Phytoplankton Community Response to Commonly Limiting Nutrients in Lake Tahoe

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ABSTRACT

Declining water clarity is seen in freshwater lakes worldwide. The declining clarity is most commonly related to anthropogenic stresses, such as eutrophication, imposed on the lakes. Eutrophication in Lake Tahoe has caused increased levels of phytoplankton growth, which is the most prominent reason for its decline in water clarity. Although Lake Tahoe is considered a phosphorus-limited system, different species of phytoplankton in the lake may respond differently to the various nutrients causing the eutrophication, making it a difficult problem to solve with a simple management approach. I used a microcosm experiment to determine if the summer species of phytoplankton have different growth responses to inputs of varying levels and combinations of nitrogen and phosphorus. I analyzed the chlorophyll-a content and the community composition of each sample to determine that nitrogen and phosphorus each had quantifiable effects on phytoplankton growth. The responses of the unique phytoplankton morphospecies present in Lake Tahoe to the nitrogen and phosphorus suggest that Lake Tahoe should not be classified as a solely phosphorus limited lake for purposes of water clarity management. To best combat the growth of phytoplankton and thus maintain the water clarity of Lake Tahoe, management efforts should be focused on both nitrogen and phosphorus.

KEYWORDS

chlorophyll-a, bioassay, limnology, Sierra Nevada, eutrophication

INTRODUCTION

Climate change and anthropogenic stresses on freshwater lakes worldwide has resulted in increasing eutrophication and thus decreasing water clarity. Eutrophication is the phenomena by which excess nutrients such as nitrates and phosphates are introduced into a system where they do not naturally occur at such high levels. Algal blooms, sudden increases in the population of algae in water bodies, are stimulated by eutrophication (Hecky et al. 2010, Domis et al. 2013, Shatwell et al. 2013) and are the main cause of the reduction of clarity in freshwater lakes as they absorb much of the light entering the water, preventing it from reaching and illuminating lower depths. Because anthropogenic sources have increased the atmospheric concentration of CO_2 by over 100 μatm (Tyrrell 2011), phytoplankton communities have shifted from carbon limitation to nitrogen and phosphorus limitations (Verschoor et al. 2013), heightening the effects of nutrient inputs and leading to more severe and widespread eutrophication. Evidence of this worldwide CO_2 impact has been recorded in Lake Victoria in Africa (Hecky et al. 2010), Lakes Constance, Geneva, Zürich, Walen, Garda, and Maggiore in Europe (Gallina et al. 2013), João Alves Reservior, Marechal Dutra Reservoir, and Santa Cruz Reservoir in South America (Chellappa et al. 2009), as well as in Lake Tahoe in North America (Schladow 2012).

Analysis of biological factors in Lake Tahoe reveals four main mechanisms by which its water clarity has historically decreased: (1) atmospheric deposition of nitrogen and particulate matter (Dolislager et al. 2012), (2) high levels of fine sediment loading through runoff (Simon 2008), (3) reduced lake water mixing resulting from increasing water temperatures (Sahoo et al. 2013), and quantitatively the most significant, (4) increased algal growth resulting from the influx of nitrogen and phosphorus generated primarily by vehicle exhaust and road dust, respectively (Sahoo and Schladow 2008, Smith and Kuchnicki 2008). Over the last five years, Lake Tahoe's decline in water clarity has been most largely attributed to the increased growth of phytoplankton from eutrophication (Schladow 2012). Despite efforts by local residents, businesses, and government agencies to restore the lake to its historic level of clarity by reducing daily input loads of nitrogen and phosphorus (Smith and Kucknicki 2008), Lake Tahoe continues to experience eutrophication.

