Phytoplankton Nutrient Response in Oyster Pond: Identification of Cyanobacteria and Potential for Future Blooms

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Abstract:

With human population on the rise, anthropogenic nutrient inputs to Cape Cod's many scenic coastal kettle ponds have increased as well. In the summer of 2016, Falmouth's Oyster Pond experienced one of the worst cyanobacterial blooms to ever affect this eutrophied body. By conducting incubation experiments of the pond water with nitrogen, phosphorus, and nitrogen and phosphorus enrichments, I was able to stimulate and measure algal growth under these varying conditions. I additionally conducted sediment incubations under the same nutrient conditions to stimulate algal growth from dormant cells in the sediment. Water incubations enriched with both nitrogen and phosphorus had a peak chlorphyll-a fluorescence of 7.63 µg/L, while water incubations enriched with phosphorus had the second most growth at 4.61 µg/L. Incubations enriched with nitrogen alone showed little to no response (2.74 µg/L). I saw similar trends in my sediment incubations. From my sediment and water incubations, I was able to identify at least five genera of cyanobacteria (*Microcystis*, *Aphanocapsa*, *Cylindrospermopsis*, *Psuedoanabaena,* and *Merismopedia*). Through single-colony PCR amplification, I confirmed suspected *Microcystis* and *Aphanocapsa* colonies as cyanobacterial species and microcystin toxin producers. Additional in-field water column profiling has shown a slight increase in the salinity of Oyster Pond over the past four months. If this upward trend continues so that salinity is between 2-4 ppt next summer, the likelihood of a freshwater cyanobacteria bloom will be minimized.

Key Words: cyanobacteria, harmful algae bloom, microcystin, *Anabaena, Aphanizomenon, Microcystis, Aphanocapsa*, eutrophic, phosphorus-limited

Introduction:

Cyanobacteria, formerly known as blue-green algae, are the oldest photoautotrophs on Earth. Though we have oxygen to thank them for, some species have the capability to do more harm than good. Unlike planktonic algal species, many cyanobacteria species have the ability to fix atmospheric nitrogen, and are therefore able to colonize phosphorus rich and nitrogen depleted waters (Ramm et al, 2014). Because cyanobacteria also have the ability to control their buoyancy, they are competitively able to adjust their position in the water column for optimal photosynthesis (Graham et al, 2009). When a surplus of nutrients occurs in freshwater ponds and lakes, this can lead to an overproduction of cyanobacteria, known as a harmful algae bloom (HAB). In addition to producing toxic secondary metabolites, these blooms can increase the turbidity of water and block sunlight to aquatic macrophytes. After the death of a bloom and aquatic plants, bacterial decomposition can lead to oxygen depleted waters, which are often characterized by mass fish kills (Paerl and Otten, 2013).

In July, 2016, Falmouth's Oyster Pond began experiencing one of the worst blue-green algal blooms ever to affect the water body. Community members were warned to keep pets and children away from the water to avoid potential toxins. This pond is particularly susceptible to blooms because of its eutrophic state (Feldott, 2016). A major factor in this season's bloom is the blockage of salt water flow into the pond from the Vineyard Sound via the Trunk River. A weir controlling the influx of water to the pond has acted to maintain a balance of saline and fresh water in the pond in previous years. Recently, the river has been taken over by phragmites, limiting its flow. This, along with the drought has led to a lag in the flushing of the pond (Kerfoot, 2016).

Five truckloads of phragmites roots were removed from the river in April, 2016. Despite these efforts, excessive growth of elodea was observed in June, and the river is likely to be reestablished with phragmites in the years to come. Because outflow was still minimal, two emergency dredgings were conducted, and proved to be successful, as outflow increased from 736 m^3 day^{-1} to 2,600 m^3 day^{-1} (Kerfoot, 2016).

Oyster Pond was a mostly freshwater system this summer, and we can therefore suspect that it was a phosphorus limited system. It is very typical for a freshwater lake or pond to be phosphorus limited due to small inputs of phosphorus relative to other nutrients under natural conditions. Even with anthropogenic inputs of phosphorus on the rise, most phosphorus that enters a freshwater system will abiotically precipitate with iron, calcium, and manganese ions present in the water and will then be occluded in the sediments. The remainder of phosphorus in a system is taken up by phytoplankton and cyanobacteria (Schlesinger and Bernhardt, 2013).

Although the bloom subsided in mid-September due to cooler temperatures, appropriate conditions could stimulate a bloom next summer. Though *Anabaena* and *Aphanizomenon* were the most prevalent genera of cyanobacteria in this past season's bloom at 17,680 cells/mL (Figure 2), *Microcystis* and *Aphanocapsa* genera were the most abundant cyanobacteria genera in my incubation experiments. *Microcystis* is one of the most common and most studied genera of cyanobacteria. This genus, along with other cyanobacteria genera (including *Anabaena*) have the ability to produce microcystin, a hepatotoxin that can lead to symptoms such as nausea, vomiting, paralysis, and acute liver failure in large doeses or with long-term exposure (NOAA, 2015).

Anabaena and *Aphanizomenon* spores (akinetes) have been known to resist desiccation and can lie dormant in pond sediment until favorable conditions arise (Cirés et al, 2013). The

resting stage cells will then recruit into the water phase where they will grow and reproduce (Stahl-Delbanco, 2003). Though *Microcystis* does not produce akinetes, it has the ability to maintain its vegetative cells while overwintering in sediment (Cirés et al, 2013). With this knowledge of the cyanobacterial life cycle, we could predict bloom reoccurrences, and which species will present in years to come assuming appropriate nutrient and salinity conditions.

Methods:

Water column profiling: I conducted basic water quality profiles at five Oyster Pond sites (Mosquito Creek, OP1, OP2, OP3, and the weir) at the start of my project on November 14, 2016 (Figure 1). I also collected a second set of measurements three weeks later, on December 5, 2016. Measurements in the first week included total ammonium (Solarzano, 1969), nitrate (Wood et al, 1967), phosphate (Murphy and Riley, 1962), and chlorophyll-a (Lorenzen, 1967) using slightly modified methods. Water samples for these measurements were collected at the surface and 0.5 meters above the pond floor at each site (OP1- 4.5 m, OP2- 3.5 m, OP3- 6 m). Only one set of samples was collected at the weir and Mosquito Creek sites. I also collected Hydrolab water profile measurements including pH, conductivity, salinity, photosynthetically active radiation (PAR), dissolved oxygen, and percent dissolved oxygen. These data were not collected at Mosquito Creek due to the minimal depth and flow of the creek. Third week measurements included just Hydrolab water profile data at sites OP1, OP2, OP3, and the weir.

Water incubations: During the first field sampling, I collected water from the Northeast cove of Oyster Pond at the Ransom road dock. After returning to the lab, I set up water incubations in one liter soda bottles after filtering the water through 250 µm mesh to remove any large zooplankton. I enriched my samples to 100 µM ammonium nitrate and 20 µM sodium phosphate. My treatments included three controls, three nitrogen enriched, three phosphorus enriched, and

three nitrogen and phosphorus enriched incubations. I incubated all of these samples on a shaker table (set to 70 rpm) in a 21°C growth chamber on a cycle of 14 hours light (185 PAR), 10 hours dark for seven days. I collected initial and final phosphate, nitrate, ammonium, and chlorophyll concentration data to determine nutrient usage and phytoplankton growth. I also conducted chlorophyll fluorescence measurements every other day throughout the duration of the incubation using a 10-AU fluorometer.

Sediment Incubations: I also collected sediment samples at the Ransom Road dock with an Ekman grab. I set up 314 cm^3 of sediment in two liter soda bottles and topped the bottles off with 1.5 liters of GF/F filtered pond water. I added ammonium nitrate and sodium phosphate to the water to reach the same concentrations as my water incubations. My treatments included three controls, three nitrogen enriched, three phosphorus enriched, and three nitrogen and phosphorus enriched sediment incubations. Two of these repetitions were exposed to an unintended heat shock where water temperatures rose from about 15 °C to 27 °C over a period of 12 hours. The third repetition was started two days later as a positive control measure. I also included a one liter bottle filled with GF/F filtered pond water as a negative control. After the initial heat shock of reps 1 and 2, these incubations were exposed to the same conditions as my water incubations, but were not incubated on a shaker table as to not disrupt the sediment. I tested the water above the sediments for initial and final phosphate, nitrate, ammonium, and chlorophyll concentrations. I also conducted chlorophyll fluorescence measurements of this water every other day.

Cyanobacteria Counts: I performed *Microcystis* and *Aphanocapsa* colony counts of Lugol's iodine preserved incubation samples from the fourth day of incubation (11/23/16) using Utermöhl settling chambers (Utermöhl, 1958). I settled 10-12.5 mL for my less concentrated water samples (usually from sediment incubations), and 5 mL for the more concentrated samples

(from water incubations) over a period of at least 12 hours. The colonies were counted with a 10x dry objective on a Zeiss Axiovert 135 inverted microscope. For both genera, I counted the number of grids covered by each colony and then found the average number of cells per colony (144 *Microcystis*, 900 *Aphanocapsa*). I then used these two values, and my initial sample volume to calculate the number of cells per mL sample water. Any pictures were taken with Zeiss Axiocam 503 mono.

Sediment Colony Counts: I conducted initial and final sediment colony counts using a Sedgewick-Rafter Counting Cell. I diluted glutaraldehyde preserved sediment samples with 59 parts water and counted 1 mL of the diluted sediment using a Zeiss Axio Imager.M2m epifluorescence microscope. From this data, I then calculated the number of colonies in one milliliter of sediment.

Single-Colony PCR: At the end of the incubation period I collected samples for PCR analysis by placing a water sample under an inverted microscope and using a capillary tube to mouth-pipet colonies of particular interest into PCR tubes. Any samples that had been previously preserved with Lugol's iodine solution were treated with 10 µL of 2.47 µM sodium thiosulfide solution to decrease the inhibiting effects of the preservative (Auinger et al, 2008). The cells were stored at -80 °C until analysis to lyse the cells. I then performed separate PCR reactions to amplify the cyanobacterial 16S rRNA gene (CYA) or microcystin synthetase gene B (McyB). The primers for the 16S rRNA gene were CYA106F and CYA781R(a) at 100 µM concentrations (Nübel et al, 1997). We used a 25 µL reaction mixture with 2.5 µL 10x buffer, 0.5 µL forward and reverse primers, 0.5 µL of 10 mM dNTPs, and 0.25 µL Taq polymerase. We used a PCR cycle of 94 °C for 5 minutes, then thirty-five cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and a final elongation stage of 72 °C for 7 minutes. For the microcystin gene, primers included McyB

P1 and McyB P2, also at 100 µM concentrations (Yang et al, 2007). This was also performed in a 25 µL reaction mixture with 2.5 µL 10x buffer, 0.5 µL forward and reverse primers, 0.5 µL of 10 mM dNTPs, and 0.25 µL Taq polymerase. I used a PCR cycle of 94 °C for 6 minutes, then thirty-five cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and a final elongation stage of 72 °C for 8 minutes. I ran out these PCR products on a 1% agarose gel in 1x TAE buffer to check for positive amplification. For samples being amplified with the McyB primers, I attempted to adjust the annealing temperature in the PCR cycle to 57 °C and 59 °C to get rid of any non-specific amplification.

