Montana Department of
ENVIRONMENTAL QUALITY

Results from the Deployment of Nutrient-diffusing Substrates in the Upper Missouri River

A project in support of the development of numeric nutrient standards for the upper Missouri River using a computer water-quality model

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ABSTRACT

The Montana Department of Environmental Quality collected data in the upper Missouri River in summer 2010 and 2011 for the purpose of developing numeric nutrient criteria using the QUAL2K model. Following initial analysis and preliminary model development, it was noted that the river had relatively high ambient soluble nutrient concentrations (mainly soluble reactive phosphate, SRP, and inorganic nitrogen) but lower-than-expected biological response (measured as the density of chlorophyll-*a* of attached algae on natural substrates). In 2013, we investigated whether other factors (pesticides/herbicides, and arsenic) may have inhibited algal growth because these would need to be given due consideration in any future nutrient-criteria development modeling. In August 2013, we completed the following: (1) sampling for 99 pesticides/herbicides using very sensitive analytical detection limits, (2) sampling for arsenic (As), which is naturally high in the upper Missouri River and has the potential to depress algal growth rates, (3) placement of nutrient diffusing substrates (Control, +N, +P, and +NP) in the river to gage a quantitative response to nutrient additions, and (4) evaluation of velocity effects (fast, slow) via different treatment designs using the diffusing substrates. Diffusing substrates indicated clearly that there was P-limitation in August 2013, which was consistent with the low SRP and elevated nitrate concentrations measured at that time. P-limitation was in sharp contrast to the situation in 2010/2011, when N:P ratios were low and suggested that the same site was likely N limited, or possibly N and P co-limited. Flows in 2013 were unusually low in contrast to 2010/2011 (which were nearer normal), and the differences in summer base flow dramatically altered ambient nutrient concentrations and reversed the limiting nutrient.

Pesticides/herbicides were not detected in quantifiable levels in any sample, therefore they were very unlikely to have influenced benthic algal growth on the diffusers or natural substrates at the study site. We found that the +NP treatments grew high densities of algae, up to an average of 306 mg Chl-*a*/m^2, which is much higher than any natural-substrate samples ever collected at this site, but not atypical for other rivers we have studied (e.g. the Yellowstone, Clark Fork). Model simulations corroborated this finding, showing that the Missouri River's native benthic algae can grow as quickly as in other rivers we have studied.

Velocity was found to play a role in the amount of algae that grew on diffusers; the +P slow diffuser treatments developed significantly less chlorophyll-*a* than the +P fast diffusers. We attribute this to boundary effects on nutrient mass-transfer, whereby algae colonizing the fast diffusers were better able to take advantage of available river nitrate than algae on the +P Slow diffusers due to a thinner diffusive boundary layer. Therefore, the lower-than-expected levels of benthic algae on natural substrates measured in 2010/2011 were possibly the result of nutrient limitation (probably N) coupled with other factors that dampen algae growth (relatively slow river velocities which can exacerbate nutrient limitation when nutrient concentrations are low).

Based on this study, we cannot state conclusively that the upper Missouri River's naturally-high As levels have no effect on algae growing on natural substrates at ambient nutrient concentrations. Few freshwater algae have been tested for As toxicity, and their sensitivities are highly variable; nevertheless, the upper Missouri River's dissolved As levels (ca. 55 µg/L) are at the low end of the detrimental-impact range from the literature. Elevated SRP concentrations negate the detrimental effects of As. But when ambient Missouri River SRP concentrations are low, as they were in 2013, there may not be enough P to negate the effects of As and As may dampen algal growth rates. If, during model development for deriving upper Missouri River numeric nutrient criteria, an effect from As is included, it

should be implemented in the model in such a manner that its effect diminishes with incrementally higher SRP-concentrations.

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ACRONYMS

Acronym	Definition
ANOVA	Analysis of Variance
DBL	Diffusive Boundary Layer
DEQ	Department of Environmental Quality (Montana)
DO	Dissolved Oxygen
DPHHS	Department of Health and Human Services
HDPE	High-Density Polyethylene
HPLC	High Performance Liquid Chromatography
PAR	Photosynthetically Active Radiation
RRL	Required Reporting Limit
SRP	Soluble-Phosphorus Concentrations
TN	Total Nitrogen
TP	Total Phosphorus
TR	Total Recoverable
Tukey's HSD	Tukey's Honest Significant Difference
USGS	United States Geological Survey

1.0 INTRODUCTION

The Montana Department of Environmental Quality (DEQ) undertook data collection in the upper Missouri River in 2010 and 2011 for the purposes of developing numeric nutrient standards for the river using the QUAL2K model, as documented in the project Quality Assurance Project Plan (QAPP) (Flynn et al., 2010). Following the initial analysis and preliminary water-quality model development, DEQ observed that the river had fairly high ambient soluble-phosphorus concentrations (SRP) and moderate nitrate levels (NO_3), but minimal accompanying biological response (**Table 1-1**). Average soluble phosphorus (P) and nitrogen (N) levels during each year were roughly at or above saturation with respect to SRP and around half-saturated for nitrate (Borchardt, 1996; Rier and Stevenson, 2006; Hill et al., 2009), meaning macronutrients were not depleted to levels expected to constrain productivity. However, the associated biological response—as reflected by benthic algal chlorophyll-*a* (Chl-*a*) levels—was well below the nuisance level of 150 mg Chl-*a*/m^2 in Suplee et al. (2009).

Table 1-1. Summary of 2010 and 2011 nutrients and benthic chlorophyll-*a*.
Ammonia was also measured, but not detected (reporting limits were 10-20 µg NH4-N/L).

Site	Year	NO_3^- (µg/L)	SRP (µg/L)	Max Chl-*a*[1] (mg/m^2)
Missouri River nr Trident	2010	41	19	40.9
	2011	39	13	50.8
Missouri River at Clarkston	2010	31	21	9.1
	2011	21	14	20.4
Missouri River above Sixteen Mile Cr near Lombard	2010	17	19	43.1
	2011	23	14	38.5
Missouri River below Toston Dam near Toston (United States Geological Survey (USGS) gage 06054500)	2010	20	19	31.8
	2011	26	13	48.3
Missouri River above York Island near Toston	2010	32	17	30.2-96.9[2]
	2011	27	12	40.8-62.1[2]
Missouri River above Canyon Ferry near Townsend	2010	31	21	26.7
	2011	8	8	14.4

[1] *The maximum Chl-a for a single replicate within the river reach using methods in DEQ (2011).*
[2] *Range reflects differing results between USGS and DEQ sampling reaches within 150 m of one another.*

Given the apparent disconnect between somewhat-elevated nutrients and response by primary producers, an addendum to the 2010 Missouri River Sampling and Analysis Plan was prepared in 2013 to further investigate and understand whether or not the river will actually respond to nutrient increases, or if other factors are affecting system primary productivity. Since nutrients appeared to be at levels conducive to substantial algal growth, but benthic algal Chl-*a* densities were lower than what we would expect, a number of physicochemical mechanisms which might influence algal response were investigated, including velocity/scour (Horner and Welch, 1981), grazing (Rutherford et al., 2000), and light (Hill, 1996).

Anecdotally, grazers (snails) were not observed at any location in the river in 2010/2011, but no qualitative measurements were made. Similarly, light and velocities should have been sufficient on the river margins for robust growth, but such a response was not observed. Consequently, other factors which dampen algal growth response may have been at play, specifically arsenic concentrations (Planas and Healey, 1978; Levy et al., 2005) which are elevated due to Yellowstone National Park's geothermal sources (Nimick and Thamke, 1998), and pesticides/herbicides, which are used throughout the basin

(see effects of pesticides/herbicides on aquatic plants at http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm).

Thus, DEQ undertook the following activities in summer 2013 to better understand the response of the river's algal community: (1) pesticides/herbicide sampling to determine if the biological response is anthropogenically muted, (2) sampling for arsenic, which is naturally high in the Missouri River and has the potential to depress algal growth, (3) placement of nutrient diffusing substrates to gage a quantitative response to nutrient additions, and (4) evaluation of grazing and velocity through different treatment designs using the diffusing substrates.

2.0 METHODS AND MATERIALS

A single sampling site was used for deployment of the nutrient diffusers, a YSI sonde, and collection of water-quality and other data (station M09MISSR05, at 46.23614; -111.46905). To minimize site disturbance which could bias samples, data collection was made sequentially so that the most sensitive parameters were collected first, in the following order: (1) chemistry parameters (e.g., water chemistry grab samples, *in situ* field measurements), (2) biological parameters (e.g., benthic algae), and (3) physical parameters (e.g., flow).

2.1 WATER CHEMISTRY SAMPLE COLLECTION

Water samples (by grab) were collected four times during summer 2013 (7/30, 8/06, 8/15, and 8/21). All water samples were collected in new high-density polyethylene (HDPE) bottles, except for pesticide/herbicide samples, for which the bottles were amber glass. Replicates were collected during each of the four sampling events for each parameter, and field blanks were made prior to departure from the field at the end of each of the four sampling events. Standard water chemistry samples (e.g., nutrients, arsenic, etc.) were analyzed by the Department of Public Health and Human Services (DPHHS) Laboratory in Helena. Pesticide/herbicide samples were analyzed by the Analytical Laboratory in Montana State University's Department of Agriculture in Bozeman. The list of nutrients, pesticides/herbicides, and other parameters, including analytical methods and required reporting limits for this project, are shown in **Table 2-1**. Details on field collection methods and preservation are discussed next.

Soluble nutrients and dissolved arsenic: For nitrate + nitrite (NO_{2+3}), soluble reactive phosphorus (SRP), and total ammonia (NH_{3+4}), water was filtered through a 0.45 µm filter and 250 ml of the filtrate was placed in a HDPE bottle and frozen until analyzed (**Table 2-2**). Filtration was accomplished with a large syringe connected to a disposal filter capsule. A small amount of the sample was wasted through the filter and the sample bottle was triple-rinsed with a small amount of filtrate before the final filtered sample was collected. Dissolved arsenic was field-filtered through a 0.45 µm filter into a 250 ml HDPE bottle (only 50 ml were necessary), preserved with nitric acid, and held on ice (not frozen -**Table 2-2**).