Although Lake Tahoe is considered a phosphorus-limited system (Gergans et al. 2011), different species of phytoplankton in the lake may respond differently to the various nutrients

causing the eutrophication (Rhee and Gotham 1980), making improving the water clarity a difficult problem to solve with a simple management approach. In general, water bodies are often considered to be either nitrogen or phosphorus limited, meaning that the balance of nitrogen and phosphorus in the system is off, and that upon addition of the less available nutrient, or limiting nutrient, there will be a greater increase in algal growth than upon addition of the nutrient that is not limiting. The challenge with this is that the responses of individual species of phytoplankton to additions of limited nutrients can differ from the response of the community as a whole. Each species has unique nutritional requirements, meaning it will use nitrogen and phosphorus at rates and quantities that may not correlate to that of the other species in the community, or to the system's larger classification of being either nitrogen or phosphorus limited (Francoeur 2001). The summertime phytoplankton community composition of Lake Tahoe consists of 51 different species of phytoplankton (Tahoe Environmental Research Center, unpublished data) and because there are so many species present, there is a high possibility that the generalizations about phosphorus limitation (Gergans et al. 2011) are not entirely appropriate (Rhee and Gotham 1980). Though extensive research by the Tahoe Environmental Research Center has shown through composite bioassay experiments that the overall phytoplankton community in Lake Tahoe responds to varying levels of nitrogen and phosphorus additions, the individual species responses to these additions have yet to be quantified.

To understand the effects of eutrophication on phytoplankton in Lake Tahoe, I used a microcosm experiment to determine if the summer species of phytoplankton have different growth responses to inputs of varying levels and combinations of nitrogen and phosphorus. The nitrogen component of the nutrient additions was ammonium nitrate and the phosphorus component was sodium phosphate, as these are the two largest components of the nitrogen and phosphorus that enter Lake Tahoe. Although this lake is a phosphorus limited system, I expected that some species may respond more to nitrogen additions than phosphorus additions, and that not each species in this experiment would respond significantly to phosphorus additions. I did expect that the individual species would have different nutritional use levels and that therefore, within each treatment group, some species would have stronger responses to nitrogen and/or phosphorus while others would have weaker responses.

METHODS

Study system

Lake Tahoe is a large alpine lake with a perimeter of 72 miles, containing an estimated 122 million acre-feet of water, located on the border of California and Nevada in the Sierra Nevada Mountain Range at an elevation of about 6,200 feet (Figure 1), making it the highest lake of such a large size in the United States. It is the second deepest lake in the United States, with a maximum depth of 501 meters and an average depth of 305 meters. It has an average surface water temperature of 5-10°C in the winter and 18-21°C in the summer, but remains at 3.9°C all year below 244 meters deep (Boughton et al. 1997). The most recent clarity readings indicate clarity of 23 meters (Tahoe Environmental Research Center, unpublished data), down from historic levels of over 30 meters just 40 years ago (Schladow 2012). The total population of residents around the lake is 53,000 (Antonucci 2010).

Figure 1: Location of Lake Tahoe within California.
(http://geology.com/topographic-physical-map/california.shtml)

Sampling site

The sampling site for this experiment, where I harvested the water and phytoplankton, is located near the center of Lake Tahoe, very close to the Tahoe Environmental Research

Center/NASA's "TB3" buoy (39° 06.662 N, 120° 04.272 W; Figure 2). I harvested the water, containing the phytoplankton, on July 24, 2013; 51 species of phytoplankton are known to be present during this season in Lake Tahoe (D. Hunter, *personal communication*).

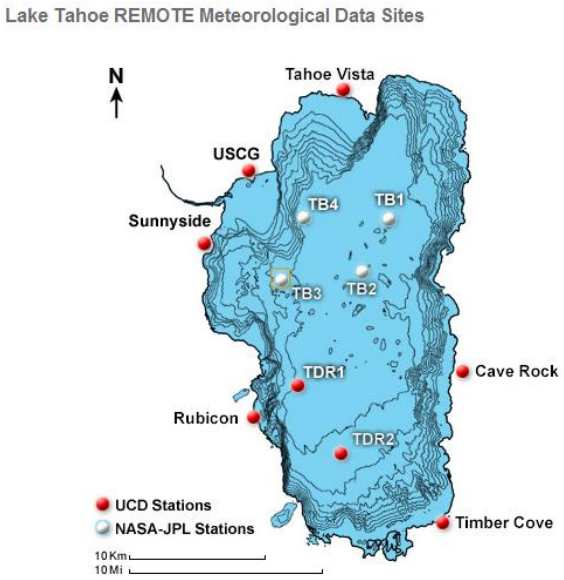

Figure 2: Location of TB3 buoy in Lake Tahoe.
(http://remote.ucdavis.edu/tahoe_location.asp#datasites)