Gel Extraction: I analyzed my PCR products on a 1% agarose gel in 1x TAE buffer. After imaging the gels under ultra-violet light, bands of interest were extracted following the protocol for a QIAquick® Gel Extraction Kit. I then analyzed sample purity on a NanoDrop 200 UV-Vis Spectrophotometer. After determining that the samples were not pure enough to send for sequencing, a second round of purification was done using a QIAquick® PCR Purification Kit.

Sanger Sequencing: 24 samples were sent to the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility.

Sequence Alignment & Analysis: I chose four forward primed sequences with high quality and aligned them with their corresponding reverse primed sequences to form contig sequences using the software Geneous. I then edited the consensus identity of the contig sequence to match the most prominent base peaks in the aligned sequences using the software MEGA 7. I also trimmed the ends of the sequences where the base signals were not that strong. I then ran each contig through the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) database and chose cyanobacteria and bacterial sequences of similar structure. I trimmed and aligned the sequences, then created a maximum likelihood phylogenic

tree. Finally, I ran a bootstrap analysis on the tree to determine the correctness of the branching pattern.

Statistical Analyses: I calculated standard error for any analyses where I had repetitions of incubation analysis procedures.

Results:

Field Observations:

Water profiles from Oyster Pond showed little variation in conductivity, pH, and dissolved oxygen throughout depth, site, and time (Figure 3). Temperature decreased by roughly three degrees from 11/14/16 to 12/5/16. Salinity increased 0.16 ppt since mid-August measurements (Figure 4). Chlorophyll-a was highest at the bottom of OP2 and was around 15 ug/L for most other sites (Figure 5). Nutrient profiles indicated the highest phosphate concentration at Mosquito creek (0.79 µM) with comparatively lower values across all the other sites (Figure 6). I saw similar trends in my nitrate data where nitrate concentrations were highest at Mosquito Creek (35.2 µM), and were much lower at the other sites (Figure 7). Ammonium concentrations, on the contrary were lowest at Mosquito Creek (2.68 µM) and at least 14 µM across all the other sites (Figure 8).

Incubation Experiments:

Both my water and sediment incubation experiments did not have a notable presence of *Anabaena* or *Aphanizomenon* genera. The cyanobacteria colonies I did observe were composed of hundreds of coccoid green cells, characteristic of *Microcystis* (Figure 9). I also observed similar looking colonies with smaller grey cells, typical of *Aphanocapsa* (Figure 10). There was also a significant presence of *Pseudoanabaena, Cylindrospermopsis* (Figure 11), and

Merismopedia (Figure 12) from my sediment water incubations (Baker et al, 2012, Prescott, 1970). Aside from cyanobacteria species, there was a large presence of diatoms in all of the incubation experiments. I also identified at least four rotifer species from my water incubations including *Brachionus calyciflorus, Filinia terminalis, Keratella cochlearis, and Keratella sp.* (Figure 13) (Haney et al, 2013).

Microcystis colonies were present in sediment taken from the Northeastern cove of Oyster Pond (Figure 9). Before incubation, colony counts averaged 4880 colonies/mL, while after the incubations there were fewer than 1000 colonies/mL across all enrichments with no significant difference in colony counts between treatments (Figure 14).

Cell counts of *Microcystis* from day four of the incubations show the most cells with the nitrogen and phosphorus enriched water (Figure 15). The control and phosphorus enriched treatments also showed a decent amount of *Microcystis* with 950 cells/mL and 1152 cells/mL respectively. Water taken from the sediment incubations had very little *Microcystis* present with no significant difference between treatments.

Cell counts of *Aphanocapsa* from day four of the incubations had much higher counts than *Microcystis* (Figure 16). I counted the most cells in the phosphorus enriched treatments for both the water and sediment water incubations (68,640 and 32,982 cells/mL respectively). The nitrogen and phosphorus enriched water had the second most colonies in the water incubation at 50,700 cells/mL.

My fluorescence data indicated the most growth with the nitrogen and phosphorus enrichments (Figures 17 & 18). This is also indicated from the chlorophyll-a data (Figure 19). The phosphorus enrichment showed the second most growth, with the control and nitrogen

enrichments showing very little growth. Fluorescence peaked around day four for the phosphorus and nitrogen and phosphorus enrichments, while control and nitrogen alone enrichments did not have a peak in fluorescence during the seven day incubation period (Figure 18).

The sediment incubations showed similar trends to the water incubation fluorescence data, however, error bars in the sediment incubation data suggest that the difference between these means is not statistically significant (Figure 20). Because the first and second reps of the sediment incubations were started two days early, when fluorescence is compared among just these, there is a significantly more growth with the nitrogen and phosphorus enrichment (Figure 20). This can also be seen in rep 3 (Figure 20). Due to a technical error with the fluorometer, I could not identify the day of peak growth from any of the sediment incubation data. Chlorophyll-a data indicated the most growth with the phosphorus enrichment (Figure 21).

In comparison of phosphate concentrations before and after incubations there was an average decrease of 5.18 µM in the water incubations for the phosphorus enriched treatment (Figure 22). In the nitrogen and phosphorus enriched treatments, there was an average decrease of 11.26 µM. In my sediment incubations, there was a complete loss of phosphate in the water across all treatments, where concentrations went from an average of 20 µM to almost zero (Figure 23).

Ammonium concentrations were greatly reduced with the water nitrogen and phosphorus enrichments (Figure 24). Comparatively, there was very minimal ammonium loss in the nitrogen alone enrichment (20 µM). Ammonium was also greatly reduced in the nitrogen and phosphorus enriched sediment incubation water (Figure 25). However, ammonium concentrations increased by 47 µM in the sediment nitrogen enriched incubation.

Finally, nitrate concentrations decreased slightly from their initial concentrations in both the nitrogen and nitrogen and phosphorus enriched incubations (Figure 26). In the sediment incubations, nitrate present in the water was reduced to almost zero µM across all treatments (Figure 27).

Identification Using Molecular Markers:

My PCR products showed positive amplification of Microcystin synthetase gene B at around 350 bp for both *Aphanocapsa* and *Microcystis* (Figure 28). An attempt to reduce the number of non-specific bands for this gene did not prove to be effective (Figure 29). In addition to this gene, I also saw positive amplification of a segment of the 16S rRNA gene at 700 bp for both *Aphanocapsa* and *Microcystis* (Figure 28).

Of my 12 sets of samples sent for sequencing, only 4 sets could be used to construct contig sequences. Bootstrap values from my phylogenic tree indicate a strong distinction of the cyanobacteria phylum from other bacterial phyla (Figure 29). Within the cyanobacteria branch, I saw two other branches which I identified as filamentous and non-filamentous species (with the exception of the placement of *Oscillatoria sp*. in the same branch as non-filamentous species. Two of my contigs fell within the non-filamentous branch of this phylum (Unknown & *Aphanocapsa* 1). My other two contigs fell outside of the cyanobacteria phylum and aligned more with *Verrucomicrobia*. The bootstrap values of the branches in my phylogenic tree suggest that some branching is accurate, while other branchings (less than a bootstrap value of 95) are more likely to be inaccurate.

Discussion:

As water cools in the Fall, stratified lakes and ponds will begin to turn over. This explains why I saw little to no difference in conductivity, pH, temperature, and dissolved oxygen at all depths and across all sampling sites. The high chlorophyll-a concentrations I saw across all Oyster Pond sites is likely left over from this season's bloom, though these are still within the New England Interstate Water Pollution Control Commission (NEIWPCC) parameters of good to fair water quality for an Eastern coastal plain pond (NEIWPCC, 2010) The extremely high and inconsistent chlorophyll-a concentrations I saw at the bottom of OP2 could have been a result of sucking up sediment/algae resting on the surface of the sediments. The elevated phosphate and nitrate concentrations at Mosquito creek are likely from anthropogenic inputs via runoff and groundwater to the stream.

The absence of *Anabaena* and *Aphanizomenon* genera in my incubation experiments could have been due to a lack of dormant cells in the sediment and water column. If this past season's most prominent blooming species made its way into Oyster Pond from an external source, such as Mosquito Creek, this could very well explain this phenomenon. Another explanation could be that *Anabena/Aphanizomenon* was overwintering in the sediment but failed to recruit to the water stage due to inadequate microcosm conditions.

The strong presence of *Microcystis* colonies in the sediments taken from Oyster Pond indicate that this colonial species is overwintering. Because I saw far fewer colonies in the sediment after incubation, these data also suggest that the colonies are recruiting to the water stage. However, this is strongly contradicted by the lack of *Microcystis* cells present in the sediment water after incubation. This could be due to the number of colonies that were coming from the sediment being diluted by 1.5 liters of water in the bottle. *Microcystis* can also take on a

unicellular stage in laboratory cultures. Though this is another possible explanation, it is less likely because *Microcystis* will usually only take on this form there is a lack of predators (Baker, 2012). Because the unicellular stage is so rare in a nature setting, this leads me to the third explanation; the zooplankton also coming out of the sediment consumed a majority of the *Microcystis* colonies.

Despite the most algal growth occurring in the nitrogen and phosphorus enriched treatments, the overwhelming amount of *Aphanocapsa* cells in the phosphorus enriched treatments suggests that this cyanobacterium has a competitive advantage over the planktonic algae in nitrogen poor but phosphorus rich conditions. This can be explained by *Aphanocapsa's* ability to fix atmospheric nitrogen (Ramm, 2014). *Microcystis* also has nitrogen fixing capabilities, so the considerably lower cell counts in the water after incubation could be due to a small sampling size (3 reps).

Not only was *Aphanocapsa* more abundant than *Microcystis*, but this genera also has much smaller cells. For every 144 *Microcystis* cells present, there were around 900 *Aphanocapsa* cells. With this information in mind, there was greater than 20,000 cells/mL present in the phosphorus and nitrogen and phosphorus enriched water incubations, and the phosphorus enriched sediment water. These are counts above the World Health Organization water quality guidelines. At these levels, there is an increased likelihood of developing cyanotoxin related symptoms for seven days following exposure to the toxins (WHO, 2003). Because *Aphanocapsa* and *Microcystis* both produce microcystin, an estimate of toxin levels would be the most accurate method of evaluating health effects during a bloom. The Massachusetts Department of Public Health (MDPH) estimates that at approximately 20,000 cells/mL, toxin levels may range between 2-4 ppb. The recommended toxin intake limit for a child is 14 ppb, so even with high

cell counts, a child would have to drink quite a few liters of water before reaching these toxicity levels. However, individuals predisposed to liver conditions may be at a higher risk (MDPH). These are only estimates of toxicity, and biochemical analyses such as ELISA can serve as a more quantitative measure of toxicity levels (Yang, 2007).

Though I did see positive amplification of microcystin synthetase gene B, microcystin can be expressed at varying toxicities. Differences in two protein amino acids of the microcystin peptide ring are largely responsible for altering the toxicity of the molecule. The most potent of these toxins is microcystin-LR with amino acids Leucine (L) and Arginine (R) (Purdue University, 1995). Once again, further biochemical analyses must be utilized to identify the specific toxin variant.