Total nutrients, total recoverable arsenic and pesticides/herbicides: Bottles were triple-rinsed with a small amount of ambient stream water prior to grabbing the final sample. Total Nitrogen (TN) and Total Phosphorus (TP) were collected in a single 250ml HDPE bottle and was immediately frozen (**Table 2-2**). Total recoverable arsenic was collected in a 250 ml HDPE bottle, preserved with nitric acid, and held on ice (not frozen – **Table 2-2**). Pesticide/herbicide samples were collected in a 500 ml amber glass bottle and held on ice (not frozen – **Table 2-2**).

Table 2-1. Analytical Methods and Required Report Limits

Parameters	***Required Method only for nutrients, arsenic, benthic chlorophyll-a and Ash Free Dry Weight based on DEQ WQPB Monitoring Suite table (DEQ, 2013). For pesticides, the ND_MTUNIVERSAL TEST was used (MSU , Department of Agriculture, Analytical Lab)***	***Required Report Limit (RRL) only for nutrients and arsenic in µg/l based on DEQ WQPB Monitoring Suite table (DEQ, 2013). For pesticides, the Limit of Quantitation (µg/l) is below the Required Report Value (RRV, in µg/l) in Circular DEQ-7 (DEQ, 2012b). RRV for each analyte is in parenthesis.***	***Comments***
Total Phosphorus (TP)	EPA 365.1	1	
Total Persulfate Nitrogen (TN)	4500-N B or C	10	
Nitrate + Nitrite-Nitrogen (NO_2+NO_3-N)	EPA 353.2	5	
Dissolved Orthophosphate (SRP)	EPA 365.1	1	
Total Ammonia as N (NH_{3+4})	EPA 350.1	5	
Total Recoverable Arsenic	EPA 200.8	1	
Dissolved Arsenic	EPA 200.8	1	
Benthic chlorophyll-*a*	A 10200 H	n/a	
Ash Free Dry Weight	A 10300 C (5)	n/a	
Acetochlor	"ND_MTUNIVERSAL" TEST	0.14(0.4)	
Acetochlor ESA	"ND_MTUNIVERSAL" TEST	0.01 (0.4)	Acetochlor metabolite
Acetochlor OA	"ND_MTUNIVERSAL" TEST	0.0042 (0.4)	Acetochlor metabolite
Alachlor	"ND_MTUNIVERSAL" TEST	0.11 (0.3)	
Alachlor ESA	"ND_MTUNIVERSAL" TEST	0.011 (0.3)	Alachlor metabolite
Alachlor OA	"ND_MTUNIVERSAL" TEST	0.0034 (0.3)	Alachlor metabolite
Aldicarb	"ND_MTUNIVERSAL" TEST	0.065 (0.4)	
Aldicarb sulfone	"ND_MTUNIVERSAL" TEST	0.022 (0.5)	Aldicarb metabolite
Aldicarb sulfoxide	"ND_MTUNIVERSAL" TEST	0.056 (0.4)	Aldicarb metabolite
AMBA (mesotrione metab)	"ND_MTUNIVERSAL" TEST	0.021 (n/a)	
Aminocyclopyrachlor	"ND_MTUNIVERSAL" TEST	0.025 (n/a)	
Aminopyralid	"ND_MTUNIVERSAL" TEST	0.015 (0.2)	
Atrazine	"ND_MTUNIVERSAL" TEST	0.0022 (0.3)	
Azoxystrobin	"ND_MTUNIVERSAL" TEST	0.0026 (0.03)	
Bentazon	"ND_MTUNIVERSAL" TEST	0.0011 (3)	
Bromacil	"ND_MTUNIVERSAL" TEST	0.0041 (0.03)	
Bromoxynil	"ND_MTUNIVERSAL" TEST	0.006 (0.3)	
Carbaryl	"ND_MTUNIVERSAL" TEST	0.004 (1)	
3-OH Carbofuran	"ND_MTUNIVERSAL" TEST	0.010 (1?)	
Chlorpyrifos	"ND_MTUNIVERSAL" TEST	0.031 (0.1)	
Chlorsulfuron	"ND_MTUNIVERSAL" TEST	0.0056 (0.02)	
Clodinafop-propargyl acid	"ND_MTUNIVERSAL" TEST	0.013 (n/a)	
Clopyralid	"ND_MTUNIVERSAL" TEST	0.022 (0.3)	
Clothianidin	"ND_MTUNIVERSAL" TEST	0.016 (n/a)	

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DEA	"ND_MTUNIVERSAL" TEST	0.0017 (0.3)	Atrazine metabolite
DEDIA	"ND_MTUNIVERSAL" TEST	0.1 (0.3)	Atrazine metabolite
DIA	"ND_MTUNIVERSAL" TEST	0.01 (0.3)	Atrazine metabolite
Dicamba	"ND_MTUNIVERSAL" TEST	0.22 (0.7)	
2,4-Dichlororophenoxyacetic Acid	"ND_MTUNIVERSAL" TEST	0.0045 (1)	
Difenoconazole	"ND_MTUNIVERSAL" TEST	0.011 (0.06)	
Dimethenamid	"ND_MTUNIVERSAL" TEST	0.003 (0.03)	
Dimethenamid OA	"ND_MTUNIVERSAL" TEST	0.0038 (0.03)	Dimethenamid metabolite
Dimethoate	"ND_MTUNIVERSAL" TEST	0.0011 (6)	
Disulfoton sulfone	"ND_MTUNIVERSAL" TEST	0.0066 (0.09 ?)	Disulfoton metabolite
Diuron	"ND_MTUNIVERSAL" TEST	0.0053 (0.5)	
Fenbuconazole	"ND_MTUNIVERSAL" TEST	0.0053 (0.2)	
Fipronil	"ND_MTUNIVERSAL" TEST	0.0012(0.004)	
Flucarbazone	"ND_MTUNIVERSAL" TEST	0.0012 (300)	
Flucarbazone sulfonamide	"ND_MTUNIVERSAL" TEST	0.00097 (300)	Flucarbazone metabolite
Flumetsulam	"ND_MTUNIVERSAL" TEST	0.029 (n/a)	
Fluroxypyr	"ND_MTUNIVERSAL" TEST	0.016 (0.1)	
Glutaric acid	"ND_MTUNIVERSAL" TEST	0.0074 (n/a)	Tralkoxydim metabolite
HA	"ND_MTUNIVERSAL" TEST	0.004 (0.3)	Atrazine metabolite
Halosulfuron methyl	"ND_MTUNIVERSAL" TEST	0.006 (n/a)	
Hexazinone	"ND_MTUNIVERSAL" TEST	0.0015 (0.02)	
Imazamethabenz acid	"ND_MTUNIVERSAL" TEST	0.0025 (40)	Imazamethabenz-methyl-ester metabolite
Imazamethabenz ester	"ND_MTUNIVERSAL" TEST	0.001 (40)	
Imazamox	"ND_MTUNIVERSAL" TEST	0.0057 (0.04)	
Imazapic	"ND_MTUNIVERSAL" TEST	0.003 (0.01)	
Imazapyr	"ND_MTUNIVERSAL" TEST	0.0035 (0.01)	
Imazethapyr	"ND_MTUNIVERSAL" TEST	0.004 (0.03)	
Imidacloprid	"ND_MTUNIVERSAL" TEST	0.0018 (0.07)	
Isoxaflutole	"ND_MTUNIVERSAL" TEST	0.13 (n/a)	
Linuron	"ND_MTUNIVERSAL" TEST	0.0054 (n/a)	
Malathion	"ND_MTUNIVERSAL" TEST	0.0012 (0.09?)	
Malathion oxon	"ND_MTUNIVERSAL" TEST	0.0012 (0.09?)	

