Project Title: Nutritional factors promoting algal blooms in the lower Chesapeake Bay

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Project Abstract:

While there is a general understanding that nutrients are important regulators of the timing and magnitude of algal blooms, the specific role of organic nutrients and mixotrophy in initiating or maintaining such phenomena is still unclear. We are therefore conducting a combined laboratory and field study to examine the role of mixotrophy in the production of algal blooms in Virginia waters. Specifically, we are determining the extent to which heterotrophic grazing or uptake of DOM supplements autotrophy over a seasonal cycle as the nutrient environment and community structure and function change. Our studies are based out of Old Dominion University in Norfolk, VA.

During this project we have conducted extensive field sampling in areas that have previously experienced algal blooms and in areas where blooms have occurred during this project year. So far we have observed that phytoplankton mixotrophs that occur in Virginia waterways can: 1) take up organic and inorganic N and C, which may allow them to out-compete other phytoplankton, and 2) compete with bacteria for organic nutrients. In addition, we have observed that, seasonally, peptide hydrolysis is an important pathway for mobilizing organic compounds that can then be used for growth. Dinoflagellate blooms occur throughout the year in the lower Chesapeake Bay tributaries but the species that bloom, change seasonally. In the Lafayette River, we have sequentially had blooms of *Heterocapsa triquetra* (winter), *Prorocentrum minimum* (spring), *Akashiwo (Gymnodinium) sanguinea* (late spring and summer), *Scrippsiella sp.* (summer), and *Cochlodinium heterolobatum* (fall). While nutrient and isotope samples are still being analyzed, we have determined that all of these species use inorganic and organic N and C and that organic C is a significant subsidy to photosynthetic C uptake for all species. In addition, a number of these species compete with bacteria for leucine and thymidine calling to question bacterial productivity estimates in estuarine systems where phytoplankton mixotrophs are abundant.

Keywords: harmful algal blooms, nutrients

Project Type: Research

Introduction

Harmful algal blooms (HABs) appear to be increasing in their geographical extent and frequency and have resulted in severe economic and public health impacts around the world, including the United States and Virginia. The late spring/early summer plankton communities of the lower Chesapeake Bay (Virginia) contain a number of potentially harmful algal bloom species (e.g., the dinoflagellates *Pfiesteria piscicida, Prorocentrum minimum, Gyrodinium galatheanum, Katodinium rotundatum, Cochlodinium heterolobatum,* and *Gymnodinium* spp.) that can reach bloom densities (Sellner and Nealley 1997). Negative economic and environmental impacts due to HABs have already been reported in Virginia's estuarine waters. Because blooms are already occurring and may increase either directly or indirectly as a result of inputs of organic or inorganic nutrients or through recycling processes, it is important to identify and understand the nutritional factors promoting blooms.

Increases in the occurrence of HABs, including dinoflagellate species in the lower Chesapeake Bay, have been attributed to changes in water quality in a variety of locations; specifically, the enrichment of estuarine waters with dissolved organic material (DOM) relative to inorganic nutrients (Paerl 1988, Lewitus et al. 1999, Glibert et al. 2001). Many of the organisms responsible for these blooms can use dissolved organic nitrogen (DON) sources to meet at least a portion of their nitrogen (N) demand for growth (Berg et al. 1997, Lewitus et al. 1999, Mulholland et al. 2002). Organisms capable of mixotrophy may have a competitive advantage when inorganic nutrients are low or when organic nutrients are present in high concentrations. There is evidence that a number of potentially harmful algal species are able to use both N and C from dissolved free amino acids (Mulholland et al., 2002; Mulholland et al., submitted). The capacity to grow using inorganic nutrients and light energy (e.g., as an autotroph) during the day AND to grow using organic substrates for both nutrients and energy (e.g., as a heterotroph) would give such mixotrophs a competitive advantage relative to strictly autotrophic phytoplankton by enabling them to: (1) access a larger nutrient pool over a longer time period (e.g., the entire 24-hour diurnal cycle); and (2) grow at high densities when other autotrophs are liable to self-shade.