The overwhelming growth of algae from the nitrogen and phosphorus enrichment shows that planktonic algal species do best in environments where there is nitrogen and phosphorus readily available. This is due to their inability to fix atmospheric nitrogen. Because I saw the greatest amounts of growth in the phosphorus and nitrogen and phosphorus enriched incubations, this suggest that Oyster Pond is a phosphorus-limited system. No matter how much ammonium and nitrate are added to the water, the plants in this system will be unable to use the nutrient to its their full advantage unless it is coupled with a source of phosphorus (Blomqvist et al, 2004).

The complete loss of phosphate in the sediment water incubations but not in the water alone incubations suggests that the phosphate concentrations are being affected by the sediment, possibly through occlusion by iron oxides (Blomqvist, 2004). The same trend can be seen with nitrate between the sediment water and water alone incubations. This is most likely an indication of denitrification by bacteria in the sediments. I was surprised to find that ammonium concentrations did not increase in the water incubations despite the presence of nitrogen-fixing

cyanobacteria and ammonium probably being released in decomposition processes. The ammonia was likely being taking up quickly by algae in my incubations. (Schlesinger & Bernhardt, 2013).

Unfortunately, the phylogenic tree I constructed from my own data and homologs from the NCBI nr database could not be used as a secondary form of cyanobacteria genera identification. This could be because the segment of the 16S rRNA gene we isolated was not long enough to determine genera, or because there was not enough species and strains in my phylogenic tree to show significant differentiation. However, this was still a useful form of analysis because I was able to confirm my PCR products as cyanobacteria. The two contigs that did not fall within the cyanobacteria phylum were likely contaminated PCR samples. These samples aligned more with *Verrucomicrobia* which is another aquatic/soil-dwelling bacteria phylum (Schlesner et al, 2006). I picked my colonies from very densely populated incubation water, so risk of contamination was very high. This could also explain why adjusting the annealing temperature for PCR with my McyB primers to eliminate non-specific amplification did not work. If the samples were contaminated with another bacterial species, positive amplification of a cyanobacteria-specific toxin is unlikely.

Though there has been a slight upward trend in salinity since the invasive phragmites blockage was removed from the weir, the Oyster Pond Environmental Trust is still at least 1 ppt away from their desired salinity of 2-4 ppt. If salinity stays below this level and nutrient conditions are similar to last summer (Table 1), we could see a reoccurring bloom of freshwater cyanobacteria. It is also essential to the health of this pond that salinity not increase too much, as the presence of sulfur can inhibit the binding of phosphate to iron, thus leaving very little oxidized iron in the sediments to act as a phosphorus sink. When the phosphorus that is usually

occluded by iron oxides is released, this can cause a form of internal eutrophication (Blomqvist, 2004).

While there are *Microcystis* colonies overwintering in the sediment of Oyster Pond, it is unclear whether or not they have the capability of blooming due to inconsistencies between sediment colony counts and cell counts in water taken from above the sediment after incubation. Because both *Microcystis* and *Aphanocapsa* have been shown to produce microcystin, the public should be warned not to swim in the water if this genera ever blooms. If a *Microcystis* bloom does occur next summer, the presence of this cyanobacterium in the sediment of ponds could be used as an indicator for potential blooms.

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Tables and Figures:

	Average	Average	Average	Average	Average	Average
	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L	µmol/L
Sample ID	Nitrate	Ammonium	Phosphate	Silicate	TDN	TN
Mosquito Creek	0.95	0.42	0.07	15.09	32.79	
Ransom	<0.04	0.36	0.12	16.36	31.30	81.05
Weir	0.14	0.56	0.24	14.85	61.05	63.99
OP1 surface	<0.04	0.39	0.06	11.14	34.84	
OP1 2M	<0.04	0.36	0.11	15.37	50.27	
OP1 BOTTOM	0.19	8.52	0.16	28.52	36.09	
OP2 SURFACE	0.25	0.58	0.08	11.79	63.85	
OP2 2M	<0.04	0.54	0.14	13.69	49.36	
OP2 BOTTOM	<0.04	0.42	0.11	12.53	17.42	
OP3 SURFACE	0.12	0.41	0.10	11.30	31.88	
OP3 4M	<0.04	0.37	0.11	11.57	18.30	
OP3 BOTTOM	<0.04	99.61	7.13	64.27	265.64	

Table 1: Nutrient data from August 2016 sampling by the Oyster Pond Environmental Trust.

Figure 1: Map of Oyster Pond sampling sites; Mosquito Creek, OP1, OP2, OP3, and the Weir.

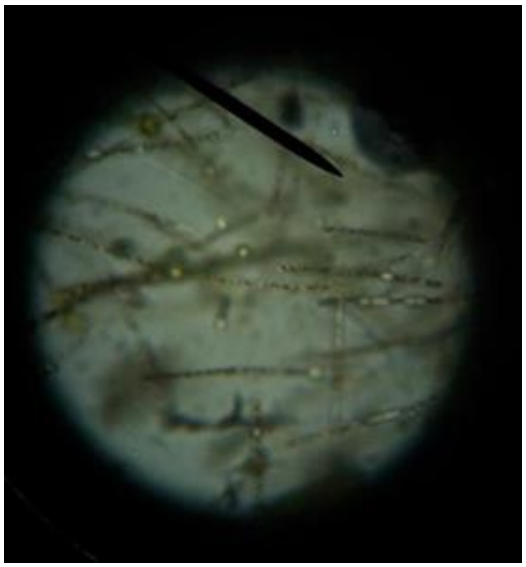

Figure 2: Anabaena or Aphanizomenon from Oyster Pond water during the peak of the bloom. Photo: Bill Kerfoot.

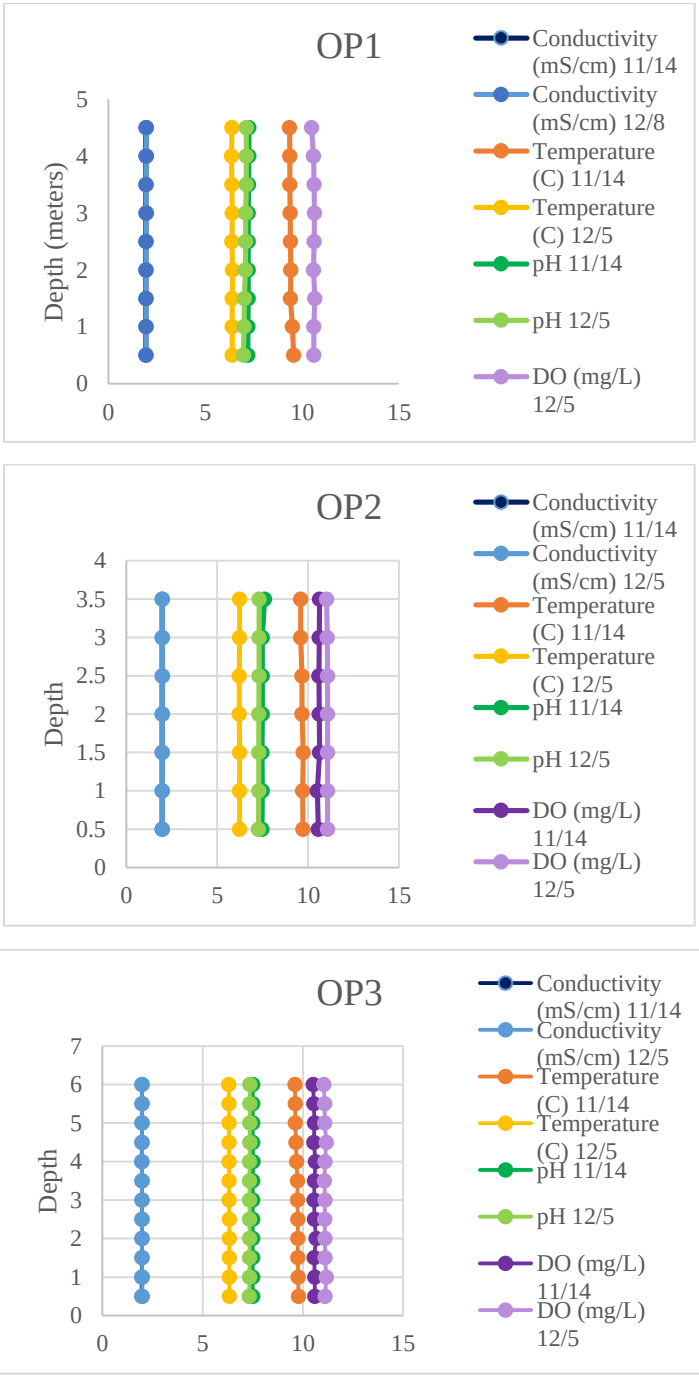

Figure 3: Changes in conductivity, temperature, pH, and dissolved oxygen (DO) profiles at three Oyster Pond sites (OP1, OP2, and OP3) between November 14 and December 5, 2016.

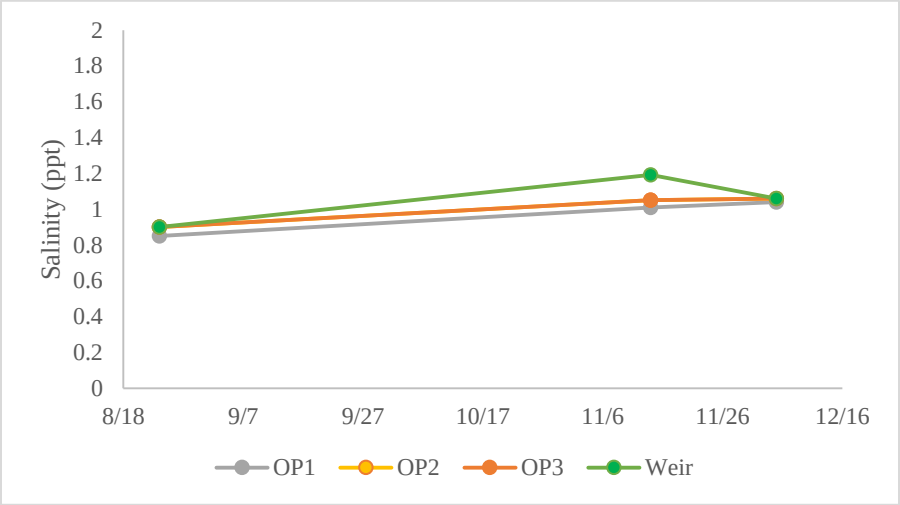

Figure 4: Average salinity of Oyster Pond sites in ppt from August 24 to December 5, 2016.