Table 2-1. Analytical Methods and Required Report Limits

Parameters	***Required Method only for nutrients, arsenic, benthic chlorophyll-a and Ash Free Dry Weight based on DEQ WQPB Monitoring Suite table (DEQ, 2013). For pesticides, the ND_MTUNIVERSAL TEST was used (MSU , Department of Agriculture, Analytical Lab)***	***Required Report Limit (RRL) only for nutrients and arsenic in µg/l based on DEQ WQPB Monitoring Suite table (DEQ, 2013). For pesticides, the Limit of Quantitation (µg/l) is below the Required Report Value (RRV, in µg/l) in Circular DEQ-7 (DEQ, 2012b). RRV for each analyte is in parenthesis.***	***Comments***
MCPA	"ND_MTUNIVERSAL" TEST	0.0023 (0.008)	
MCPP	"ND_MTUNIVERSAL" TEST	0.0022 (0.007)	
Metalaxyl	"ND_MTUNIVERSAL" TEST	0.0035 (0.04)	
Methomyl	"ND_MTUNIVERSAL" TEST	0.0016 (1)	
Metolachlor	"ND_MTUNIVERSAL" TEST	0.012 (0.2)	
Metolachlor ESA	"ND_MTUNIVERSAL" TEST	0.0025 (0.2)	Metolachlor metabolite
Metolachlor OA	"ND_MTUNIVERSAL" TEST	0.021 (0.2)	Metolachlor metabolite
Methoxyfenozide	"ND_MTUNIVERSAL" TEST	0.0023 (n/a)	
Metsulfuron methyl	"ND_MTUNIVERSAL" TEST	0.01 (0.08)	
Nicosulfuron	"ND_MTUNIVERSAL" TEST	0.011 (0.03)	
NOA 407854	"ND_MTUNIVERSAL" TEST	0.0052 (200)	Pinoxaden metabolite
NOA 447204	"ND_MTUNIVERSAL" TEST	0.01 (200)	Pinoxaden metabolite
Norflurazon	"ND_MTUNIVERSAL" TEST	0.02 (n/a)	
Norflurazon desmethyl	"ND_MTUNIVERSAL" TEST	0.02 (n/a)	Norfluraxon metabolite
Oxamyl	"ND_MTUNIVERSAL" TEST	0.010 (1)	
Parathion methyl oxon	"ND_MTUNIVERSAL" TEST	0.012 (0.2?)	
Phorate sulfone	"ND_MTUNIVERSAL" TEST	0.0061 (n/a)	
Phorate sulfoxide	"ND_MTUNIVERSAL" TEST	0.0015 (n/a)	
Picloram	"ND_MTUNIVERSAL" TEST	0.14 (1)	
Prometon	"ND_MTUNIVERSAL" TEST	0.001 (0.002)	
Propiconazole	"ND_MTUNIVERSAL" TEST	0.01 (70)	
Prosulfuron	"ND_MTUNIVERSAL" TEST	0.005 (0.02)	
Pyrasulfotole	"ND_MTUNIVERSAL" TEST	0.0093 (0.07)	
Pyroxsulam	"ND_MTUNIVERSAL" TEST	0.013 (0.09)	
Saflufenacil	"ND_MTUNIVERSAL" TEST	0.01 (n/a)	
Simazine	"ND_MTUNIVERSAL" TEST	0.0026 (0.5)	
Sulfentrazone	"ND_MTUNIVERSAL" TEST	0.035 (n/a)	
Sulfometuron methyl	"ND_MTUNIVERSAL" TEST	0.0025 (0.02)	
Sulfosulfuron	"ND_MTUNIVERSAL" TEST	0.0054 (30)	
Tebuconazole	"ND_MTUNIVERSAL" TEST	0.007 (0.04)	
Tebuthiuron	"ND_MTUNIVERSAL" TEST	0.0011 (0.002)	
Tembotrione	"ND_MTUNIVERSAL" TEST	0.018 (n/a)	
Terbacil	"ND_MTUNIVERSAL" TEST	0.0024 (0.02)	
Terbufos sulfone	"ND_MTUNIVERSAL" TEST	TBD (0.07)	Terbufos metabolite
Tetraconazole	"ND_MTUNIVERSAL" TEST	0.0039 (n/a)	

Table 2-1. Analytical Methods and Required Report Limits

Parameters	***Required Method only for nutrients, arsenic, benthic chlorophyll-a and Ash Free Dry Weight based on DEQ WQPB Monitoring Suite table (DEQ, 2013). For pesticides, the ND_MTUNIVERSAL TEST was used (MSU , Department of Agriculture, Analytical Lab)***	***Required Report Limit (RRL) only for nutrients and arsenic in µg/l based on DEQ WQPB Monitoring Suite table (DEQ, 2013). For pesticides, the Limit of Quantitation (µg/l) is below the Required Report Value (RRV, in µg/l) in Circular DEQ-7 (DEQ, 2012b). RRV for each analyte is in parenthesis.***	***Comments***
Thiamethoxam	"ND_MTUNIVERSAL" TEST	0.02 (n/a0	
Thifensulfuron	"ND_MTUNIVERSAL" TEST	0.011 (90)	
Tralkoxydim	"ND_MTUNIVERSAL" TEST	0.0051 (n/a)	
Tralkoxydim acid	"ND_MTUNIVERSAL" TEST	0.005 (n/a)	Tralkoxydim metabolite
Triallate	"ND_MTUNIVERSAL" TEST	0.3 (5)	
Triasulfuron	"ND_MTUNIVERSAL" TEST	0.0055 (0.03)	
Triclopyr	"ND_MTUNIVERSAL" TEST	0.011 (0.5)	
Triticonazole	"ND_MTUNIVERSAL" TEST	0.016 (0.1)	

Table 2-2. Sampling Volumes, Containers, Preservation, and Holding Times.

Analyte	**Sample Volume**	**Container**	**Preservation and Storage**	**Holding Time**
Pesticides	500 ml	Amber Glass	No preservative, cool to ≤6°C (on ice)	14 days
TN, TP	250 ml	HDPE Bottle	Freeze	45 days
NO_2+$_3$, NH_{3+4}, SRP	250 ml	HDPE Bottle	Field filter 0.45 µm, Freeze	45 days
Dissolved Arsenic	250 ml	HDPE Bottle	Field filter 0.45 µm, 1.5 ml conc. HNO_3, cool to ≤6°C (on ice)	180 days
Total Recoverable Arsenic	250 ml	HDPE Bottle	1.5 ml conc. HNO_3, cool to ≤6°C (on ice)	180 days
Benthic chlorophyll-*a*	n/a	Ziploc bag (hoop), Petri dish (template), or centrifuge tube (core)	Freeze	45 days

2.2 FIELD WATER-QUALITY MEASUREMENT: INSTRUMENTS

Real-time measurement of field water-quality parameters (dissolved oxygen (DO), pH, conductivity, temperature, turbidity, and Chl *a*) was undertaken continuously by a deployed YSI 6600 V2-4 sonde from July 30th through August 21st, 2013. Calibration of the sonde was completed in the Helena field laboratory prior to deployment according to the manufacturer's instructions (YSI Incorporated, 2009). The sonde logged data every 15 min and was equipped with wipers that cleaned the sensor surfaces every 15 min to prevent biofouling. The deployed sonde was cleaned by hand of growth and snagged filamentous algae and checked for other issues such as failed sensors, low battery power, etc. every week. Any necessary maintenance to the instruments was carried out in the field, as needed, followed by calibration.

2.3 NUTRIENT DIFFUSING SUBSTRATES AND TREATMENTS

Nutrient diffusing substrates (Marcarelli et al., 2009) were deployed on racks in the river on July 30th and were left *in situ* for 22 days. Four different nutrient treatments were evaluated using the methods in Marcarelli *et al*. (2009). These were: (1) a Control consisting of an agar containing no nutrients, (2) an N-addition made from $NaNO_3$ to produce a 0.5 moles N/L agar, (3) a P-addition made from K_2HPO_4 to produce a 0.2 moles P/L agar, and (4) an N + P addition with the same molar strengths as indicated before. In the laboratory, 48 ml polystyrene vials were filled with substrate (hot 2% agar solution) which hardens at room temperature to a gelatin-like consistency. While hot, the designated agar solutions received a quantity of nutrient salts commensurate with the aforementioned concentrations. The agar was increased to a 3% concentration for the +NP diffusers, to assure hardening. After agar solidification, vials were capped with 2.7-cm diameter fritted glass disks (Leco Corporation, St. Joseph, MI, USA), and the glass frits were held in place with snap-on caps with holes in them which exposed 2.84 cm^2 of the glass frit to the water (**Figure 2-1**).

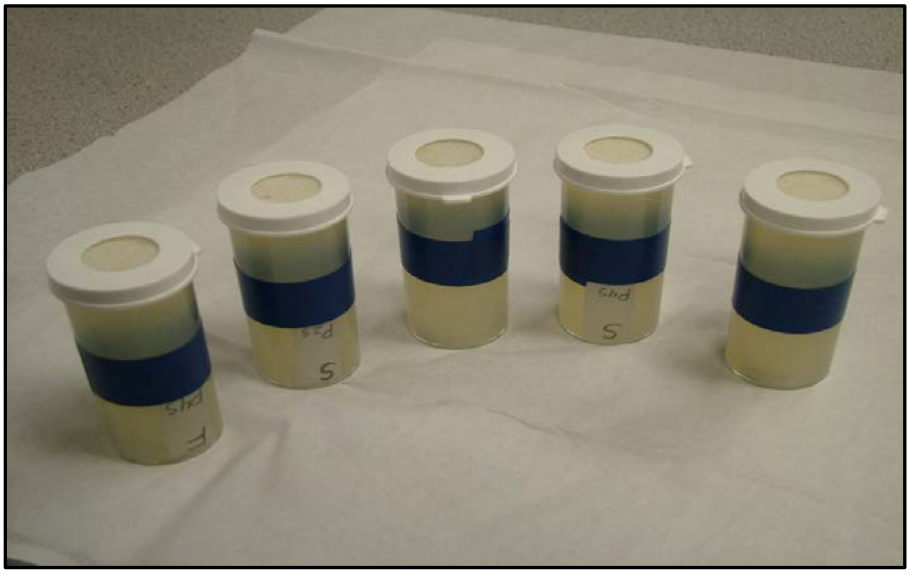


Figure 2-1. Nutrient diffusing substrate vials.
The holes in the snap on caps expose an area of 2.84 cm^2.

The experimental design called for a matrix consisting of the three nutrient treatments (+P, +N, +NP) and a Control, screened and unscreened diffusers within each nutrient treatment, and two different deployment locations to capture the effect of two different river velocities (Fast, Slow; **Figures 2-2, 2-3**) Every other diffuser vial along a nutrient treatment row was equipped with a screen (screen size: 1.0 mm^2) to prevent grazing of the developing algae by fish and macroinvertebrates (**Figure 2-2**). One rack (Fast) was placed near the right riverbank where initial mean river velocity was 0.21 m/s, and the other rack (Slow) was placed closer to the left riverbank where velocity was 0.10 m/s. Oncoming flow velocity was measured with a Marsh-McBirney Flow Mate meter set to thirty second averaging. Velocity (as reported above) was measured at 0.6 tenths depth (Rantz, 1982) and also just at the plane at the top of the deployed diffusing substrates where velocities were very similar to the ones in the riverbanks. To assure similar incident photosynthetically active radiation (PAR) reached all diffusers, both racks were situated so that the water depth (diffuser glass frit to water surface) was as similar as practicable; 27-30 cm depth range for the Fast rack, 27 cm depth for the Slow rack. We did not measure irradiance at these

locations. (The Fast rack had a slight incline upstream to downstream.) The apparatus used for river deployment is shown in **Figure 2-2**.

