to ascertain the effects of nutrients on that region's streams. This document is the result of that study.

The effect of increased nutrients on stream water quality and beneficial uses is relatively complex. It is mediated through interactions of autotrophic and heterotrophic organisms. The proximate stressors that are known to harm waterbody beneficial uses may be several steps removed from the ultimate cause of the problem—elevated nutrients (**Figure 1-1**). Due to these complexities, it can be difficult to extrapolate laboratory- or mesocosm-scale studies of nutrient effects in flowing waters to the whole stream scale. For these reasons we decided to carry out a nutrient addition study *in situ* in a stream of the Northwestern Great Plains; we believed that a whole-stream fertilization study would provide the most accurate understanding of nutrient effects on uses of the region's prairie streams.

[2] Standards (i.e., water quality standards) are criteria that have been adopted into law to protect waterbody beneficial uses.

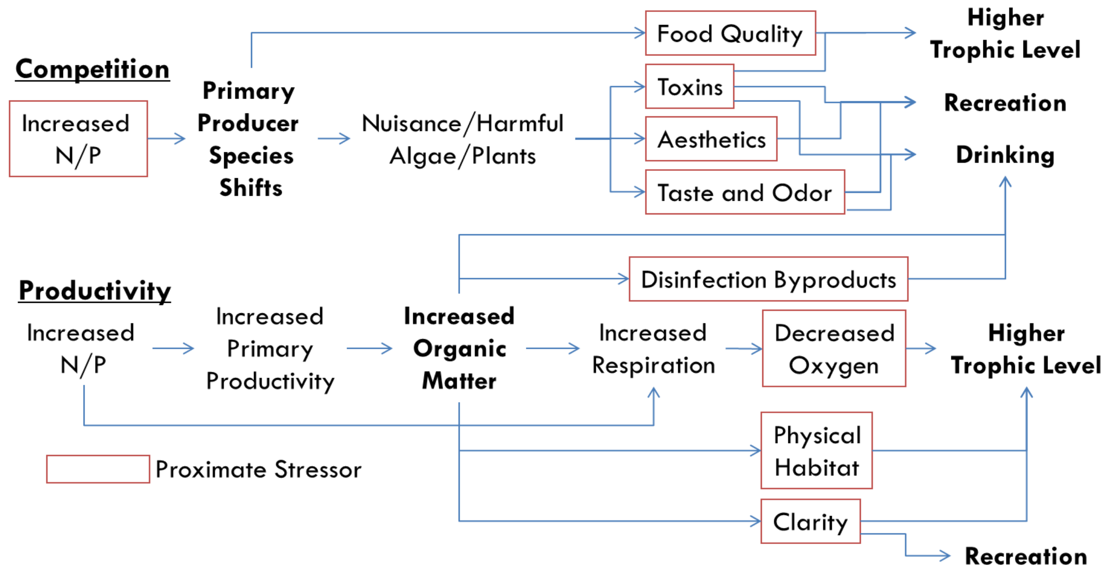

Figure 1-1. Flowchart Exemplifying the Relationships between Increased Nutrient Concentrations and Stream Beneficial Uses.

Beneficial use impacts are usually several steps removed from increased nutrients, which are the ultimate cause of the changes. This model/flowchart was developed as part of the Stream Nutrient Enrichment Workshop (U.S.Environmental Protection Agency 2014).

2.0 BACKGROUND

This study followed standard scientific method (formulation of a question, hypothesis, prediction, testing, analysis, and conclusion). The vast majority of this report addresses the last three elements (testing, analysis, and conclusion). Here, we present our questions, hypotheses, and predictions, which were developed at the beginning of the study in 2009. The experimental unit in the study was a length of stream and the water flowing through it (i.e., a stream reach). Stream reaches were then assigned to different treatments (control, low-level nutrient dose, high-level nutrient dose).

2.1 QUESTIONS, HYPOTHESES, AND PREDICTIONS

Questions

The following two questions were included in the project QAPP (Montana Department of Environmental Quality 2010).

1. If nitrogen and phosphorus are added to the stream during the growing season (July-Sept) in order to bring the concentrations up to levels DEQ currently believes are appropriate as regional nutrient criteria, what will be the effect on the stream's dissolved oxygen and pH patterns, benthic and phytoplankton algal density and composition, and macroinvertebrate density and composition?

2. If nitrogen and phosphorus are added to the stream during the growing season (July-Sept) in order to bring the concentrations to levels somewhat beyond what DEQ believes are appropriate as regional nutrient criteria, what will be the effect on the dissolved oxygen and pH patterns, benthic and phytoplankton algal density and composition, and macroinvertebrate density and composition?

Based on earlier work (Suplee 2004; Suplee et al. 2008), DEQ has a fairly good idea what total nitrogen concentration criteria for eastern Montana prairie streams ought to look like. That work also showed nitrate to be very important in these streams, however the exact nitrate loading and concentration that these streams could tolerate was not clear. We bracketed some nitrate tolerance estimates and these estimates formed the basis of the two nutrient dosing rates (low, high) that were applied.

Hypotheses

We hypothesized that in the reach of prairie stream receiving an increase in nutrients up to the level defined in question 1 above, we would see changes in the dissolved oxygen (DO) and pH patterns, benthic and phytoplankton algal density and composition, and macroinvertebrate density and composition. However, we did not necessarily expect those changes would be so pronounced as to constitute "harm to use", i.e., a quantifiable and demonstrable impact to the legally-defined designated beneficial uses of the stream. Readers should note that the stream is classified C-3 and beneficial uses are bathing, swimming, and recreation, growth and propagation of non-salmonid fishes and associated aquatic life, waterfowl, and furbearers.

In contrast, we did expect to measure and document harm to the stream's beneficial uses in the reach receiving an increase in nutrients up to the level defined in question 2. In this more highly-dosed reach, we also expected to see the nutrient induced effects on the stream extend further downstream (compared to the lower dosed reach in question 1) because nutrients in excess of the assimilative capacity of the local biota would move downstream and continue to manifest effects.

Predictions

In 2009 we discussed several predictions which we anticipated would result from the addition of nutrients to the stream. Specifically:

1) ***Reach receiving low nutrient dose***
    a) *Higher daily highs and lower daily lows for pH and DO, but the daily DO lows would not drop below minimum standards, for example the 1-day minimum in Circular DEQ-7 (Montana Department of Environmental Quality 2012).*
    b) *An increase in benthic and phytoplankton algae density, but the benthic algae reach average would remain below the nuisance threshold of 150 mg Chla/m^2 (Suplee et al. 2009).*
    c) *An increase in the probability of nutrient impairment according to the warm-water diatom increaser taxa metric (Teply 2010a; Teply 2010b), but still with an overall probability of impairment less than 50%.*
    d) *Based on the macroinvertebrates, a decrease in the plains MMI score but still above the recommended impact threshold of 37 (Montana Department of Environmental Quality, Water Quality Planning Bureau 2006).*

2) ***Reach receiving high nutrient dose***
    a) *Higher daily highs and lower daily lows for pH and DO, with daily DO lows dropping below the minimum standards, for example the 1-day minimum in Circular DEQ-7 (Montana Department of Environmental Quality 2012). The pH might show change in excess of the standard, in that it might rise to values greater than 9.0 or it could change more than 0.5 standard units compared to natural—natural in this case being the pH measured in the study control reach.*
    b) *An increase in benthic and phytoplankton algae density, with benthic algae reach averages exceeding the nuisance threshold of 150 mg Chla/m^2 (Suplee et al. 2009).*
    c) *An increase in the probability of nutrient impairment according to the warm-water diatom increaser taxa metric (Teply 2010a; Teply 2010b), with a change showing the probability of impairment to be greater than 50%, i.e., indicative of an excess nutrient problem per the current DEQ SOP (Montana Department of Environmental Quality 2011a).*
    d) *Based on the macroinvertebrates, a possible decrease in the plains MMI to a level below the recommended threshold of 37 (Montana Department of Environmental Quality, Water Quality Planning Bureau 2006)*
    e) *Some of the impacts listed here in bullet 2, but probably not all, would reach levels that could readily be identified as "harm to beneficial use".*

Regarding 2d above, we were less certain that the macroinvertebrate community would actually exceed the threshold for the plains MMI. This is because macroinvertebrates have longer lifespans than, say, diatoms, and the study duration (8-12 weeks) might have been too short for changes large enough to exceed the MMI threshold to occur. Diatom algae have generation times measured in weeks or less, whereas macroinvertebrates operate on time scales of weeks to years (Hering et al. 2006a; Hering et al. 2006b). Per 2e, we knew that nutrient impacts on beneficial uses are mediated through complex interactions of autotrophic and heterotrophic organisms (see **Introduction**), therefore the predictability of the outcomes becomes more uncertain. Because of this, we were not certain that all the parameters we measured would exceed defined thresholds, but we were fairly certain that at least some of them would.

3.0 METHODS AND MATERIALS

3.1 STREAM SITE SELECTION

The study was carried out on a reach of Box Elder Creek east of Mill Iron, MT (**Figure 3-1**; latitude -104.1387, longitude 45.8458, measured in NAD83). Box Elder Creek is classified C-3. It is an intermittent-to-perennial, 5th-order stream located in Carter County (southeastern Montana), where it flows northeast across prairie landscape from its headwaters until exiting Montana and entering South Dakota. The stream is wholly contained within HUC 10110202. Summer baseflow is typically around 7 CFS (0.2 m^3/s).

The stream site was selected because it is on state-owned land and has been classified by DEQ as a reference site since the early 1990s (Bahls et al. 1992). In 2009, an NRCS riparian assessment (Pick et al. 2004) carried out by DEQ and Carter County Conservation District staff (including a trained range botanist) deemed the site to have a sustainable rating. The site's water quality had been sampled repeatedly by DEQ during the past decade (2001, 2005, 2006, and 2007). From these data, the reference reach was known to have fairly low N and P concentrations. The stream is sodium-sulfate dominated with a strong buffering capacity (total alkalinity about 400 mg/L as $CaCO_3$); however, the anions carbonate and bicarbonate are almost as abundant as sulfate. Sodium-sulfate and sodium-bicarbonate dominated streams are extremely common in the region, therefore Box Elder Creek's basic water chemistry is typical of the area. From headwaters to the site, the stream is wholly contained within ecoregions with prairie-like characteristics (i.e., it does not have mountain water influences on its water-quality as does, for example, the Yellowstone River).

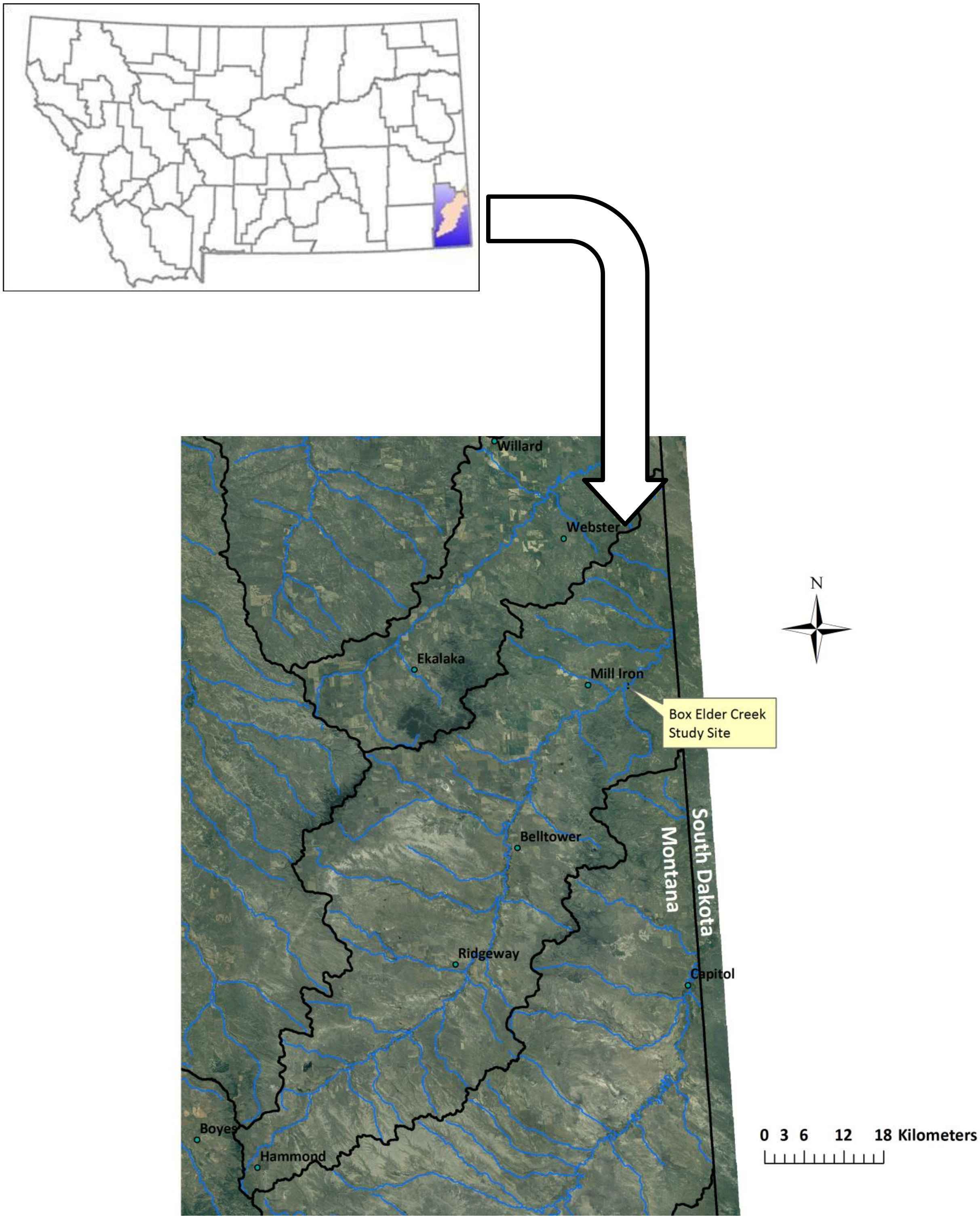

Figure 3-1. Upper Panel. Map of Montana (with Counties) Highlighting the Box Elder Creek Watershed in Carter County. Lower Panel. Closer View of the Watershed, Showing the Study Site at the Northern end of the Watershed, near the Montana-South Dakota border.
The stream is located in Hydrologic Unit Code (HUC) 10110202.

3.2 Study Design: Before After Control Impact Paired (BACIP)

The basic study design is a Before After Control Impact Paired (BACIP) study (Stewart-Oaten et al. 1986). BACIP designs are used when the ability to have standard treatment replicates (e.g., five prairie reference streams that receive nutrient dosing and five different prairie reference streams that are untreated and serve as controls) is not feasible. The design involves comparing measured stream characteristics before and after an impact in a single stream, in this case the impact being nutrient addition. To account for effects of natural changes over time (e.g., weather effects), a stream reach that receives no impact is *paired* with one or more reaches that will (Smith, 2002).

In this study, there was one no-impact reach (Control Reach), and two impact reaches; a Low Dose Reach and a High Dose Reach (**Figure 3-2**). The Control Reach was the most upstream, followed immediately downstream by the Low Dose Reach. The High Dose Reach began 900 m downstream from the terminus of the designated Low Dose Reach.

BACIP designs comprise specific statistical approaches and a brief overview of the method is warranted here. The statistical design and testing followed Stewart-Oaten et al. (1986) wherein, for any given parameter (e.g., daily dissolved oxygen minima), the mean (or median) of the differences (**D**) between the Control Reach and an experimental reach (e.g., the Low Dose Reach) in the Before period are compared, via Student's T-test or (preferably) the non-parametric Mann-Whitney test[3], to the mean (or median) of the **D**s for the same parameter in the After period. An example of the data handling is given in **Table 3-1**, which is example data for dissolved oxygen (DO) to illustrate the concept. Note in **Table 3-1** that it is the calculated values of **D** that are used in the statistical test (Before *vs.* After), not the original values measured in the Low- and High Dose reaches. It is by this mechanism that natural changes in DO (or any parameter) that occur in the impacted reaches are separated from experimentally induced changes; the Control Reach acts as a covariate, and it is assumed that DO in the experimental reaches would have been the same as DO in the Control Reach if the experimental reaches had not received their respective perturbations. In the **Table 3-1** example, there is no statistical difference (Mann-Whitney test, alpha = 0.05) in median **D** for the Low Dose Reach between the Before and After periods, whereas the difference between the Before and After periods in the High Dose Reach is significant ($p = 0.01$).

[3] Using a non-parametric test is more robust when the data may or may not fit a normal distribution function (Conover, 1999).

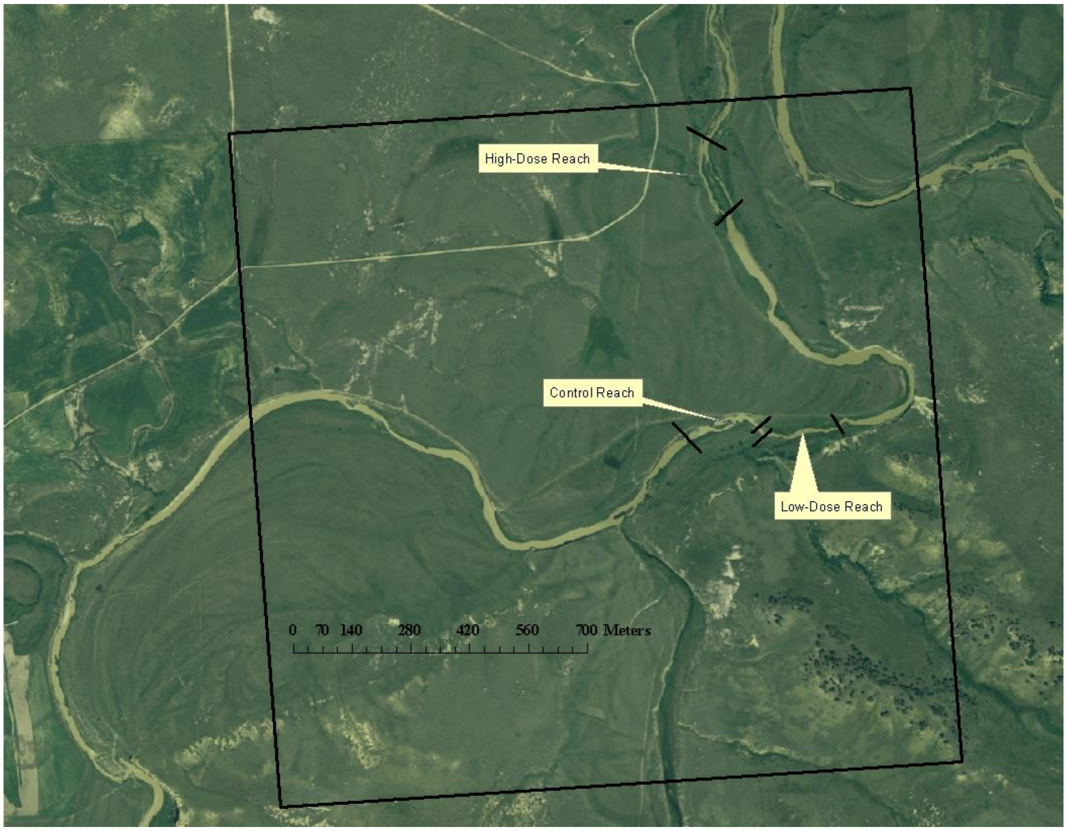

Figure 3-2. Close-up View of the Box Elder Creek Study Site.
Stream flow is from left to right. The black outlined box is the boundary of the state-owned parcel. Start and end points of the Control, Low Dose, and High Dose reaches are shown by black lines perpendicular to stream flow.

Table 3-1. Illustration of BACIP-design statistical testing.
Fictitious dissolved oxygen concentrations in the Control, Low, and High Dose Reaches. A Mann-Whitney or T-test is carried out on the **D** values from the Before *vs.* After periods.

		Daily Dissolved Oxygen Minima (mg/L)				Calculated Difference (D)	
PERIOD	**Sampling Event**	**Control**	**Low Dose**	**High Dose**		**Difference (D): Control – Low Dose**	**Difference (D): Control – High Dose**
BEFORE	1st	6.0	6.0	6.0		0.0	0.0
	2nd	5.5	5.3	5.5		0.2	0.0
	3rd	6.0	5.9	6.1		0.1	-0.1
	4th	7.0	7.0	7.1		0.0	-0.1
	5th	6.5	6.4	6.5		0.1	0.0
					Median:	***0.1***	***0.0***
AFTER	1st	6.0	5.9	5.4		0.1	0.6
	2nd	6.0	6.0	5.0		0.0	1.0
	3rd	7.0	7.0	6.2		0.0	0.8
	4th	7.3	7.2	6.4		0.1	0.9
	5th	6.0	6.0	5.0		0.0	1.0
					Median:	***0.1***	***0.9***

In the results that follow, BACIP statistical testing—regardless of which parameter is addressed—will follow the procedures just described. In addition to the BACIP statistical analyses, other inferential statistical tests (as well as descriptive statistical summaries) of data will be presented throughout. Where we had *a priori* knowledge or expectations about an inferential test (e.g., we expect a particular biometric to manifest higher values in the After period compared to Before) we ran one-sided tests. We have considered statistical tests significant when p-values are ≤0.05 and marginally significant when >0.05 but ≤0.1. We selected this more graded alpha-error significance threshold to help reduce beta error. Beta error is important as it represents the chance of declaring a truly significant difference as insignificant; if there is a reasonable chance that nutrients had an effect on the stream water quality or biota, we want to be able to consider it. Because sample size of the **D** values is low (often n = 4), the remaining option to reduce beta error is to increase alpha error, which has been done by considering results to be at least marginally significant if p <0.1.

3.3 Lengths of the Study reaches and Stream Features in each Reach

The BACIP design anticipates that the control and impact reaches are in fairly close proximity so that they experience the same climate and weather, and that they be as physically similar as possible in terms of geomorphology, geology, and hydraulics such that natural influences manifest in each reach about the same way. All three reaches were contained within the one square mile (259 hectare) state-owned parcel (**Figure 3-2**).

To establish reasonably similar reaches, in 2009 we identified the proportions of riffle, pool, and glide along Box Elder Creek within the state parcel, along stretches of the stream where we estimated the study reaches were likely be placed. Along this longitudinal extent of stream (777 m), stream features were identified every 3.05 m as either riffle, pool, or glide. The 777 m reach examined was found to be 15% riffle, 24% pool, and 60% glide.

Stream nutrient spiraling calculations (Newbold et al. 1981; Newbold et al. 1982; Ensign and Doyle 2006; Mulholland et al. 2002; Kohler et al. 2008) were used to approximate the stream length necessary for the added nutrients to have time to be taken up by aquatic flora and microorganisms within each experimental reach. Estimates were quite variable, but calculations indicated 200 m of stream length was appropriate.

Based on the spiraling calculations, the goal was that each of the study reaches would be approximately 200 m in length and would encompass riffle, pool, and glide in proportions approximately equal to that typical for the stream as a whole (i.e., 15% riffle, 24% pool, and 60% glide, per above). After carefully reviewing the longitudinal extent of stream within the state parcel, each reach was identified. At the transects where data-collection would be occurring, the layout of the Control Reach comprised 9% riffle, 18% pool, and 73% glide, whereas the Low Dose was 18% riffle, 18% pool, and 64% glide and High Dose Reach was 18% riffle, 27% pool, and 55% glide. The need to balance stream features against the realities and constraints of the study area led to proportions of stream features that differed from the ideal and, in addition, slightly varied reach lengths (Control Reach length of 150 m, Low Dose Reach of 200 m, and High Dose Reach of 200 m). The three reaches were benchmarked and had reach midpoints of: 45.8460, -104.1407, (Control); 45.8458; -104.1387, (Low Dose); and 45.8514, -104.1414, (High Dose).

3.4 Nutrient Dosing: Forms of the Additions, Dose Rates, and the Delivery System

Two reaches (Low Dose, High Dose) each received different nutrient concentration additions. The intent was to add nutrients to the Low Dose Reach at concentrations approaching but short of our best estimation of a "harm to use" level, while the High Dose Reach was targeted to receive a dose higher than this (about four times higher).

3.4.1 Determining the Chemical Forms and the Dosing Rates

We had to decide the form by which nutrients were to be added to the stream. Because nitrate has been shown to be a key limiting nutrient in Montana prairie streams (Suplee 2004), and nitrate is known to increase in regional ground and stream water due to human activity (Nimick and Thamke 1998), it was concluded that nitrogen should be added as nitrate. Sodium nitrate ($NaNO_3$) and dipotassium phosphate (P source; K_2HPO_4) were selected after a review of what others have used in similar studies (Perrin and Richardson 1997; Ferreira et al. 2006), along with consideration of Box Elder Creek's base water chemistry. Sodium and potassium concentrations are high in Box Elder Creek (about 305 mg Na/L and 8 mg K/L during summer baseflow), and calculations showed that to elevate N and P to levels suitable for the experiment the dissolved counter ions (Na and K) in the additions would increase ambient stream Na and K by <1%. Therefore, the counter ions' potential effects on the experiment were negligible. Further, the K_2HPO_4 solution had a pH of about 9.0, very close to Box Elder Creek's typical pH of 8.5. (In contrast, another candidate P source, KH_2PO_4, would have had a solution pH of 4.5.) Each of the compounds was reasonably safe to transport and store and very soluble in water, so concentrated drip solutions could be easily made.

The scientific literature was consulted to derive the dosing rates. A body of work in prairie streams from the Konza Prairie Biological Station (Kansas) was the most applicable to Box Elder Creek (Tate 1990; Dodds et al. 1996; Kemp and Dodds 2001; Kemp and Dodds 2002; Dodds and Oakes 2006; O'Brian and Dodds 2008). O'Brian and Dodds (2008) find that a Michaelis-Menten curve adequately describes N uptake by a stream, and the half-saturation constant (Ks) for their study stream was 27 µg N/L. Ks is the concentration at which the soluble N uptake rate in the stream is half of the maximum (Vmax). In effect, Ks is a nutrient concentration at which stream primary productivity is still constrained by nutrient concentrations. At approximately five times Ks, nutrients are reported to be saturated and further increases in nutrients will not further increase Vmax (Chapra, 1997). Also considered was a large number of laboratory and field-derived Ks values for algae (phytoplankton and benthic algae); the median Ks for that dataset was 67 µg N/L (United States Environmental Protection Agency 1985). The median Ks for soluble P for the same EPA dataset was 15 µg P/L. These and other information were considered in developing the following:

- The dose rate for the Low Dose Reach was targeted to achieve 40 µg NO_3-N/L and 6 µg SRP/L[4] at the headwaters of the study reach. These concentrations included the ambient stream N and P concentrations, which were about 3 µg NO_3-N/L and 4 µg SRP/L (2009 data). We assumed complete mixing near the point of nutrient addition. (It was not expected that 40 µg NO_3-N/L and 6 µg SRP/L would persist to the end of the 200 m study reach; the target concentrations were the goal at the headwaters, after mixing.) The final soluble N:P ratio would be 6.7 (by mass), close to the Redfield ratio of 7:1 (Redfield 1958), which provided balanced resource availability (i.e., neither N nor P would be strongly limiting).

- The dose rate for the High Dose Reach was targeted to achieve 150 µg NO_3-N/L and 23 µg SRP/L. Again, this was the target for the reach headwaters after mixing and included natural background. We included an estimate of residual nitrate that would arrive to the headwaters of

[4] All SRP (soluble reactive phosphorus) concentrations discussed in this report are "as P".

the High Dose Reach due to the dosing of the Low Dose (it was assumed no residual P would arrive to the High Dose Reach). We assumed an additional 2 µg NO_3-N/L above ambient background for this purpose. The target dose rate should have, theoretically, brought the stream close to N saturation (i.e., five time Ks). The selected dosing concentrations were intended to maintain a soluble N:P ratio at 6.5 by weight, very close to Redfield ratio and therefore providing balanced resource availability (i.e., neither N nor P would be strongly limiting).

3.4.2 Nutrient Delivery System

The nutrient dosing equipment comprised two polyethylene tanks at each experimental reach, one tank for $NaNO_3$ solution and one for K_2HPO_4 solution (**Figure 3-3**). Each tank was color coded (white and blue for N and P, respectively). The solution concentrations used are shown in **Table 3-2**. The reduction in P-solution strength at the Low Dose Reach on August 23, 2010 (**Table 3-2)** was necessary because declining stream flow could not be matched by simply reducing the P delivery rate (the delivery rate was already very slow). Batches of nutrient solution were made offsite by weighing $NaNO_3$ or K_2HPO_4 using an Ohaus 15-kg balance (0.5 g readability), dissolving the salts in appropriate volumes of distilled water in carboys, and then transporting the carboys to the site to fill the tanks. For quality control, soluble nutrient samples were collected directly from the filled High Dose and Low Dose tanks on 3 different dates throughout the study and provided to the Montana Department of Public Health & Human Services (DPHHS) Environmental Laboratory for analysis.

Table 3-2. Target Concentrations of Nutrient Salt Solutions in the Dosing Supply Tanks for each Experimental Reach.

The equivalent grams of N or P per liter provided by each solution are also shown.

	Experimental Reach							
	Low Dose				High Dose			
Time Period	grams $NaNO_3$/L	g N/L	grams K_2HPO_4/L	g P/L	grams $NaNO_3$/L	g N/L	grams K_2HPO_4/L	g P/L
Aug 8th to Aug 23rd	300	49.4	25	4.45	400	65.9	50	8.89
Aug 23rd onward	300	49.4	15	2.67	400	65.9	50	8.89

Figure 3-3. The Nutrient Supply Tanks for the High Dose Reach.
Note that the tanks are located on a high terrace above the stream, allowing gravity feed of the solutions to the instream drip assembly. A similar arrangement was established for the Low Dose Reach.

Figure 3-4. The Nutrient-dripper Assembly, Located Mid-channel at the Head of a Riffle.
The assembly comprised an N supply line and a P supply line, control valves, and a perforated PVC tube into which the two solutions dripped. It was secured by a fencepost pounded into mid channel.

Figure 3-3 shows the tanks on the terrace above the High Dose Reach, surrounded by stock panels to prevent interference by cattle and wildlife (Low Dose tanks were similarly protected). Nutrient solution was delivered to the stream by gravity feed[5] via ½ inch ID reinforced vinyl tubing which was buried about 15 cm underground. Near the stream, the vinyl tubing was also placed inside PVC pipe to provide extra protection from hoof shear or potential stream scour. The vinyl delivery tubing (inside PVC pipe) was buried under the streambed until mid-channel. At mid-channel, the vinyl tubes re-emerged, were attached to needle valves used to control the drip rate, and then additional vinyl tubing was placed on the delivery side of the needle valves and placed inside a perforated PVC tube. The entire assembly was attached to a fencepost (**Figure 3-4**, previous page, and **Figure 3-5**). The perforated PVC pipe allowed the nutrient solutions to drip into the stream without the possibility of being licked by cattle or wildlife. The lengths of vinyl tubing on the delivery side of the needle valves were cut (and periodically adjusted) so that they ended just above the water surface; this assured that dripped solution made it to the stream and did not dry on the sides of the PVC pipe. Early tests with colored dye showed that stream flow very rapidly washed the dripped solution out from the inside of the PVC tube and into the stream.

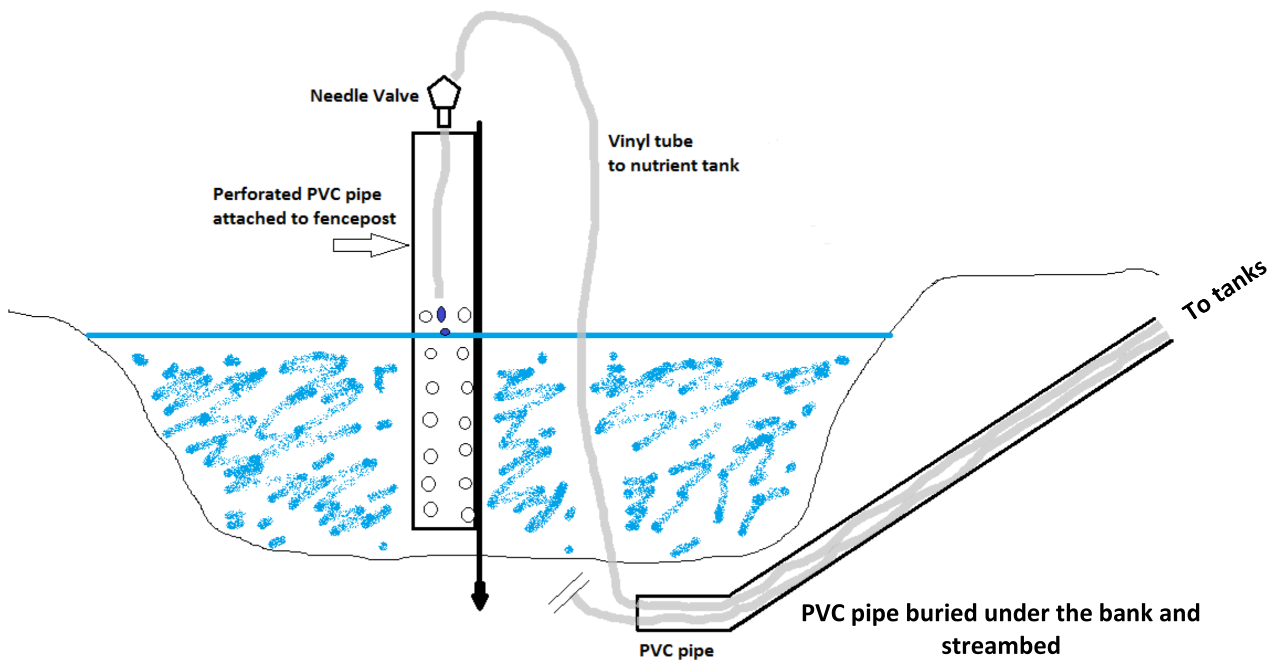

Figure 3-5. Cross-section Diagram of the Instream Nutrient-dripper Assembly.
For simplicity, only one vinyl nutrient-delivery tube is shown inside the perforated PVC pipe, but *in situ* both were there. Lengths of the vinyl extension tubes on the delivery end of the needle valves (inside the perforated PVC pipe) were maintained just above the water level, as shown.

The experiment assumed nearly instantaneous mixing of stream water and dripped tank solutions, so to achieve this the location of the dripper arrays and the positioning of the Low- and High Dose reaches were carefully selected. For both the Low- and High Dose reaches the most upstream reach feature was a riffle, and the dripper arrays were placed in the stream just upstream of these riffles to provide fastest mixing.

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5 The tanks were at first configured as Mariotte's bottles (McCarthy 1934), which provide constant drip rates regardless of changing liquid levels in the tanks. However we found that the Mariotte configuration only works for rigid-walled vessels and was not practical for plastic tanks, and therefore switched to gravity feed.

3.4.3 Regulating the Nutrient Dose Rate

Drip rates from the tanks to each experimental reach were varied according to stream flow in order to maintain the target dose rates. We assumed that background nutrient concentrations in the stream in 2010 would be similar to what was measured in August and September 2009 (3 µg NO_3-N/L and 4 µg SRP/L), and also assumed (as noted above) a slightly higher N background (5 µg NO_3-N/L) at the head of the High Dose Reach to account for residual nitrate from the Low Dose Reach. In 2009 we measured flow repeatedly at a location in the Low Dose Reach and at a location in the High Dose Reach and found no significant difference between them ($p = 0.129$; paired t-test). As a result, we only made flow measurements in 2010 in the Low Dose Reach but applied the measured values to both study reaches.

Flow was measured regularly during the dosing period (August-October 2010), on average about every four days. A computer spreadsheet had been developed which, when stream flow was input, provided the drip rate needed to maintain the target dosages. Using a laptop and the spreadsheet, the appropriate drip rates were determined (based on the most currently-measured stream flow) and then the drip rates were adjusted via the needle valves. At the stream, at each experimental reach, the N and P drip rates (ml/min) were measured using a graduated cylinder for drip volume and a wristwatch with a second hand for time. Drip volumes/times were measured at least twice before locking the valve in position.

3.5 Data Collection

A full suite of physical, chemical, and biological data collections was undertaken during the study. Methods for each are described below.

3.5.1 Physical Measurements

Stream flow was obtained using a Marsh-McBirney FlowMate meter set to thirty second averaging, with the velocity measurements being taken at twenty equally-spaced points across the channel using the 0.6 m depth method (Rantz 1982). Flow was measured a total of thirty three times from 2009 to 2011, twenty one of those being taken in 2010 alone, during the nutrient dosing.

The main reach features of each experimental reach (riffle, pool, glide; see **Section 3.3**) were documented each year of the study. Basic wetted channel geometry was also quantified annually, in late September. To do this, stream depth was measured at five equidistant locations across each sampling transect (i.e., transects A to K) within each study reach, and stream width at the same locations was also recorded. However, in 2009 these measurements were only collected from three longitudinally-spaced transects in the High-Dose reach and four longitudinally-spaced transects in the Control and Low Dose reaches.

Weather data were collected during summer and fall all three years of the project using a HOBO Weather Station and Logger. The station recorded air temperature, wind speed and direction, solar radiation, and relative humidity. The centers of the anemometer cups were positioned 2 m above the ground surface and the station referenced to true north. The station was placed in the same location all three years, near the upstream boundary of the Control Reach in an open grassy area with no trees or large topographic features in the immediate vicinity.

3.5.2 Chemical/Water Quality Measurements

In all three years of the study, water-quality data were collected from each of the study reaches (Control, Low Dose, High Dose). In 2009 and 2011, water samples were collected near the middle of each study reach. In 2010, water-quality samples were collected towards the downstream end of each experimental reach to capture the largest effect of the treatments (i.e., collection occurred at or very close to transect A in each reach). In addition, in 2010, data were collected just upstream of the headwaters of the High Dose Reach during nutrient dosing in order to document arriving water quality and to ascertain if any residual nutrients from the Low Dose Reach (located upstream) were influencing the High Dose Reach.

Soluble Nutrients: For nitrate + nitrite (NO_{2+3}), ammonia, and SRP samples, well-mixed stream water was collected and filtered through a 0.45 um filter. Then, 250 ml of the filtrate was placed in a HDPE bottle and frozen until analyzed; summary information is in **Table 3-3**. Filtration was accomplished with a large syringe connected to a disposable filter capsule. A small amount of deionized water followed by a small amount of the sample was wasted through the 0.45 um filter before the filtered sample was collected. All reusable gear was acid washed (10% HCl) and triple rinsed in deionized water between uses. All sample bottles were new or were acid washed in 10% HCl and triple-rinsed in deionized water. Sample bottles were pre-rinsed with a small amount of the filtered sample before collecting the final filtered sample. All samples were analyzed by the DPHHS Environmental Laboratory in Helena, MT.

Total Nutrients, TSS & TDS, Turbidity, Common Ions, and Biochemical Oxygen Demand (BOD). Summary information is shown in **Table 3-3**; all samples were collected from well-mixed parts of the stream. A 250 ml HDPE bottle was collected for TP and TN and was immediately frozen. Common ions were collected in two 250 ml HDPE bottles and held on ice (not frozen). One bottle was analyzed for cations (including hardness and cation/anion balance) and the other for anions (including total alkalinity). The cation sample was preserved with nitric acid and held on ice, while the anion sample was held on ice without acid preservation. TSS, TDS, and turbidity were collected in a 1000 ml HDPE bottle and held on ice (not frozen). All sample bottles were new or were acid washed in 10% HCl and triple rinsed with de-ionized water. Five day biochemical oxygen demand (BOD_5) samples were collected in 1000 ml HDPE bottles and held on ice (not frozen), and delivered to the analytical laboratory within 48 hrs. All samples were analyzed by the DPHHS Environmental Laboratory in Helena, MT.

Sestonic CNP samples. To measure sestonic (suspended particulate) carbon (C), N and P content, known volumes of stream water were filtered through GF/F filters. For each sampling event at least one filtered sample was collected from each study reach (in some cases duplicates were collected), including the site just upstream of the High-Dose reach which was only sampled in 2010. Samples were stored in 50 cc centrifuge tubes (or in small petri dishes) on ice (not frozen). For each study reach, equal volumes of water were filtered on a pair of filters. Vacuum on the filters was kept below 9.0 inches Hg to prevent cell rupture and loss of their contents into the filtrate (Wetzel and Likens, 1991). At the LCG Water Laboratory in Helena, one of the filters (for C & N analysis) was placed on a filter holder and rinsed with 10% HCl until it stopped fizzing, to remove inorganic carbonates (Nieuwenhuize et al., 1994). Fifty ml tap water was then pulled through the filter to remove the acid, and the filter was dried at 105 ^{o}C. The remaining filter (for P analysis) was dried directly. Dried CNP samples were analyzed by the Agricultural Analytical Services Laboratory at Penn State University, State College, PA (**Table 3-3**). Lower reporting limits for sestonic CNP varied by the volume filtered, and ranged as follows: C—0.15 to 1.08 mg/l (median: 0.26 mg/l); N—0.10 to 0.72 mg/l (median: 0.17 mg/l); and P—5 to 38 µg/l (median: 8 µg/l).

Table 3-3. Analytical Methods and Lower Reporting Limits for Nutrients and Other Samples.

Analyte	Method	Lower Report Limit
Total Phosphorus (TP)	EPA 365.1	1 µg/l
Total Nitrogen (TN)	Standard Methods 4500-N B or C	5 or 10 µg/l
Nitrate + Nitrite (NO_2+NO_3-N)	EPA 353.2	1 or 5 µg/l
Total Ammonia (NH_3+NH_4-N)	EPA 350.1	5 µg/l
Soluble Reactive Phosphorus (SRP)	EPA 365.1	1 µg/l
Total Suspended Solids (TSS)	EPA 160.2	1000 µg/l
Total Dissolved Solids (TDS)	EPA 160.1	1000 µg/l
Specific Conductance	Standard Methods 2510 B	1 µmho/cm
Sulfate	EPA 300.0	1000 µg/l
Chloride	EPA 300.0	1000 µg/l
Alkalinity (Bicarb., Carb.)	EPA 310.2/A2320 B	1000 µg/l
Calcium, Magnesium, Potassium, Sodium	EPA 200.7	10 or 200 µg/l
Total Hardness as $CaCO_3$	Standard Methods 2340 B (Calculated)	1000µg/l
Sodium Absorption Ratio (SAR)	Calculated	
Cation-Anion Milliequivalent	Standard Methods 1050 A	
BOD_5	Standard Methods 5210 B	4000 µg/l
Chlorophyll *a*, corrected for pheophytin	Standard Methods 10200 H	Variable, dependent on area sampled/volume filtered
Ash Free Dry Mass (AFDM)	Standard Methods 10300 C (5)	0.01 g/m^2 (hoop) and 0.8 g/m^2 (template)
Sestonic Carbon and Nitrogen	High temperature induction furnace*	Variable, dependent on volume filtered
Sestonic Phosphorus	Ashing followed by molybdate P method†	Variable, dependent on volume filtered

*American Society of Agronomy (1998)
†(Solorzano and Sharp, 1980)

Continuous Monitoring via Deployed Instruments. Yellow Springs Instruments (YSI) 6600 V2-4 sondes were deployed all years of the study and were the principle means by which temperature, dissolved oxygen, pH, conductivity, and turbidity were measured in the stream. Logging was set at 15 minute intervals. In 2009 one sonde was placed in each reach (Control, and what would become the Low- and High Dose reaches), and the sondes were located near the middle of each study reach in glides with laminar flow. In 2010 the Low Dose and High Dose reaches each received two sondes; one placed in the same location as in 2009, and another near the terminus of the 200 m study reach. The Control Reach in 2010 received one sonde at the same location as 2009. In 2010 (during dosing) a sonde was also located just upstream of the High Dose Reach in order to monitor water quality as it arrived to the headwaters of the High Dose Reach; the intent was assess any residual water quality effects from the Low Dose Reach (located upstream). In 2011, each of the three study reaches again received one sonde each, in the same locations as in 2009.

Sondes were deployed using "sturgeon"-type platforms that held the sondes in a horizontal position 12 cm off the streambed (**Figure 3-6**). Sonde probes always faced the rear of the deployer. There was one exception to the use of the sturgeon-type deployers; in 2010 the downstream sonde of the High Dose Reach was deployed vertically on a mid-channel fencepost, with its probes positioned at a comparable depth from the stream bottom.