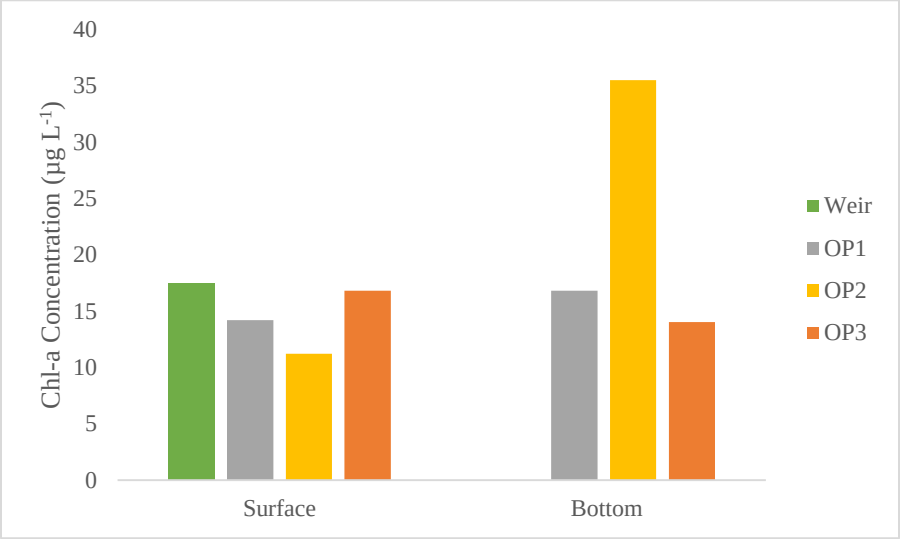

Figure 5:Surface and bottom chlorophyll-a measurements across Oyster Pond sites.

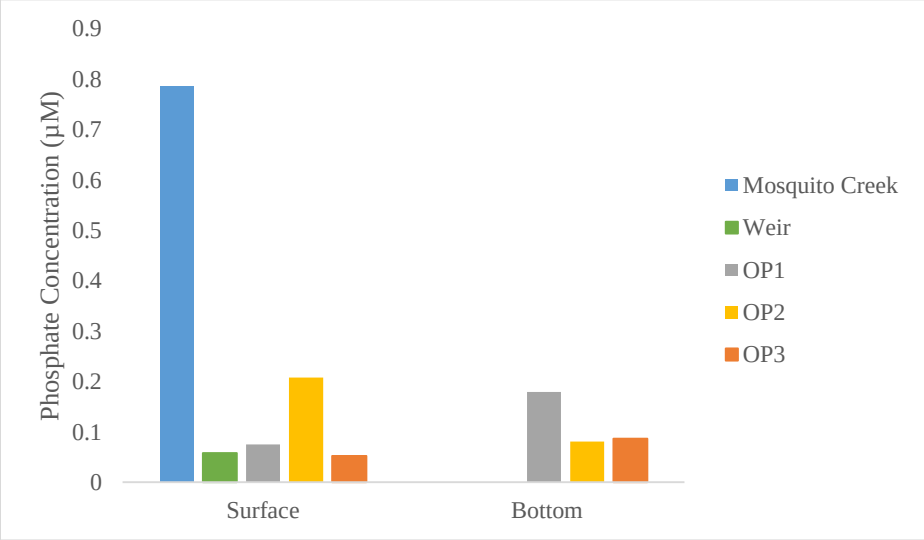

Figure 6:Surface and bottom phosphate concentrations across Oyster Pond sites.

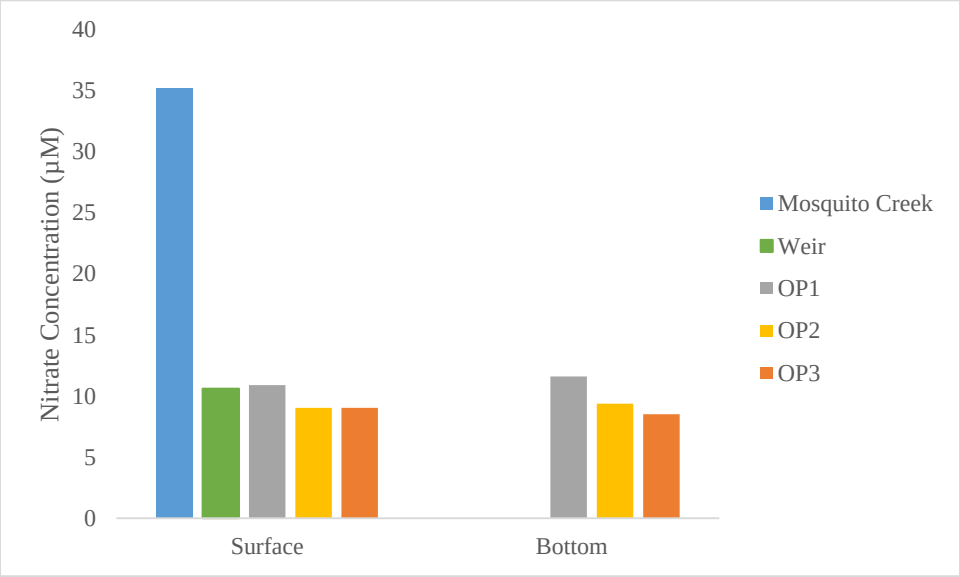

Figure 7: Surface and bottom nitrate concentrations across Oyster Pond sites.

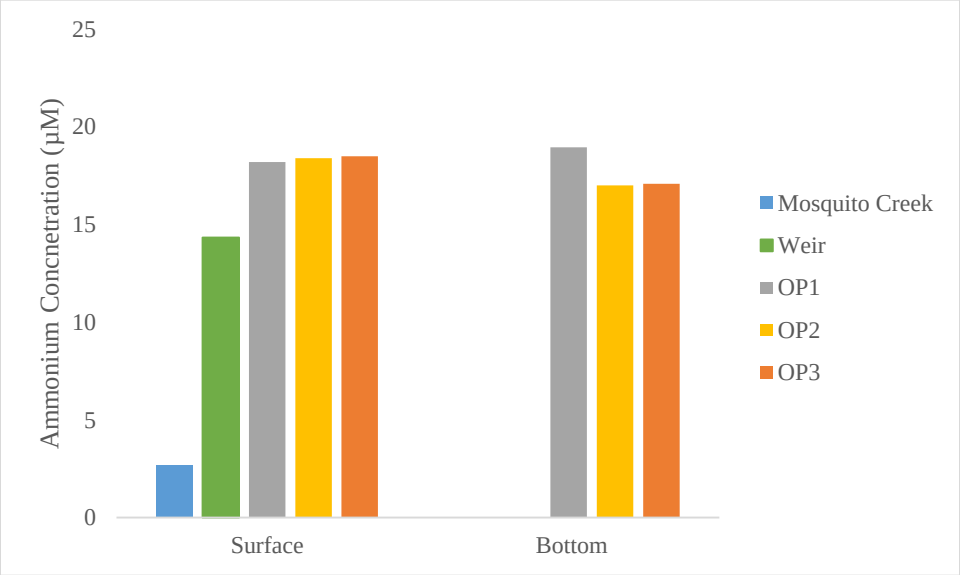

Figure 8: Surface and bottom ammonium concentrations across Oyster Pond sites.

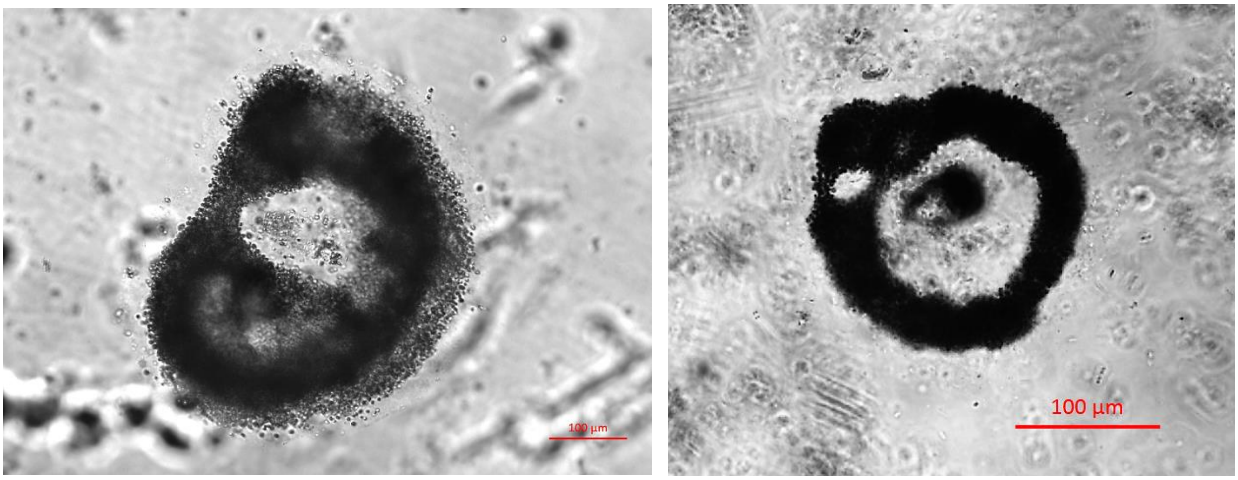

Figure 9: Microcystis from control water incubation (left) and from initial sediment (right).

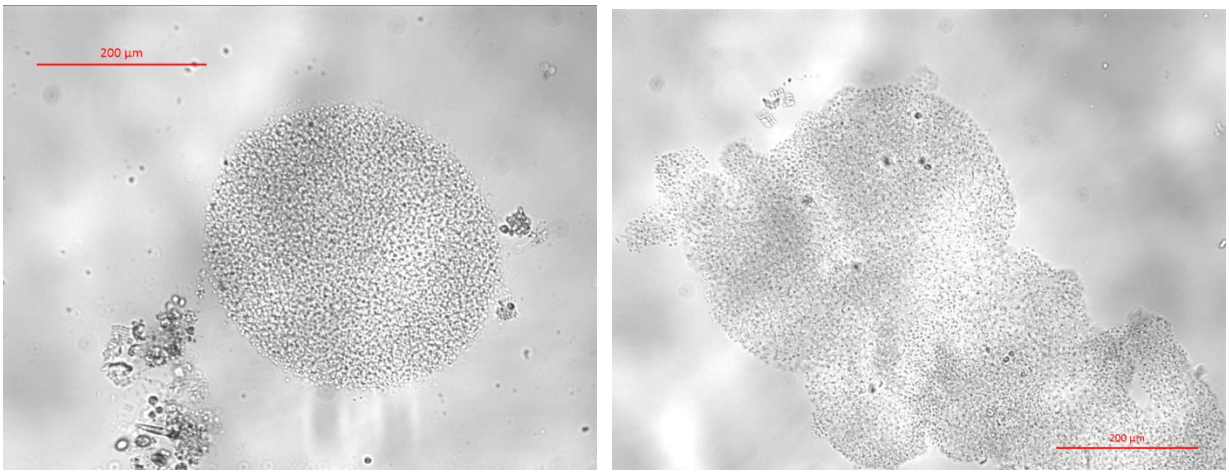

Figure 10: Aphanocapsa colonies from nitrogen and phosphorus enriched water incubations

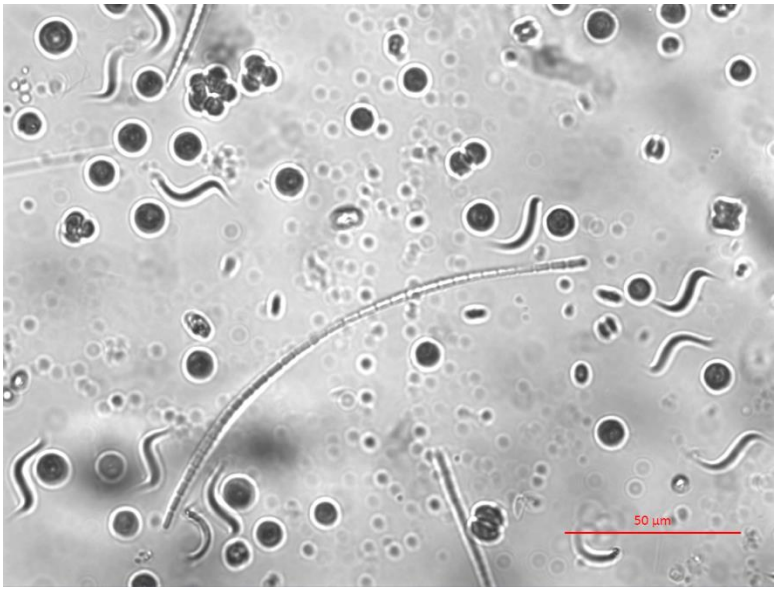

Figure 11: Pseudoanabaena (center) and Cylindrospermopsis (s-shaped) from nitrogen and phosphorus enriched sediment water.