2.4 BENTHIC CHLOROPHYLL-*A*

Benthic Chl-*a* was collected from natural substrates at the site (and at the same reach evaluated in previous years) using methods in DEQ's Chl-*a* Standard Operating Procedure (i.e., a maximum of 16 replicates collected at evenly-spaced locations across a transect, with 11 in the wadeable zone (Montana Department of Environmental Quality, 2011). This was done at the end of the diffuser deployment period on 8/21 to evaluate the biomass on natural substrates compared to the biomass measured on the nutrient-diffusing substrates (which were deployed 22 days earlier). Natural substrate samples are collected using either the template, hoop, or core methods (Montana Department of Environmental Quality, 2011) depending on the dominant substrate and/or algae type present. In this case, only template samples were collected; the algal material was scraped, captured on GF/F (glass fiber) filters, and placed in petri dishes and wrapped in foil. All samples were immediately frozen on dry ice until analyzed (**Table 2-2)**.

For the diffusers, the fritted glass disks from each vial from the different nutrient treatments (Control, +N, +P, +B) were carefully placed in petri dishes using forceps, wrapped in foil, and placed on dry ice for Chl-*a* analyses using the same laboratory methods as for the natural-substrate samples. Chl-*a* was analyzed by the DPHHS Laboratory in Helena using high performance liquid chromatography (HPLC).

Digital photographs were taken of the nutrient diffusers on each visit. The objective of these photos was to document visible changes in the attached flora as time passed (**Figure 2-4)**.

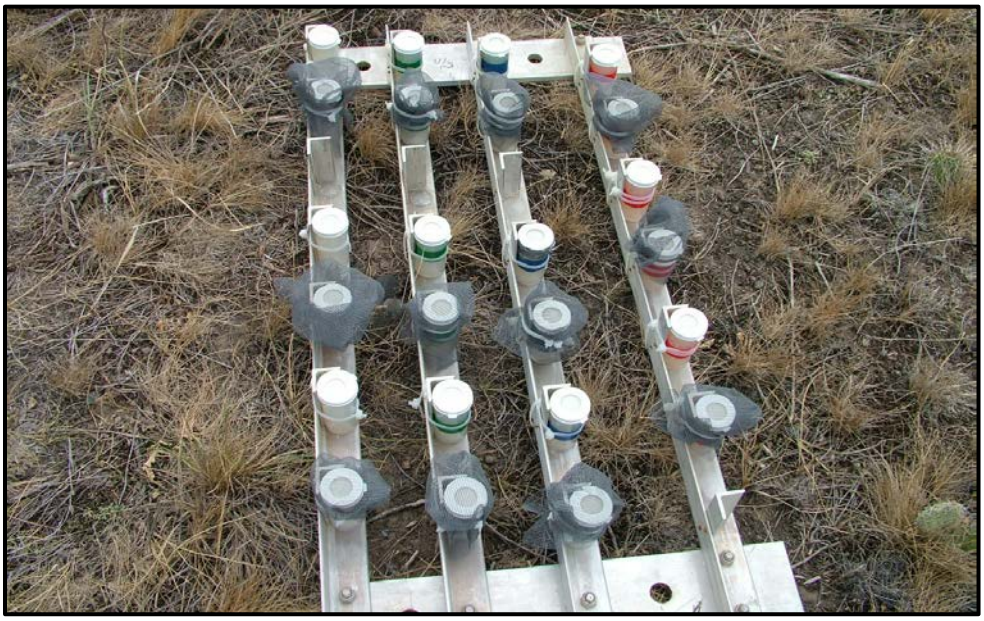


Figure 2-2. Rack used to deploy nutrient diffusing substrates in the Missouri River, just prior to river deployment.

Each rack had four treatments (Control, +N, +P, +NP) arrayed along the long axis of the rack, with two sub-treatments each (screened, not screened). The purpose of the screens was to prevent grazing by fish and macroinvertebrates. One rack was deployed in a higher-velocity location (Fast) and a second in slower-velocity location (Slow).

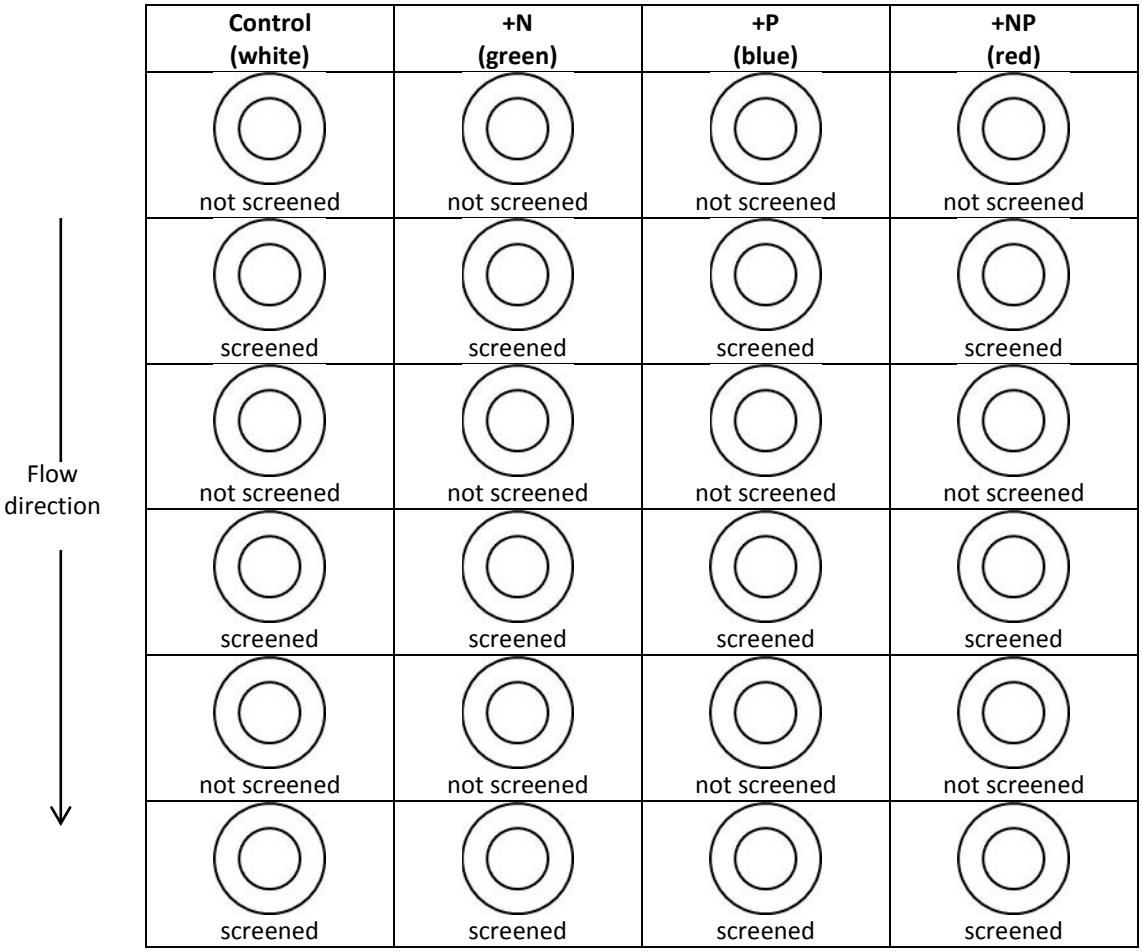


Figure 2-3. Layout of nutrient diffusers and treatments.
One rack configured as above was deployed in a higher velocity location and another (configured the same) in a lower-velocity location.

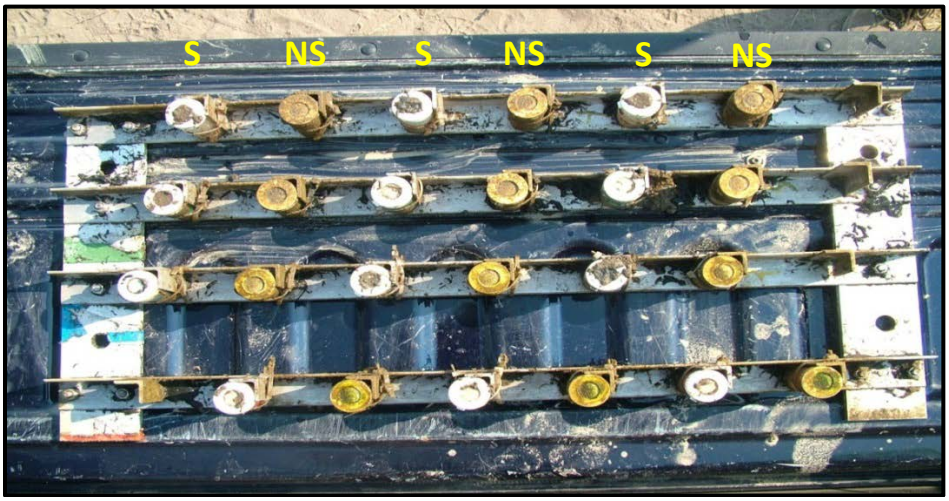


Figure 2-4. Rack showing visual observations of algal growth amongst different nutrient treatments.
S (screened); NS (not screened). Photograph was taken at the end of the project.

2.5 DATA HANDLING, AND STATISTICAL ANALYSES TO EVALUATE TREATMENT EFFECTS

All water quality samples were collected so that there was a routine sample and an accompanying field duplicate collected at the same time. The average of the routine sample and its duplicate were calculated and the values are presented in **Section 3.0** (Results).

All statistical analyses were carried out in MiniTab version 16 and tests were considered significant when p-values were ≤ 0.05. Differences in algal biomass (as Chl-*a*) among treatments at the Fast and at the Slow deployment locations were carried out using one-way analysis of variance (ANOVA). ANOVA tested, for example, whether Chl-*a* biomass was significantly different among unscreened Control, +P, +N, and +NP treatments of the Fast rack. If a significant difference was observed among treatments, Tukey's Honest Significant Difference (HSD) test was then used to calculate which treatments were significantly greater than others, thereby indicating which nutrient(s) were limiting (Francoeur et al., 1999). Tukey's HSD is a post-hoc test, meaning it is only performed after an ANOVA test has shown a significant difference among treatments. In additional to testing each velocity location (Fast, Slow) separately, the diffuser data from the two velocities were combined to carry out a global analysis of the effects of the treatments. The objective was to see if nutrient addition patterns showed any commonality among treatments in spite of differing water velocities.

