Lower Mill Pond Food Web Study 2020

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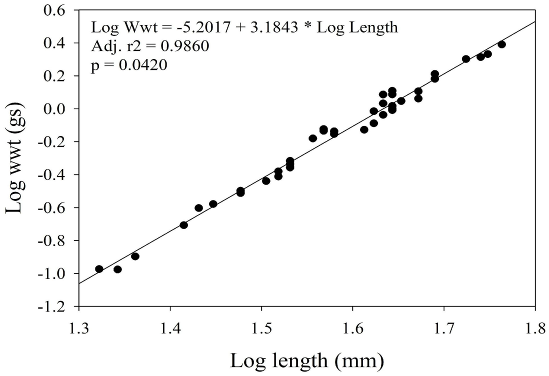
Introduction

In 2019, a study to determine the influence of biotic variables on the aerosolization of cyanotoxins was conducted in Lower Mill Pond which is at the terminus of the Mill Ponds Complex in Brewster, MA exiting via Stony Brook to the nearby estuary. Stony Brook is the largest herring run in the Cape Cod North region, and supports one of the largest freshwater spawning sites of *Alosa pseudoharengus*. The early larval and juvenile forms of *Alosa* are known to be planktivorous, where heavy feeding upon their preferred food source of large crustacean zooplankton often results in changes to their composition and size structure. A change in the zooplankton can influence the composition and size structure of the cyanobacterial population. This may increase the exposure of juvenile *Alosa* to cyanotoxins via direct ingestion. Depletion of the crustacean biomass by >95% resulted in an increase in the rotifer biomass, where an increase in the amount of potentially edible (<50 µm) cyanobacterial biomass was observed. Cyanobacteria that are <50 µm in size, specifically those 2-10 µm in size, are subject to the process of aerosolization. Within 15 - 30 days of their estimated spawning date, *Alosa* shifted their feeding strategy from planktivory to benthic detritivory for the majority of their life history, although this did not appear to affect their condition. Mean MC (0.003 µg·g−1 dwt) and BMAA (4.49 µg·g−1 dwt) concentrations in the muscle tissue of out-migrating juveniles exported freshwater cyanotoxins and created a potential transfer to consumer of 0.0012 µg MC and 1.85 µg BMAA. The occurrence of juvenile *Alosa pseudoharengus* appears to be coupled to the sequential increases of cyanobacterial biomass via its influence on the zooplankton community. In 2020, a grant from the Brewster Ponds Coalition supported the continuation of this project to 1) determine if similar changes in zooplankton and cyanobacterial composition and size structure would be observed and 2) validate the 2019 sample collection procedures and data interpretations (i.e metrics) thereby allowing the BPC Citizen Science program to evaluate other waterbodies.

Assessment of juvenile *Alosa pseudoharengus* condition

The mean lengths of A. pseudoharengus specimens increased from 31.0 to 47.3 mm between July 10 and Oct 2 while the mean wet weights progressively increased from 0.49 to 1.33 grams during the same time period. The averages for length and weight during entire study period was 40.8 mm and 0.97 grams respectively. The Fulton's Condition Index was used to assess their condition, where the standard weight equation (W_s) confirmed this isometric relationship. (See Figure 1). It has been previously noted that this method has been developed for specimens greater than 180 mm in length.



Bioaccumulation of cyanotoxins in *Alosa pseudoharengus*

Toxin analysis using the ELISA technique indicated that both cyanotoxins MC and BMAA were present in the gut rinse throughout the collection period of 10 July -2 Oct, 2020, ranging from 0.065 to 1.54 µg L^{-1} for total microcystins and 543.3 to 6744.6 µg L^{-1} for BMAA. Both cyanotoxins accumulated in the fish muscle tissue where concentrations of microcystins and BMAA varied throughout the sampling season with mean concentrations for microcystins of 0.001 µg·g$_{-1}$ dwt and BMAA of 5.475 µg·g$_{-1}$ dwt. There were no differences in muscle tissue concentration between collection dates for total microcystins (Figure 2) with the highest concentration 0.002 µg g-1 dwt being observed on 2 October. There were significant differences in BMAA muscle tissue concentration between 10 July and 2 Oct (Figure 3) with the highest concentration of 7.225 µg g^{-1} dwt. being observed on 10 July. The potential transfer to consumers (gut + muscle) varied seasonally, with mean transfer of microcystins of 0.0003 µg (Figure 4) and BMAA of 1.09 µg (Figure 5). The transf [illegible] ocystins to consumers was highest on 2 October (0.0006 µg), which was significantly higher than all ot [illegible] g dates. The transfer of BMAA to consumers was highest on 18 September (1.88 µg), which was significantly higher than all other sampling dates with the exception of 4 September.

Figure 2. Microcystin concentrations in *Alosa* muscle tissue.