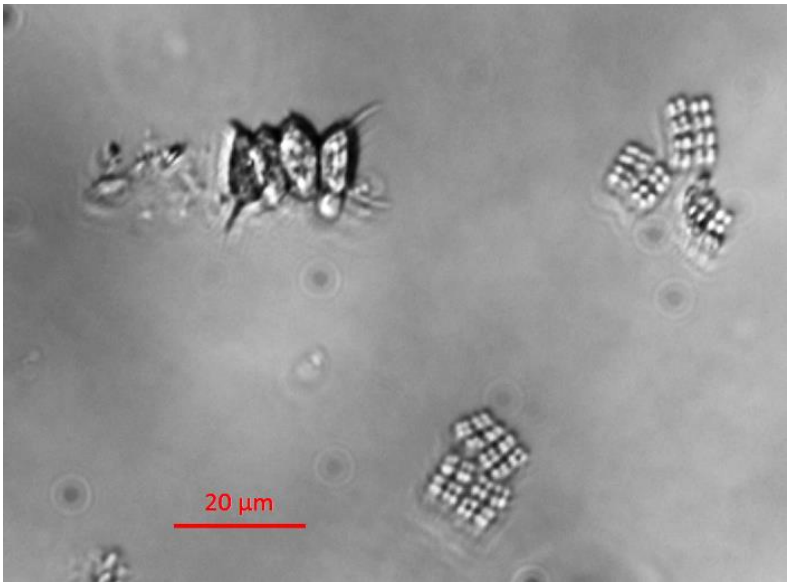

Figure 12: Merismopedia (top right and bottom center) from phosphorus enriched water incubation.

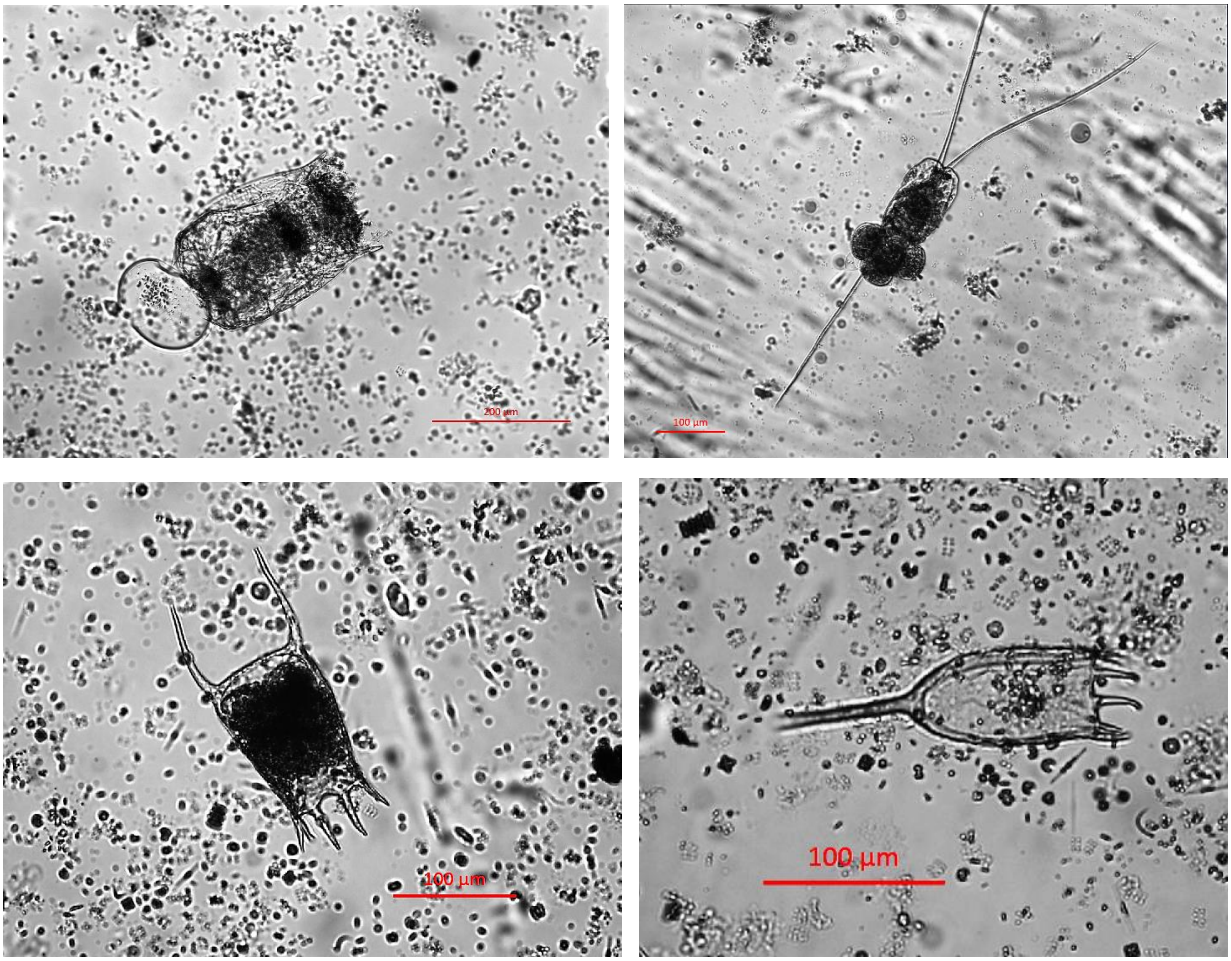

Figure 13: Brachionus calyciflorus and egg (top left). Filinia terminalis and eggs (top right). Keratella sp. (bottom left). Keratella cochlearis (bottom right).

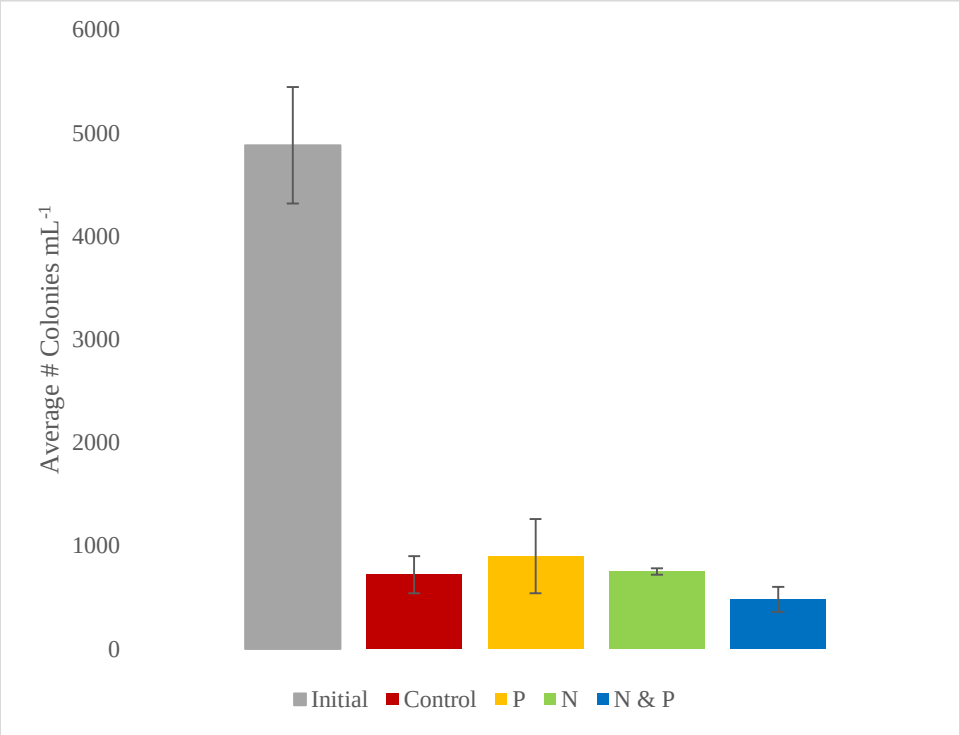

Figure 14: Average Microcystis colony counts per 1 mL sediment before and after incubations.

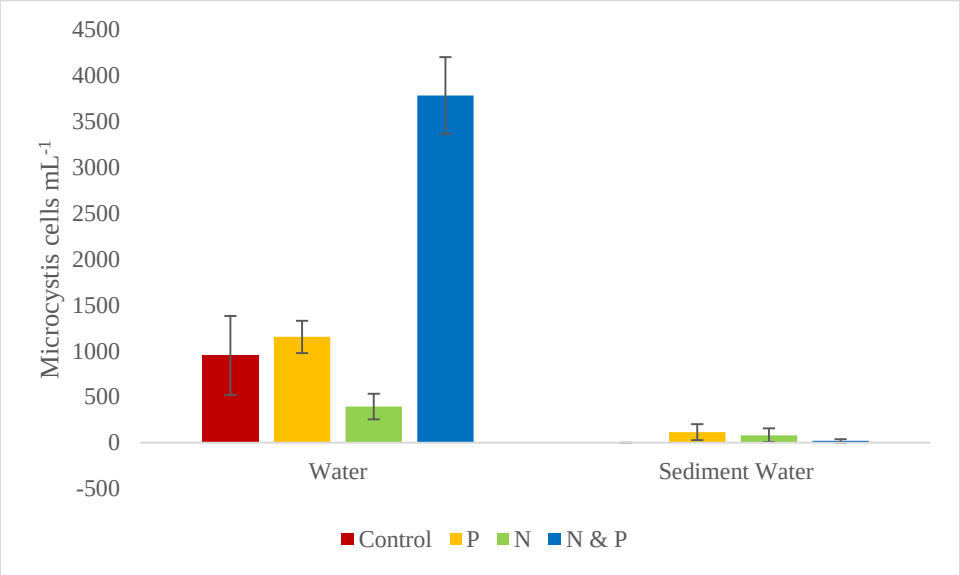

Figure 15: Average Microcystis cell counts per 1 mL of water at the peak of incubation growth.

