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## Chapter 5

# Algal Nutrient Limitation throughout the Little Bear River Watershed [by] Jared Baker

### SUMMARY

The objective of this study was to use a 5 day bioassay experiment to assess whether nitrogen or phosphorus limited the growth of algae in the Little Bear River watershed. Four sites were sampled along the river in September 2012. The locations of the sites were south of Avon (Station 2), near Paradise, UT (Station 6), downstream of Hyrum Reservoir (Station 7), and downstream of the Waste Water Treatment Facility in Wellsville (Station 10). Chlorophyll *a* analysis was conducted prior to, after 2.5 days, and at the conclusion of the 5 days. Varying combinations of nitrogen and phosphorus were added to water samples from each site and these were incubated in 125-ml flasks with 150  $\mu\text{M m}^{-2}$  lighting and at 15°C. ANOVA was used to determine nutrient limitations within samples. Chlorophyll concentrations measured at the conclusion of the experiment indicated that both nitrogen and phosphorus limited algal growth at Stations 2 and 10 while phosphorus alone was limiting at Stations 6 and 7.

### INTRODUCTION

The Little Bear River is located near Logan Utah and is a 58.6 km long tributary of the Bear River. Before entering the Bear River, the Little Bear River travels through moderately pristine areas, then through agricultural land, into Hyrum Reservoir, and then to Cutler Marsh. This ecosystem is impacted by anthropogenic influences consisting of about 182,000 acres, including rangeland, pasture, and cropland. In this watershed there is approximately 21,024 acres of irrigated land. The area supports wildlife, but the majority of the animals are domesticated. The river also receives effluent from the Waste Water Treatment Facility in Wellsville. These are all a part of human effects on the Little Bear River watershed (Little 2012). There has been limited research on the many chemical, biological, and physical factors that impact this ecosystem. However, past studies suggest highly variable levels of these factors throughout the river. This is likely due to the varying degrees of pollution and agriculture.

In managing the water quality, it is very useful to identify what nutrient(s) limit algal growth (Holmboe et al. 1999). If managers are aware of what nutrients are already present, one can adjust land use practices to potentially alter stream productivity. As a result, one could influence the amount and types of fish within the watershed. Another more common use would be to reduce the amount of the limiting nutrient going into a system to reduce eutrophication. Thus, understanding the nutrient limitations of the Little Bear River is essential to management and preservation.

Based on the above reasons, I studied the potential nutrient limitations of phytoplankton production in the Little Bear River. I conducted a bioassay experiment in order to determine the nutrient limitations at four locations. In-stream nutrient measurements were recorded by Jason Fuller and compared against my results. This data was essential for compiling the results I gathered through chlorophyll *a* analysis.

According to the Utah Department of Environmental Quality Division of Water Quality TMDL (Total Maximum Daily Load) Section, the "Little Bear drainage shows signs of water quality deterioration both above and below Hyrum Reservoir" (Little 2012). Although phosphorus is the nutrient most frequently addressed in Utah and other States, both phosphorus and nitrogen frequently limit algal growth in lakes (Lewis and Wurtsbaugh 2008).

## **STUDY AREA AND METHODS**

Bioassay experiments were done to determine which nutrient or nutrients limited phytoplankton growth. The bioassays were completed within "1 week to minimize the effects of temporal changes on the bioassay responses" (Marcarelli and Wurtsbaugh 2007). I conducted a 5 day bioassay experiment and used water samples from four sites along the Little Bear River.

I chose to use Stations 2, 6, 7, and 10 to get a good representation of the longitudinal gradient of the Little Bear River (See site map in Executive Summary). Water samples were gathered from four locations: south of Avon (Station 2), in the middle near the town of Paradise (Station 6), downstream of Hyrum Reservoir (Station 7), and downstream of the Waste Water Treatment Facility in Wellsville (Station 10).

The first location, just south of Avon, was chosen based on both its accessibility and the fact that it is fairly pristine and has less anthropogenic impact than the other sites. The next sample was taken near Paradise where there is a higher anthropogenic influence. A sample just downstream of Hyrum Reservoir was selected to potentially show the effects of the reservoir on the nutrient regime. Lastly, the fourth sample was collected downstream of the Waste Water Treatment Facility in Wellsville where nutrients, and in particular phosphorus were expected to be high.

