ALGAL GROWTH RESPONSE TO NUTRIENT LOADING ON THE LOWER BOISE RIVER, IDAHO

by

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The following individuals read and discussed the thesis submitted by student Ian Penn, and they evaluated his presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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DEDICATION

I dedicate this thesis to my family and friends who supported me through this process.

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ABSTRACT

Nitrate and phosphate concentrations in the Lower Boise River become increasingly elevated with distance downstream. While there is correlative evidence that there is algal growth from elevated nutrients, no one has formally evaluated the algal growth response from nutrient loading. We quantified algal biomass in response to increased nutrient concentrations by sampling benthic algal biomass from natural substrata (rocks) and artificial substrata that also assessed nutrient limitation along a 64 mile stretch from Diversion Dam to the city of Parma. This stretch of river exhibited an increase of in-stream nitrate (0.01 mg/L to 3.40 mg/L) and phosphate (below detection to 0.56 mg/L) from upstream to downstream. Samples were collected from August to October of 2013. We observed low values for algal biomass accrual rate on the unamended artificial substrata in the upper section of the Boise (above Lander Street, Mile 12) of 1.41 – 1.60 mg chlorophyll a/m^2/day. Accrual rate values increased to 7.03 – 9.88 mg chlorophyll a/m^2/day near Caldwell (Mile 41), than declined to 6.42 mg chlorophyll a/m^2/day at the confluence with the Snake River. Trends in nutrient limitation were similar for both August and October, showing lack of algal biomass response to nutrient additions (nutrient limitation) downstream of the Lander wastewater treatment plant discharge (Mile 12). This observation corresponds to higher algal biomass growth downstream. The increased algal biomass growth in the lower Boise is interpreted to be in response to nutrient loading, primarily from wastewater discharge. In the lowermost portion of the Boise River (below Mile 55), a decline in algal biomass is observed,

presumably due to light limitation by turbid water in the lower portion of the river. Reductions in nutrients in this section of the river may not reduce algal biomass. Above Lander wastewater treatment plant (Mile 12), nitrate limitation is observed. This suggests that declines in phosphorus loading alone in the lower Boise River may not reduce algal biomass levels to those observed above the Lander wastewater treatment plant.

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LIST OF ABBREVIATIONS

cm	centimeter
ft	foot
ft/s	feet per second
mg/m^2	milligram per square meter
mg	milligram
mg/L	milligram per liter
mL	milliliter
mol/L	molar concentration
µm	micrometer
µg/L	microgram per liter
µS/cm	microSiemen per centimeter
WWTP	wastewater treatment plant

CHAPTER ONE: INTRODUCTION

Problem

Increased nutrient loading to rivers is a concern due to its effects on water quality, beneficial uses, and aquatic life (Dodds & Welch, 2000; Smith, Joye, & Howarth, 2012; Suplee, Watson, Teply, & McKee, 2009). Nutrient concentrations in the Lower Boise River become increasingly elevated with distance downstream (MacCoy, 2004; Yelen, 2015). Elevated nutrient (nitrate, phosphate, or both) concentrations have been demonstrated to increase algal biomass in rivers and streams (Francoeur, 2013; Hill & Knight, 1988; Tank & Dodds, 2003). Previous evaluations of algal biomass in the Lower Boise River have led to the assumption that a biological response is occurring due to nutrient loading. However, no one has evaluated the response of benthic algal (algae attached to aquatic substrata) growth to nutrient loading. This study was conducted to evaluate whether the distribution and nutrient limitation of algal biomass was influenced by nutrient loading.

What Is Known about Nutrients and Algae in the System

The U.S. Geological Survey (USGS) has conducted water quality and biological studies on the Lower Boise River, some of which include benthic algal biomass samples (the predominant form of aquatic plant in the Lower Boise River) (Etheridge, 2013; MacCoy, 2004; Mullins 1998, 1999a). Previously sampled in-stream nitrate and phosphate concentrations in the Lower Boise River suggest that nutrient concentrations in this system are generally consistent year after year, showing overall spatial trends of

increasing concentration downstream (MacCoy, 2004; Yelen, 2015). Total phosphorus concentrations in the lower section of the Boise River are considered impaired by nutrient enrichment according to the U.S. Environmental Protection Agency (DEQ, 2015). Mean total nitrogen concentrations, although not considered impaired, exceeded the upper range of the U.S. Environmental Protection Agency's ecoregional nutrient criteria of 0.90 mg TN/L between 1994 and 2002, in the lower section of the river (DEQ, 2015; EPA, 2000; MacCoy, 2004). Literature suggests that chlorophyll a (an indicator of algal biomass) values >100 mg/m^2 may present nuisance algae growth conditions in rivers (Dodds, Smith, & Zander, 1997; Welch, Jacoby, Horner, & Seeley, 1988). The Idaho Department of Environmental Quality has proposed a mean monthly numeric Total Maximum Daily Load (TMDL) target of $\leq$ 150 mg/m^2 chlorophyll a to describe nuisance aquatic growth. This TMDL is for sections of the Lower Boise River that are impaired by nutrient enrichment (phosphorus), according to the U.S. Environmental Protection Agency to support recreational and biological beneficial uses (DEQ, 2015). The USGS (Etheridge, 2013; MacCoy, 2004) reported chlorophyll a (an indicator of algal biomass) increased in a downstream direction. This downstream increase in chlorophyll a, however, was determined with a relatively small size of downstream sample locations (n = 5), potentially missing important information between locations. Turbidity in the water column was presumed to reduce algal biomass levels at the most downstream end of the river (Etheridge, 2013; MacCoy, 2004). Historically, observed values for benthic algal biomass in the Lower Boise River often exceed algal biomass nuisance (excessive) thresholds suggested in the literature (Etheridge, 2013; MacCoy, 2004). Nutrient enrichment and nuisance algal biomass growth may potentially cause management issues

in the Lower Boise River, such as general water quality and aesthetic impairment, as well as adverse effects on aquatic animal communities (Bourassa & Cattaneo, 1998; Dodds & Welch, 2000; Miltner & Rankin, 1998; Welch et al., 1988).

The potential adverse effects of nutrient enrichment and excessive (or nuisance) algal biomass growth on aquatic animal communities are an important component of aquatic ecosystem management. The stimulation of excessive (or nuisance) algal biomass from enrichment of the nutrients nitrogen and phosphorus can subsequently deplete dissolved oxygen in an aquatic ecosystem when decomposition of the dead algal biomass by microbial respiration occurs (EPA, 2015a). This process, known as eutrophication, can be illustrated by two general equations. The first equation shows the photosynthetic production of algal biomass from the enrichment of nitrogen and phosphorus (Strumm & Morgan, 2013).

Photosynthetic Production of Algal Biomass

$$106CO_2 + 16NO_3^- + HPO_4^{2-} + 122H_2O + 18H^+ \rightarrow \{C_{106}H_{263}O_{110}N_{16}P_1\} + 138O_2$$

The second equation demonstrates the decomposition of the excessive (or nuisance) dead algal biomass and the use (and potential depletion) of dissolved oxygen by microbial respiration.

Destruction of Algal Biomass (Microbial Respiration)

$$\{C_{106}H_{263}O_{110}N_{16}P_1\} + 138O_2 \rightarrow 106CO_2 + 16NO_3^- + HPO_4^{2-} + 122H_2O + 18H^+$$

In extreme cases, the depletion of dissolve oxygen can have adverse effects on aquatic animal communities like invertebrates and fish (Welch, 1992).

Nutrient Limitation

Protecting aquatic systems from excessive (or nuisance) algal biomass growth may be achieved by controlling the limiting nutrient in the system (Biggs et al., 2007; Smith, Tilman, & Nekola, 1999; Suplee, Watson, Dodds, & Shirley, 2012; Sosiak, 2002). Redfield (1958) showed that carbon, nitrogen, and phosphorus were the three essential nutrients for algal growth. Nitrogen and phosphorus are typically the nutrients that limit algal biomass growth, which has led to extensive field research on these nutrients (Francoeur, 2013; Wold & Hershey, 1999). The concept of single nutrient limitation comes from Liebig's (Von Liebig, 1843) Law of the Minimum, which states that "growth is limited by the resource that is supplied at the lowest rate relative to the demands of the plant." Applied originally to individual plants, this concept has been, in more recent times, applied to multispecies algal biomass communities that may contain species that are limited by different nutrients (Francoeur, 2013). Algal biomass growth has been shown to be limited by nitrogen and phosphorus individually, as well as co-limited by nitrogen and phosphorus, leading to some observed discrepancies between Liebig's Law of the Minimum and co-limitation results (Francoeur, 2013; Larned, 2010). Because of these discrepancies, and the potential of nutrient limitation to change spatially and temporally within a river system, it has been suggested by some that controlling both nitrogen and phosphorus provides the greatest likelihood of protecting aquatic systems (EPA, 2015a; Francoeur, 2013). Phosphorus has been shown to limit algal growth with phosphate in ranges from 0.003 to 0.05 mg/L, but typically occurs at concentrations ≤0.015 mg/L (Bothwell, 1985, 1989; Newbold, 1992). In separate nitrogen limitation studies, nitrate was found to limit algal growth below concentrations of 0.10 mg/L and

0.055 mg/L (Grimm & Fisher, 1986; Lohman, Jones, & Baysinger-Daniel, 1991). If nutrient limitation occurs, an increase in these nutrients may produce an algal biomass growth response and possible nuisance growth conditions (Smith et al., 1999).

Because nutrient concentrations can vary across a landscape, as a result of both landscape and in-stream processes, the relative contribution of and limitation by nitrogen and phosphorus can change spatially and temporally within the same watershed (EPA, 2015a). Factors that may influence the source and supply of nutrients to rivers include human disturbance, land-use practices, geology, hydrology, soil processes, landscape vegetation, and atmospheric loading (Meyer et al., 1988). These factors, along with precipitation patterns, groundwater inputs, and in-stream biological activity contribute to nutrient concentrations and their spatial and temporal variation within a river system (Allan & Castillo, 2007). Nutrients that limit algal growth have been shown to vary spatially and temporally within a river system (Francoeur, 2013; Tate, 1990).

Additional Factors That Influence Algal Biomass

Aside from nutrients, the factors that can limit the development of algal biomass in rivers include: light (Hill, 1996), biological grazing (Steinman, 1996), temperature (DeNicola, 1996), and physical disturbance caused by increased flow (Peterson, 1996). Biggs (1996) categorized these factors (along with nutrients) into ones regulating processes of algal biomass development and ones regulating the counteracting processes of algal biomass loss. For example, the level of resources (like light and nutrients) is the main factor that leads to algal biomass development. The interaction of temperature and these resources influences the rate of algal growth. Disturbance is the main factor that influences algal biomass loss, and to a much lesser extent, the grazing of algal biomass

by invertebrates and fish (Biggs, 1996). Lohman, Jones, and Perkins (1992) demonstrated how disturbance (flooding) decreased algal biomass through scouring, but nuisance algal biomass was established soon after at nutrient enriched sites.

What Information Can Be Gained about the System

Because the protection of aquatic systems from nuisance algal biomass growth may be achieved by controlling the limiting nutrient in the system, evaluation of the algal biomass response to nutrients (or nutrient limitation) is important for managing aquatic ecosystems (Smith et al., 1999; Suplee et al., 2012; Sosiak, 2002). Redfield (1958) suggested that when the carbon (C), nitrogen (N), and phosphorus (P) atomic ratio was 106:16:1, oceanic phytoplankton growth was balanced. The Redfield ratio provides a benchmark for assessing nutrient limitation (Borchardt, 1996), and has been used to assess nutrient limitation in the water column of rivers (Grimm & Fisher, 1986). Past studies conducted in the Lower Boise River have estimated nutrient limitation using the ratio (N:P) of in-stream nitrate and phosphate concentrations (MacCoy, 2004). A summary from a workshop of recognized experts on aquatic ecology; however, states that the most rigorous method for assessing algal biomass response to nutrients (or nutrient limitation) is to conduct nutrient diffusing substrata (NDS) assays (Biggs et al., 2007). These artificial substrata with nutrient enrichment (nutrient diffusing substrata) have been used in different river systems to assess the algal biomass response to nutrients (or nutrient limitation) (Lowe, Fairchild, & Richardson, 1985; Lowe, Golladay, & Webster, 1986; Pringle & Bowers, 1984).