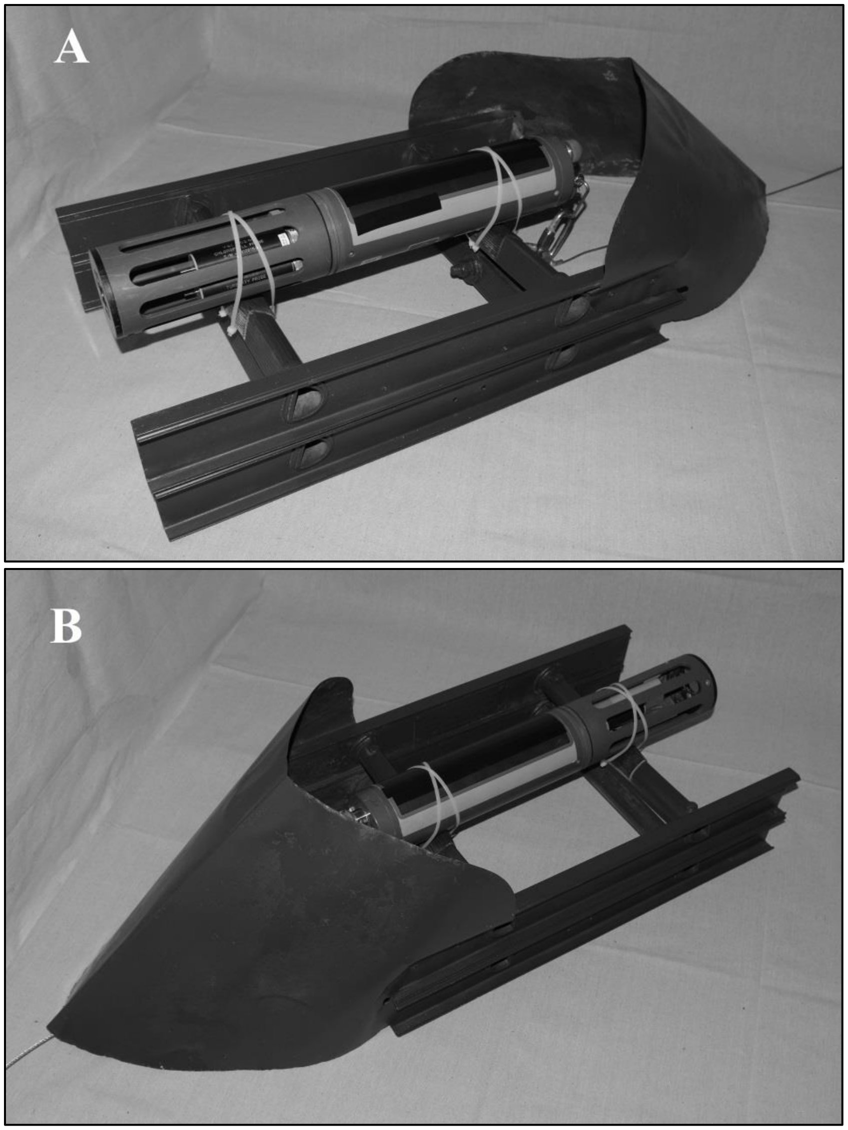

Figure 3-6. The "sturgeon" sonde deployer.
(A) Looking from the back towards the front. (B) Looking at the unit from the front, where there is a large deflector which faces the oncoming flow.

Quality Control for YSI calibration and allowable drift from calibration for the measured parameters are detailed in Section 4.0 of the project QAPP (**Appendix A**). During deployment the sondes were inspected and cleaned routinely (especially in 2010) to replace failed probes, recalibrate as necessary, and to clear the instruments of any biofouling that might have interfered with the measurements. **Table 3-4** shows the deployment, cleaning/servicing, and retrieval schedule for deployed YSI sondes for all years of the project.

Table 3-4. Deployment, Cleaning/Servicing, and Final Retrieval of YSI Sondes During the Study.

YSI Activity	Year, Sites, and Date of Activity					
	2009					
	Control	**Low Dose**	**High Dose**			
Deployment	25-Jul	25-Jul	25-Jul			
Cleaning/Service	31-Aug	29-Aug	29-Aug			
Retrieval	27-Sep	27-Sep	27-Sep			
	2010					
	Control	**Low Dose**		**Just upstream of High Dose**	**High Dose**	
		Upstream	**Downstream**		**Upstream**	**Downstream**
Deployment	18-Jul	17-Jul	17-Jul	30-Jul	17-Jul	17-Jul
Cleaning/Service	23-Aug	24-Aug	23-Aug	24-Aug	24-Aug	24-Aug
Cleaning/Service	9-Sep	7-Sep	8-Sep	8-Sep	7-Sep	8-Sep
Cleaning/Service	24-Sep	24-Sep	23-Sep	*none*	24-Sep	24-Sep
Cleaning/Service	*none*	5-Oct	*none*	5-Oct	5-Oct	5-Oct
Retrieval	8-Oct	8-Oct	8-Oct	7-Oct	7-Oct	7-Oct
	2011					
	Control	**Low Dose**	**High Dose**			
Deployment	26-Aug	26-Aug	26-Aug			
Cleaning/Service	*none*	22-Sep	22-Sep			
Retrieval	24-Sep	24-Sep	23-Sep			

3.5.3 Biological Data Collection

In DEQ's SOPs, biological data collection (e.g., benthic chlorophyll *a*, macroinvertebrates) is undertaken at 11 transects evenly spaced along a defined reach. A reach is normally defined as 40 times the wetted width, with the wetted width being measured near mid-reach. For this study, we modified DEQ's 11-transect method so that (1) the transects and data collection were kept within the pre-defined 150 m (Control) or 200 m (Low Dose, High Dose) reaches rather than be defined as a 40 times the stream's wetted width, and (2) the 11 transects were placed along each reach so that the targeted reach-features would be sampled. (See **Section 3.3** for a discussion of the proportion of stream features—riffle, pool, or glide—at the Box Elder Creek study site.) Because we targeted reach features, transects were not always evenly spaced within a study reach; however, transect spacing was optimized to provide as much linear distance between each transect as possible while still capturing the targeted stream features.

3.5.3.1 Benthic Algae, Macrophyte, and Periphyton Collection, Observation, and Data Analysis

Other than the changes in reach layout described at the start of **Section 3.5.3**, aquatic flora were collected per Department SOPs (Montana Department of Environmental Quality 2011b). Beginning at the most downstream transect in each reach, samples were collected moving upstream following a right-left-center-repeat process. Benthic algal chlorophyll *a* (Chl*a*) and ash free dry mass (AFDM) were collected at each of the eleven transects and for this study we also kept the macrophytes (when encountered) for determination of chlorophyll *a* and AFDM. However, we did not analyze or report AFDM data for any samples collected via the core method as these samples mostly contain previously-deposited organic material, not the current year's biomass. In each study reach individual transect samples were kept separate from other transect samples, and were immediately frozen on dry ice and protected from light. In addition, a duplicate of the reach-wide quantitative floral biomass was collected at the Low Dose Reach in September 2009. This involved collecting the 11 benthic flora biomass samples along the reach using the right-left-center method, followed by a repetition of the entire process but starting from a different initiation point (right bank for the first sampling, center for the duplicate); by doing so duplicates were not collected at the same spot. All benthic algal biomass samples were analyzed in the laboratory of Dr. Vicki Watson (University of Montana). There, Chl*a* was determined with hot ethanol extraction followed by spectrophotometric measurement (Sartory and Grobbelaar 1984), and AFDM via standards methods (American Public Health Association, 1998). Lower reporting limits for benthic Chl*a* vary according to the surface area sampled (**Table 3.3**), and ranged from 0.1 mg Chl*a*/m^2 (all hoop samples) to 2.7-18 mg Chl*a*/m^2 (for templates and cores).

Visual assessments of stream bottom floral biomass were undertaken at all 11 transects in each study reach and this work corresponded in time with the quantitative data collection described above. At each transect, the observer used a standard form from the SOP (Montana Department of Environmental Quality 2011b)(and found at the end of **Appendix A**) to evaluate percent cover, dominant color, and growth status of plant groups (microalgae, filamentous algae, macrophytes, and moss), and also to record microalgae thickness and filament length of filamentous algae[6]. The visual evaluation was carried out at a much broader scale compared to the quantitative biomass collection, as the observer considered the condition from right bank to left within a 10 m long sub-reach centered on each transect (5 m upstream, 5 m downstream of the transect). Prior to data analysis, the form's numeric categories were transformed as follows: plant cover ratings (1-4) were converted to the midpoint value of the associated % ranges (e.g., rating level 1 became 5%, 3 became 39.5%); microalgae thickness descriptors (Thin, Medium, Thick) were converted to 0.25 mm, 1.75 mm, and 3 mm, respectively; filamentous algae length (Short, Long) was converted to 1 cm and 2 cm, respectively. In most cases (68%), notes were also taken regarding the actual length of filamentous algae filaments. Where recorded, the maximum lengths of filaments were substituted for 2 cm (2 cm being the default value associated with Long filaments).

Qualitatively collected periphyton samples were obtained from the same locations where the benthic chlorophyll *a* sub-samples were collected, and these samples were composited into a single reach-wide sample bottle and preserved with 2-3% formalin solution (final concentration). Samples were submitted to the Philadelphia Academy of Natural Sciences for enumeration of soft-bodied algae and diatom species following DEQ methods (Montana Department of Environmental Quality 2011a).

For all samples (and observations) described in this subsection, in 2009 sampling occurred four times between July and the end of September. During the nutrient dosing phase of the study in 2010, sampling occurred about every two weeks starting in late July. In 2011 sampling occurred twice (August, September).

3.5.3.2 Phytoplankton Chlorophyll a

Stream water samples for phytoplankton chlorophyll *a* were collected in each study reach at times corresponding to the collection of benthic algae described in **Section 3.5.3.1.** Duplicate samples were filtered on to GF/F filters (vacuum held below 9 inches Hg), immediately frozen on dry ice, and protected from light. Samples were analyzed by the same laboratory and method used for benthic algae. Samples were analyzed in the laboratory of Dr. Vicki Watson (University of Montana). Lower reporting limits/detection limits for phytoplankton Chl*a* vary according to the volume filtered (**Table 3.3**) and ranged from 2.5 to 10 µg Chl*a*/l (average lower reporting limit: 4.6 µg Chl*a*/l).

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[6] The visual assessment process was enhanced in spring 2010 to its present form, as found in the cited SOP and at the end of **Appendix A**. The earlier form (used only in 2009) did not contain categories for floral color, growth status, microalgae thickness, or filamentous algae length.

Chlorophyll *a* was also monitored by each of the deployed YSI 6600 sondes (see **Section 3.5.2**). Howeverm the YSI sonde Chl*a* measurements are relative, however, and need to be calibrated against field-collected water sample Chl*a* results from the same locations. Instrument drift for the YSI chlorophyll *a* measurements were determined using Rhodamine WT as the initial and final calibration testing dye.

3.5.3.3 Macroinvertebrate Collection and Data Analysis

All macroinvertebrate samples were collected following Department SOPs (Montana Department of Environmental Quality, Water Quality Planning Bureau 2006) and EMAP reachwide sampling protocols (Lazorchak et al., eds., 1998), but with the modification described at the start of **Section 3.5.3**. The macroinvertebrate samples were collected from the same transects (but not the same locations on each transect) as the periphyton samples. Samples were provided to a DEQ-approved laboratory for identification and counting (*Rhithron* Associates, Missoula, MT). In addition to standard taxa IDs and counts, samples received an additional sorting of an equivalent number of grids, followed by ashing of the macroinvertebrates from the extra grids. These provide macroinvertebrate biomass per unit area of stream bottom (g AFDM/m^2). In 2009 sampling occurred three times (July – September). In 2010 five sampling events were completed, one (July) in the Before period and four in the After period. In 2011 sampling occurred twice (August, September).

The expected response of individual taxa to nutrient dosing was estimated based on their Montana tolerance values (Montana Department of Environmental Quality, Water Quality Planning Bureau 2006). Taxa with tolerance values between 0 and 5 were categorized as decreasers (i.e., they were expected to decrease in number as a response to nutrient dosing), and those with values from 6 to 10 as increasers. The expected response of macroinvertebrate metrics and harm-to-use thresholds was based on DEQ SOPs (Montana Department of Environmental Quality, Water Quality Planning Bureau 2006).

It resulted that many taxa (e.g., Baetis sp., Heptageniidae) were not consistently observed during paired sampling events (e.g., Baetis sp. was counted in the Control Reach sample of 7/25/2009, but not found in the Low Dose Reach sample on the same date). As a result, there were fewer overall **D** values for individual taxa, reducing the power of the BACIP statistical testing. As an operational minimum, at least three **D** values in the Before period and three D values in the After period was considered necessary to run Mann-Whitney tests for individual taxa.

4.0 RESULTS

4.1 WEATHER

The BACIP "Before" and "After" statistical analyses are restricted to 2009 and 2010 data (see BACIIP example, **Table 3-1**). 2011 data are considered follow-up/recovery data. Therefore, weather data summaries are provided here in two sets (2009 and 2010 data, and 2009, 2010, and 2011 data) so that weather conditions specific to the BACIP period can be viewed on their own accord. The 2009 and 2010 data summaries cover a longer record (two months), extending from late July to the end of September in each year, as shown in **Table 4-1** and **Figure 4-1**. The only common time period for weather data across all three years of the study was August 26th to September 24th (less than one month), and these data are shown in **Table 4-2** and **Figure 4-2**.

Table 4-1. Weather Conditions in 2009 and 2010. Data are the averages for the date range shown.

Year	Date Range	Air Temperature (°C)	Solar Radiation (µmol quanta/m^2/sec)	Dew Point (°C)
2009	7/29 to 9/28	17.6	956	8.4
2010	7/29 to 9/28	17.2	1004	9.2

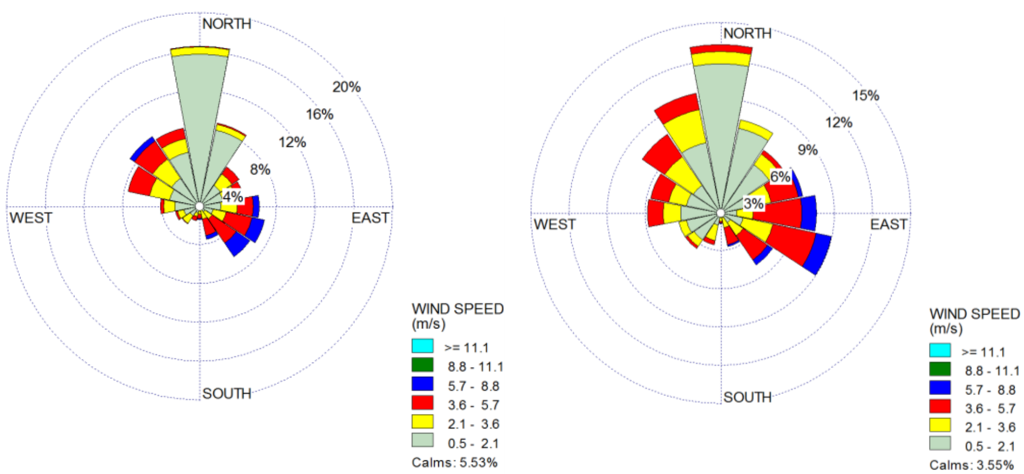


Figure 4-1. Paired Wind Rose Data for Box Elder Creek, 7/29 to 9/28, in 2009 and 2010.
Left panel, 2009. Right panel, 2010. Wind direction vectors are shown as blowing to.

Table 4-2. Weather Conditions in 2009, 2010, and 2011. Data are the averages for the date range shown.

Year	Date Range	Air Temperature (°C)	Solar Radiation (µmol quanta/m^2/sec)	Dew Point (°C)
2009	8/26 to 9/24	17.8	926	8.0
2010	8/26 to 9/24	14.0	830	7.3
2011	8/26 to 9/24	15.3	984	5.8

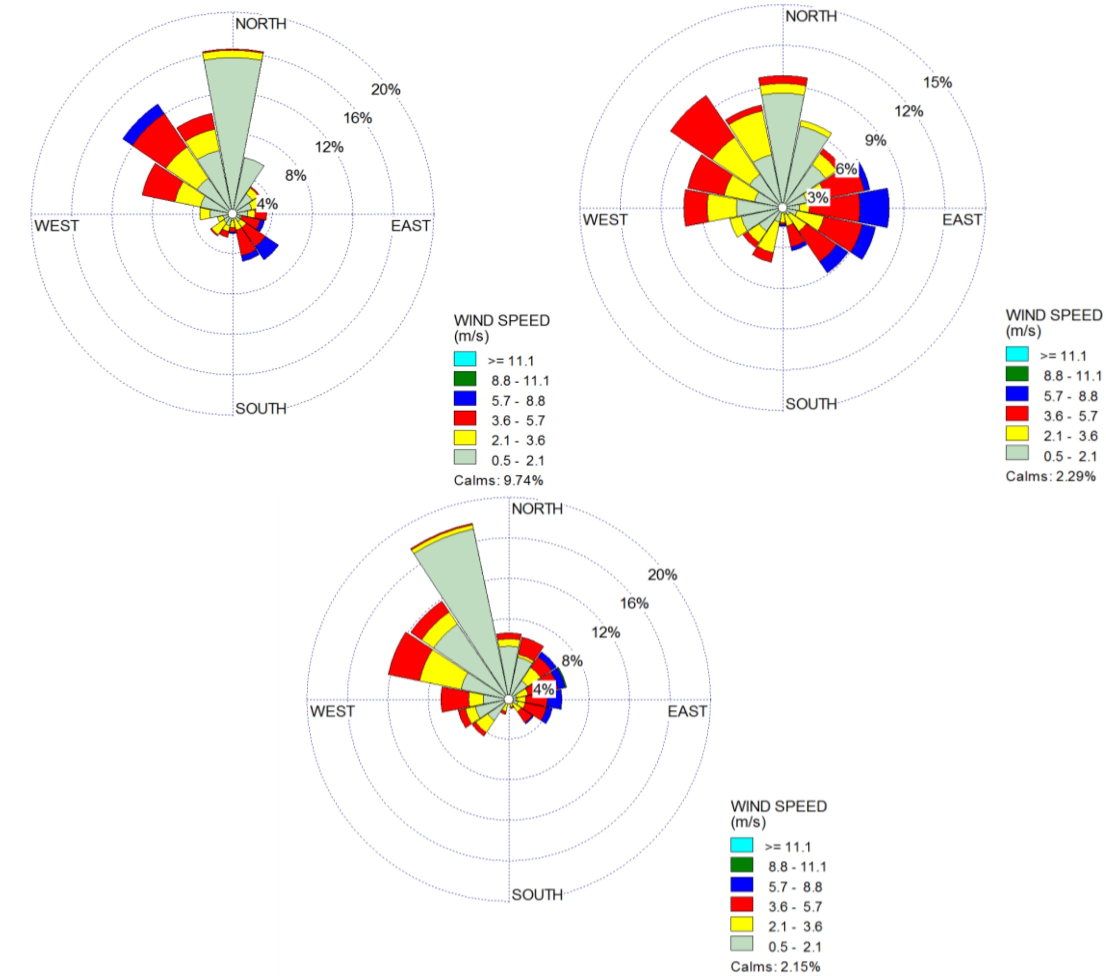


Figure 4-2. Wind Rose Data for Box Elder Creek, 8/26 to 9/24, 2009, 2010, and 2011.
Upper left panel, 2009. Upper right panel, 2010. Lower Panel, 2011. Wind direction vectors are shown as blowing to.

In the time period during which the bulk of the nutrient dosing occurred (August and September 2010), weather conditions were, on average, quite similar to the same time period in 2009 (**Table 4-1**; **Figure 4-1**). Average air temperature, dew-point temperature[7], and solar radiation were all very similar; 2010 was somewhat sunnier, with fewer overcast days as indicated by the higher solar radiation value. Prevailing winds usually blew from the south to the north at low velocities, whereas higher-velocity winds in both years generally came from either the southeast or northwest in roughly equal measure. 2010 tended to be windier and with a greater proportion of high-velocity winds.

Late August to late September data for all three years (**Table 4-2** and **Figure 4-2**) show somewhat more variability. Wind speed and direction in 2010 in particular was more variable, whereas both 2009 and 2011 showed a fairly substantial proportion of low-velocity vectors blowing to the north or north-northwest. 2009 was somewhat warmer, whereas (based on dew point) 2011 somewhat drier than the other two years of the study.

[7] For comparison, the dew point in Houston, Texas (a hot, very humid city) is often around 23 ^{o}C, whereas at the Box Elder Creek study site it was usually around 9 ^{o}C; thus, the air at the study site was much drier.

4.2 Channel Geometry and Stream Flow

4.2.1 Wetted Channel Geometry

Average wetted widths and depths for the study reaches for each year of the study are shown in **Table 4-3**. Values reported from 2010 and 2011 are more accurate than 2009, because they are based on all eleven transects from each study reach. Wetted channel dimensions varied somewhat from year to year, in part due to variations in flow (**Table 4-3**) but also due to observed morphological changes that occurred in spring 2011. At that time, a very large spring flow event occurred which moved the channel within its floodplain. At some locations, lateral channel movement was at least 1 to 2 m, based on bank pins which had been placed in 2009. Overall water depth of all three reaches across the study period was fairly similar (ca. 24 cm, on average), and was never less than 18 cm.

Table 4-3. Wetted Channel Geometry for the Three Study Reaches, 2009-2011.

Study Reach	Year	Measurement Date	Flow (m^3/sec)	Average Wetted Width (m)	Average Water Depth (cm)
Control	2009	9/29/2009	0.07	11.6	25
Control	2010	9/22/2010	0.18	9.1	18
Control	2011	9/24/2011	0.22	11.4	30
Low Dose	2009	9/29/2009	0.07	8.7	19
Low Dose	2010	9/22/2010	0.18	9.3	25
Low Dose	2011	9/24/2011	0.22	11.2	35
High Dose	2009	9/29/2009	0.07	12.3	28
High Dose	2010	9/22/2010	0.18	10.7	21
High Dose	2011	9/24/2011	0.22	15.4	21

4.2.2 Stream Flow

Measured stream flow ranged from 0.07 to 2.0 m^3/sec. A high-flow event occurred towards the middle of July 2010 which—due to safety concerns—was too high to measure, but we estimated that it may have peaked at roughly 15 m^3/sec. Mid-summer to fall baseflow was commonly around 0.2 m^3/sec, however 2009 showed lower flows than the other years, and averaged 0.12 m^3/sec.

Table 4-4. Stream Flow Measured in Box Elder Creek during the Study.

YEAR							
2009			2010			2011	
Date	Flow (m^3/sec)*		Date	Flow (m^3/sec)†		Date	Flow (m^3/sec)†
25-Jul	0.18		26-Jul	2.01		27-Aug	0.39
12-Aug	0.18		27-Jul	1.30		24-Sep	0.22
30-Aug	0.09		28-Jul	1.14			
15-Sep	0.06		29-Jul	0.74			
28-Sep	0.07		30-Jul	0.59			
			31-Jul	0.59			
			1-Aug	0.53			
			7-Aug	0.31			
			9-Aug	0.29			
			22-Aug	0.17			
			24-Aug	0.14			
			27-Aug	0.13			
			1-Sep	0.11			
			7-Sep	0.17			
			9-Sep	0.14			
			19-Sep	0.19			
			22-Sep	0.18			
			24-Sep	0.31			
			26-Sep	0.23			
			1-Oct	0.22			
			5-Oct	0.13			

*Average of paired values measured at the Low Dose and High Dose reaches.
†Measured at the Low Dose Reach.

4.3 Chemical/Water Quality Measurements

This section covers the grab-sample water quality measurements of the study. Continuously monitored data will be covered in the next section. Unless reported otherwise, data handling included converting all below detection values to ½ their reporting limit prior to inclusion in statistical summaries (Suplee et al. 2007), and reducing to a single average value the routine and field-duplicate samples (i.e., those collected at the same study reach at the same time) prior to inclusion in monthly or annual averages.

4.3.1 Quality Control Check on the Nutrient Solutions in the Dosing Tanks

Laboratory-analyzed nutrient concentrations from samples collected from the nutrient dosing tanks were compared to the calculated tank concentrations as computed from the solution preparations. Similarity was quite good, as the percent difference between the two was always less than 10% and was, on average, about 5% (**Table 4-5**). In almost all cases the tank solutions—based on the laboratory measurements—were at a lower concentration than the calculated concentration.

Later in this document we will report the nutrient concentrations achieved in the Low- and High Dose reaches resulting from the mixing of dripped tank solutions and ambient stream water. Whenever possible, we will make these calculations using the average concentrations measured in the tanks (i.e., the laboratory-analyzed values) instead of the calculated concentrations, as we believe the directly-measured tank concentrations are the most accurate.

Table 4-5. Percent Difference Between Calculated and Measured Nutrient Concentrations in the Dosing Tanks.

Experimental Reach Tanks	Tank Sampling Date	Tank Nutrient	Calculated Tank Concentration (g N or P/L)	Measured Tank Concentration (g N or P/L)	Percent Difference
Low Dose	8/29/2010	NO_3 as N	49.4	45.8	-7.3%
Low Dose	9/25/2010	NO_3 as N	49.4	46.1	-6.7%
High Dose	8/9/2010	NO_3 as N	65.9	61.9	-6.1%
High Dose	9/25/2010	NO_3 as N	65.9	62.0	-5.9%
Low Dose	8/29/2010	PO_4 as P	2.67	no data	n/a
Low Dose	9/25/2010	PO_4 as P	2.67	2.55	-4.5%
High Dose	8/9/2010	PO_4 as P	8.89	9.18	3.3%
High Dose	9/25/2010	PO_4 as P	8.89	8.72	-1.9%

4.3.2 Common Water-quality Parameters

Box Elder Creek was dosed with fairly low concentrations of nutrients, and it was expected that the counter-ions of the nutrients salts (potassium, K, and sodium Na) would have no measureable effect on the stream's ambient levels of those dissolved constituents (see **Section 3.4.1**). To confirm this, 2010 measured concentrations of K and Na are here compared between the Control Reach and the two experimental reaches. We evaluate K and Na concentrations using the BACIP statistical design. For K, in 2010 during dosing (i.e., the After period), concentrations in the Control reach averaged 7.99 mg/L, in the Low Dose reach they averaged 7.94 mg/L, and in the High Dose Reach they averaged 7.82 mg/L. Based on BACIP analysis, there was no significant difference in K concentrations between the Before and After periods (one-sided Mann-Whitney test; **Table 4-6**) for the Low Dose and High Dose reaches.

For sodium (Na), results were nearly identical. During 2010 dosing the Control- and Low Dose reaches averaged 301 and 296 mg/L, respectively, and the High Dose Reach averaged 294 mg/L. The BACIP analysis indicated there was no significant difference in Na concentrations between the Before and After periods (one-sided Mann-Whitney) for either the Low- or High Dose reaches. These data confirm our pre-study expectation that the added nutrient salts would have no measurable effect on the background K and Na concentrations in the stream.

Table 4-6. BACIP-arrayed Potassium (K) Data for the Control, Low Dose, and High Dose reaches.

Date	Period	Sampling Event	Control	Low Dose (mg/L)	High Dose (mg/L)	Difference (D) Control - Low Dose	Difference (D) Control - High Dose
7/24/2009	Before	1(2009)	7.925	7.88	7.88	0.045	0.045
8/12/2009	Before	2 (2009)	8.135	7.81	8.25	0.325	-0.115
8/29/2009	Before	3 (2009)	8.235	5.86	8.28	2.375	-0.045
9/26/2009	Before	4 (2009)	8.05	7.99	8.11	0.06	-0.06
7/16/2010	Before	5 (2010)	9.8	10.6	10.1	-0.8	-0.3
8/25/2010	After	1 (2010)	8.41	8.44	8.31	-0.03	0.1
9/7/2010	After	2 (2010)	8.19	7.98	7.7	0.21	0.49
9/22/2010	After	3 (2010)	7.37	7.39	7.45	-0.02	-0.08

Other common water quality measurements were made as well (alkalinity, carbonate, bicarbonate, hardness, sulfate, chloride, total dissolved solids (TDS), and total suspended solids (TSS). A review of these water-quality parameters indicated that they did not vary in any obvious way among the three study reaches during the dosing period in 2010, and were generally similar among the three study reaches during any given sampling event. Month-to-month variation was noted, undoubtedly driven by variables in the watershed (flow changes driven by precipitation events, for example). Since dosing had no apparent effect on these variables, for brevity, the annual averages for these common parameters are presented only for the Control Reach (**Table 4-7**).

Table 4-7. Statistical Summaries for Common Water-quality Parameters in the Control Reach. Concentration data shown are the annual average, followed by minimum and maximum in parentheses. Data were collected between July and October of each year.

Water-quality Parameter (and units)	Sample Size (by year)			Concentration (by year)		
	2009	2010	2011	2009	2010	2011
Total suspended solids (mg/L)	4	5	2	17 (10-26)	19 (14-24)	10 (10-10)
Total dissolved solids (mg/L)	4	5	2	1144 (911-1345)	1142 (1040-1210)	1488 (1465-1510)
Hardness (Ca, Mg) (mg/L)	4	5	2	242 (229-258)	276 (242-384)	353 (349-353)
Total alkalinity (mg/L as $CaCO_3$)	4	5	2	434 (346-527)	412 (315-486)	474 (462-485)
Carbonate (mg/L as $CaCO_3$)	4	5	2	27 (1-48)	28 (6-43)	29 (25-34)
Bicarbonate (mg/L as $CaCO_3$)	4	5	2	407 (334-480)	383 (298-444)	444 (428-460)
Sulfate (mg/L)	4	5	2	520 (446-598)	535 (477-627)	689 (669-708)
Chloride (mg/L)	4	5	2	7 (4-9)	7 (6-9)	10 (10-10)

As has been observed in previous years, Box Elder Creek was a sodium sulfate dominant stream during the study, with strong buffering capacity (total alkalinity > 400 mg/L as $CaCO_3$). It resulted that, from water quality point of view, 2009 and 2010 were fairly similar to one another, whereas 2011 had higher concentrations of dissolved constituents and lower total suspended solids (**Table 4-7**). For example, there is no significant difference between 2009 and 2010 for TSS or TDS concentrations, whereas there is a significant difference for both of these water-quality parameters between 2010 and 2011 (two-sided T-test, unequal variance, p = <0.01 each for TSS and TDS). The fact that 2009 and 2010 are so similar is ideal for this BACIP study design, as those are the two years used for BACIP statistics.

4.3.3 Nutrient Concentrations in the Control Reach

Average, minimum and maximum concentrations for the four measured nutrients are compared, by year, in **Table 4-8**. Field blanks associated with these data were nearly all non-detects, with only a few exceptions, and those were mostly right above the reporting limit. As we have observed on other projects (Suplee 2004), ammonia hits in field blanks were the ones highest above the reporting limit (ammonia reporting limit were 1 to 10 µg/L as N; field blanks of 16 and 17 µg /L as N were measured once in 2010 and once in 2011, respectively).

We assumed that background soluble nutrient concentrations in the stream in 2010 would be very similar to what was measured in August and September of 2009 (i.e., 3 µg NO_3-N/L and 4 µg SRP/L), and as can be seen in **Table 4-8** this was the case. (Note: the single high SRP value in 2009 of 47 µg/L had not been included among the 2009 data used to project 2010 stream conditions because it was collected early, in July 2009.) Concentrations among total nutrients were quite similar in 2009 and 2010, although 2011 had notably lower average TP concentrations.

Table 4-8. Descriptive Statistics for Nutrient Concentrations in the Control Reach.
Data shown are the annual average, followed by minimum and maximum in parentheses. Data were collected only between July and October of each year.

Year	Nutrient Concentration (µg/L)				
	Total N	**NO_{2+3}-N**	**Ammonia-N**	**Total P**	**SRP**
2009	473 (364-593)	4 (2-6)	7 (3-13)	43 (23-70)	14 (3-47)
2010	465 (368-622)	3 (3-3)	15 (8-23)	40 (34-44)	3 (2-5)
2011	441 (369-513)	7 (3-12)	7 (3-11)	24 (17-31)	2 (1-3)

4.3.4 Nutrient Concentrations in the Low Dose Reach

4.3.4.1 Calculated dosing concentrations at the upstream end of the Low Dose Reach

The dose rate for the Low Dose Reach was targeted to achieve concentrations of 40 µg NO_3-N/L and 6 µg SRP/L near its headwaters, after mixing (see **Section 3.4.1**). Ambient Box Elder Creek nitrate concentrations in 2010 averaged exactly what was anticipated (4 µg/L), whereas ambient SRP concentrations in 2010 were a bit lower than projected (3 µg/L instead of the projected 4 µg/L; **Table 4-8**). Stream flow and nutrient drip rates and concentrations were integrated over the course of the experiment (49 and 51 days for N and P, respectively) to determine the experiment-long doses achieved, given the ambient 2010 soluble nutrient concentrations observed at the Control Reach (**Table 4-8**). Low-Dose reach concentrations actually achieved were 38.6 µg NO_3-N/L and 4.4 SRP/L, with an associated N:P ratio (by mass) of 8.8:1.

4.3.4.2 Measured nutrient concentrations in the Low Dose Reach

Summary nutrient data for the Low Dose Reach are shown in **Table 4-9**. Note that for 2010, the summaries only reflect samples collected during the time dosing was occurring (there was a pre-dosing July sample and a post-dosing October sample which were not included in the table). In 2009 and 2011, the Low Dose nutrient concentrations are very similar to the Control Reach (as one would expect), although the 2011 TN concentration is quite a bit lower in the Low Dose Reach. In 2010, the dosing effect is quite evident for NO_{2+3}, where the Low-Dose reach average concentration (32 µg NO_{2+3}-N/L) is close to the season-long achieved dose-rate concentration of 38.6 µg NO_3-N/L[8]. Further, the BACIP analysis (**Table 4-10**) shows that stream NO_{2+3} concentrations in the After (i.e., dosing) period was significantly greater than in the Before period (one sided Mann-Whitney, p = 0.02). Based on BACIP analyses identical in design to **Table 4-10**, there was no significant difference in nutrient concentrations between the Before and After periods for TN, ammonia, TP, or SRP.

[8] Recall that these samples were collected at the end of the 200 m study ready, therefore the concentration has been influenced by assimilation by flora and would be expected to be lower than the initially-mixed concentration.

Table 4-9. Descriptive Statistics for Nutrient Concentrations in the Low Dose Reach.
Data shown are the annual average, followed by minimum and maximum in parentheses. Data were collected between July and October (2009 and 2011), but for 2010 only samples collected during dosing (Aug-Oct) are shown.

	Nutrient Concentration (µg/L)				
Year	**Total N**	**NO_{2+3}-N**	**Ammonia-N**	**Total P**	**SRP**
2009	482 (363-647)	4 (3-5)	5 (3-11)	40 (23-54)	16 (3-53)
2010	446 (406-492)	32 (22-39)	21 (16-25)	43 (35-52)	5 (3-7)
2011	302 (197-407)	8 (3-13)	3 (3-3)	22 (14-29)	1 (1-2)

Table 4-10. BACIP-arrayed NO_{2+3} Data (µg NO_{2+3}-N/L) for the Control- and Low Dose reaches.
Mann-Whitney test carried out on the Before *vs.* After D values.

Date	Period	Sampling Event	Control (µg/L)	Low Dose (µg/L)	Difference (D) Control - Low Dose
7/24/2009	Before	1(2009)	5.5	5	0.5
8/12/2009	Before	2 (2009)	2.25	3	-0.75
8/29/2009	Before	3 (2009)	6	4	2
9/26/2009	Before	4 (2009)	3.5	4	-0.5
7/16/2010	Before	5 (2010)	2.5	5	-2.5
8/25/2010	After	1 (2010)	2.5	36	-33.5
9/7/2010	After	2 (2010)	2.5	38.5	-36
9/22/2010	After	3 (2010)	2.5	22	-19.5

4.3.5 Nutrient Concentrations Just Upstream of the High Dose Reach

Average, minimum and maximum concentrations just upstream of the headwaters of the High Dose Reach during dosing are given in **Table 4-11**. We assumed that an average residual of 2.0 µg NO_{2+3}-N/L would arrive to the headwaters of the High Dose Reach from the Low Dose, and this turned out to be the case. The average concentration just upstream of the High Dose Reach was 5 µg NO_{2+3}-N/L (**Table 4-11**) and the dosing-period average in the Control Reach was 3 µg NO_{2+3}-N/L (**Table 4-8**). Also, as we anticipated, SRP just upstream of the High Dose Reach was the same as ambient background observed in the Control Reach. Note that an average of 27 µg NO_{2+3}-N/L were taken up between the end of the Low Dose Reach and the head of the High Dose Reach, a distance of 900 m of stream. This decline in nitrate is consistent with our field observations, as we documented increased algal growth during dosing along the interim reach of stream between the Low- and High Dose reaches; the increased algae was almost certainly what reduced the nitrate concentrations.

Table 4-11. Descriptive Statistics for Nutrient Concentrations Just Upstream of the High Dose Reach.
Data shown are the 2010 dosing-period average, followed by the minimum and maximum in parentheses.

	Nutrient Concentration (µg/L)				
Year	**Total N**	**NO_{2+3}-N**	**Ammonia-N**	**Total P**	**SRP**
2010	466 (408-497)	5 (2.5-10)	18 (9-22)	46 (41-52)	3 (2-5)

4.3.6 Nutrient Concentrations in the High Dose Reach

4.3.6.1 Calculated dosing concentrations at the upstream end of the High Dose Reach

The nutrient dose rate for the High Dose Reach was targeted to achieve concentrations of 150 µg NO_3-N/L and 23 µg SRP/L near its headwaters, after mixing (**Section 3.4.1**). As noted in **Section 4.3.5** above, nitrate concentrations in 2010 arriving to the High Dose Reach headwaters averaged exactly what was anticipated (5 µg/L) whereas ambient SRP concentrations were slightly lower than projected (3 µg/L instead of the projected 4 µg/L; **Table 4-11**). Stream flow and nutrient drip rates and concentrations were integrated over the course of the experiment (55 and 53 days for N and P, respectively) to determine the experiment-long doses achieved, given the ambient soluble nutrient concentrations observed just upstream of the High Dose Reach (**Table 4-11**). High Dose Reach concentrations actually achieved were 118.7 µg NO_3-N/L and 15.6 SRP/L, with an associated N:P ratio (by mass) of 7.6:1.

4.3.6.2 Measured nutrient concentrations in the High Dose Reach

Nutrient data summaries for the High Dose Reach are shown in **Table 4-12**. Note that for 2010, the summaries only reflect samples collected during the time dosing occurred (there was a pre-dosing July sample and a post-dosing October sample which were not included here). In 2009 and 2011, the High-Dose nutrient concentrations are fairly similar to the Control Reach in the corresponding years. In 2010, the dosing effect for NO_{2+3} is evident, as the High Dose Reach average concentration (48 µg NO_{2+3}-N/L) is much higher than the Control reach NO_{2+3} average the same year (3 µg/L; **Table 4-8**). Supporting this, the BACIP analysis (**Table 4-13**) showed that stream NO_{2+3} concentrations in the After (i.e., dosing) period were significantly greater than in the Before period (one sided Mann-Whitney, p = 0.02). Based on BACIP analyses identical in layout to **Table 4-13**, there was also significantly greater TN and TP in the After period compared to the Before period (one sided Mann-Whitney, p = 0.04 and 0.02, respectively); there were no significant differences for SRP. For ammonia, there was a marginally significant *decrease* in concentrations in the After period (two sided Mann-Whitney, p = 0.07), contrary to our expectations. In 2010, there appears to have been a longitudinal increase in TP concentration, presumably due to the dosing. In the Control Reach (most upstream) TP averaged 40 µg/L, in the Low Dose Reach it was 43 µg/L, it increased to 46 µg/L just upstream of the High-Dose reach, and was 52 µg/L at the end of the High-Dose reach. No longitudinal trend was evident for TN.

Table 4-12. Descriptive Statistics for Nutrient Concentrations in the High Dose Reach.
Data shown are the annual average, followed by the minimum and maximum in parentheses. Data were collected between July and October (2009 and 2011), but for 2010 only samples collected during dosing (Aug-Oct) are shown.

Year	Nutrient Concentration (µg/L)				
	Total N	**NO_{2+3}-N**	**Ammonia-N**	**Total P**	**SRP**
2009	492 (401-653)	4 (1-7)	9 (3-18)	41 (21-65)	20 (2-67)
2010	538 (485-595)	48 (8-80)	17 (13-23)	52 (48-59)	6 (5-8)
2011	447 (334-559)	3 (3-3)	3 (3-3)	22 (18-26)	2 (1-3)

Table 4-13. BACIP-arrayed NO_{2+3} Data (µg $_{NO2+3}$-N/L) for the Control- and High Dose reaches.

Date	Period	Sampling Event	Control (µg/L)	High Dose (µg/L)	Difference (D) Control - High Dose
7/24/2009	Before	1(2009)	5.5	7	-1.5
8/12/2009	Before	2 (2009)	2.25	4	-1.75
8/29/2009	Before	3 (2009)	6	4	2
9/26/2009	Before	4 (2009)	3.5	0.5	3
7/16/2010	Before	5 (2010)	2.5	5	-2.5
8/25/2010	After	1 (2010)	2.5	8	-5.5
9/7/2010	After	2 (2010)	2.5	80	-77.5
9/22/2010	After	3 (2010)	2.5	55.5	-53

4.4 Continuously-monitored Water Quality Measurements

The deployed instruments were frequently checked and cleaned throughout the study (see **Table 3-4**). Post-deployment, all data received a QC review using standardized *a posteriori* methods. Data that were suspect were flagged, as were data that had drifted beyond the project's drift criteria. Suspect data that were flagged were excluded from the figures and analysis presented below. Details on the *a priori* and *a posteriori* QC methods can be found in the project QAPP in **Appendix A**.

4.4.1 Temperature and Dissolved Oxygen

Table 4-14 shows the average monthly water temperatures recorded by deployed sondes in each year of the study. Limited data were also available in July and October, but these datasets were short and have not been presented. Average water temperatures were typically in the low 20s in August, and between 15 and 18^{o} C in September. For any given month during a given year, there was very little variation in water temperature among the study reaches. Similarly, there was generally little difference in 2010 monthly averages between sondes positioned in the upstream vs. downstream locations within the same study reach (the Low Dose Reach in September being something of an exception to this).

Table 4-14. Average Water Temperature (degrees C) Recorded by the Deployed Sondes.

Year	Month*	Control Reach (one sonde location only)	Low Dose Reach		High Dose Reach	
			Upstream Sonde	Downstream Sonde	Upstream Sonde	Downstream Sonde
2009	August	20.4	20.5	n/a	20.5	n/a
2009	September	18.2	18.4	n/a	18.3	n/a
2010	August	22.3	21.7	22.2	22.3	22.1
2010	September	15.2	14.5	15.2	15.2	15.0
2011	August	Limited data†	Limited data†	n/a	Limited data†	n/a
2011	September	16.4	16.1	n/a	16.4	n/a

*Due to flagged data or early retrieval, monthly averages may be somewhat shy of a complete 30-day dataset.
†Data were only available 8/26 to 8/31 and are not presented.

Figure 4-3 presents the entire DO dataset for the Control, Low Dose, and High Dose reaches in 2009, a year prior to when nutrient dosing occurred in 2010. A basic assumption of a BACIP study is that conditions among the study reaches should be largely comparable in advance of the impact (the impact being the 2010 nutrient dosing). As can be seen, in 2009 the season-long DO patterns among the three reaches were very similar. On close comparison one will find that one reach may have had somewhat higher or lower daily DO highs and lows, but overall, the three manifest essentially the same seasonal DO patterns. In no case did DO fall below Montana's standards for a C-3 stream (i.e., 5 mg/L for early life stages).

DO saturation was calculated for the 2009 datasets, using the reaches' elevation above sea level (921 m, Control and Low Dose reaches; 917 m, High Dose Reach) and the ambient water temperatures measured by the sondes. Calculated DO saturation fell between 7 and 10 mg DO/L, and was generally higher near the end of September as water temperatures dropped. In summer and fall 2009, measured DO (**Figure 4-3**) in the three reaches oscillated fairly tightly around saturation, with daily highs rising higher than saturation by about 1 mg DO/L, and daily lows dropping lower than saturation by about 0.5 mg DO/L.