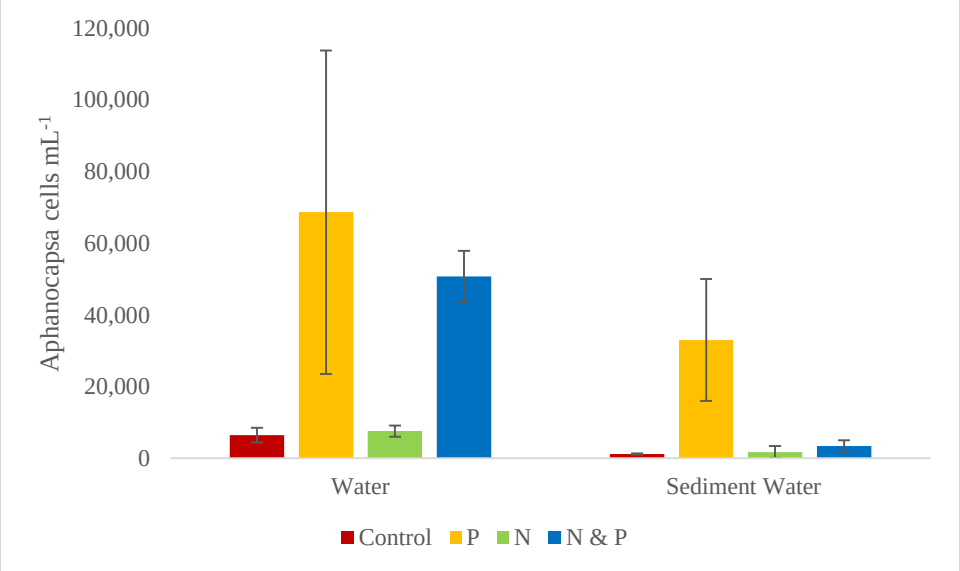

Figure 16: Average Aphanocapsa cell counts per 1 mL of water at the peak of incubation growth.

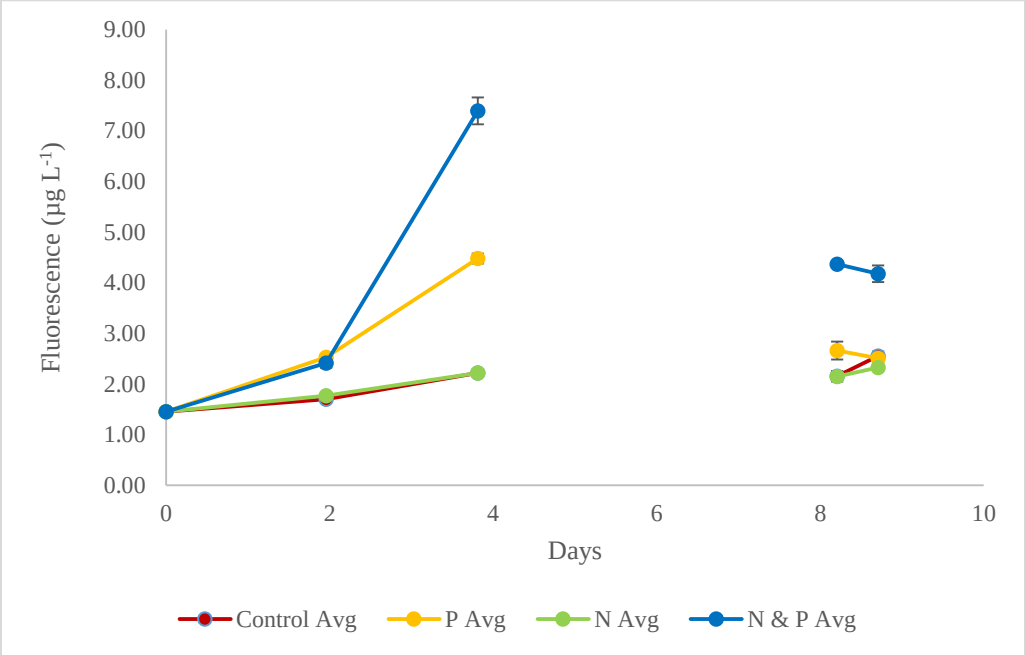

Figure 17: Average fluorescence for water incubation #1over eight days.

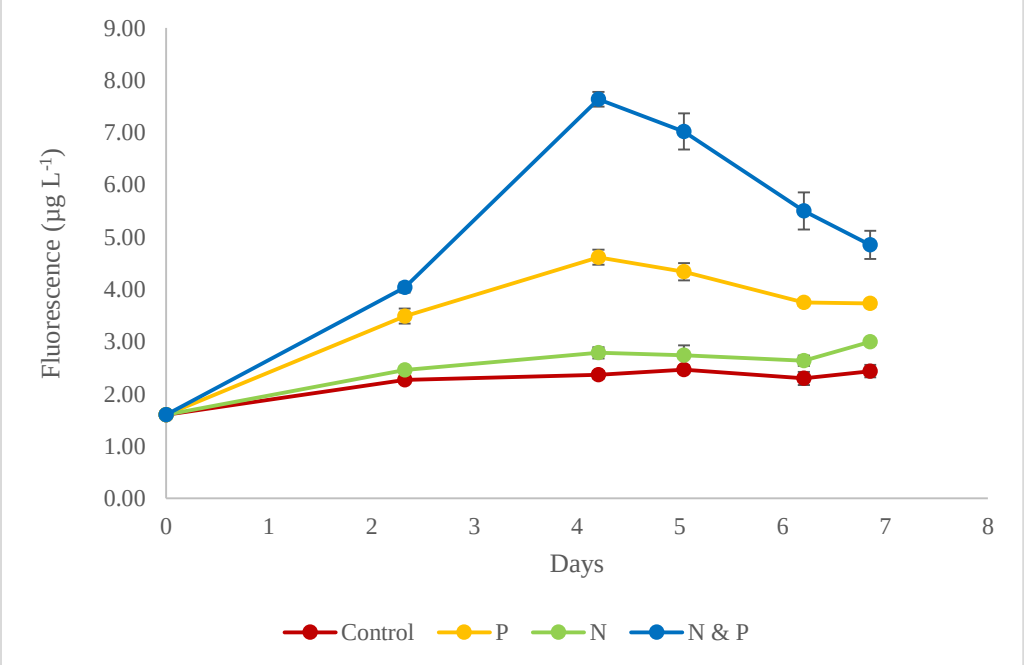

Figure 18: Average fluorescence for water incubation #2 over seven days.

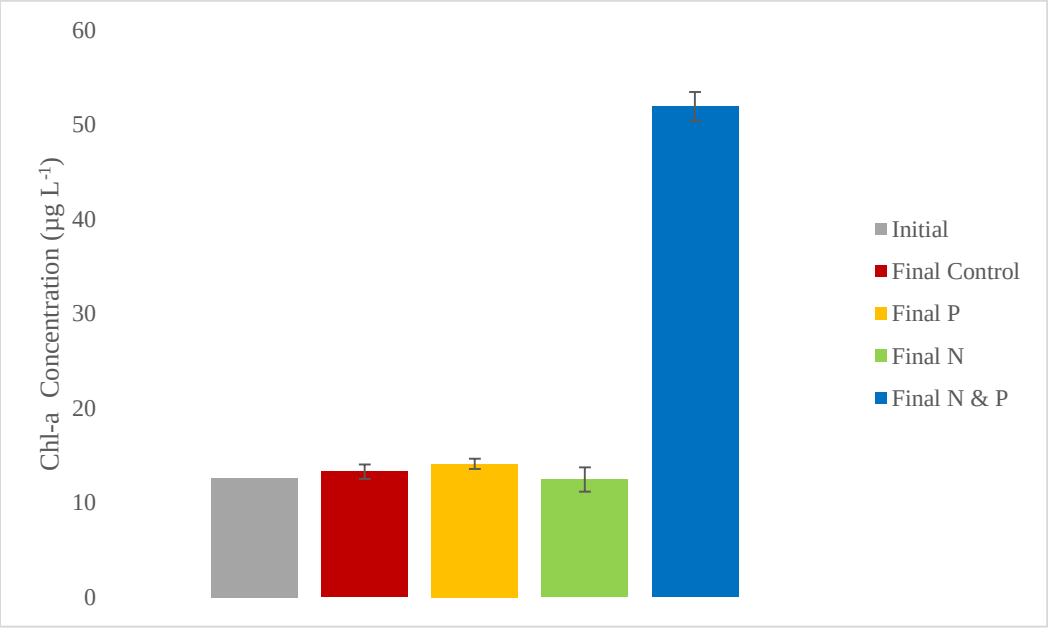

Figure 19: Initial and average final chlorophyll-a concentrations from water incubation #1.

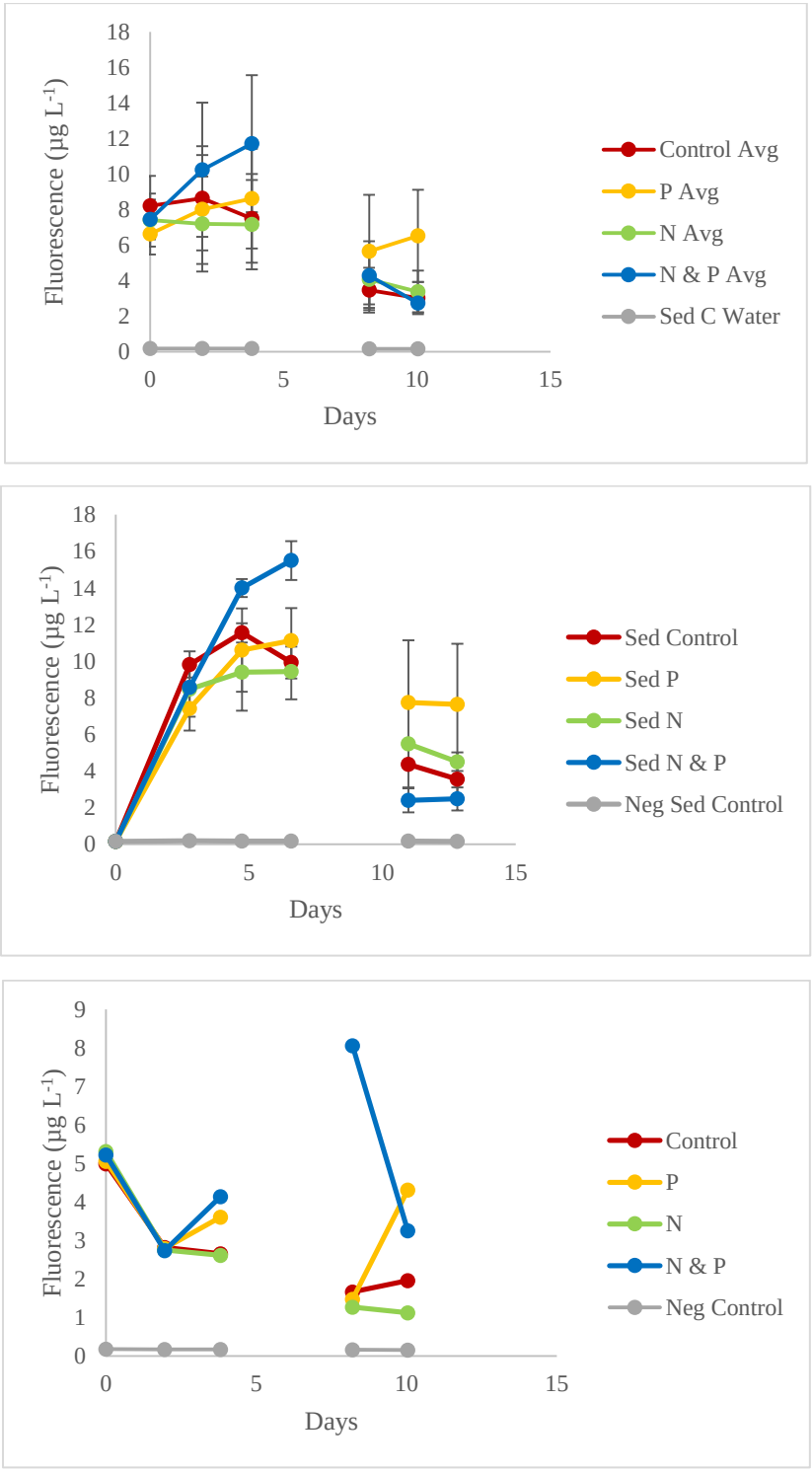

Figure 20: Average fluorescence in the sediment incubations across 10 days (top). Average fluorescence for sediment incubations reps 1 & 2 over 12 days (middle). Average fluorescence for sediment incubations rep 3 over 10 days (bottom).