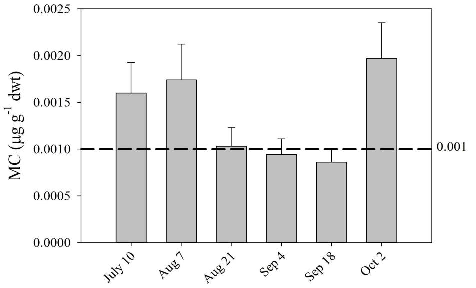


Figure 3. BMAA concentrations in *Alosa* muscle tissue.

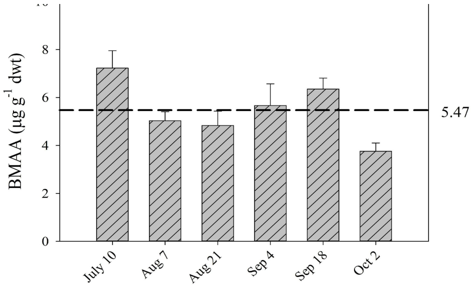


Figure 4. Transfer of microcystins to consumer

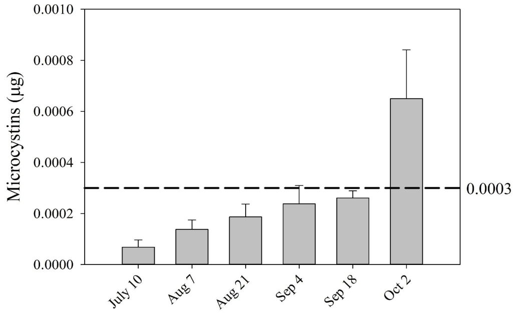
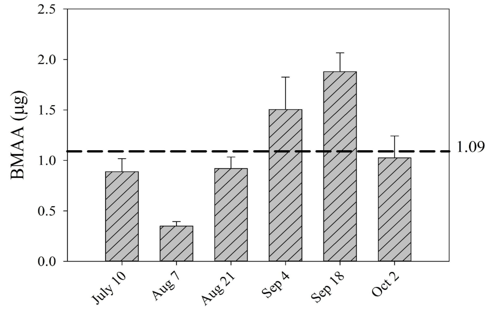


Figure 5. Transfer of BMAA to consumer



The bioaccumulation of both cyanotoxins MC and BMAA in the muscle tissue suggest that the exposure pathways could include direct ingestion as detrital material or to a lesser extent from passive ingestion from the water column. Sediment samples were collected from the littoral and pelagic zones of Lower Mill Pond, where concentrations in the littoral zone were higher than the pelagic zone with the exception of Site #2. Littoral zone microcystin concentrations were higher along the northeastern shoreline Sites #5, 6 and 7 (Figure 6) while the BMAA concentrations were more variable (Figure 7). Average concentrations of 0.53 µg g-1 dwt MC and 0.34 µg g-1 dwt BMAA in sediment from the littoral zone were observed during the study period. The bioaccumulation factors (MC BAF and BMAA BAF) were calculated as the ratio between muscle tissue and littoral sediment where values of MC BAF 0.002 and BMAA BAF 15.9 were observed. BAF values <1.0 indicate biodilution while values >1.0 indicate biomagnification. In Lower Mill Pond for 2020, microcystins biodiluted while BMAA biomagnified.

Figure 6. Microcystins concentrations in sediment samples.

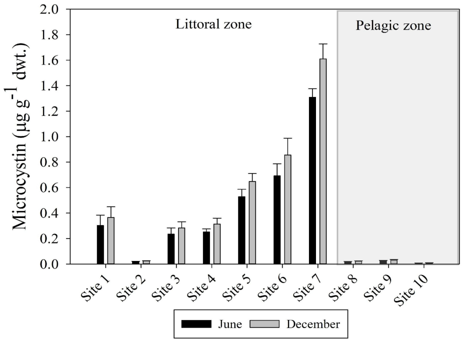
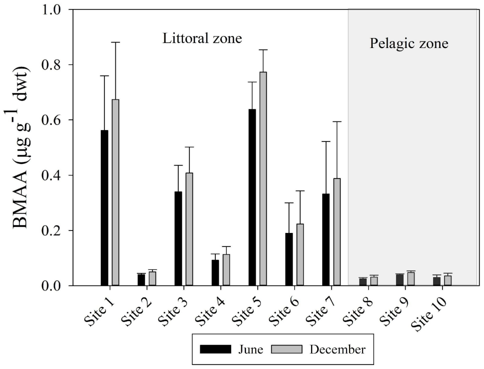


Figure 7. BMAA concentrations in sediment samples.



