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N:P Ratios and Temperature Affect Phosphate Uptake Rate in Phytoplankton Assemblages of a Cooling Water Reservoir

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ABSTRACT

Phytoplankton affinities for substrate uptake are influenced by temperature as well as nutrient concentration and may vary in response to environmental gradients. Newton Lake provides the opportunity to evaluate uptake rates by naturally occurring phytoplankton assemblages as influenced by heated effluent from a coal-fired power plant. We evaluated phosphate (PO_4-P) assimilation by assemblages from warm arm, forebay, and cold arm sites, which represent locations along a thermal gradient. Samples were collected and returned to the laboratory for *in vitro* determination of PO_4-P uptake at ambient temperature in response to nutrient additions. Treatment N:P ratios of 2.5:1 to 17.1:1 were intended to range from nitrogen to phosphorus limiting conditions. Under ambient conditions, PO_4-P uptake rates did not differ significantly among phytoplankton assemblages. When phosphate was limiting, uptake rates by warm arm and forebay assemblages were similar, but greater than that of the cold arm. At exceedingly high PO_4-P concentrations, any patterns of site-specific differences in uptake were not discernable.

INTRODUCTION

More than 80% of the natural landscape in Illinois has been converted to farmland (Rhoads and Herricks, 1996). With this change in landuse, a large proportion of waterways have been channelized, drained, and riparian zones removed in order to decrease flooding while increasing the amount of arable land for row crop agriculture (Infante et al., 2006; Talmage et al., 2002). Best management practices (BMPs) such as contour plowing, reduced tillage, buffer strips, and grassed waterways are very important to preserving stream integrity, but when not employed can increase the severity of agricultural disturbance (Allan and Castillo, 2007; Royer et al., 2006; Meador and Goldstein, 2003; Rabeni and Jacobson, 1999).

Impacts of agricultural practices also affect downstream bodies of water such as reservoirs. In agriculturally impacted regions, large quantities of sediment and nutrients flow into the reservoir. Watershed derived sediment and nutrients entering the reservoir can create varying abiotic conditions along the longitudinal gradient (Wetzel, 2001). Therefore, phytoplankton assemblages may respond directly to such environmental variability.

In conjunction with increased sediment and nutrient inputs, rising water temperatures are of concern in agricultural watersheds due to removal of the riparian zones and soil compaction (Allan and Castillo, 2007; Sheehan and Rasmussen, 1999). Increased temperature can have profound impacts on aquatic communities which are adapted to narrow temperature ranges. Also, at high temperatures, the solubility of dissolved oxygen in water decreases resulting in hypoxic conditions. These increases in temperature and hypoxia may stress and ultimately extirpate aquatic communities (Herbert and Steffensen, 2005; Larson and Larson, 1996). Subsequently, eutrophication and algal blooms caused by nutrient loading may further exacerbate these conditions (Morgan et al., 2006; Royer et al., 2006).

Phytoplankton taxa exhibit various affinities for the uptake of a limiting substrate at different temperatures, and assemblage structure among thermally diverse habitats may differ along a spatial gradient (Raven and Geider, 1988). Numerous experiments have been conducted to determine the effect of temperature on unialgal cultures. For example, Rhee and Gotham (1988) found that above 15°C *Scenedesmus* sp. exhibits an increase in substrate uptake rate with a decrease in temperature. Conversely, several studies have documented an increase in nutrient uptake with an increase in temperature (Lim et al., 2006; Reay et al,. 2001; Viner, 1984; Eppley, 1972). In marine phytoplankton, Goldman (1977) found that substrate uptake rates varied widely with regard to taxon. Though laboratory conditions of unialgal cultures may not mirror natural conditions of a reservoir, it is important to consider temperature as a major influence on the differentiation of zonal phytoplankton assemblages.

Monitoring and management of a reservoir may require understanding of the manner in which thermal variation affects trophic states and seasonal cycles. To our knowledge, little research has been conducted to determine whether phytoplankton assemblages vary in their uptake of nutrients across a temperature gradient within a reservoir. Newton Lake, a power plant reservoir in east-central Illinois, provides the opportunity to test the nutrient kinetics of naturally occurring phytoplankton assemblages across a thermal gradient. Therefore, the objectives of this project were to 1) determine which nutrient was in limitation across the thermal gradient and 2) determine if the natural phytoplankton assemblages at three points along the gradient differed with respect to uptake rate of the limiting nutrient.