At each site, a 2 L Nalgene bottle was used to collect samples. In the laboratory, 17.5 ml of water from Hyrum Reservoir was added to each sample in order to ensure that there was phytoplankton present. These samples were then divided into 125-ml Erlenmeyer flasks. These flasks were each filled with 100 ml of water from the various sampling locations. I used 12 Erlenmeyer flasks for each site: three flasks as the control, three had phosphorus introduced, three had nitrogen introduced, and the last three had both phosphorus and nitrogen introduced. I used three replicates because I modeled this part of my experiment after a bioassay of phytoplankton with sockeye salmon lakes of Idaho (Wurtsbaugh et al. 1997).

The concentrations of nutrients that were added were based on those found by Abbott et al. (2008) in Cutler Reservoir: 0.82 mg/L phosphorus and 1.27 mg/L nitrogen. From these measurements, I added 0.5 mg/L phosphorus and 4.0 mg/L nitrogen to the appropriate treatments (Figure 1). The flasks were put in a climate controlled room at 15 °C with 150  $\mu\text{M}/\text{m}^2$  light intensity and a 12:12 light: dark cycle. The samples were labeled and randomly placed on a shaker table to agitate the water. This was done to emulate water movement. The algae needed to remain suspended, as they would be in a natural setting.

Chlorophyll concentrations were measured initially, after 2.5 days, and on day 5. Each day, 10-ml aliquots from each of the 48 flasks was filtered on Gelman A/E filters with a nominal pore size of 1.0  $\mu\text{m}$ . The filters were frozen and then extracted for 24 hours in 95 percent ethanol in the dark. The concentration of extracted chlorophyll was measured with a Turner 10AU fluorometer equipped with a

Welschmeyer filter set that does not require acidification (Welschmeyer 1994).

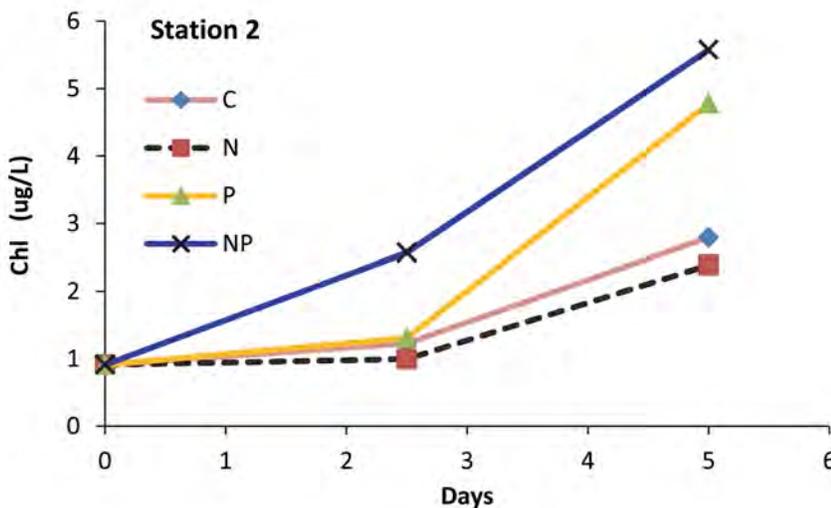
ANOVA was used to determine significant differences in phytoplankton production between treatments, for each site. A p-value of 0.05 or less was considered significant. Microsoft Excel was used for this analysis.