Objectives

The objective of this study was to evaluate the response of algal biomass to nutrient loading in the Lower Boise River (Idaho, USA). We examined sites along the longitudinal reach to determine if the distribution of the algal biomass and its nutrient limitation status were potentially being influenced by point sources of nutrients. Chlorophyll a on both artificial and natural substrata were used to estimate algal biomass and address key questions: (1) What is the distribution of nutrient limitation measured by the algal biomass (chlorophyll a) response to additions of nitrate (NO_3-N), phosphate (PO_4-P), and NO_3-N + PO_4-P via nutrient diffusing substrata? (2) What is the distribution of algal biomass along the longitudinal reach? (3) Does the algal biomass exceed nuisance growth levels accepted in the literature?

CHAPTER TWO: MATERIALS AND METHODS

Study Site

The Lower Boise River sub-basin drains approximately 1,290 square miles of rangeland, forests, agriculture, and urban lands (DEQ, 2015; Etheridge, 2013). The approximately 65 mile long Lower Boise River is a seventh-order stream that flows northwest from Lucky Peak Dam, east of the city of Boise, and empties into the Snake River near the Oregon-Idaho border (Figure 1; DEQ, 2015). Human disturbances within the watershed include, and are not limited to, alteration of the hydrologic regime, nutrient loading, channel bottom, and riparian vegetation (Etheridge, 2013; MacCoy, 2004).

Land use is predominantly urban adjacent to the river, flowing downstream from the Lucky Peak dam to just below the city of Caldwell (site 8 in Figure 1), where land use becomes predominantly agricultural (Etheridge, 2013). Multiple tributaries and agricultural return drains discharge into the main stem of the Lower Boise River, joining the river in the downstream portion of the watershed below the city of Middleton (below site 7 in Figure 1). These tributaries and return drains can act as conduits for nutrient loading into the Boise River. Nutrients from agricultural practices (i.e., application of fertilizer and animal feeding operations) and other non-point sources may move over the surface of the ground as runoff into these conduits and into the river (Etheridge, MaCoy, & Weakland, 2009). These non-point sources may also contribute to nutrient loading in the Boise River by infiltration into the groundwater and subsequent discharge into the river (Etheridge et al., 2009). The USGS indicates that non-point sources (including

groundwater) contribute phosphorus loads directly to the Boise River and indirectly via groundwater discharge to agricultural tributaries and return drains (Etheridge, 2013).

Four wastewater treatment plants discharge into the main stem of the Lower Boise River at different points within the watershed (Figure 1; DEQ, 2015). Wastewater treatment plants, along with other point sources of nutrients (i.e., industrial wastewater discharge), are single locations where nutrients are discharged into a waterbody like the Boise River (EPA, 2015b). While phosphorus loading from human disturbances and geologic material upstream of Lucky Peak Dam (upstream of the city of Boise, Figure 1) contribute relatively little to downstream concentrations, USGS modeling results suggest that point sources (particularly wastewater treatment plants) represent the largest contribution of phosphorus to the Lower Boise River year round (Etheridge, 2013).

Sites in the Lower Boise River were located as follows: sites 1, 2, and 3 were chosen at the start of the urban area (city of Boise, Figure 1), upstream of the first major input of wastewater. Site 4 was chosen downstream of the first wastewater discharge (Lander wastewater treatment plant, Figure 1). Site 5 was chosen on the southern channel of the one major channel split in the Lower Boise River, to sample downstream of the West Boise wastewater treatment plant. Sites 6 and 7 were spaced downstream between the West Boise and Middleton wastewater treatment plants. Site 8 was downstream of the Middleton wastewater treatment plant. Site 9 was downstream of the Caldwell wastewater treatment plant. Sites 10, 11, and 12 were spaced as evenly as possible (access permitting) downstream to the city of Parma, near the confluence with the Snake River. At all study sites, experiments were conducted within run habitats (areas usually

between riffles and pools that have no turbulence at the river's surface), in the main channel of the river.

Figure 1. Location sites on the Boise River for algal biomass and environmental conditions sampling during both August and October 2013 nutrient diffusing substrata deployments and September 2013 natural substrata sampling. Red triangles indicate sampling sites. Yellow circles indicate the locations of wastewater treatment plants (WWTP) that discharge directly into the main-stem of the river.

Nutrient Limitation Experiment Design

Nutrient diffusing substrata experiments were conducted (deployed) at all sites in August and October of 2013 to assess the potential for nutrient limitation of the algal biomass community in the Lower Boise River. Nutrient diffusing substrata were designed (with some modification) according to Tank, Bernot, and Rossi-Marshall (2006). Holding racks for nutrient diffusing substrata were constructed using five pieces of 12 inch length (1.5" by 1.5" wide) steel L bar welded onto two pieces of 12 inch length steel (1" wide) flat bar (Figure A.1). At the ends of the two steel flat bars, a 0.5 inch diameter hole was drilled in order for stakes to fasten the holding rack to the river bed. Nutrient diffusers were built with 2 oz. hinged polyethylene containers (U.S. Plastic Corporation, Lima, OH, USA, item no. 66178) filled with a combination of 2 % agar and nutrients. A fritted glass disc (Leco Corporation, St. Joseph, MI, USA, item no. 528-042) was placed atop

each agar enriched treatment to serve as a growth substrate. The hinged lid received a drill hole (1.9 cm diameter), which allowed the diffusion of nutrients and the growth of algae on the fritted glass disc. The lid was secured down over the disc, aligning the hole in the lid with the fritted glass disc. Nutrient treatments included: phosphorus, nitrogen, nitrogen + phosphorus, and control. Nutrient treatments were amended with a concentration of 0.5 mol N/L as KNO_3. Because of the need to achieve a pH of 7 in each nutrient treatment solution, two compounds of phosphate were needed as a buffering solution to create a total of 0.2 mol P/L: 0.12 mol P/L as K_2HPO_4 and 0.08 mol P/L as KH_2PO_4. The 2% agar control treatments were not enriched with nutrients, or unamended. Five replicates of each treatment were randomly secured with silicon onto each steel holding rack (Figure A.1) and fastened to the stream bed using half foot long rebar stakes. Each holding rack was attached to the streambed for between 21 and 26 days, which allowed for acceptable algal biomass development and nutrient diffusion over the entire algae accrual period (Francoeur, Biggs, Smith, & Lowe, 1999; Rugenski, Marcarelli, Bechtold, & Inouye, 2008; Tank & Dodds, 2003; Winterbourn, 1990). While most nutrient diffusing substrata studies have found that the nutrient concentrations within the diffusers, similar to what were used in this study, have been sufficient to attain nutrient limitation results over their algae accrual periods, other authors have found that nutrient concentrations were depleted after 6 days or could not confirm whether their substrates released enough nitrogen throughout the experiment (Capps et al., 2011; Corkum, 1996).

The protocol used for the collection and preservation of nutrient diffusing substrata is a modification of a USGS protocol for algal biomass (Hambrook Berkman &

Canova, 2014). Following algal biomass development, nutrient diffusing substrata were collected from the river, wrapped in aluminum foil, and kept on dry ice until storage at -20°C. The USGS recommends analysis of chlorophyll a 24 days after collection, but will analyze samples in excess of this holding time, adding a qualifier to the data (Hambrook Berkman & Canova, 2014). A past study noted that freezing algae samples at -20°C for three months decreased chlorophyll a, but not significantly (Wasmund, Topp, & Schories, 2006). Algal biomass growth on the nutrient diffusing substrata in this study was analyzed for chlorophyll a from three to five months after collection following an adapted EPA protocol (Arar & Collins, 1997). The extraction of chlorophyll a from each disc was done in a centrifuge tube with 90% acetone (Arar & Collins, 1997; Tank et al., 2006). All algal biomass samples were analyzed for chlorophyll a and corrected for pheophytin using a BioTek SynergyMx flourometer in the Department of Biological Sciences at Boise State University. A three or four point standard calibration curve was created for each fluorometric analysis to calculate chlorophyll a concentrations. A recommended instrument detection limit of 0.05 µg/L chlorophyll a in 90 % acetone was determined by this protocol (Arar & Collins, 1997). Laboratory method precision was tested by analyzing replicates of field samples during each fluorometric analysis. Variability that may result from the collection, processing, storage, and analysis of algal biomass samples was assessed by using the replicate unamended nutrient diffusing substrata samples (Hambrook Berkman & Canova, 2014). EPA protocol 445.0 (Arar & Collins, 1997) states an upper concentration limit of 250 µg/L. Concentrations from this study generally exceed this concentration limit. Using the area drilled in a nutrient diffusing substrata lid where algae could grow, chlorophyll a (µg/L) concentrations from each disc were

converted to chlorophyll a density (mg/m^2), which is an indicator of algal biomass (Hambrook Berkman & Canova, 2014).

Natural Substrata Sampling

To gain information about the distribution and possible nuisance growth of algal biomass on the natural substrata in the Lower Boise River, we sampled benthic algae from streambed rocks once from study sites in September 2013. Collection and preservation of natural substrata samples followed a modification of a USGS protocol for algal biomass (Hambrook Berkman & Canova, 2014). At all study sites, five randomly chosen rocks from one of the five individual transects spaced ten meters apart were scraped of algae into a bucket. This algae collection process was repeated for each transect. The composite algal slurry from each transects rocks was homogenized using a Vortex Blender (GSI Outdoors, Spokane, WA, USA), sub-sampled (5-10 mL aliquots) onto three different 0.7 µm Whatman glass-fiber filters (GE Healthcare Bio-sciences, Pittsburgh, PA, USA, cat. # 1825047), and frozen on dry ice until storage at -20°C. Holding times for natural substrata samples were similar for the nutrient diffusing substrata samples above. Variability that may result from the collection, processing, storage, and analysis of algal biomass samples was assessed using replicate filtered samples (Hambrook Berkman & Canova, 2014). Chlorophyll a concentrations (µg/L) were analyzed as discussed above for unamended substrata but with differences in laboratory sample preparation for chlorophyll a extraction due to substrate type. Each filter was placed in a tissue grinder with 90 % acetone and ground to a slurry. After samples were ground for one minute in an ice bath, contents were transferred from the tissue grinder to a 15 mL centrifuge tube (extract and filter). The extraction of

chlorophyll a from each filter was done in a 15 mL centrifuge tube with 10 mL of 90% acetone. Following chlorophyll extraction, all samples were brought to room temperature, centrifuged at 1000 g, and analyzed for chlorophyll a concentration (µg/L). Using tracings of sampled rocks to determine planar area (Bergey & Getty, 2006), chlorophyll a density (mg/m^2) (an indicator of algal biomass) was able to be converted from chlorophyll a concentration.

Monitoring of Environmental Variables

Monitoring of the environmental conditions at each site was done for the August and October 2013 nutrient diffusing substrata experiment deployments and the natural substrata algal biomass sampling in September 2013 (considered 3 total sampling events). For the nutrient diffusing substrata experiments, variables were measured at the time of deployment and retrieval of each diffuser rack. The two instantaneous variable measurements from the nutrient diffusing substrata deployment and retrieval were averaged with the assumption that these two measurements would represent the average for that variable during the experiment. The averages for all the variables during one nutrient diffusing substrata experiment would be considered one sampling event, for a total of two sampling events for the nutrient diffusing substrata experiments. For the natural substrata algal biomass sampling, five transects at each site were established ten meters apart. Environmental variables for the natural substrata sampling event were measured once during the time of sampling at the center transect (3rd from the ends) at each site. Temperature and conductivity were measured using a handheld multi-probe (YSI 556 MPS model) system (YSI Incorporated, Yellow Springs, OH, USA). A top setting wading rod and flowmeter (Hach/Marsh McBirney, Loveland, CO, USA) were

used to take measurements of depth and mean water-column velocity (at 60-percent depth) following an adaptation of a USGS protocol (Fitzpatrick et al., 1998). While the depth and mean water-column velocity measurements for the nutrient diffusing substrata experiments were taken at each rack site, measurements for the natural substrata sampling were taken at the center transect at each site. Percent open canopy cover (an estimate of light availability) was measured using a clinometer (Nikon Forestry Pro) following a protocol modified from the USGS (Fitzpatrick et al., 1998). Three individual clinometer measurements at each site were averaged for use in the calculation of percent open canopy cover. Percent open canopy cover was measured one year, to the month, after the August nutrient diffusing substrata deployment.