Because temperature is known to increase rates of reaction, our hypothesis was that uptake rate of the limiting nutrient would increase with increasing temperature. Furthermore, Elliot et al. (2006) showed that with an increase in temperature, algal growing season expanded and timing of seasonal blooms changed with regard to species. Therefore, relative to the distinct thermal zones of Newton Lake, we reasoned that there would be a lengthy growing season in the warmer areas of the reservoir relative to the cold area, and that species-specific spring and summer blooms will occur at different times in each of these areas. Altered normal seasonal succession of taxa may cause variation in uptake rate among sites along the temperature gradient. That is, due to thermal disturbance, community structure may vary among sites at given times; this may lead to assemblage-wide differences in uptake rate.

METHODS

Study Site

Newton Lake, located in Jasper County in eastern Illinois, is a reservoir with a surface area of 718 hectares and a maximum depth of 12 meters. A coal burning power plant utilizes this lake as a source for cooling water. Water is drawn from the eastern (cold) arm of the lake and discharged into the western (warm) arm of the lake (Ameren, 2008). Three sample collection locations were used for this experiment with one located in the warm arm, one in the cold arm, and one in the forebay (Figure 1).

Preliminary Nutrient Determinations

Prior to the experiment, carbon, nitrogen, and phosphorus levels were determined for each location in order to establish which nutrient most likely limited phytoplankton productivity. Water samples were collected and taken to the laboratory where analyses were performed using standard methods (APHA, 1995). Inorganic carbon was established from total alkalinity by titration. Ammonia, nitrate and nitrite, and total phosphate and soluble reactive phosphate were determined using the phenate method, cadmium reduction method, and the ascorbic acid method, respectively. Because total alkalinity exceeded 48 mg $CaCO_3$ L^{-1}, we excluded carbon as a potential limiting nutrient (Moyle, 1949). The Redfield ratio (Redfield, 1958) suggests that phosphorus limitation occurs at N:P ratios greater than 7:1, as was the case in Newton Lake.

Experimental Protocol

Water samples were collected from the photic zone at each of the three locations on the following day using an integrated sampler fitted with 80-μm mesh Nytex® on the inlet, so as to exclude zooplankton. Depth of the photic zone was determined as 2x Secchi depth. All water samples were placed into acid-washed 2-L cubitainers and transported back to the laboratory. Sample aliquots were dispensed into clear 60-mL borosilicate vials which are certified to be free of any contaminants.

Three replicates of each sample were treated with one of seven different PO_4-P spikes making a total of 42 vials per location. PO_4-P spikes were formulated based on the preliminary nutrient concentrations (Table 1). Vials were incubated in the laboratory under ambient light in 40-L aquaria maintained within ± 1.3 °C of the temperature from which the samples were collected.

For each location, 42 vials were divided into short and long incubation time treatments of 21 vials apiece; the former incubation periods ranging from 32 to 55 min., the latter ranging from 212 to 245 min. Each incubation time treatment was further subdivided into three replicates of seven PO_4-P treatments. Following the short and long incubation periods, soluble reactive phosphorus in each sample was determined after passage through 0.45-µm pore nitrocellulose syringe filters to remove phytoplankton. For each location, rate of disappearance of PO_4-P was used as a surrogate for average uptake velocity (ν) as mg PO_4-P mg^{-1} ash-free dry weight hr^{-1} and was calculated using the following formula:

$$v = \frac{\sum_{i=1}^{3}\left[\sum_{j=1}^{3}\left(ss_i - s_j\right)\right]}{\sum_{i=1}^{3}\left[\sum_{j=1}^{3}\left(tt_i - t_j\right)\right]}\left(\frac{1}{VSS}\right)$$

where

ss = phosphate concentration at end of incubation,
s = phosphate concentration at beginning of incubation,
tt = time at end of incubation,
t = time at beginning of incubation, and
VSS = volatile suspended solids.

Statistical Analyses

All data were analyzed utilizing SAS 9.1 software. An analysis of variance (ANOVA) was used to determine if there were significant differences among uptake rates across locations for each concentration. Duncan post hoc tests were performed to determine where differences occurred. Significance was determined as $P \leq 0.05$.

RESULTS

An array of descriptive physiochemical data were collected at all three study locations the day of the experiment (Table 1). Secchi depths for the warm arm, cold arm, and forebay were 27.45, 25.35, and 26.85, respectively. Cursory examination revealed that phytoplankton assemblages were similar at all three sample locations.