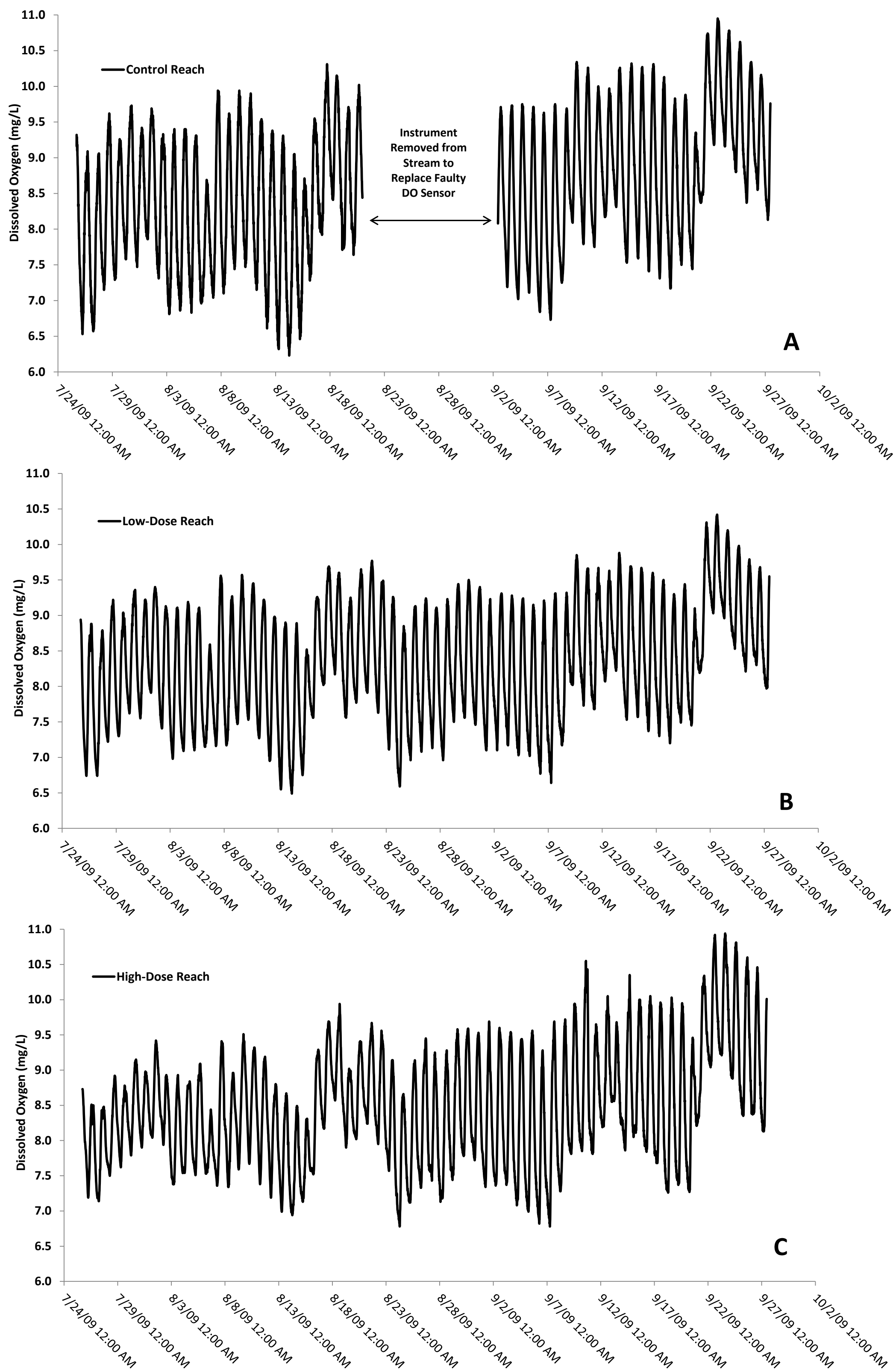


Figure 4-3. DO in the Study Reaches, 2009.

(A) Control Reach. (B) Low Dose Reach. (C) High Dose Reach. No nutrient dosing occurred in 2009.

Figure 4-4 presents the entire DO dataset for the Control- and Low Dose reaches in 2010, before and after dosing occurred. Prior to dosing, the pattern of daily highs and lows observed in the Control- and Low Dose reaches overlapped almost perfectly. About 16 days after dosing began, both Low Dose sondes began recording increasingly higher daily DO highs compared to the Control Reach, corresponding with the increased algal biomass we observed. At the upstream Low Dose sonde, nightly DO lows remained essentially matched to the Control Reach (**Figure 4-4A**). However, in the downstream Low Dose sonde, the nightly DO lows became lower, relative to the Control reach (**Figure 4-4B**). For both sondes, the greatest differences in DO occurred close to mid-September.

Figure 4-5 shows the entire DO dataset for the Control- and High Dose reaches in 2010, before and after dosing occurred. Prior to dosing, the pattern of daily highs and lows observed in the Control- and High Dose reaches overlapped to a very high degree, although the High Dose sondes show slightly higher daily DO highs. After dosing, DO response was much more rapid than in the Low Dose Reach, and the upstream High Dose sonde recorded very high DO concentrations (nearly 27 mg/L) during the peak period around mid-September (**Figure 4-5A**). In contrast to the similarity in DO patterns recorded by the two sondes in the Low Dose Reach, the two sondes in the High Dose Reach each recorded very different DO patterns. The upstream High Dose sonde shows a distinct period near the end of the deployment when DO crashed **(Figure 4-5A**), and for the remaining ten days of the deployment DO remained quite low, at times dropping to close to 1 mg DO/L. Concentrations at this time fell below Montana's DO standards for aquatic life (i.e., 5 and 3 mg DO/L for juvenile and adult aquatic life, respectively). These data—restricted to the end of the growing season/beginning of fall senescence—represent the only place or time in the three years of the study where DO fell below Montana's DO standards. In contrast, the downstream High Dose sonde (**Figure 4-5B**) did not demonstrate this pronounced DO decline late in the season, but instead, continued to show elevated daily DO relative to the Control Reach. The largest differences between Control and downstream High-Dose DO concentrations occurred (like they did for the upstream High Dose sonde) in September, and as October progressed, these differences diminishing.

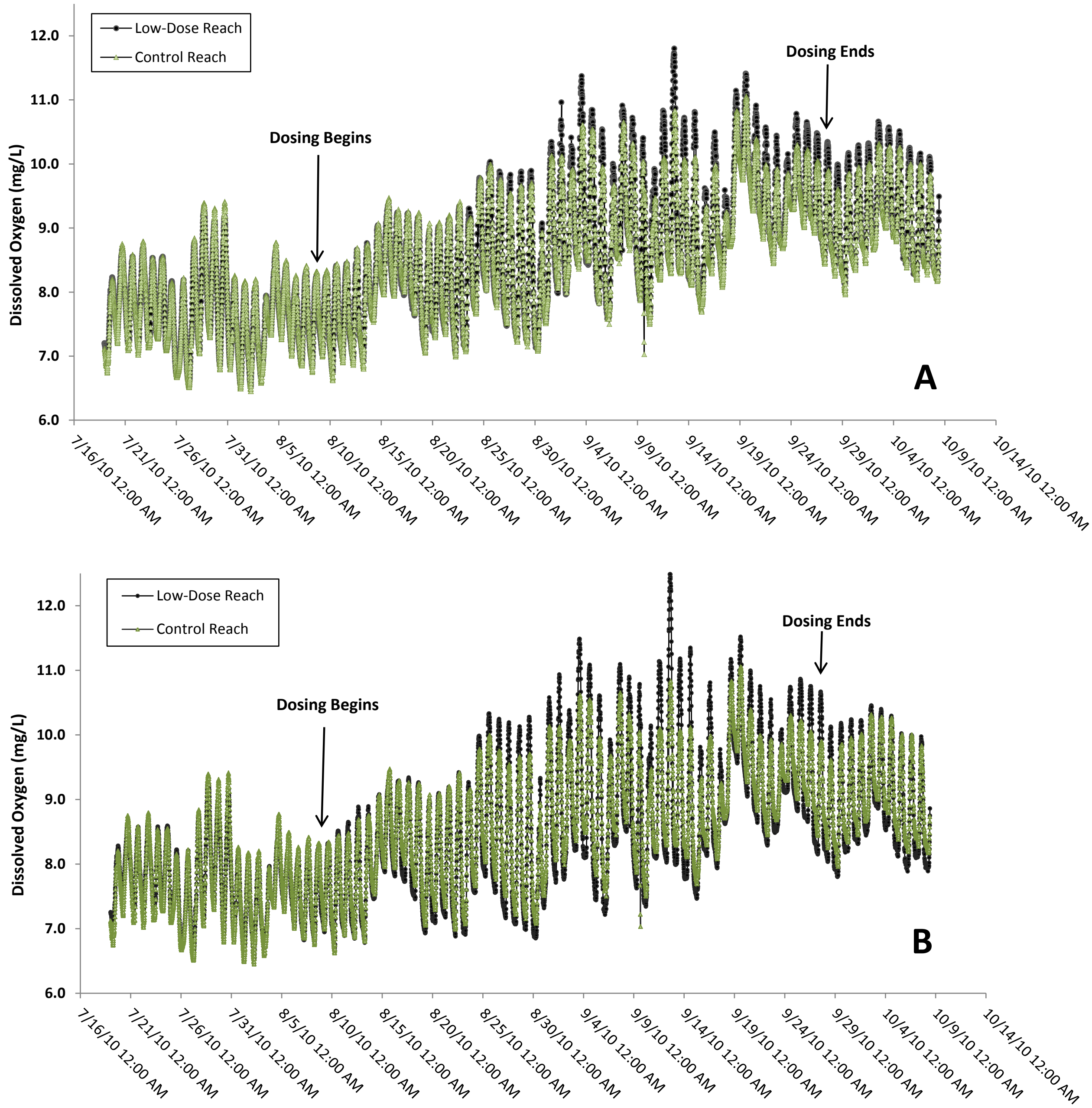
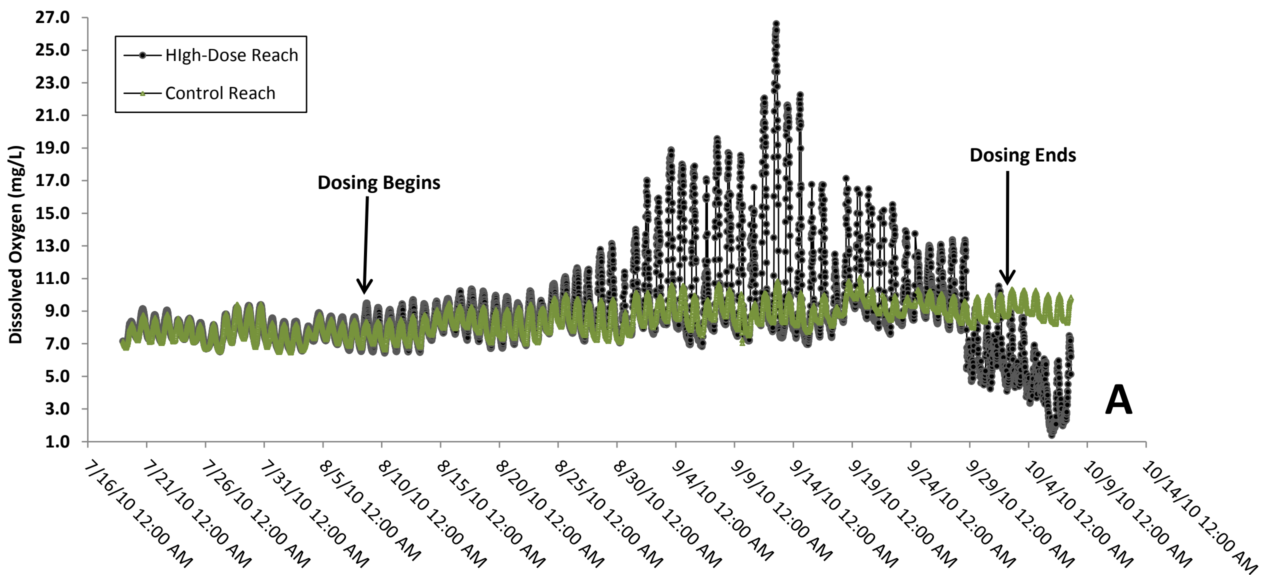


Figure 4-4. Dissolved Oxygen Patterns Recorded by Sondes Located in the Control- and Low Dose Reaches in 2010.

(A) Control Reach data (green) and data from the "upstream" Low Dose Reach sonde (black). The "upstream" sonde was positioned about mid-reach within the Low Dose Reach. (B) Control Reach data (green) and data from the "downstream" Low Dose Reach sonde (black). The "downstream" sonde was located about 200 m downstream from the Low Dose nutrient-dripper assembly, near the end of the Low Dose Reach.



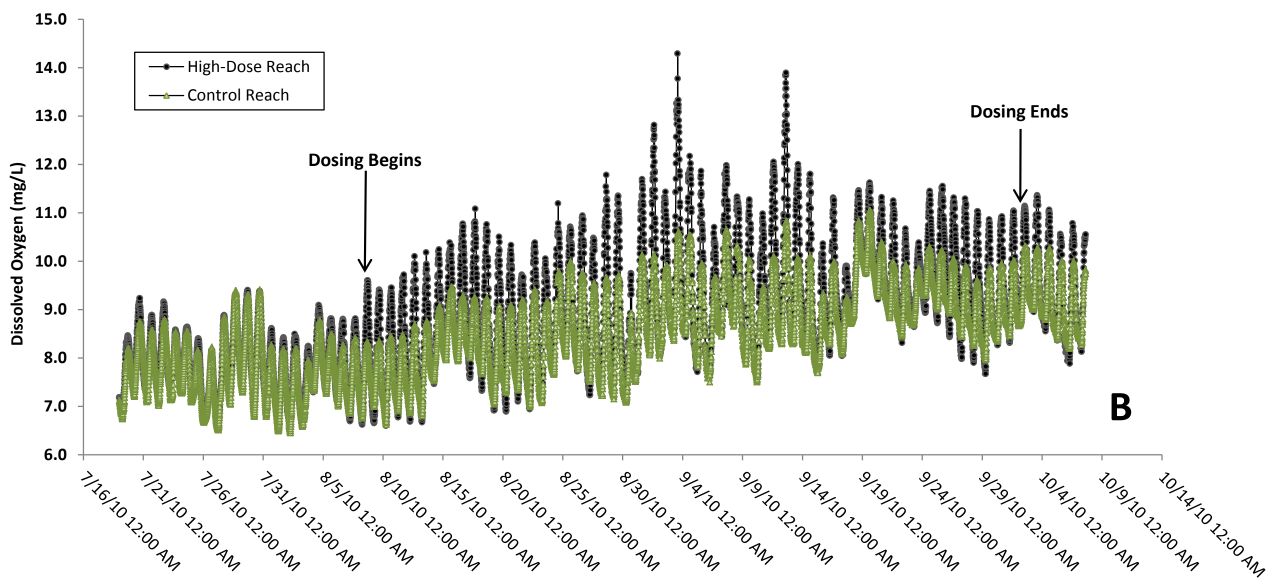


Figure 4-5. Dissolved Oxygen Patterns Recorded by Sondes Located in the Control- and High Dose Reaches in 2010.

(A) Control Reach data (green) and data from the “upstream” High Dose Reach sonde (black). The “upstream” sonde was positioned about mid-reach within the High Dose Reach. (B) Control Reach data (green) and data from the “downstream” High Dose Reach sonde (black). The “downstream” sonde was located about 200 m downstream from the High Dose nutrient-dripper assembly, near the end of the High Dose Reach.

In 2011, YSI sondes were again deployed to measure DO. In 2011 no dosing occurred. The DO patterns of the three reaches largely resemble one another, as they did in 2009 (**Figure 4-6**). There is no indication that the dosing effects of the previous year caused a lingering effect on the DO patterns of the stream. Just like 2009, in no case in 2011 did DO fall below Montana’s standards for this C-3 stream (i.e., 5 mg/L for early life stages).

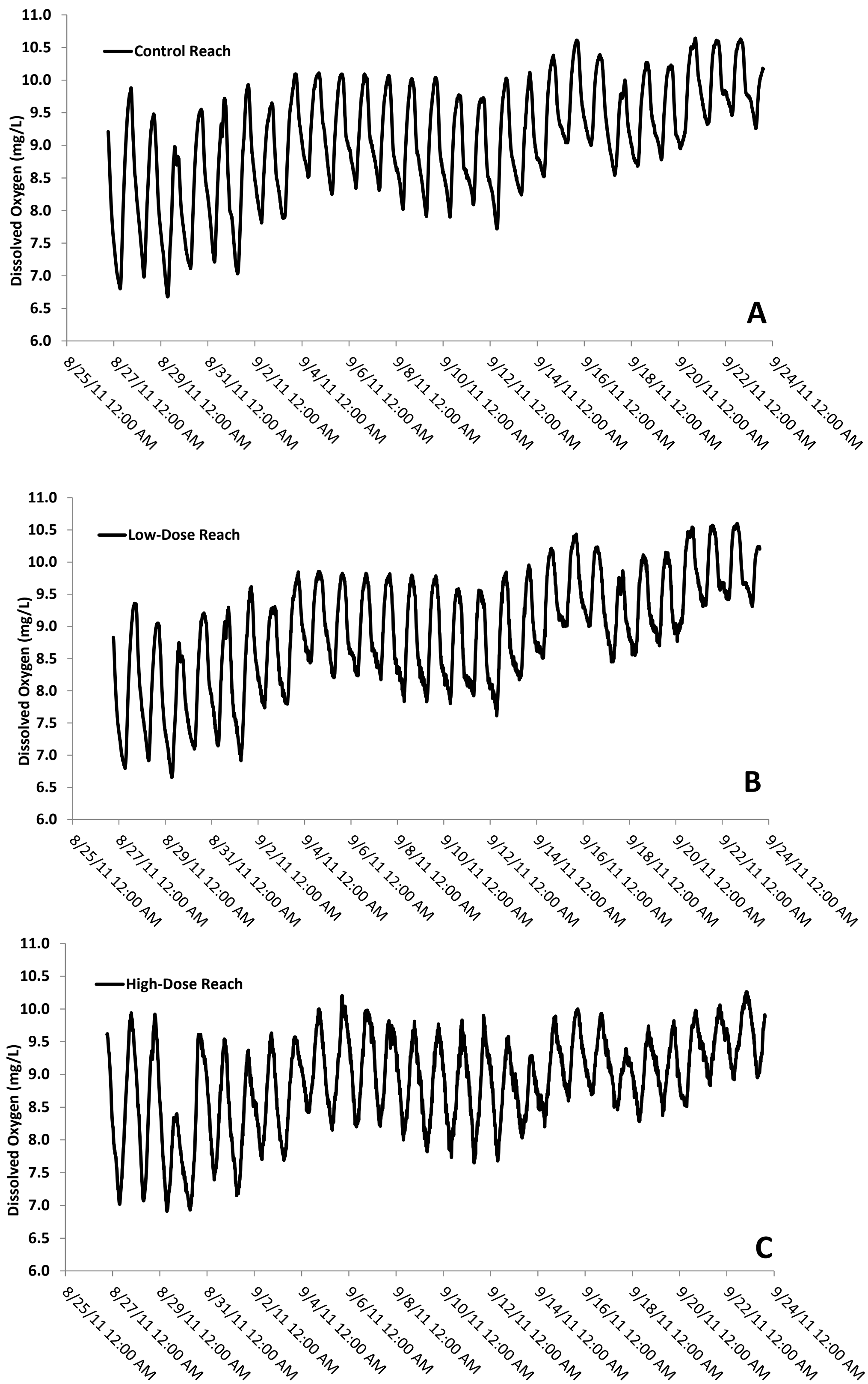


Figure 4-6. DO in the Study Reaches, 2011.
(A) Control Reach. (B) Low Dose Reach. (C) High Dose Reach. No nutrient dosing occurred in 2011.

4.4.1.1 DO Delta and BACIP Analysis

Daily dissolved oxygen delta (daily DO maximum – daily DO minimum) is a useful index of a waterbody's photosynthetic production and state of eutrophication, as first noted by Odum (1956). Others have found that DO delta is related to harm to aquatic life. In Minnesota, strong positive correlations are found between the percent tolerant fish and the magnitude of a stream or river's DO deltas. At DO deltas <4.5 mg/L, tolerant fish are usually <10% of the total fish population, but when DO deltas are > 4.5 mg/L tolerant fish become a substantial proportion of the population (Heiskary and Bouchard 2015).

Because of its demonstrated importance, DO delta was calculated for each day during the Before and After periods for each of the sondes in Figures **4-3**, **4-4** and **4-5**[9]. (Recall that the Before period includes DO data recorded in 2009, i.e. **Figure 4-3** data, and DO data in 2010 prior to dosing. The After period does not include 2011 data.) Using the BACIP-design statistical test (i.e., same layout as shown in **Table 3-1**), there were significantly higher DO deltas in the After period compared to the Before period, for both of the Low Dose sondes and for both of the High Dose sondes (one sided Mann-Whitney, p << 0.001). In some cases DO delta was as high as 16.7 mg/L (After period, High Dose Reach). These results demonstrate conclusively that the daily DO highs increased in magnitude and the daily DO lows decreased in magnitude as a direct result of the addition of nutrients to the stream.

4.4.2 pH

Figure 4-7 presents the entire pH dataset for the Control, Low Dose, and High Dose reaches in 2009, a year prior to when nutrient dosing occurred. An assumption of the BACIP study is that water-quality conditions among the study reaches should be largely comparable in advance of the impact (the impact here being nutrient dosing). But for the 2009 pH data, there were obvious differences between observations in the Control Reach *vs*. the Low- and High Dose reaches. The Control Reach values were around 8.3 to 8.4 at the beginning, and tended to decline somewhat over the course of the deployment (**Figure 4-7A**). In contrast to the Control Reach, measured pH values in both the Low- and High Dose reaches started at 8.4 and tracked one another closely, and their measured pH values increased over the deployment (**Figures 4-7B, C**). Nothing in the post-deployment QC would suggest that there was a problem with the Control Reach pH probe (initial calibration was only 0.05 pH units below the calibration standard, and drift from calibration over the deployment period was 0.01 pH units up). There is a large data gap in the Control Reach data due to the period of instrument repair (**Figure 4-7A**), and a period of time near the end of the deployment in the Low Dose Reach when that sonde's pH probe failed (**Figure 4-7B**). Overall, there is more disparity between Control, Low Dose, and High Dose pH values then was observed in DO concentrations over the same time period in 2009.

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[9] Montana does not have a water quality standard for DO delta. Nevertheless, daily DO delta is used as an assessment tool for evaluating ambient stream conditions (Suplee and Sada de Suplee 2011). According to the assessment method, DO delta values > 5.3 are indicative of eutrophied conditions in prairie streams.

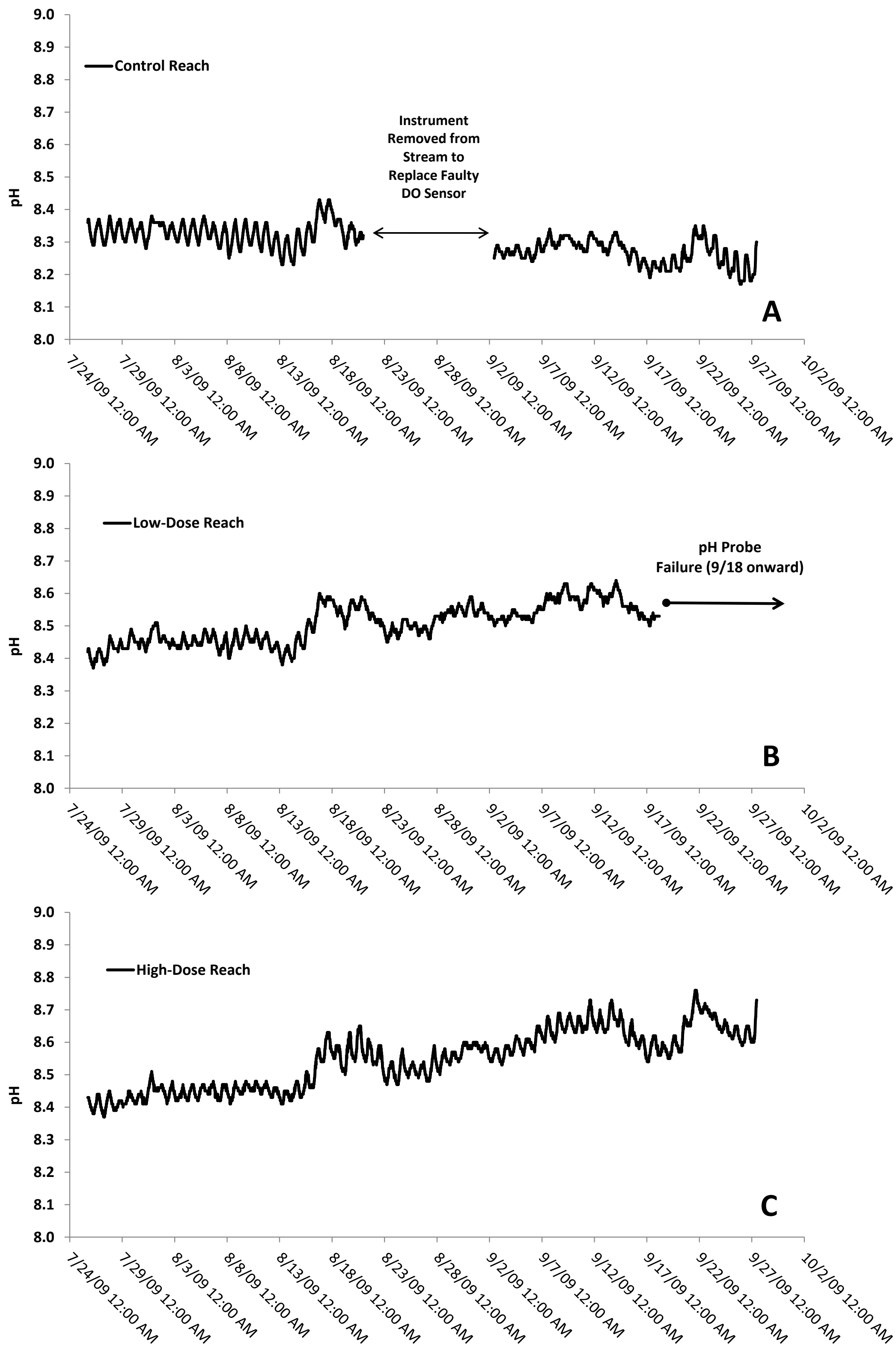


Figure 4-7. Values of pH in the Study Reaches, 2009.
(A) Control Reach. (B) Low Dose Reach. (C) High Dose Reach. No nutrient dosing occurred in 2009.

Montana's standard for pH is quasi-narrative, and for Box Elder Creek (C-3 class; ARM 17.30.629(2)(c)) it applies as follows: "induced variation of hydrogen ion concentration (pH) within the range of 6.5 to 9.0 must be less than 0.5 units. Natural pH outside this range must be maintained without change." Thus, for Box Elder Creek, water quality standards are exceeded when a human-induced change is larger than ± 0.5 pH units from ambient <u>or</u> if the pH is moved outside of the range of 6.5 to 9.0[10]. In this study, a comparison between the Control Reach pH values and the Low- and High Dose pH values provided a means to evaluate dosing effects relative to this standard. In this study we only calculated induced daily changes for the <u>maximum</u> pH values (e.g., High Dose maximum daily pH minus Control maximum daily pH). This is because analysis of human-induced pH change in the Yellowstone River shows that the daily maximum pH is always a higher value than the daily minimum (Suplee et al. 2015) and would therefore be the more limiting standard. Statistical evaluation of these data will be presented momentarily.

Plots of the pH patterns in the Control, Low Dose, and High Dose reaches in 2010, both before and after dosing are shown in **Figures 4-8** and **4-9.** The pH recorded by the upstream Low Dose sonde (**Figure 4-8A**) was, from beginning of deployment, about 0.2 units *lower* than the Control Reach. This difference increased to about 0.4 units by the end of the deployment. There is nothing in the calibration logs for the upstream YSI of the Low Dose Reach, or for the Control Reach, that readily explains the results in **Figure 4-8A**. Both units were within 0.04 pH units of their respective calibration standards prior to deployment, and each sonde showed about the same positive upward drift in pH over the course of the deployment (+0.06 and +0.08 pH units for the Control sonde and upstream Low Dose sonde, respectively). In contrast, the downstream Low-Dose sonde tracked the Control Reach sonde pH values fairly closely and there were no issues with the instrument (**Figure 4-8B**).

Plots of the pH patterns in the Control- and High Dose reaches in 2010, both before and after dosing, are shown in **Figures 4-9**. In the High Dose Reach, there was a very high degree of overlap with the Control Reach data up to the time when dosing began (**Figures 4-9A, B**). After that, the High Dose sondes recorded pH values that were both higher in magnitude and of greater daily amplitude than was observed in the Control Reach. The highest pH values (8.89-8.96) were recorded by both High-Dose sondes during the first 10 days of September. These daily pH peaks occurred near to or somewhat earlier than the corresponding DO peaks measured by the same sondes (see **Figure 4-5**). The greatest nutrient-induced pH change (relative to the Control Reach) in 2010 was 0.25 pH units, and therefore within the allowable increase according to Montana's water-quality standards. But the induced change did increase pH to the brink of 9.0. Overall, the addition of nutrients in the High Dose Reach pushed the allowable pH maximum right to the allowable limit (i.e., pH of 9.0), but did not quite exceed the limit.

In 2011, YSI sondes were again deployed to measure pH, as part of the recovery and follow-up monitoring of the study. In 2011 no dosing occurred. The pH patterns of the three reaches largely resemble one another (**Figure 4-10**). The main disparity in 2011 is in the High Dose Reach, where overall pH trends are the same as for the Control- and Low Dose reaches but the absolute values are about 0.1 pH units higher. There is no indication that the dosing effects of the previous year caused a lingering effect on the pH patterns of the stream (i.e., early September data do not approach pH values of 9.0, as they did in 2010 in the High Dose Reach).

[10] Nutrients influence pH indirectly. Algae and aquatic plants (autotrophs) increase in abundance due to elevated nutrients. During the day, autotrophs consume carbon dioxide which is dissolved in the water, causing the pH to increase (pH in waterbodies is often highest late in the afternoon). At night, autotrophs (as well as insects, fish, etc.) release carbon dioxide, which lowers pH. In this manner, increased nutrients lead to higher diel pH changes.

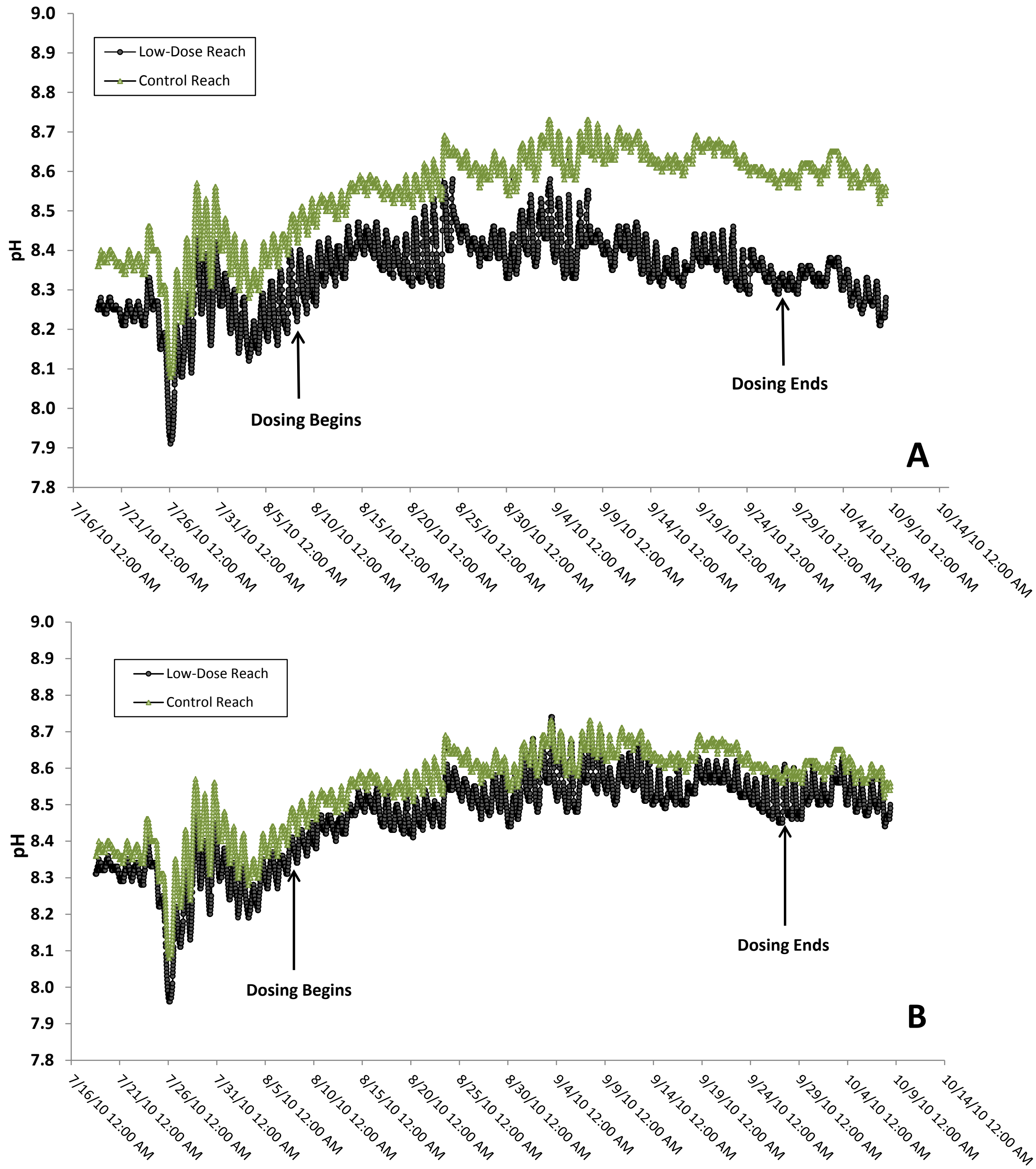
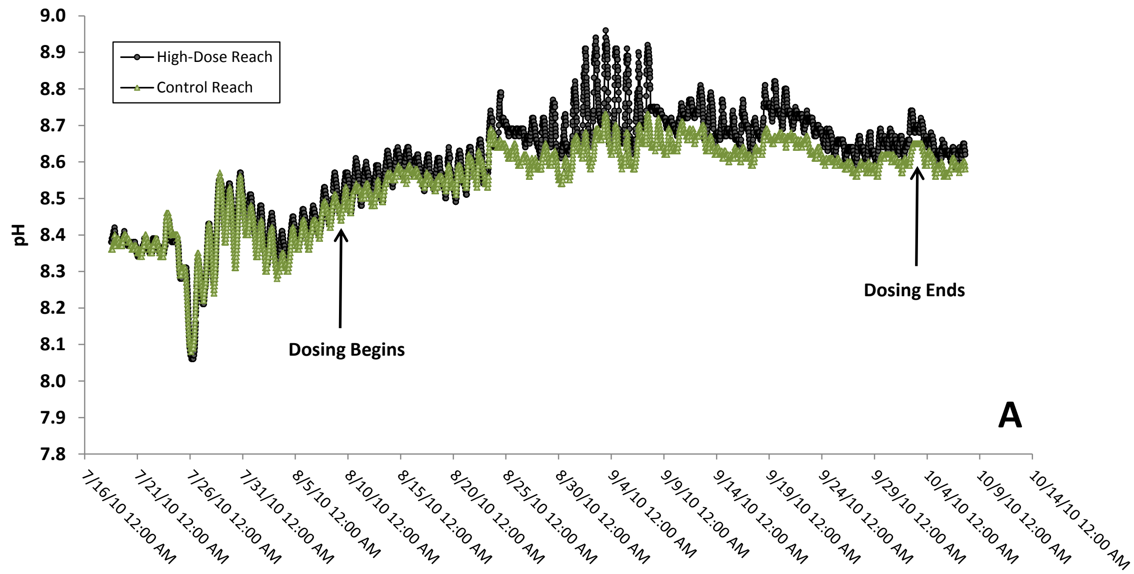


Figure 4-8. Patterns of pH Recorded by Sondes Located in the Control- and Low Dose Reaches in 2010. (A) Control Reach data (green) and data from the "upstream" Low Dose Reach sonde (black). The "upstream" sonde was positioned about mid-reach within the Low Dose Reach. (B) Control Reach data (green) and data from the "downstream" Low Dose Reach sonde (black). The "downstream" sonde was located about 200 m downstream from the Low-Dose nutrient-dripper assembly, near the end of the Low Dose Reach.



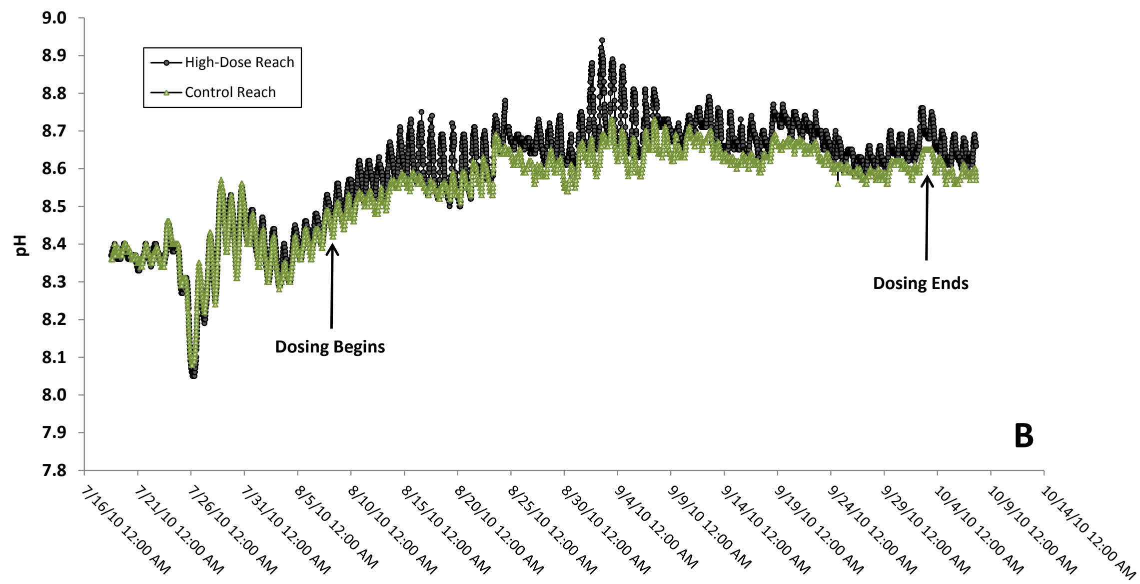


Figure 4-9. Patterns of pH Recorded by Sondes Located in the Control- and High Dose Reaches in 2010. (A) Control Reach data (green) and data from the "upstream" High Dose Reach sonde (black). The "upstream" sonde was positioned about mid-reach within the High Dose Reach. (B) Control Reach data (green) and data from the "downstream" High Dose Reach sonde (black). The "downstream" sonde was located about 200 m downstream from the High Dose nutrient-dripper assembly, near the end of the High Dose Reach.

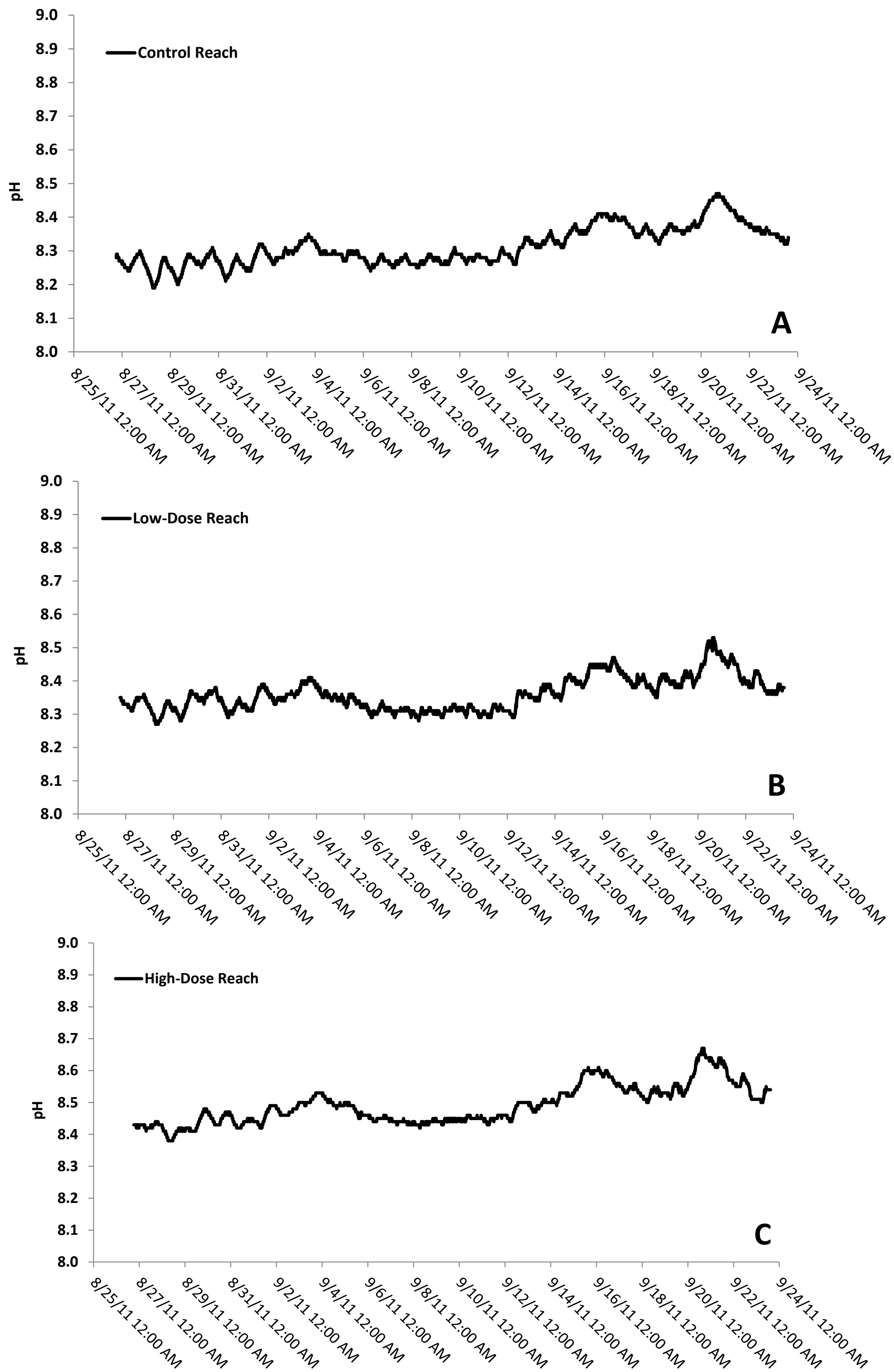


Figure 4-10. Values of pH in the Study Reaches, 2011.

(A) Control Reach. (B) Low Dose Reach. (C) High Dose Reach. No nutrient dosing occurred in 2011.

4.4.2.1 Daily Maximum pH and BACIP Analysis

Daily maximum pH (for both sondes in the Low Dose and both sondes in the High Dose) minus the daily maximum pH for the Control Reach sonde was calculated for the Before and After periods for data shown in **Figures 4-7, 4-8,** and **4-9**. (Recall that the Before period includes pH data recorded in 2009, i.e. **Figure 4-7** data, and pH data in 2010 prior to dosing. The After period does not include 2011 data.) Using the BACIP-design statistical test there was a significant difference in daily pH maximum between the Before and After periods for all sondes in the Low- or High Dose reaches (two sided Mann-Whitney, p-values ranging from <0.001 to 0.02). However, the results are *backwards* from what we would expect (BACIP **D** values were greater in the Before period); this was driven by the fact that measured pH in the Control Reach tended to move downward throughout summer 2009, while during the same time period pH in the Low- and High Dose reaches tended to move upwards (**Figure 4-7**), increasing the calculated daily differences; this strongly influenced the aggregate Before dataset.

The differences in maximum daily pH between the Control and experimental reaches were relatively modest during the study (< 0.4 pH units at the very most), and it was evident that unknown sources of variability were affecting our pH probes and measurements, and there were instrument failure issues in 2009 as well (discussed above). If only the 2010 Before and After pH data are considered (i.e., excluding all 2009 Before data), the statistical results change. For the Low Dose Reach, there continues to be no significant difference between Before and After periods for the upstream sonde, however for the downstream sonde there are significantly higher daily pH maximums in the After period compared to the Before period (one sided Mann-Whitney, p <<0.001). In the High Dose Reach, there are significantly higher pH maximums in the After period than in the Before for both sondes (one sided Mann-Whitney, p << 0.001). Because we have excluded 2009 data, strictly speaking, this last set of statistical tests is not consistent with our standard BACIP statistical approach and should be considered with caution; however, it is consistent with a visual inspection of **Figure 4-9**.

4.4.3 Phytoplankton Chlorophyll-*a*

Consistent with experience on the Yellowstone River (Flynn and Suplee 2013), the phytoplankton Chl*a* data recorded by the YSI sondes were quite noisy, with intervals of major interference (such as when drifting filamentous algae were snagged on the units). Given the degree of noise and interference, we chose not to carry out analyses on the sondes' Chl*a* data. However, we collected 87 phytoplankton Chl*a* grab samples over the course of the study (**Table 4-15**), and BACIP statistics were completed on these data. For the Low Dose Reach, we could not reject the null hypothesis that the Before period was the same as the After period (one-sided Mann-Whitney). For the High Dose Reach, there was no significant difference (one sided Mann-Whitney) between the Before and After periods. Concentrations of phytoplankton just upstream of the High Dose Reach ranged from 3.5 to 5 µg/L in 2010, and were essentially of the same magnitude as values measured elsewhere that year. In 2011, concentrations were again very similar among the three study reaches (**Table 4-15**) and similar to concentrations observed in the Control Reach throughout the study. It resulted that phytoplankton Chl*a* concentrations were quite low overall (study average = 6 µg/L), and compared to the much greater biomass of attached algae (discussed next) phytoplankton would have contributed very minimally to stream DO and pH patterns. For this reason, data regarding phytoplankton CNP content will not be addressed in this report.

Table 4-15. Average Phytoplankton Chlorophyll-*a* Concentration in Box Elder Creek.
Difference values used in BACIP statistics are shown in the last two columns on the right.