## RESULTS

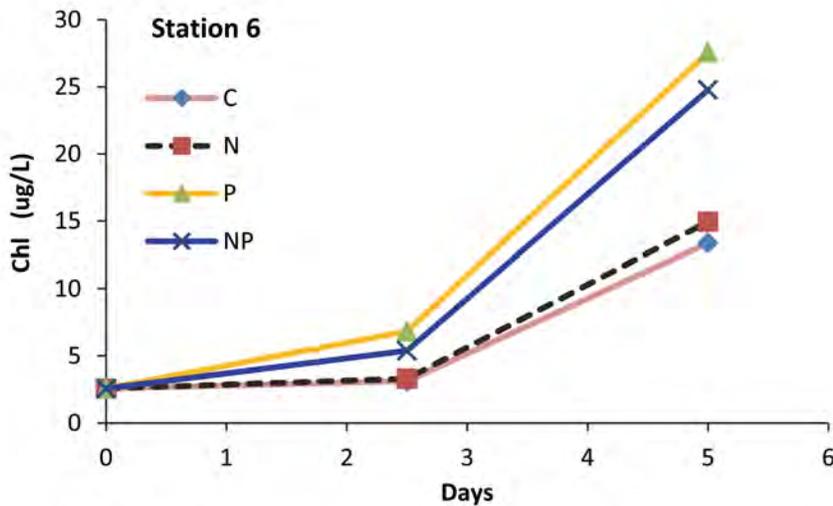
Although each sample was kept at the same temperature, the chlorophyll a responded differently in each treatment. Changes in phytoplankton production at Station 2 did not appear to be significant between treatments for either two 2.5 or 5 day assay periods. At Stations 6, 7 and 10 there were significant differences between treatments after 2.5 days, however, at 5 days only Station 7 produced significantly different results.

In the treatment utilizing water from high in the watershed (Station 2) chlorophyll concentrations increased the most in the N+P treatment after both 2.5 and 5 days, but these results were not significant (Figure 1; ANOVA,  $p = 0.49, 0.27$  for 2.5 and 5 days, respectively). Chlorophyll a concentrations in the phosphorus treatment were also statistically insignificant. Also note that mean chlorophyll concentrations in the Control treatment increased nearly 2.5 fold by the end of the 5 day bioassay. At Station 2, N and P appear to be co-limiting, but the lack of statistical significance warrants caution in this interpretation.

In water from Station 6 chlorophyll a concentrations in the control, nitrogen, phosphorus, and N+P samples all extended upwards over time (Figure 2). The chlorophyll a concentrations at Station 6 increased the most in the phosphorus treatment after both 2.5 days and 5 days, but treatments were only significantly different on day 2.5 (Figure 2; ANOVA,  $p = 0.01, 0.26$  for 2.5 and 5 days, respectively). At Station 6 phosphorus appeared to be the primary limiting nutrient for algal growth.



**Figure 1.** Chlorophyll a concentrations in four nutrient treatments of the laboratory bioassay, utilizing Little Bear River water from Station 2 located south of the town of Avon, Utah. The location is 3.45 km downstream. Chlorophyll a concentrations increased the most in the N + P treatment after both 2.5 and 5 days, but these results were not significant.