3.0 RESULTS

3.1 AMBIENT CONDITIONS DURING THE STUDY: FLOW, VELOCITY, YSI SONDE DATA, AND BENTHIC ALGAL BIOMASS

Figure 3-1 shows the flow data about 10 km upstream of the study site during August 2013, as measured by the U.S. Geological Survey at their gage at Toston (USGS 06054500). As can be seen, the summer during which we carried out the study was well below the median long-term flow (about half of the median daily statistic). Mid-August flows in 2013 were 1,200 ft^3/s (34 m^3/s). Based on McCarthy (2004), this flow is close to the July-October seasonal 14Q10.

Velocity measured at the two diffuser deployment sites (Fast, Slow) declined during the 22-day deployment. At the Fast location, flow declined from an initial value of 0.21 m/s to 0.13 m/s. At the Slow location, water velocity declined from 0.10 m/s to 0.03 m/s. It is expected that these velocities generally patterned that of the flow over the 7/30 – 8/21 monitoring period.

For measurement of *in situ* conditions, all YSI sonde probes were within the quality control criteria for allowable drift from calibration (Flynn et al., 2010) and worked properly throughout deployment, except for pH, which stopped working on August 12th. Probes showed no indication of external interference (e.g., snagged algae) with the possible exception of Chl-*a*, which showed a marked increase near the end of August. Variation in daily DO concentration (i.e., daily maximum − daily minimum) was fairly high (deltas of 7.5 mgO_2/L were typical) and DO fell below the state water-quality standard (8.0 mg DO/L) every night (typical values were around 5.5 mg DO/L at dawn; **Figure 3-2**).

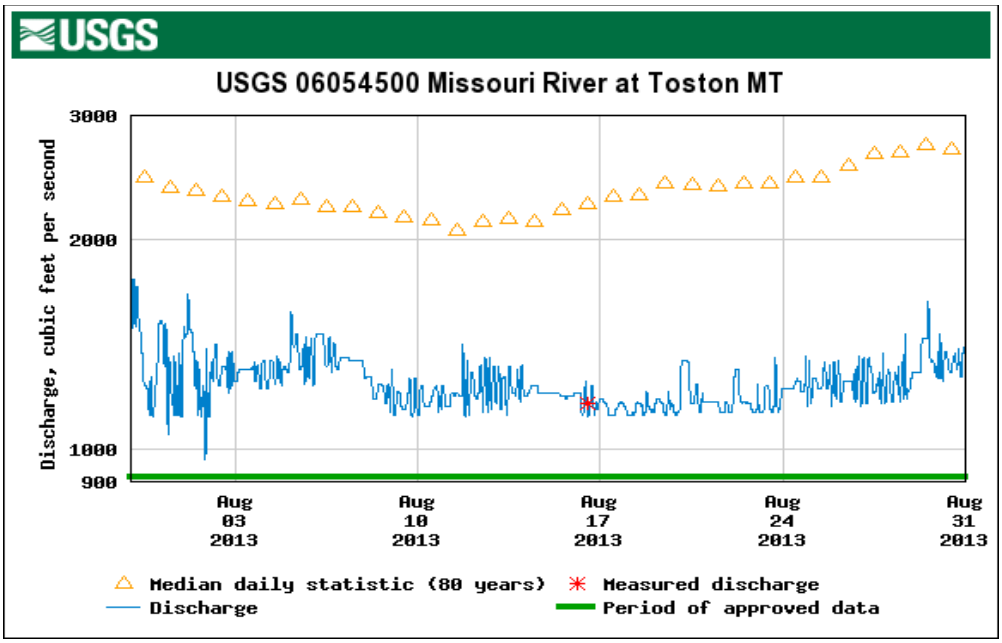


Figure 3-1. Flow measured by the U.S. Geological Survey at their gage at Toston, about 10 river km upstream of the study site.

Data and figure are from the National Water Information System website at http://waterdata.usgs.gov/mt/nwis .

During the time that the pH probe was functioning (note: there was a period of malfunction from 08/12 - 08/21, 2013), the daily change in pH was typically from a low of 8.0 to a little over 9.0 S.U., which is a range that is generally suitable for fish. Turbidity was usually < 10 Nephelometric Turbidity Units in August 2013, with some notable short-term increases between the 11th and 14th of August and again around the 21st, although we do not know what the cause of these increases were. Water temperature averaged 20.7 ^{o}C (**Figure 3-2**).

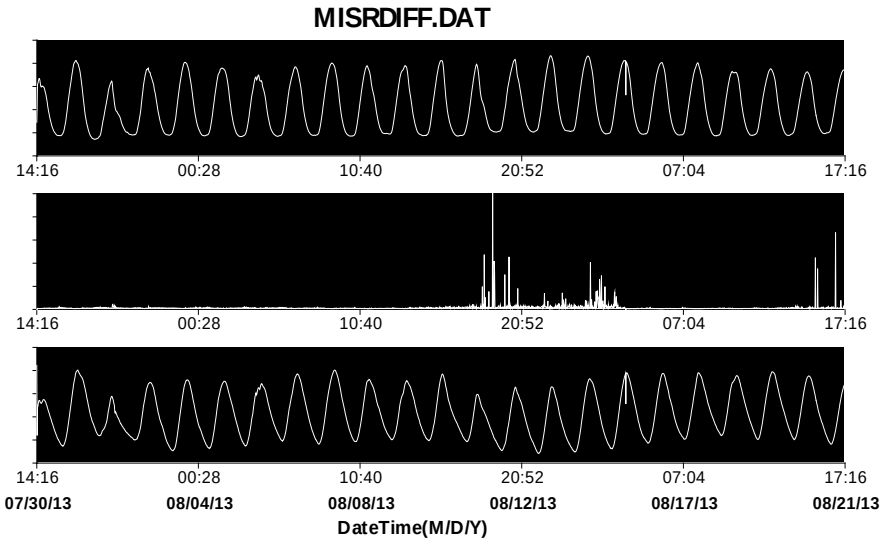


Figure 3-2. Dissolved oxygen (DO), turbidity, and temperature measured by YSI sonde at the study side during August 2013.

Benthic algal biomass was measured in the wadeable regions of the river (depths ≤ 1 m) on 8/21. The wadeable zone density averaged 26.8 mg Chl-*a*/m^2 (replicate range: 0.12 to 64.2 mg Chl-*a*/m^2).

3.2 PRESENCE OF PESTICIDES/HERBICIDES AND ARSENIC IN THE MISSOURI RIVER

Among the ninety-nine pesticides/herbicides or associated break-down products analyzed for, none were detected at concentrations above the project required reporting limits (RRL) in **Table 2-1**. Only two pesticides (2,4-Dichlororophenoxyacetic Acid, and Prometon) were even detected, and then only at concentrations below the RRL (i.e., too low to quantify). The herbicide 2,4-Dichlororophenoxyacetic Acid was detected below the RRL on all four sampling dates (7/30, 8/06, 8/15, and 8/21), whereas Prometon was detected only on 7/30 and 8/06. There were no pesticide/herbicide detections of any kind in any of the four field blanks collected concurrently with the river pesticide/herbicide samples.

Arsenic, as anticipated (Nimick et al., 1998), was detected on all four sampling occasions. Almost all of the arsenic was in dissolved form, as illustrated by the following comparisons between total recoverable (TR) and dissolved (D) forms which were collected simultaneously: July 30th—61 µg/L (TR), 59 µg/L (D); Aug 6th—56 µg/L (TR), 54 µg/L (D); Aug 15th—52 µg/L (TR), 53 µg/L (D); and Aug 21st — 53 µg/L (TR), 53 µg/L (D). No arsenic was detected above the project RRL in any of the field blanks.

3.3 AMBIENT NUTRIENT CONCENTRATIONS

A summary of the ambient nutrient concentrations during the study is provided in **Table 3-1**. Measurement of concentrations above RRLs and reproducibility of field-duplicates was excellent, except for ammonia. For ammonia, there was a huge disparity between the routine and duplicate samples collected on July 30th; the routine sample—with a concentration of 187 µg/L—was most likely contaminated, as it was so much greater than all the remaining ammonia samples collected during the course of the study. Overall, average nutrient concentrations in 2013 showed a high N:P ratio (19:1 for total nutrients, 56:1 using the soluble nutrients NO_{2+3} and SRP), which is in sharp contrast to what was encountered in summer 2010 and 2011 at the same site (**Table 1-1**), i.e., when NO_{2+3} concentrations were an order of magnitude lower and SRP concentrations were an order of magnitude higher. Correspondingly, the soluble nutrient N:P ratios in 2010 and 2011 were low, 1.9 and 2.3, respectively. Thus it appears that the limiting nutrient can differ between years.

Table 3-1. Ambient Nutrient Concentrations Measured During the Study.

	Nutrient concentration (µg/L)				
Sampling Date	**Total P**	**SRP**	**Total N**	**NO_{2+3} -N**	**Ammonia -N**
July 30, 2013	24	3	512	252	97†
August 6, 2013	28	<1	468	188	12
August 15, 2013	23	6	501	191	<5
August 21, 2013	27	6	529	268	<5
Average*:	**26**	**4**	**503**	**225**	**6**

* For values below the RRL, 1/2 the RRL was used to calculate the average.

† There was a huge disparity between the routine ammonia sample (187 µg/L) and its simultaneously-collected field duplicate (7 µg/L). Results from this date were therefore not included in the study average (bottom of table).

3.4 NUTRIENT DIFFUSING SUBSTRATES

The Chl-*a* density (mg Chl-*a*/m^2) measured on each diffuser is shown in **Figures 3-3A, 3-3B.** The screens placed on the screen-equipped diffusers were problematic as they very rapidly (within hours) became clogged with fine sediments that shaded the diffuser surfaces beneath them. The screens were cleaned during each sampling visit, but this proved ineffective due to the rapid rate at which the screens became clogged again. As can be seen in **Figures 3-3A, 3-3B,** the density of benthic algae that developed on the screened diffusers was very similar to the Control diffusers (either screened or unscreened).