Sampling Date	Period	Sampling Event	Control (µg/L)	Low Dose (µg/L)	High Dose (µg/L)	Difference (D) Control - Low Dose	Difference (D) Control - High Dose
7/24/2009	Before	1(2009)	13.0	8.5	10.0	4.5	3.0
8/12/2009	Before	2 (2009)	6.7	6.6	5.6	0.1	1.2
8/29/2009	Before	3 (2009)	6.4	7.3	6.7	-1.0	-0.4
9/26/2009	Before	4 (2009)	2.9	3.8	6.7	-1.0	-3.8
7/17/2010	Before	5 (2010)	3.5	4.5	8.5	-1.0	-5.0
8/25/2010	After	1 (2010)	5.8	4.0	5.5	1.8	0.3
9/8/2010	After	2 (2010)	6.5	5.7	6.3	0.8	0.2
9/22/2010	After	3 (2010)	4.5	5.0	7.3	-0.5	-2.8
10/5/2010	After	4 (2010)	2.0	3.9	6.0	-1.9	-4.0
8/26/2011	n/a	–	7.0	9.5	10.0	–	–
9/23/2011	n/a	–	4.8	4.8	4.0	–	–

4.5 Attached (Benthic) Plant Biomass

4.5.1 Quantitative Measurement of Attached Algae

Attached (benthic) algal biomass was quantified as Chl*a* and AFDM (see **Section 3.5.3.1**). Data quality was very high in that (1) we were able to collect and analyze all eleven Chl*a* replicates from each study reach during all sampling events in all years of the study, with a single exception (High Dose Reach, 8/27/2011; n = 10 replicates, no sample for transect I), and (2) the averages of the reach-wide Chl*a* biomass duplicates were very similar to one another (Low Dose Reach, 9/28/2009; routine measure = 22 mg Chl*a*/m^2, field duplicate = 31 mg Chl*a*/m^2). As a result of nutrient dosing, there was a large increase (as much as an order of magnitude) in benthic algal Chl*a* in the After period in 2010 (**Table 4-16; Figure 4-11**). Using the BACIP-design statistical testing, in the High Dose Reach there was significantly higher benthic Chl*a* in the After period compared to the Before period (one-sided Mann-Whitney, $p = 0.01$). However, the difference between the Before and After periods was not significant in the Low-Dose Reach (one-sided Mann-Whitney). By fall of 2010, the High Dose Reach had developed a level of benthic Chl*a* (127 mg Chl*a*/m^2; **Table 4-16)** which exceeds DEQ's harm-to-use assessment threshold of 125 mg Chla/m^2 (Suplee and Sada de Suplee 2011; Suplee and Flynn 2014). In 2011 benthic algal Chl*a* density in the Control Reach was similar to 2009 and 2010, while density in the Low- and High Dose reaches in 2011 dropped to levels lower than those observed in 2009 and even lower than the 2011 Control Reach (**Figure 4-11)**.

Table 4-16. Average Benthic Algal Chlorophyll *a* Density in Box Elder Creek.
Difference values used in BACIP statistics are shown in the last two columns on the right.

Sampling Date	Period	Sampling Event	Control (mg/m^2)	Low Dose (mg/m^2)	High Dose (mg/m^2)	Difference (D) Control - Low Dose	Difference (D) Control - High Dose
7/24/2009	Before	1(2009)	20.2	18.6	17.9	1.5	2.3
8/13/2009	Before	2 (2009)	49.7	32.2	39.8	17.5	9.9
8/30/2009	Before	3 (2009)	49.5	28.0	39.5	21.5	10.0
9/28/2009	Before	4 (2009)	48.5	26.6	70.0	21.9	-21.5
7/17/2010	Before	5 (2010)	7.9	4.9	7.8	3.0	0.1
8/26/2010	After	1 (2010)	12.5	77.9	111.4	-65.5	-98.9
9/8/2010	After	2 (2010)	16.8	72.0	115.6	-55.2	-98.8
9/22/2010	After	3 (2010)	27.3	14.3	87.3	13.0	-60.1
10/6/2010	After	4 (2010)	60.0	29.5	127.2	30.5	-67.2
8/27/2011	n/a	–	34.8	14.1	16.5	–	–
9/24/2011	n/a	–	47.1	15.1	10.5	–	–

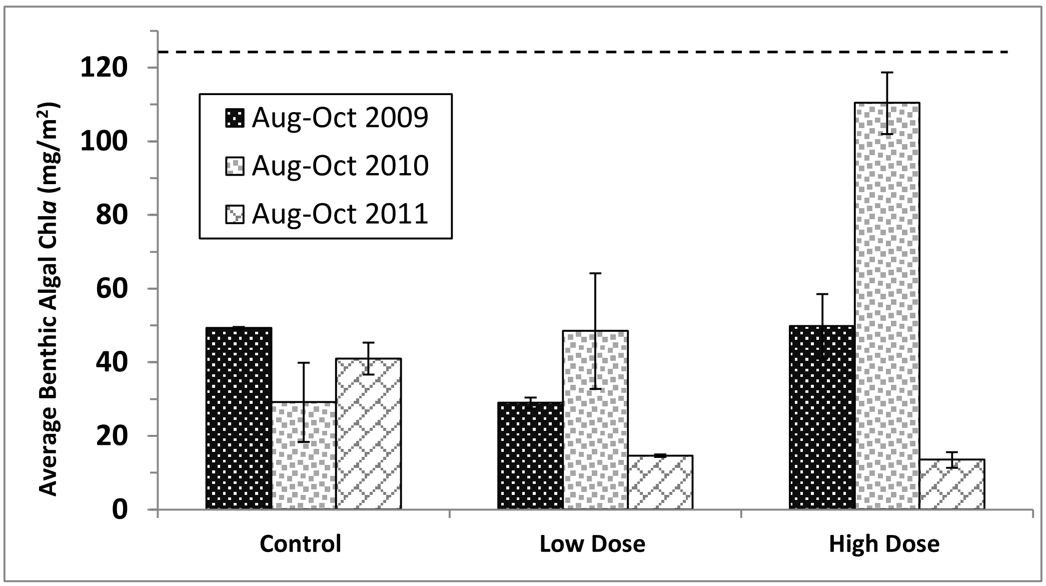


Figure 4-11. Average Benthic Algal Chla over a Common Period (August to October) in the Three Study Reaches.
Error bars are one standard deviation of the mean across the time period at the given location. For the 2010 data, only data collected during the After period are displayed. Horizontal dashed line is DEQ's harm-to-use assessment threshold of 125 mg Chl*a*/m^2.

As noted in Methods, core-type samples are not included in the benthic algal AFDM dataset; core samples comprised 26% of replicates collected during the study. For the remaining 74% of the transect replicates (i.e., templates and hoops), the results are shown in **Table 4-17**. As a result of nutrient dosing, there was a demonstrable increase in benthic algal AFDM in the After period in 2010 (**Table 4-17**). Using the BACIP design, in both the Low Dose and High Dose reaches there was significantly more benthic AFDM in the After period compared to the Before period (one-sided Mann-Whitney, p = 0.01 for both reaches).

Table 4-17. Average Benthic Algal AFDM Density in Box Elder Creek.
Difference values used in BACIP statistics are shown in the last two columns on the right.

Sampling Date	Period	Sampling Event	Control (g/m^2)	Low Dose (g/m^2)	High Dose (g/m^2)	Difference (D) Control - Low Dose	Difference (D) Control - High Dose
7/24/2009	Before	1(2009)	9.2	6.6	10.0	2.6	-0.8
8/13/2009	Before	2 (2009)	29.4	22.3	26.6	7.0	2.8
8/30/2009	Before	3 (2009)	21.9	14.9	15.7	7.0	6.1
9/28/2009	Before	4 (2009)	28.3	16.2	33.6	12.2	-5.3
7/17/2010	Before	5 (2010)	14.5	5.5	6.8	8.9	7.7
8/26/2010	After	1 (2010)	5.1	26.7	25.9	-21.6	-20.8
9/8/2010	After	2 (2010)	13.7	39.1	33.8	-25.4	-20.1
9/22/2010	After	3 (2010)	23.3	59.6	36.8	-36.3	-13.5
10/6/2010	After	4 (2010)	25.0	93.0	33.0	-68.0	-8.0
8/27/2011	n/a	–	16.5	60.2	29.1	–	–
9/24/2011	n/a	–	30.7	8.3	6.3	–	–

Figure 4-12 compares benthic algal AFDM among the three study reaches over a sampling period common to all study years (August to October). During nutrient dosing (2010), both the Low- and High Dose reaches exceeded DEQ's harm-to-use assessment threshold of 35 g AFDM/m^2 as defined in Suplee and Sada de Suplee (2011)(**Table 4-17; Figure 4-12**), whereas the Control Reach remained well below 35 g AFDM/m^2 that year and in 2009 and 2011. The Low Dose Reach exceeded the assessment threshold in 2010, but it did so in 2011 as well, when no dosing occurred (**Table 4-17**).

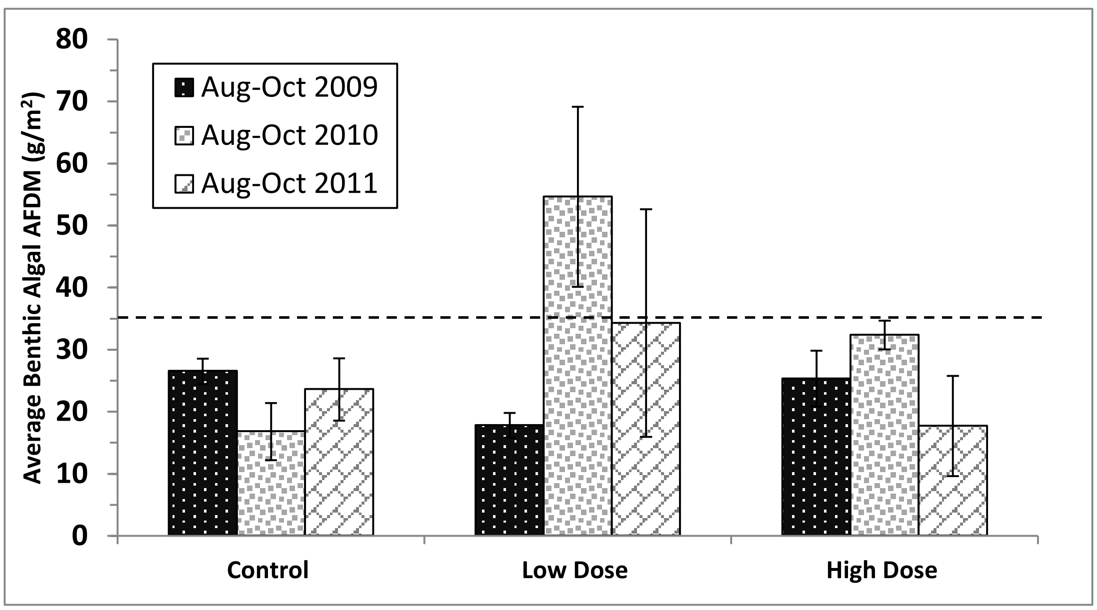


Figure 4-12. Average Benthic Algal AFDM over a Common Period (August to October) in the Three Study Reaches.
Error bars are one standard deviation of the mean across the time period at the given location. For 2010, only data from the After period are shown. Horizontal dashed line is DEQ's harm-to-use assessment threshold of 35 g AFDM/m^2.

4.5.2 Visual Evaluation of Aquatic Flora

Visually-assessed data common to the 2009, 2010, and 2011 periods are % streambed coverage by filamentous algae, % streambed coverage by macrophytes, and % streambed coverage by moss. The standard BACIP-design statistical analyses (2009 and early 2010 data as Before, post-dosing 2010 data as After) were applied to these data and are presented below. The visual assessment form used to record visual data was enhanced in advance of the 2010 field season to include details such as length of filamentous algae strands, thickness of the microalgae mats on the streambed, and growth status of the plants (Growing, Mature, Decaying). These data were only collected in 2010 and 2011 and do not lend themselves to our standard BACIP statistical analysis. Nevertheless, they provide many interesting and insightful findings pertaining to the dosing study, and will be presented later in **Section 4.5.2.1.**

Tables 4-18, 4-19, and 4-20 summarize observed % streambed cover in the Control, Low Dose, and High Dose reaches for filamentous algae, macrophytes, and moss, respectively. Filamentous algae coverage of the streambed (**Table 4-18**) increased significantly in the After period in both the Low- and High Dose reaches as a results of nutrient dosing (one-sided Mann-Whitney, p = 0.01 and 0.03, respectively). In the High Dose Reach, filamentous algae streambed coverage increased from an average of 9% to over 30% during nutrient additions. Correspondingly, there was a significant *decrease* in macrophyte streambed coverage in the After period in the High Dose Reach compared to the Before period (**Table 4-19**; two-sided Mann-Whitney, p = 0.02)[11]. This statistical result corroborates our general observation in the High Dose Reach that filamentous algae increased their coverage of the stream bottom at the expense of the macrophytes during nutrient dosing. In contrast, there was no significant difference between the Before and After periods for macrophyte coverage in the Low Dose Reach (two-sided Mann-Whitney, p = 0.11). In general, moss was always a very small percentage (usually 1% or less) of the plants covering the stream bottom (**Table 4-20**). There was no significant difference between the Before and After periods for moss coverage in the Low Dose Reach (two-sided Mann-Whitney, p = 0.59). However, there was a marginally significant *decrease* in moss coverage in the High-Dose Reach in the After period compared to the Before period (**Table 4-20**; two-sided Mann-Whitney, p = 0.08).

Table 4-18. Average Filamentous Algae Coverage (%) of the Stream Bed in the Study Reaches.
Difference values used in BACIP statistics are shown in the last two columns on the right.

Sampling Date	Period	Sampling Event	Control (% cover)	Low Dose (% cover)	High Dose (% cover)	Difference (D) Control - Low Dose	Difference (D) Control - High Dose
7/25/2009	Before	1(2009)	8	11	2	-3.0	6.4
8/12/2009	Before	2 (2009)	20	18	9	1.8	10.9
8/30/2009	Before	3 (2009)	5	4	5	0.4	-0.5
9/28/2009	Before	4 (2009)	5	6	5	-1.8	-0.5
7/17/2010	Before	5 (2010)	1	0	0	0.1	0.6
8/26/2010	After	1 (2010)	10	22	15	-12.3	-5.2
9/8/2010	After	2 (2010)	11	34	10	-23.8	0.0
9/24/2010	After	3 (2010)	6	32	15	-26.3	-9.2
10/6/2010	After	4 (2010)	12	44	30	-32.6	-18.7
8/27/2011	n/a	–	1	1	1	–	–
9/25/2011	n/a	–	3	3	1	–	–

[11] Because we were uncertain as to whether nutrient dosing would be expected to increase or decrease macrophyte and moss streambed coverage, we ran two-sided tests.

Table 4-19. Average Macrophyte Coverage (%) of the Stream Bed in the Study Reaches.
Difference values used in BACIP statistics are shown in the last two columns on the right.

Sampling Date	Period	Sampling Event	Control (% cover)	Low Dose (% cover)	High Dose (% cover)	Difference (D) Control - Low Dose	Difference (D) Control - High Dose
7/25/2009	Before	1(2009)	5	2	6	2.3	-1.8
8/12/2009	Before	2 (2009)	5	13	10	-8.4	-5.2
8/30/2009	Before	3 (2009)	6	20	7	-13.2	-0.5
9/28/2009	Before	4 (2009)	5	6	5	-1.4	0.0
7/17/2010	Before	5 (2010)	0	0	0	0.0	0.0
8/26/2010	After	1 (2010)	3	0	0	2.3	2.3
9/8/2010	After	2 (2010)	3	1	1	1.6	1.6
9/24/2010	After	3 (2010)	2	1	1	1.1	1.1
10/6/2010	After	4 (2010)	4	2	3	2.2	0.8
8/27/2011	n/a	–	2	2	1	–	–
9/25/2011	n/a	–	4	1	3	–	–

Table 4-20. Average Moss Coverage (%) of the Stream Bed in the Study Reaches.
Difference values used in BACIP statistics are shown in the last two columns on the right.

Sampling Date	Period	Sampling Event	Control (% cover)	Low Dose (% cover)	High Dose (% cover)	Difference (D) Control - Low Dose	Difference (D) Control - High Dose
7/25/2009	Before	1(2009)	1	0	3	0.5	-2.7
8/12/2009	Before	2 (2009)	0	0	0	0.0	0.0
8/30/2009	Before	3 (2009)	0	0	0	0.0	0.0
9/28/2009	Before	4 (2009)	2	1	2	0.5	-0.5
7/17/2010	Before	5 (2010)	0	0	0	0.0	0.0
8/26/2010	After	1 (2010)	0	0	0	0.0	0.0
9/8/2010	After	2 (2010)	1	0	0	0.5	0.5
9/24/2010	After	3 (2010)	0	0	0	0.0	0.0
10/6/2010	After	4 (2010)	2	0	0	1.0	1.0
8/27/2011	n/a	–	1	0	0	–	–
9/25/2011	n/a	–	1	0	0	–	–

Figure 4-13 shows graphically the changing relationship between filamentous algae coverage and macrophyte coverage over the course of the study. In 2009, there was a fair degree of consistency between the three reaches (in terms of % coverage) and fairly balanced proportions of filamentous *vs.* macrophyte coverage (**Figure 4-13A**). Throughout 2010, filamentous and macrophyte coverage in the Control Reach was very similar to 2009, however filamentous algae coverage dramatically increased in the Low- and High Dose reaches, and macrophyes decreased (**Figure 4-13B**). In 2011, there was a uniform proportion of the two plant groups across the three study reaches (**Figure 4-13C**), and filamentous algae coverage was well below that observed in earlier years (3% coverage, *vs.* about 8% in past years in the Control Reach). The extremely high, scouring flows in spring 2011 are likely responsible for reducing the base level of these plants in 2011.

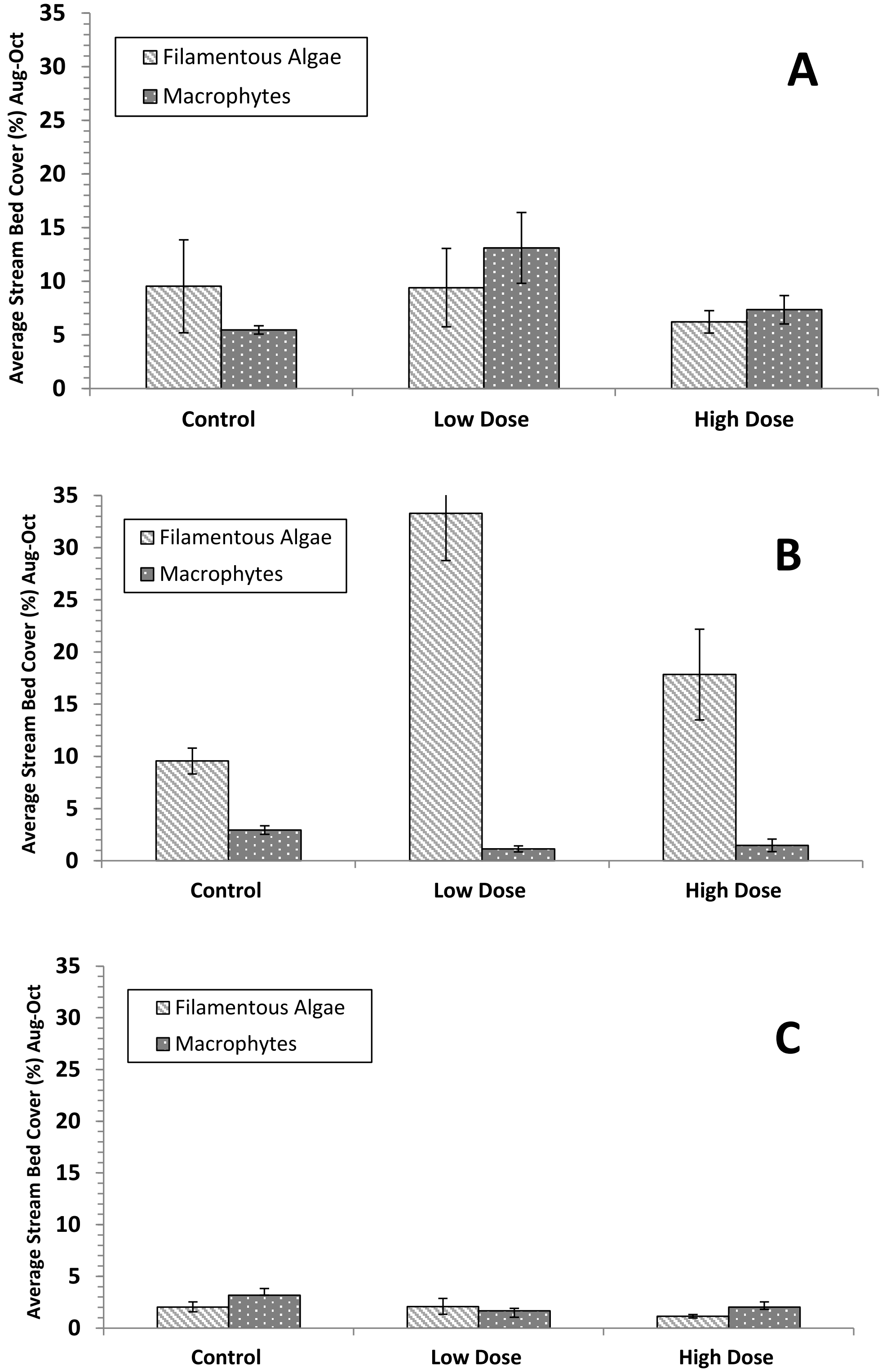


Figure 4-13. Average Stream Bed Cover by Filamentous Algae and Macrophytes over a Common Period (Aug to Oct).

(A) 2009. (B) 2010. (C) 2011. Error bars are one standard deviation of the mean across the time period at the given location. For 2010, only data from the After period are shown.

4.5.2.1 Benthic Flora Data Collected Only in 2010 and 2011

Starting in field season 2010, we began to systematically record additional information about the benthic flora of the stream, and those data are presented here. Details on the criteria for these visual assessments can be found in the SOP (Montana Department of Environmental Quality 2011b) and at the end of **Appendix A**. The combined coverage of the streambed by filamentous algae, macrophytes, and moss never exceeded 35% even during dosing, and was usually far below this (ca. 12%) in the Control Reach or in other reaches when dosing was not occurring (**Tables 4-18** to **20**). Thus, the majority of the streambed was actually covered by a film of microalgae (mix of diatoms, very short green filaments, *Nostoc sp*., etc.) whose thickness on the streambed varied over time.

As shown in **Figure 4-14**, during dosing in 2010 the average thickness of the attached microalgae increased in the Low- and High Dose reaches up to eight times that which was observed in the Control Reach. The High Dose Reach consistently developed the thickest microalgae mats. In August 2011 (when no dosing occurred), we observed microalgae thickness in all three reaches comparable to all reaches in July 2010 (before dosing) and to the Control Reach for the remainder of 2010. However, by September 2011, microalgae thickness increased in all three reaches to about 1.2 mm, much thicker than any level observed in the Control Reach prior to that time; still, the 2011 microalgae thickness was still well below the peak microalgae thickness observed during dosing in 2010 in the Low- and High Dose reaches.

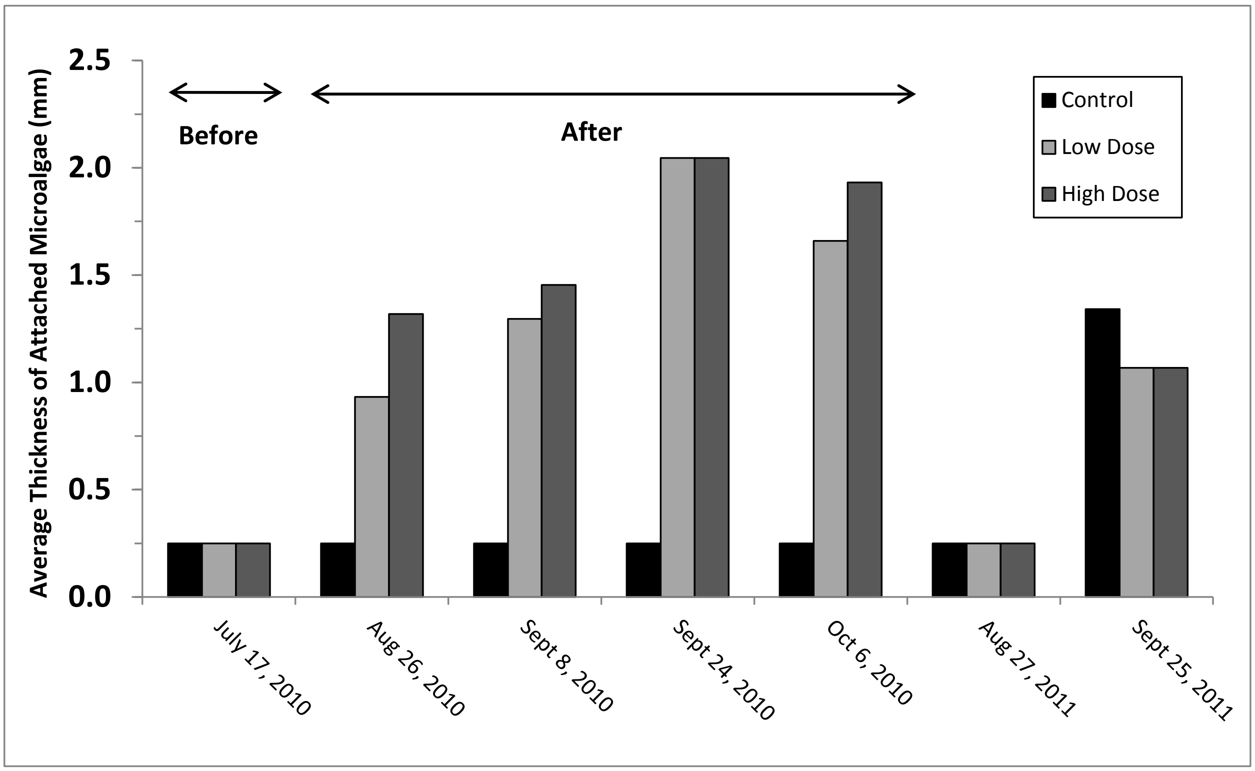


Figure 4-14. Average Thickness of Attached Microalgae in 2010 and 2011.

As noted earlier, filamentous algae were always a minority of the floral coverage of the streambed. Where filamentous algae were observed, however, length of the filaments was recorded. The maximum lengths that algal filaments achieved in each reach are shown in **Figure 4-15**. The data in **Figure 4-15** are for sampling events where filamentous algae was observed on at least two of the eleven transects in any given reach; for this reason there are no data for the Before period (there were no or only a single observation of filamentous algae on July 17th).

Relative to the Control Reach, filamentous algae filament-lengths were usually much longer (sometimes 3X longer) in the Low- and High Dose reaches during dosing, and long filaments were observed in the dosed reaches much later into the year. In the first week of September 2010, filament lengths in the Control Reach peaked, and were fairly similar to the two dose reaches (9/8/2010; **Figure 4-15**). As 2010 progressed, filaments still attached to the streambed in the Control Reach were seen to be progressively shorter[12]. In contrast, algal filament lengths in the High-Dose Reach continued to increase, ultimately peaking in early October at 1¼ meters in length (125 cm; **Figure 4-15**). The pattern in the Low Dose Reach was more varied, but the Low Dose Reach also had peak filament lengths in October (which were, at that time, >2X longer than the Control Reach). In 2011 (when no dosing occurred), algal filaments never exceeded 30 cm and they were fairly equal in length among the three reaches.

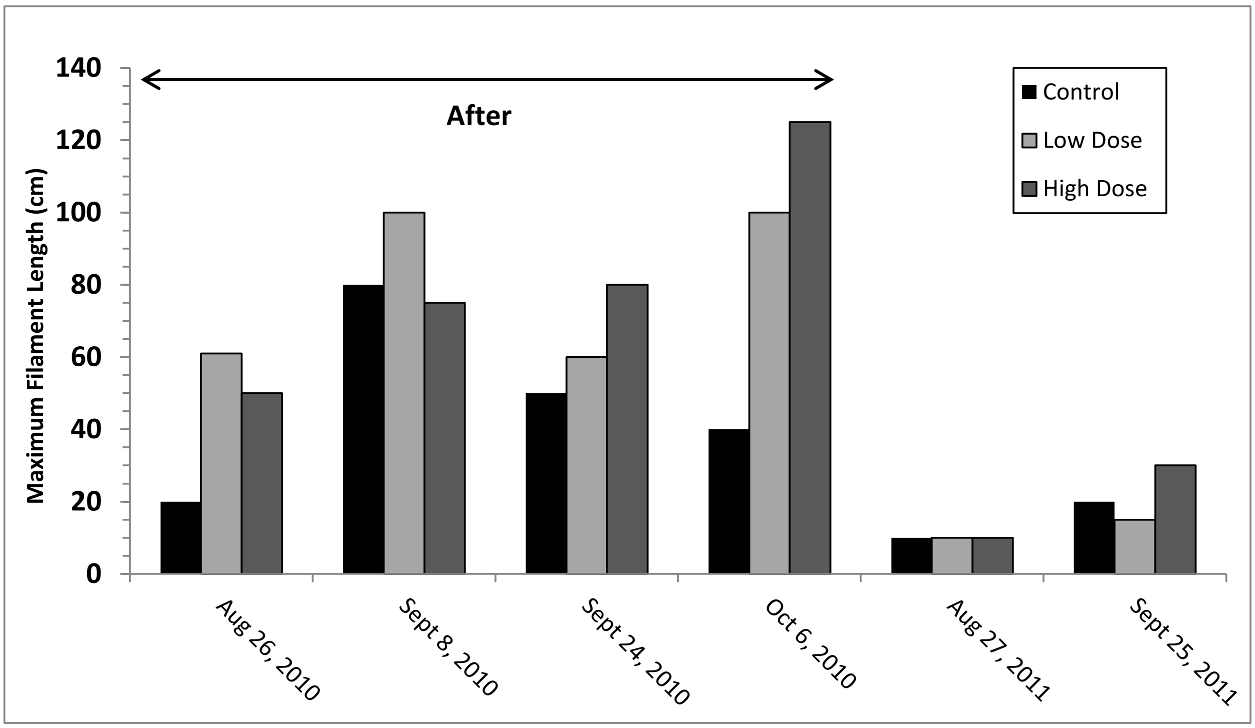


Figure 4-15. Maximum Length of Filamentous-algae Filaments in 2010 and 2011.

The growth status of the aquatic flora was also visually estimated. Plants were classified as appearing to be Growing, Mature, or Decaying. These data are most informative when viewed alongside the dissolved oxygen patterns, and have therefore been superimposed on the DO data (**Figure 4-16**).

[12] Throughout the study, filaments in all three reaches were continually breaking off and drifting downstream. The visual assessments were made on the attached filaments.

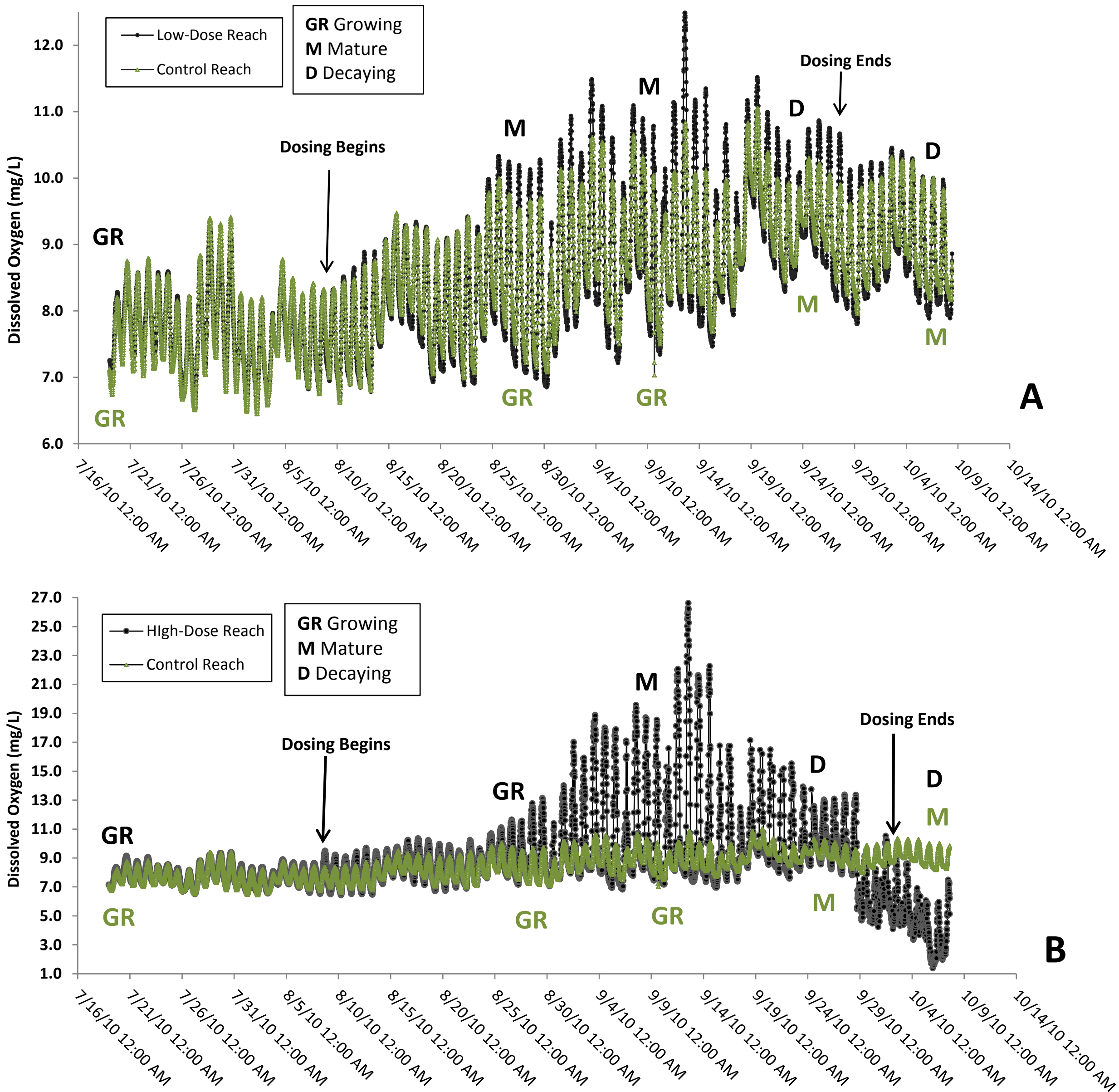


Figure 4-16. Growth Status of Attached Microalgae Superimposed on the DO Data (same DO Data Presented in Figures 4-4A and 4-5B).

(A) Low Dose Reach (downstream YSI) and Control Reach. (B) High Dose Reach (upstream YSI) and Control Reach. Growth status letters (GR, M, D) corresponding to the Control Reach are green, letters associated with the dosed reaches are black. The growth status letters are positioned on the date on which the visual assessment of growth was made.

In the Low Dose Reach, microalgae were initially (in July) categorized as Growing but began to appear Mature by late August, and remained Mature through the first ten days of September (**Figure 4-16A**). The late August to mid-September period is when DO delta (daily high minus daily low; i.e., an index of primary productivity) was greatest in the Low Dose Reach. By late September and beyond, Low Dose Reach microalgae were categorized as Decaying and this corresponds to the period when DO delta in the Low Dose Reach was declining from its seasonal high. In the High Dose Reach there was a sharp increase in DO delta in mid-September, followed by a DO crash in early October (**Figure 4-16B**). It is apparent that that pattern of growth, maturity, and decay correspond well with the DO patterns recorded in the High Dose Reach. Growing corresponds to the period when DO delta was steadily increasing, Mature matches the period of peak primary productivity, and Decay aligns with the period when algae where decomposing and consuming stream oxygen and causing the DO crash which started in late September.

Over the same time period, the Control Reach manifested a somewhat different appearance from the dosed reaches (green letters, Figures **4-16A**, **B**). In the Control Reach, attached microalgae appeared to be Growing all the way into mid-September, and were only classified as Mature by late September. A Decaying phase was never noted; Control Reach microalgae appeared to be no more than Mature even when evaluated in early October.

4.5.2.2 Other Observations on the Stream's Flora During Nutrient Additions

During nutrient dosing, and for both the Low- and High Dose reaches, filamentous algae (mainly *Cladophora*) achieved very long lengths (**Figure 4-15**) but were largely restricted to riffle areas where water velocity was fastest. Using photos, we documented the changes in filamentous growth over time at the 1st riffle below the Low Dose dripper assembly (**Figure 4-17)**. In glides and pools, where velocities were lower, microalgae clearly dominated. Medium and Thick microalgae developed in glides even where water depths where over 0.5 m deep and light was probably becoming a co-limiting factor.

Prior to the beginning the study, we derived the length of our nutrient addition reaches (200 m) based on nutrient spiraling calculations. However, it was very obvious during the study that effects from the added nutrients extended beyond 200 m. We observed enhanced algal growth, particularly filamentous algae in riffles, up to 700 m downstream of the terminus of the Low Dose Reach. But by the start of the High Dose Reach (a full 900 m downstream of the terminus of the Low Dose), no notable changes in the appearance of the stream from Before to After were observed; field notes indicate that microalgae levels looked essentially like the Control Reach.

Downstream of the High Dose Reach, it was evident during dosing that the higher nutrient concentrations were continuing to manifest effects far downstream from the end of the 200 m study reach. We visited areas of the stream 1,700 m (1.7 km) downstream of the High Dose Reach terminus and observed heavy growths of filamentous algae in riffles and Medium and Thick microalgae. Whether or not these effects manifested even further downstream is unknown, as we did not have permission to enter the private property beyond.

Finally, we made some general observation pertaining to *Chara* spp. (commonly called stonewort or muskgrass). These plants were a fairly common component of the stream flora but were greatly depressed in number in the nutrient-dosed reaches compared to the Control Reach, and also compared to the pre-dosing period. *Chara* spp. are a branched form of algae, are an important component of natural aquatic ecosystems (DiTomaso and Healy 2003), and are often associated with clean water.

Figure 4-17. Photo Series of Filamentous Algae Growth over Time at the 1st Riffle below the Low Dose Dripper.

4.6 PERIPHYTON (DIATOM ALGAE)

Much of the evaluation of the study's diatom autecological data is provided in Bollman et al. (2014), with addition analysis from Teply (2013). Results from those reports are summarized here. Figures and tables from the original reports have been reproduced (and cited), with permission from the authors.

4.6.1 Diatom Assemblage Patterns in the Control Reach, 2009 to 2011

Patterns in the diatom assemblage were evaluated for the Control Reach because these assemblages provide the background against which changes in the dosed reaches are judged. In general, diatom taxa assemblages in the Control Reach were most similarly related to one another by the year in which they were sampled, rather than by the month of sampling (Bollman et al. 2014). **Figure 4-18** shows the 1st and 2nd principal components, per Principal Components Analysis[13]. The year in which samples were collected can be seen to loosely group taxa assemblages together (collectively, the two components explain 38.3% of the variance in the compositional data). A notable outlier was the sample collected in July 2010 (upper right corner of the figure), which was dominated by *Nitzschia gracilis* and high frequencies of *Thalassiosira pseudonana*. The July 2010 sample was distinct from all other Control Reach samples.

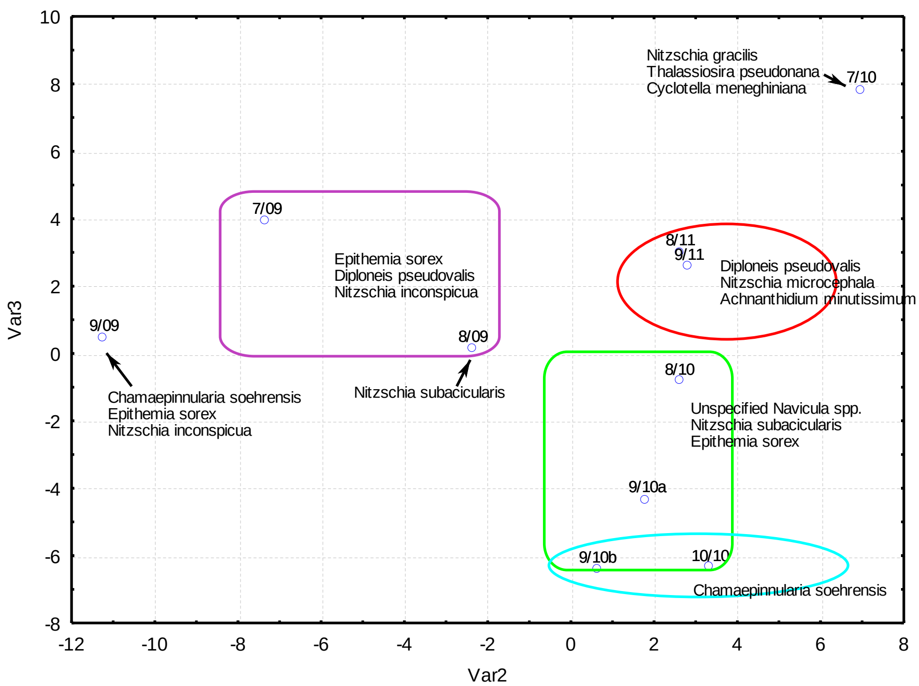


Figure 4-18. First and Second Principal Components of Principal Components Analysis for Diatom Assemblages from the Control Reach (2009-2011).

Major groupings are outlined by colored lines. After Bollman et al. (2014).

[13] An exploratory statistical technique that organizes community data based on relative abundances of taxa. The technique produces a graphical display in which similar assemblages are plotted close to one another and less similar assemblages are plotted farther away. Principal Components Analysis is organized such that the 1st principal component is the axis that accounts for the greatest amount of variation in the matrix, the 2nd principal component accounts for the 2nd largest amount, and so on through the complete matrix of taxa.

4.6.2 Individual Diatom Taxa Responses to Nutrient Dosing

Among the 215 diatom taxa (*Genus species*) that were identified during the study, two taxa (*Navicula recens*, *Epithemia sorex*) showed significant responses to nutrient additions which were clearly concordant with the dosing levels. A third diatom, *Amphipleura pellucida*, showed what appears to be a delayed effect of nutrient additions. These diatoms are discussed here.

By far the clearest response to nutrient dosing was manifested by *Navicula recens*. *Navicula recens* is an alkaliphilous, alpha-mesosaprobous, eutraphentic, moderately motile species. Given its attribute designation, it would be reasonable to expect this diatom to increase in abundance in nutrient-enriched conditions (Bollman et al. 2014), and the graph of its relative abundance in samples bears this out (**Figure 4-19**). In the Control Reach, abundance of *N. recens* remained stable at between 0.3% and 2%. In the High Dose Reach, its abundance from “background” levels of 1-5% jumped to 30-33%, remaining elevated above Control Reach abundances until October 2010. Abundances of this taxon in the Low Dose Reach responded similarly, but with diminished amplitude, increasing from values similar to the Control Reach to 5-12% during the dosing period (Bollman et al. 2014). No other diatom taxon exhibited a pattern so closely related to the dosing levels. BACIP-design statistical testing of the relative increase in abundance of *N. recens* in both the Low- and High Dose reaches were significant for both reaches (one sided Mann-Whitney, p = 0.029 and 0.014, respectively); that is, there were significantly more *N. recens* in the After period compared to the Before period.

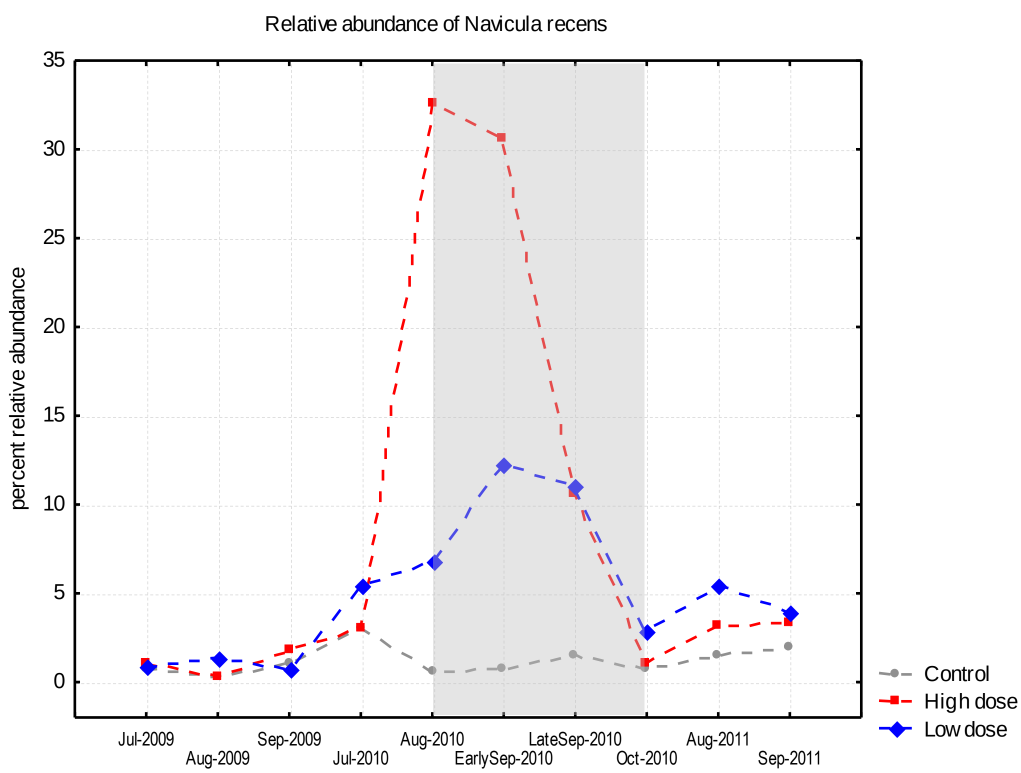


Figure 4-19. Percent Relative Abundance of *Navicula recens* During the Study Period.
The nutrient dosing period in 2010 is shown in gray. After Bollman et al. (2014).

