Analysis of artificial substrates for collection and identification of benthic cyanobacteria in the Ohio River

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Abstract

Benthic cyanobacteria are an emerging concern in aquatic biology because of their potential to contribute to harmful algal blooms, which can have adverse effects on human and ecosystem health. As opposed to their counterparts, planktonic cyanobacteria, research on benthic cyanobacteria and their contributions to harmful algal blooms is limited. Because of the limitations associated with sampling benthic algae, the development of artificial substrates as a collection device can be a viable way to make benthic algae research more accessible and efficient. The goal of this study is to construct an artificial substrate that will successfully support cyanobacteria growth, while also quantifying and identifying the benthic cyanobacteria population present in the Ohio River. Plates containing four substrates—unglazed clay tile, rock-imitating clay tile, nylon mesh, and masonite—of the same surface area were constructed, fixed in two foot increments on ropes, and lowered into the Ohio River for a total of three weeks. After two weeks, the unglazed clay tiles had fallen off of all but two of the plates and were therefore not counted in this study. After three weeks, the substrates were removed from the river, scraped using a nylon-bristled brush, and rinsed into a sorting tray. The contents from the scraped sample were then transferred into plastic test tubes, preserved with Lugol's Iodine, and placed in a refrigerator until analysis. The samples were then quantified using a Sedgewick-Rafter counting chamber. While each substrate successfully supported cyanobacteria growth, masonite was the most effective of the substrates, collecting the most cells per square inch, presenting the highest average cyanobacteria concentration, and supporting the growth of both *Oscillatoria* and *Lyngbya*. Both of the genera observed on the artificial substrates have been known to cause benthic harmful algal blooms, which suggests that cyanobacteria blooms observed in the Ohio River could have a benthic component.

Introduction

Benthic cyanobacteria are an emerging concern in aquatic biology because of their potential to contribute to harmful algal blooms, which can have adverse effects on human and ecosystem health. This is a particular concern in the Ohio River, which has recently experienced significant blooms in 2015 and 2019. Benthic cyanobacteria are blue-green algae that live attached to substrates like rocks, docks, and aquatic vegetation. Although benthic cyanobacteria tend to grow on river bottoms, they can experience periods of growth and subsequent detachment, causing mats of cyanobacteria to become free-floating (Sabater, S, et al., 2003). Furthermore, current research in the field of harmful algal blooms is primarily centered around planktonic cyanobacteria, and less is known about their benthic counterparts (Bouma-Gregson, Keith, et al., 2017). Specifically, there is sparse data on the benthic cyanobacteria growth occurring in the Ohio River.

Because of the growth patterns of benthic cyanobacteria, effective collection can be difficult without diving. By using an artificial substrate, diving can be avoided, making the collection of benthic algae more accessible and convenient. This study primarily aims to construct an artificial substrate that will successfully support cyanobacteria growth, while also quantifying and identifying the benthic cyanobacterial population present in the Ohio River.

Methods

Fifteen substrate plates were constructed by gluing two 1.75"x5.25" pieces of masonite in an "x" formation and attaching a 1.75"x1.75" square of unglazed clay, rock-imitating clay, and nylon mesh to three ends of the masonite. Nothing was attached to the fourth end of the masonite "x" in order to create a square of exposed masonite as the fourth substrate. This created a plate in which four substrates of the same surface area could sit at the same depth in the water (Figure 1).

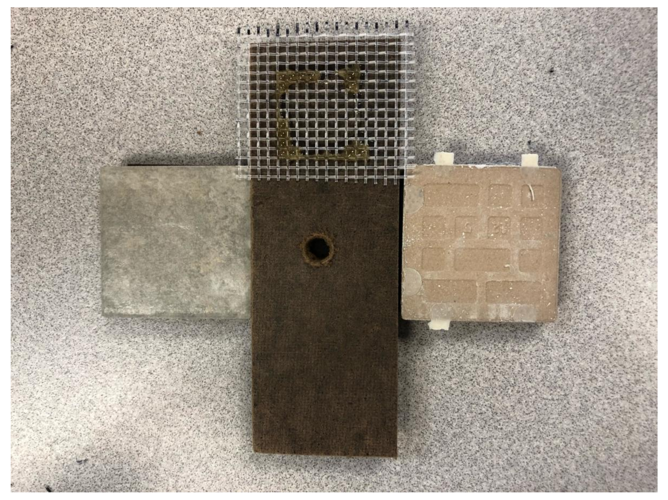

Figure 1. Substrate plate with all four initial substrates attached

Figure 2. How substrates were fixed in the river

Five of the constructed plates each were then attached to three ropes by drilling holes in the middle of each plate and fixing them in place in increments of two feet by tying knots just above and below the plates. An anchor was attached to one end of the rope and the substrates were lowered into the water at the far upstream end of the boat jetty wall at the Thomas More Biology Field Station, which is located at mile 481 on the Ohio River. They were positioned so that the top substrate was 1 foot beneath the surface. The remaining end of each rope was secured to another anchor on top of the concrete wall, which was tied with another piece of rope to a metal button on the wall (Figure 2).