One way ANOVA was run on the Fast and Slow screened diffusers, and it was found that there was no significant difference (α=0.05) among treatments (Control, +N, +P, +NP) for either the Fast or Slow locations (p = 0.72 and p = 0.08, respectively). The purpose of the screens was to prevent fish and snail grazing, but in fact the screens diminished light to the point where little algal growth could occur. Very obvious increases in Chl-*a* occurred among treatments without screens (**Figures 3-3A, 3-3B**), even though they were potentially subject to grazing which would tend to decrease algal density and mute the inter-treatment differences. It is clear that the screens failed to perform as hoped and simply shaded out algal growth. Therefore, additional statistical analysis will only be carried out on the unscreened diffusers, and the screened diffusers will not be considered further. In the case of the unscreened diffusers, algal biomass compared to the Controls (**Figures 3-3A, 3-3B**) was higher in many treatments, with the highest Chl-*a* value occurring on the +NP diffusers at the Slow deployment location (average: 306.3 mg Chl-*a*/m^2).

(A) Fast Deployment Rack

Flow direction ↓

Control	+N	+P	+NP
48.4 not screened	**49.2** not screened	**209.0** not screened	**166.0** not screened
15.5 screened	**18.6** screened	**17.4** screened	**15.9** screened
23.7 not screened	**47.6** not screened	**123.0** not screened	**191.0** not screened
34.0 screened	**21.2** screened	**17.2** screened	**18.9** screened
29.8 not screened	**51.0** not screened	**111.0** not screened	**186.0** not screened
14.5 screened	**16.8** screened	**16.3** screened	**14.2** screened

Figure 3-3A. Chlorophyll-*a* Density (mg Chl-*a*/m^2) Measured on the Nutrient Diffusers after 22 days of Deployment at the Fast Velocity Location.

(B) Slow Deployment Rack

Flow direction

Control	+N	+P	+NP
35.6 not screened	**33.0** not screened	**78.4** not screened	**314.0** not screened
31.2 screened	**29.0** screened	**29.5** screened	**30.0** screened
23.8 not screened	**44.5** not screened	**74.9** not screened	**323.0** not screened
26.0 screened	**26.5** screened	**35.6** screened	**19.6** screened
23.4 not screened	**77.5** not screened	**62.8** not screened	**282.0** not screened
33.3 screened	**30.8** screened	**43.4** screened	**21.1** screened

Figure 3-3B. Chlorophyll-*a* Density (mg Chl-*a*/m^2) Measured on the Nutrient Diffusers after 22 days of Deployment at the Slow Velocity Location.

Focusing now only on the unscreened diffusers, ANOVA tests for the two diffuser velocity deployments (Fast, Slow) showed that there were significant differences ($p < 0.001$, $p < 0.001$, respectively) among treatments (Control, +N, +P, +NP) at each in terms of the density of Chl-*a* that grew on the diffusers. Similarly, the ANOVA for the global analysis—which combined the results of the Fast and Slow deployments— showed a significant difference ($p < 0.001$) in Chl-*a* density among the treatments.

Because all ANOVA tests showed significant differences among unscreened treatments in terms of their Chl-*a* density, the post-hoc Tukey's HSD was run for the diffuser treatments for the Fast rack, the Slow rack, and the combination of both Fast and Slow treatments. The results are presented in **Tables 3-2, 3-3,** and **3-4**. Grouping significance was accepted at the 95% confidence level.

Table 3-2. Post-hoc Tukey's HSD for Differences in Chl-*a* on the Diffuser

Treatments in the Fast Water-velocity Location.				
Rack Deployment Description	**Treatment**	**n**	**Mean Chl-*a* (mg/m^2)**	**Grouping***
Fast	Control	3	34.0	B
Fast	+N	3	49.3	B
Fast	+P	3	147.7	A
Fast	+NP	3	181.0	A

*Means that do not share a letter are significantly different.

Table 3-3. Post-hoc Tukey's HSD for Differences in Chl-*a* on the Diffuser

Treatments in the Slow Water-velocity Location.				
Rack Deployment Description	**Treatment**	**n**	**Mean Chl-*a* (mg/m^2)**	**Grouping***
Slow	Control	3	27.6	C
Slow	+N	3	51.7	BC
Slow	+P	3	72.0	B
Slow	+NP	3	306.3	A

*Means that do not share a letter are significantly different.

Table 3-4. Post-hoc Tukey's HSD for Differences in Chl-*a* on the Diffuser

Treatments for the combined Fast and Slow Water-velocity Locations.				
Rack Deployment Description	**Treatment**	**n**	**Mean Chl-*a* (mg/m^2)**	**Grouping***
Fast and Slow combined	Control (Fast)	3	34.0	C
Fast and Slow combined	Control (Slow)	3	27.6	C
Fast and Slow combined	+N (Fast)	3	49.3	C
Fast and Slow combined	+N (Slow)	3	51.7	C
Fast and Slow combined	+P (Fast)	3	147.7	B
Fast and Slow combined	+P (Slow)	3	72.0	C
Fast and Slow combined	+NP (Fast)	3	181.0	B
Fast and Slow combined	+NP (Slow)	3	306.3	A

*Means that do not share a letter are significantly different.

As can be seen in **Tables 3-2, 3-3, and 3-4**, there are significantly different groups of treatments. At the Fast deployment site, the Control and +N treatments were indistinguishable from one another, but were significantly different from the +P and +NP treatments (which themselves formed a group). At the Slow deployment location, things were more complex. There, the Control and +N treatments were indistinguishable from one another and formed a group, while the +N and +P treatments formed another group, with the +N treatment as a member of both of the above. The +NP treatments stood out alone, and developed the highest average Chl-*a* of any of the treatments (306.3 mg Chl-*a*/m^2).

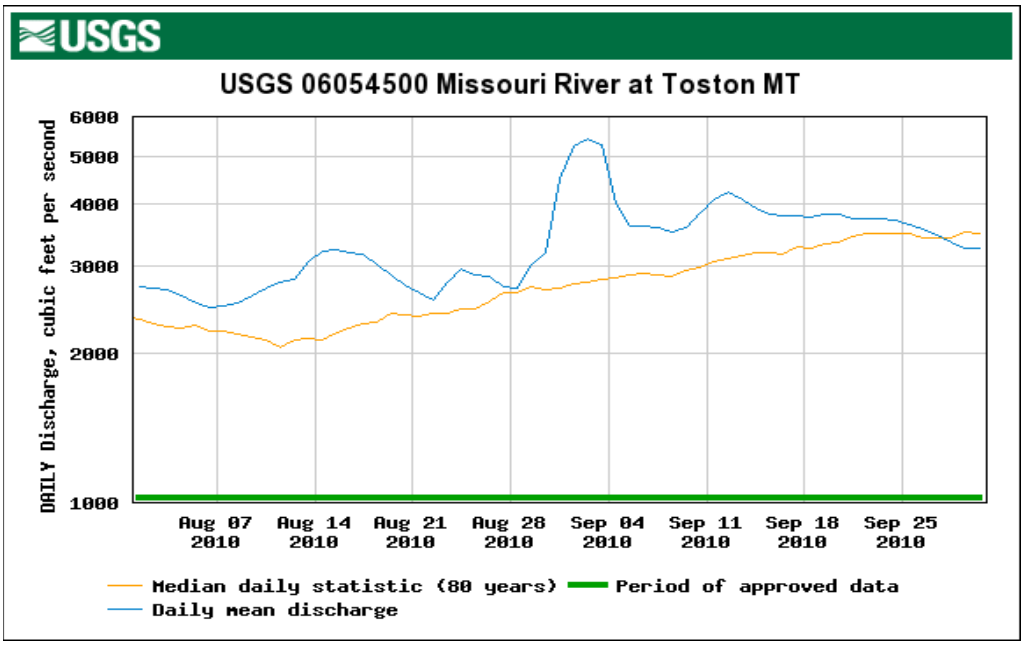
The combined analysis (i.e., all treatments from the Slow and Fast racks) revealed that there was a large group of treatments which are indistinguishable from one another (those labelled "C"; **Table 3-4**). This large group consists of all of the Controls and +N treatments, and one of the +P treatment (Slow). Again, the Slow +NP diffusers stand out (group A) because they have significantly higher Chl-*a* than all other treatments. A third group (B) consists of the Fast +P and Fast +NP treatments; this group had Chl-*a* higher than the C group but lower than the A group.