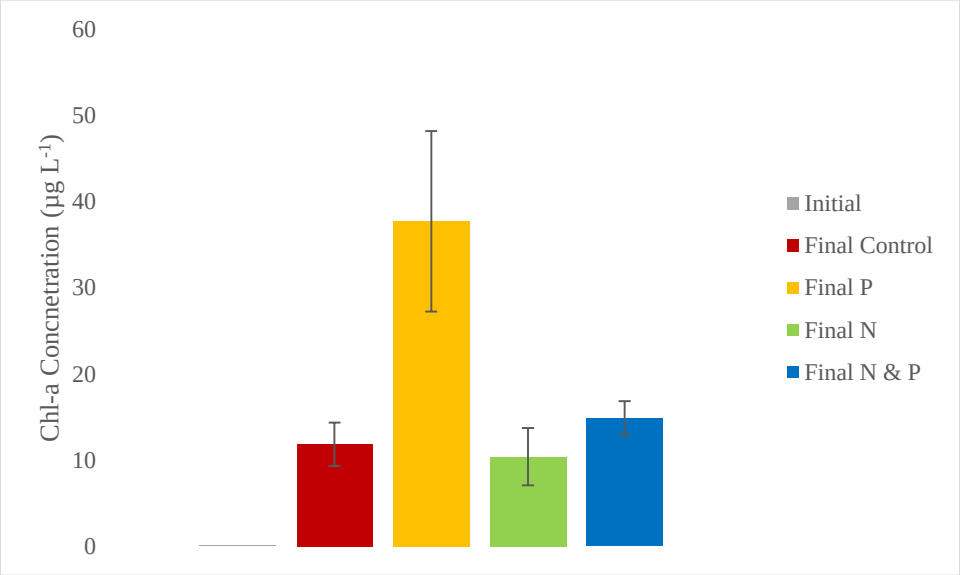

Figure 21: Initial and average final chlorophyll-a concentrations from the sediment incubation water.

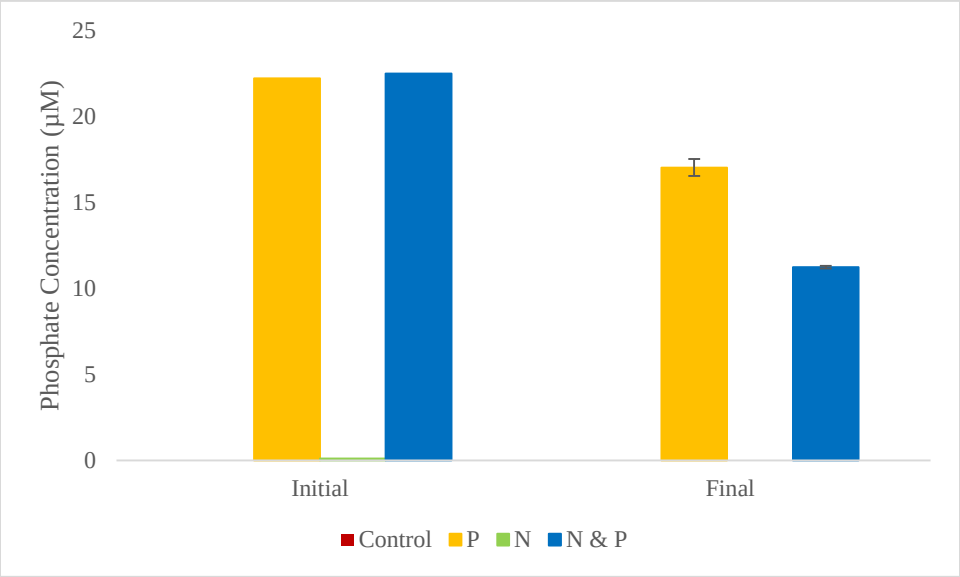

Figure 22: Initial and final phosphate concentrations in the water incubations.

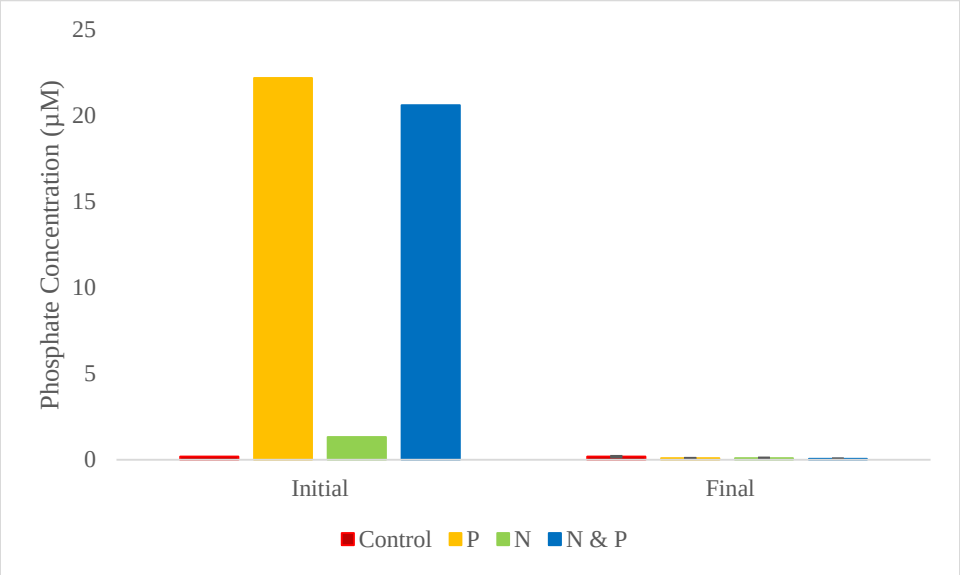

Figure 23: Initial and final phosphate concentrations in the sediment incubations.

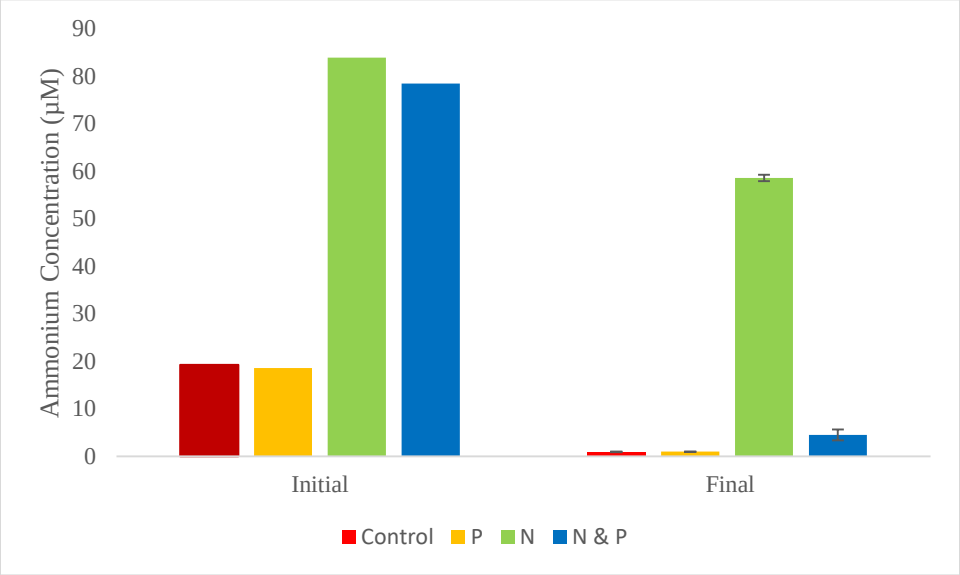

Figure 24: Initial and average final ammonium concentrations in the water incubations.

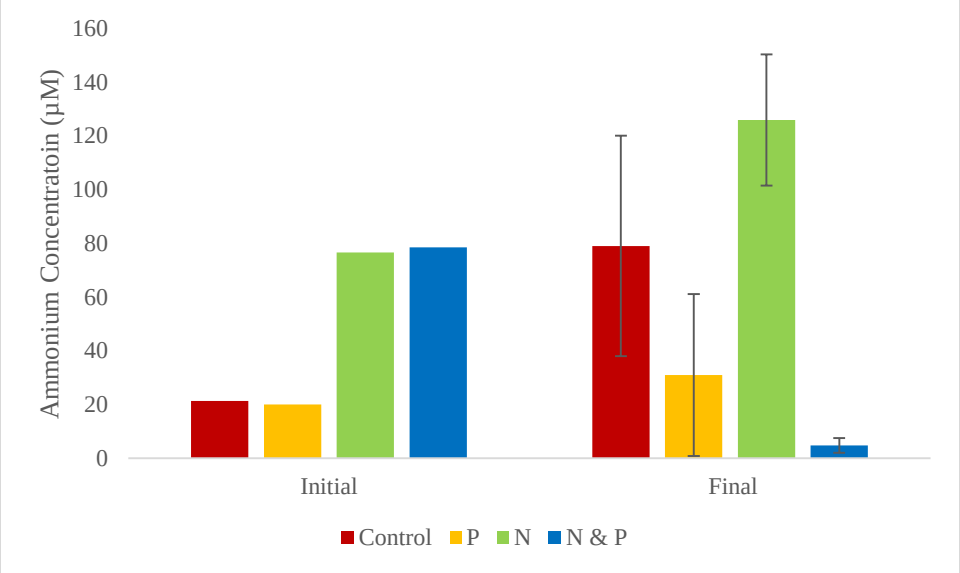

Figure 25: Initial and average final ammonium concentrations in the sediment incubations.

