

# **Dreissenid Mussels as Homeostatic Filter Feeders and Nutrient Excreters: Implications for Harmful Algal Blooms (HABs) and Nutrient Cycling Across Trophic Gradients**

**Primary Investigator(s):** Hank Vanderploeg - NOAA GLERL, Juli Dyble Bressie

**Co-Investigators:** Orlando Sarnelle, Steve Hamilton, Geoff Horst - Michigan State University, Tom Johengen- CILER, University of Michigan, Miguel Dionisio Pires – Deltares, Alan Wilson - Auburn University

## **Overview**

During 2005-2008 we did experiments to examine the roles of homeostatic filtering and nutrient excretion in dreissenids in promoting toxic *Microcystis* blooms of particular strains (Vanderploeg et al. 2001, 2002) using a variety of state of the art and novel tools at different sites in Saginaw Bay, Lake Erie, and experimental mesocosms in Gull Lake, a Zebra mussel infested lake at the MSU Biological Station. Much of this work was supported by funding to Sarnelle and Vanderploeg by the NSF-NOAA ECOHAB Program (2005-2007). Work during 2007 was particularly intense and focused on two major approaches to understand the causal mechanisms of toxic *Microcystis* bloom promotion:

- During 3 1-week trips to Gull Lake we examined mussel feeding, nutrient excretion, and selective rejection of algae, *Microcystis*, and particular *Microcystis* strains (DNA) and toxic gene expression in mesocosms having different concentrations of mussels and added nutrients. (This was the 3rd and last year for funding mesocosm work). In addition, *Microcystis* genetic structure and toxic gene expression was sampled from 30 mesocosms throughout the summer 2007 and also in 2008.
- We did experiments with cultured colonial *Microcystis* strains and *Microcystis* in natural seston in which we manipulated colony size by breaking large colonies into small colonies to test the hypothesis that rejection of *Microcystis* by mussels depends on the colony size and toxicity (Vanderploeg et al. 2001).

We were very excited about all these experiments particularly the work at Gull Lake in 2007, because the mussels were in good health in the mesocosms (they all died in 2005) and *Microcystis* did occur in many of the mesocosms. During 2008 most of our effort focused on processing samples collected in these experiments: algal counting, toxin analysis, genetic analysis, and nutrient analyses. Over 30 experiments were performed at Gull Lake in 2007. All but the genetic analyses were completed in 2008. In 2009, we propose to finish genetic analyses, organize the data base, statistically analyze the results, and prepare manuscripts.



**Figure 1:** *The mesocosms on Gull Lake. During summer 2007, we measured mussel filtering, nutrient excretion, and selection for different genetic strains of Microcystis in 10 mesocosms treated with different concentrations of mussels and nutrients during 3 1-week trips to Gull Lake. Our colleagues at MSU monitored weekly changes in phytoplankton and zooplankton composition, Microcystis abundance, and microcystin (Microcystis toxin) in all 30 mesocosms, and sent us material to evaluate changes in Microcystis genetic structure and toxin gene expression.*

### **Proposed Work**

- Assemble database for all variables and process measured at Gull Lake and in related experiments.
- Perform statistical analyses
- Finish genetic analyses on 2008 samples
- Prepare manuscripts

The data set to be analyzed for this work is large. To finish up the Gull Lake sample analyses from more than 30 short-term feeding and excretion experiments, 6 long-term feeding experiments, and 20 pseudofeces rejection experiments were done in 2007 and other experiments (described below), we needed to make the following measurements: PO<sub>4</sub> (SRP) and ammonia sampled from excretion experiments; phytoplankton composition, microcystin, DNA, C, N, and P sampled from seston used for all feeding experiments; dry weight and C, N, and P of all mussels used in experiments; and composition of phytoplankton, microcystin, DNA sampled from all control and experimental bottles from long-term feeding experiments and “rejection” experiments.

Most of the analyses for nutrients, microcystin, and phytoplankton are nearly completed, and we are assembling a database from which to look at factors affecting mussel feeding and nutrient excretion with the goal of understanding of how this contributes to toxic *Microcystis* blooms in the Great Lakes.

In addition *Microcystis* DNA was sampled weekly in the mesocosms during both summer 2007 and 2008. We have already done the analysis of *Microcystis* community composition for 2007, including both quantifying the percentage of the *Microcystis* community that is comprised of toxin-producing strains and characterizing the changes in community composition in response to the dreissenid and nutrient treatments. In order to publish this work, we need to do a similar analysis on the 2008 experiments. This will include using quantitative PCR to determine the percentage of toxic *Microcystis* colonies in 30 mesocosms at the initial time point and 30 days after the addition of nutrients and dreissenids as well as characterizing the change in community composition using DNA sequencing. *Microcystis*-specific PCR primers for the ITS (internal transcribed spacer region) between the 16S-23S genes will be employed.