The concentrations of MC and BMAA in water samples

The cyanotoxin concentrations for MC and BMAA were normalized to the amount of biomass (phycocyanin) as toxin per unit pigment (micrograms per milligram). This metric provides a broader description of the exposure potential based upon the community size structure, and suggests that the greatest concentration of cyanotoxins within the edible (<50 µm) and bloom forming (BFC) fractions occurred early in the spring (May 16) and in early July (July 10) as depicted in Figures 8 and 9. The increases in the edible fraction could indicate time periods when the toxicity of aerosols were at their highest concentrations.

Figure 8. Microcystin concentrations in water samples.

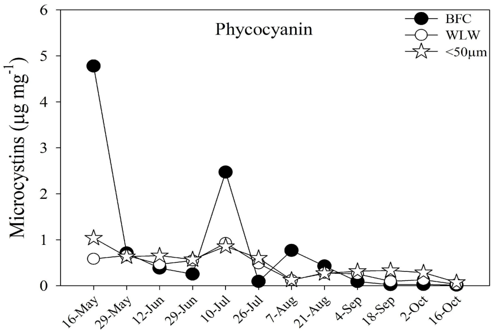
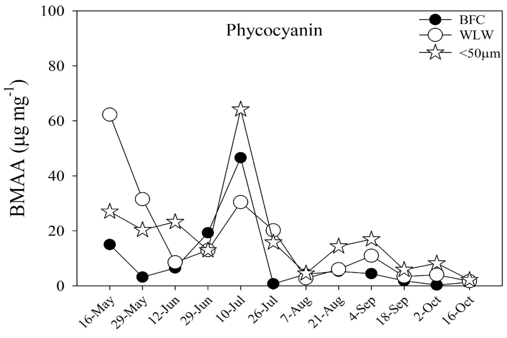


Figure 9. BMAA concentrations in water samples.



There were significant linear relationships between cyanobacterial biomass and microcystins (Figure 10) and BMAA (Figure 11) concentrations. The relationship between phycocyanin and microcystin was similar to that documented in Lower Mill Pond in 2018. This is the first description of the relationship between phycocyanin and BMAA for any sites being monitored for cyanobacteria by the Brewster Ponds Coalition and the Association to Preserve Cape Cod.

Figure 10. Relationship between cyanobacterial biomass and microcystin concentrations.

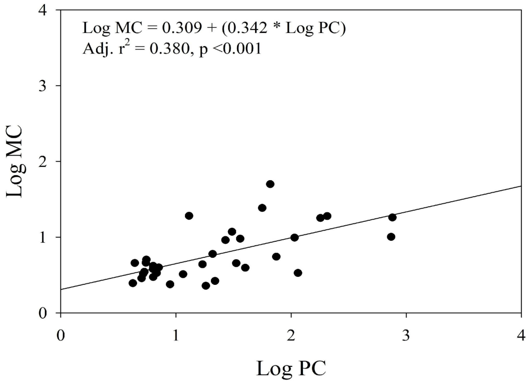
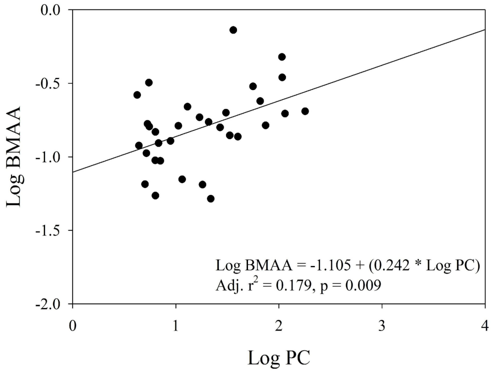


Figure 11. Relationship between cyanobacterial biomass and BMAA concentrations.



Interactions between *Alosa pseudoharengus* and planktonic populations

The zooplankton biomass in Lower Mill Pond exhibited strong seasonal patterns with varying distributions of the crustacean and rotifer grazers, and Asplanchna spp. Over the entire study period (2019-2020), the biomass of the crustacean grazers was negatively correlated with the rotifer grazer biomass and the edible cyanobacterial biomass. Specifically, in Lower Mill Pond the reduction of crustacean grazers allowed for proliferation of rotifer grazers (Figure 12) and edible cyanobacteria (Figure 13). The rotifer grazers were positively correlated with the edible cyanobacteria during the study period. The presence of the juvenile A. pseudoharengus forced a redistribution of the zooplankton biomass from crustacean to rotifer grazers resulting in increased edible (<50 µm) cyanobacterial biomass in the absence of crustacean grazing pressure. Over the entire study period, linear regression analysis confirmed a positive relationship between the rotifer grazer and edible cyanobacterial biomass suggesting top-down control. Increased biomass coupled with inefficient utilization of edible algae (<50 µm) by small planktonic herbivores, including rotifers, appears to have occurred where heavy Alosa planktivory existed.

Figure 12. Distribution of zooplankton biomass.