Data collection methods

Phytoplankton collection and microcosm set up

To determine how eutrophication is impacting the phytoplankton species present during the summer season in Lake Tahoe, I conducted a microcosm experiment using methods adapted from Allen et al. (2011). From the depths of 5, 20, 40, 60, 75, and 90 meters, I collected 4 gallons of water each, for a total of 24 gallons of water. This water was harvested from the Tahoe Environmental Research Vessel near the TB3 buoy using 3 liter Van Dorn bottles, and filtered on-board as I collected it using an 80-micrometer mesh filter to remove any zooplankton. I homogenized the water in a large vessel then placed 1,350 milliliters of the homogenized water into each of 54 2.5 liter buckets. To mediate the water temperature of the small buckets on land, I placed them in a cooling pool of regular water.

Nutrient treatments

This experiment had six nutrient treatment groups. I prepared the nutrient stock solutions, a 10 mg N/liter nitrogen stock solution (using Fisher Scientific Certified ACS ammonium nitrate, NH_4NO_3) and a 5 mg P/liter phosphorus stock solution (using Fisher Scientific Certified ACS sodium phosphate dibasic anhydrous, Na_2HPO_4) then applied treatments to each microcosm for a total of 9 replicates each: control (no nutrient addition), 20 ppb nitrogen (2.7 ml N-stock), 2 ppb phosphorus (.54 ml P-stock), 10 ppb phosphorus (2.7 ml P-stock), 20 ppb nitrogen + 2 ppb phosphorus (2.7 ml N-stock + .54 ml P-stock), and 20 ppb nitrogen + 10 ppb phosphorus (2.7 ml N-stock + 2.7 ml P-stock). After adding the nutrients, I incubated the microcosms for six days, then took 330 milliliter aliquots (approximately) from each microcosm and placed them in a dark cooler for future processing.

Sample processing for chlorophyll-a content

To determine the relative phytoplankton biomass of each treatment group, I processed the aliquots for chlorophyll-a content with a spectrophotometer. I adapted the methods for this analysis from McCarthy et al. (2011). I filtered 100 milliliters of water from each aliquot onto Whatman GF/D glass microfiber filers with 2.7 μm pores, adding a small amount of magnesium carbonate during filtration to each to preserve the cells, then immediately froze the filters. I later thawed the filters, suspended the phytoplankton cells and analyzed each filter with the spectrophotometer at 750 nm and 664 nm, then added .1 milliliter of .1N hydrochloric acid (Ricca Chemical Company, HCl) and analyzed them again at 750 nm then 665 nm. I corrected each 664 nm and 665 nm absorbance reading output by the spectrophotometer by subtracting its respective (pre or post acidification) 750 nm reading from it. I used the corrected values in a formula that yielded the chlorophyll-a content of each aliquot (Eq. 1).

$$Chlorophyll\ {}^{ug}/_{L} = \frac{26.7\ (corr.664 - corr.665) x\ volume\ of\ extract\ in\ L}{\frac{[volume\ of\ sample\ in\ L]}{1000}\ x\ 1\ cm}$$

Equation 1: Equation used to calculate chlorophyll-a content of samples.

Sample processing for phytoplankton community analysis

To determine the differences in algal community composition in the different nutrient treatments, I identified the phytoplankton present through classifying them into morphospecies groups and quantifying their abundance in each sample. After I removed the 100 milliliters of water from each aliquot for processing with the spectrophotometer, I preserved the remaining 220 milliliters (approximately) by adding .75 milliliters of Lugol's Solution (Fisher Scientific iodine solution) to each. To increase the concentration of cells present in the small volume of water I used for identification purposes, I settled 50 milliliters of each sample down to 15 milliliters then down to 2 milliliters over a two week period. I then used a Palmer Counting Cell, which allows for viewing of 0.1 milliliter of liquid at a time, to view the phytoplankton beneath the microscope. To improve the accuracy of the identifications, I classified the phytoplankton I saw under the microscope into 12 morphospecies groups based off their appearances. I used a photographic guide I generated from a species list provided by the Tahoe Environmental Research Center, paying special attention to the size and shape of each cell, the presence of flagella, and the internal contents of each cell, to assign species to each morphospecies group (Table 1). It is important to note that not all of the 51 species known to be in Lake Tahoe during this season were present in my study. I identified the morphospecies of phytoplankton in .025 milliliter of water from each aliquot. I used an Olympus CH microscope and 400x total magnification to identify the phytoplankton morphospecies.