Methods cont.

The substrates remained in the water for the three week period of June 20 - July 11 and were monitored daily and adjusted as needed as the river level fluctuated so that the top plate remained 1 foot beneath the surface. Weekly water quality measurements were taken using a YSI probe and secchi disc throughout the sampling timeframe.

After three weeks, the substrates were removed from the river, scraped using a nylon-bristled brush, and rinsed into a sorting tray. The contents from the scraped sample were then transferred into plastic test tubes, preserved with Lugol's Iodine, and placed in a refrigerator until analysis.

The preserved samples were quantified using a Sedgewick-Rafter counting chamber in which twenty of the 1,000 squares in the chamber were counted. In each sample, the number of cells of diatoms, green algae, and cyanobacteria were counted and documented, and the cyanobacteria present were identified to the genus level.

Results

Cyanobacteria were successfully collected on various substrates and at various depths. At week two, the unglazed clay square had fallen off on all but two of the fifteen plates, so this substrate was not scraped or quantified. Upon macroscopic observation, the algal growth on the nylon mesh and masonite appeared filamentous (Figures 4 and 5), where a film of algae covered the rock-like clay substrate (Figure 3). Quantitatively, the masonite substrate collected the most cyanobacteria and displayed the highest average percent density of cyanobacteria in comparison to the nylon mesh and the clay. On both the nylon mesh and masonite substrates, the highest concentrations of cyanobacteria were observed at 7 and 9 feet, where no cyanobacteria were observed on the clay substrate at these depths. The sample containing the highest average ratio of cyanobacteria per square inch of surface area was the masonite at 9 feet. The highest average density of cyanobacteria also occurred on the masonite substrate at 9 feet. Furthermore, the clay substrate collected only the genus *Oscillatoria*, and the nylon mesh and masonite collected both *Oscillatoria* and *Lyngbya*.