In-stream water grab samples were collected and used to analyze nitrate (NO_3-N), orthophosphate (PO_4-P), and total suspended solids concentrations (TSS). Grab samples were generally taken in the channel thalweg at approximately 30 cm depth, integrating water from the water column. Triplicate field samples were taken for each sampling event to conduct testing of the variability between samples that may exist due to the entire method process, including collection, heterogeneity of water, and analysis of samples. Total suspended solids samples were preserved and analyzed according to U.S. Environmental Protection Agency (EPA, 1983) protocol 160.2. The procedures for nutrient sampling and preservation of grab samples are a modification of a USGS protocol (USGS, 2006). All nitrate and orthophosphate samples were analyzed three to six days after field collection, which is longer than the EPA recommended holding time of 48 hours (Lachat Application Group, 2013). Sample degradation was possible but

unlikely due to samples being kept on ice during collection and at recommended temperatures during storage (Lachat Application Group, 2013).

In-stream nutrient concentrations were analyzed using the Lachat Quikchem 8500 ion-chromatograph in the Biogeochemistry Laboratory at Boise State University following an EPA 300.00 equivalent protocol (Lachat Application Group, 2013). All nutrient samples were filtered through individual 0.45 micron nylon filters. A six point calibration curve was used for every analysis, including the repeated use of one calibration standard throughout a single analysis to test for instrument accuracy. One field sample, from each sampling event, was split into three lab samples to conduct testing of laboratory method precision. Field blanks were analyzed to test for possible method contamination. The method 300.0 recommended detection limits for nitrate and orthophosphate are 0.003 mg/L and 0.016 mg/L, respectively (Lachat Application Group, 2013). The estimated instrument detection limits for nitrate and orthophosphate were 0.006 mg/L and 0.019 mg/L, respectively (Johannesson, 2005). Since the estimated instrument detection limits for nitrate and orthophosphate are higher than the recommended method detection limits, these higher concentrations are what will be used as the detection limits for this study. From this point on, when the word phosphate is used to describe measured in-stream phosphate from this study, it is referring to orthophosphate.

Statistical Analyses

To determine if the algal biomass community at each site was nutrient limited, a two-way ANOVA was used to analyze the response of algal biomass (mg chlorophyll a/m^2) to additions of nitrate and phosphate. The two-way ANOVA used nitrate

(present/absent) and phosphate (present/absent) as factors and allowed the determination of the significant interaction response of algal biomass when nitrate and phosphate (N + P) were added together. Hochberg post-hoc adjustment ($P < 0.05$) was used to differentiate mean algal biomass (mg chlorophyll a/m^2) differences among nutrient treatments. A lettering system was used for graphical purposes above each response of mean algal biomass (mg chlorophyll a/m^2) to the addition of nutrients to symbolize statistical differences or similarities. Mean algal biomass (mg chlorophyll a/m^2) responses to nutrient treatments, all with the letter "a", were statistically the same. Mean algal biomass (mg chlorophyll a/m^2) responses to nutrient treatments with different letters (i.e., "a", "b", "c") were not statistically the same. Guidance regarding nutrient limitation classification was taken from Tank and Dodds (2003).

To assess whether algal biomass downstream of the Lander wastewater treatment plant was statistically different compared to upstream, sites downstream of the Lander plant were grouped and compared to sites grouped upstream of the Lander plant. Because nutrient diffusing substrata racks, during a single deployment, were left in the river for differing lengths of time, chlorophyll a density accrual time differences from unamended substrata were normalized to help assess the spatial distribution of algal biomass in the Lower Boise River (Stevenson, 1996). This assessment of the general spatial distribution of algal biomass was accomplished by comparing the mean of the algal biomass accrual rate (mg chlorophyll a/m^2/day) from grouped sites downstream of the Lander plant with the grouped sites upstream, using a one-way ANOVA ($P < 0.05$) with site as a fixed factor. This analysis was conducted for the October 2013 nutrient diffusing substrata deployment only due to sample availability. The same grouped site one-way ANOVA (P

< 0.05) was assessed using the September 2013 natural substrata, except this analysis was conducted using algal biomass (mg chlorophyll a/m^2).

To evaluate if algal biomass among sites downstream of nutrient loading (below Lander plant) were statistically different, individual one-way ANOVA were conducted using the mean algal biomass accrual rate (mg chlorophyll a/m^2/day) from unamended substrata and mean algal biomass (mg chlorophyll a/m^2) from natural substrata (rocks). A Hochberg adjustment for multiple comparisons was used for each analysis ($p < 0.05$). Individual one-way ANOVA were conducted for each individual nutrient diffusing substrata deployment (August and October) and the natural substrate (rocks) data. Although the assumption of autocorrelation was tested and verified for all ANOVA models, the hydrological dependence of the sites downstream may still be present in the outcome variables.

Values for algal biomass (mg chlorophyll a/m^2) and algal biomass accrual rate (mg chlorophyll a/m^2/day) were ln-transformed to normalize distributions prior to statistical analyses. Residuals for linear models were tested for and all assumptions were verified. All levels of statistical significance were set at $\alpha = 0.05$ unless otherwise stated. Statistical analyses were conducted using R Studio version 0.98.1091.

CHAPTER THREE: RESULTS

Measured Environmental Conditions

A qualitative analysis of the physicochemical variables measured in the Lower Boise River during both nutrient diffusing substrata experiment deployments and the natural substrata sampling was conducted. A general downstream gradient among some of the measured variables in the Lower Boise River was indicated (Figure 1; Table 1.1, 1.2). For example, in-stream nitrate and phosphate concentrations in this study increased from sites upstream to sites downstream by as much as 245 times and approximately 9 times, respectively (Figure 1; Table 1.1, 1.2). These concentrations are generally

Table 1.1 Physical and chemical variables sampled at each site during the August and October 2013 nutrient diffusing substrata deployments on the Lower Boise River. All variables (except canopy) at each site are mean values calculated from instantaneous samples acquired during deployment and retrieval of nutrient diffusing substrata. Estimated instrument detection limits for nitrate and orthophosphate are 0.006 mg/L and 0.019 mg/L, respectively (Johannesson, 2005). (Abbreviations: TSS (Total Suspended Solids); Cond (Conductivity); Canopy (Percent Open Canopy Cover).)

AUGUST								
Site	NO_3-N (mg/L)	PO_4-P (mg/L)	TSS (mg/L)	Temp (°C)	Cond (µS/c)	Depth (ft)	Velocity (ft/s)	Canopy (%)
1	0.02	0.01[a]	2.1[b]	17.40	80	1.40	1.68	71
2	0.01	0.01[a]	1.3[b]	18.88	79	1.80	1.46	66
3	0.01	0.01[a]	2.8[b]	19.23	79	1.40	2.50	52
4	0.22	0.01[a]		20.05	95	1.90	2.35	82
5	1.27	0.15	4.6	20.38	130	1.65	3.55	46
6	0.44	0.06	7.0	21.17	133	1.60	2.60	57
7	1.09	0.12	9.0	22.79	204	1.25	2.44	64

Site	NO_3-N (mg/L)	PO_4-P (mg/L)	TSS (mg/L)	Temp (°C)	Cond (µS/c)	Depth (ft)	Velocity (ft/s)	Canopy (%)
8	1.54	0.14	12	21.99	273	1.40	2.85	75
9	2.12	0.18	16	23.19	392	1.40	2.13	82
10	1.92	0.15	31	23.21	421	1.20	2.72	90
12	1.76	0.16	32	26.15	448	0.70	2.13	82
OCTOBER								
1	0.02	0.01[a]	1.3[b]	14.87	119	1.60	1.12	71
2	0.02	0.01[a]	1.3[b]	14.07	92	2.00	0.53	66
3	0.02	0.01[a]	1.0[b]	14.13	92	1.40	1.68	52
4	0.46	0.06	1.2[b]	13.46	122	1.60	1.60	82
5	3.40	0.56	3.1[b]	15.47	211	1.80	1.63	46
6	1.30	0.19	3.9[b]	14.13	180	1.15	1.43	57
7	2.49	0.23	4.1	13.42	260	1.15	2.21	64
8	2.54	0.15	10	12.11	324	1.80	2.88	75
9	2.96	0.17	7.2	12.17	382	1.75	3.66	82
10	3.23	0.17	13	11.33	411	1.35	3.50	90
12	3.20	0.16	13	10.77	445	1.60	2.44	82

[a]: Indicates below estimated instrument detection limit for nutrient; [b]: Indicates below practical range of determination for total suspended solids concentration (4 mg/L).

Table 1.2 Physical and chemical variables sampled at each site during the September 2013 natural substrata algal biomass sampling on the Lower Boise River. All variables (except canopy) at each site are values from samples acquired during retrieval of algal biomass samples. Estimated instrument detection limits for nitrate and orthophosphate are 0.006 mg/L and 0.019 mg/L, respectively (Johannesson, 2005). (Abbreviations: TSS (Total Suspended Solids); Cond (Conductivity); Canopy (Percent Open Canopy Cover).)

Site	NO_3-N (mg/L)	PO_4-P (mg/L)	TSS (mg/L)	Temp (°C)	Cond (µS/cm)	Depth (ft)	Velocity (ft/s)	Canopy (%)
1	0.02	0.02	1.7[b]	17.42	98	2.10	1.80	71
2	0.01	0.01[a]	2.0[b]	17.16	84	2.25	1.31	66
3	0.01	0.02		18.31	84	1.70	2.82	52
4	0.20	0.01[a]	1.5[b]	18.03	105	1.75	2.49	82
5	1.78	0.22	3.4[b]	19.24	151	1.50	2.82	46
6	0.74	0.08	4.0	18.33	143	1.70	1.87	57
7	1.00	0.11	5.7	22.38	196	1.20	2.46	64
8	1.63	0.12	13	22.44	270	1.60	3.28	75

Site	NO_3-N (mg/L)	PO_4-P (mg/L)	TSS (mg/L)	Temp (°C)	Cond (µS/cm)	Depth (ft)	Velocity (ft/s)	Canopy (%)
9	2.09	0.15	13	21.18	353	1.50	2.95	82
11	2.38	0.15		21.41	395	1.70	1.84	87
12	2.45	0.15	29	20.06	440	2.30	2.00	82

[a]: Indicates below estimated instrument detection limit for nutrient; [b]: Indicates below practical range of determination for total suspended solids concentration (4 mg/L).

consistent with past downstream concentration trends that occur year over year (Figure 1.2, 1.3; Yelen, 2015). Measured in-stream nitrate and phosphate concentrations upstream of the Lander wastewater treatment plant were relatively low, with most phosphate concentration values below this study's estimated instrument detection limit (Figure 1.2, 1.3; Table 1.1, 1.2). Potential increases in in-stream nutrient concentrations downstream of most of the wastewater treatment plants were qualitatively identified, with a noticeable change higher in in-stream nutrient concentrations beginning downstream of the Lander plant (site 4) (Figure 1.2, 1.3; Table 1.1, 1.2). A qualitative analysis of the data indicates that the in-stream nutrient concentrations measured during the October nutrient diffusing substrata sampling event were generally higher at most sites than those measured during the August nutrient diffusing substrata sampling event and the natural substrata sampling event (Table 1.1, 1.2). Irrigation return water may have contributed to these lower nutrient concentration samples.