Table 1: Phytoplankton species in each morphospecies group.

Morphospecies Group	Phytoplankton Species in this Group
Morphospecies 1	*Botryococcus braunii*
	Gloeobotrys limneticus
Morphospecies 2	*Synedra acus var. radians*
	Fragilaria capucina
	Aulacoseira italic
	Planktonema lauterbornii
Morphospecies 3	*Cryptomonas sp.*
	Rhodomonas lacustris
	Cosmarium abbreviatum
Morphospecies 4	*Chromulina sp.*
	Kephyrion culpiforne
	Kephrion rubric-claustri
Morphospecies 5	*Staurastrum longipes*
	Ankistrodesmus spiralus
	Dinobryon cyst
	Chrysolykos planctonicus
	Chrysolykos skujaii
Morphospecies 6	*Navicula pupa*
Morphospecies 7	*Chrysomulina parva*
Morphospecies 8	*Peridinium inconspicuum*
	Gymnodium lantzchii
	Gymnodium cnecoides
Morphospecies 9	*Cyclotella gordonensis*
Morphospecies 10	*Carteria sp.*
Morphospecies 11	*Microcystis elachista*
Morphospecies 12	*Diploneis smithii var. pumila*

Data analysis methods

Algal biomass

To determine which nutrient(s) had the most quantifiable effects on which species, I intended to use ANOVA tests on the chlorophyll-a readings. I selected the method of ANOVA analysis to show differences between and within treatment groups. Statistical analysis was conducted using R and R Commander (R Development Core Team 2010).

Community composition

To determine the differences in community composition of the phytoplankton morphospecies between the treatment groups, I conducted Non-metric Multidimensional Scaling

(NMS) tests. The NMS tests revealed similarities and differences in the abundances of each morphospecies in each treatment group. I also determined parameters such as species evenness, richness, and diversity for each treatment group. Statistical analysis was conducted using R and R Commander (R Development Core Team 2010, Fox 2005).

RESULTS

Chlorophyll-a

The results of the chlorophyll-a calculations were inconclusive. Plugging the absorbance readings of each sample from the spectrophotometer analysis into the chlorophyll-a content equation yielded negative values. The readings could not be further analyzed as anticipated, as a negative content of chlorophyll-a in the samples indicates that a problem occurred. Given this situation, these values were not used for analysis and not considered in the conclusions drawn from this study.

Phytoplankton community

The 20 ppb N + 2 ppb P treatment group had the highest evenness, the 20 ppb N + 10 ppb P treatment group had the highest richness with 10 morphospecies, and the two combination groups (20 ppb N + 2 ppb P and 20 ppb N + 10 ppb P) were the most diverse, with the same Shannon Diversity Index of H = 1.59. I found that morphospecies 3 and morphospecies 4 had the widest variation in prevalence amongst the different treatment groups, as they were found in all 6 treatment groups, morphospecies 6, morphospecies 7, morphospecies 10, morphospecies 12 had the least amount of variation, as they showed up in only one or fewer treatment groups (Table 2).

NMS tests yielded plots that revealed abundance correlations between the treatment groups and the morphospecies found in each. In the NMS plot showing morphospecies groupings by treatment, the control group lies across the ordination, each uniquely phosphorus treated group is pulled to the left of the ordination, and every group involving nitrogen, including the combination treatment groups, lies on the right side of the ordination (Figure 3). The NMS plot of the morphospecies responses to the treatments shows that each species responded to the treatments

differently; the closer the morphospecies groups lie to each other, the more similarly they responded to the nutrient additions, and the farther apart they are from each other, the more differently they responded (Figure 4).

Table 2: Relative abundances of each morphospecies in each treatment group.