While the use of DOM as a C and N source may allow certain phytoplankton to out-compete other algal taxa, it presents a new challenge in that phytoplankton mixotrophs must now compete with bacteria for organic nutrients.

Elevated DOM concentrations can result from direct inputs or indirectly, as a result of nutrient cycling. Increases in nitrate (NO_3^-) inputs due to agricultural runoff and groundwater can stimulate micro- or macroalgal production. Elevated DOM concentrations can occur during and subsequent to NO_3^--fueled blooms as a result of direct release and degradation. Developing management strategies to reduce nutrient inputs or removing or safely sequestering nutrients once they have entered sensitive aquatic system may be a key to avoiding blooms of undesirable or harmful algae.

In addition to the direct uptake of DOM, a number of algal mixotrophs can feed directly on bacteria and other phytoplankters. Species such as *Prorocentrum minimum* and *Gyrodinium galatheanum* have been found in association with low levels of dissolved inorganic nutrients and feeding on the genus *Cryptomonas* spp. (Stoecker et al. 1997, Li et al. 2000a, 2000b). An added advantage of this type of mixotrophy is the ability of these species to directly eat their competitors (e.g., for nutrients, light, and/or prey).

Methods

We conducted a combined laboratory and field study to examine the role of mixotrophy in the production of algal blooms in Virginia waters. The specific hypotheses that were tested during this study are: 1) the abundance of bloom-forming mixotrophs increases when inorganic N concentrations are low and organic nutrient concentrations are high, and 2) the relative degree by which autotrophic growth is supplemented by heterotrophic grazing and uptake of DOM increases when inorganic nutrient concentrations are low or light is limited by turbidity or cell density. In order to address these hypotheses we:

- Determined seasonal and interannual changes in: inorganic N uptake, photosynthetic CO2 fixation, relative C and N uptake from organic compounds and grazing by mixotrophs as the nutrient and light environment change at field sites impacted by algal blooms.

- Conducted laboratory culture experiments under controlled condition to determine the role of nutrients and light in regulating heterotrophic behavior in selected mixotrophic species.

We used stable isotopes and fluorescently labeled substrates to trace both C and N uptake and examine pathways of organic material mobilization by different size-fractions in natural populations and by individual species and groups of species in cultures. Uptake of inorganic and organic N and C were assessed using the methods previously described (Glibert and Capone 1993, Mulholland and Capone 2000, Mulholland et al. 2002). Nutrients were analyzed using standard methods (Grasshoff et al. 1999). Extracellular peptide hydrolysis was being measured using fluorescently-labeled organic compounds as described in Mulholland et al. (2002). Grazing was measured by the dilution technique (Landry et al. 1995) and by direct counts of laboratory cultures in which a known concentration of prey is added (Li et al. 2000b).

Results

This study began in July 2002 and we intend to continue working to answer these questions pending additional funding. Between July 2002 and August 2003, we experienced a large number of algal blooms in the lower Chesapeake Bay tributaries. Because we have only finished sampling for this project, we are still analyzing results and so in this report we will summarize both quantitatively (where possible) and qualitatively our results to date. We expect at least two publications citing support from VWRRC once results have been fully analyzed.

During the summer of 2002, we encountered blooms of *Prorocentrum minimum* and *Gyrodinium galatheanum* in the Elizabeth River, the cryptophyte, *Cryptomonas* spp. co-occurred with the *Gyrodinium* bloom. During the fall of 2002, there was a bloom of *Cochlodinium heterolobatum* in the Lafayette River. This winter, spring and early summer, 2003, we experienced blooms of *Heterocapsa triquetra, Prorocentrum minimum* and *Akashiwo (Gymnodinium) sanguinea* and *Scrippsiella* sp., respectively. During the fall, 2002, we incorporated these studies into my Biological Oceanography Laboratory class and conducted additional field sampling on C. *heterolobatum* and culture experiments on P. *minimum* and *A. sanguinea*. All of the cultures used during these studies were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) or through Horn Point Laboratory (University of Maryland) and are maintained on either L1 or f/2 media under a 12 hour light and 12 hour dark light regime. Nutrient studies using cultured populations were conducted after altering the nutrient concentration in the growth media and pre-conditioning cells to the new nutrient environment (for at least 3 growth cycles prior to any experimental manipulation).