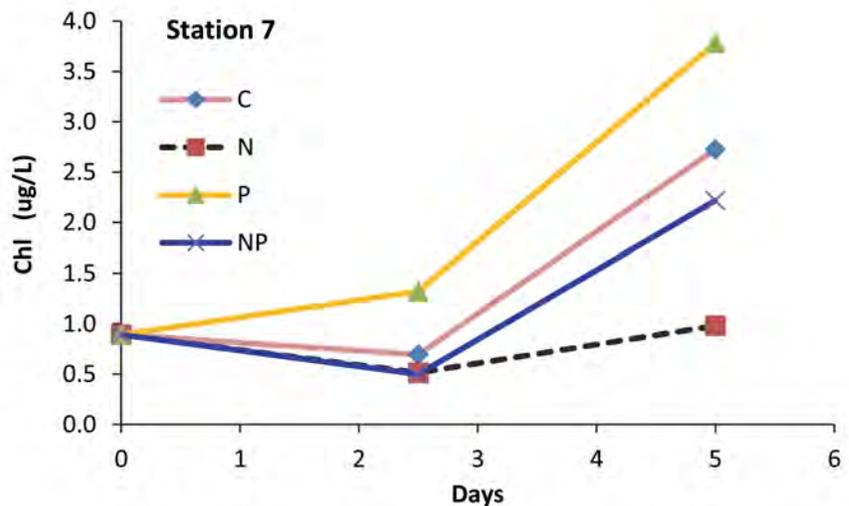


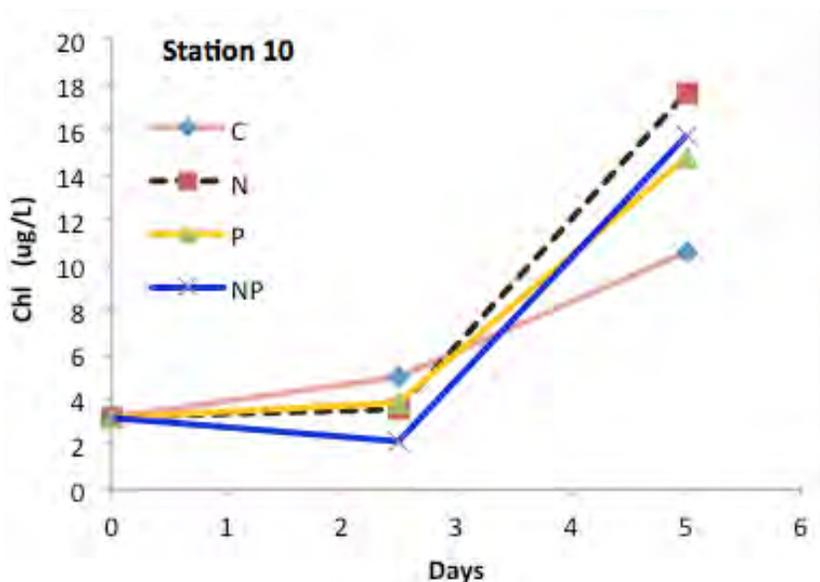
**Figure 2.** The chlorophyll a concentration in the control, nitrogen, phosphorus, and N + P samples for bioassays of Station 6 water. By day 2.5 the chlorophyll a appears to have increased the most with phosphorus added. After 5 days this is still the case and is reiterated in the above graph.

In the assay with water below Hyrum Reservoir (Station 7) the chlorophyll a concentration in the control, nitrogen, phosphorus, and N+P samples all rose. By day 2.5 the chlorophyll a increased the most in the P treatment. The P treatment still produced the best phytoplankton growth on day 5. These results were significant for both days 2.5 and day 5 (Figure 3; ANOVA,  $p = 0.001, 0.004$  for 2.5 and 5 days respectively). At Station 7, P appeared to be the limiting nutrient.

Lastly, in the water from Station 10 the chlorophyll a concentration in the control, nitrogen, phosphorus, and N+P samples all increased. However, by day 2.5 chlorophyll levels had changed little from the initial condition and mean levels in the nutrient treatment were all below the mean control level. After 5 days the phosphorus + nitrogen, and the phosphorus treatments increased markedly. However, results were only significant at 2.5 days (Figure 4; ANOVA,  $p = 0.02, 0.07$  for 2.5 and 5 days, respectively). The marginally significant response on day 5 suggests that both N and P may have been limiting nutrients for the phytoplankton.