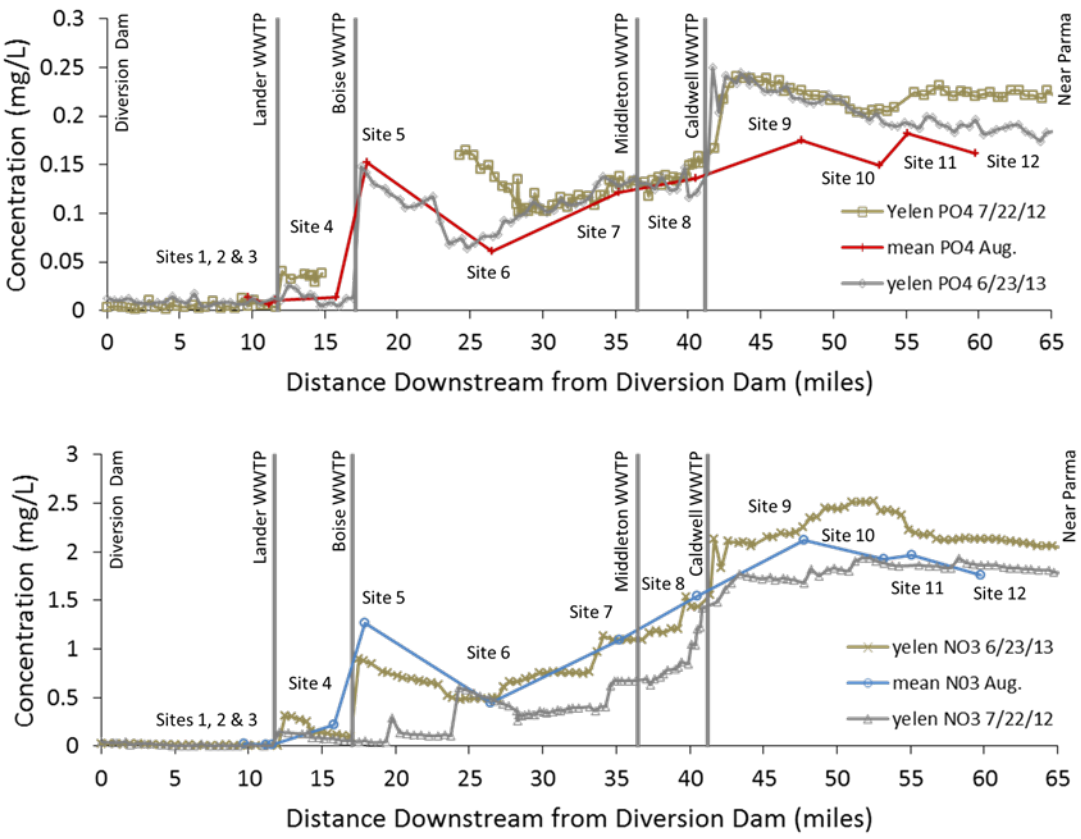

Figure 1.2 Mean in-stream nutrient concentrations at each site during the August 2013 nutrient diffusing substrata deployment (red and blue lines). Included is nutrient concentration data from Master's student Brian Yelen (brown and grey lines) (Yelen, 2015).

Non-detect nutrient concentration values from field blanks suggest no signs of contamination from nutrient field sampling practices (Appendix B). Four out of the five variability analyses of field triplicate samplings for nitrate and phosphate showed no higher than a 4% RSD (Relative Standard Deviation), but one sampling showed 11.71% and 17.87% RSD, respectively (Appendix B). These results suggest that the variability between samples that may exist due to the entire method process, including collection, heterogeneity of water, and analysis of samples, was generally low. However, the potential for higher variability existed during the August nutrient diffusing substrata retrieval sampling (Table B.2). Instrument accuracy had percent average errors that

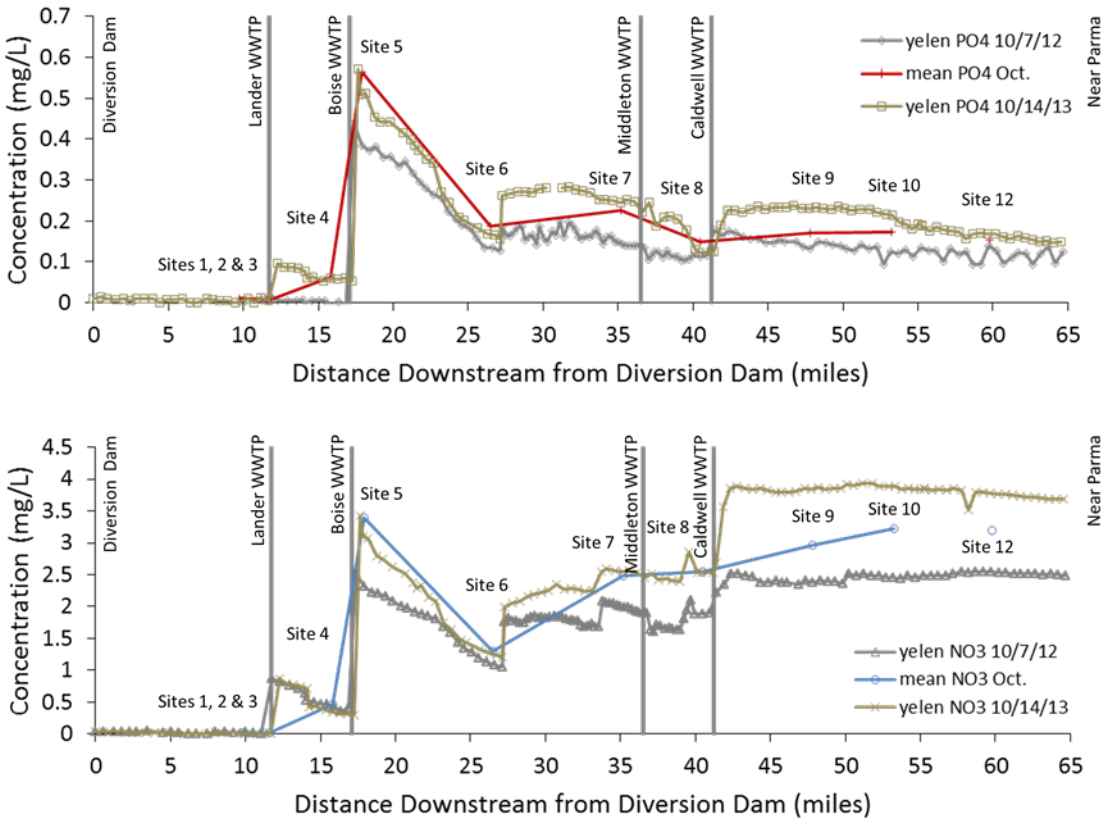

Figure 1.3 Mean in-stream nutrient concentrations at each site during the October 2013 nutrient diffusing substrata deployment (red and blue lines). Included is nutrient concentration data from Master's student Brian Yelen (brown and grey lines) (Yelen, 2015).

ranged from 0.67% to 11% for nitrate and phosphate, for all nutrient concentration analyses (Appendix B). The laboratory method precision results suggest that nitrate variability ranged between 3.84% and 18.54% RSD, but phosphate for most samplings was unable to be calculated due to concentrations below detection (Appendix B). Because the sample chosen (site 3) to be analyzed for the laboratory method precision had nitrate concentrations near the nitrate estimated instrument detection limit for this study, the variability was potentially increased. We saw reduced laboratory method precision results for nitrate when samples with higher concentrations were used, like in

the October 2013 deployment (nutrient diffusing substrata) and the natural substrata sampling (Table B.3, B.5).

Other measured variables showed a general downstream gradient. For example, each sampling event (nutrient diffusing substrata experiments and natural substrata sampling) showed total suspended solid concentrations beginning below the practical range of determination at sites upstream, then increasing downstream, resulting in a range of concentrations from 13 to 32 mg/L for all sampling events at the farthest downstream site (site 12) (Table 1.1, 1.2). The gradient in measured temperature among sites downstream for each sampling event differed. Temperatures measured at sites during the August nutrient diffusing substrata and natural substrata sampling events demonstrate a potentially small increase in temperature downstream (Table 1.1, 1.2). However, the temperature measured during the October nutrient diffusing substrata sampling event demonstrated a potentially small decrease in temperature moving downstream from 14.87 °C to 10.77 °C (Table 1.1). The gradient in measured mean water-column velocity among sites downstream for each sampling event differed. The mean water-column velocity measured during the October nutrient diffusing substrata sampling event is the only sampling that demonstrated a general increase from upstream to downstream, increasing from 1.12 ft./s to 2.44 ft./s (Table 1.1). There was an increasing gradient for measured conductivity downstream among sites for each sampling event (Table 1.1, 1.2).

Measured variables that did not demonstrate a general downstream gradient include depth and percent open canopy cover. The depth at which the nutrient diffusing substrata were placed and the depth the natural substrata were sampled was relatively consistent among sites, with most sites ranging from 1.15 ft. to 2.30 ft. (Table 1.1, 1.2).

Percent open canopy cover was similarly open at most sites (64-87 % open canopy) except sites 3, 5, and 6 where canopy was more closed (46-57 % open canopy) (Table 1.1, 1.2).

Nutrient Diffusing Substrata Loss

Due to nutrient diffusing substrata loss (vandalism or other means) or error in labelling, certain sites have been eliminated from sampling and analysis.

Nutrient Limitation of Algal Biomass

Two deployments of the nutrient diffusing substrata experiment were conducted to assess the nutrient limitation of the algal biomass community in the Lower Boise River. The October deployment was more complete because of missing samples in August. Algal biomass (mg chlorophyll a/m^2) was determined to be nitrate-limited at sites upstream of the Lander wastewater treatment plant (sites 1, 2, and 3) during the October nutrient diffusing substrata deployment (Figure 1.4; Table 1.3). The algal biomass responded to the nitrate treatment enrichment showing a significant increase in algal biomass over the unamended substrata (control) (Figure 1.4; Table 1.3). No nutrient treatment (NO_3-N, PO_4-P, NO_3-N + PO_4-P) enrichment limited the algal biomass downstream of the Lander plant during the August and October nutrient diffusing substrata deployments (except site 7 in August) (Figure 1.4, 1.5; Table 1.3). Algal biomass at site 7 during the August nutrient diffusing substrata deployment was found to be simultaneously limited by phosphate and suppressed by nitrate (Figure 1.5; Table 1.3). The addition of nitrate and phosphate suppressed algal biomass at sites downstream (sites 6, 8) of the Lander plant (Figure 1.4, 1.5; Table 1.3).

Table 1.3 Nutrient limitation status at each site for August and October deployments of nutrient diffusing substrata on the Lower Boise River, 2013. Statistics presented determined using a Two-way ANOVA ($\alpha = 0.05$) with Hochberg post hoc adjustment.

Site	Deployment	N (p-val)	P (p-val)	NxP (p-val)	Nutrient Limitation
1	August	-	-	-	-
	October	<0.01	0.92	0.12	N limitation
2	August	-	-	-	-
	October	<0.001	0.19	0.60	N limitation
3	August	-	-	-	-
	October	<0.001	0.63	0.28	N limitation
4	August	0.55	0.85	0.37	NS
	October	0.26	0.64	0.01	NSWA
5	August	-	-	-	-
	October	0.81	0.15	0.36	NS
6	August	0.96	0.29	<0.001	Cosuppression by N and P
	October	-	-	-	-
7	August	<0.001	0.01	0.94	P limitation, N suppression
	October	-	-	-	-
8	August	0.016	0.066	0.65	N suppression
	October	<0.01	0.15	0.39	N suppression
9	August	-	-	-	-
	October	0.37	0.92	0.15	NS
10	August	0.87	0.69	0.74	NS
	October	-	-	-	-
11	August	-	-	-	-
	October	-	-	-	-
12	August	-	-	-	-
	October	0.57	0.43	0.63	NS

NS: not significant, -: not analyzed, NSWA: No Significance When Adjusted using Hochberg

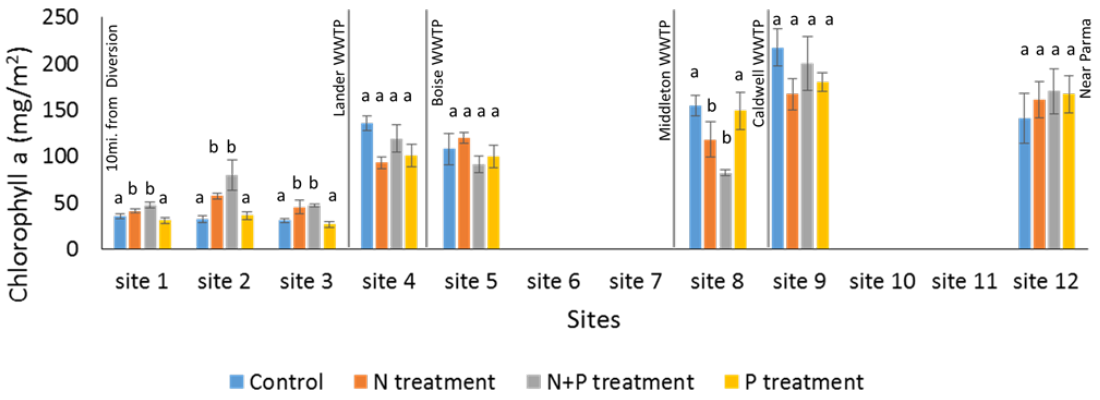

Figure 1.4 Results of October, 2013 nutrient limitation for Lower Boise River sites. Mean algal biomass (mg chlorophyll a/m^2) from nutrient diffusing substrata treatments: control, nitrate (N), phosphate (P), and nitrate (N) + phosphate (P). Each graphed treatment (bar) at each site has n = 5 (with some exceptions where samples were missing) and indicates the mean chlorophyll a ± standard error. Different letters above bars at each site indicate significant effects determined by ANOVA results (α < 0.05). Sites are arranged from upstream (site 1) to downstream (site 12) with locations of wastewater treatment plants (WWTP) that discharge directly into the main-stem river.