### **Scientific Rationale**

Experimental work at GLERL (Vanderploeg et al. 2001) has shown that it is likely that Zebra mussels promoted *Microcystis aeruginosa* blooms in Saginaw Bay and Lake Erie through the mechanism of selective rejection during the filtration and pseudofeces production process. In addition this same work showed that the rejection process was strain specific. The major focus of this project is to follow up on these results to examine the role of the mussels in promoting toxic *Microcystis* blooms across different trophic gradients, as this promotion appears to vary with trophic state of the lake (Raikow et al. 2004; Sarnelle et al. 2005). Part of this may be related to the viability of toxin production as predation escape mechanism at different conditions of nutrient availability. Perhaps, as Vanderploeg et al. (2001, 2002) suggest, the toxicity option is effective when algal growth rate is low, which would be expected at low P concentrations. Whatever the explanation, the role of mussels in promoting toxic *Microcystis* blooms is required for forecasting blooms and their toxicity.

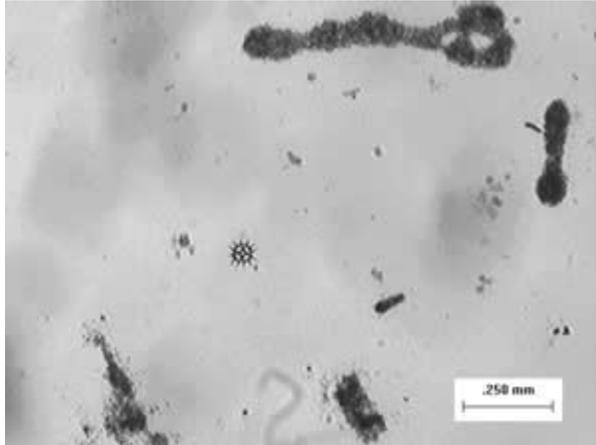
### **Gull Lake Work**

In mesocosms or the open lake we did 4 kinds of experiments, the last 3 of which represent novel approaches:

1. "Standard" short-term (2 h) that examined feeding in two size fractions using the size-fractionated chlorophyll method (Vanderploeg et al. 2001) followed immediately by experiments that quantified SRP and ammonia excretion
2. Long-term (12-16 h) feeding experiments that allowed mussels to remove all "desirable" algae to see what is left over with a particular focus on looking at what *Microcystis* strains (DNA) remained, their toxicity, and toxic gene expression.
3. Two feeding experiments to evaluate the importance of colony size of Gull Lake *Microcystis* to mussel rejection by creation of small colonies by sonication of large colonies into small colonies.

4. Direct determination of algae and *Microcystis* strains (microscope and DNA analyses) rejected as pseudofeces by mussels during excretion experiments).

The mussels exhibited a broad range of filtering rates in the different mesocosms and *Microcystis* became dominant in some mesocosms. *Microcystis* was the major dominant found in pseudofeces collected from excretion experiments (Figure 2). Much work will be required to analyze the samples and complete analyses.



**Figure 2:** Photomicrograph of algae from pseudofeces collected during excretion experiment. As was typical of most experiments, *Microcystis* colonies dominated. Note the lone *Pediastrum* colony (green alga) indicated by the arrow.

### **Experimental with Manipulated Colonies**

Experiments in which we manipulated colony size of different laboratory isolates showed that microcystin toxicity (measured by ELISA) may not be the explanation for unpalatability, because small colonies of non-toxic *Microcystis* (created from large colonies by sonication) were rejected.

### **Governmental/Societal Relevance**

Since water quality is vital to society for drinking, recreation, and fisheries among other things, it is necessary to predict how dreissenid mussels process seston and associated nutrients and promote HABs.

### **Relevance to Ecosystem Forecasting**

Dreissenid mussels are an integral part of Great Lakes ecosystems (Figure 1). No serious models or forecasts of food web dynamics, water quality, fisheries recruitment, or HABs can be developed until we have a reasonable model of how mussels process seston and excrete nutrients, especially in shallow regions of Great Lake such as Lake Erie. With the mussels as probable actors in HAB bloom promotion, the usual environmental predictors (temperature, nutrients) of HABs and HAB toxin concentration would be expected to have very low predictive power without the inclusion of an accurate assessment of mussel impacts.

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