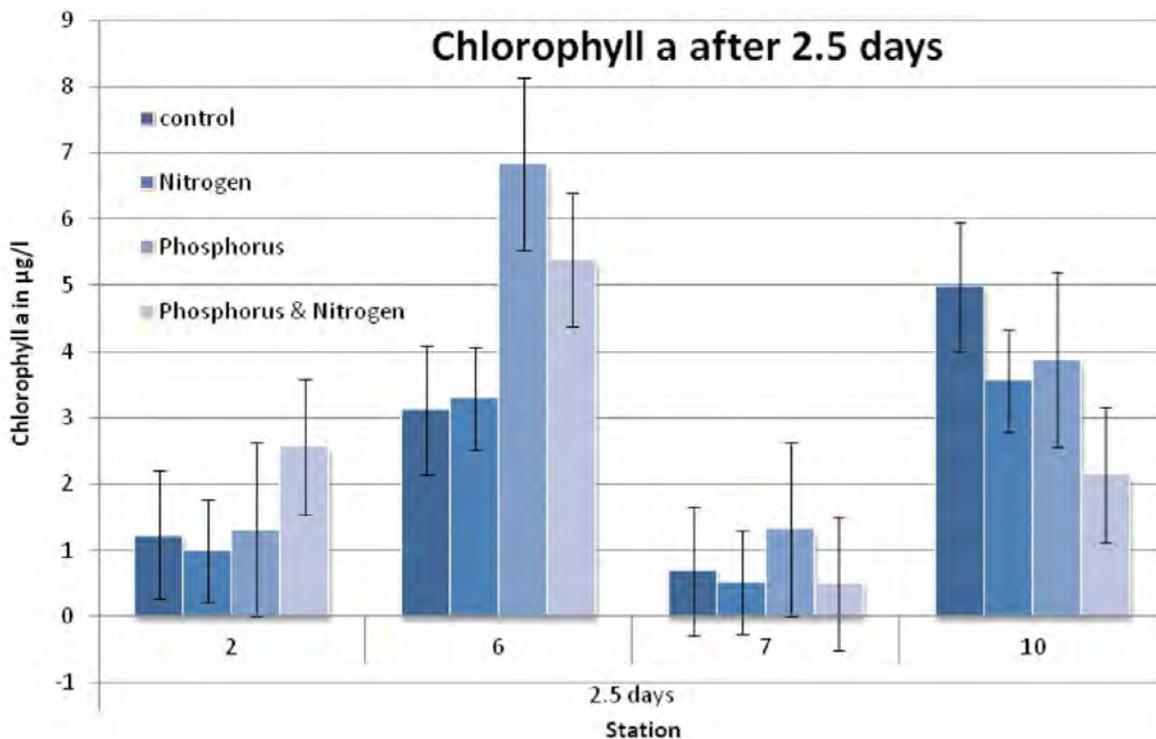
**Figure 3.** Chlorophyll a concentrations in the control, nitrogen, phosphorus, and N + P bioassays using water from Station 7. By day 2.5 the chlorophyll a thrived the most with phosphorus added. After 5 days this is still the case and is reiterated in the above graph.





**Figure 4.** Chlorophyll a concentrations in the control, nitrogen, phosphorus, and N + P bioassay treatments using Station 10 water.

Figure 5 summarizes the results from all of the treatments on day 2.5. Phosphorus appears to most commonly stimulate chlorophyll production, with the exception of Station 2 where both N and P were the only stimulatory treatment. Station 10 is interesting because the control had the highest levels of chlorophyll a. This may be as a result of toxic levels of phosphorus and nitrogen.



**Figure 5.** Chlorophyll a concentrations after 2.5 days of incubation for Stations 2, 6, 7, and 10.

## **DISCUSSION**

The Marcarelli et al. (2002) study did not include multiple stations along a river. My study is unique in that aspect. Most nutrient level studies are in regard to lakes and/or large bodies of water (e.g. Abbott et al. 2008, Lewis and Wurtsbaugh 2008, Lewis and Wurtsbaugh 2011, Holmboe et al. 1999, Morris and Lewis 1988, and Wurtsbaugh et al. 1997).

According to the nutrient data from Jason Fuller, Hyrum Reservoir acts as a nutrient sink where nitrate flowing down the Little Bear River was trapped. The bioassay design I used is not useful for determining sinks or sources of nutrients, but rather just the relative response to different nutrient additions.

Due to the impact of humans on this watershed, I hypothesized that nutrient limitation would decrease with downstream movement. This did not appear to be true. At Stations 2, 6, and 7 chlorophyll levels in the most response treatments were approximately double those of the controls, suggesting relatively constant nutrient limitation at these sites (Figure 5). Only at Station 10 where nutrient levels were very high was there a lack of significant response to any nutrient addition. In fact, the nutrient-amended treatments all had lower levels than the controls, albeit not significantly below the controls (Figure 5)

Given the lack of studies done on the Little Bear River another bioassay experiment could be done to test whether or not seasonal variations in nutrient limitation exist, and whether my results can be replicated. Variability between replicates was high in the treatments and this made it difficult to determine if there were responses or not. An analysis of seasonal variation could be insightful for understanding the composition, biomass, and production of phytoplankton (Morris et al. 1988). Finally, since periphyton dominates chlorophyll levels in the Little Bear River (see Fisher (this report)) it would be useful in future studies to analyze nutrient limitation of these benthic algae.

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