4.0 DISCUSSION

Summer 2013 had a particularly low base-flow in the upper Missouri River, as illustrated in **Figure 3-1**. This is in contrast to 2010 and 2011, when summer flows were near or above the long-term median at the time that nutrients and benthic algae were sampled in August and September of those years; (**Figures 4-1A, 4-1B**). Effects of the 2013 low-flow on water quality and productivity, as measured by dissolved oxygen (DO), are notable. At the study site in 2011, DO deltas (change from early-morning low to daily high) were on the order of 4 to 5 mgO_2/L, whereas in 2013 they were typically 7.5 mg/L. Similarly, instantaneous DO never fell below 6.43 mg/L in 2011 and was more commonly above 7.0 mg/L, but it was near 5.5 mg/L most nights in August 2013 (**Figure 3-2)**. Therefore the reduced flow

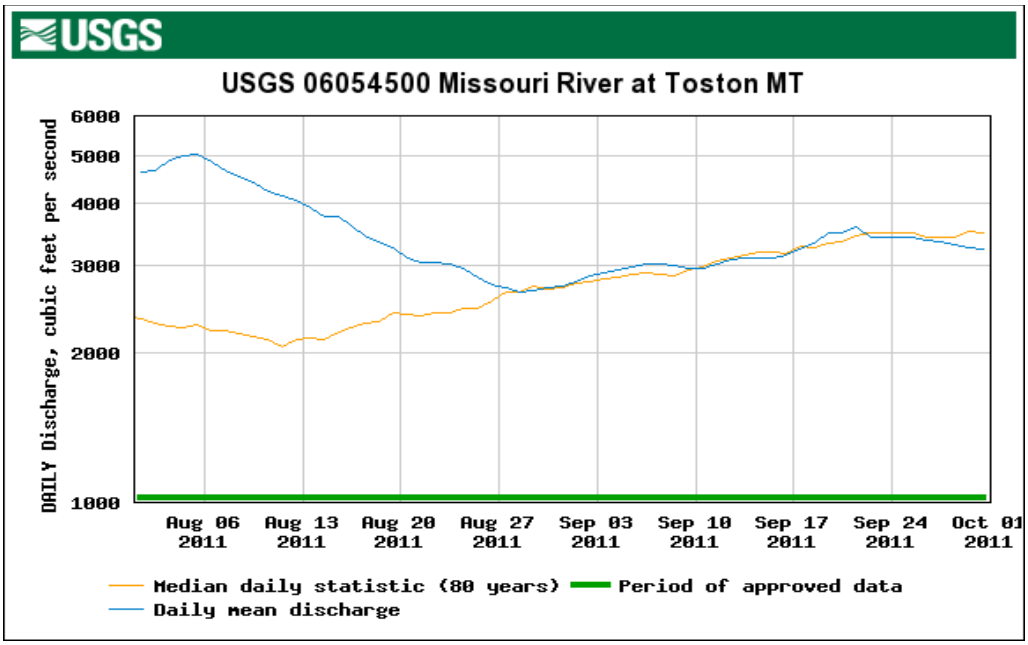
conditions in 2013 are likely responsible for the drastically different nutrient concentrations observed, whereby the ratio of influent groundwater to upstream contributing baseflow is greatly increased.

For example, in summer 2010/2011, total N (TN) averaged 280 µg/L, but was 503 µg/L in summer 2013; virtually all of the increase in TN in 2013 can be attributed to increased NO_{2+3}. Since groundwater can be a dominant source of nitrate in base-flow dominated streams in agricultural areas (Tesoriero et al., 2009), and during the low flow of 2013 groundwater certainly made up a greater portion of the Missouri River's flow, it is likely that the shift in concentration is attributable to a similar load of nitrate, but a much smaller flow volume. In 2010/2011, soluble N:P ratios (by mass) were typically near 2:1 and nitrate concentrations were relatively low (30 µg/L as N), whereas in 2013 soluble N:P ratios were 56:1—the change being driven by the order-of-magnitude increase in nitrate (with a contemporaneous halving of SRP concentrations). These fundamental water quality changes influenced the results observed in the diffuser study, and are addressed throughout the discussion below.



A

Figure 4-1A. Flow measured by the U.S. Geological Survey at their gage at Toston, about 10 river km upstream of the study site August and September 2010.
Data and figures are from the National Water Information System website at http://waterdata.usgs.gov/mt/nwis .



B

Figure 4-1B. Flow measured by the U.S. Geological Survey at their gage at Toston, about 10 river km upstream of the study site August and September 2011.
Data and figures are from the National Water Information System website at http://waterdata.usgs.gov/mt/nwis.

4.1 Interpretation of the Unscreened Nutrient Diffuser Results

The Control diffusers contained no added nutrients, and regardless of where they were placed (either Fast or Slow locations) they developed over the course of 22 days a biomass of algae (Chl-*a*/m^2) very similar to that which was measured on the river's natural substrates in the wadeable zone (depths ≤ 1m). Benthic Chl-*a* on the Control diffusers averaged 30.8 mg chl-*a*/m^2 (interquartile range 23.7 to 34.1) and the wadeable-zone natural substrate algal biomasses averaged 26.8 mg Chl-*a*/m^2 (interquartile range 21.0 to 32.4). In contrast, some of the nutrient-spiked diffusers (e.g., +NP Slow) developed much higher biomasses of algae than we have ever observed on natural substrates at this site. It is clear that providing additional nutrients benefited the growth of attached algae in this study.

At the Fast deployment location, Tukey's HSD clearly shows that the addition of P resulted in significantly greater benthic Chl-a, but the addition of N did not. At the Slow location, the results are less clear; adding P was a benefit to algal growth but there was potentially some benefit from the N-only addition too. What was very clear at the Slow location is that the addition of both N and P resulted in, by far, the highest biomass of the study (> 300 mg Chl-a/m^2). This is consistent with a zone where drifting algae and algal spores are constantly present for colonization (immigration) and where very little scour of accrued biomass can occur.

Analyzing the combined Fast and Slow results (**Table 3-4**) using Tukey's HSD showed that all Controls and all +N-only treatments are indistinguishable, meaning that adding N was not a benefit, and P was the most limiting nutrient in the river at this site in 2013. Whenever P was added (alone or with N), there was a significant increase in Chl-*a*—with one exception, the +P Slow treatment. This single exception has a plausible explanation.

Studies involving diatoms and closely-attached mats of algae suggest that velocities ranging from 0.2 to 0.6 m/s are near optimal for a wide range of conditions (Horner and Welch, 1981; Biggs et al., 1998; Horner et al., 1990; Horner et al., 1983). Outside of this velocity range, slower and faster velocities most likely hinder algae growth due to nutrient limitation or scour and detachment, respectively. Increasing water velocity (up to a point) is known to benefit lotic benthic algae growth due to the fact that mass transfer through the diffusive boundary layer (DBL) is enhanced, yet detachment through shear stress is minimal. For example, in stagnant water, very low algal growth occurs (Hondzo and Wang, 2002) and growth rates generally benefit from increasing velocity and from increasing nutrient mass-transfer primarily by reducing the thickness of the DBL at the periphyton-water interface. Since the flux of nutrients to the cell wall is enhanced, so too is subsequent uptake and growth (Biggs and Stokseth, 1996; Biggs et al., 1998). Conversely, at higher velocities detachment via sloughing becomes dominant and results in a net loss of biomass (Biggs et al., 1998).

These findings are consistent with our study results. Velocities at the Slow location (0.10 m/s to 0.03 m/s) were always well below optimal, and therefore algae growing on the +P Slow diffusers were not able to leverage the unlimited P supply from the diffusers because they did not receive river nitrate at an optimal rate; these diffusers experienced partial N limitation. However, at the Fast diffuser location, velocity was initially 0.21 m/s and ambient river nitrate was being delivered at near-optimal levels so colonizing algae took full advantage of the unlimited P. Still, we note that the +P Slow diffuser did develop higher Chl-*a*/m^2 than any of the Controls or +N-only additions, so the added P did impart some growth advantage—just not as much as on the +P Fast diffusers.

Algae colonizing the +NP diffusers were not reliant on river N or P concentrations, and the +NP Slow diffusers grew the highest Chl-*a* observed in the study. But why did the +NP Slow diffuser grow significantly more Chl-*a* (41% more; **Table 4-4**) than the +NP Fast diffuser? Grazing by fish is a possibility. Schools of small fish were always observed holding near and around the diffusers of the Fast rack, which was 4 m from the right bank. In contrast, fish were never observed around the Slow rack, which was placed 40 m from the left bank. A number of fish commonly found in this reach of the Missouri (white sucker, longnose dace, carp, and longnose sucker; MT Fish, Wildlife and Parks MFISH database) graze attached algae (Brown, 1971). The species observed near the diffusers were not identified, but may very well have been juveniles of algae-grazing fish. Another possibility is that the Slow diffusers experienced less sloughing than at the Fast location. McIntire (1966) and Horner et al. (1983) observed significantly higher algae sloughing rates (emigration) from fast laboratory troughs than slow ones. Therefore it is possible then that slow-velocity environments may tend to accrue higher standing crop biomasses (given ample nutrients) than faster locations due to increased potential for immigration, and less emigration.

Another possible explanation is that algal growth was dampened by the elevated arsenic (As) concentrations measured in the upper Missouri River (ca. 55 µg As/L, dissolved). Arsenic competes with phosphate as a chemical analogue and interferes with various metabolic pathways in cells that phosphate is involved with (see summary of effects in Sanders, 1979). Given the low ambient P concentrations observed in 2013, it is possible that this type of interference was occurring. Research on toxicity of As to freshwater algae is limited, but concentrations at the low end of the detrimental-impact range are reported to be around 48 to 75 µg As/L (Planas and Healey, 1978; Levy et al., 2005) which overlap with ambient As concentrations measured in the Missouri River. However the detrimental effects of As are mitigated with increasing phosphate concentrations. For a marine alga, phosphate concentrations from 12-22 µg PO_4-P/L (Sanders, 1979) neutralized the growth-inhibiting effects of 5 to 25 µg As/L (Sanders, 1979). This marine alga is apparently more sensitive to As than the freshwater alga

studied by Planas and Healy (1978). Higher concentrations of phosphate (489 µg PO_4-P/L) are reported to alleviate the effects of As on some freshwater algae species (Levy et al., 2005). In the +NP diffusers, phosphate was provided to colonizing algae at what amounts to an unlimited supply; therefore, it seems unlikely that water-column As inhibited algal growth on the +P diffusers, given the mitigating concentrations of phosphate just discussed. The most parsimonious explanation for the lower Chl-*a* of the +NP Fast diffusers (181 mg Ch*a*/m^2) compared to the +NP Slow diffusers (306 mg Chl-*a*/m^2) is (1) grazing by the observed fish or (2) a velocity-sloughing effect.