	Control	**20 ppb N**	**2 ppb P**	**10 ppb P**	**20 ppb N + 2 ppb P**	**20 ppb N + 10 ppb P**
Morphospecies 1	8.51%	7.69%	9.60%	5.93%	8.61%	10.0%
Morphospecies 2	0%	0%	4.80%	3.39%	3.97%	1.54%
Morphospecies 3	34.0%	40.0%	37.6%	35.6%	36.4%	36.2%
Morphospecies 4	42.6%	44.6%	37.6%	42.4%	34.4%	35.4%
Morphospecies 5	10.6%	4.62%	1.60%	5.93%	8.61%	6.92%
Morphospecies 6	0%	0%	0%	0%	0%	1.54%
Morphospecies 7	0%	0%	0%	0%	0%	0.77%
Morphospecies 8	0%	1.54%	0.80%	4.24%	2.65%	1.54%
Morphospecies 9	0%	0%	7.20%	1.69%	2.65%	1.54%
Morphospecies 10	0%	0%	0%	0%	0%	0%
Morphospecies 11	4.26%	1.54%	0.80%	0.85%	1.99%	4.62%
Morphospecies 12	0%	0%	0%	0%	0.66%	0%

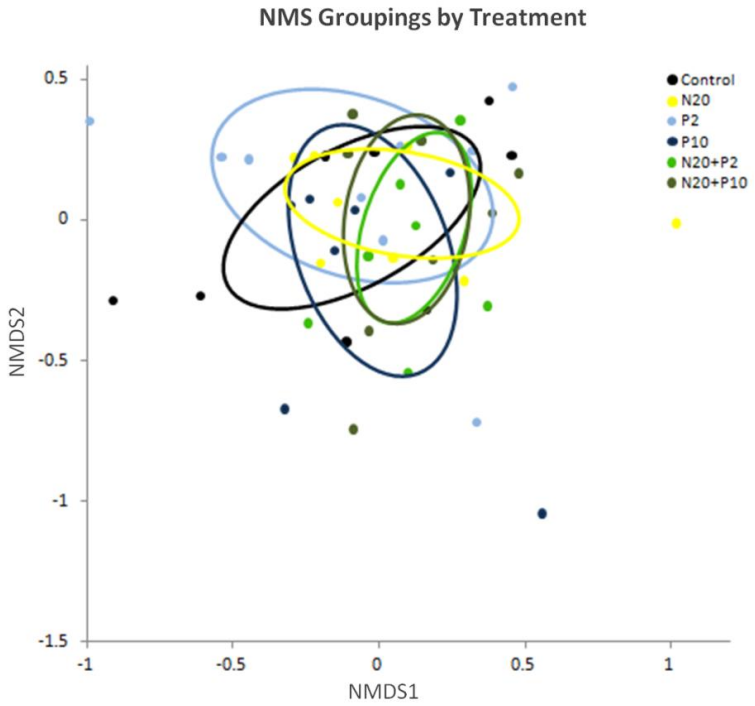

Figure 3: NMS plot showing correlations of morphospecies abundance amongst the treatment groups.

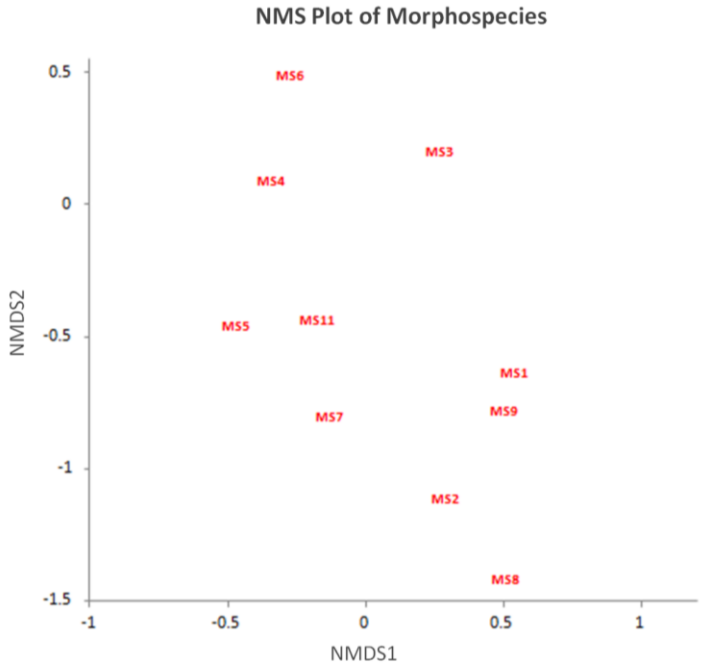

Figure 4: NMS plot showing correlations between morphospecies responses to nutrient treatments.