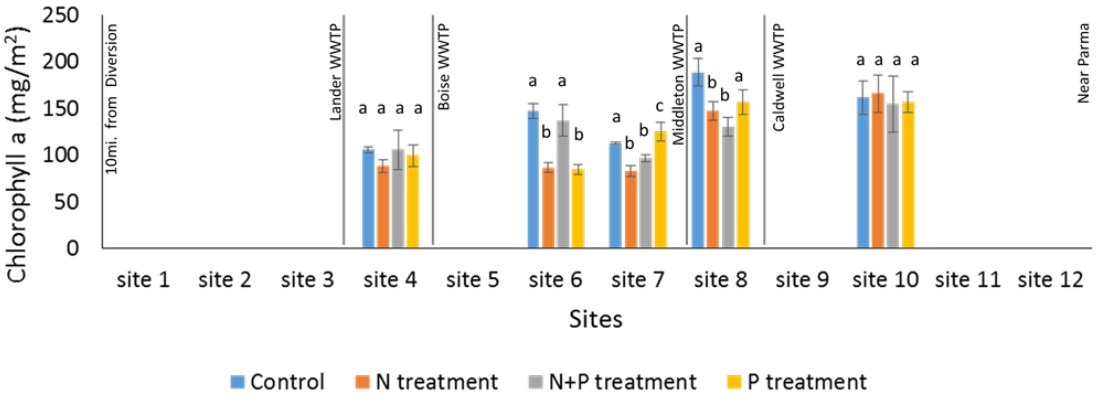

Figure 1.5 Results of August, 2013 nutrient limitation for Lower Boise River sites. Mean algal biomass (mg chlorophyll a/m^2) from nutrient diffusing substrata treatments: control, nitrate (N), phosphate (P), and nitrate (N) + phosphate (P). Each graphed treatment (bar) at each site has n = 5 (with some exceptions where samples were missing) and indicates the mean chlorophyll a ± standard error. Different letters above bars at each site indicate significant effects determined by ANOVA results (α<0.05). Sites are arranged from upstream (site 1) to downstream (site 12) with locations of wastewater treatment plants (WWTP) that discharge directly into the main-stem river.

Distribution of Algal Biomass

Mean algal biomass (mg chlorophyll a/m^2) in the Lower Boise River on unamended (nutrient diffusing substrata) and natural substrata ranged from 30.9 mg/m^2 to 217 mg/m^2 and 26.8 mg/m^2 to 127 mg/m^2, respectively (Table 1.4). While the lowest observed mean algal biomass values on unamended substrata occurred at sites upstream of the Lander wastewater treatment plant, the highest values occurred at sites downstream of the Lander plant (Figure 1; Table 1.4). Spatial variation in algal biomass was shown to

Table 1.4 Mean algal biomass (mg chlorophyll a/m^2) and mean algal biomass accrual rates (mg chlorophyll a/m^2/day) from August and October 2013 unamended substrata (nutrient diffusing substrata) in the Lower Boise River. Mean algal biomass (mg chlorophyll a/m^2) from September 2013 natural substrata (rocks) in the Lower Boise River. (Coefficient of Variation (%)).

Site	Chlorophyll a Accrual Rate (mg/m^2/day)	Chlorophyll a (mg/m^2)	Chlorophyll a (mg/m^2)
August	**Unamended Substrata**	**Unamended Substrata**	**Natural Substrata (Sept)**
1	-	-	54.3 (29.0)
2	-	-	46.0 (20.5)
3	-	-	26.8 (31.9)
4	4.08 (7)	106 (7)	101 (8)
5	-	-	82.7 (-)
6	5.89 (10)	147 (10)	81.8 (30.3)
7	4.52 (2)	113 (2)	40.1 (51.7)
8	7.55 (17)	189 (17)	127 (26)
9	-	-	43.4 (29.7)
10	6.48 (25)	162 (25)	-
11	-	-	44.9 (30.1)
12	-	-	6.84 (29.7)
October			
1	1.6 (18.1)	35.2 (18.1)	
2	1.47 (27.9)	32.4 (27.9)	
3	1.41 (16.8)	30.9 (16.8)	
4	6.18 (12)	136 (12)	
5	5.13 (31)	108 (31)	
6	-	-	
7	-	-	

Site	Chlorophyll a Accrual Rate (mg/m^2/day)	Chlorophyll a (mg/m^2)	Chlorophyll a (mg/m^2)
October	**Unamended Substrata**	**Unamended Substrata**	**Natural Substrata (Sept)**
8	7.03 (16)	155 (16)	
9	9.88 (20)	217 (20)	
10	-	-	
12	6.42 (38)	141 (38)	

-: Indicates missing data

occur between grouped sites upstream and downstream of the Lander plant. The upstream group included sites 1, 2, and 3, while the downstream group included sites 4 -12. The mean algal biomass accrual rate from grouped October unamended substrata sites downstream of the Lander plant were significantly greater than the grouped sites upstream (one-way ANOVA, $F(7,30) = 50.20$, $p = < 0.001$; Figure 1.6; Table C.1). Similarly, the mean algal biomass from grouped September natural substrata sites downstream of the Lander plant were significantly greater than the grouped sites upstream (one-way ANOVA, $F(10,41) = 31.65$, $p = < 0.001$; Figure 1.7; Table C.1).

Significant differences in algal biomass were shown to occur among individual sites downstream of the Lander plant. The mean algal biomass accrual rate differed among some sites downstream of the Lander plant for each individual analyses (August and October nutrient diffusing substrata) of the unamended substrata (one-way ANOVA, October: $F(7,30) = 50.20$, $p = < 0.001$; August: $F(4,17) = 11.00$, $p = < 0.001$; Figure 1, 1.6; Table D.1). Similarly, the mean algal biomass differed among some sites downstream of the Lander plant for the natural substrata (one-way ANOVA, $F(10,41) = 31.65$, $p = < 0.001$; Figure 1, 1.7; Table D.1). Qualitative analysis of unamended and natural substrate data indicates that the development of algal biomass at each site may

differ between substrate types (Table 1.4; Porter, Cuffney, Gurtz, & Meador, 1993; Tuchman & Stevenson, 1980).

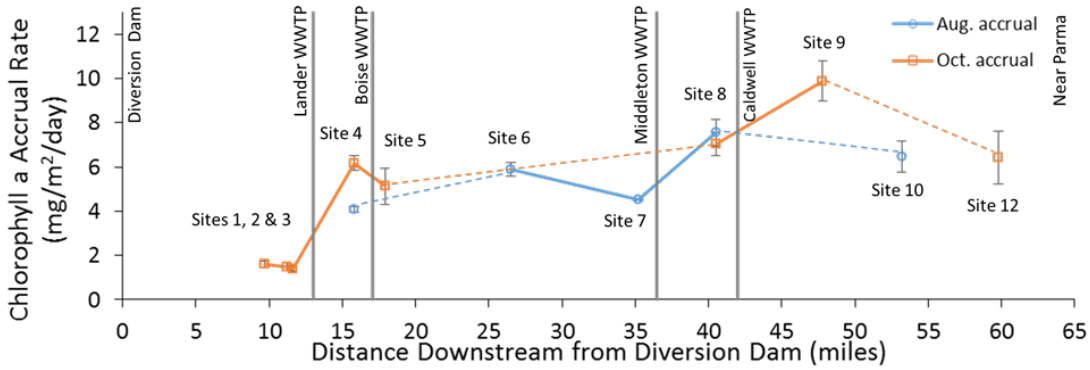

Figure 1.6 Mean algal biomass accrual rate (mg chlorophyll a/m^2/day) from August and October 2013 unamended substrata (nutrient diffusing substrata) along the longitudinal reach of the Lower Boise River. Included are locations of wastewater treatment plants (WWTP) that discharge directly into the main-stem of the river. The mean algal biomass accrual rate from October grouped sites downstream of the Lander wastewater treatment plant is significantly larger than the grouped sites upstream (F(7,30) = 50.20, p = < 0.001, Table C.1). The mean algal biomass accrual rate differs among some sites downstream of the Lander plant for both the August and the October unamended substrata (October: F(7,30) = 50.20, p = < 0.001, August: F(4,17) = 11.00, p = < 0.001, Table D.1).

Quality assurance results for algal biomass (Table E.1, E.2) developed on nutrient diffusing substrata from both the August and October deployments suggest that the variability associated with substrata within the same treatment group is consistent with past studies (Matlock, Storm, Smolen, & Matlock, 1999). The variability associated with the laboratory method for nutrient diffusing substrata suggests a percent relative standard deviation in the range of 0.1 to 18% (Table E.1, E.2). The variability calculated from natural substrata algal biomass in this study is consistent with the variability calculated from past natural substrata algal biomass samples from the Lower Boise River (Table E.3; Mullins, 1999b). The laboratory method variability associated with the natural

substrata algal biomass in this study is similar to the nutrient diffusing substrata, with a percent relative standard deviation in the range of 0.9 to 15.67% (Table E.3).

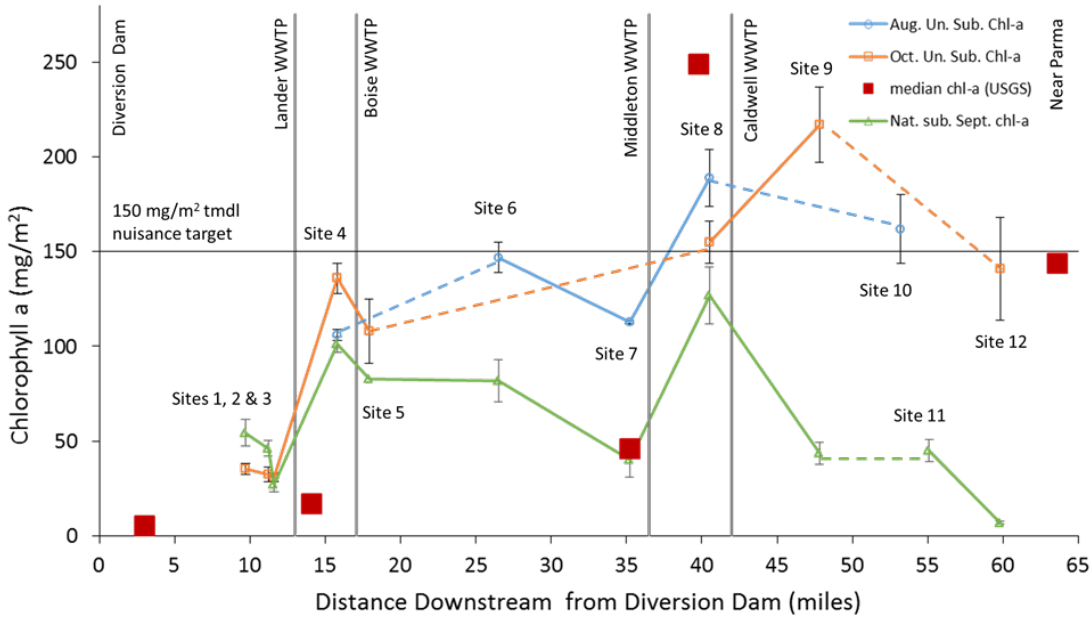

Figure 1.7 Mean algal biomass (mg chlorophyll a/m^2) from August and October 2013 unamended substrata (nutrient diffusing substrata), and from September 2013 natural substrata sampling along the longitudinal reach of the Lower Boise river. Red squares indicate the median historical (1995-2013) algal biomass (from natural substrata) recorded by the USGS at sites along the Boise River. The 150 mg/m^2 line indicates the proposed Total Maximum Daily Load algal biomass nuisance target for the Lower Boise River. Included are locations of wastewater treatment plants (WWTP) that discharge directly into the main-stem of the river. Chlorophyll a estimates indicate potential nuisance algal biomass growth downstream of the Lander wastewater treatment plant. The mean algal biomass from grouped natural substrata sites downstream of the Lander wastewater treatment plant is significantly larger than the grouped sites upstream ($F(10,41) = 31.65$, $p = < 0.001$; Table C.1).

