Primary Productivity and Lake Health: Examination of Phytoplankton Growth Rate Regulations in Keuka Lake via Short-term Microcosm Experiments

Extended Abstract
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Introduction:

Lakes are ecological systems that can integrate changes throughout their watersheds resulting from anthropogenic activity. Consequently, investigation of biological processes within lakes can elucidate the effects of watershed activity on lake health. Phytoplankton (i.e. suspended algae) - the primary producers of pelagic systems – are a conduit through which watershed activity can affect lake health. Due to their location within the pelagic food web, phytoplankton abundance and diversity in part determines the abundance and diversity of organisms belonging to higher trophic levels (e.g. zooplankton, mollusks and fish).

Historically, various groups have studied algal abundance in Keuka Lake using survey techniques. This approach provides snapshots of change, but does not illuminate the mechanisms that regulate phytoplankton growth. Our research group is thus seeking to quantify phytoplankton growth rates in response to changes in nutrients, light and grazing (i.e. phytoplankton consumption by zooplankton and zebra mussels) by developing short-term microcosm experimental techniques. Ultimately, phytoplankton growth rates generated from these experiments will be used to formulate predictive models to further our understanding of how watershed activity impacts the health of Keuka Lake via effects of primary productivity.

Between June of 2005 and May of 2006, we have conducted a series of nine experiments to investigate the effects of light, nutrients and grazing on phytoplankton growth rates. Whole water samples were collected from the Keuka College access point and incubated for 96 hours within an environmental chamber. By placing samples within an environmental chamber, temperatures were effectively controlled at ambient levels. Phytoplankton abundance was measured using fluorometry, and fluorescence measurements were used to derive daily growth rates. To detect significant differences among treatments, results were analyzed using one and two-way ANOVAs in conjunction with a Tukey post-hoc test.

Nutrient Addition Bioassays:

Three nutrient addition bioassays were conducted in June, July and September of 2006. During these experiments, the limiting effects of both phosphorous (PO$_4^{3-}$) and nitrogen (NO$_3^-$) along with nutrient co-limitations (+NP) were tested. Temperatures were controlled according to measured ambient values, and ranged from 21-25 °C. 500mL Erlenmeyer flasks were used as experimental vessels, and sample volumes were controlled at 450mL among all replicates.

The results of the June bioassay suggested that a limiting effect is observable when the ambient concentration of phosphate (~2.0 µg L$^{-1}$) is tripled. Thus, phosphate was added to samples to obtain a phosphorous concentration of 6.0 µg L$^{-1}$ within our phosphorous addition treatments (+P) during the July and September experiments. Nitrogen addition treatments (+N) for these two experiments had a final nitrate concentration of 220.0 µg L$^{-1}$. During the
July and September experiments, mean growth rates in the +P treatments were significantly higher than the control while growth rates observed in the +N and +NP treatments were not (Fig. B and C). The results of these experiments suggest that phytoplankton populations in Keuka Lake are limited by phosphorous rather than nitrogen at the end of the algal growing season (i.e. July –September).

Light Effects on Growth Rates:

To determine whether or not phytoplankton growth in Keuka Lake is limited by light, we conducted several experiments in which irradiance was manipulated using shade cloths. The first light-manipulation experiment was carried out in January of 2006. Zero (control), one and three layers of shade cloth were used to manipulate light among treatments (see Fig. 2 for irradiance values). Temperature was held constant at 4°C, and the sample volume was controlled at 450mL. The mean growth rate within the control treatment (0.30 day⁻¹) is significantly lower than the mean growth rates in the 1-layer and 3-layer treatments. This finding reveals that phytoplankton in Keuka Lake may experience photo-inhibition during
winter months. Mean phytoplankton growth rates were higher among treatments receiving lower levels of irradiance.

### Light Effects on Growth Rates

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<th>Shade Cloth Layers</th>
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</tr>
<tr>
<td>1</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>0.20</td>
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</table>

**Figure 2**: Results analyzed using 1-Way ANOVA and error bars indicate +/-1 standard deviation. A (*) symbolizes significantly different mean growth.