DISCUSSION

The responses of the unique phytoplankton species present in Lake Tahoe to the nitrogen and phosphorus additions suggest that Lake Tahoe should not be classified as solely a phosphorus limited lake for purposes of water clarity management. Analysis of the phytoplankton community composition of each treatment group supported these conclusions. Each morphospecies of phytoplankton responded differently to nitrogen versus phosphorus. Both nitrogen and phosphorus resulted in growth responses in the morphospecies. Given that both nutrients caused increases in phytoplankton growth, management efforts should emphasize mitigating any nutrient input to restore historic water quality and clarity.

Effects on algal biomass

The results of the chlorophyll-a analysis were inconclusive, due to the negative values yielded by the chlorophyll-a content equation, so I am unable to draw conclusions about the effects of the nutrient additions on algal biomass. The most likely source of the error in this portion of the experiment was that too little water was filtered for analysis with the spectrophotometer. The low volume of water filtered along with the fact that Lake Tahoe is not an overly productive lake mean that there may have been too small an amount of chlorophyll-a in the samples for the absorbance readings and the chlorophyll-a content equation to yield plausible values.

Phytoplankton community

There were greater amounts of phytoplankton present in the treatment groups involving nutrient additions than in the control groups, which supports the conclusions found by Sahoo and Schladow (2008) that nutrient influx into Lake Tahoe is causing increased levels of phytoplankton, and thereby contributing to decreasing clarity. In accordance with ideas of multi-nutrient limitation (Lewis and Wurtsbaugh 2008, Francouer 2001), the plots generated by the NMS tests revealed that different morphospecies did have different responses to the varying nutrient treatments, as some morphospecies responded most to nitrogen, some to phosphorus, and some to the combination groups. The particulars of which morphospecies were most affected by the additions were

dependent on their unique nutritional needs (Hecky and Kilham 1988), as different species have different optimum nutrient ratios for growth.

Since Lake Tahoe has historically been considered a phosphorus limited lake, it was expected that I saw the highest number of morphospecies respond most prominently to the phosphorus treatments. The morphospecies groups in which I saw the greatest response to phosphorus were two, eight, and nine. These groups include such species as *Cyclotella gordonensis*, *Peridinium inconspicuum*, *Gymnodium lantzchii*, *Synedra acus var. radians*, *Fragilaria capucina*, *Aulacoseira italic*, and *Planktonema lauterbornii*. Two of the species in these groups, *Cyclotella gordonensis* and *Peridinium inconspicuum,* are known to be sensitive to the upwelling of nutrients in lakes, particularly to the upwelling of phosphorus, which would explain their prominent response to this nutrient addition (Forrest et al. 2002, Townsend et al. 2012).

As I saw certain morphospecies groups respond more significantly to phosphorus than the other nutrients, I also saw groups that responded to nitrogen more than any of the other nutrients. The two morphospecies groups that responded most significantly to nitrogen were three and four. Interestingly, these were the groups that made up the highest relative abundance in every sample. The species in these morphospecies groups are *Cryptoponas sp*., *Rhodomonas lacustris*, *Cosmarium abbreviatum*, *Chrysomulina parva*, *Chromulina sp*., *Kephyrion culpiforne* and *Kephrion rubric-claustri*. It is known that the growth of one of the species in this group, *Cosmarium abbreviatum,* is hindered by the presence of phosphorus (Wilson et al. 2007). Given that the water used in this study was taken directly from Lake Tahoe, it must have phosphorus in it already, which leads me to believe that the addition of nitrogen to a system like this is the only thing that would encourage the growth of a species like this.