We also surveyed a large portion of our culture collection for the ability to hydrolyze larger organic compounds via peptide hydrolysis and their ability to take up thymidine and leucine, compounds commonly used to assess bacterial productivity. Results suggest that both natural and cultured populations can hydrolyze peptides and thereby mobilize large organic compounds and regenerate small, usable organic substrates. In addition, leucine and thymidine uptake by phytoplankton was been observed in our culture studies and this may be important in environments where bacteria and phytoplankton compete for organic growth substrates. DOM utilization by bacteria and bacterial productivity may be seriously overestimated when phytoplankton mixotrophs are abundant. This has been a major finding of this study and results were presented at the Estuarine Research Federation meeting in Seattle earlier this month.

During field investigations, water was collected within and outside of bloom areas to characterize the nutrient environment and N and C uptake by phytoplankton and bacteria. Analyses completed to date show that organic and inorganic N and C was taken up by phytoplankton-sized cells and bacteria. Preliminary results demonstrate that extracellular peptide hydrolysis rates contribute to the mobilization of organic matter rendering it usable by organisms.

Discussion

Research on harmful algal blooms in Virginia waters has been limited to date. Therefore, these are among the only data on how nutrient cycles affect algal blooms in the Virginia's Chesapeake Bay tributaries. This research represents an important contribution to both state and regional water quality initiatives as well as to a general understanding about how phytoplankton mixotrophs bloom in estuarine systems. This study has allowed us to expand our work investigating the role of nutrients and organic material in the formation of algal blooms in

Virginia waters and to make discoveries of general importance in aquatic ecology and biological oceanography.

While we are still analyzing samples and compiling results from this study, we have observed that there is substantial uptake of organic compounds during bloom events. At the same time, we are trying to continue to sample as blooms occur in local waters. This has been a tremendous undertaking and we will continue to seek funding to expand and continue this research. We have found that some cultured populations grew equally well when either organic (urea) or inorganic (NH_4' or $N03^-$) nitrogen was supplied in the culture medium. In addition, preliminary results suggest that peptide hydrolysis is an important pathway for mobilizing large organic compounds (e.g., proteins and peptides) in natural and cultured systems. This indicates that cells are capable of regenerating small usable organic substrates from large compounds that cannot be directly taken up by cells.

We have also found that organic compounds are important sources of N AND C for bloom-forming phytoplankton mixotrophs and that they compete with bacteria for compounds previously considered to be "bacterial nutrients". These are major findings and have been the subject of several presentations at national meetings. Different species demonstrated differing capacities for inorganic versus organic N and C uptake and that may, in part, affect when they bloom seasonally.

Because organic enrichment has been implicated in the bloom of a variety of potentially harmful species at a variety of sites, it is crucial that we understand the processes and pathways through which DOM becomes available to these organisms so that we can: (1) mitigate the effects of blooms that result from natural or cultural eutrophication of water bodies, and (2) prevent their appearance in locations that have not previously experienced blooms or their economic and environmental repercussions.

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Student Support

Ms. Andrea Rocha has been supported on this project for 3 terms and Ms. Sue Reynolds has been supported for 1 term on this project. Sue Reynolds graduated with an MS degree in Chemical Oceanography at ODU in the Department of Ocean, Earth and Atmospheric Sciences. Ms. Rocha has successfully defended her thesis prospectus that includes preliminary data collected during this project and has presented her results to date a the Estuarine Research Federation meeting in Seattle. She intends to complete her thesis project and graduate in December of this year. In order to facilitate her on-going research, we received a no-cost extension for this project. She is currently being supported as a TA while she finishes her analyses. In addition, 2 other students in our department have participated in sampling events conducted as part of this project.