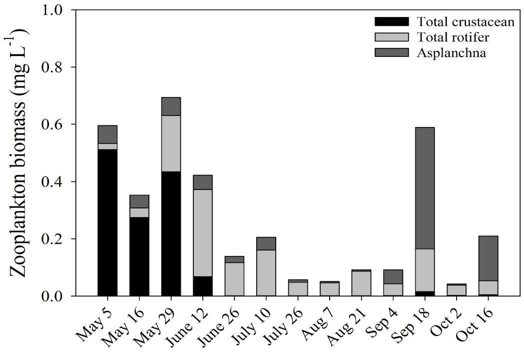
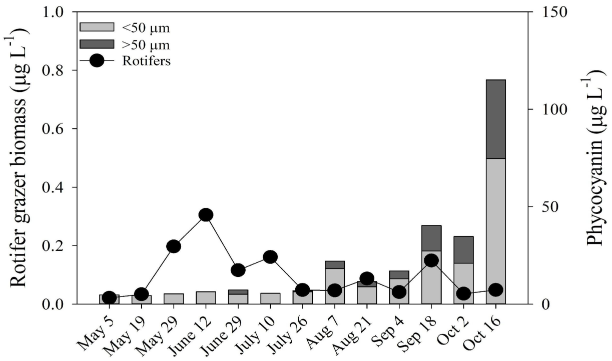


Figure 13. Zooplankton and cyanobacterial biomass.



The total crustacean biomass (excluding copepodites) was consistently dominated by the small bodied cladoceran *B. longirostris*. The mean crustacean length maxima of 0.6 mm was observed on 5 May with lengths then declining to 0.3 mm on 26 June, remaining at zero from 10 July to 21 August, until 4 Sep through 16 October when an average length of 0.3 mm was noted. A positive predator: panfish ratio of 0.1 was calculated for 5 May, and was negative or zero thereafter, suggesting that predators were absent from Lower Mill Pond. A comparison of these results from Lower Mill Pond (Figure 14) with previously published data (Figure 15) demonstrates the need to develop a broader assessment metric that extends past considerations of crustaceans to include rotifers.

Figure 14. Predator:panfish ratio in Lower Mill Pond. Large circles indicate data points within range of Mills et al.

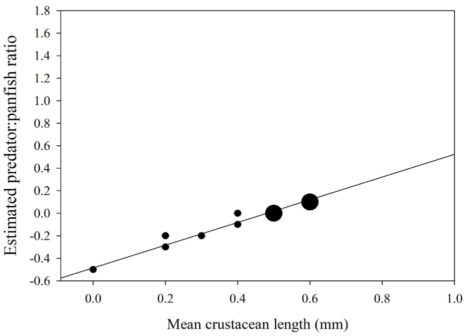
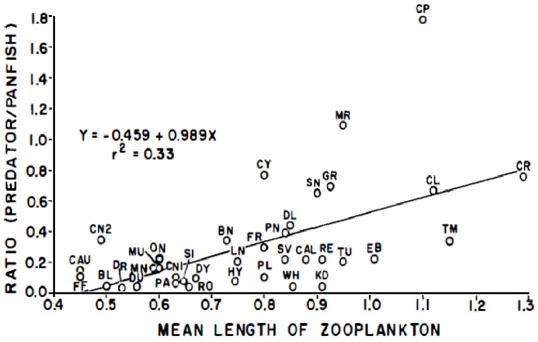


Figure 15. Predator:panfish ratio from Mills et al. 1987.



Conclusion

During this study, we observed similar response of the zooplankton population to the presence of juvenile *Alosa pseudoharengus* where removal of crustaceans allowed for the proliferation of rotifers. The changes in the zooplankton community ultimately influenced the composition of the cyanobacterial population, creating an imbalance in the system. Zooplankton samples could be used as an indicator of ecological integrity of the water body. Typically, evaluation of zooplankton body lengths is conducted, however this requires trained personnel and is time consuming, creating barriers for this type of application. In cases where rotifers are the only zooplankton in the waterbody, the predator:panfish ratio needs to be revised. The change in the zooplankton community structure can be easily observed by visual assessment of the sample collected from the base of the “Pocket ZAPPR”, a piece of equipment included in the citizen monitoring kit. Citizen scientists could easily conduct these visual surveys on a regular basis to determine if this particular source of imbalance was present.

The monitoring protocol currently uses fractionation of water samples (<50 µm) to evaluate that portion of the cyanobacterial population that are edible, and subject to the influence of the zooplankton. This particular sample served as the critical metric to further describe these interactions.and verify the presence of an aquatic trophic cascade. Although aerosols were not evaluated during 2020, the relative proportion of the <50 µm fraction to the WLW could be a useful metric for describing the potential for this process to take place on other waterbodies.