Treatments had a unique influence on phytoplankton assemblages at each sample location (Figure 2, Table 3). Under ambient conditions, PO_4-P uptake rates were indistinguishable among phytoplankton assemblages at all three locations. Though significant differences in uptake rate were not apparent between locations in treatment 2, the phytoplankton assemblage in the cold arm appeared to exhibit a lower uptake rate than warm arm or forebay assemblages. For treatments 2 and 3, as relative phosphorus availability increased, uptake rate of the cold arm assemblage decreased. Additionally, cold arm assemblage uptake rate was lower than that observed in the other two assemblages, though the difference was only significant for treatment 3. Uptake rates for treatments 4 and 5, those with moderate N:P ratios ranging from 6.6:1 to 9.1:1, were not significantly different among the assemblages. Treatments 6 and 7, which had very high relative phosphorus availability, produced no discernable pattern in uptake rate, although a significant difference was observed between cold and warm arm for treatment 6. For all treatments with significant differences in uptake rate, warm and cold arm grouped independently, whereas forebay samples were associated with either the warm or cold arm samples, depending upon treatment (Table 3).

DISCUSSION

Nutrient Limitation and the Redfield Ratio

Environmental stoichiometry aids in determining which nutrient may limit phytoplanktonic taxa. Redfield (1958) suggested that a ratio of 40C: 7N: 1P is useful for determining the nutrient which is in short supply relative to need. In experiments utilizing *Selenastrum capricornutum*, Chiaudani and Vighi (1974) demonstrated that phosphorus limitation occurred at N:P ratios in excess of 10:1, and that nitrogen limitation resulted when ratios were below 5:1. At N:P ratios between 5-10:1, they demonstrated that either N or P could be limiting depending upon other environmental factors. Our treatments were formulated to approximate this general categorical division of nutrient limitation.

Uptake Under P-limitation (Treatments 2, 3)

Uptake of PO_4-P by phytoplankton was found to be a function of temperature and concentration. Because no differences in PO_4-P uptake were found among the three assemblages under ambient conditions, differences in uptake rate in experimental treatments could be attributed to phosphate augmentation. Adaptation of phytoplankton to ambient conditions may explain the apparent decreased uptake rate by cold arm assemblages in treatments 2 and 3. Forebay and warm arm assemblages likely exhibited a real increase in uptake rate, whereas uptake rate of the cold arm assemblage was perceived to have decreased. This may have been an artifact of our methodology. According to Nalewajko and Lean (1980), measurement of uptake as PO_4- P remaining in the filtrate rather than the particulate fraction may cause a significant departure from the Michaelis-Menten equation. In actuality, cold arm assemblage uptake rate likely remained constant, but appeared to decrease since it was calculated as a function of PO_4-P availability. Likewise, uptake by forebay and warm arm assemblages may have appeared constant over a range of concentrations if assemblages were able to increase uptake rate to match PO_4-P availability.

Though temperature of the forebay (13.28°C) and cold arm (12.00°C) relate more closely with one another than with the warm arm (21.80°C), the forebay assemblage functions in a manner similar to that of the warm arm assemblage. Suttle and Harrison (1988) showed that *Scenedesmus* sp. under N-limitation and *Asterionella formosa* under PO_4-P limitation exhibit optimal uptake rates at 15°C and 19°-20°C, respectively. In the same sense, distinct assemblages also may have optimum temperatures for nutrient uptake as determined by their constituent species (Reay et al., 2001). Uptake rates of PO_4-P for assemblages in the warm arm and forebay could be similar in the event that ambient temperatures fall on either side of the optimum.

Uptake in Absence of P Limitation (Treatments 4, 5, 6, 7)

Nutrient additions in treatments 4 through 7 produced unrealistically high phosphate concentrations resulting in fluctuations in PO_4-P uptake rates that are not easily interpretable. Fuhs et al. (1972) and Spijkerman and Coesel (1998) suggested that such concentrations can cause deviations from standard uptake models and are unpredictable. Some phytoplankton occurring at low N:P ratios may modify their nutrient uptake and thereby alter environmental stoichiometry (Ketchum, 1939). Phytoplankton not only absorb PO_4-P, but also continuously exchange it with the extracellular environment (Kuenzler and Ketchum, 1962). Moreover, Ketchum (1939) demonstrated that uptake of PO_4-P was dependent upon nitrate concentrations. Changes in the N:P ratio may result in either absorption or release of PO_4- P; thus, speculation on differences among treatments may be fruitless.

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Table 1. Physiochemical descriptors of Newton Lake locations collected on the day of sampling.