### Nutrient-Light Interaction Experiments:

In natural environments, variables that regulate phytoplankton growth are concurrently present and thus may interactively affect phytoplankton growth. In April of 2006, two experiments (starting 4/10 and 4/24) were conducted to investigate light and nutrient interactions. These experiments consisted of four treatments in which light and nutrients were simultaneously manipulated. The light regime consisted of two layers (Low light) and zero layers (high light) of shade cloth. Two treatments received additions of both phosphorous and nitrogen while the remaining two treatments did not receive any additional nutrients. Within +NP treatments, the phosphate and nitrate concentrations were 6.00 µg L⁻¹ and 220.0 µg L⁻¹ (tripling ambient concentrations) respectively, and each treatment was replicated three times while temperature held constant at 8 °C. We were unable to detect an interaction effect using a two-way ANOVA, and thus we analyzed nutrient and light effects independently. The mean growth rates within the +NP treatments for both experiment were not significantly different from the mean rates within treatments having ambient concentrations (Fig. 3, A and C). Nutrient additions did not appear to increase growth rates suggesting phytoplankton growth is not limited by nutrients at the beginning of the growing season (i.e. April). The results of each experiment once again reveal the occurrence of photo-inhibited growth. Mean growth rates within the low light treatments were significantly higher than mean growth rates within the high light treatments.

We were able to demonstrate that our replicated irradiance levels correspond to ambient light conditions using solar irradiance values measured in the field. The irradiances of both the high (60 µE/m² day⁻¹) and low light (14 µE/m² day⁻¹) treatments did not surpass surface irradiance levels and were above 1% of the surface irradiance, or the irradiance at the compensation depth (Fig. 3, C). Our methods allowed for successful replication of lighting conditions experienced by natural populations of phytoplankton in Keuka Lake.
Figure 3 (A,B,C): Nutrient-Light Interaction Experiments analyzed using a Two-Way ANOVA. Error bars show +/-1 standard deviation. Interaction p-values for 4/10 and 4/24 are 0.123 and 0.078 respectively. No nutrient addition effect detected (4/10, $p = 0.178$; 4/24, $p = 0.126$). Figure C produced using solar irradiance values measured in the field. Curve showing average irradiance constructed using a non-linear arithmetic average.
**Light Allocation Effects on Phytoplankton Growth:**

Within a 24 hour period wave action, cloud cover, changes in the sun’s position and water column mixing are source of light heterogeneity. Upon demonstrating the photo-inhibition of phytoplankton growth in three separate experiments, during which ambient lighting conditions were successfully replicated, we became interested in examining phytoplankton sensitivity this heterogeneous lighting environment. By attempting to control the total number of photons received by a treatment during a 24 hour period while manipulating the manner in which those photons were partitioned among 24 hrs, we aimed to determine if the fluctuation of light during the photo-period impacts the growth of phytoplankton in Keuka Lake.

We designed an experiment with three treatment groups: a control in which light was held constant at 10.40 µE/m² sec⁻¹, a low-pulse treatment and a high-pulse treatment. Within the low-pulse treatment, irradiance was pulsed between 2.84µE/m² sec⁻¹ and 12.68µE/m² sec⁻¹ 5.5hrs after the beginning of the replicated day period by switching between five and two layers of shade cloth. Within the high-pulse treatment, the irradiance varied between these same values, but once every 2.7hrs. To better control the total 24 hour irradiance the length of the photo-period varied between treatments: the control treatment experienced a photo-period of 11.5hrs while the pulsed treatments were exposed to light for only 11hrs. In comparison to the total 24 hour irradiance in the control, total 24 irradiance within the low-pulse treatment varied by 30% and by 18% within the high-pulse treatment. After the experiment was completed, all samples were filtered using an 83µm plankton net and the filtrate’s fluorescence was compared to that of the whole-water sample as a means of examining differences in community structure based on size. Nearly 100% of our fluorescence was accounted for by the filtrate samples, and suggests there was no change in the size-based community structure among treatments.