Figure 3. Clay substrate, 1 foot

Figure 4. Masonite substrate, 1 foot

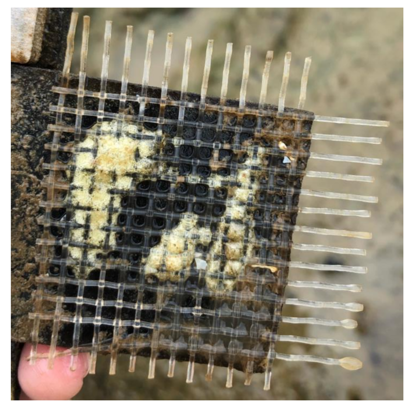

Figure 5. Nylon mesh substrate, 1 foot

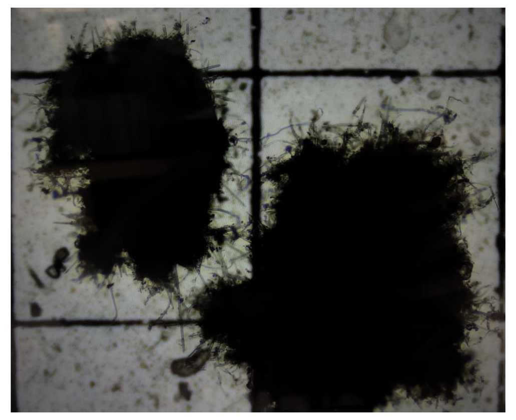

Figure 6. Cyanobacteria clump found on masonite substrate, 9 feet

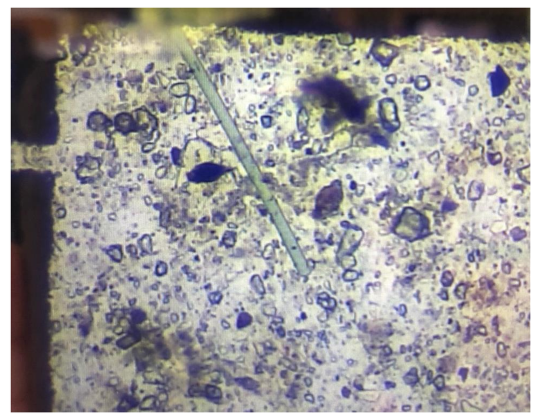

Figure 7. *Oscillatoria* found on clay substrate, 1 foot

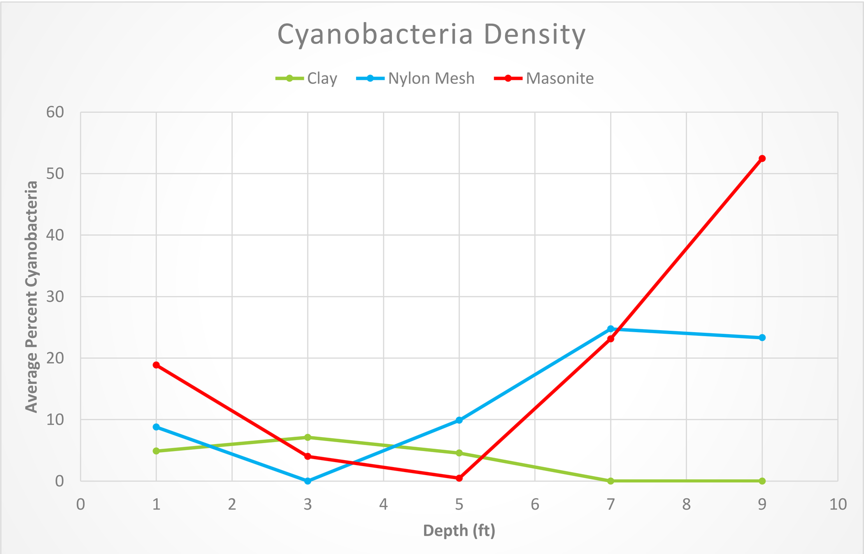

Figure 8. Cyanobacteria density vs. depth of substrate

Depth (ft)	Clay	Nylon Mesh	Masonite
1	4.883%	8.782%	18.87%
3	7.102%	0%	4.005%
5	4.566%	9.886%	0.4614%
7	0%	24.74%	23.13%
9	0%	23.31%	52.45%

Table 1. Cyanobacteria density vs. depth of substrate

Results cont.

Depth (ft)	Clay	Nylon Mesh	Masonite
1	9,573	17,430	137,000
3	9,279	0	5,127
5	870.7	6,580	1,633
7	0	28,690	18,570
9	0	8,770	427,100

Table 2. Average cyanobacteria cell abundance per square inch vs. depth of substrate

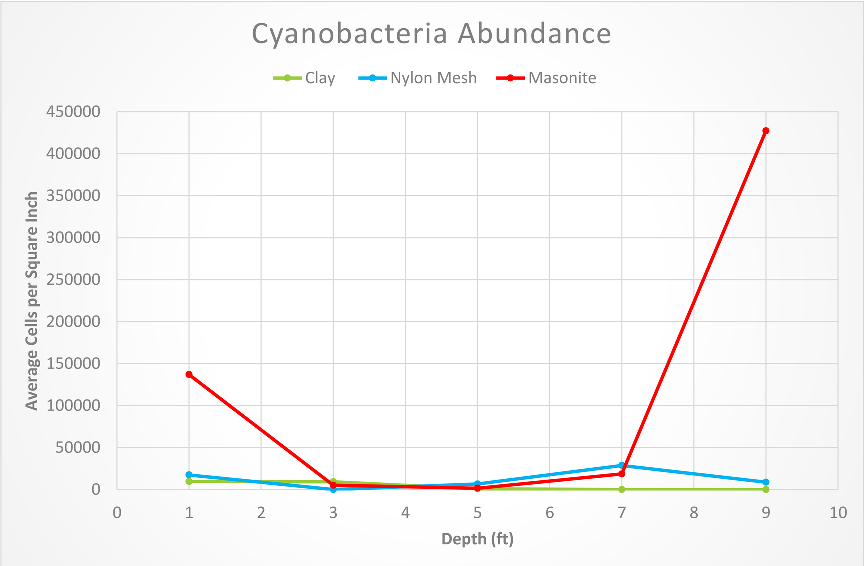

Figure 9. Cyanobacteria abundance per square inch vs. depth of substrate

Conclusion

Each substrate that was evaluated in this study successfully supported cyanobacteria growth. Overall, masonite was the most effective substrate, collecting the most cells per square inch, presenting the highest average cyanobacteria density, and supporting the growth of both *Oscillatoria* and *Lyngbya*. Furthermore, both of the genera observed on the artificial substrates have been known to cause benthic harmful algal blooms; *Oscillatoria* and *Lyngbya* both harness the potential to produce cyanotoxins that threaten domestic and wild animal health and make water sources unusable by humans for drinking and recreation (Bouma-Gregson, Keith, et al., 2017). This evidence suggests that cyanobacteria blooms observed in the Ohio River could have a benthic component, which calls for further research on the composition of historic algal blooms in this area, as well as the toxicity of the specific species of benthic cyanobacteria observed in the Ohio River during this sampling period.

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