Epithemia sorex is the most common diatom in the class Rhopalodiales occurring in samples in this study. Since it harbors nitrogen-fixing cyanobacteria, it would be reasonable to expect its abundance to diminish when N increases in concentration and, in turn, favors competition from other diatoms

(Bollman et al. 2014). *E. sorex* dominated several samples in each reach, especially those collected in 2009. Beginning in September 2009, before the onset of dosing, *E. sorex* abundance fell in all three reaches (**Figure 4-20**). During 2010 dosing period, the Control Reach had the highest abundances of *E. sorex*, followed in turn by the Low Dose Reach and then the High Dose Reach; this pattern tracks the expected response of the organism to dosing. The BACIP analysis indicated a marginally-significant response (one sided Mann-Whitney, p = 0.073) of this diatom in the High Dose Reach (Bollman et al. 2014). In 2011, *E. sorex* abundance in the previously-dosed reaches remained slightly below frequencies observed in the Control Reach, and abundance in all three reaches was generally lower than what was observed in previous years.

As if exhibiting a delayed response to dosed nutrients at lower levels only (i.e., in the Low Dose Reach), *Amphipleura pellucida* (**Figure 4-21**) exhibited an increase in abundance beginning in September 2010, which persisted through late September and October, returning to "background" levels similar to the Control Reach by 2011. The High Dose Reach showed a mild increase in numbers of *A. pellucida* in late September/early October 2010. This diatom is alkaliphilous, a nitrogen autotroph, with an oligo-mesotraphentic trophic status (Bollman et al. 2014). The BACIP statistical analysis for the Low Dose Reach yielded a significant Before *vs.* After response (one sided Mann-Whitney, p = 0.029), whereas the High Dose did not.

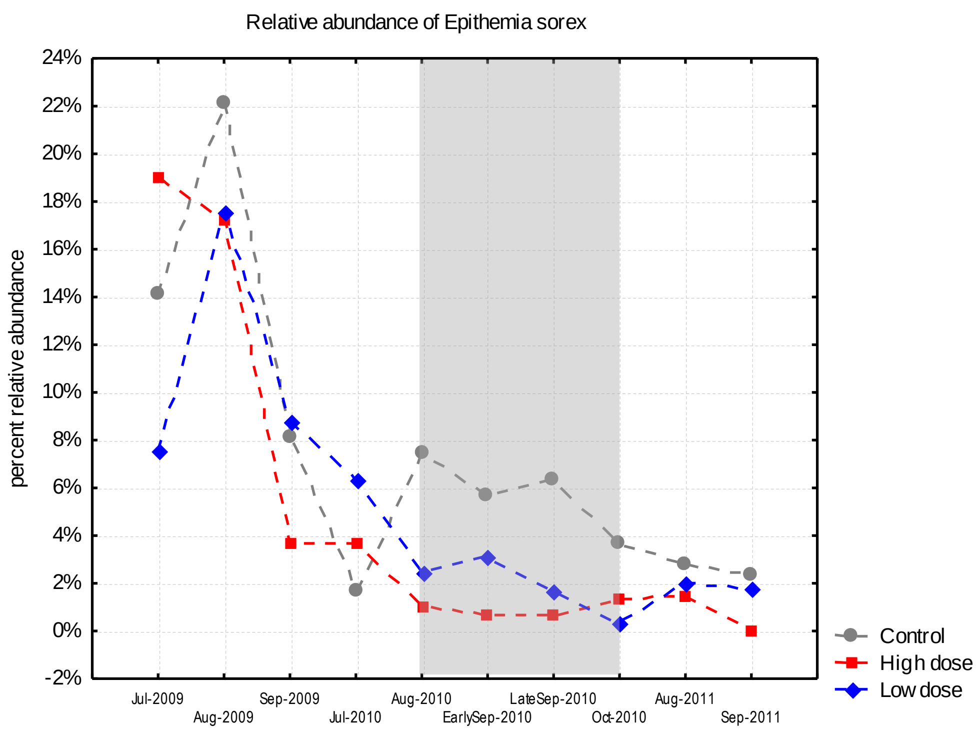


Figure 4-20. The percent relative abundance of *Epithemia sorex* during the study period.
The nutrient dosing period in shown in gray. After Bollman et al. (2014).

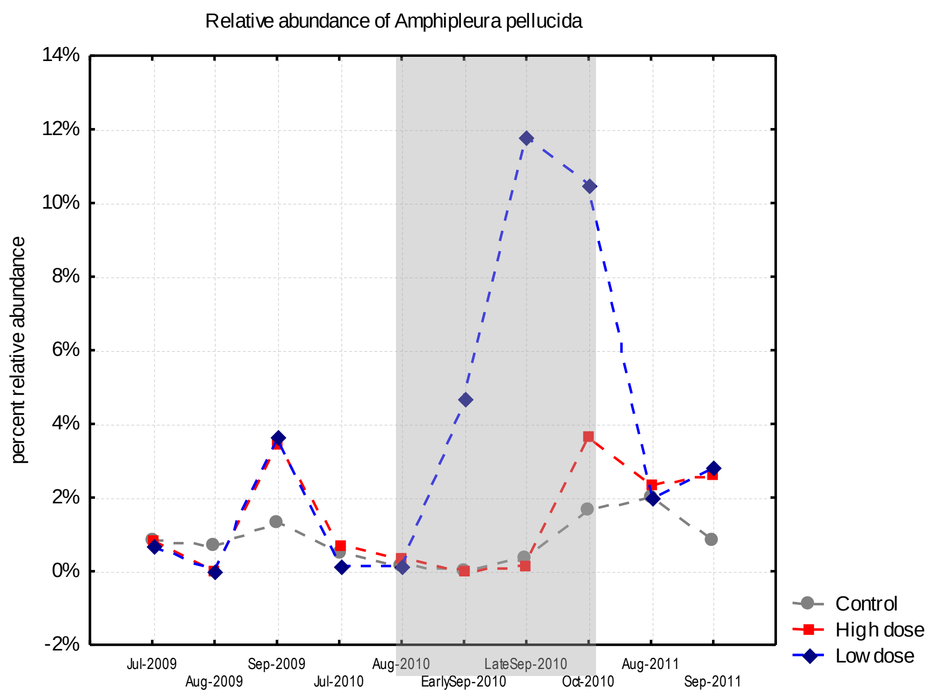


Figure 4-21. The percent relative abundance of *Amphipleura pellucida* during the study period. The nutrient dosing period in shown in gray. After Bollman et al. (2014).

Based on BACIP-design statistical analysis, there were eight other diatom taxa that responded significantly ($p \leq 0.05$) or marginally significantly ($p \leq 0.1$) to nutrient dosing, either in the Low Dose Reach, High Dose Reach, or both. These were *Navicula cryptotenella*, *Navicula germainii*, *Nitzschia acicularis*, *Nitzschia intermedia*, *Nitzschia pumila*, *Nitzschia reversa*, *Rhoicosphenia abbreviata*, and *Tabularia tabulata*. For some (*Navicula germainii*; *Nitzschia pumila*) the response was confusing as the taxa increased in the High Dose Reach and the Control Reach in the After period, whereas in the Low-Dose Reach abundance remained essentially unchanged in the After period. In other cases, in the After period the taxon increased in the High Dose Reach but decreased in the Low Dose Reach (*Nitzschia acicularis*), the taxon responded in the reverse direction from what was expected (*Navicula cryptotenella*), or response was clearly stronger in the Low Dose Reach compared to the High Dose (*Tabularia tabulata*). For complete details on these taxa, see Appendix B and C in Bollman et al. (2014).

Overall, the taxon that showed the most concordant response to nutrient dosing was *Navicula recens*, followed by *Epithemia sorex* (which decreased, as expected, concordant with dosing magnitude).

4.6.3 Diatom Metrics and their Response to Nutrient Dosing

There are many diatom metrics in the U.S. which could be used to evaluate eutrophication of streams (Potapova and Charles 2007; Porter et al. 2008). A subset of fourteen of these was judged as having good probability of responding to nutrient dosing in this study, and were examined in detail. In addition, a genus-level metric was tested (i.e., diatom identification is only required to genus level in order to use the metric). Results are shown in **Table 4-21**. There were ten metrics that responded significantly (or marginally so) in the High Dose Reach, but only two in the Low Dose Reach. The genus-level index showed no significant response in either reach. Those that responded significantly (or marginally so) for both the Low- and High Dose reaches—and in the expected direction—were the ‘Rhopalodiales taxa percent’ and the ‘Polysaprobous taxa percent’ of VanDam et al. (1994).

'Rhopalodiales taxa percent' was one of the two metrics that significantly responded to nutrient dosing and responded as expected. The Rhopalodiales order of diatoms harbor nitrogen-fixing cyanobacteria as symbionts, and their abundance is thought to be negatively associated with the availability of N. There is also evidence that this diatom order is positively correlated with solutes and conductivity in prairie streams. Six taxa (*Rhopalodia gibba, R. abbreviata, R. brebissonii, Epithemia adnata, E. sorex, and E. turgida*) represented this group in samples collected for this study (Bollman et al. 2014). Rankings of the dominant taxa collected from Box Elder Creek indicate that *Epithemia sorex* was particularly abundant, especially in samples collected in 2009. High relative abundance of taxa in the Rhopalodiales suggests that biologically available N is limiting relative to P (Bollman et al. 2014). During the dosing period, there was a decrease in abundance of Rhopalodiales taxa: the High Dose Reach generally supported fewer of these taxa than the Low Dose Reach from August through July (**Figure 4-22**). The pattern is consistent with the expected response. BACIP-design statistical testing indicated that there were fewer Rhopalodiales in the After period compared to the Before period, and this was marginally significant for both the High and Low Dose reaches (one sided Mann-Whitney, $p = 0.1$ and 0.057, respectively; **Table 4-21**). Note that the response of this biometric, comprising several taxa from the Rhopalodiales order, is consistent with the behavior of the individual taxon from the group (*Epithemia sorex*) which was discussed earlier in **Section 4.6.2**.

Table 4-21. Diatom Metrics, with Expected Responses and Observed BACIP Responses to Different Levels of Nutrient Dosing.
After Bollman et al. (2014).

Metric	Source*	Expected response	Observed response	Difference: Control minus Low Dose (Mann-Whitney p value)	Statistical significance (* = p ≤0.1, **= p ≤0.05)	Difference: Control minus High Dose (Mann-Whitney p value)	Statistical significance (* = p ≤0.1, **= p ≤0.05)
Low dissolved oxygen taxa percent	1	increase	decrease	0.171		0.442	
Nitrogen autotroph taxa percent	1	increase	mixed	0.557		0.014	**
Nitrogen heterotroph taxa percent	1	increase	mixed	0.657		0.03	**
Pollution Tolerance Index	1	decrease	decrease	0.9		0.557	
Polysaprobous taxa percent	1	increase	increase	0.014	**	0.073	*
Rhopalodiales percent	1	decrease	decrease	0.1	*	0.057	*
Shannon diversity (H)	1	unknown	decrease	0.2		0.7	
Species richness	1	unknown	decrease	0.121		0.1	*
Low nitrogen taxa percent	2	decrease	decrease	0.171		0.014	**
High nitrogen taxa percent	2	increase	mixed	0.9		0.014	**
Siltation taxa percent	1	unknown	increase	0.657		0.014	**
Percent tolerant taxa	3	increase	increase	0.171		0.014	**
Generic index	4	decrease	decrease	0.557		0.557	

*Source: 1. Van Dam et al. (1994), Bahls (1993); 2. Potapova & Charles (2007); 3. Stevenson et al. (2008); 4. Wu (1999).

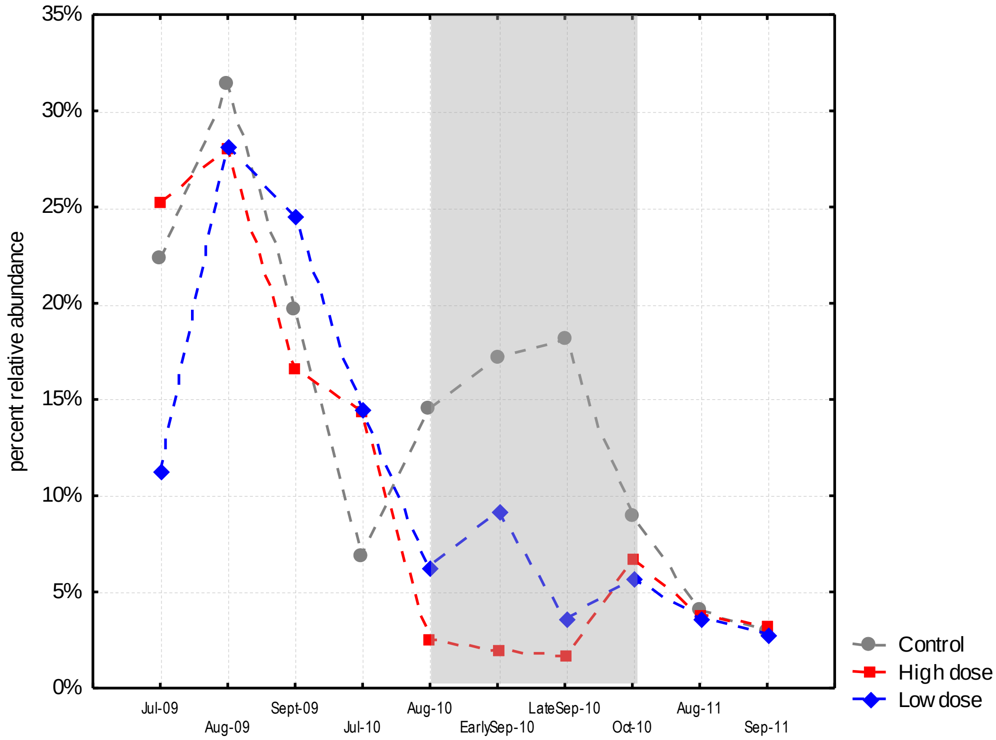


Figure 4-22. The 'Rhopalodiales Taxa Percent' Diatom Metric.
The nutrient dosing period in shown in gray. After Bollman et al. (2014).

The 'Polysaprobous taxa percent' was the second biometric that significantly responded to nutrient dosing *and* responded as expected (**Figure 4-23**). This metric is calculated using diatoms following Van Dam's saprobity classes: alpha-mesosaprobous (O_2 saturation 25-70%, BOD 4-13 mg/L), alpha-meso/polysaprobous (O_2 saturation 10-25%, BOD 13-22 mg/L), and polysaprobous (O_2 saturation <10%, BOD >22 mg/L). Among the taxa that significantly influenced the performance of this metric in the study were *Navicula recens* and *Nitzschia filiformis*, which dominated or strongly dominated all samples collected in the High Dose Reach during the dosing period: both are classified as alpha-mesosaprobous (Bollman et al. 2014). Although present throughout the study in the Control Reach (**Figure 4-19**), *Navicula recens* was never abundant there and *Nitzschia filiformis* was even less common. Polysaprobous taxa were present in relatively low frequencies (ca. 30%) in all reaches in 2009, increasing dramatically beginning in August 2010 in the High Dose Reach and in September 2010 in the Low Dose Reach, while frequencies in the Control reach remained lower (Bollman et al. 2014). In both the High- and the Low Dose Reach, the abundance of these taxa dropped to levels not much different than the Control Reach in 2011. The behavior of the Polysaprobous taxa percent metric was suggestive of an expected response to the dosed nutrient enrichment. BACIP-design statistical analysis indicated that there were significantly (or marginally so) more Polysaprobous taxa in the After period compared to the Before period for both the Low- and High Dose reaches (one sided Mann-Whitney, $p = 0.014$ and 0.073, respectively).

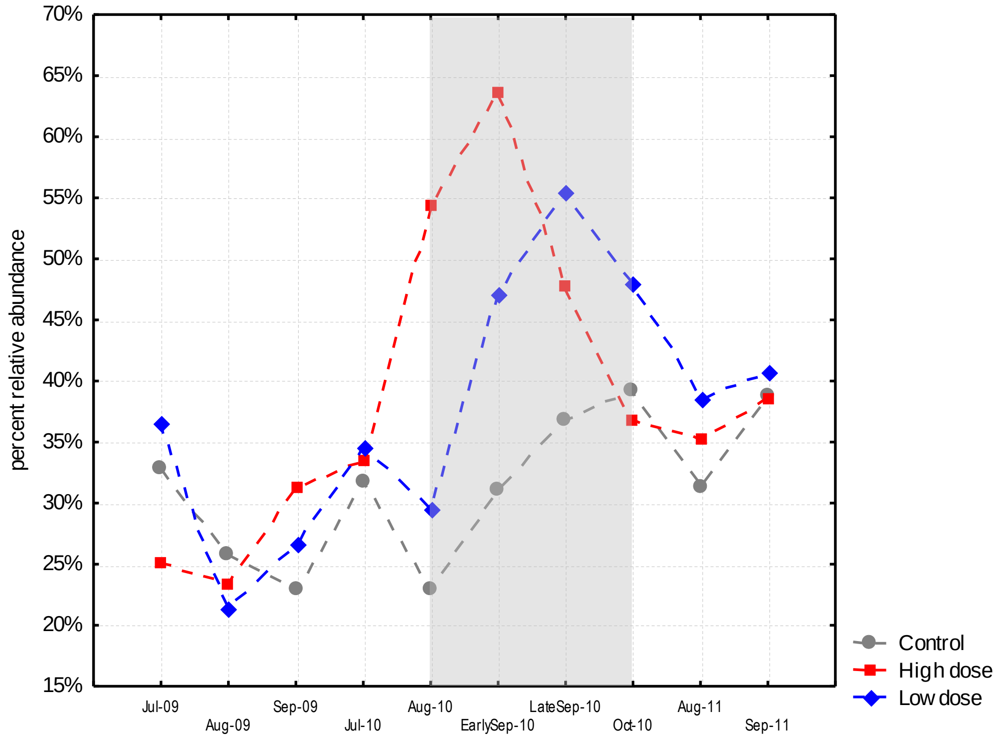


Figure 4-23. The 'Polysaprobous Taxa Percent' Diatom Metric.
The nutrient dosing period in shown in gray. After Bollman et al. (2014).

There were six other diatom metrics that showed significant ($p < 0.05$) differences in the After period compared to the Before period, but only in the High Dose Reach (**Table 4-21**). Although they only responded significantly in one of the two dosed reaches, two of these metrics ('Low nitrogen taxa percent' and 'High nitrogen taxa percent') warrant further discussion here, as N limitation is common in Montana prairie streams (Suplee 2004).

Potapova and Charles (2007) designated low nitrogen taxa as those which indicated N concentrations less than or equal to 0.2 mg/L: the designations are specific to the Central and Western Plains or reflect findings for all diatom samples in the U.S. Geological Survey's NAWQA database. The metric behaved in the expected way for the High Dose Reach in the dosing period, falling to low frequencies between August and September, 2010 (Bollman et al. 2014). While the Low Dose Reach exhibited a drop in abundance of these taxa in August 2010, frequencies increase over the next months of 2010, exceeding the frequencies recorded for the Control Reach in late July (**Figure 4-24**). In the High Dose Reach, the BACIP-design statistical test indicated that the response was significant (one sided Mann-Whitney, $p = 0.014$), but it was not in the Low Dose Reach (**Table 4-21**).

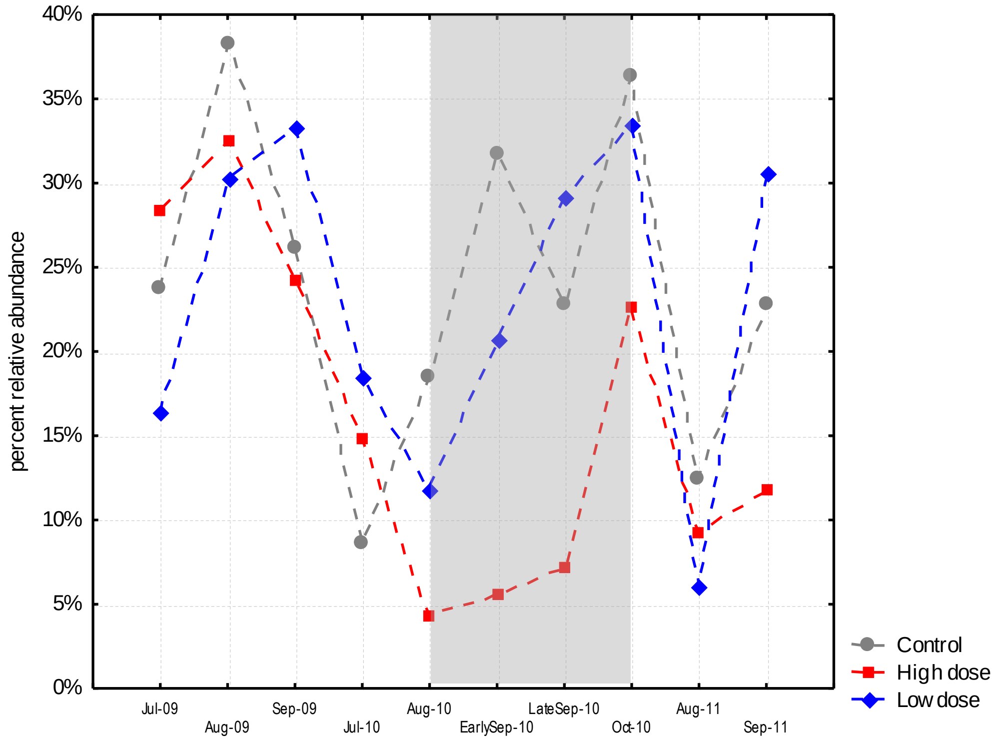


Figure 4-24. The 'Low Nitrogen Taxa Percent' Diatom Metric.
The nutrient dosing period in shown in gray. In this case, it was expected that there would be a decline in these diatoms' abundances in the After period. Differences between the Before and After periods were only significant in the High Dose Reach. After Bollman et al. (2014).

High nitrogen taxa were described by Potapova and Charles (2007) as taxa that indicate N concentrations greater than or equal to 3.0 mg/L: similar to the low nitrogen percent metric, these taxa designations are specific to the Central and Western Plains or reflect findings for all samples in the NAWQA database. The metric behaved in the expected way, since it is reasonable to expect these nutrient-tolerant taxa to increase in abundance with increasing nutrient concentrations (Bollman et al. 2014). All 3 reaches harbored greater abundances of these taxa beginning in July 2010, compared to 2009 (**Figure 4-25**). The High Dose Reach exhibited a continued increase in numbers of high nitrogen taxa over the following month, and both High- and Low Dose reaches continued to support greater abundances compared to the Control Reach until late in 2011 (Bollman et al. 2014). The BACIP-design statistical test showed there were significantly more of the High nitrogen taxa in the After period compared to the Before period in the High Dose Reach (one sided Mann-Whitney, $p = 0.014$), but there was no significant differences in the Low Dose Reach (**Table 4-21**).

For a detailed discussion of the other four diatom metrics that responded uniquely in the High Dose Reach, see Bollman et al. (2014).

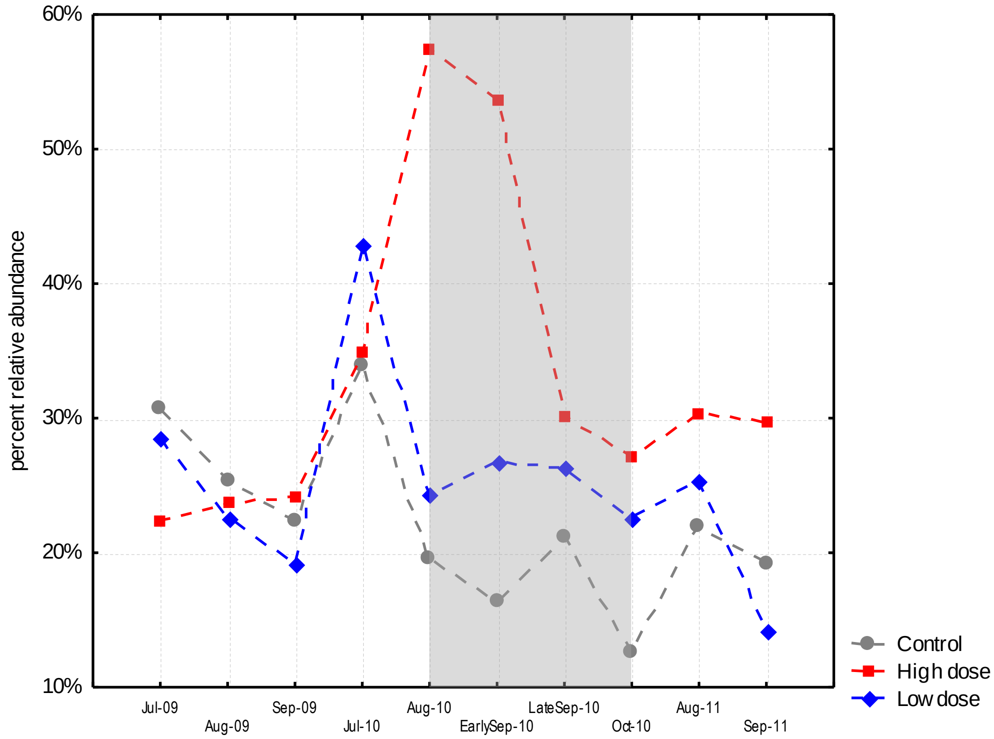


Figure 4-25. The 'High Nitrogen Taxa Percent' Diatom Metric.
The nutrient dosing period in shown in gray. After Bollman et al. (2014). Differences between the Before and After periods were only significant in the High Dose Reach.

As of this writing, one of DEQ's main tools for assessing eutrophication in prairie streams is via the warm-water nutrient increaser taxa metric (Suplee and Sada de Suplee 2011; Montana Department of Environmental Quality 2011a). The metric outputs a probability, based on the diatom population, that the stream is impaired by excess nutrients. Results are shown in **Table 4-22**. BACIP-design statistical tests were carried out on the output of this biometric. There was no significant difference between the Before and After periods in the Low-Dose Reach (one sided Mann-Whitney test). In the High-Dose Reach, there was a marginally significant difference between the Before and After periods (one sided Mann-Whitney, p = 0.056); i.e., there was greater indication of nutrient impairment in the After period compared to the Before period. The warm-water nutrient increaser metric showed a high degree of variability in the Control Reach over the study, with individual sampling events indicating a probability of impairment ranging from 27.6% to 68.2%; the average impairment probability in the Control Reach for the entire study was 46.8%. DEQ is currently using >51% as the impairment threshold (Montana Department of Environmental Quality 2011a) so, on average, the Control Reach was shown to not have a nutrient problem based on this metric. But given that this Box Elder Creek site is a well-established reference site one would not expect the probability of impairment at the Control Reach to exceed 50% during any sampling event, yet individual sampling events in excess of 50% occurred five times (half the sampling events).

Teply (2013) approached the BACIP statistics for the warm-water nutrient increaser taxa metric in a slightly different way. For the High Dose Reach, he input the percent relative abundance of warm-water nutrient increaser taxa (e.g., 13.2%, Control Reach, July 2009) into the BACIP table in lieu of the % probability of impairment, as we have done in **Table 4-22**. In the High Dose Reach, the After period had marginally significantly greater abundance of increaser taxa in the After period compared to the Before period (one sided, $p < 0.08$). What is clear here is that either way this diatom metric's data are handled, the statistical result is essentially the same.

Table 4-22. Probability of Nutrient Impairment Based on DEQ's Warm-water Nutrient Increaser Diatom Taxa Metric.

Difference values used in BACIP statistics are shown in the last two columns on the right.

Sampling Date*	Period	Sampling Event	Control (% probability)	Low Dose (% probability)	High Dose (% probability)	Difference (D) Control - Low Dose	Difference (D) Control - High Dose
7/25/2009	Before	1(2009)	50.6	46.6	45.6	4.0	5.0
8/13/2009	Before	2 (2009)	65.1	56.1	56.4	9.0	8.7
9/26/2009	Before	3 (2010)	56.1	45.1	42.0	10.9	14.1
7/18/2010	Before	4 (2010)	68.2	86.1	86.1	-18.0	-18.0
8/26/2010	After	1 (2010)	47.0	61.7	80.0	-14.8	-33.0
9/8/2010	After	2 (2010)	37.8	33.9	56.7	3.9	-18.9
9/22/2010	After	3 (2010)	50.6	35.0	47.7	15.6	2.9
10/8/2010	After	4 (2010)	27.6	45.1	37.4	-17.6	-9.9
8/27/2011	n/a	–	37.4	50.0	46.6	–	–
9/24/2011	n/a	–	28.0	27.6	53.1	–	–

*No diatom taxa samples were collected on the 8/30/2009 sampling event.

4.7 Macroinvertebrates

Macroinvertebrate samples were collected in all three years of the study and reach-scale sample duplicates were collected during the BACIP period (2009 and 2010) in all three reaches (duplicate dates: 7/25/2009, Control; 7/18/2010, Control; 9/9/2010, Low Dose; and 9/26/2009, High Dose). Results from the macroinvertebrate sample analyses are shown in the next two sections. No clear patterns emerged from the macroinvertebrate biomass (AFDM) data and those data are not further addressed in this report.

4.7.1 Individual Macroinvertebrate Taxa Responses to Nutrient Dosing

We tested 71 individual taxa for their response to nutrient dosing. In the great majority of cases there were insufficient data (<3 matched observations in the Before and After periods) to allow for BACIP statistics, based on our operational minimums defined in Methods (**Section 3.5.3.3**). However fourteen taxa were collected in sufficient abundance—and on enough occasions—to meet our minimum, and these are shown in **Table 4-23**. An interesting result was that the observed responses were, as often as not, backwards from the expected response (as determined *a priori* using taxa tolerance values). The taxa *Thienemannimyia* (tolerance value 5), a genus of non-biting midges in the subfamily Tanypodinae of the bloodworm family Chironomidae, provided perhaps the most clear response to dosing in the expected manner (**Figure 4-26**).

Table 4-23. Macroinvertebrate Taxa, with Expected Responses (Based on Tolerance Values) and Observed BACIP Responses to Different Levels of Nutrient Dosing.

Taxa	Response			Difference: Control minus Low Dose (Mann-Whitney p value)	Statistical significance (* = p ≤0.1, **= p ≤0.05)	Difference: Control minus High Dose (Mann-Whitney p value)	Statistical significance (* = p ≤0.1, **= p ≤0.05)	Notes
	Expected	Observed LD	Observed HD					
Ablabesmyia sp.	increase	increase	–	0.500		–	–	High Dose ***n*** too low
Caenis sp.	Increase	decrease	decrease	0.057	*†	0.171		
Cheumatopsyche sp.	decrease	increase	decrease	0.029	**	0.171		
Cladotanytarsus sp.	increase	increase	increase	0.050	**	0.100	*	
Cryptochironomus sp.	increase	decrease	decrease	0.200		0.200		
Dicrotendipes sp.	increase	increase	increase	0.171		0.200		
Dubiraphia sp.	increase	decrease	decrease	0.029	**†	0.443		
Hydropsyche sp.	decrease	decrease	increase	0.314		0.343		
Hydropsychidae	decrease	increase	–	0.100	*†	–	–	High Dose ***n*** too low
Polypedilum sp.	increase	decrease	decrease	0.443		0.343		
Pseudochironomus sp.	decrease	decrease	decrease	0.343		0.429		
Simulium sp.	decrease	increase	increase	0.008	**†	0.171		
Tanytarsus sp.	increase	decrease	–	0.200		–	–	High Dose n too low
Thienemannimyia sp.	decrease	decrease	decrease	0.057	*	0.100	*	

†Response is significant (or marginally significant) but backwards from the expected response.

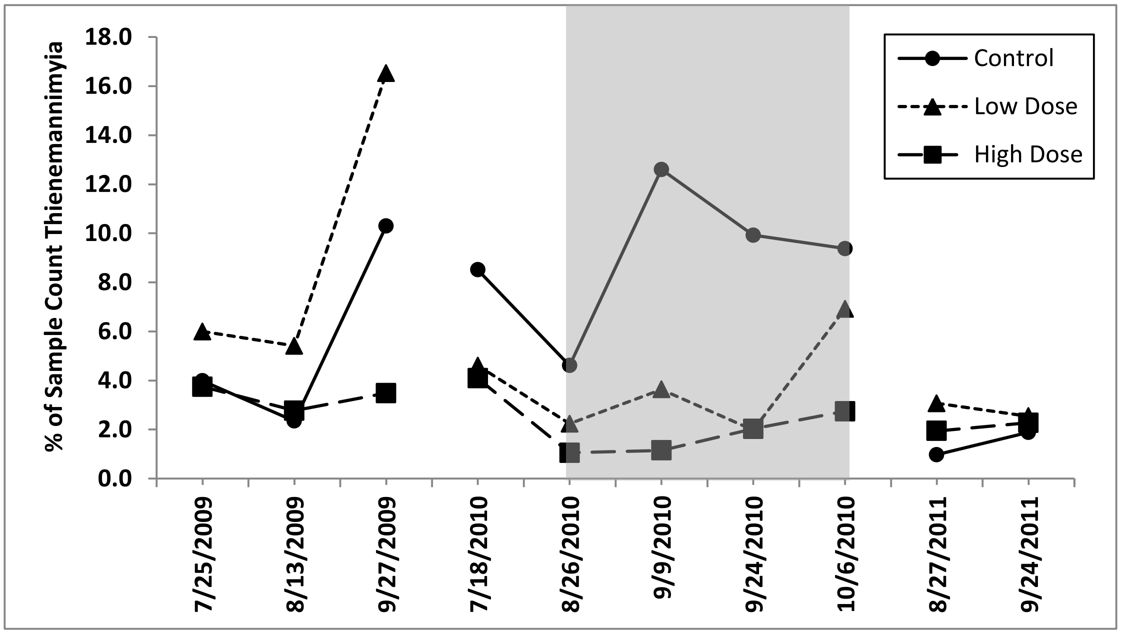


Figure 4-26. Percent Relative Abundance of *Thienemannimyia* sp. During the Study.
The nutrient dosing period in shown in gray.

Thienemannimyia's decrease in response to nutrient dosing was marginally significant and concordant with the levels of nutrient dosing (**Figure 4-26**). *Cladotanytarsus* sp. (a genus of European non-biting midges in the subfamily Chironominae of the bloodworm family Chironomidae) increased significantly and as expected, and the increases were concordant with the dosing levels (**Table 4-23**; **Figure 4-27**). In contrast, the taxa *Simulium* sp. (a genus of black flies with tolerance value 5, designated as a decreaser) *increased* significantly in the Low Dose Reach (**Figure 4-28**), however the response was not significant in the High Dose Reach (**Table 4-23**).

The manner in which we processed the data (i.e., one-to-one database joins between the Control Reach data and an experimental reach's data, joins being made on sampling date and taxa name) created the possibility of excluding some potentially meaningful outcomes for individual taxa. Specifically, if there was consistent observation of a decreaser taxa in the Before period in both the Control and an experimental reach, followed by the complete absence of the organism in the After period in the experimental reach, this might indicate that nutrient dosing had completely extirpated the decreaser organism. (The converse situation for increaser taxa is similar). Our data processing method would not pick up the non-existent After data in the experimental reach and would preclude BACIP statistical analysis. However we hand-checked the data and it resulted that these scenarios were uncommon, as only five out of 71 taxa (Acarina, *Ithytrichia* sp., *Stempellina* sp., and *Stenelmis sp.*, decreasers; *Orthocladius* sp., increaser) demonstrated the pattern just described. Of these, results from three (*Ithytrichia* sp., *Stempellina* sp., and *Stenelmis* sp.) are probably not meaningful. For these three organisms, two (of four) sampling events in the Before period did not have simultaneous observation of the organisms in the Control and the paired experimental reach; this indicates that the organisms were not particularly common to begin with, and their absence in the After period may simply be due to their overall rarity. Of the remaining two, Acarina (a decreaser) was not observed in the After period in either experimental reach *nor* in the Control Reach; thus it would be impossible to conclude that its absence from the experimental reaches in the After period was due to nutrient dosing. *Orthocladius* sp. (an increaser), in contrast, was observed sporadically in low numbers in samples from the Before period in all three reaches (**Figure 4-29**), and then increased in number concordant with the dosing levels (the highest counts being observed in the High Dose Reach in the After period). This suggests that *Orthocladius* sp. may be a good indicator organism which increases with nutrient dosing, though we cannot test this statistically using the BACIP method. *Orthocladius* sp. is a genus of non-biting midges in the subfamily Orthocladiinae of the bloodworm family Chironomidae.

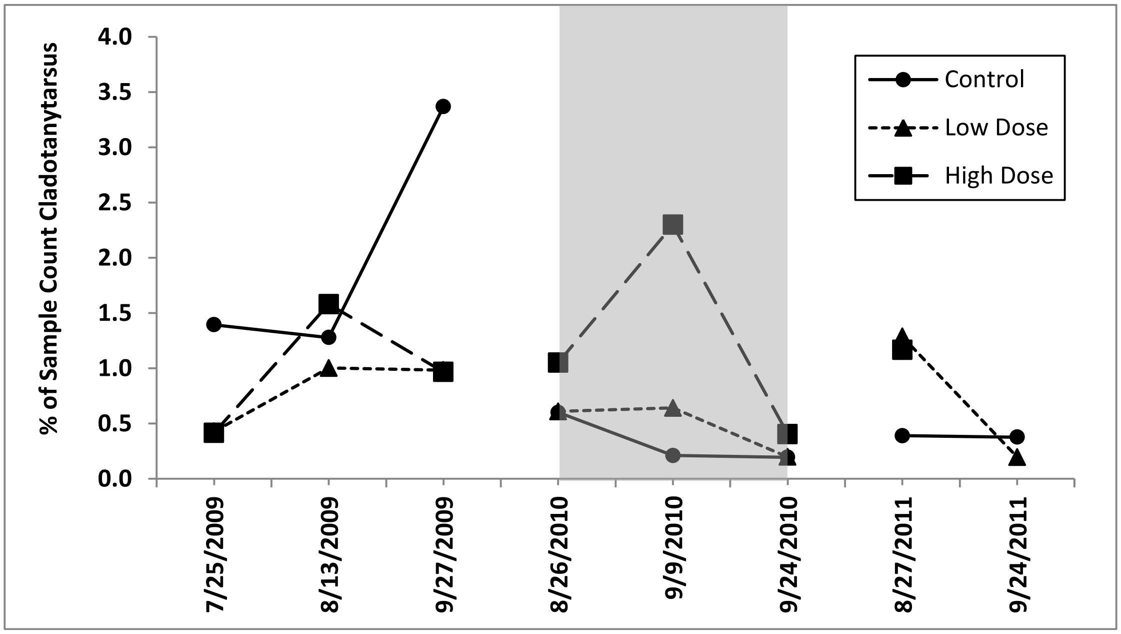


Figure 4-27. Percent Relative Abundance of *Cladotanytarsus* sp. During the Study.
The nutrient dosing period in shown in gray.

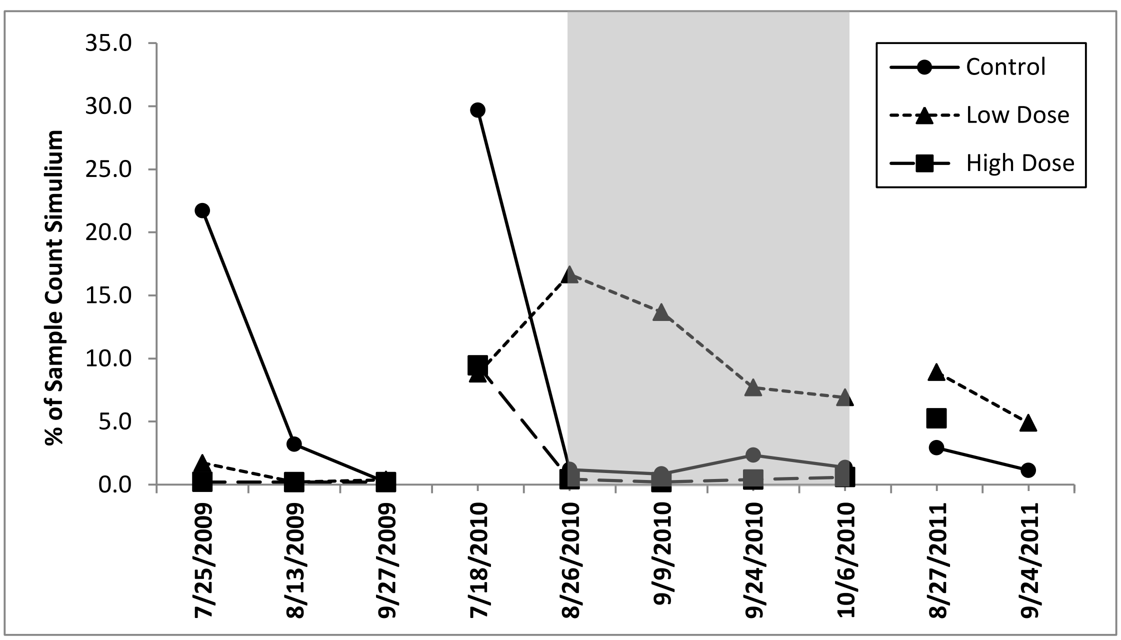


Figure 4-28. Percent Relative Abundance of *Simulium* sp. During the Study.
The nutrient dosing period in shown in gray.

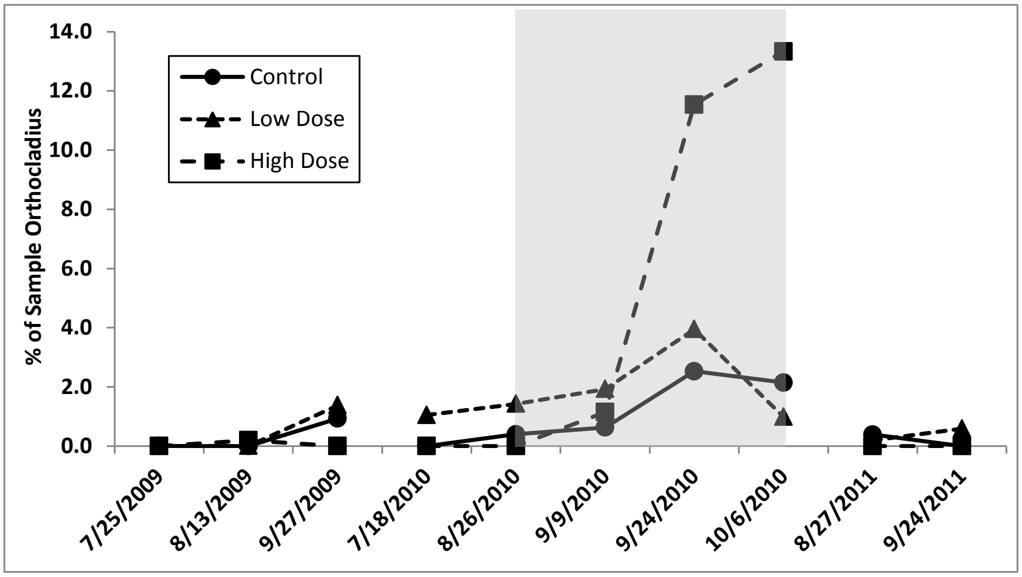


Figure 4-29. Percent Relative Abundance of *Orthocladius sp*. During the Study.
The nutrient dosing period in shown in gray. This figure differs from the other macroinvertebrate figures in that the graph includes samples where the sample count of the organism was zero; see text for explanation.

4.7.2 Macroinvertebrate Metrics and their Response to Nutrient Dosing

We tested twelve macroinvertebrate metrics which DEQ has used for stream assessment or which we believed would have a good probability of showing response to nutrient dosing (**Table 4-24**). Like the individual taxa, observed macroinvertebrate metrics responses were often backwards from the expected response. However, macroinvertebrate metrics showed significant ($p \leq 0.05$) responses far more often than was the case for individual macroinvertebrate taxa. The Montana Plains MMI, a metric used by DEQ in the 2000s to assess Montana prairie streams, responded significantly and concordantly to nutrient dosing (**Figure 4-30**). Note in **Figure 4-30** that in the High Dose Reach metric scores declined to the impact-level threshold (and in the Low Dose Reach they came close), whereas in the Control Reach, during all years of the study, metric scores were well above the impact threshold.