Using a one-way ANOVA, we were unable to detect a significant difference among mean growth rates of the three treatments. However, our results do suggest the trend that continuous steady light better sustains phytoplankton growth than pulsed light (Fig 4). Based on these results, we will continue to develop this experimental design for there is reason to believe light allocation may affect phytoplankton growth. We will be working to further develop this experimental design in order to understand the sensitivity of phytoplankton growth in response to the heterogeneity of the natural lighting environment.

**Figure 4:** Results analyzed using 1-Way ANOVA with \( p<0.05 \) indicating significance. Error bars represent +/-1 standard deviation. No significant difference detected \((p = 0.592)\).
Grazer-Light Interaction Experiment:

Whole-water samples collected during February of 2006 were highly concentrated with zooplankton, both Copepods and Cladocerans, which we speculate resulted from a sudden release from diapause. To capitalize on our possession of a rare source of zooplankton, we conducted a two-way experiment to examine the effects of grazing and light on phytoplankton growth. This experiment consisted of four treatments, and all samples were filtered to remove zooplankton to allow for consistent sample composition among treatments. The filtrate containing zooplankton was pipetted back into treatments while the other two treatments did not receive zooplankton additions. Two treatments were covered with a single layer (high light) of shade cloth while the remaining two were placed under three layers (low light). Each treatment was replicated 3 times with a volume of 450mL, and temperature was controlled at 4.2°C. Statistical analysis using a two-way ANOVA reveals the absence of a light and grazer interaction. We did not observe a significant difference between mean growth rates of low-light and high-light treatments, but the presence of zooplankton significantly reduced phytoplankton growth rates (Fig. 5). We attribute our inability to observe photo-inhibition to the smaller separation of irradiance values between high and low light treatments during this experiment in comparison to previous light manipulation experiments.

**Figure 5:** Results analyzed using 2-Way ANOVA. Error bars represent +/-1 standard deviation. We were unable to detect an interaction effect ($p = 0.131$) or a light effect ($p = 0.25$). Mean growth rates within grazing treatments significantly lower ($p = 0.014$).
Discussion:

This series of experiments demonstrates that we have begun developing a methodology that can be used to effectively illuminate the mechanisms that drive changes in primary productivity within Keuka Lake. Through these experiments, we have been able to quantify changes in phytoplankton growth in response to nutrients, grazing and light.

The results of our nutrient addition bioassays suggest that phytoplankton populations in Keuka Lake are more limited by phosphorous than nitrogen. Bioassays conducted during July and September exhibited a phosphorous limiting effect while mean growth rates in nitrogen addition treatments were no significantly different than mean rates within the control. We have also demonstrated that nutrient limitations vary seasonally; while we observed a phosphorous limitation at the end of the phytoplankton growing season (i.e. early fall) nutrient and light interaction experiments carried out at the beginning of the growing season (i.e. early spring) suggested nutrients were not limiting. This temporal variation is to be expected because runoff and lake turnover in the early spring produce higher concentrations of nutrients whereas in the summer, nutrient concentrations have been depleted due to lake stratification and summer productivity. Results of the light manipulation experiments with the exception of our light-grazing interaction experiment imply that phytoplankton in Keuka Lake experience photo-inhibition during the winter and early spring months. Completion of similar experiments in the fall will elucidate possible temporal variation in this light effect. Lastly, the results of the light-grazer interaction experiment indicate that we can use this experimentation to quantify zooplankton grazing rates.

We have demonstrated that nutrients, light and grazing are variables that regulate phytoplankton growth in Keuka Lake. Moreover, we have exhibited that this methodology can be used to quantify seasonal changes in phytoplankton growth regulation by these variables. The validation of our successful replication of ambient growing conditions using survey data suggest that the results of our experiments potentially reflect lake processes. Phytoplankton are central to health of Keuka Lake, and our short-term microcosm experiments enable us to better understand what regulates phytoplankton growth. Future work at the Center for Aquatic research will seek to further develop this methodology in order to formulate predictive models and to gain a more holistic understanding of phytoplankton growth limitations. Ultimately, by combining surveys, experimentation and modeling we can increase our understanding of the role played by phytoplankton in maintaining lake health to better protect the Keuka Lake watershed.