4.2 Pesticides/Herbicides in the Upper Missouri River

A very large number of pesticides/herbicides (97 of 99 tested) were completely absent from the river in 2013, with the exception of 2, 4-Dichlororophenoxyacetic Acid (2,4-D)and Prometon, which were each detected but at levels below our very sensitive quantitation limits. The herbicide 2,4-D is commonly used to control broadleaf weeds. Prometon is another herbicide which is often applied before weed emergence and can control most annual and many perennial broadleaf weeds and grasses for a full growing season or longer (Cornell University Pesticide Management Education Program; http://pmep.cce.cornell.edu). The herbicide 2,4-D has acute toxicity to nonvascular and vascular aquatic plants at concentrations of 66 and 330 µg/L, respectively, whereas Prometon has acute toxicity on nonvascular and vascular aquatic plants at concentrations of 98 and 624 µg/L, respectively (U.S. Environmental Protection Agency, http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm). Given that concentrations of 2,4-D and Prometon were at unquantifiable concentrations below 0.0045 µg/L and 0.001 µg/L, respectively, we can safely conclude that these two compounds had no effect on algal growth in the upper Missouri River in 2013. It is unlikely they had any effect in 2010/2011 either, given the greater potential for dilution from the associated flow conditions. We did not consider multiplicative effects, though based on the quantification levels, these would be unlikely as well.

4.3 Nutrient Limitation in 2010/2011 *vs.* 2013

One of most interesting results from this study was that nutrient limitation may not be consistent from year to year in the same river in the same location. In fact it may vary inter-annually according to flow conditions or other factors. The nutrient diffuser experiments indicate that P was clearly the limiting nutrient in August 2013, which is consistent with findings from many waterbodies across the United States. It also aligns well with other empirical evidence at the site, such as ambient water N:P ratios, which were 19:1 (by mass) for total nutrients and 56:1 for soluble nutrients (**Table 3-1**). Thus during 2013, SRP concentrations were fairly low in the river (average 4 µg P/L) and were at low enough concentrations that SRP is probably at or below a saturating concentration (Freeman, 1986; Welch et al., 1989). Both the total and soluble N:P ratios are far above the Redfield mass ratio of 7:1 (Redfield, 1958; Hillebrand and Sommer, 1999), which further strengthen the weight-of-evidence for P-limitation.

We unfortunately did not carry out a nutrient-diffuser study in 2010/2011 at this Missouri River site, but we do have ambient river nutrient data from which to draw analogous comparisons. In 2010/2011 soluble nutrient N:P ratios were very low, 1.9 and 2.3, respectively whereas total nutrient N:P ratios were much closer to Redfield; in 2010 the N:P ratio was 12:1; in 2011, 8.5:1. Dodds (2003) cautions about concluding which nutrient is limiting based only on soluble fractions. Nevertheless, giving consideration to both the soluble and total N:P ratios, the relatively high SRP concentrations in 2010/11 (ca. 15 µg P/L), which were likely saturating (Bothwell, 1988; Freeman, 1986; Welch et al., 1989), and the fairly low nitrate levels, which were near reported half-saturation constants (Rier and Stevenson,

2006; O'Brian and Dodds, 2008), we believe that the river in 2010/2011 was N limited, or might possibly have been N and P co-limited. Thus, the nutrient-limitation status of the river essentially flipped between the two study periods and the importance of nutrient controls may differ between different years and flow conditions.

4.4 Do Algae Grow in the Upper Missouri River as Quickly as We Have Measured in Other Montana Rivers?

The nutrient-diffuser data indicate that benthic algae Chl-*a* densities can grow to substantial levels in the absence of nutrient limitation. Chl-*a* densities measured on some diffusers (e.g., +NP Fast, +NP Slow) exceeded any Chl-*a* values we have so far measured on natural substrates at the study site. But how does the Chl-*a* growth rate under unlimited nutrient conditions over 22 days compare to growth rates we have used for modeling other rivers, for example the Yellowstone River? To make a comparison, we used the transect AT2K model for benthic algae which is shown in Figure 5 of Flynn et al. (2013). The model accounts for regional summertime daylight available for algal growth, water depth above the growing surface (the diffuser's glass frits in this case), and water temperature. By applying measured water quality conditions for the above-listed input variables during 2013 (e.g., sunlight from an adjacent meteorological station and measured water temperature), and then using the same rate coefficients used elsewhere in the state (Flynn et al., 2013)[1] and finally, assuming no nutrient or light limitation, it can be seen that maximum simulated biomasses under these conditions are very similar to that observed on the +NP Slow diffusers (i.e., 306.3 mg Chl-*a*/m^2; **Figure 4-2**). We used the unscreened +NP Slow diffusers because they had unlimited nutrient supplies and no apparent reduction in Chl-*a* due to fish grazing.

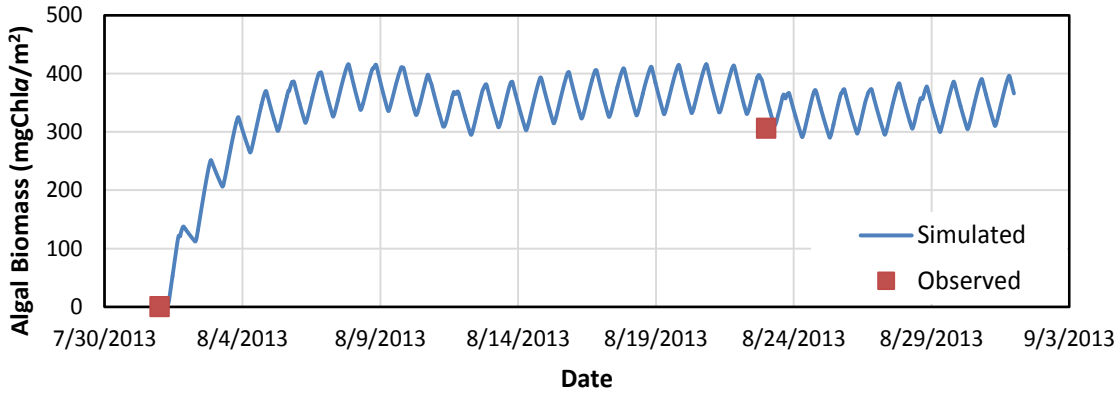


Figure 4-2. Modeled Benthic Algal Growth (quantified as mg Chl-*a*/m^2) over the 22 day period when the diffusers were deployed, based on the Zero-order Model in Flynn et al. (2013).
The blue line is the modeled simulation, assuming no nutrient or light limitation beyond ambient solar conditions (no loss through attenuation of water column), and the two red squares are the starting and ending average Chl-*a* densities measured on the +NP Slow diffusers.

Generally, results in **Figure 4-2** suggest that under unlimited resource conditions, coefficients used in calibrating the lower Yellowstone River AT2K model correspond to the growth rate of attached algae on the +NP Slow diffusers. In this regard, indigenous benthic algae in this part of the Missouri River, given an unlimited supply of nutrients, are capable of growing as quickly as algae in other Montana rivers we

[1] Zero-order algal growth rate = 400 mg Chl-*a*/m^2/day, first-order respiration rate = 0.2/day and death = 0.3/day.

have examined, and developing nearly the same steady-state biomass. The model suggests that peak biomass may have already been reached 12 days after deployment (**Figure 4-2**); indeed, field notes from 8/15 (15 days after deployment) state that "a clear evidence of green algal growth on the +P and +NP diffusers" had already developed on the unscreened diffusers of the Slow rack. However, it is still unclear how the interaction between As and suppression of algal growth rate at ambient water quality conditions may interact. Further research is needed on this topic.

5.0 CONCLUSION

Based on the results of the diffuser study, we arrived at the following conclusions:

1. Phosphorus was the limiting nutrient at the study site in the upper Missouri River in August 2013. This is based on the Chl-*a* growth patterns observed among the diffusers, and the low ambient SRP concentrations measured in the river water. This is in contrast to summer 2010/2011, when ambient water N:P ratios at the same location indicate that N was limiting, or possibly that there was N and P co-limitation.

2. The maximum amount of algae that grew on the diffusers developed on the unscreened +NP Slow diffusers. These diffusers developed 41% more Chl-*a* than the next highest-growth treatment, the unscreened +NP Fast diffusers. This occurred because either (1) the algae on the +NP Fast diffusers were subject to grazing by small fish which were consistently observed around the diffuser rack, whereas at the +NP Slow diffusers no fish were ever observed, or (2) the higher velocities at the Fast location lead to partial sloughing and resultant loss of Chl-*a*. Our literature search indicated that the elevated arsenic levels in the upper Missouri River played no role in hampering algal growth on these or other P-addition diffuser treatments, though we were unable to confirm this effect on the Controls.

3. At this study site, pesticides and herbicides in the water of the Missouri River were undetected —or detected at such low concentrations—that they are believed to have had no effect on benthic algal growth on either the diffusers or natural substrates.

4. Model simulations suggest that naturally-occurring benthic algae have similar growth rates in this part of the Missouri River as in other rivers we have studied (e.g., the Yellowstone River), given an unlimited supply of nutrients.

5. The lower-than-expected levels of benthic algae on natural substrates that were measured in the past (2010/2011) are most likely the result of nutrient limitation (probably N-limitation), but possibly may be due to inhibition by the river's As concentration. Further investigation on the relationship between As, inorganic P concentration, and algal growth rates is needed, either through literature review or experimental studies, to determine if there are As inhibitive effects at lower orthophosphate concentrations.

Based on this study, we cannot state conclusively that the upper Missouri River's naturally-high As levels have no effect on algae growing on natural substrates at ambient nutrient concentrations. So far, few freshwater algae have been tested for As toxicity, and their sensitivities are highly variable (48 to 202,000 µg As/L; Planas and Healey, 1979; Levy et al., 2005). What is known is that the upper Missouri River's dissolved As levels (ca. 55 µg/l) fall within the toxic-effects range (albeit at the low end). When

Missouri River SRP concentrations are low, as was observed in 2013, SRP concentrations may not be adequate to negate the effects of As and As may (1) exert a dampening effect on benthic algae growth rates and/or (2) a selection pressure that permits As-insensitive algae species to thrive. These As-insensitive algae may have slower growth rates than As-sensitive flora.

If, during future model development for the purposes of developing numeric nutrient criteria in the upper Missouri River, an effect from As is included, it should be implemented in the model in such a manner that its effects diminish with incrementally higher SRP-concentration simulations. The literature will have to be used to provide reasonable ranges of SRP which dampen the As effects.

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