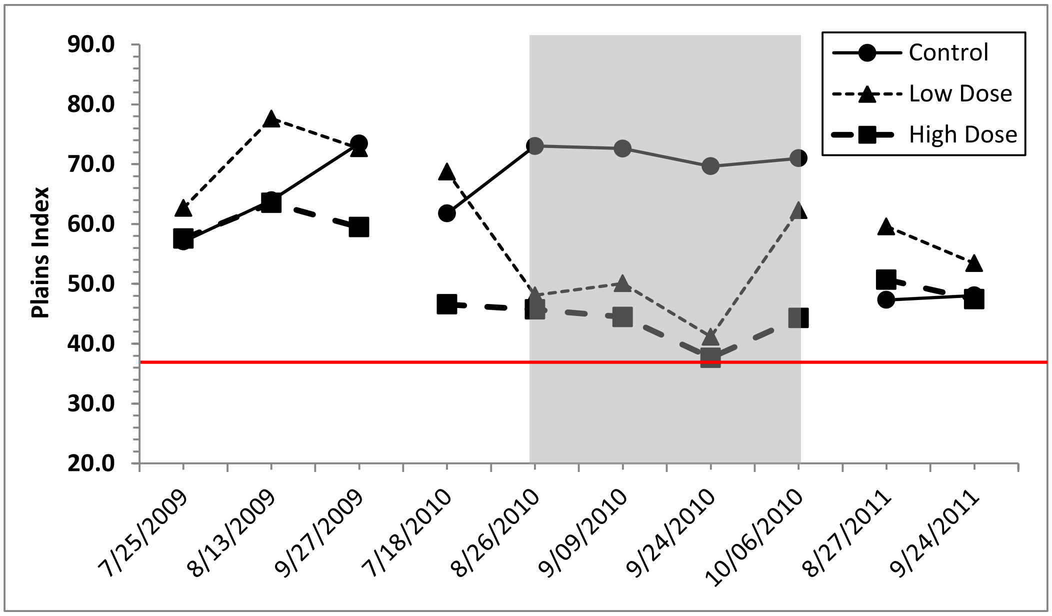


Figure 4-30. Response of the Montana Plains MMI During the Study.
The nutrient dosing period in shown in gray. The red horizontal line shows the harm threshold as established by DEQ (Montana Department of Environmental Quality, Water Quality Planning Bureau 2006); metric scores lower than 37 indicate there is impact to the stream's macroinvertebrate population.

The O/E metric also responded significantly (and as expected) to nutrient dosing (**Figure 4-31**). This metric has the interesting property that elevated nutrients can result in scores which are too low as well as scores which are too high (note the two red lines in the figure). Unlike the Plains MMI metric, however, O/E metric was highly variable across time, with nearly half the Control Reach samples being in one or the other non-compliant score ranges. The FiltCollPct and EPTnoHBPct metrics also responded as expected, and were equally significant in their responses to nutrient dosing (**Figures 4-32, 4-33**).

Clear responses that were backwards from expected were manifested by the metrics associated with macroinvertebrate predators. PredPctM, TanypodPct, and PredatorTax **(Table 4-24)** all showed significant decreases during the dosing period, although these metrics had been expected *a priori* to increase in response to nutrients. The PredatorTax response during the study is shown in **Figure 4-34.** Like the O/E metric, Control Reach scores of the PredPctM, TanypodPct, and PredatorTax were quite variable over time.

Table 4-24. Macroinvertebrate Metrics, with Expected Responses and Observed BACIP Responses to Different Levels of Nutrient Dosing.

Metric Code	Metric Description	Response			Difference: Control minus Low Dose (Mann-Whitney p value)	Statistical significance (* = p ≤0.1, **=p ≤0.05)	Difference: Control minus High Dose (Mann-Whitney p value)	Statistical significance (* = p ≤0.1, **= p ≤0.05)
		Expected	Observed LD	Observed HD				
PlainsIndex	Multimetric stream-condition index, applicable to Montana plains regions	decrease	decrease	decrease	0.014	**	0.014	**
O/E	Proportion of observed taxa to expected taxa (expected based on reference streams)	decrease	decrease	decrease	0.022	**	0.03	**
Montana HBI	Hilsenhoff Biotic Index, with MT-specific tolerance values	increase	decrease	increase	0.156		0.235	
EPTPct	% of the sample in the Epheroptera, Plecoptera, and Trichoptera orders	decrease	increase	decrease	0.443		0.171	
NonInsPct	% of the sample not insects	increase	decrease	increase	0.557		0.557	
PredPctM	% of the sample comprised of predatory insect taxa	increase	decrease	decrease	0.029	** †	0.443	
EPTnoHBPct	% of the sample composed of Ephemeroptera, Plecoptera, and Trichoptera excluding Hydropsychidae and Baetidae families	decrease	decrease	decrease	0.014	**	0.014	**
MidgePct	% of the sample comprised of midge taxa	increase	decrease	increase	0.243		0.243	
EPTTax	Number of Epheroptera, Plecoptera, and Trichoptera taxa in the sample	decrease	decrease	increase	0.243		0.243	
TanypodPct	% of sample in the Tanypodinae (a subfamily within the family Chirinomidae)	increase	decrease	decrease	0.014	** †	0.057	* †
PredatorTax	Number of predatory invertebrate taxa in the sample	increase	decrease	decrease	0.014	** †	0.014	** †
FiltCollPct	% of the sample comprised of filtering and collecting invertebrates	increase	increase	increase	0.014	**	0.014	**

†Response is significant (or marginally significant) but backwards from the expected response.

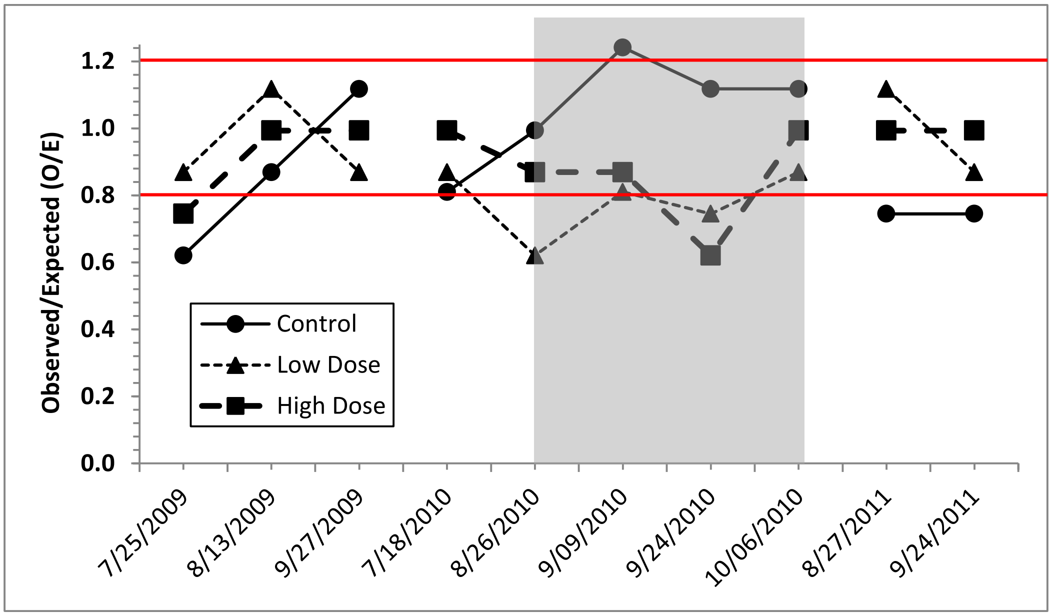


Figure 4-31. Response of the Observed/Expected (O/E) Metric During the Study.
The nutrient dosing period in shown in gray. The red horizontal lines show the harm threshold as established in DEQ SOPs (Montana Department of Environmental Quality, Water Quality Planning Bureau 2006); metric scores lower than 0.8 or higher than 1.2 indicate there is impact to the stream's macroinvertebrate population.

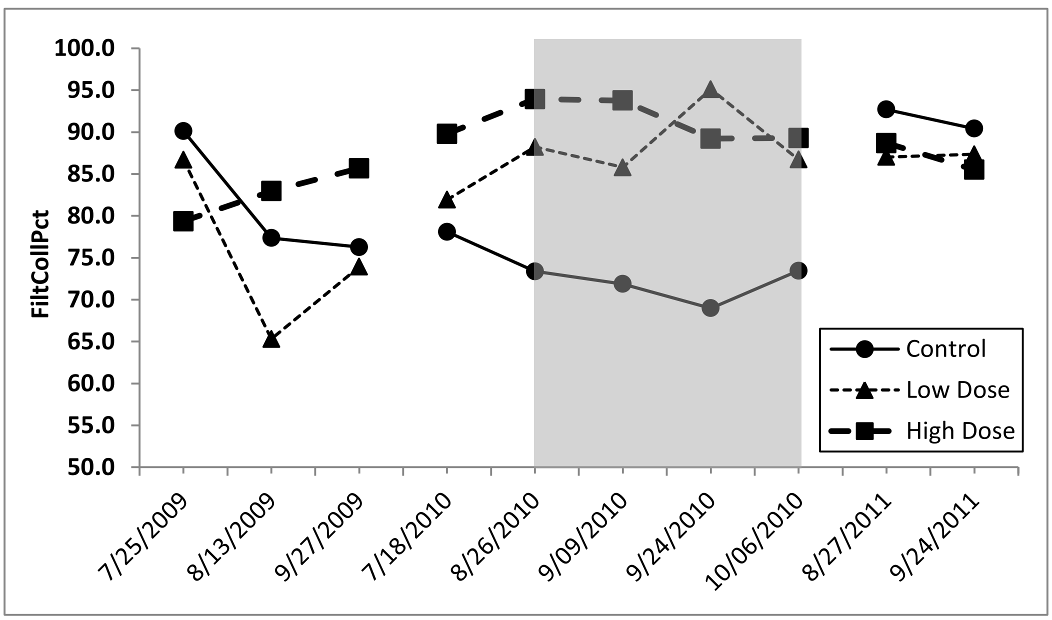


Figure 4-32. Response of the Percent Filterer and Collector (FiltCollPct) Metric During the Study.
The nutrient dosing period in shown in gray.

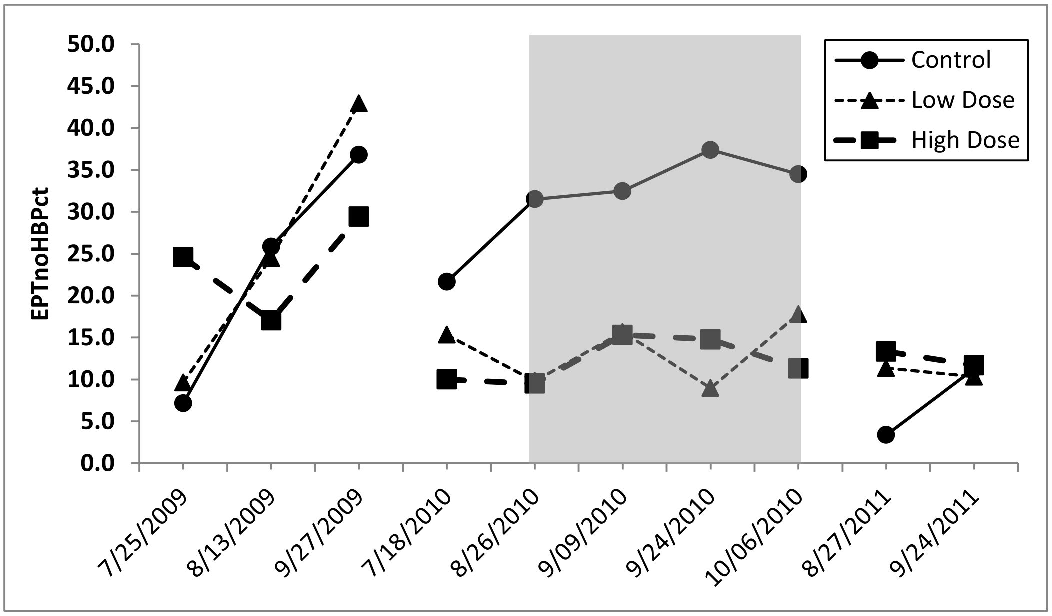


Figure 4-33. Response of the Metric Comprising % EPT but Excluding Hydropsychidae and Baetidae Families (EPTnoHBPct), During the Study.
The nutrient dosing period in shown in gray.

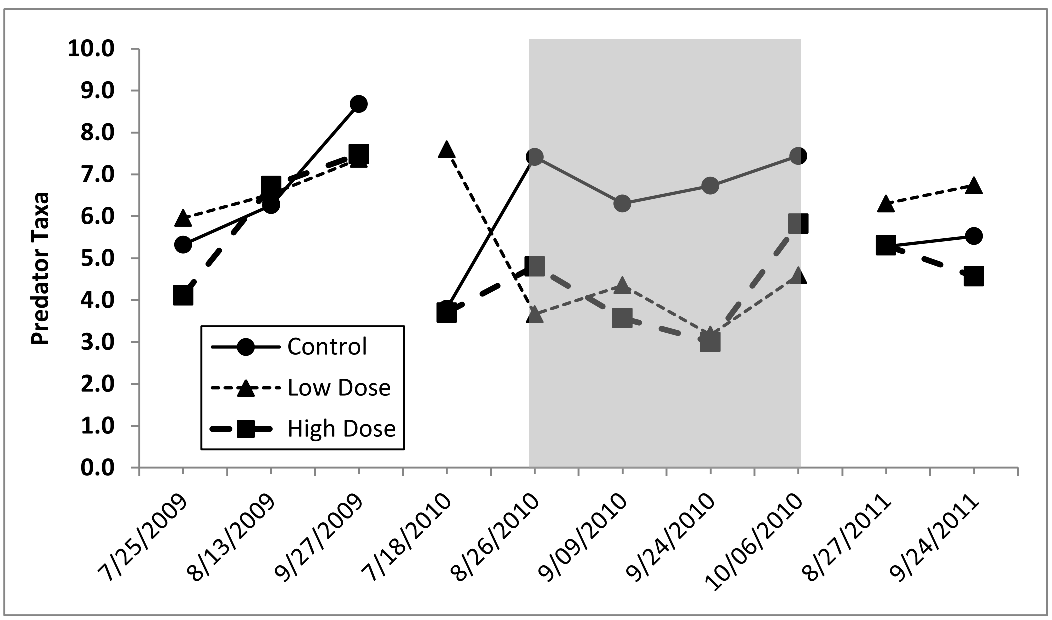


Figure 4-34. Response of the Predator Taxa Metric (PredatorTax) During the Study.
The nutrient dosing period in shown in gray.

5.0 Discussion

From the outset, our major purposes in carrying out this study were to (1) better understand the effects of nutrient enrichment in prairie streams of eastern Montana, and (2) collect data which lent themselves best to the interpretation of harm to beneficial uses of these streams. The latter was a particularly important goal because—as a regulatory agency—DEQ is constantly striving to understand the linkage between pollutants and the effects they manifest on legally defined and adopted stream uses and their associated water quality standards. Thus, we invested considerable energy in collecting good measurements of DO and pH, benthic algae density, diatoms, and macroinvertebrates, because these parameters can be linked to Montana's surface water quality standards and were expected to be affected by nutrient enrichment.

One of the most interesting findings of the study was that—contrary to what is commonly asserted in the introductory sections of many scientific papers on stream eutrophication—DO never fell to low levels concurrently with peak algal growth during nutrient enrichment. Daily DO highs in the Low- and High Dose reaches always equaled and usually exceeded (sometimes by a large margin) those of the Control Reach, but the corresponding daily DO lows in the dosed reaches were very similar to the Control Reach (**Figures 4-4, 4-5**). Water temperatures were lower at night, thus slowing nighttime plant respiration, but there was almost certainly also an effect from dark respiration. Dark respiration is a low-level, base respiration rate that is maintained by plants at night. Studies show that attached algae can have a much higher respiration rate in full light (or for several hours in darkness, after having been exposed to strong light) than they do in the dark, and this operates above and beyond the temperature effect (Yallop 1982; Graham et al. 1996). In fact, recent computer-simulation modeling of *Cladophora* incorporates a dark respiration rate in order to achieve better model fit to empirical data (Tomlinson et al. 2010). Our data support the notion that in streams, elevated benthic algal biomass which produces high daytime DO concentrations may not necessary cause equally low nighttime DO concentrations, and this is probably the case because (1) lower nighttime temperatures reduced respiration rates, and (2) the plants switch to a lower, dark respiration rate as night progresses, reducing the DO demand.

Although exceedence of DO standards concurrent with elevated algae biomass and peak primary productivity were not observed, the *ultimate* impacts of high algal biomass accumulation on stream DO were clearly manifested in the High Dose Reach. There, starting in late September, DO fell at times close to 1 mg DO/L, resulting in violations of Montana's DO standards (the 1-day minimum of 3 mg DO/L for adult aquatic life; Montana Department of Environmental Quality, 2012). The evident cause of this seasonally-manifested low DO was large masses of senesced algae decomposing on the stream bottom. The algae which had developed during the previous months of the growing season did impact DO, but the impact occurred later, out-of-sync with peak algal growth and photosynthesis. There appears to be little discussion of this out-of-sync phenomenon in the scientific literature, and even less quantitative documentation. Jewell (1971) notes in streams in England that "At the end of the growing season, or when the weeds are killed, their decomposition may exert heavy demands on the oxygen resources of a water." Novotny and Bendoricchio (1989) observe that "oxygen deficiency is highest and most troublesome in streams where shallow productive zones are followed be deeper sections". The latter statement largely corroborates what we observed, where senesced algae accumulated in the slower-flowing and deeper glides of the High Dose Reach.

The low DO we measured in fall 2010 was not continuous along the High Dose Reach. The entire bed of the High Dose Reach accumulated some senesced algae (per observation) starting in late September, but the extremely low DO concentrations recorded by the upstream YSI where not recorded by the downstream YSI at end of the same reach (**Figure 4-5**). There were 98 m between the upstream High Dose YSI and the downstream High Dose YSI, and stream geomorphology greatly altered the manifested DO patterns recorded at these two locations. The stream channel where the downstream High Dose YSI was located was much wider (18.8 m *vs.* 7.4 m) and slightly shallower (56 cm *vs.* 65 cm) than the upstream High Dose YSI locale. The upstream YSI location also had taller, more sheltering banks. We believe that wind mixing over the much larger surface area (and shallower depth) was responsible for maintaining higher DO at the downstream end of the High Dose Reach in late September 2010. Downstream from the terminus of the 200-m long High Dose Reach, areas of deep, narrow glides and pools again occurred along Box Elder Creek. We speculate that very low DO was manifested in these locations as well, leading to a longitudinal series of low DO zones (**Figure 5-1**) wherever geomorphic features allowed low DO to occur (i.e., in locations that were narrow, deeper, and with sheltering banks).

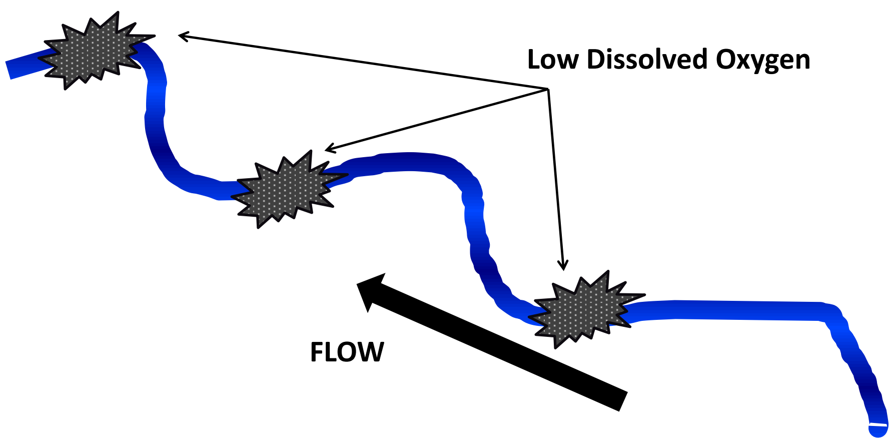


Figure 5-1. Longitudinal Zones of Low Dissolved Oxygen, as Suggested by Findings in the High Dose Reach.
The low DO zones are posited to result from senesced, decaying algae which accumulate where stream geomorphology results in narrow, deeper, and minimally-reaerated zones.

Although very low DO was measured by the upstream High Dose YSI in late September, it is unlikely that a uniform DO concentration existed from bottom to surface. The senesced, decaying algae manifested a strong DO demand which radiated *from* the stream bottom. As modeled by Suplee and Flynn (2014), DO was almost certainly zero on the bottom and near saturation at the surface, and declined linearly from surface to bottom[14]. As such, average DO at mid-depth was calculated to be 4.5 mg/L (also below Montana's water-quality standards for juvenile fish). Many macroinvertebrates and sessile organisms would not be able to take advantage of the better DO higher up in the water column, however, and were certainly killed by the near-zero DO concentrations on the streambed. The effect would also cause other macroinvertebrates to drift downstream to more suitable locations with higher DO concentrations.

[14] Conversely, the unusually high DO concentrations recorded in mid-September by this sonde (**Figure 4-5A**) were probably not uniform surface to bottom. Rather, the highest DO concentrations would be near the bottom where attached algae were photosynthesizing, and these concentrations would grade to near-saturation at the surface.

The senesced algae were observed to be laying loosely on the streambed in areas with low velocity. In the Suplee and Flynn memo (2014), the bottom-radiating DO demand generated by these decaying algae was referred to as “senesced algae oxygen demand”, to distinguish it from true sediment oxygen demand (SOD) which is associated with the DO consuming properties of organic material in a stream’s sediments (Edwards and Rolley 1965). Although not measured directly, we know that SOD was not a significant DO sink in Box Elder Creek, as evidenced by the near-saturation levels of water-column DO consistently maintained in the Control Reach throughout the study (**Figures 4-3A, 4-4A,** and **4-6A**).

Changes in pH were also observed as a result of nutrient additions. The most relevant observations were that (1) pH in the High Dose Reach arrived to but did not quite exceed Montana’s pH standard of 9.0 (**Figure 4.9A**), and (2) the highest pH values in the High Dose Reach were observed during the first 10 days of September 2010, a bit earlier than the corresponding DO peaks. Results from bullet 1 indicate that there is a demonstrable relationship between elevated nutrient concentrations and Montana’s pH standard; indeed, the pH standard was the key driver for establishing nutrient standards for the Yellowstone River using the QUAL2K water-quality model (Suplee et al. 2015). Note that Box Elder Creek has very high pH buffering capacity (400 mg/L as $CaCO_3$), and in a similarly-dosed stream with somewhat less buffering the standard would probably have been exceeded.

A concerning aspect of the pH data was related to QC problems associated with inexplicable directional drift and small (but problematic) differences in initial readings after deployment. In a well-buffered stream such as Box Elder Creek, diel pH changes will tend to be minimal and so small variations in instrument readings and behavior can diminish one’s ability to discern meaningful changes (i.e., the signal to noise ratio was low). Our results indicate that great care must be taken when measuring pH over extended periods if meaningful results are to be expected. YSI (2010) recommends that a pH probe’s millivolt output should be within ± 50 millivolts of the standard values associated with three pH calibration buffers (standard values are +180, 0, and -180 millivolts corresponding to pH buffers 4, 7, and 10, respectively). The company indicates that if a pH probe is near the outer bound of the allowable millivolt range (say, +48), the probe should probably not be used for extended deployment. We did not check this prior to our deployments, and some of our pH probes had been in service a number of years at the time of this study. It is possible that our results were affected by aging pH probes. We recommend that in future long-term deployments, pH probes be checked per YSI’s recommendations and replaced with new ones prior to deployment if they are close to their allowable millivolt limits.

Increased nutrients are expected, first and foremost, to influence the primary productivity of a stream (Odum 1956; Cole 1973). Accordingly, we monitored a number of different aspects of the benthic floral community. The effects of nutrient dosing on the benthic floral community of Box Elder Creek were highly consistent and in alignment with the level of dosing (see summary, **Table 5-1**). As a result of nutrient additions, benthic algae (both microalgae and filamentous) increased in areal coverage, thickness, and length, and depressed the growth of benthic macrophytes and moss. We also observed anecdotally that *Chara* sp. abundance was reduced during dosing. Others report similar findings in whole stream nutrient-addition studies. Perrin et al. (1987), Greenwood and Rosemond (2005), Sabater et al. (2005), and Veraart et al. (2008) all report significant increases in benthic algal standing crop as a result of their nutrient additions. Cole (1973) observes that *Chara* died out in his study stream as thick plant beds developed above the *Chara* and shaded them out, which is essentially the same phenomenon we observed in Box Elder Creek.

Table 5-1. Summary of Effects on Benthic Flora Resulting from Nutrient Additions.
Dashes indicate no significant result.

	Observed Effect Resulting From Nutrient Dosing	
Benthic Flora Measurement	**Low Dose**	**High Dose**
Benthic Algae Chl*a* Density (mg/m^2)	–	increase
Benthic Algae AFDM Density (g/m^2)	increase	increase
Streambed Cover by Filamentous Algae	increase	increase
Maximum Length of Filamentous Algae Filaments*	longer	longest
Thickness of Microagae Mats*	thicker	thickest
Streambed Cover by Macrophytes	–	decrease
Streambed Cover by Moss	–	decrease

*This measurement could not be evaluated using BACIP statistics but was, nonetheless, an unambiguous result.

In the present study, moss significantly decreased in abundance in the High Dose Reach due to the increased growth of benthic algae (which proliferated due to dosing). In contrast, Slavik et al. (2004) observed the gradual *domination* of their study stream (in arctic Alaska) by moss as a direct result of long-term nutrient additions. But it took eight years of nutrient dosing before this occurred in Slavik et al.'s study stream. Moss domination in a Montana prairie stream is unlikely, as we have never observed any other prairie stream dominated by moss. Nevertheless, it is possible that long-term nutrient additions in Box Elder Creek could result in gradual changes in the floral structure different from those we observed. If we were to have carried out our study long-term, and spring runoff events were generally large and caused scouring of the streambed each spring, we speculate that floral changes similar to what we observed would be the long-term response to increased nutrients. But if spring runoff events were more modest we speculate, per findings in Suplee (2004), that one would observe increased macrophyte (e.g., *Potamogeton pectinatus*) density and coverage, which would intermix with the filamentous algae and which, together, would further shade and inhibit microalgae and *Chara*.

We also noted that closely-attached microalgae mats and long streamers of filamentous algae occupied different ecological niches in Box Elder Creek. Long streamers of filamentous algae were only dominant in the riffles—where the highest water velocities were found—while the microalgae mats occupied the remaining, lower-velocity areas. This is consistent with Flynn (2014) who shows that *Cladophora* was most prevalent at higher velocities (0.68 m/s) and was absent at lower velocities (0.42 m/s) in the Clark Fork River. Freeman (1986) and Flinders and Hart (2009) also find that *Cladophora* biomass is consistently higher at higher water velocities.

Diatoms—being primary producers—were expected to respond to nutrient dosing, and this was in fact the case. But among those we evaluated there were relatively few taxa or composite metrics for which a reliable, consistent and expected response was observed in our study. *Navicula recens* and *Epithemia sorex* were the two diatom taxa that showed the clearest behaviors vis-à-vis nutrient dosing. We found no reference to *Navicula recens* in the whole-stream fertilization literature, however stream or multiple-stream studies correlating diatoms with nutrients show that *Navicula recens* is significantly associated with eutrophic conditions for both N and P (Van Dam et al. 1994; Potapova et al. 2003; Potapova and Charles 2007; Ponader et al. 2007). During non-dosing periods, *Navicula recens* ranged from 1-5% of sample counts, whereas during dosing it rose to 10-32% of the dosed reach samples. This behavior lends itself well to establishing thresholds for purposes of plains stream assessment; i.e., streams whose diatom samples have >10% *Navicula recens* could have excess nutrients.

Similar to the case for *Navicula recens*, we could not locate references to *Epithemia sorex* in the whole-stream fertilization literature either. *Epithemia sorex* is intolerant of elevated organic N, and is generally associated with fairly high oxygen saturation (Van Dam et al. 1994). Perhaps somewhat contradictorily, it is also found to commonly grow on *Cladophora* (Mpawenayo and Mathooko 2005; Charles and Christie 2011). Our results indicate that elevated nutrients (probably N) reduced the population size of *E. sorex* in accordance with the dosing level, even though *Cladophora* was significantly more prevalent in the dosed reaches (**Table 5-1**). Our results indicate that *E. sorex* is more impeded by increased N than it is encouraged by increased *Cladophora*. However, its relative abundance in samples was highly variable during non-dosing periods over the course of the study (**Figure 4-20**), which diminishes its usefulness as a general eutrophication assessment tool.

Regarding diatom population metrics, 'Rhopalodiales taxa percent' and the 'Polysaprobous taxa percent' of VanDam et al. (1994) were the two diatom metrics that showed consistent behavior across the Low and High Dose reaches—and in the expected direction. 'Rhopalodiales taxa percent' comprises six taxa which harbor nitrogen-fixing bacteria (*E. sorex* is one of the six) and so this metric corroborates the findings for *E. sorex*. Unfortunately, the metric showed such wide natural variability over the course of the study (**Figure 4-22**) it would be difficult, based on these results, to identify a "too low a percentage Rhopalodiales taxa" threshold that could be used for eutrophication assessment. In contrast, the 'Polysaprobous taxa percent' metric demonstrated fairly consistent proportions in samples during non-dosed periods (20-40%), but these taxa rose to 45-60% in samples collected during dosing. This behavior lends itself well to developing assessment thresholds. Although this metric is not currently included in DEQ's eutrophication assessment methodology, it was part of the Pollution Index which was used by DEQ in the past (Bahls 1993) and, further, this metric was identified as useful for assessing DO concentrations in Montana plains streams (Bahls et al. 2008). It may be time to reconsider including this metric in DEQ's assessment toolbox.

Finally, the 'High nitrogen taxa percent' showed a strong response in the High Dose Reach, reaching percentages far above levels observed in non-dosing periods (**Figure 4-25**). Potapova and Charles (2007) describe this metric as associated with N concentrations greater than 3.0 mg/L, which was certainly not the case here (Box Elder Creek's concentrations were much lower). Nevertheless, the High Dose Reach N concentration was high enough to trigger a response, and the metric is probably more sensitive to N than stated in the literature. This metric appears to have merit as an assessment tool for situations where stream N levels are fairly elevated.

The nutrient increaser taxa metric (Teply 2010a; Teply 2010b; Montana Department of Environmental Quality 2011a) showed a high degree of variability in the Control Reach (**Table 4-22**). In the Control Reach, which is a long-established plains reference site (Bahls et al. 1992; Suplee et al. 2005), one would expect consistently low probability of nutrient impairment. But individual sample results from the Control Reach ranged from 28% to 68% probability of impairment over the three years study. (Recall that >51% probability indicates nutrient impairment, based on current assessment methods.) This is disconcerting, given the structure of DEQ's assessment methodology (Suplee and Sada de Suplee 2011). At present, DEQ's assessment methodology requires only two diatom samples per assessment reach, and the result from each sample is considered on its own merits (results are not averaged). Thus, one sample, e.g. '62% probability of nutrient impairment', could tip the balance of an assessment. But our results show that the warm-water nutrient increaser metric can be quite variable over time even in a plains reference stream. Although the study-long average of 47% probability of impairment in the Control Reach indicates, on average, no exceedance of DEQ's assessment threshold, 33% of individual samples from the Control Reach *do* indicate impairment. Kelly et al. (2009) find that results from several diatom samples (as many as six), averaged, were necessary to properly characterize a stream's impairment status. Diatoms are fast-growing organisms and are sensitive to environmental change, but the diatom community is also inherently variable in its composition (Kelly et al. 2009). Our results bear this out. The implication of this is that the current DEQ assessment methodology, which makes decisions on just a few diatom samples, should be updated. Averaging same-site samples together will address this concern, but at the same time diatom samples are among our most expensive biometrics on a per-sample cost; requiring large numbers of replicates to be averaged for an assessment reach could become cost prohibitive. During the next major update to the nutrient assessment methodology, these competing concerns (desire for more accurate diatom assessment results *vs.* the cost to do so) should be carefully weighed in order to arrive at an optimum solution.

Macroinvertebrates are part of a stream's secondary productivity, i.e. comprising herbivores and predatory taxa. As such, the macroinvertebrates are at least one step removed from the nutrient additions (**Figure 1-1**); it was probably changes in Box Elder Creek's DO and pH patterns and food sources that mainly affected them. The present study showed a number of individual macroinvertebrate taxa and macroinvertebrate metrics responded significantly and in the expected direction. However, most of these showed high variability in magnitude in the Control Reach or in the other reaches during non-dosing periods, such that easily identifiable harm thresholds for assessment purposes could not be readily derived. One exception to this was found, the Plains MMI (Montana Department of Environmental Quality, Water Quality Planning Bureau 2006). This index is comprised of the following individual metrics: EPT taxa, % Tanypodinae, % Orthocladiinae of Chironomidae, Predator Taxa, and % Filterers and Collectors. Several of these sub-component metrics responded significantly to nutrient dosing in this study; some of them (% Tanypodinae, Predator Taxa) responded significantly but *backwards* from expected.

The reasonably-patterned behavior of the Montana Plains Index in this study (**Figure 4-30**) was driven most by the % Orthocladiinae of Chironomidae and the % Filterers and Collectors sub-component metrics, which—individually—showed strong (and expected) responses (**Table 4-24**; **Figure 4-29**; **Figure 4-32**). Although we could not test the Orthocladius metric via BACIP statistics, recall that our analyses indicated that *Orthocladius* sp. responded strongly (increased) in response to nutrient dosing in the High Dose Reach (**Figure 4-29**), similar to the pattern shown by the diatom metric 'High nitrogen taxa percent'. The other sub-component metrics likely moderated or muted the overall volatility of the Plains MMI. Others report that the Plains MMI has reasonable consistency and repeatability; impaired *vs.* unimpaired decisions (i.e., above or below the threshold of 37) differed between Montana samples in 18.3% of repeated-sample pairs (Stribling et al. 2008). Plains-streams only pairs, with a 15% difference, performed even better (Stribling et al. 2008). We note here that the O/E metric has a similar repeatability between repeated-sample pairs (19.5%) (Stribling et al. 2008), but in the present study the high variability of O/E scores *over time* in the Control Reach was an undesirable characteristic of the metric (**Figure 4-31**).

The correlation between nutrients and macroinvertebrates has been analyzed in Montana using broad-scale correlation analysis between extant nutrient concentrations and macroinvertebrate data from statewide streams (Tetra Tech 2010). In TetraTech's study, interestingly, the Plains MMI was not found to significantly correlate with nutrients in the plains region, a counter-intuitive finding given that the metric was developed to address (in part) this type of pollution in this region. It is also contrary to our controlled nutrient-addition study, where we found that the Plains MMI performed well. Other findings in TetraTech (2010) did align with ours, however. For example, the EPTnoHBPct metric (which should decrease as nutrients increase) decreased in this study, as it did in TetraTech's analysis.

Whole-stream fertilization studies which include macroinvertebrate analyses in prairie streams are very uncommon. We located one study (Ocon et al. 2013), carried out in the pampas grasslands of Argentina, using a BACI design. They dosed their stream with N and P for one year, continuously, therefore their study is more informative as to longer-term effects. Ocon et al. (2013) find that macroinvertebrates of the 'filtering collectors' type significantly increased in response to nutrients, as did we (**Table 4-24)**. They also find that predator taxa increased greatly in response to nutrient addition, though the increase was not significant (they only report that the BACI p-value was >0.05, but their graph shows a very evident response). Their findings regarding predator taxa are in line with what we expected *a priori* in our study (**Table 4-24**), whereas our actual findings were the reverse (predators decreased significantly due to dosing). It is quite possible that the significant decrease in macroinvertebrate predators in our study is an artifact of the study's timespan. It takes some time for the predator taxa to respond to their shifting food base; in Ocon et al. (2013) the predator taxa only showed marked increases after nearly 6 months of nutrient dosing.

Returning to the flora, there are varied definitions of eutrophication, but the following has particular relevance to findings in this study: "*The process by which a body of water acquires a high concentration of nutrients, especially phosphates and nitrates. These typically promote excessive growth of algae. As the algae die and decompose, high levels of organic matter and the decomposing organisms deplete the water of available oxygen, causing the death of other organisms, such as fish. Eutrophication is a natural, slow-aging process for a water body, but human activity greatly speeds up the process*"(Art, 1993). In the present study the addition of nutrients not only increased algal primary productivity and led to depletion of DO (seasonally) by decomposing flora, it apparently caused the flora to run through their seasonal cycle of growth, maturation and decay more quickly (**Figure 4-16**). The accelerated aging process induced by eutrophication is usually associated with lakes, but our results indicate that it demonstrably occurs in flowing waters as well, at least on an annual basis.

We based the study's soluble nutrient dosing concentrations on a Michaelis-Menten model and an estimate of the stream's overall nitrogen Ks (nutrient uptake half-saturation constant) from the literature. The Low Dose Reach dose was targeted to attain Ks, estimated to be 40 µg NO_3-N/L in Box Elder Creek, and this concentration was essentially achieved. The High Dose Reach dose was planned to be five times the Ks based on Chapra's (1997) rule of thumb that, at about five times Ks, inorganic soluble nutrients are saturated and further increases in nutrients will not further increase the uptake rate (Vmax); at that point nutrient uptake is kept in check by other finite resources (space for algae to grow, for example). It ended up that the High Dose Reach achieved <u>three</u> times the target Ks, and at that concentration (119 µg NO_3-N/L) harm was clearly shown in the present study (summary in **Table 5-2**)[15]. Chapra (1997) states that at five times Ks nutrients are saturated, but it is evident from his Michaelis-Menten curve (reproduced in **Figure 5-2**) that three times Ks is still a fairly saturated concentration (well past the part of the curve where growth rate is proportional to nutrient concentration). Tying these concepts together, the present study shows that soluble concentrations at the overall Ks of a stream will cause ecological changes but not necessarily harm, whereas concentrations three times higher than Ks will cause harm[16].

[15] And at the same time that this nitrate concentration was achieved, phosphorus was not limiting because we added it at the Redfield Ratio.

[16] This statement assumes that the K_s we drew from the prairie-stream literature was approximately correct for Box Elder Creek, because we did not measure K_s directly during our study.

Table 5-2. Summary of Harms to Beneficial Uses Documented in the Study, Resulting from Nutrient Dosing.

		Demonstrable Impact to Beneficial Use Resulting From Nutrient Dosing	
Measurement	**Linked Beneficial Use**	**Low Dose**	**High Dose**
Dissolved Oxygen Concentration	Fish and Aquatic Life	None	Impacted
pH	Fish and Aquatic Life	None	Borderline impact
Benthic Algal Density (Chl*a*)*	Fish and Aquatic Life, Recreation	None	Impacted
Benthic Algal Density (AFDM)*	Fish and Aquatic Life, Recreation	Impacted	Impacted
Diatom Nutrient-increaser Metric	Fish and Aquatic Life	None	Borderline impact
Macroinvertebrate Metric (Plains MMI)	Fish and Aquatic Life	None	Borderline impact

*These measurements and thresholds have more specificity to western MT streams, and the recreation use.

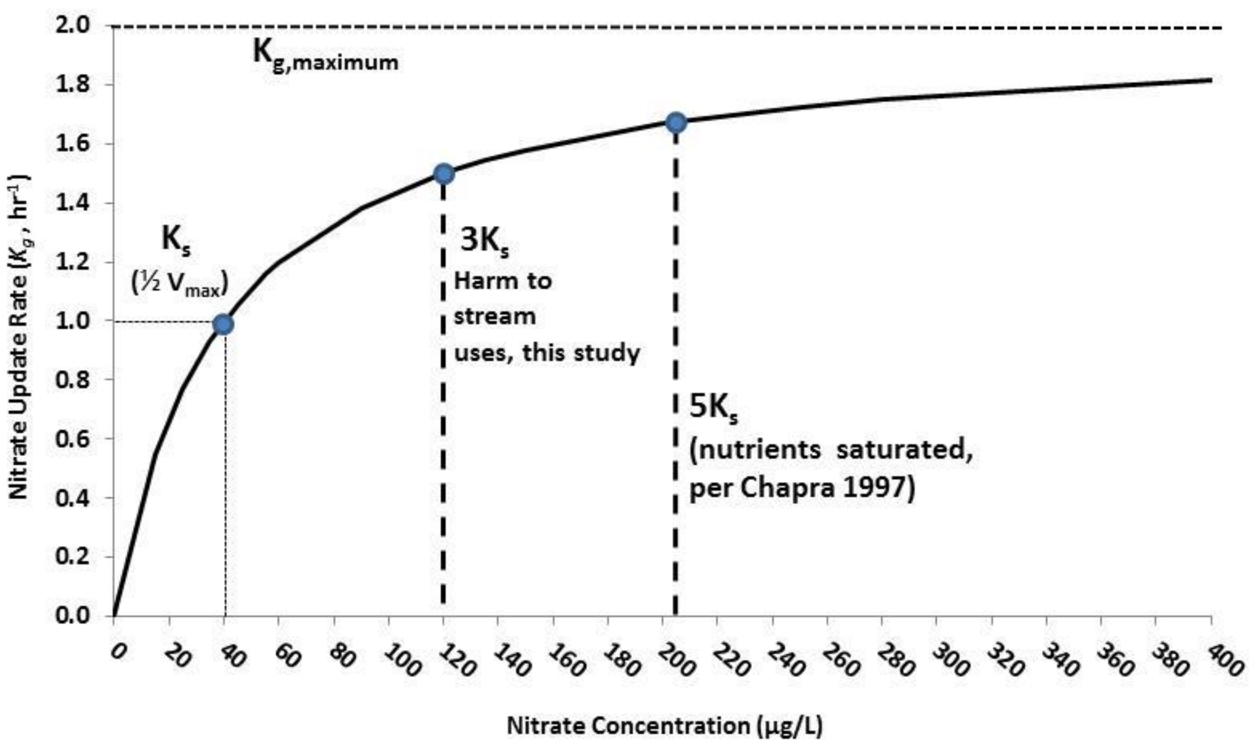


Figure 5-2. Michaelis-Menten Relationship between Nutrient Concentration (X-axis) and Nutrient Uptake Rate (Y-axis), from Figure 32.2 in Chapra (1997).

In the present study, harm to beneficial uses was estimated to have occur at three times Ks. We assume that our estimate of Ks (40 µg NO_3-N/L), drawn from the prairie stream (and other) literature, is reasonably accurate for Box Elder Creek.

These results can be used to back calculate a range of total nutrient concentration criteria that are appropriate for this particular stream. Soluble to total nutrient concentration ratios are highly variable, so whichever ratio we choose as the final conversion is an educated guess. In eastern Montana streams, very low soluble:total ratios have been recorded (Suplee 2004). In Box Elder Creek, the ambient soluble:total ratios were also low (**Table 4-8**), ranging from 0.02-0.04:1 (N) and 0.08-0.33:1 (P). A soluble:total ratio of 0.25:1 is common in the Clark Fork River (Suplee and Watson 2013). In the Missouri River, a soluble:total ratio for P was 0.15:1, and for N it was 0.45:1 (Suplee et al. 2014).

If ecological change in Box Elder Creek—but no harm—is shown at 39 µg NO_3-N/L and 4.4 µg SRP/L, and harm is shown at 119 µg NO_3-N/L and 16.0 µg SRP/L, then soluble criteria for this stream should be set somewhere between. Given the shape of the Michaelis-Menten relationship (**Figure 5-2)**, concentrations at about 1.5 (to perhaps as high as 2) times Ks are appropriate as criteria[17]. This equates to soluble criteria of 60-80 µg NO_3-N/L and 7-9 µg SRP/L for this reach of Box Elder Creek.

Converting these to total nutrient criteria using reasonable midpoints for the soluble:total ratios discussed above (i.e., 0.1:1 for N, P), potential total criteria would range from 600-800 µg TN/L and 70-90 µg TP/L. These criteria presume, at least for N, that most of the TN would be organic N, as is currently the case in Box Elder Creek. Suplee and Watson (2013) review several dose-response studies for this ecoregion, and state that total nutrient criteria could be selected within the range of 540-1,830 µg TN/L (a wider range than our calculations) and 70-150 µg TP/L (also a wider range). Candidate criteria concentrations for wadeable streams are found in the scientific literature, and range from 210-1700 µg TN/L and 10-90 µg TP/L (Suplee et al. 2015). Low ambient soluble:total ratios in prairie streams are undoubtedly part of why higher total nutrient standards (compared to western MT) are established in this region (see standards in Circular DEQ-12A); reference prairie streams have relatively high total nutrients but low soluble nutrients, and so soluble nutrients are still near concentrations which limit eutrophication. This implies that waste water treatment plant (WWTP) discharges whose permits are only established as total nutrient concentrations have the potential to impact prairie streams, because WWTP outfalls have considerably higher soluble:total ratios (i.e., more soluble nutrients per unit total nutrient) than the receiving streams. Indeed, Chapra et al. (2014) find that soluble nutrient concentrations from WWTPs must be kept at very low levels if water quality improvement is to be expected. Actual instream conditions downstream of WWTPs should be monitored as more nutrient permits are established in this region.

The Effects of Grazing. This nutrient-dosing study presented an opportunity to consider the effects of land use on water quality. The only land use of this state-owned, one-square mile parcel is cattle grazing. For the entire period over which we have been familiar with the site (since 2000), it has been used to graze cattle. Our study began in summer 2009 and if any grazing occurred in 2009, it must have been initiated after we finished our field work at the end of September (we never saw cattle that year). In 2010, cow/calf pairs were brought to the parcel the week of September 13th (our records), and were apparently kept there until about November 1st (Carter County NRCS office). In 2011 cow/calf pairs were brought in again, sometime in September (likely mid-September). The parcel is leased for an allowable 150 animal unit months (AUMs)[18]. We observed roughly 40-60 cow/calf pairs brought to the site in

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[17] Again, this assumes our original estimate of Ks for N for this prairie stream (40 µg/L) was reasonable.