Variate	Warm Arm	Forebay	Cold Arm
Temperature (°C)	21.80	13.28	12.00
Specific Conductance (mS)	277	263	243
pH	7.40	7.09	7.25
Dissolved O_2 (mg O_2 L^{-1})	14.00	9.61	14.40
Total Phosphorus (mg PO_4-P L^{-1})	0.178	0.194	0.189
Soluble Reactive Phosphorus (mg PO_4-P L^{-1})	0.095	0.084	0.099
NO_2+NO_3 (mg L^{-1})	0.821	0.828	0.807
NH_4-N (mg L^{-1})	0.035	0.069	0.113

Table 2. Nitrogen availability relative to phosphorus for the warm, cold, and forebay sites of Newton Lake. Treatment 1 represents ambient relative to available nitrogen. Treatments 2-7 represent ambient plus phosphorus spike.

Treatment	Warm Arm	Cold Arm	Forebay
1	17.1	12.8	13
2	11.9	10.1	9.9
3	10.3	9	8.7
4	9.1	8.3	7.9
5	7.3	7	6.6
6	5	5	4.7
7	2.5	2.8	2.5

Table 3. ANOVA comparing influence of treatment on phytoplankton assemblage uptake rate for cold arm (C), warm arm (W), and forebay (F). Duncan post-hoc tests were performed on treatments with a significant $P \leq 0.05$.

Treatment	df	F value	*P*	Duncan grouping
1	2	1.6	0.2237	n/s
2	2	3.19	0.0593	n/s
3	2	31.27	<0.0001	W,F ≠ C
4	2	4.41	0.0234	W ≠ F,C
5	2	0.21	0.8149	n/s
6	2	7.83	0.0024	W,F ≠ C
7	2	0.72	0.4971	n/s
Error	22			

Figure 1. Map of Newton Lake in east-central Illinois, depicting three sampling locations. The warm arm (W), cold arm (C), and forebay (F) were sampled at N38.92444° W88.30254°, N38.91108° W88.29025°, and N38.89286° W88.30637°, respectively.

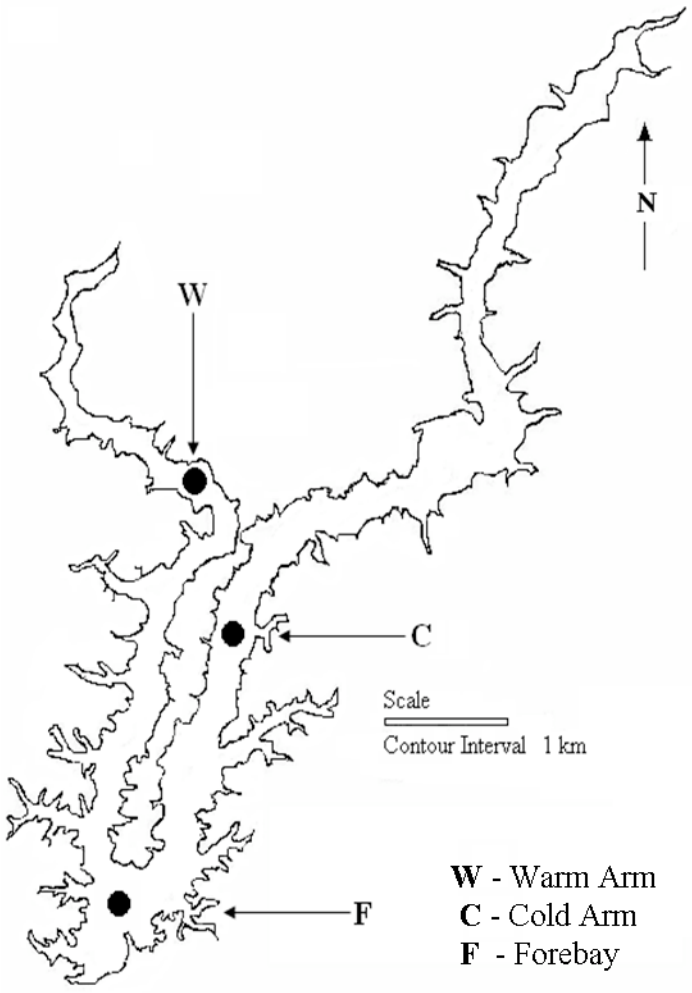

Figure 2. Mean uptake rate of phosphorus (mg PO_4-P mg VSS^{-1} hr^{-1}) for each treatment by location within Newton Lake (± standard error).