CHAPTER FOUR: DISCUSSION

Algal Biomass and its Nutrient Limitation

Nutrient Limitation Upstream of Nutrient Loading

Based on the nutrient diffusing substrata results, algal biomass growth is generally nitrate-limited upstream of the first wastewater discharge location (Lander plant) (Table 1.3, Figure 1.4). These results are consistent with low in-stream mean nitrate concentrations at these sites as well as literature threshold values found to stimulate algal biomass growth (Table 1.1; Bothwell, 1985, 1989; Grimm & Fisher, 1986; Horner, Welch & Veenstra, 1983; Lohman et al., 1991). Furthermore, these results are consistent with other studies, including the nutrient diffusing substrata study conducted in southeast Idaho, where nitrate was the most common limiting nutrient in the system (Marcarelli, Bechtold, Rugenski, & Inouye, 2009; Tank & Dodds, 2003; Grimm & Fisher, 1986). A lack of response in algal biomass from additions of phosphate (either alone or in combination with nitrate) at sites upstream of the Lander plant suggests nitrate is the limiting nutrient and that phosphate is not secondarily limiting (Table 1.3, Figure 1.4; Grimm & Fisher, 1986). In other words, when nitrate is added, phosphate does not become depleted and limited, therefore suggesting that phosphate is above the limiting concentration and is sufficiently elevated to support growth of nitrate enriched algal biomass (Grimm & Fisher, 1986). Phosphate concentrations that typically limit algal biomass growth ($\leq$ 0.015 mg/L) indicate that measured in-stream mean phosphate

concentrations upstream of the Lander plant (at detection level of 0.019 mg/L) are most likely high enough to saturate algal biomass growth (Table 1.1; Newbold, 1992).

No Nutrient Limitation Downstream of Nutrient Loading

Algal biomass growth is generally not nutrient limited downstream from the first wastewater discharge location (Lander plant) (Table 1.3; Figures 1.4, 1.5). Elevated in-stream mean nutrient concentrations measured downstream of the Lander plant suggest algal biomass growth is saturated with nutrients and the general lack of nutrient limitation may be due to these elevated concentrations. Nitrate has been found to limit algal growth below concentrations of 0.10 mg/L and 0.055 mg/L (Grimm & Fisher, 1986; Lohman et al., 1991). All in-stream mean nitrate concentrations measured downstream of the Lander plant are above 0.10 mg/L, indicating potential saturation of algal biomass growth downstream of the Lander plant (Figure 1; Table 1.1). Phosphorus has been shown to limit algal growth with phosphate concentrations in the range of 0.003 to 0.05 mg/L (Bothwell, 1985, 1989; Horner et al. 1983). All in-stream mean phosphate concentrations measured downstream of the Lander plant (except August site 4) are above 0.05 mg/L, indicating potential saturation of algal biomass growth downstream of the Lander plant (Figure 1; Table 1.1). According to these generally accepted nutrient concentration thresholds, most algal biomass growth downstream of the Lander plant may be considered saturated with in-stream nutrients, leading to a lack of nutrient limitation. The nutrient diffusing substrata experiment results demonstrate the potential influence of nutrient loading, beginning below the Lander plant, on the spatial distribution of algal biomass nutrient limitation, as well as the potential influence on the spatial distribution of algal biomass.

Increased Algal Biomass Downstream of Nutrient Loading

The measured October in-stream nitrate and phosphate concentrations increased 23 times and approximately 3 times downstream of the Lander wastewater treatment plant, respectively (Figure 1; Table 1.1). Concurrently, a significant increase in mean algal biomass accrual rate from grouped sites downstream (sites 4-12) of the Lander plant compared to upstream sites (sites 1-3) is demonstrated using the October unamended substrata (nutrient diffusing substrata) (Figure 1.6, Table C.1). Similarly, a significant increase in mean algal biomass from grouped sites downstream (sites 4-12) of the Lander plant compared to upstream sites (sites 1-3) is demonstrated using the September natural substrata (Figure 1.7, Table C.1). These observations are consistent with a report by Etheridge (2013) that states that effluent from the Lander plant may promote algal biomass growth. These observations indicate algal biomass and its nutrient limitation is most likely influenced by nutrient loading, beginning downstream of the Lander wastewater treatment plant.

Additional Factors That May Influence Algal Biomass and Nutrient Limitation

Statistically significant differences in algal biomass in the river section downstream of the Lander wastewater treatment plant suggest other factors likely influence algal growth and potentially influence nutrient limitation. Potential additional variables that may be important include light availability, temperature, velocity, invertebrate grazing, and flooding (Francoeur, 2013; Scrimgeour & Chambers, 1997).

Light Availability (Canopy Cover)

Past studies have demonstrated that reduced light availability by riparian vegetation, rather than nutrients, has been shown to limit algal biomass growth (Hill &

Knight, 1988; Winterbourn, 1990). In our study, sites 3, 5, and 6 have percent open canopy cover values relatively lower than the rest of the sites (Table 1.1). Therefore, the nutrient limitation results at sites 5 and 6 may be influenced by light availability (Table 1.3). The significant nutrient limitation result at site 3 in October (Table 1.3), however, suggests that site 3 has adequate light availability for algal growth.

Turbidity

Previous studies have shown that sediment in the water column that increases turbidity may potentially reduce algal biomass due to light attenuation, deposition, and/or scouring (Cline, Short, & Ward, 1982; Figueroa-Nieves, Royer, & David, 2006; Van Nieuwenhuyse & LaPerriere, 1986; Wood & Armitage, 1997). Site 12 (near the city of Parma) has a relatively lower mean algal biomass (September natural substrata) value and a relatively higher total suspended solids concentration than other natural substrata sites (Figure 1.8), suggesting a potential influence of total suspended solids on algal biomass growth. It should be noted that a higher discharge event before the September natural substrata sampling near site 12 (Parma USGS Gaging Station) may have potentially influenced the natural substrata algal biomass value at site 12 (Figure G.1). Visual inspection during samplings suggests the river becomes more turbid downstream. This is consistent with past finding by the USGS, which states that light limitation owing to high turbidity limits algal biomass growth near the city of Parma (this study's site 12) (Etheridge, 2013). Therefore, along with elevated nutrients, total suspended solids in the water column may contribute to the lack of nutrient limitation results at site 12 by limiting algal biomass growth.

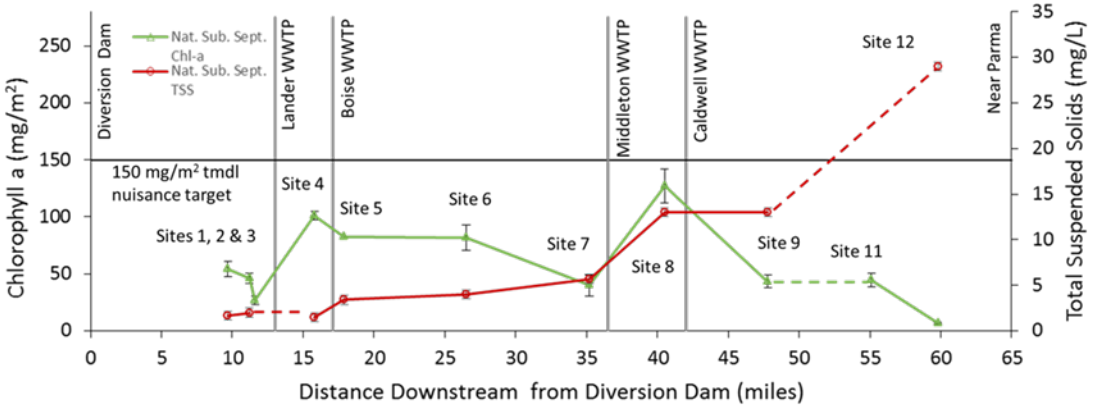

Figure 1.8 Mean algal biomass (mg chlorophyll a/m^2) and total suspended solids concentrations from the September 2013 natural substrata sampling. The relatively lower mean algal biomass and relatively higher mean total suspended solids concentration at site 12 may indicate an inhibitory influence of total suspended solids on algal biomass growth. Included are locations of wastewater treatment plants (WWTP) that discharge into the main-stem of the river.

Water Velocity

Water velocity can influence algal biomass through the offsetting mechanisms of biomass enhancement through increased nutrient transport to cells and reduction of biomass through sloughing (Borchardt, 1996; Stevenson, 1996). It is unlikely that a majority fraction of the algal biomass at any site was lost due to sloughing because no high velocity flood events occur during any nutrient diffusing substrata experiment (Figure G.1; Biggs & Close, 1989).

Temperature

Temperature is a variable that has been shown to positively influence algal biomass growth in some systems (Francoeur et al., 1999). A qualitative analysis of the in-stream mean water temperatures measured during the nutrient diffusing substrata sampling events suggests that temperatures were lower at all sites in October than in August (Table 1.1). Because significant nutrient limitation occurred in August at higher

temperatures, and in October at lower temperatures (Table 1.1, 1.3), nutrient limitation results were most likely not influenced by temperature in this system.

Grazers

Although invertebrate grazers have been shown to influence the results of nutrient diffusing substrata studies (Lohman et al., 1991), we do not try to protect our nutrient diffusing substrata from this possibly limiting factor. In an eight-year study, MacCoy (2004) stated that the concentration of chlorophyll a did not seem to be limited by the excessive grazing of algal biomass by macro-invertebrates. Invertebrate grazers are rarely observed on nutrient diffusing substrata when they are retrieved from the river during this study. Therefore, we make the assumption that grazers do not strongly influence nutrient limitation results.

August Site 7 Does Not Follow Overall Trends

The nutrient limitation at site 7 in August does not follow the overall trend in the Lower Boise River (Table 1.3; Figure 1.4, 1.5). It is unclear why this nutrient limitation result did not follow the overall trend, but potential reasons are offered here. First, algal species composition may vary with the addition of different nutrient treatments at each site, potentially altering the limitation status by stimulating growth of taxa with higher chlorophyll a concentrations (Bernhardt & Likens, 2004; Lowe et al., 1986; Pringle & Bowers, 1984). Second, micronutrients (Fe, B, Mn, Zn, Co, Mo, EDTA) may become limiting in the elevated nitrate and phosphate conditions, resulting in a significant limitation result at site 7 (Pringle, Paaby-Hansen, Vaux, & Goldman, 1982).

Evidence of Growth Suppression Downstream of Nutrient Loading

Suppression of algal biomass with the addition of nutrients occurs downstream of the Lander wastewater treatment plant in relatively elevated nutrient conditions (Table 1.1, 1.3; Figure 1.4, 1.5). These results are consistent with past studies where nitrogen and/or phosphorus suppressed algal biomass (Bernhardt & Likens, 2004; Francoeur, 2013; Hill & Knight, 1988; Marcarelli et al., 2009; Tank & Dodds, 2003). Bernhardt and Likens (2004) have proposed several potential mechanisms by which nutrient enrichment might lead to the suppression of algal biomass. These mechanisms may include: (1) grazing invertebrates feed on high-nutrient periphyton grown on N and P enriched substrates; (2) nutrient levels are toxic to stream periphyton; (3) nutrient addition differentially promotes growth of periphyton taxa with lower chlorophyll concentrations or (4) the addition of nutrients could stimulate bacterial growth, that inhibit periphyton growth (p. 24). This study has insufficient field data to support any one explanation for the suppression of algal biomass according to these mechanisms. A final potential explanation of observed suppression is the use of K_2HPO_4 as a phosphate source in diffusers; Potassium has been shown to have a toxic effect on certain species of algae, inhibiting growth (Lowe et al., 1985; Lehman, 1976).