[18] AUMs are standardized units which allow for calculation of available forage from a parcel of land and the amount of forage needed by particular animals over time. One AUM is the forage required by one animal unit for one month. A mature cow, for example, equals one animal unit.

2010. Cow/calf pairs in which the calf is older than four months are commonly assigned an AUM of 1.32 (Lacey, 1993; Pratt and Rasmussen, 2001). An AUM of 1.32 should be applicable to the pairs we saw, since calving usually starts no later than mid-April in Montana and so the calves would have been ≥ 4 month old when we saw them. We estimate that the cow/calf pairs observed in 2010 equate to [1.32 AUMs/pair X 60 pairs X 1.5 months] = 119 AUMs. Thus, our calculation indicates that in 2010 there were less AUMs occurring (119) than the lease allowed for (150).

We then calculated what the parcel's available grass forage was (as AUMs), based on the soil types and their relative areas within the state parcel, using a GIS (Natural Resources Conservation Service, 2003) and NRCS recommended methods (i.e., native rangeland AUMs, soil type coefficients for below-normal precipitation) which can be found at http://www.nrcs.usda.gov/wps/portal/nrcs/detailfull/mt/technical/landuse/pasture/?cid=nrcs144p2_057059 .

There were 21 soil types in the parcel, although most of the area comprised soils classified 7B, 75C, 83A, 83C, and 186C. Four of these (7B, 83A, 83C, and 186C) were types of sandy loams, while 75C was "Archin-Absher complex, 2-8% slopes" (NRCS website above). Based on GIS, the parcel produces enough forage to support 162.6 AUMs during below-normal precipitation, so the allowable grazing lease of 150 AUMs is appropriate, and slightly conservative (92% of the theoretically-available forage during dry years). If our estimate of 119 AUMs of actual grazing use in 2010/2011 is correct, then the parcel was being grazed at about 73% of the available forage for a dry year and 79% of that allowed by the lease.

In 2010 and 2011 we observed that the cow/calf pairs moved around a great deal, using the entire parcel which was fenced around its perimeter; they were seen grazing with approximately equal intensity along the banks of the Control, Low Dose, and High Dose reaches. During their presence, there was a notable reduction in the height of the upland grass, and near the stream the cattle substantially grazed down the sedges and rushes, trampled the banks somewhat, and left manure along the banks and in the stream. A review of project photos of the riparian areas showed that, by fall, riparian plant stubble was about 10 cm tall, with numerous areas remaining where greater plant height was still available (see 2011 photos in **Figure 4-17**). This level of riparian stubble height is generally considered appropriate for soil conservation purposes (Clary and Leininger 2000). Corroborating this, in 2009 an NRCS riparian assessment (Pick et al. 2004) carried out by DEQ and Carter County Conservation District staff (including a trained range botanist) concluded that the site had a sustainable rating.

In this study we have documented many water quality effects from the controlled addition of nutrients; however, we could not discern specific water quality effects due to the presence of livestock. Stream impacts in the western U.S. due to excess livestock grazing have been well documented, and parameters such as nutrient concentrations, TSS, bacteria, and BOD are commonly affected (Gary et al. 1983; Owens et al. 1989; Owens et al. 1997; Agouridis et al. 2005; Haan et al. 2006). We reviewed the temporal patterns of the nutrient concentration data in our study, and could not discern any pattern suggesting that nutrient concentrations (including ammonia) increased due to the arrival of the cattle. TSS did not show any discernable effect when the cattle were introduced in 2010 or 2011 (**Figure 5-3**). Nor could we assign definitive effects on BOD due to cattle (**Figure 5-4**). In 2010 BOD was generally variable, and increased sharply after the cattle were brought in; but BOD did not remain high after the arrival of the cow/calf pairs; in fact BOD was below detection on October 7th, 2010 in spite of stream flow being among the lowest measured values that year (**Table 4-4**). In 2011 BOD in the Control Reach increased notably after cattle arrived, but in the other two reaches (where cattle activity was equally common) there was minor or no change in BOD. Overall, the high variability in BOD makes it impossible to say with any certainty whether or not the cattle influenced stream BOD. Finally, note that in 2010 two of DEQ's primary biometrics used for assessing plains streams (nutrient-increaser diatom metric and macroinvertebrate-based Plains MMI; **Table 4-22**; **Figure 4-30**) show no indication that metric scores declined in a patterned way corresponding with the presence of cattle from 9/13/2010 onward.

Studies documenting strong, negative impacts from grazing on water quality (cited above) usually refer to continuous, year-round grazing. Seasonal (rotational) grazing is found to induce greatly reduced impacts on water quality. For example, Clary (1999) reports that light to medium grazing for short durations did not negatively impact an Idaho stream. Similarly, Saunders and Fausch (2009) find that sites managed for wildlife use only (no cattle) had similar amounts of terrestrial and adult aquatic invertebrate inputs (which provided food for fish) as did sites managed for rotational cattle grazing, whereas macroinvertebrates were reduced in sites grazed by cattle year-round. Our study supports the notion that rotational (seasonal) cattle grazing, when undertaken at an intensity somewhat lower than the site's maximum available dry-year forage, does not significantly impact a perennial prairie stream's water quality.

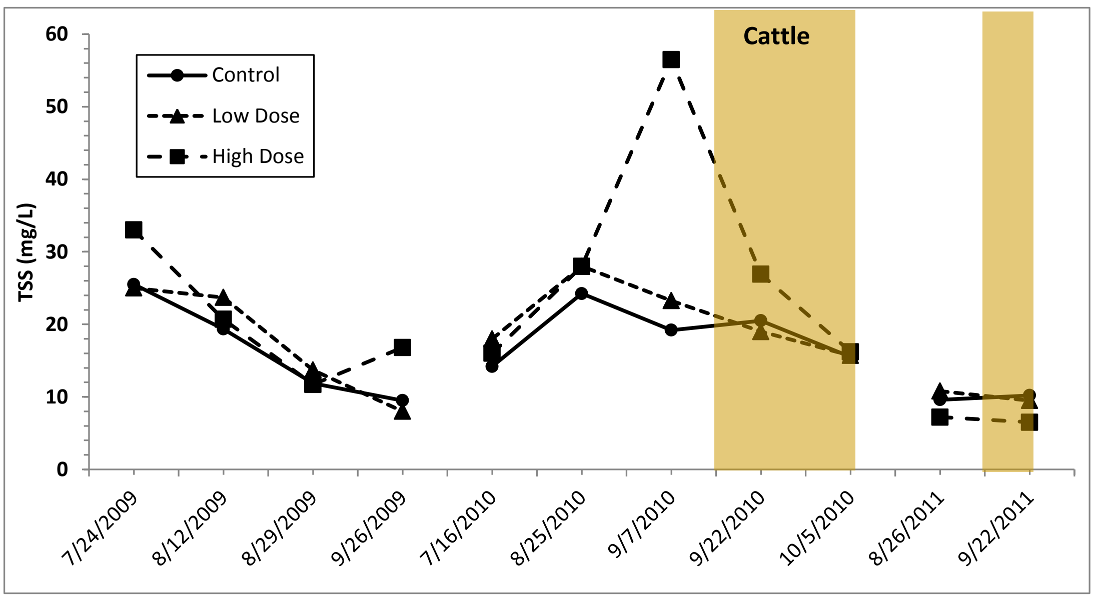

Figure 5-3. Stream Total Suspended Sediment (TSS) Concentrations During the Study.
The periods when cattle were present at the sites are shown by the tan colored regions. If cattle were brought to the site in 2009, it occurred after we finished our sampling in late September of that year.

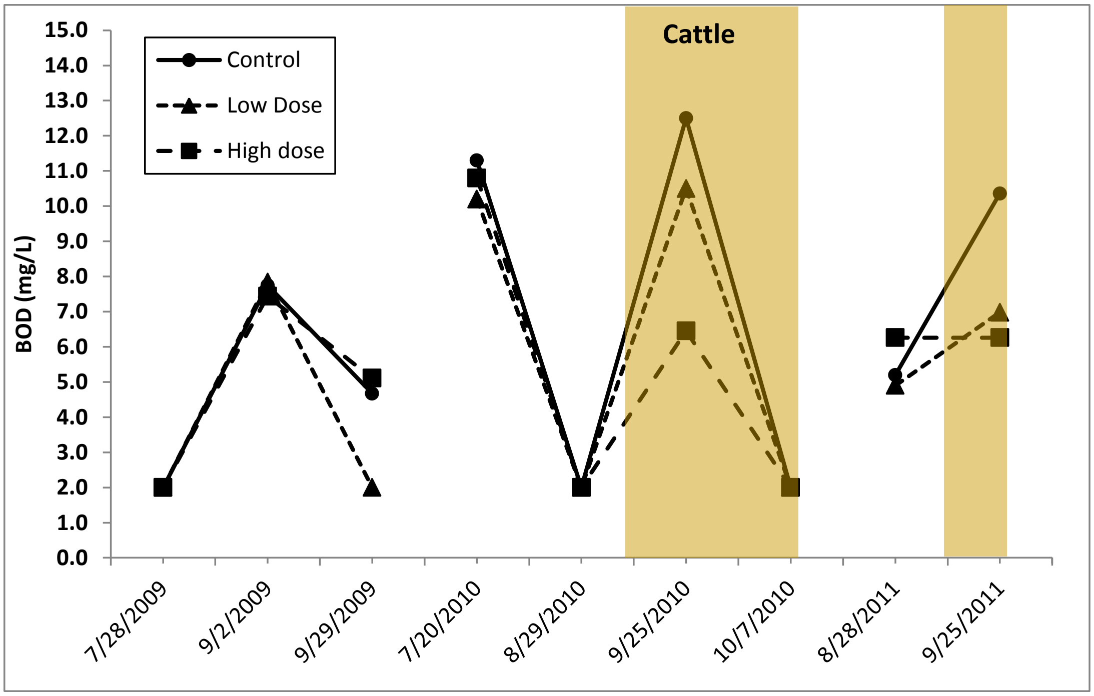

Figure 5-4. Stream Biochemical Oxygen Demand (BOD) During the Study.

The periods when cattle were present at the sites are shown by the tan colored regions. Note: BOD was not collected in August 2009, or on 9/7/2010. If cattle were brought to the site in 2009, it would have occurred after we finished our sampling in late September of that year.

6.0 Conclusions and Recommendations

Using a BACIP study design, harm to beneficial stream uses (aquatic-life, and to a lesser extent recreation) was clearly demonstrated when soluble nutrients were added to a reference prairie stream in southeastern Montana. In the High Dose Reach, growing season soluble nutrient concentrations were raised to 119 µg NO_3-N/L and 15.6 µg SRP/L (average Control Reach concentrations were 3.0 µg NO_3-N/L and 3.0 µg SRP/L) and harm to beneficial uses were observed. In the Low Dose Reach, where growing season soluble nutrient concentrations were only increased to 39 µg NO_3-N/L and 4.4 µg SRP/L, ecological changes were documented, but definitive harm to beneficial uses was largely absent (summary, **Table 6-1**). Impacts documented in the High Dose Reach included seasonal DO concentrations below state standards, pH increases bordering on exceeding standards, development of nuisance attached algae levels, and a decline in macroinvertebrate metric scores to the threshold DEQ has used to indicate impairment of that biotic community (**Table 6-1**). DO impacts in the High Dose Reach were seasonal, occurring in the early fall when large volumes of algae senesced and these decaying algae exerted a strong DO demand in the stream; the DO demand radiated from the stream bottom and was longitudinally patchy. Most of these findings were in alignment with our original predictions, outlined in **Section 2.1.**

Table 6-1. Summary of Harms to Beneficial Uses Documented in the Study, Resulting from Nutrient Dosing.

		Demonstrable Impact to Beneficial Use Resulting From Nutrient Dosing	
Measurement	**Linked Beneficial Use**	**Low Dose**	**High Dose**
Dissolved Oxygen Concentration	Fish and Aquatic Life	None	Impacted
pH	Fish and Aquatic Life	None	Borderline impact
Benthic Algal Density (Chl*a*)*	Fish and Aquatic Life, Recreation	None	Impacted
Benthic Algal Density (AFDM)*	Fish and Aquatic Life, Recreation	Impacted	Impacted
Diatom Nutrient-increaser Metric	Fish and Aquatic Life	None	Borderline impact
Macroinvertebrate Metric (Plains MMI)	Fish and Aquatic Life	None	Borderline impact

*These measurements and thresholds have more specificity to western MT streams, and the recreation use.

The Low Dose Reach received nutrient doses equivalent to the stream's estimated half-saturation constant (Ks) for N and P, and major ecological changes—but little demonstrable harm—were documented. But as seen in **Figure 4-17**, some parts of the Low Dose Reach developed a great deal of algae which could easily be characterized as nuisance, per Suplee et al. (2009). DEQ does not make impairment decisions based on algae from a single stone, but rather, on 11 samples (averaged) from a length of stream that is usually 200-300 m long (Montana Department of Environmental Quality 2011b). *On average*, the Low Dose Reach had chlorophyll *a* levels below nuisance. But a lack of definitive harm in the Low Dose Reach should not diminish the fact that the added nutrients greatly changed the character and aesthetic appearance of the Low Dose Reach. If not harmed, it was certainly degraded.

The High Dose Reach received N and P doses equal to three times the estimated Ks, and impacts to stream uses occurred. Based on these findings for soluble nutrients, we estimate that concentrations on the order of 1.5 times a stream's overall Ks are appropriate as protective criteria; this finding is in line with those of Chapra et al. (2014). In Box Elder Creek, 1.5 times Ks equates to 60 µg NO_3-N/L and 7 µg SRP/L. This stream, being located in the Northwestern Great Plains, has adopted *total* nutrient standards of 1,300 µg TN/L and 150 µg TP/L (see Circular DEQ-12A). Based on the range of potential total nutrient criteria derived for Box Elder Creek in this study, we conclude that the stream's currently-adopted TN and TP may be too high, and this reach of Box Elder Creek would be better suited with a more stringent standards, on the order of 600 µg TN/L and 70 µg TP/L. To remain protective, it is presumed that the bulk of the nutrients within these totals would be organic in nature, not soluble nutrients such as nitrate or orthophosphate.

The introduction of cattle (cow/calf pairs) into the study site each fall had no demonstrable impact on the stream's water quality. Much more deleterious effects, by far, were induced by our additions of N and P, particularly at the levels established in the High Dose Reach. During the study, we estimated that the parcel was grazed at 73% of the available dry-year forage and 79% of that allowed by the grazing lease. It was grazed seasonally for only a couple of months each fall. This type and intensity of grazing practice appears to allow for long-term sustainability of the water quality in this perennial prairie stream.

Based on the findings of this study, we here provide several recommendations which should inform future DEQ monitoring and assessment work in eastern MT plains streams (and beyond). For pH, pre-deployment calibration of YSI sondes should include an evaluation of the remaining life of the pH probes (following manufacturer's methods). Changes in pH, even when affected by strong eutrophication effects, can be fairly small, and can result in low signal-to-noise ratios; this can best be addressed by careful pre-deployment calibration and testing of the probes.

We found that the Aquatic Plant Visual Assessment Form (Montana Department of Environmental Quality 2011b) was quite effective for documenting significant changes to the stream which resulted from nutrient dosing. Although these aquatic plant visual assessment forms have been used by DEQ since 2010 (and earlier versions of them date back much before this), this is the first systematic testing of their data as far as we know. We recommend they continue to be used for assessing streams. They produced useful, semi-quantitative data, data which could be used to discern statistically significant differences in stream condition, at very low cost. A similar conclusion was reached during a recent workshop hosted by EPA (U.S.Environmental Protection Agency 2014) and in the United Kingdom (Kelly et al. 2016). We also found good consistency and reproducibility in DEQ's quantitative benthic algal biomass methods (chlorophyll *a* and AFDM), and the changes we recorded using these methods were concordant with dosing levels (less so for AFDM). These methods have long been used by DEQ and their continued use is recommended.

DEQ currently uses the warm-water nutrient increaser diatom taxa metric (Montana Department of Environmental Quality 2011a) to assess wadeable prairie streams in eastern Montana. This study showed that metric scores from different sampling events from the same site can be, even in a reference stream, quite variable over time. Over short time periods (weeks), metric scores fluctuated above and below DEQ's impairment threshold, whereas averaging results from a site's sampling events resulted in a metric score in line with what we expected from this reference stream. Thus, this study indicates that it is advisable to collect more than one diatom periphyton sample from a site and average the increaser taxa results. Others have found that as many as six diatom replicates at a site may be needed (Kelly et al. 2009), but unfortunately diatom samples are relatively expensive to process. During the next update of the nutrient assessment methodology (Suplee and Sada de Suplee 2011), these competing issues (need for more accurate diatom assessment results *vs.* the cost to do so) should be carefully weighed in order to arrive at the best solution.

The present study suggests that other diatom metrics besides the warm-water increaser taxa can (and probably should) be incorporated into the plains streams eutrophication assessment methods. The percent relative abundance of *Navicula recens* (**Figure 4-19**) may be a good addition to the suite of tools one uses to assess prairie streams. Proportions of this organism in samples were fairly constant over the three years, except during periods when the dosing treatments were in place. The organism responded concordantly with the dosing levels. The Polysaprobous and High Nitrogen Taxa metrics (**Figures 4-23, 4-25**) also look useful, as they are relatively stable over time and change concordantly with the dosing levels. The High Nitrogen Taxa metric appears to be best suited to detect high levels of N, as it was designed to do.

Two macroinvetebrate metrics stand out as having good potential for eutrophication assessment of plains streams. The Plains MMI (**Figure 4-30**) manifested the characteristics one would look for; it was fairly stable over time except when dosing occurred, at which point metric scores declined significantly and concordantly with dosing, and in the case of the High Dose Reach scores declined to DEQ's impact threshold (score of 37). Also of value is the proportion of *Orthocladius* sp. in a sample, which increased markedly during dosing. These organisms are also sub-components of the Plains MMI which may explain, in part, why that multi-metric index worked well. We recommend these metrics be incorporated into future eutrophication assessment methods for Montana prairie streams.

Lastly, it is worth pointing out that the results from this study are most meaningful for this particular segment of Box Elder Creek, or regional prairie streams which are most like it: gravel bottomed, perennial, fairly low turbidity, with limited riparian canopy. As the recommendations we provide here are carried to other prairie streams with different characteristics (e.g., ones that are intermittent, more turbid, or have muddier substrates), it will undoubtedly be found that some of the biometrics do not behave as they did in Box Elder Creek. The need to continue to study the most diverse and complex streams in Montana—the prairie streams—will continue.

7.0 ACKNOWLEDGEMENTS

We would like to thank the Board of the Carter Country Conservation District for their willingness to be involved with this work. Without their cooperation it would not have been completed. Similarly, we appreciate the cooperation of Clayton Jardee (Carter County rancher and leasee of the state-owned study parcel). The following DEQ staff contributed significantly to the completion of this study: Steve Fernandes, Katie Makarowski, Paul Kusnierz, Al Nixon, and Chace Bell. We would also like to acknowledge the contributions of Wayne Yost and Rebecca Wolenetz (Ekalaka NRCS Field Office). Thanks also to Tina Laidlaw (U.S. EPA) for providing helpful comments via her peer review of the draft of this document.

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Appendix A

BOX ELDER CREEK NUTRIENT ADDITION STUDY: A PROJECT TO PROVIDE KEY INFORMATION FOR THE DEVELOPMENT OF NUTRIENT CRITERIA IN MONTANA PRAIRIE STREAMS

Quality Assurance Project Plan (QAPP)

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Mindy McCarthy (QA Officer)	Date

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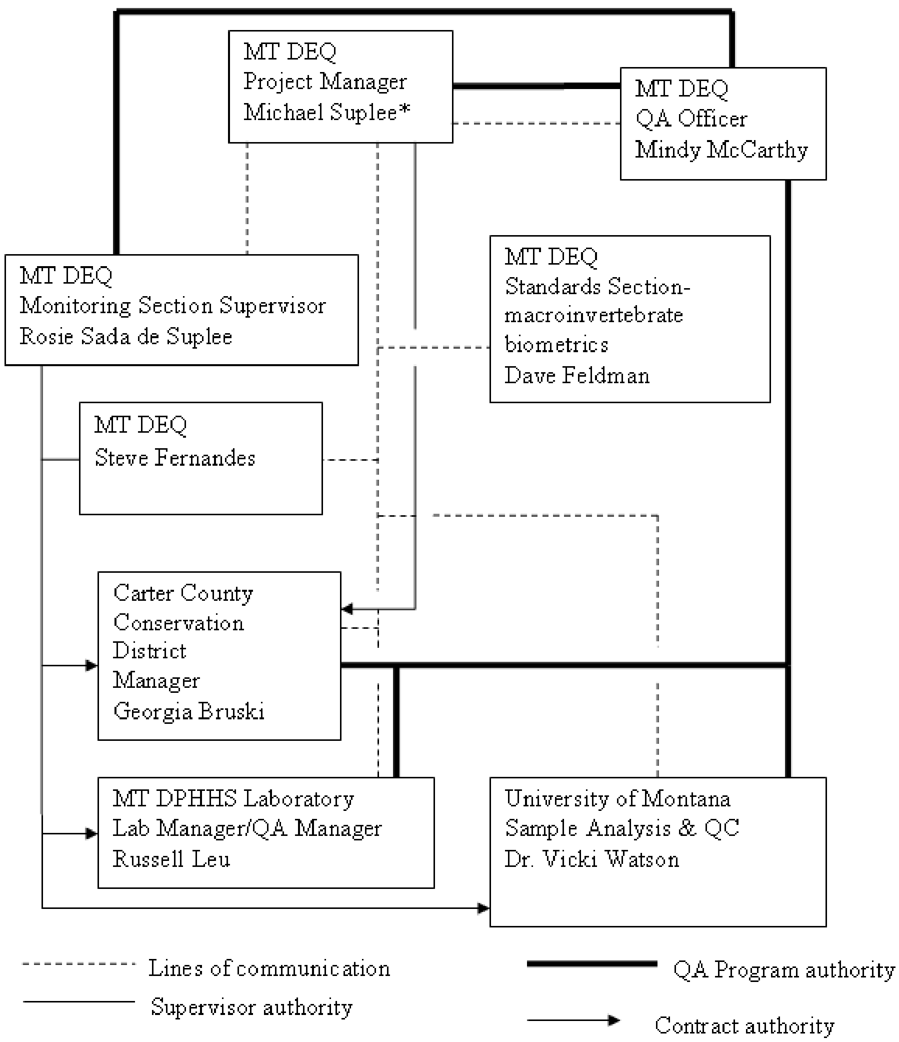
1.0 Project/Task Organization

This quality assurance project plan (QAPP) addresses the collection and analysis of data from Box Elder Creek, which will receive medium-term nutrient (nitrogen and phosphorus) additions in summer/fall 2010. This work is being undertaken for the purpose of collecting critical information needed for developing nutrient criteria for eastern MT prairie streams. Most field data collection will be done by staff of the Montana Department of Environmental Quality (DEQ). However, the Carter County Conservation District will also play a key role in day-to-day maintenance of the study and data collection. Sample analyses will be undertaken by the University of Montana and the Montana Department of Public Health and Human Services Environmental Laboratory. Michael Suplee, Ph.D., will provide overall project oversight. The following chart shows he roles of the various entities and their relationship to one another.



* In the field, Suplee will have general management authority for sampling decisions affecting the crew.

2.0 Introduction

2.1 Background

In Montana, designated beneficial uses of state surface waters include growth and propagation of fish and associated aquatic life, drinking water, agriculture, industrial supply, and recreation (ARM 17.30.621 through 629). The over enrichment of waterbodies by nutrients (usually nitrogen [N] and phosphorus [P]) can result in undesirable water-quality changes that can impair these beneficial uses (Freeman 1986; Arruda and Fromm 1989; Welch 1992; Dodds *et al.* 1997). Since 2001, DEQ has been working to develop numeric nutrient criteria for all state surface waters. The intent of these criteria is to protect waterbodies and their associated beneficial uses from the adverse effects of nutrient over-enrichment (i.e., eutrophication). DEQ has made good progress in nutrient criteria development for wadeable streams and small rivers in the mountainous regions of the state, and is nearly ready to recommend criteria for a large river segment (lower Yellowstone River). However, nutrient criteria development for wadeable prairie streams is not as advanced. Prairie streams, as a group, have been much less studied than gravel-bottom trout streams (Mathews 1988; Dodds 1997), and DEQ currently has only one study (Suplee 2004; and the related Appendix A in Suplee *et al*. 2008) which was carried out in prairie streams specifically for the purpose of developing nutrient criteria. Herein, we propose a dose-response study in a prairie stream that is certain to yield information critical for the development of scientifically-defensible nutrient criteria for these stream types.

2.2 Problem Definition

Stressor-response studies examine the relationship between a variable that has the potential to cause a water quality problem (stressor) and the specific effect that it manifests (response). Here, the stressors of interest are nutrients and the responses are the measurable impacts, i.e. harm, to a stream or river's beneficial use(s). When it comes to developing stream and river numeric nutrient criteria, some of the most useful studies have been those carried out in the field (i.e., not exclusively in laboratories). This is because the way nutrients manifest their effects in streams changes in response to regional environmental factors, and these factors are not easily replicated in the laboratory. Nutrient stressor-response studies carried out in the field vary by the degree of control the researcher has over the study, and can be broadly categorized (from most to least controlled) as: artificial stream studies, whole-stream fertilization experiments, and observational experiments. Observational experiments or studies are those in which the researcher seeks to define a quantitative relationship between an ecological response variable (e.g., daily dissolved oxygen minima) and a gradient of an environmental condition (e.g., total N), but does not involve any direct manipulation of the streams under study.

All three of these study types have been available in the scientific literature as DEQ has worked on nutrient criteria for gravel-bottom salmonid streams (e.g., Perrin *et al.* 1987;

Watson *et al.* 1990; Dodds *et al.* 1997; Mebane 2010). Together, these studies support and corroborate one another. DEQ also has, for gravel-bottom salmonid streams, a good understanding of what constitutes a nuisance algae condition to the public (Suplee *et al.* 2009). This knowledge lends itself to the establishment of a harm-to-use threshold.

In contrast, DEQ currently has only one stressor-response study (observational) in the prairies, a study that relates nutrient concentrations to undesirable impacts on dissolved oxygen (DO) concentrations (see Appendix A; Suplee *et al.* 2008). In that study it was observed that DO minima fell below regional dissolved oxygen standards when total nitrogen (TN) concentrations became elevated. Several aspects of the study give DEQ good confidence in it (e.g., the reference sites all had low TN concentrations, and the correlation between TN and DO was significant). However, the correlation coefficient was (as for many observational studies) fairly low, and DO was inferred by the type of diatom taxa collected from the streams as opposed to being measured directly by instrument. Nutrient work has been carried out in a prairie stream in Kansas (e.g., Kemp and Dodds 2002; O'Brian and Dodds 2008), but the studies are very short term (nutrient additions of hours) and did not measure the types of parameters (e.g., DO) that readily lend themselves to harm-to-use evaluation and criteria setting. DEQ would prefer to have another stressor-response study in regional prairie streams to corroborate the earlier findings. Therefore: the intent of the project described in this QAPP is to undertake another stressor-response study in prairie streams but this time, in lieu of an observational study, we intend to carry out a controlled whole-stream fertilization study. A whole-stream fertilization study should provide the most experimental control with the most environmental realism. To our knowledge, no summer-long whole-stream nutrient addition study has ever been carried out in a prairie stream.

We have selected for this project a reach of Box Elder Creek near Mill Iron, MT, which is currently considered a reference prairie stream site by DEQ (Suplee *et al.* 2005).

3.0 Project/Task Description

3.1 Primary Question, Objectives

The project outlined in this QAPP is designed to answer the following questions, *given that* the current base condition of Box Elder Creek is in reference condition:

1. If nitrogen and phosphorus are added to the stream during the growing season (July-Sept) in order to bring the concentrations up to levels DEQ currently believes are appropriate as regional nutrient criteria, what will be the affect on the stream's dissolved oxygen and pH patterns, benthic and phytoplankton algal density and composition, and macroinvertebrate density and composition?

2. If nitrogen and phosphorus are added to the stream during the growing season (July-Sept) in order to bring the concentrations to levels somewhat beyond what DEQ believes are appropriate as regional nutrient criteria, what will be the affect on the dissolved *oxygen and pH patterns, benthic and phytoplankton algal density and composition, and macroinvertebrate density and composition?*

Response parameters in 1 and 2 above were specifically mentioned because it is very likely that changed nutrient concentrations will affect them, and there are existing standards (pH, dissolved oxygen) that can be used to set the endpoints for nutrient control. Others, such as changes in macroinvertebrate populations, may lend themselves to future biological assessment tools or standards.

Based on earlier work (Suplee 2004; Suplee *et al.* 2008) DEQ has a fairly good idea what total nitrogen concentration criteria for these types of streams ought to look like. That work also showed nitrate to be very important in these streams, however the exact nitrate loading rate that these streams can tolerate is not clear. We have bracketed some nitrate tolerance estimates (more on this, Section 3.2.3), and these estimates form the basis of the two dosing rates that will be applied. Between the two dosing rates, DEQ should be able to derive reasonably accurate nutrient criteria for wadeable prairie streams.

Three other objectives will also be addressed with this study. First, DEQ is working on a "nutrient-increaser" diatom taxa biometric; it is currently being refined using the same methods used to develop it (Teply and Bahls 2005). The biometric is comprised of specific diatom taxa that respond in a predictable way to nutrients in prairie streams. The present study can help to independently verify that the 11 diatoms on the nutrient-increaser list do in fact increase in the presence of quantified increases in nutrients. Second, we can compare the nutrient criteria concentrations derived from this study to nutrient concentrations found in other regional reference prairie streams. If the pattern already observed holds, i.e. harm-to-use nutrient concentrations equate to concentrations in the upper percentiles (e.g., 85^{th}, 90^{th}) of reference stream data, it would lend further support to the stressor-response/reference data comparison approach presented in Suplee *et al.* (2007). Third, the study will help DEQ better understand how macroinvertebrate communities react to nutrient enrichment in prairie streams. Prairie streams have long been problematic for the development of reliable macroinvertebrate biometrics. This study can inform future macroinvertebrate biometric development for these stream types.

In addition, we are collecting enough data that we may be able to carry out an independent test of the QUAL2K water quality model (Chapra and Pelletier 2003). DEQ is currently using this EPA-supported model to derive nutrient criteria for the Yellowstone River, and will likely use it again on other streams and rivers for TMDLs, etc. In theory, the model should be able to accurately simulate the affects nutrient additions have on dissolved oxygen, pH, and algal growth in this stream; since DEQ will have actually measured these effects, the study can act as an independent cross-check of the model's capabilities. This will be very helpful to both DEQ and others who are using this model.

3.2 Project Design

This section presents the outline of the study design and parameters to be measured.

3.2.1 Study Location, BACIP Design

The study is to be carried out on a reach of Box Elder Creek east of Mill Iron, MT (Fig. 3.1; latitude -104.1387, longitude 45.8458). The stream site was selected because it is a perennial 5th order stream on state-owned land which has been known as a reference site since 1992 (Bahls *et al*. 1992; Suplee *et al.* 2005). (More details on selection rationale are found in SAP [2009].) Summer baseflow is about 2.5 CFS (2009 data). From headwaters to the site, the stream is wholly contained within ecoregions with prairie-like characteristics (i.e., it does not have mountain influences on its water-quality as does, for example, the Yellowstone River). Base water chemistry shows it is sodium-sulfate dominated with a strong buffering capacity (total alkalinity about 400 mg/L as $CaCO_3$); its overall chemical characteristics are similar to the Little Powder River. DEQ has been working with the Carter County Conservation District on water quality issues in their region for some years, and the Conservation District is a partner with DEQ on this project (more on this later).

The basic study design is a Before After Control Impact Paired (BACIP) study (Stewart-Oaten *et al*. 1986; Smith 2002). BACIP designs are employed when the ability to have treatment replicates (e.g., 5 prairie streams that receive nutrient dosing, and 5 different prairie reference streams that are untreated and serve as controls) is not feasible. The design involves comparing measured stream characteristics before and after an impact in a single stream, in this case the impact being nutrient addition. To account for problems of natural change, a stream reach that will receive no impact is paired with one or more reaches that will. In this study, there is one no-impact reach (Control reach), and two impact reaches; a Low-Dose reach and a High-Dose reach (Fig 3.2). The Control reach is the most upstream, followed immediately downstream by the Low-dose reach. The High-Dose reach is about 900 m downstream from the terminus of the Low-Dose reach. The statistical design and testing will follow Stewart-Oaten *et al.* (1986) wherein, for any given parameter (e.g., daily dissolved oxygen minima), the mean of the differences (**D**) between the Control and the Low-Dose reach in the before period will be compared, via Student's T-test, to the mean of the Ds for the same parameter in the after period. The same will be done for the High-Dose reach.

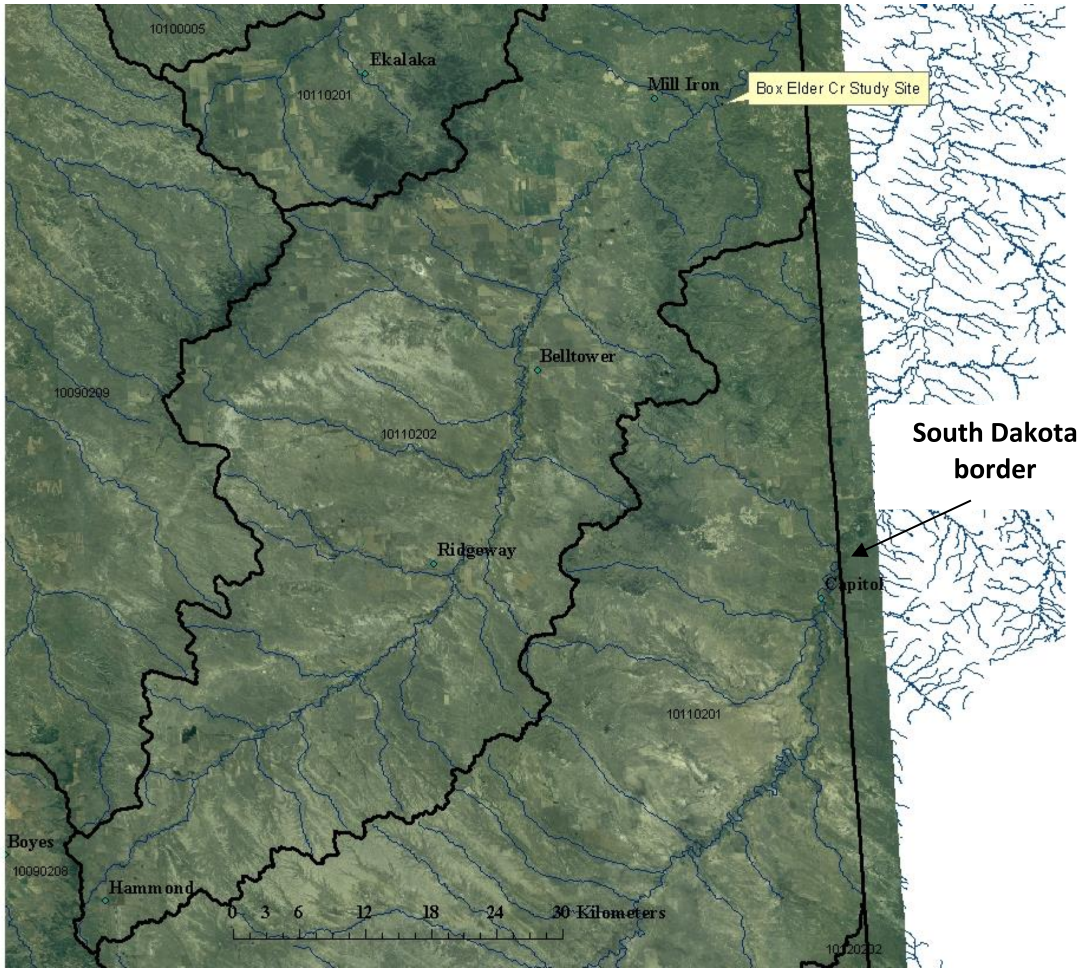


Figure 3.1. Map of southeastern corner of Montana, showing Box Elder Creek, its watershed, and the study site. The stream is wholly contained within HUC 10110202.

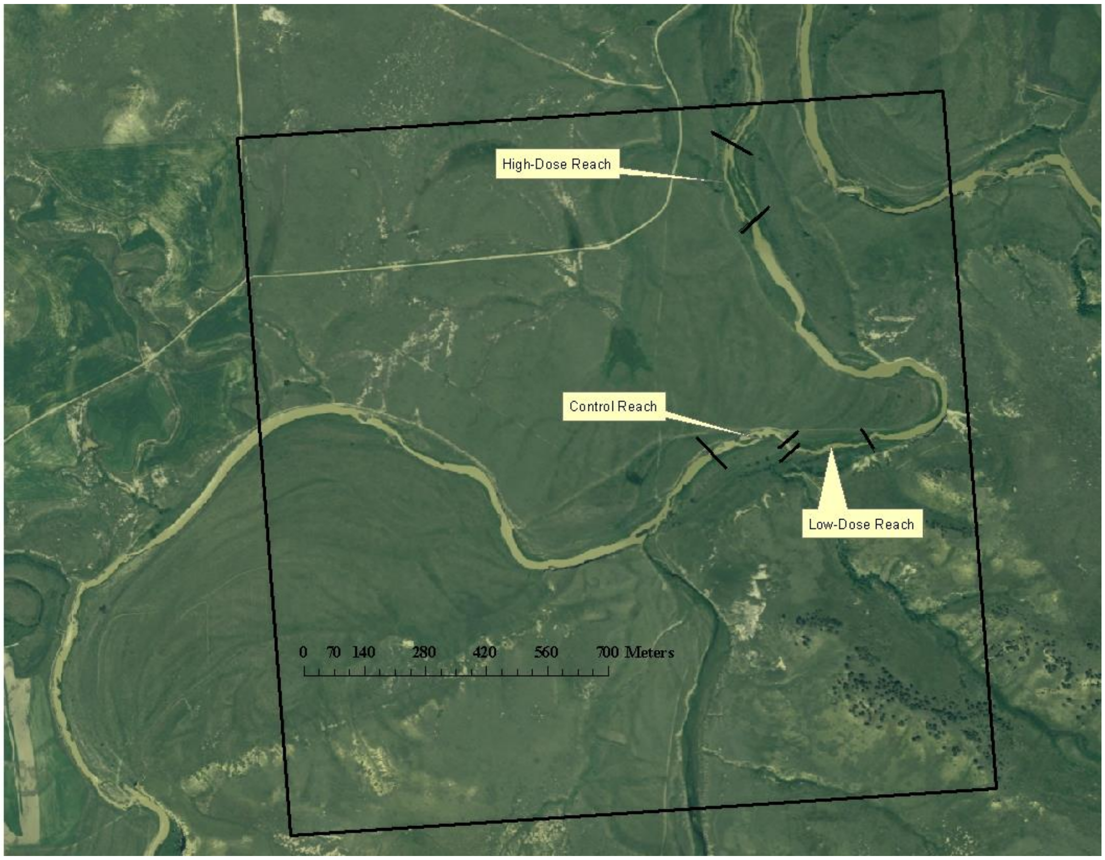


Figure 3.2. Box Elder Creek study site. Stream flows from left to right. The black outlined box is the boundary of the state-owned parcel. Start- and end-points of the Control, Low-Dose, and High-Dose reaches are shown by black lines perpendicular to stream flow.

The results might look something like Table 3.1 on the following page, which is fictitious data for dissolved oxygen (DO) presented purely to illustrate the concept. Note in Table 3.1 that it is the calculated values of **D** that are used in the T-test (before *vs.* after), not the original values measured in the Low- and High-Dose reaches. It is by this mechanism that natural changes in DO (or any parameter) that occur in the impacted reaches are separated from experimentally induced changes; the Control reach acts as a co-variate, and it is assumed that DO in the experimental reaches would have looked like DO in the Control reach if the experimental reaches had not received their respective perturbations. In the Table 3.1 example, there is no statistical difference in **D** at the Low-Dose reach between the before and after periods, whereas the difference in the before and after periods at the High-Dose reach is highly significant ($p << 0.001$). Actual DO data collected in 2009 shows that DO patterns in the three reaches are nearly identical. Thus, the expected high level of homogeneity among the reaches in the before period has been observed, and supports continued use of the same three reaches going forward.

Table 3.1 Illustration of the BACIP design concept. Fictitious dissolved oxygen concentrations in the Control, Low- and High-Dose reaches are shown in the before and after impact periods. T-test is carried out on the differences (**D**).

		Daily Dissolved Oxygen Minima (mg/L)				Calculated Difference (**D**)	
PERIOD	Sampling Event	Control	Low Dose	High Dose		Difference (D): Control - Low Dose	Difference (D): Control - High Dose
BEFORE	1st	6.0	6.0	6.0		0.0	0.0
	2nd	5.5	5.3	5.5		0.2	0.0
	3rd	6.0	5.9	6.1		0.1	-0.1
	4th	7.0	7.0	7.1		0.0	-0.1
	5th	6.5	6.4	6.5		0.1	0.0
					Mean:	**0.1**	**0.0**
AFTER	1st	6.0	5.9	5.4		0.1	0.6
	2nd	6.0	6.0	5.0		0.0	1.0
	3rd	7.0	6.8	6.2		0.2	0.8
	4th	7.3	7.2	6.4		0.1	0.9
	5th	6.0	6.0	5.0		0.0	1.0
					Mean:	**0.1**	**0.9**

3.2.2 2009 Reach Layouts and Preliminary Data Collection ('Before' Data)

The BACIP design anticipates that the control and impact reaches are in fairly close proximity so that they experience the same weather conditions, and that they be as physically similar (geomorphometry, hydraulics) as possible so that various natural influences manifest in each reach about the same way. In 2009 DEQ laid out three similar reaches along Box Elder Creek, and collected much of the 'before' data (SAP 2009) that will be compare against the 2010 'after' data. The three reaches were benchmarked and have reach midpoints of: Control (-104.1407, 45.8460); Low-Dose (-104.1387, 45.8458); and High-Dose (-104.1414, 45.8514).

Stream nutrient spiraling calculations (Newbold *et al.* 1981; Newbold *et al.* 1982; Mulholland *et al.* 2002; Ensign and Doyle 2006; Kohler *et al.* 2008) were used to estimate the stream length necessary for the added nutrients to have time to be taken up by biota within each experimental reach. These calculated to approximately 200 m. The need to balance inherent stream characteristics against the realities of the study area led to a Control reach length of 150 m, a Low-Dose reach of 200 m, and a High-Dose reach of 200 m. We selected three reaches within the state parcel that had similar proportions of pools, riffles, runs, and flow. The Control reach has (at the data-collection transects) 18% riffle, 18% pool, and 64% run. The Low-Dose and High-Dose reaches each have 18% riffle, 9% pool, and 73% run. Runs tend to dominate in many prairie streams due to their low-gradient nature. We slightly modified the 11-transect method for biological collections (see DEQ Chl*a* and macroinvertebrate SOPs) in that we placed the 11 transects within the pre-defined reaches (150 or 200 m) rather than have them be dictated as 40 times the mean wetted width of the reach, per Lazorchak *et al.* (1998). Periphyton samples, benthic Chl*a*, and macroinvertebrates were collected at transects. Also measured was dissolved oxygen, nutrients, BOD_5, etc. In 2009, YSI 6600 sondes were deployed about midreach of each reach, in a run of sufficient depth (1 m) to assure the instruments would remain submerged as flows decreased. Table 3.2 provides an inventory of data collected in 2009.

Table 3.2. Inventory of data collected in 2009 in Box Elder Creek as part of the before dataset.

BACIP Period	Month & Days	Reach	Macro-invertebrate samples (composite)	Peri-1 mod samples (composite)	Benthic Chl *a* (No. replicates)	Phytoplankton Chl*a*	Continuous Monitoring (DO, pH, temperature, SC, turbidity)	Nutrients (N & P), TSS, TDS, common ions	BOD_5	Visual Assessment of Bottom Algae Cover	Weather Station (air temp, wind speed/direction, solar radiation, relative humidity)
Before	July 23-26	Control	2	1	11	2	24/7, 15 min time-step	1	1	1	24/7, 15 min time-step
Before	July 23-26	Low Dose	1	1	11	2		1	1	1	
Before	July 23-26	High Dose	1	1	11	2		1	1	1	
Before	August 11-13	Control	1	1	11	2		1		1	
Before	August 11-13	Low Dose	1	1	11	2		1		1	
Before	August 11-13	High Dose	1	1	11	2		1		1	
Before	August 24-29	Control	1	1	11	2		1	1	1	
Before	August 24-29	Low Dose	1	1	11	2		1	1	1	
Before	August 24-29	High Dose	1	1	11	2		1	1	1	
Before	September 24-30	Control	1	1	22	2		1	1	1	
Before	September 24-30	Low Dose	2	1	11	2		1	1	1	
Before	September 24-30	High Dose	1	1	11	2		1	1	1	

The reach layouts and basic data collection will be carried forward into 2010 so that the data are balanced and comparable. In addition, in 2010, the boundary conditions of the High-Dose reach will be continuously monitored for DO, pH, temperature, SC, turbidity, and phytoplankton Chl*a* (via YSI sonde), and periodically sampled for nutrient concentrations and phytoplankton Chl*a*, to ascertain the influence (if any) of the Low-Dose nutrient additions on the High-Dose reach. Much more detail on 2010 sample locations, numbers, parameters, etc. will be provided in the SAP to be written under this QAPP. The SAP should be ready by June 2010.