Evidence from this study that there are certain morphospecies that respond differently to nutrient additions due to physiological differences, along with the fact that there were also certain morphospecies that flourished in the combination treatments, supports the concept that lakes can be limited by more than one nutrient. I did not see statistically significant results in this study, but that could be partially due to the fact that predicting algal responses to nutrient additions is very challenging because of all the biological factors they are susceptible to, as well as the environmental stochasticity they are exposed to (Watson et al. 1997). In spite of the challenges associated with studying phytoplankton, they are a key facet of aquatic communities and thus important to understand. In spite of the uncertainty, it is reasonable to conclude that increases in

available nutrients led to increases in algal biomass, and thus that mitigating against nutrient inputs into water is an essential component of preventing water clarity loss.

Effects on water clarity

The disproportionate responses seen between the different morphospecies in this experiment, combined with information from previous studies on phytoplankton taxonomy allow for estimations of which species might have the most negative effects on Lake Tahoe clarity. Recent studies (Schladow 2012, Winder et al. 2009) give details of why the species Cyclotella has been of highest concern in Lake Tahoe in recent years. Cyclotella has a comparatively small body size to the other species of phytoplankton in the lake, only a few micrometers in diameter, and thus is able to remain suspended in the upper parts of the water columns in Lake Tahoe, allowing them to absorb and obscure more of the light coming into the lake than other species. Cyclotella also favors high nutrient environments, so as nutrient inputs continue to increase, Lake Tahoe will become better suited for Cyclotella. This study showed that Cyclotella responded most significantly to the presence of phosphorus, in the 2 ppb phosphorus treatment group particularly, thus given their high impact on water clarity and this response, phosphorus should be a management priority.

Limitations and future directions

Given this study's restricting budget and time frame, the most prominent limitation was that it could not be performed in a system that could more closely mirror that of Lake Tahoe. Although the microcosms were designed to mimic the lake's conditions, it was challenging to control was the temperature of the mesocosms; consequently they had a much higher temperature than average lake water. Similarly, I did not have the equipment to do the recommended in-vivo testing on the chlorophyll-a content of each microcosm, which would have yielded more accurate results than the method I used. Increasing the volume of the microcosms would enable accurate assessment of the chlorophyll-a content as well. Seasonal differences in nutrient limitation, as Trommer et al. (2013) suggests, could also have been a source of the differences between the results of this study and other studies.

Abundance of phytoplankton also differs seasonally. This study was conducted in the summer and there could have been different conditions this summer than other summers, leading to an altered environment for the phytoplankton which could have resulted in different responses. Anderson et al. (2002) argued that the same nutrient loads under different conditions yield different results. Avoidance of these limitations will yield more ecologically sound results in future studies.

This study focused specifically on Lake Tahoe, with nutrients known to affect this lake, so it cannot be directly replicated to other lakes. A similar study design could be applied to other lakes that are threatened by eutrophication such as Lake Victoria (Hecky et al. 2010), Lake Constance, Lake Geneva, Lake Zürich, Lake Walen, and Lake Garda (Gallina et al. 2013). Additionally, further research could be done on each individual species of phytoplankton present in Lake Tahoe to quantify nutritional needs. Another expansion upon this study would be to apply it at a larger scale, with a wider range of nutrient additions to fully assess the responses of the phytoplankton across a large spectrum of nutrient concentrations and combinations.

Broader implications and conclusions

Lake management efforts should not be based off the idea of a sole limiting nutrient in the water body. This study showed that although Lake Tahoe is regarded as a phosphorus limited lake, nitrogen also had a quantifiable effect on the phytoplankton species present in the study, indicating that the concentrations of both nutrients in this lake should be a concern for algal growth.

To best combat the growth of phytoplankton and thus maintain the water clarity of Lake Tahoe, management efforts should be focused on both nitrogen and phosphorus. My recommendation is consistent with reasoning used by Gikuma-Njuru et al. (2013), who found Lake Victoria to be phosphorus limited and recommended that phosphorus loading be a management priority. The increases in growth in the nutrient treated groups as compared to the control group which received no addition indicate that all the nutrients need to be managed for.

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