Does the Algal Biomass Exceed Nuisance Growth Levels?

Nuisance algal biomass thresholds are established to prevent aesthetic and biological health impacts on streams. Welch et al. (1988) proposed that chlorophyll a densities of 100 - 150 mg/m^2 would represent nuisance conditions for algal biomass growth. The Idaho Department of Environmental Quality has accepted a $\leq$ 150 mg/m^2

total maximum daily load target for algal biomass in the nutrient impaired lower sections of the Boise River (DEQ, 2015).

Unamended artificial and natural substrata are often used in water quality assessment protocols to indicate nuisance conditions (McPherson, Gill, & Moreland, 2005; Tuchman & Stevenson, 1980), although the development of algal biomass communities likely differ. In this study, both are examined. The mean chlorophyll a density values (mg/m^2) at all unamended artificial substrata sites downstream of the Lander plant are above the 100 mg/m^2 nuisance threshold (Table 1.4, Figure 1.7). Nearly 50% of the unamended artificial substrata sites downstream of the Lander plant potentially exceed the 150 mg/m^2 nuisance algal biomass threshold (Table 1.4, Figure 1.7).

Mean algal biomass on the natural substrata exceed the 100 mg/m^2 nuisance condition threshold at two sites downstream of the Lander plant, but never exceed the 150 mg/m^2 total maximum daily load target (Table 1.4, Figure 1.7). These results are consistent with previously observed exceedances of the 100 mg/m^2 nuisance threshold in the Boise River (Etheridge, 2013; MacCoy, 2004). Historically, algal biomass (natural substrata) values have also exceeded the 150 mg/m^2 total maximum daily load target (Etheridge, 2013; MacCoy, 2004).

The elevated algal biomass values downstream of the Lander plant indicate nutrient enrichment according to the framework of Dodds (2006). Therefore, estimates of algal biomass suggest that nutrient conditions downstream of the Lander plant promoted nuisance growth, consistent with the observed threshold violation.

CHAPTER FIVE: CONCLUSIONS

Influence of Nutrient Loading on Algal Biomass in the Lower Boise River

Our study demonstrates that phosphate and nitrate loading, primarily from wastewater discharge beginning downstream of the Lander wastewater treatment plant, is most likely influencing the nutrient limitation status and producing an increase in algal biomass. Increased loading of both nitrate and phosphate, downstream of the Lander plant, would not likely result in an increase in algal biomass due to general nutrient saturation. In fact, increased nutrient loading downstream of the Lander plant may actually reduce algal biomass at certain sites, due to suppression.

Additional factors (especially total suspended solids, and riparian cover) may also limit algal biomass growth in this system. In the lower section of the river, where sediment loading is highest, light availability likely limits algal biomass growth. In this reach of river, decreased nutrient levels may not produce declines in algal biomass.

Observed algal biomass estimates violate generally accepted nuisance thresholds, indicating nuisance algal growth conditions downstream of the Lander wastewater treatment plant.

Management, Phosphorus Limitation, and Nuisance Algal Biomass Growth

Water quality managers proposing control of phosphorus loading in the Boise River to manage algal biomass growth may be interested in the observed nitrate limitation and associated absences of phosphate limitation found upstream of the Lander wastewater treatment plant. This indicates that lowering phosphorus loading, without also

reducing nitrate, may still lead to some degree of increased algal growth. This observation is consistent with the recommendation that reducing both nitrogen and phosphorus, as opposed to reducing phosphorus alone, will more effectively control algal biomass in many systems (Biggs et al. 2007; EPA, 2015a; Suplee & Watson, 2013).

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APPENDIX A

Example of Nutrient Diffusing Substrata in Holding Rack

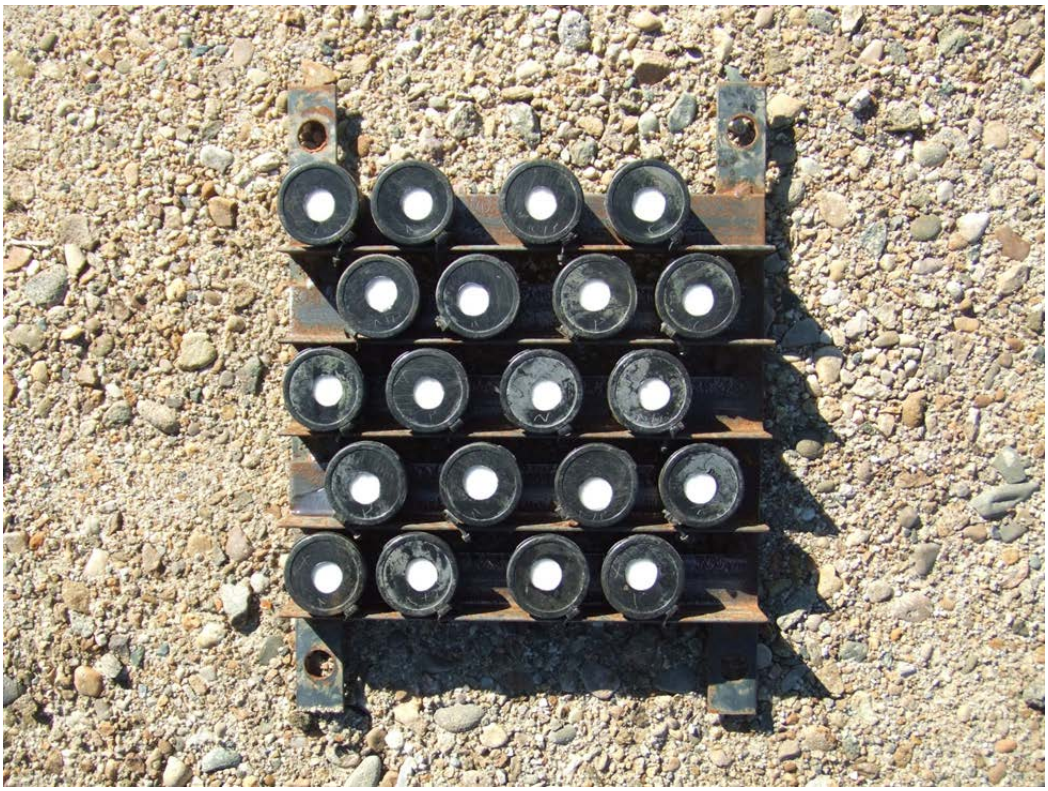

Figure A.1 Nutrient diffusing substrata secured to holding rack.

APPENDIX B

Nutrient Concentration Quality Assurance

Table B.1 Nutrient concentration quality assurance data from August, 2013 nutrient diffusing substrata deployment. Estimated instrument detection limits for nitrate and orthophosphate are 0.006 mg/L and 0.019 mg/L, respectively (Johannesson, 2005).

Nutrient Diffusing Substrata Deployment (August, 2013 deployment)					
Field Triplicate			**Lab Method Precision**		
	NO_3-N (mg/L)	PO_4-P (mg/L)		NO_3-N (mg/L)	PO_4-P (mg/L)
Site 12			Site 3		(below detection)
(a)	1.64	0.16	(a)	0.008	
(b)	1.64	0.15	(b)	0.011	
(c)	1.65	0.15	(c)	0.01	
mean	1.64	0.15	(d)	0.008	
st.dev.	0.01	0.01	(e)	0.007	
%RSD	0.35	3.77	(f)	0.008	
			(g)	0.008	
			(h)	0.01	
			(i)	0.01	
			mean	0.01	
			st.dev.	0.001	
			%RSD	15.35	
Field Blanks			**Instrument accuracy (calibration standard)**		
	NO_3-N (mg/L)	PO_4-P (mg/L)		NO_3-N (mg/L)	PO_4-P (mg/L)
FB-1	0.004	0.007	Standard d		
			Avg % Error	1.2	2

Table B.2 Nutrient concentration quality assurance data from August, 2013 nutrient diffusing substrata retrieval.

Nutrient Diffusing Substrata Deployment (August, 2013 Retrieval)					
Field Triplicate			**Lab Method Precision**		
	NO_3-N (mg/L)	PO_4-P (mg/L)		NO_3-N (mg/L)	PO_4-P (mg/L)
Site 12			Site 3		(below detection)
(a)	1.62	0.136	(a)	0.011	
(b)	2	0.188	(b)	0.012	
(c)	2	0.19	(c)	0.008	
mean	1.87	0.171	(d)	0.01	
st.dev.	0.22	0.031	(e)	0.01	
%RSD	11.71	17.87	(f)	0.008	
			(g)	0.012	
			(h)	0.011	
			(i)	0.007	
			mean	0.01	
			st.dev.	0.002	
			%RSD	18.54	
Field Blanks			**Instrument accuracy (calibration standard)**		
	NO_3-N (mg/L)	PO_4-P (mg/L)		NO_3-N (mg/L)	PO_4-P (mg/L)
FB-1	N/A	N/A	Standard d		
			Avg % Error	N/A	N/A

Table B.3 Nutrient concentration quality assurance data from October, 2013 nutrient diffusing substrata deployment.

Nutrient Diffusing Substrata Deployment (October, 2013 deployment)					
Field Triplicate			**Lab Method Precision**		
	NO_3-N (mg/L)	PO_4-P (mg/L)		NO_3-N (mg/L)	PO_4-P (mg/L)
Site 12			Site 3		(below detection)
(a)	2.8	0.153	(a)	0.027	
(b)	2.77	0.148	(b)	0.028	
(c)	2.8	0.15	(c)	0.029	
mean	2.79	0.15	(d)	0.026	
st.dev.	0.02	0.003	(e)	0.027	
%RSD	0.62	1.67	(f)	0.028	
			(g)	0.029	
			(h)	0.027	
			mean	0.028	
			st.dev.	0.001	
			%RSD	3.84	
Field Blanks			**Instrument accuracy (calibration standard)**		
	NO_3-N (mg/L)	PO_4-P (mg/L)		NO_3-N (mg/L)	PO_4-P (mg/L)
FB-1	0.005	-0.002	Standard d		
			Avg % Error	1.7	11

Table B.4 Nutrient concentration quality assurance data from October, 2013 nutrient diffusing substrata retrieval.

Nutrient Diffusing Substrata Deployment (October, 2013 Retrieval)					
Field Triplicate			**Lab Method Precision**		
	NO_3-N (mg/L)	PO_4-P (mg/L)		NO_3-N (mg/L)	PO_4-P (mg/L)
Site 12			Site 3		(below detection)
(a)	3.6	0.162	(a)	0.008	
(b)	3.61	0.16	(b)	0.009	
(c)	3.6	0.159	(c)	0.007	
mean	3.60	0.16	(d)	0.008	
st.dev.	0.01	0.002	mean	0.008	
%RSD	0.16	0.95	st.dev.	0.001	
			%RSD	10.21	
Field Blanks			**Instrument accuracy (calibration standard)**		
	NO_3-N (mg/L)	PO_4-P (mg/L)		NO_3-N (mg/L)	PO_4-P (mg/L)
FB-1	0.007	0.001	Standard d		
			Avg % Error	5.8	7

Table B.5 Nutrient concentration quality assurance data from September, 2013 natural substrata sampling.

Natural Substrata (September, 2013)					
Field Triplicate			**Lab Method Precision**		
	NO_3-N (mg/L)	PO_4-P (mg/L)		NO_3-N (mg/L)	PO_4-P (mg/L)
Site 12			Site 8		
(a)	2.45	0.147	(a)	1.66	0.12
(b)	2.46	0.145	(b)	1.66	0.119
(c)	2.45	0.145	(c)	1.65	0.118
mean	2.45	0.15	(d)	1.65	0.116
st.dev.	0.01	0.001	(e)	1.65	0.122
%RSD	0.24	0.80	(f)	1.51	0.106
			(g)	1.66	0.117
			(h)	1.58	0.112
			(i)	1.66	0.115
			mean	1.63	0.12
			st.dev.	0.05	0.005
			%RSD	3.20	4.12
Field Blanks			**Instrument accuracy (calibration standard)**		
	NO_3-N (mg/L)	PO_4-P (mg/L)		NO_3-N (mg/L)	PO_4-P (mg/L)
FB-1	0.002	0.003	Standard d		
			Avg % Error	0.67	7.8

APPENDIX C

Comparison of Algal Biomass Using Grouped Sites

Table C.1 Results of One-way ANOVA comparing mean algal biomass of grouped sites upstream of the Lander wastewater treatment plant with grouped sites downstream, on the Lower Boise River. Individual analyses were conducted using the mean algal biomass accrual rates (mg chlorophyll a/m^2/day) of the October 2013 unamended substrata (nutrient diffusing substrata) and using the mean algal biomass (mg chlorophyll a/m^2) of the September 2013 natural substrata. Grouped mean values are shown and an asterisk represents a significant difference. Sites in each group, for each analysis, are shown and are connected by an underline. If the two groups are significantly different they are not connected by a third underline. (α = 0.05).