3.2.3. Nutrient Dosing: Forms of the Additions, Dose Rates

An important first step was to determine the chemical form by which nutrients are to be added. Because nitrate has been shown to be a key limiting nutrient in regional prairie streams (Suplee 2004), and nitrate is known to increase in regional ground- and stream water due to human activity (Nimick and Thamke 1998), it was concluded that nitrogen should be added as nitrate. Sodium nitrate ($NaNO_3$) and dipotassium phosphate (P source; K_2HPO_4) were selected after a review (e.g., Perrin and Richardson 1997; Ferreira *et al.* 2006) of what others have used in similar studies, along with consideration of Box Elder Creek's base water chemistry. Sodium and potassium concentrations are high in Box Elder Creek (about 305 mg Na/L and 8 mg K/L during baseflow), and calculations showed that to elevate N and P to levels suitable for the experiment, the counter ions (Na and K) would increase ambient stream Na and K by < 1%. Therefore, their potential effect on the experiment is negligible. Further, the K_2HPO_4 solutions will have a pH of about 9.0, very close to Box Elder Creek's typical pH of 8.5. (In contrast, another candidate P source, KH_2PO_4, would have a solution pH of 4.5.) Also, each of the compounds is reasonably safe to transport and store (more on this, Section 7.2). Each is also very soluble in water, so concentrated drip solutions can easily be made.

As noted, two reaches will receive nutrient additions and the plan is to add nutrients to the Low-Dose reach at concentrations close to potential criteria, while the High-Dose reach will receive a dose something higher than this. Since a TN-DO relationship for prairie streams is already described (Suplee *et al.* 2008), data were first examined to see if TN could be readily related to nitrate concentrations. No easy way to relate total N concentrations to soluble nitrate concentrations was identified for these streams. Prairie stream datasets (Suplee *et al.* 2008) were examined, but no clear patterns were found; therefore the scientific literature was consulted. There exists a body of scientific whole-stream nutrient addition studies, including an entire text devoted to the topic (Stockner 2003), but that literature is largely focused on salmonid streams or streams with large allochthonous inputs (i.e., leaf litter load). A body of work in prairie streams from the Konza Prairie Biological Station (Kansas) was much more pertinent and useful (e.g., Tate 1990; Dodds *et al.* 1996; Kemp and Dodds 2001; Kemp and Dodds 2002; Dodds and Oakes 2006; O'Brian and Dodds 2008). O'Brian and Dodds (2008) find that a Michaelis-Menten curve adequately describes N uptake by the stream, and the half-saturation constant (Ks) for the entire study stream was measured as 27 µg N/L. Ks is the concentration at which the soluble N uptake rate in the stream is half of the maximum (Vmax). In effect, it is a nutrient concentration at which stream primary productivity is still controlled by nutrient concentrations. At approximately five times Ks, nutrients are saturating and further increases in nutrients will not further increase Vmax (Chapra 1997). Also considered was a large number of laboratory and field-derived Ks values for algae (phytoplankton and benthic algae); the median Ks for that dataset was 67 µg N/L (USEPA 1985). The median Ks for soluble P for the same dataset was 15 µg P/L. These and other information were considered in developing the following:

- The dose rate for the Low-Dose reach will be set to maintain 40 µg NO_3-N/L and 6 µg SRP/L[19] at the headwaters of the study reach. This concentration includes the ambient stream N and P concentrations, which typically run about 3 µg NO_3-N/L and 4 µg SRP/L, and assumes complete mixing near the point of nutrient addition. (It is not expected that 40 µg NO_3-N/L will persist to the end of the 200 m study reach; the target concentrations are what is to be achieved at the headwaters after mixing.) The final soluble N:P ratio will be 6.7 (by weight),

[19] All SRP (soluble reactive phosphorus) concentrations discussed in this QAPP are "as P".

very close to the Redfield ratio (Redfield 1958), which should prevent the stream from switching over to P limitation.

- The dose rate for the High-Dose reach will be set to maintain 150 µg NO_3-N/L and 23 µg SRP/L. Again, this is the target for the reach headwaters after mixing. This concentration includes an estimate of N and P concentrations that will arrive at the headwaters of the High-Dose reach as influenced by the Low-Dose study (assumed to be about 5 µg NO_3-N/L and 4 µg SRP/L). This dose rate should, theoretically, bring the stream close to N saturation (i.e., fives time Ks). It will also maintain a soluble N:P ratio at 6.5 by weight, which again is very close to Redfield ratio and will prevent the stream from switching to P limitation.

3.2.4. Nutrient Dosing Equipment

Detailed site notes (floodplain bench heights, distance to stream, etc.) were taken in 2009 so that the equipment for 2010 could be determined. The dosing equipment will comprise two plastic tanks at each experimental reach, one tank for the $NaNO_3$ solution and one for the K_2HPO_4 solution. Each will be color coded (blue *vs.* white). Solution strengths used in the tanks will be the same regardless of which reach they are on. The N solution will be 400 g $NaNO_3$/L and the P solution 35 g K_2HPO_4/L[20]. The tanks will be arranged as Mariotte's bottles (McCarthy 1934), which will assure a constant drip rate regardless of changing liquid levels in the tanks. Drip rates will be checked every other day at the commencement of the study (more, Section 7.1). The mass of each compound needed for the entire study was estimated based on mean 2009 flow (4.2 CFS) and ambient 2009 stream nutrient concentrations. Tanks will be secured on the high bench above the stream and the solutions will pass to the stream by gravity feed via flexible tubing, ending in needle valves used to control the addition rates. The needle valve will drip into a diffuser array that will help disperse the solution across the channel laterally.

Dosing rates at each site must be varied according to ambient nutrient concentrations and flow. Nine inch Parshall flumes will be located at the Low-Dose and High-Dose reaches in the same riffle where the nutrients are dripped in, so that the daily flow can be determined (via look-up table) and the drip rates adjusted accordingly. (Permits for flume installation were acquired in 2009.) An easy to use write-protected Excel spreadsheet (DoseCalc_CarterCD_FNL.xls) has been prepared for making the daily drip-rate adjustment calculations. The tanks have been sized, based on the highest measured flow in 2009 (7 CFS), such that they can deliver nutrient solution to the stream for 5 days without replenishment.

Safety features are also being built into the dosing equipment design. A flow meter and shutdown valve assembly will be installed on each tank. The system runs on 12v DC and battery charge will be maintained by solar panels. If the system were to go to gravity free-flow (e.g., an animal chews a hole in the flexible line), the flow meter will induce the shutdown valve to close and prevent further nutrient solution from entering the stream. Similarly, glass tubing connections with low-shear breaking points will be installed inline on the flexible lines, near the

[20] These bulk compound concentrations equate to 65.9 g N /L and 6.22 g P/L.

tanks. If an unanticipated high flow event were to occur and carry the instream equipment (flumes, dripper arrays, hoses) away, the glass tubes will shear off and not allow the tanks to be dragged downstream. Breaking of the glass connector tubes will also cause the flow valve to trigger the aforementioned shutdown valve, ceasing all tank flows. A list of special materials to be purchased for the nutrient solution delivery and safety systems discussed above and in Section 3.2.3 is shown in Appendix A.

3.2.5. Planned Sampling Schedule

Table 3.3 below outlines the planned sampling schedule and data collection for 2010.

Table 3.3. 2010 schedule for Box Elder Creek data collection. Numbers represent number of samples to be collected. Data collection includes one before event, and four after events.

BACIP Period	Month & Days	Reach	Macro-invertebrate samples (composite)	Peri-1 mod samples (composite)	Benthic Chl *a* (No. replicates)	Phytoplankton Chl*a*	Continuous Monitoring (DO, pH, temperature, SC, turbidity, Chl*a*)	Phytoplankton CNP	Nutrients (N & P), TSS, TDS, common ions	BOD_5	Visual Assessment of Bottom Algae Cover	Weather Station (air temp, wind speed/direction, solar radiation, relative humidity)
Before	~July 12	Control	2	1	11	2	24/7, 15 min time-step	1	1	1	Biweekly	24/7, 15 min time-step
Before	~July 12	Low Dose	1	1	11	2		2	1	1		
Before	~July 12	High Dose	1	1	11	2		1	1	1		
Nutrient Dosing Begins about July 15th												
After	~ Aug 3	Control	1	1	11	2		2	1	1		
After	~ Aug 3	Low Dose	1	1	11	2		2	1	1		
After	~ Aug 3	High Dose	1	1	11	4		4	1	1		
After	~ Aug 22	Control	1	1	11	2		2	1			
After	~ Aug 22	Low Dose	1	2	11	2		2	1			
After	~ Aug 22	High Dose	1	1	11	4		4	1			
After	~ Sept 9	Control	1	1	22	2		2	1	1		
After	~ Sept 9	Low Dose	2	1	11	2		2	1	1		
After	~ Sept 9	High Dose	1	1	11	4		4	1	1		
After	~Sept 30	Control	1	1	11	2		2	1	1		
After	~Sept 30	Low Dose	2	1	11	2		2	1	1		
After	~Sept 30	High Dose	1	1	11	4		4	1	1		

Additional sampling is planned for summer 2011 and 2012, although nutrient additions to the stream will end for good in 2010. The subsequent sampling events will collect somewhat less data than 2010 and also focus on 'after' sampling of aquatic macroinvertebrates. Aquatic macroinvertebrates may continue to manifest changes in the following summer(s) due to their longer life spans; we expect other parameters (DO patterns, algal Chl*a*, etc.) to return to pre-2010 patterns by 2011. Reduced water chemistry sampling will occur, and DO will be monitored only at the 2009 locations. Details of the 2011 and 2012 sampling will be presented in their respective SAPs.

3.2.6. Real-time Water Quality Measurements

Continuous measurements (15-min increments) of pH, DO, temperature, SC, turbidity, and Chl*a* using YSI 6600 V2-4 sondes will be made in six locations in 2010 (three instruments were deployed in 2009). At the Control reach, a YSI will be deployed in the same place and manner as 2009. In the Low-Dose reach, one YSI will be deployed in the 2009 location and another at the terminus of the 200 m reach. The intent of the 2nd downstream YSI is to see if, over the 200 m distance of the experimental reach, DO has changed relative to the upstream YSI. It will also serve as a backup in case of instrument failure. In the High-Dose reach, a YSI will be deployed just upstream of the nutrient dripper array to measure boundary conditions that may be influenced by the Low-Dose nutrient additions. A 2nd YSI will be placed in the 2009 location, and a 3rd at the terminus of the 200 m reach. No DEQ SOPs exist for long-term instrument deployment therefore data quality objectives for their use will be presented in Section 4.1.

3.2.7. Water Column Measurements

Most water quality measurements are routine and will be adequately detailed in the upcoming SAP or in existing DEQ QAPPs (e.g., DEQ 2005). However, some non-standard analytical measurements are important to measure and need to be addressed. The QUAL2K model, for example, prompts the user for the stoichiometry (C:N:P ratio) and mass of suspended organic matter ("seston"; living and detrital organic material), therefore samples for these will be collected and analyzed since model testing is an ancillary project objective. See the upcoming SAP for details on sample collection procedures.

Limited real time measurements of ambient stream phosphate and (possibly) nitrate concentrations will be carried out. Also, the nutrient addition solutions will be carefully prepared using DI water and checked periodically using a field instrument (Hach DR890 Colorimeter). Data quality objectives for these are presented in Section 4.2.

3.2.8 Benthic Measurements

This section discusses measurements associated with the stream bottom.

3.2.8.1 Macroinvertebrate Collection and Processing

Macroinvertebrate samples will be collected from each study reach following the EMAP-RW (Environmental Monitoring and Assessment Protocol- Reach wide) protocol (Lazorchak *et al.* 1998). The one difference is that the 11 transects will be spaced along the length of each study reach rather than be spaced as a function of stream wetted width. After the samples are collected and sent to the contractor for processing, the contractor will follow the procedures outlined in the DEQ SOP to process and identify macroinvertebrates (DEQ 2006). The mass (weight) of macroinvertebrates in each sample will be determined to ascertain the influence of the nutrient additions on macroinvertebrate biomass. This will be measured as Ash Free Dry Weight (AFDW). To do this, the sample processor must first remove the first 500 (±10%) macroinvertebrates from each sample and count the number of grids from the Caton Tray used in the first processing run. The macroinvertebrates removed from the first run will be sent to the taxonomist for identification. Next, the processor must remove all of the macroinvertebrates from the same number of previously-unprocessed grids from the same sample; these are to be sent to the oven and ashed. The number of macroinvertebrates and grids from the first and second run will be recorded and provided to DEQ with the project deliverables.

3.2.8.2 Periphyton and Benthic Chl*a* Collection

Chlorophyll *a* monitoring will generally follow the DEQ SOP provided at http://www.deq.state.mt.us/wqinfo/QAProgram/SOP%20WQPBWQM-011v4_final.pdf. The one difference is that the 11 transects will be spaced along the length of each study reach rather than be spaced as a function of stream wetted width. Benthic Chl*a* samples will be taken using either the template, hoop, or core method, depending upon what is appropriate for each sub-location. Planktonic Chl*a* samples will be taken following the phytoplankton method described in the SOP, and all of these samples will be preserved and stored according to SOP methods. Results of the benthic algae sampling will be expressed as Chl*a* and AFDW per m^2 of stream bottom, and macrophyte biomass (if any are encountered) will be collected and analyzed, and expressed as mg/m^2 of Chl *a*, dry weight, and AFDW.

Within each study reach (Control, Low-Dose, High-Dose), a qualitative composite periphyton sample will be taken for species presence following DEQ's "PERI-1 mod" method except that (as for Chl *a*) the reach length and distance between transects will be established by the length of the study unit not stream wetted width. Periphyton material will be placed in a 50 ml centrifuge tube and preserved with formalin. Samples will be provided to a DEQ-approved laboratory for identification and counting.

3.2.8.3 Visual Estimates of Benthic Algae and Macrophyte Coverage

Algal growth is likely to change rapidly after nutrient additions begin and may outstrip the planned quantitative sampling intervals shown in Table 3.3. Therefore, weekly visual estimates will be undertaken following the 11 transects visual estimation method of EMAP (Lazorchak *et al.* 1998; USEPA 2001), in each study reach. A custom form was developed from approaches used by DEQ in its older Aquatic Plant Field Sheet, its current Fish Cover/Other Form, and the NIWA (2000) SHMAK periphyton assessment protocol; the new form is shown in Appendix B. Data collected via this new form are compatible with DEQ's Fish Cover/Other Form (used in 2009), there are just more pieces of information collected. Much of this work will be carried out by staff of the Carter County Conservation District who will receive training on its use (more on this, Section 7.1).

3.2.9 Hydraulic Measurements

Parshall Flumes (9") will provide accurate real-time flow measurement at the Low-Dose and High-Dose reaches (USBrRec 1967). The Low-Dose reach is in such close proximity to the Control reach that Control reach flows can reasonably be considered the same as Low-Dose flows. Because the flumes are being installed, we are not intending to measure flow using open-channel methods as we did in 2009, except at initial installation as a flow cross-check. A U.S. Army Corps of Engineers 404 permit and a MT Fish, Wildlife and Parks 124 permit for flume installation were both acquired in 2009, and each permit is good through 2010.

The QUAL2K model testing outlined in Section 3.1 will also require some channel dimension values. At each of the three reaches, during each DEQ visit, 3 cross-sections representative of the reach as a whole will be measured for water depth at 5 equidistant points across the channel. This was carried out in 2009 as well. These data will also allow for the determination of phytoplankton Chl*a* on an area basis (i.e., mg Chl*a*/m^3 can be converted to mg Chl*a*/m^2).

3.2.10 Meteorological Measurements

An independent weather station will be installed by DEQ alongside Box Elder Creek in the same location used in 2009. The station (HOBO Weather Station & Logger) will measure air temperature, wind speed and direction, solar radiation, and relative humidity. These data can be used to compare 2009 weather conditions (when most of the before data were collected to 2010 weather conditions (when most of the after data will have been collected).

3.2.11 Nutrient Spiraling Length Measurements

As presented in Section 3.2.2, nutrient spiraling calculations based on literature values were used to estimate the necessary stream length for each experimental reach. Once the dosing equipment is setup at Box Elder Creek, DEQ will have in place everything necessary to carry out a direct measure of this stream's nutrient spiraling length. It would be remiss not to carry out this simple measurement which should take less than a day and will be completed prior to the time the actual nutrient dosing begins.

The method of Payn *et al.* (2005) will be followed at one location (High-Dose reach). This method involves adding a small amount of a harmless conservative tracer (bromine, Br^-) to the sodium nitrate drip solution described in Section 3.2.4. The nutrient solution is dripped into the headwaters of the High-Dose reach and the bromine concentration monitored at the bottom of the reach with an ion-selective probe (which can be attached to an ordinary pH meter). Once the bromine concentration at the monitoring point reaches the pre-calculated value (ca. 1-2 hr), it indicates that complete mixing (laterally and longitudinally) has occurred. At this point nitrate samples are collected every 20 meters along the reach (10 total samples). The process is then repeated 2 more times, each subsequent nutrient addition slightly higher than the previous. The data can then be used to calculate the nitrate spiraling length based on the longitudinal decreases in nitrate concentration that occur during each of the three dosing events (Payn *et al.* 2005). Further details will be presented in the SAP.

3.2.12 Sediment Oxygen Demand (Optional)

Sediment oxygen demand (SOD) measurements will be helpful for the independent testing of the QUAL2K model discussed in Section 3.1. The model generates an SOD based on internal algorithms, but measured SOD can be plotted alongside those data for cross-check/validation. If time allows, SOD will be measured in duplicate cores using the extracted-core method (Edberg and Hofsten 1973). SOD will be measured in opaque core tubes collected from the Control reach and (if time allows) the High-Dose reach as well. All SOD values will be corrected for the water-column oxygen demand (WOD) of the water above the sediment cores (Suplee and Cotner 1995). WOD will be measured in a similar opaque core but containing no sediment; see pages 9-10 of the 2007 Yellowstone River QAPP and the SAP "HUNGRY HORSE RESERVOIR, AND SWAN AND WHITEFISH LAKES SEDIMENT OXYGEN DEMAND SAMPLING PROJECT — 2009".

4.0 Quality Objectives and Criteria

4.1. Quality Criteria for YSI 6600 Sondes Deployed Long-term

Long Term Deployment of YSI 6600 Sondes. Six YSI 6600 sondes will be deployed along the stream and continuously record data for up to 77 days. Each instrument will be calibrated in the laboratory prior to deployment, and checked again for instrument drift upon retrieval. The Alliance for Coastal Technologies (ACT) is a third-party organization that carries out performance verification studies for these (and other) instruments in rigorous, long-term field deployments around the U.S. (*see reports and organization information at*: http://www.act-us.info/evaluation_reports.php) We have used their "Performance Verification Statement" reports to develop quality criteria for the sondes. These ACT reports discuss, on a probe-type by probe-type basis, the period of time until biofouling begins to interfere with instrument measurements. Days-to-interference from biofouling vary, but typically fall in the range of 14-35 days (ACT 2007). Biofouling was not a problem in 2009 in Box Elder Creek but increased algal growth in the experimental reaches could change this dynamic. The sondes will be checked for biofouling weekly and cleaned (as needed) by Carter County Conservation District staff. DEQ will also check the sondes during their periodic visits (ca. every 19 days), and data collected to that point will be down loaded to a laptop for safe keeping.

Instrument drift during the deployment period is an equally important issue, and is addressed below, by measurement type.

pH. No ACT drift criteria for pH were located. However, pH is usually a stable parameter after calibration and drift is small. The pH drift will be measured and recorded as part of routine instrument setup and retrieval.

Dissolved Oxygen. Accurate DO measurement is key to this study, and DEQ is using the best available probes for this purpose, YSI's ROX™ optical DO sensors. These sensors became available from YSI in 2006 and show no significant drift over the 1-2 month deployment timeframes during which they were tested (YSI 2007). ACT has carried out studies on the older polarographic probes (ACT 2004) but has not yet carried out test of the ROX™ optical sensors (phone conversation with ACT staff, March 24, 2010). Therefore, the quality criterion for DO concentration data collected over the sampling period using ROX™ optical sensors will continue to be the same as that used in the Yellowstone QAPP. Instrument drift will be ≤ 0.2 mg DO/L, using (preferably) the single point, air-saturated water technique or alternatively the single-point, water-saturated air technique (YSI 2009).

Turbidity. In an ACT test at 7 sites around the country with deployment times ranging from 29-77 days, instrument drift (5 NTU, initial standard calibration) ranged from 0-17%, with a mean drift of 8% (ACT, 2007). The quality criterion for turbidity data collected over the sampling period in our study is that instrument drift, from initial calibration at 12.7 NTU, will be ≤ 10% (YSI has calibration solution of 12.7 NTU which is as close to the 5 NTU as they provide).

Chlorophyll a. In another ACT test at 5 of the 7 sites mentioned above, Chl *a* (using Rhodamine WT as the initial calibration dye) drift during deployment ranged from 31-63% "pre-cleaning" of the probe, and from 0.8 to 18% (mean 7%) "post-cleaning" of the probe (ACT 2006). (Keeping this probe clean clearly diminishes drift.) The quality criterion for Chl *a* data collected over the sampling period in our study is that instrument drift from calibration (using Rhodarmine WT) will be ≤ 10%, post-cleaning.

4.2. Quality Criteria for Other Measurements

Routine Water Quality Measurements. All quality assurance and quality control (QA/QC) requirements followed by DEQ will be instituted for this project. This includes use of standard site visit forms and chain of custody forms for all samples. The QA/QC requirements for water quality samples, etc. are described in detail in DEQ (2005), and are sufficiently covered that repeating them here is not needed.

Real-time Measurement of Nitrate and Phosphate Concentrations.
1. Nitrate. To maintain the target nutrient-dose concentrations, measurement of stream flow in real time and knowledge of stream ambient N and P concentrations are required. I carried out a series of tests on DEQ's portable Hach DR890 Colorimeter since it can be used to measure N and P concentrations in real time in the field. Its capabilities (detection limits, precision) were then compared to already-collected N and P data analyzed in approved laboratories. For nitrate-N, the Hach DR890 Low-Range procedure is generally too coarse for our purposes. Data collected in 2005 and 2009 (n=20) and analyzed by certified laboratories (e.g., DPHHS) show that the Box Elder Creek research site has a mean of 2.5 µg NO_3-N/L ± 2.0 µg NO_3-N/L (1 SD), and a maximum of 7.0 µg NO_3-N/L. These concentrations are well below the Hach DR890's estimated detection limit of 10-20 µg NO_3-N/L and tested precision (coefficient of variation, CV) of ± 35%. Therefore, we will instead use the mean of the prior-collected nitrate concentrations (3.0 µg NO_3-N/L) as the assumed ambient concentration arriving at the headwaters of the Low-Dose reach (Table 4.1). Given the 900 m of stream (and biological uptake) between the terminus of the Low-Dose reach and the headwaters of the High-Dose, it is very unlikely the High-Dose reach will receive nitrate concentrations of 40 µg NO_3-N/L (the target concentration in the Low-Dose reach). Instead, I expect concentrations to again run very close to the Hach's capabilities. Therefore, I will generally assume that the High-Dose reach receives 5 µg NO_3-N/L (Table 4.2), just slightly elevated from the value used for the Low-Dose.

If time allows, DEQ staff will periodically check the nitrate concentrations just above the High-dose reach, using the Hach instrument, to assure that they are below the Hach's detection limit (and, therefore, that our assumption is holding). If the Hach test reveals the High-dose reach is receiving nitrate ≥ than 40 µg NO_3-N/L, we will repeat the test 2 additional times and then decide if the assumed ambient value should be changed (or not). Inability to reproduce consistent results (i.e., CV > 35%) will lead to a decision to not adjust the ambient assumptions. Based on the decision, instructions will be left with the Carter County Conservation District staff on how to proceed (i.e., what concentration to input in the Excel spreadsheet) until the next DEQ visit which will occur about 19 days later.

2. Phosphate. Similar to nitrate, knowledge of ambient soluble reactive phosphate (SRP) is needed. SRP concentrations are more variable in Box Elder Creek, usually in July, when they can be an order-of-magnitude higher than in August and September. I found the Hach DR890

Low Range phosphorus analysis test easier to use, faster, and more consistent than the nitrate analysis. The method has an estimated detection limit of 20 µg SRP/L and a measured precision (CV) of ± 25% near the detection limit. DEQ will measure SRP concentrations during its periodic visits, at the headwaters of both the Low-Dose and High-Dose reaches. If measured SRP is < 30 µg SRP/L, the default values in Tables 4.1, 4.2 will be used. (Table 4.1 and 4.2 default values were derived after a review of the Box Elder Cr SRP dataset's means and medians, considered together and by month). If measured SRP is found to be ≥30 µg SRP/L, we will repeat the test 2 additional times and then decide if the assumed ambient value should be changed (or not). Inability to reproduce consistent results (i.e., CV > 25%) will lead to a decision to not adjust the ambient input assumptions. Based on the decision, instructions will be left with the Carter County Conservation District staff on how to proceed (i.e., what concentration to input) until the next DEQ visit which will occur about 19 days later.

Table 4.1. Default values for the Low-Dose reach when Hach DR890 concentrations are less than the instrument's capabilities

Month	Nitrate (mg N/L)	Reactive Phosphorus (mg P/L)
July	0.003	0.007
August	0.003	0.004
September	0.003	0.004

Table 4.2. Default values for the High-Dose reach when Hach DR890 concentrations are less than the instrument's capabilities.

Month	Nitrate (mg N/L)	Reactive Phosphorus (mg P/L)
July	0.005	0.007
August	0.005	0.004
September	0.005	0.004

Nutrient Dosing Solutions. After the nutrient dosing solutions have been added to the stream, rapid uptake by biota will make instream measurement an ineffective means to validate that the target dosing concentrations have been achieved. Instead, great care will be exercised in the preparation of the nutrient solutions. The solutions will be prepared in a controlled environment in Ekalaka using a high-quality Ohaus 15 kg balance and distilled water, after which the solutions will be driven to the research site in 20 L carboys and poured into the dripper tanks. During DEQ's visits, samples of the solution preparations will be periodically collected, diluted appropriately, and measured for nitrate and SRP using the Hach DR890 Mid-Range tests. Since the solution strengths are known, it will be very easy to assure that the tested solutions are diluted so that they fall midway in the test ranges (e.g., the nitrate Midrange test range is 0-5 mg NO_3-N/L.), Samples will also be periodically collected for analysis by the Montana DPHHS laboratory in Helena, MT, however these results will not be available for months and will only be useful for back-calculating actual dosing rates.

4.3. Quality Criteria for Core-sample SOD

In spite of its importance to DO dynamics, SOD measurement is not in Standard Methods (APHA 1998); however, there is a significant body of literature on the topic (see review by Bowman and Delfino 1980). Bowman and Delfino (1980) defined 3 criteria for acceptable SOD measurements: (1) consistency; (2) reproducibility; and (3) efficiency. Consistency refers to the ability of the investigator to adhere to the prescribed SOD measuring technique. Consistency will be addressed by adherence to the techniques outlined in the upcoming SAP. Reproducibility addresses replicate variability. We will measure SOD in duplicate chambers at each site, with a CV target of ± 20%, which is considered good (Bowman and Delfino 1980). WOD (used to correct gross SOD) will be measured in the dark and incubated at ambient stream temperatures using a YSI 85 (see Hungry Horse SAP). Efficiency refers to the ability to make a sufficient number of measurements over a relatively short time period. We intend to be able to complete each set of SOD measurements within 2-8 hours of initiation, based on similar work in the Yellowstone River in 2006. If the longer timeframe (i.e. 8 hrs) is needed, these will be run overnight so that SOD measurement will not consume the working hours required to complete other project tasks.

5.0 Data Review, Validation and Verification

Data generated during this project will be stored on the DEQ site visit/Chain of Custody field forms, and in laboratory reports obtained from the laboratory. Site Visit/Chain of Custody forms will be properly completed for all samples. Written field notes, field forms (photo log, site information), and digital photos will be processed by DEQ staff following QA/QC procedures to screen for data entry errors. All water quality monitoring data collected will be submitted to DEQ using the most current upload process. Direction concerning the upload process can be accessed at the Bureau's Data Management's web pages: http://www.deq.mt.gov/wqinfo/datamgmt/index.asp, or by contacting the Bureau's Data Management Section Water Quality Metric Database Manager (contact information is available at: http://svc.mt.gov/deq/staffdir.asp#ppa). Electronic Data Deliverables (EDD) must meet the acceptance criteria set forth by DEQ. Signed hard-copy results for analytical results will be provided to DEQ. Data will be processed with Excel and with Minitab release 14. ArcView version 9 ArcMap will be used for GIS applications. The GPS coordinate system datum will be NAD 1983 State Plane Montana, in decimal degrees, to at least the third decimal. All data generated during this project will be available to the public.

6.0. Assessment and Response Actions

The QA program under which this project operates includes independent checks obtained for sampling and analysis (i.e., laboratory quality assurance processes). The DEQ QA officer may perform audits of field operations and laboratory activities during the course of the project. The QA officer has the authority to stop work on the project if problems affecting data quality that will require extensive effort to resolve are identified.

Any changes to the SAP that may result after the project is initiated will be documented and included as an addendum to the SAP. Project responsibilities for individuals directly involved in the project are shown in Table 5.1 below. The project manager (M. Suplee) will communicate all significant changes in field protocols or sampling locations to relevant DEQ staff, the DEQ QA officer, and Carter County Conservation District staff. The likely impacts of these changes on project success will be discussed on a case-by-case basis, and the project adjusted/modified to continue to meet the objectives in this QAPP, as needed.

Table 6.1. Project personnel and their responsibilities in the field.

Name	Organization	Project Responsibilities
Michael Suplee	MT DEQ	Project management/data collection
Rosie Sada de Suplee	MT DEQ	Supervision of her field staff/data collection
Steve Fernandes	MT DEQ	Data collection
Dave Feldman	MT DEQ	Macroinvertebrate methods/data collection
Georgia Bruski	Carter County Conservation District	Preparation of nutrient solutions, adjustment of tank drip rates, data collection

7.0 Special Training/Certification

7.1 Training of Carter Country Conservation District Staff

Georgia Bruski of the Carter Country Conservation District will carry out much of the day-to-day operation of the experiment during the times that DEQ is not onsite. It is anticipated that she will go the research site every other day in July and early August, tapering off to about every 3 days later in the summer. To assure that she can properly carry out her work, DEQ will carry out training in early July 2010 which will cover the following subjects:

- Proper storage and safe handling of dipotassium phosphate and sodium nitrate compounds (per Section 7.2 below)
- Proper setup and use of the analytical balance
- Proper procedure for preparing nutrient solutions (including safety considerations)
- Method for determining stream flow from Parshall flume gage readings
- Proper use of the Excel spreadsheet to calculate daily nutrient drip rates
- Adjustment of daily drip rates
- Procedure for re-setting the nutrient dripper shutdown system if it trips
- Checking and cleaning of YSI 6600 sondes
- Collection of algae density data using the visual algal density form (Appendix B)

We anticipate that all these activities will require about a week of training. The project manager (M. Suplee) is planning to stay onsite for as many days after the nutrient dosing has begun as are needed to assure that all tasks are clear and that work is proceeding smoothly.

7.2 Safe Handling of Compounds (from Material Safety Data Sheets)

The Material Safety Data Sheets for both dipotassium phosphate and sodium nitrate will be clearly posted at the location in Ekalaka where the compounds are being stored. A summary of the key concerns for each compound are shown below. No eating or drinking is to take place at the storage facility. A gravity-fed eyewash station will be setup at the storage facility.

Dipotassium phosphate. Fire Hazard: This compound is non-combustible. Human Health: It poses little or no threat to human health. When handling the compound, latex (or similar) gloves and protective eyewear (goggles) should be worn to avoid irritation of the skin and eyes. If it gets on the skin, remove it with plenty of soap and water. If eye contact should occur, flush eyes with plenty of water and seek medical attention if needed.

Sodium nitrate. Fire Hazard: This compound is stable and a negligible fire hazard, but is an oxidizer and has an explosion potential. It should be stored in a cool, dry place separate from other combustible, organic, or readily-oxidizable materials. Avoid generating dust. Human Health: Sodium nitrate is an oxidizer and may cause irritation to skin and eyes. DO NOT swallow. When handling use gloves made of neoprene or rubber. Wear chemical safety goggles and protective clothing (a laboratory coat will be provided by DEQ). A NIOSH approved dust mask (e.g., NIOSH 95) should be worn when handling this material. Avoid generating dust. If skin contact occurs, wash area with soap and water and get medical attention. If eye contact occurs, flush with water and get medical attention; also seek medical attention if material is inhaled.

8.0 Documents and Records

Beginning in early 2011, a detailed technical report document will be prepared which will describe the findings of the study, including a summary of the approaches taken (i.e., this QAPP and the SAP). The report will be updated with addendums when the 2011 and 2012 sampling results are available. It is anticipated that nutrient criteria for prairie streams will be recommended as part of the report. As such, a detailed discussion will be made describing the linkages between nutrients and effect variables (DO, pH), as well as direct effects on aquatic life (e.g., macroinvertebrates). The nitrogen and phosphorus criteria derived from the study will be compared to available literature values and will be thoroughly discussed in the report. The criteria will likely include soluble N (nitrate), and the report will provide details on how to monitor and implement a soluble nutrient criterion. It is also very likely that one or two articles will be prepared and submitted to peer-review scientific journals.

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Appendix A

EQUIPMENT AND CHEMICAL PURCHASES SPECIFIC TO THE PROJECT

Appendix A. Equipment/supply purchases for the entire project in calender year 2010.

Item	No. Units	Supplier	Supplier Part No.	Unit Price	Total Price
0.25 GPM normally-open flow switch	4	Cole-Parmer	R-32774-60	$73.50	$294.00
Spray gun, 0.5" NPT(F)	4	Cole-Parmer	R-98515-03	$144.00	$576.00
40/60 amp Beuler Bosch-type relay	12	AEI Components	BU5084M	$2.20	$26.40
Wire, circuit boxes, etc.	1	Local		$100.00	$100.00
Misc PVC pipe nipples	1	Local		$75.00	$75.00
Misc. PVC pipe for barrels, dripper array	1	Local		$175.00	$175.00
Closed head polyethylene drum, 55 gallon	2	Lab Safety Supply	5BB-16268	$99.00	$198.00
Closed head polyethylene drum, 20 gallon	2	Lab Safety Supply	5BB-41376	$40.00	$80.00
Flexible PVC reinforced tubing 0.5", 25 ft roles	10	Cole-Parmer	R-06601-03	$47.50	$475.00
0.5" NPT(F) PVC needle valve	5	Cole-Parmer	R-03245-64	$54.00	$270.00
0.5" NPT (M) X 0.5" barb, polypropylene	1	Cole-Parmer	R-30610-46	$10.50	$10.50
0.5" barb X 0.5" barb straight connector, polypropylene	1	Cole-Parmer	R-30610-03	$8.50	$8.50
Glass shearing-tube connectors (0.5" OD)	12	Kansas State University		$6.25	$75.00
Wood, nails, misc. hardware	1	Local		$300.00	$300.00
Ohaus 15 kg balance (0.5 g readability) 115 Vac, w/ battery	1	Cole-Parmer	R-11100-03	$420.00	$420.00
Ohaus 10 Kg calibration mass	1	Cole-Parmer	R-10151-36	$153.00	$153.00
Pounds sodium nitrate ($NaNO_3$) powder, food grade	1958	Chemical MT Co.		$0.95	$1,860.10
Pounds potassium phosphate (K_2HPO_4) powder, food grade	211	Chemical MT Co.		$4.00	$844.00
Freight charges, both chemicals	2	Chemical MT Co.		$60.00	$120.00
Sodium Bromide, 500 g bottle	1	Fisher Scientific	S255-500	$56.44	$56.44
Br- ion probe solid state	1	Cole-Parmer	R-27504-02	$199.00	$199.00
Oakton Ion 6 pH meter kit	1	Cole-Parmer	R-35613-74	$475.00	$475.00
Heavy duty carboy with handles, widemouth, 5.25 gallon	15	Cole-Parmer	R-62507-20	$45.00	$675.00
Polycarbonate scoop, 6" X 3"	2	Cole-Parmer	R-66600-10	$6.25	$12.50
Gallons DI water for HD, LD, N & P solutions-all summer	1038	AquaSystems, Laurel MT	In 5 gallon bottles	$1.40	$1,453.20
				Total:	**$8,931.64**

Appendix B

VISUAL FIELD ASSESSMENT FORM FOR ALGAE AND AQUATIC PLANT GROWTH (MODIFIED FISH COVER/OTHER FORM)

Explanations:

Techniques for using most aspects of the Fish Cover/Other Form are found in other documents. The following are to assist with the parts of the form that have been supplemented.

Predominant Color: The colors of aquatic plants are clues to their identity, state of growth, and health of the aquatic ecosystem. Record the predominant color of the plants or algae from the pick list, using the letter codes.

Condition: Aquatic plants go through seasonal cycles of growth, maturity, and decay. The condition of a plant or algae will indicate the approximate stage of this seasonal cycle. It can also help explain cases where, for example, AFDW to Chl*a* ratios are found to be unusually high. Growing plants and algae show new growth and bright colors. Mature plants and algae are larger but have more subdued colors because of age, epiphytes, and sediment deposits. Decaying plant and algae display a loss of both pigmentation and physical integrity. Record conditions as Growing, Mature, or Decaying on the form using the letter codes.

Thickness or Length Category: Non-filamentous algae can be present on stones and fine sediment surfaces and can develop a fairly wide array of Chl*a* levels depending upon the matt thickness. The categories (Thin, Medium, Thick) will help corroborate Chl*a* and AFDW measurements collected and also show the progression of algal growth at a site. Increasing filament length of filamentous algae has been generally associated with recreation impacts (Biggs 2000; Suplee et al. 2009). Highly enriched waters tend to grow long filaments, sometimes 1-2 meters or more in length. Record filamentous algae filament lengths as Short or Long on the form. When filaments are >2 cm in length, record their approximate lengths in the Comments section.

Waterbody: ______________________
Site Visit Code: ______________
Date: ______ Reach: EMAP layout
Visit No.: ______

Transect Letter:	A			
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%) **Actual** Cover in channel (circle one)	**G** = Green **GLB**=Green/light brown **LB**= Light brown **BR** = Brown/reddish **DBB** =Dark brown/black **Predominant Color**	**Gr** = Growing **M** = Mature **D** = Decaying **Condition**	**Thin** = < 0.5 mm thick **Medium** = 0.5-3 mm thick **Thick** = > 3 mm thick **Short** = < 2 cm long **Long** = >2 cm long **For microalgae & filamentous algae:** Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			
COMMENTS				

Transect Letter:	B			
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%) **Actual** Cover in channel (circle one)	**G** = Green **GLB**=Green/light brown **LB**= Light brown **BR** = Brown/reddish **DBB** =Dark brown/black **Predominant Color**	**Gr** = Growing **M** = Mature **D** = Decaying **Condition**	**Thin** = < 0.5 mm thick **Medium** = 0.5-3 mm thick **Thick** = > 3 mm thick **Short** = < 2 cm long **Long** = >2 cm long **For microalgae & filamentous algae:** Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			
COMMENTS				

Waterbody: ______________________
Site Visit Code: ____________
Date: ______ Reach: EMAP layout ____________
Visit No.: ______

Transect Letter: C				
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	G = Green GLB=Green/light brown LB= Light brown BR = Brown/reddish DBB =Dark brown/black	Gr = Growing M = Mature D = Decaying	**Thin** = < 0.5 mm thick **Medium** = 0.5-3 mm thick **Thick** = > 3 mm thick **Short** = < 2 cm long **Long** = >2 cm long
	Actual Cover in channel (circle one)	**Predominant Color**	**Condition**	**For microalgae & filamentous algae:** Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			
COMMENTS				

Transect Letter: D				
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	G = Green GLB=Green/light brown LB= Light brown BR = Brown/reddish DBB =Dark brown/black	Gr = Growing M = Mature D = Decaying	**Thin** = < 0.5 mm thick **Medium** = 0.5-3 mm thick **Thick** = > 3 mm thick **Short** = < 2 cm long **Long** = >2 cm long
	Actual Cover in channel (circle one)	**Predominant Color**	**Condition**	**For microalgae & filamentous algae:** Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			
COMMENTS				

Waterbody: ______________
Site Visit Code: ________
Date: ______ Reach: EMAP layout
Visit No.: ______

Transect Letter: E				
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	**G** = Green **GLB**=Green/light brown **LB**= Light brown **BR** = Brown/reddish **DBB** =Dark brown/black	**Gr** = Growing **M** = Mature **D** = Decaying	**Thin =** < 0.5 mm thick **Medium** = 0.5-3 mm thick **Thick** = > 3 mm thick **Short** = < 2 cm long **Long** = >2 cm long
	Actual Cover in channel (circle one)	**Predominant** Color	**Condition**	**For microalgae & filamentous algae:** Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			
COMMENTS				

Transect Letter: F				
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	**G** = Green **GLB**=Green/light brown **LB**= Light brown **BR** = Brown/reddish **DBB** =Dark brown/black	**Gr** = Growing **M** = Mature **D** = Decaying	**Thin =** < 0.5 mm thick **Medium** = 0.5-3 mm thick **Thick** = > 3 mm thick **Short** = < 2 cm long **Long** = >2 cm long
	Actual Cover in channel (circle one)	**Predominant** Color	**Condition**	**For microalgae & filamentous algae:** Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			
COMMENTS				

Waterbody: ______________
Site Visit Code: ______________
Date: ________ Reach: EMAP layout
Visit No.: ________

Transect Letter: G				
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	**G** = Green **GLB**=Green/light brown **LB**= Light brown **BR** = Brown/reddish **DBB** =Dark brown/black	**Gr** = Growing **M** = Mature **D** = Decaying	**Thin** = < 0.5 mm thick **Medium** = 0.5-3 mm thick **Thick** = > 3 mm thick **Short** = < 2 cm long **Long** = >2 cm long
	Actual Cover in channel (circle one)	**Predominant Color**	**Condition**	**For microalgae & filamentous algae:** Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			
COMMENTS				

Transect Letter: H				
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	**G** = Green **GLB**=Green/light brown **LB**= Light brown **BR** = Brown/reddish **DBB** =Dark brown/black	**Gr** = Growing **M** = Mature **D** = Decaying	**Thin** = < 0.5 mm thick **Medium** = 0.5-3 mm thick **Thick** = > 3 mm thick **Short** = < 2 cm long **Long** = >2 cm long
	Actual Cover in channel (circle one)	**Predominant Color**	**Condition**	**For microalgae & filamentous algae:** Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			
COMMENTS				

Waterbody: ______
Site Visit Code: ______
Date: ______ Reach: EMAP layout
Visit No.: ______

Transect Letter: I				
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	G = Green GLB=Green/light brown LB= Light brown BR = Brown/reddish DBB =Dark brown/black	Gr = Growing M = Mature D = Decaying	Thin = < 0.5 mm thick Medium = 0.5-3 mm thick Thick = > 3 mm thick Short = < 2 cm long Long = >2 cm long
	Actual Cover in channel (circle one)	Predominant Color	Condition	For microalgae & filamentous algae: Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			
COMMENTS				

Transect Letter: J				
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	G = Green GLB=Green/light brown LB= Light brown BR = Brown/reddish DBB =Dark brown/black	Gr = Growing M = Mature D = Decaying	Thin = < 0.5 mm thick Medium = 0.5-3 mm thick Thick = > 3 mm thick Short = < 2 cm long Long = >2 cm long
	Actual Cover in channel (circle one)	Predominant Color	Condition	For microalgae & filamentous algae: Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			
COMMENTS				

Waterbody: ______________
Site Visit Code: ______________
Date: ________ Reach: EMAP layout
Visit No.: ________

Transect Letter: K				
FISH COVER/OTHER	0 = Absent (0%) 1 = Sparse (< 10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)	G = Green GLB=Green/light brown LB= Light brown BR = Brown/reddish DBB =Dark brown/black	Gr = Growing M = Mature D = Decaying	Thin = < 0.5 mm thick Medium = 0.5-3 mm thick Thick = > 3 mm thick Short = < 2 cm long Long = >2 cm long
	Actual Cover in channel (circle one)	**Predominant Color**	**Condition**	**For microalgae & filamentous algae:** Record thickness or length category
Microalgae	0 1 2 3 4			
Filamentous Algae	0 1 2 3 4			
Macrophytes	0 1 2 3 4			
Moss	0 1 2 3 4			
Woody Debris >0.3 m (large)	0 1 2 3 4			
Brush/Woody Debris <0.3m (small)	0 1 2 3 4			
Live Trees or Roots	0 1 2 3 4			
Overhanging Veg. =<1m of surface	0 1 2 3 4			
Undercut Banks	0 1 2 3 4			
Boulders	0 1 2 3 4			
Artificial Structures	0 1 2 3 4			

COMMENTS