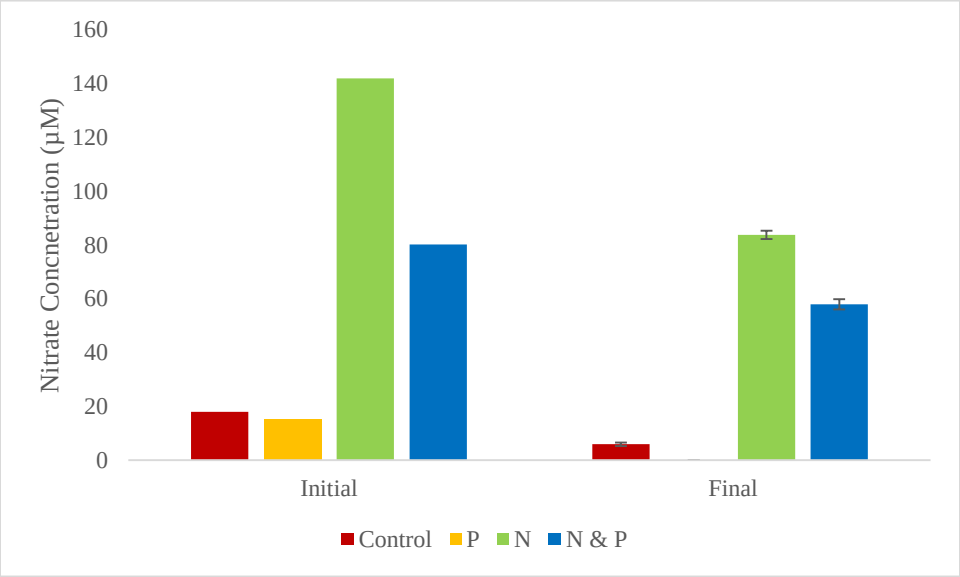

Figure 26: Initial and average final nitrate concentrations in the water incubations.

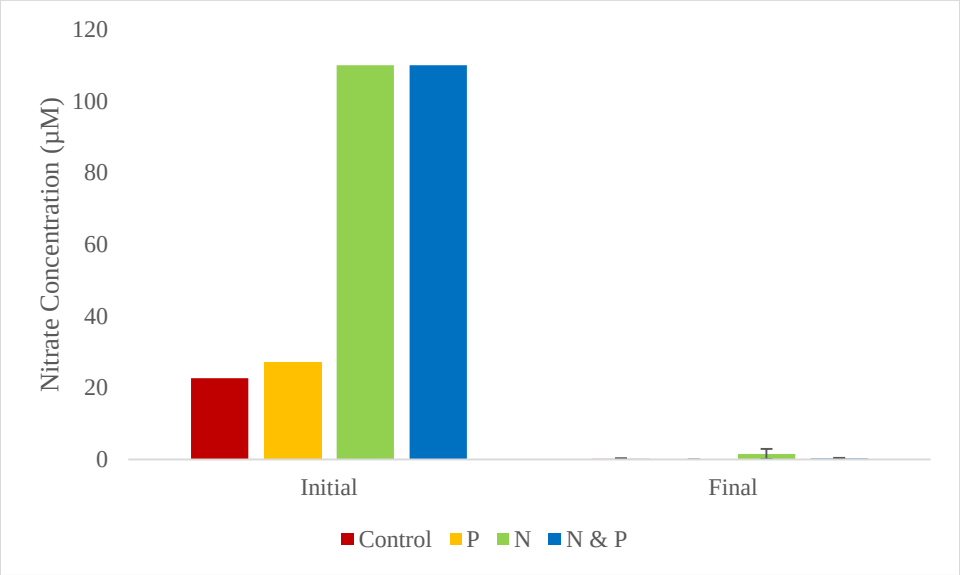

Figure 27: Initial and final nitrate concentrations in the sediment incubations.

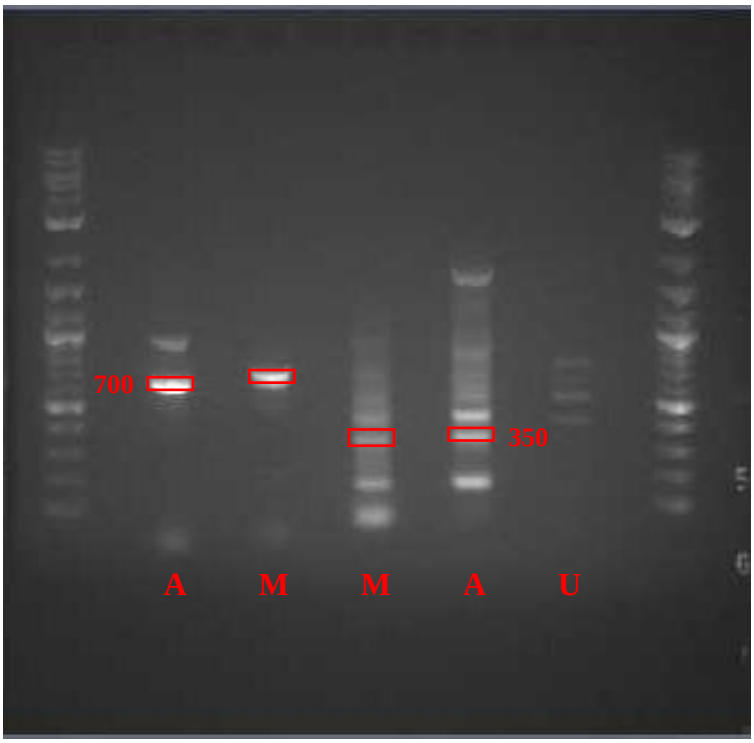

Figure 28: Positive amplification of the CYA 16S rRNA gene at 700 bp from Microcystis (M) and Aphanocapsa (A) PCR products (left). Positive amplification of the McyB gene at 350 bp from Microcystis and Aphanocapsa PCR products (right). No amplification in the unknown PCR product (U).

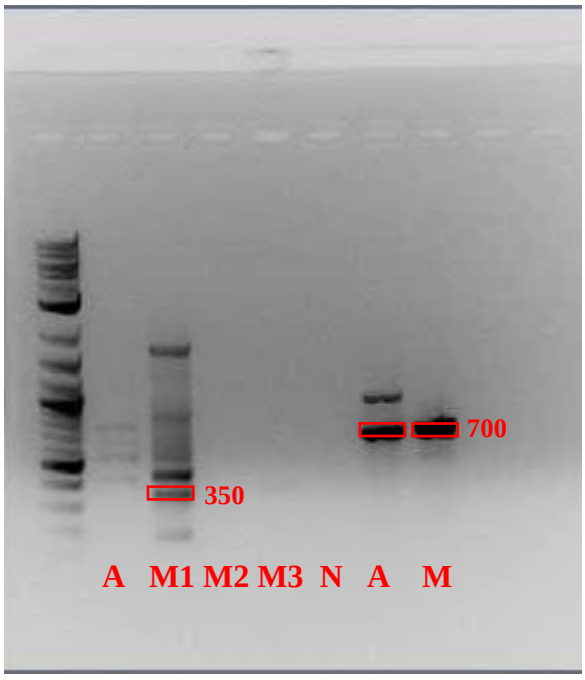

Figure 29: Positive amplification of the McyB gene at 350 bp (left) in microcystis (M1). An attempt to adjust the annealing temperature of the McyB PCR cycle (M1= 57 °C, M2= 59 °C). Negative control (N) and CYA gene (right) positively amplified at 700 bp in aphanocapsa and microcystis.

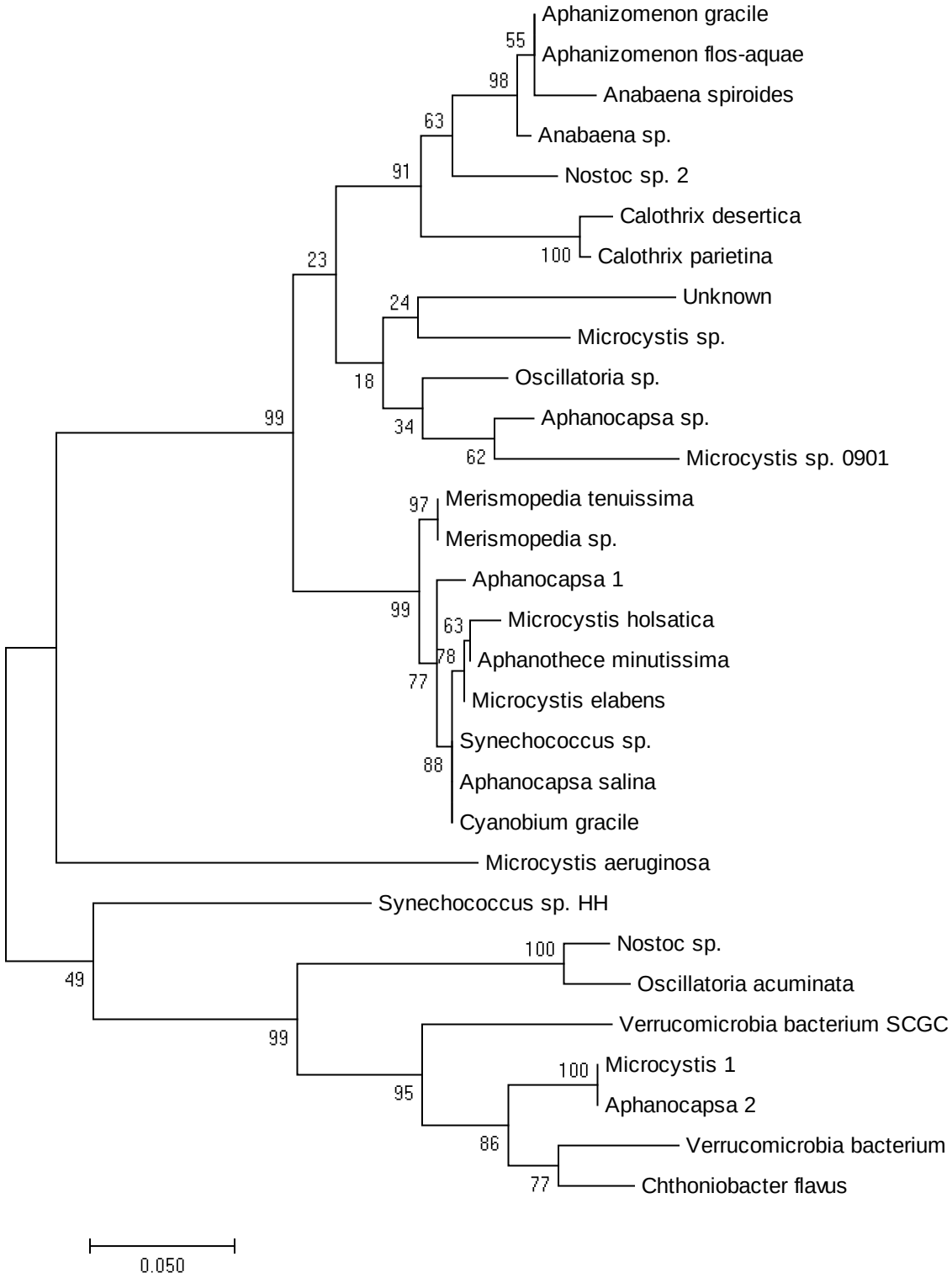

Figure 30: Phylogenic tree of sequenced 16S rRNA products compared with NCBI BLAST sequence segments.