SS	df	F-value	P	Grouped Sites
October one-way ANOVA (unamended substrata)				
22.17	7	50.20	<0.001	1 2 3 4 5 8 9 12
Mean of Grouped Sites				
Upstream		Downstream		
32.8		151*		
September one-way ANOVA (natural substrata)				
31.04	10	31.65	<0.001	1 2 3 4 5 6 7 8 9 11 12
Mean of Grouped Sites				
Upstream		Downstream		
42.4		66.0*		

*indicates significant difference

APPENDIX D

Comparison of Algal Biomass Using Individual Sites

Table D.1 Summary of individual One-way ANOVA of the algal biomass at sites downstream of the Lander wastewater treatment plant, on the Lower Boise River. Individual analyses used mean algal biomass accrual rate (mg chlorophyll a/m^2/day) from unamended substrata (nutrient diffusing substrata) and mean algal biomass (mg chlorophyll a/m^2) from natural substrata. Hochberg multiple comparison tests were performed to determine differences between sites. Underlines below sites show sites that are not significantly different ($P \leq 0.05$).

Source of Variation	SS	df	F-value	p-value	Site
August (unamended substrate)					4 7 6 10 8
Site	1.09	4	11.00	<0.001	
October (unamended substrate)					5 4 12 8 9
Site	22.17	7	50.20	<0.001	
September (natural substrate)					7 9 11 5 6 8 4 12
Site	31.04	10	31.65	<0.001	

APPENDIX E

Algal Biomass Quality Assurance

Table E.1 Algal biomass (mg/m^2) quality assurance data for the August, 2013 nutrient diffusing substrata deployment.

Nutrient Diffusing Substrata Deployment (August, 2013)			
Field Replicate Range		**Lab Replicate Range**	
Smallest Example of Field Variability		Smallest example of Lab Variability	
Site 7 unamended substrata		Site 7 N substrata	
mean	113	mean	76.0
st.dev.	2	st.dev.	0.7
%RSD	2	%RSD	0.9
Largest Example of Field Variability		Largest example of Lab Variability	
Site 4 N+P substrata		Site 10 N+P substrata	
mean	106	mean	130
st.dev.	41	st.dev.	23
%RSD	39	%RSD	18

Table E.2 Algal biomass (mg/m^2) quality assurance data for the October, 2013 nutrient diffusing substrata deployment.

Nutrient Diffusing Substrata Deployment (October, 2013)			
Field Replicate Range		**Lab Replicate Range**	
Smallest Example of Field Variability		Smallest example of Lab Variability	
Site 3 N+P substrata		Site 2 N substrata	
mean	47.4	mean	46.7
st.dev.	3.0	st.dev.	0.1
%RSD	6.4	%RSD	0.1
Largest Example of Field Variability		Largest example of Lab Variability	
Site 2 N+P substrata		Site 4 unamended substrata	
mean	79.7	mean	115
st.dev.	36.2	st.dev.	11
%RSD	45.5	%RSD	9

Table E.3 Algal biomass (mg/m^2) quality assurance data for the September, 2013 natural substrata sampling.

Natural Substrata (September, 2013)			
Field Replicate Range		**Lab Replicate Range**	
Smallest Example of Field Variability		Smallest example of Lab Variability	
Site 4		Site 1	
mean	101	mean	48.6
st.dev.	9	st.dev.	0.5
%RSD	8	%RSD	0.9
Largest Example of Field Variability		Largest example of Lab Variability	
Site 7		Site 12	
mean	40.1	mean	4.82
st.dev.	20.7	st.dev.	0.76
%RSD	51.7	%RSD	15.67

APPENDIX F

Algal Biomass Values (Chlorophyll a) for Nutrient Diffusing Substrata Treatments

Table F.1 Algal biomass values (mg/m^2) for each nutrient treatment of August 2013 nutrient diffusing substrata deployment.

Nutrient Diffusers	N	C	N+P	P
Site 4				
(a)	68.6	100	85.3	112
(b)	111	101	117	89.2
(c)	82.4	115	64.2	114
(d)	94.7	108	159	59.6
(e)	86.8			124
count	5	4	4	5
Mean	88.7	106	106	99.8
St. Deviation	15.6	7	41	25.8
Coef. Var. (%)	17.7	7	39	25.9

Nutrient Diffusers	N	C	N+P	P
Site 6				
(a)	95.1	161	175	87.8
(b)	87.6	139	132	98
(c)	77.4	130	94	70.7
(d)		159	147	82.4
(e)				
count	3	4	4	4
Mean	86.7	147	137	84.7
St. Deviation	8.9	15	34	11.4
Coef. Var. (%)	10.2	10	25	13.4

Nutrient Diffusers	N	C	N+P	P
Site 7				
(a)	70.5	112	104	111
(b)	90.6	115	89.6	143
(c)	76.0	115	92.8	120
(d)	93.9	110	101	
(e)				
count	4	4	4	3
Mean	82.8	113	96.9	125
St. Deviation	11.3	2	6.8	17
Coef. Var. (%)	13.6	2	7.0	13

Nutrient Diffusers	N	C	N+P	P
Site 8				
(a)	140	160	113	176
(b)	134	175	145	105
(c)	155	161	117	162
(d)	180	225	162	172
(e)	126	223	118	170
count	5	5	5	5
Mean	147	189	131	157
St. Deviation	21	33	21	30
Coef. Var. (%)	14	17	16	19

Nutrient Diffusers	N	C	N+P	P
Site 10				
(a)	107	120	130	141
(b)	187	208	207	135
(c)	190	170	82.7	172
(d)	179	122	200	191
(e)		190		146
count	4	5	4	5
Mean	166	162	155	157
St. Deviation	39	40	59	24
Coef. Var. (%)	24	25	38	15

Table F.2 Algal biomass values (mg/m^2) for each nutrient treatment of October 2013 nutrient diffusing substrata deployment.

Nutrient Diffusers	N	C	N+P	P
Site 1				
(a)	41.5	34.6	45.6	21.7
(b)	40.1	24.9	36.7	37.5
(c)	42.9	37.2	49.1	31.2
(d)	34.1	42.1	49.9	33.2
(e)	47	37.3	55	
count	5	5	5	4
Mean	41.1	35.2	47.3	30.9
St. Deviation	4.7	6.4	6.8	6.7
Coef. Var. (%)	11.4	18.1	14.4	21.6

Nutrient Diffusers	N	C	N+P	P
Site 2				
(a)	65.5	31.6	67	34.2
(b)	58.1	38.7	47.9	36.4
(c)	54.5	21.8	129	48.8
(d)	46.7	43.9	106	37
(e)	60.7	25.9	48.7	24
count	5	5	5	5
Mean	57.1	32.4	79.7	36.1
St. Deviation	7.1	9.0	36.2	8.8
Coef. Var. (%)	12.4	27.9	45.5	24.5

Nutrient Diffusers	N	C	N+P	P
Site 3				
(a)	40.6	37.4	43.5	30.3
(b)	43.7	29.6	50.8	35
(c)	65.1	30	46.9	26.1
(d)	30.8	23.6	48.2	26.1
(e)		34		15.9
count	4	5	4	5
Mean	45.1	30.9	47.4	26.7
St. Deviation	14.5	5.2	3.0	7.1
Coef. Var. (%)	32.1	16.8	6.4	26.5

Nutrient Diffusers	N	C	N+P	P
Site 4				
(a)	83.2	146	104	75.1
(b)	91.5	130	175	88.2
(c)	115	159	86.6	136
(d)	81.2	130	105	126
(e)	94.2	115	126	81.1
count	5	5	5	5
Mean	93.0	136	119	101
St. Deviation	13.4	17	34	28
Coef. Var. (%)	14.5	12	29	27

Nutrient Diffusers	N	C	N+P	P
Site 5				
(a)	110	140	92.6	113
(b)	132	60.5	68.5	97
(c)	104	110	80	56.8
(d)	134	120	94	123
(e)	120		122	111
count	5	4	5	5
Mean	120	108	91.4	100
St. Deviation	13	34	20.0	26
Coef. Var. (%)	11	31	22.0	26

Nutrient Diffusers	N	C	N+P	P
Site 8				
(a)	132	129	80.3	183
(b)	180	181	89.7	176
(c)	63.7	147	84	146
(d)	111	134	73	168
(e)	102	182	84.3	71.2
count	5	5	5	5
Mean	118	155	82.3	149
St. Deviation	43	25	6.2	46
Coef. Var. (%)	36	16	7.5	31

Nutrient Diffusers	N	C	N+P	P
Site 9				
(a)	167	211	212	198
(b)	221	273	245	149
(c)	153	213	120	189
(d)	178	238	148	164
(e)	116	152	274	201
count	5	5	5	5
Mean	167	217	200	180
St. Deviation	38	44	65	23
Coef. Var. (%)	23	20	32	13

Nutrient Diffusers	N	C	N+P	P
Site 12				
(a)	179	186	175	115
(b)	229	78	185	177
(c)	129	116	103	212
(d)	150	185	218	208
(e)	118			125
count	5	4	4	5
Mean	161	141	170	167
St. Deviation	45	53	48	45
Coef. Var. (%)	28	38	28	27

APPENDIX G

Discharge in the Lower Boise River

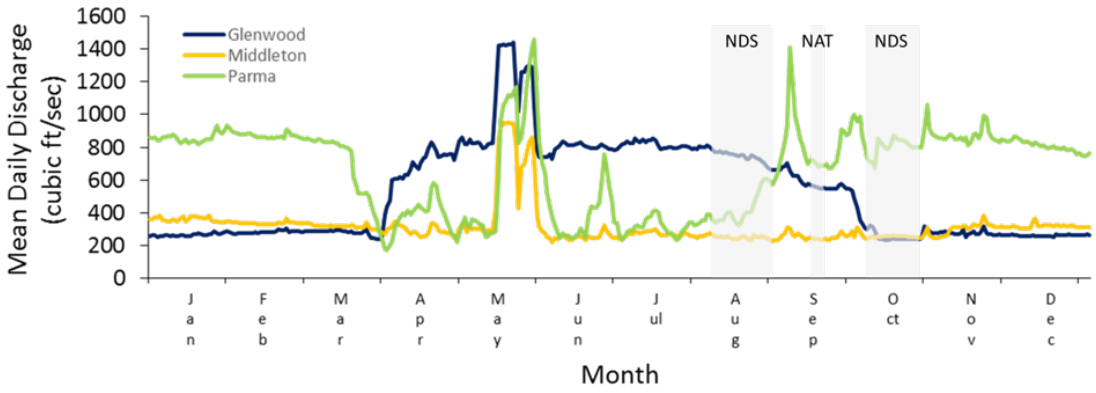

Figure G.1 Mean daily discharge from USGS gaging stations during August and October, 2013 nutrient diffusing substrata deployments (NDS), and September, 2013 natural substrata sampling (NAT) (represented by grey boxes).

APPENDIX H

Coordinates (Latitude and Longitude) of Sampling Sites

Table H.1 Latitude and longitude of algal biomass and environmental conditions sampling sites.

site	Latitude	Longitude
1	43°37'2.29"N	116°13'29.08"W
2	43°38'3.60"N	116°14'25.71"W
3	43°38'13.73"N	116°14'38.29"W
4	43°40'14.47"N	116°18'30.13"W
5	43°40'34.24"N	116°20'40.27"W
6	43°40'56.88"N	116°28'40.53"W
7	43°41'42.31"N	116°37'2.98"W
8	43°40'50.15"N	116°41'29.09"W
9	43°43'17.61"N	116°47'35.93"W
10	43°43'58.05"N	116°53'12.18"W
11	43°44'46.60"N	116°54'43.34"W
12	43°46'39.62"N	116°58'17.07"W