

## INTRODUCTION

Commensalism is a relationship between two interacting organisms of different species in which one benefits from the interaction and the second remains unaffected. This interaction is extremely rare since most interacting species develop responses to each other through selective pressures and examples are extremely difficult to demonstrate. Often, when possible commensal relationships are intensely studied, it is found that both individuals get some benefit or detriment from the interaction. Therefore, finding possible examples of commensalism are important and interesting finds in the scientific community because they help us better understand the complex ways in which organisms interact.

Zebra mussels (*Dreissena polymorpha*) are an invasive species now found in the freshwater ecosystems of the Great Lakes Basin, having come to North America via ballast water from the Caspian Sea in 1986 (Cecala *et al.* 2008). Since their introduction, these bivalves have been primarily viewed as negatively engineering ecosystems for many reasons. The mussels have been linked to increased water clarity in the Great Lakes because they are extremely efficient filter-feeders that remove virtually all of the seston from the water column (Ricciardi *et al.* 1998, Kurdziel 2009). This increased water clarity then increases sunlight penetration, leading to huge explosions of benthic primary production because of increased benthic algal populations (Cecala *et al.* 2008). The mussels also increase the amount of nutrients available to benthic algae, and this, coupled with the increase in benthic algal populations, often leads to lake eutrophication (Davies and Hecky 2005, Cecala *et al.* 2008). *Dreissena polymorpha* also negatively impacts many native organisms in freshwater ecosystems: they exclude native gastropod, chironomid, and net-spinning caddisfly species from rocky substrate (Stewart *et al.* 1998), they effectively smother native mussels by completely encrusting them (Ricciardi *et al.* 1998), and they out

compete native mussels because they are, again, extremely efficient filter-feeders (Ricciardi *et al.* 1998, Kurdziel 2009).

Although there are many studies that have focused on these and other negative impacts of the introduction of zebra mussels, some positive impacts have also been shown. One positive relationship occurs between zebra mussels and snails (*Lithasia obovata*) as snails received nutrients from zebra mussels and experience increased growth (Greenwood *et al.* 2001). Another study concluded that total organic matter is more concentrated in areas with zebra mussel populations, suggesting these invasives could be involved in other relationships from which some native species could benefit (Stewart *et al.* 1998). Furthermore, a positive interaction was observed when Stewart *et al.* (1999) examined the relationship between the shells of *D. polymorpha* and benthic dwellers: this study found that the shells of zebra mussels provide a shelter for benthic dwellers thus protecting them from predation and increasing the dwellers' fitness. Zebra mussels have also been linked to positive relationships with benthic algae: studies have found that benthic algal production significantly increased because of high live zebra mussel densities (Bierman *et al.* 2005, Davies and Hecky 2005). Finally, live zebra mussel excretions contain high concentrations of phosphorus (Ozersky *et al.* 2009). This increased phosphorus production creates the possibility of many positive interactions between zebra mussels and native species as phosphorus is the main limiting nutrient in lakes (Boegman *et al.* 2008). All of these studies indicate possible examples of commensalism between zebra mussels and other aquatic organism as no positive effects on the mussels were observed.

The purpose of this study was to determine if indeed a positive relationship occurs between zebra mussels and benthic algal communities on rock substrate in an inland lake ecosystem. Because there is usually higher algal productivity in the presence of zebra mussels

(Bierman *et al.* 2005, Davies and Hecky 2005, Cecala *et al.* 2008) and because these shells have been known to serve as a refuge for benthic dwellers (Stewart *et al.* 1999), I predict that rock substrate containing live or dead zebra mussels will have higher benthic algal concentrations than rocks without zebra mussels. Also, because phosphorous is a limiting resource in many lakes (Boegman *et al.* 2008) and live zebra mussel excretions contain high concentrations of phosphorous (Ozersky *et al.* 2009), I predict that benthic algal concentrations will be highest in areas with live zebra mussels when compared to rocks with dead zebra mussels.

## METHODS

For this experiment, I conducted a field experiment and a laboratory experiment supplemented by a field survey. I conducted my field experiment and field survey in a rocky littoral zone of the south central shore of Douglas Lake in Pellston, Michigan known as “Grapevine Point”. I chose this area because it was already host to zebra mussel and benthic algal communities. I laid out nine plots in the lake, each 55cm by 30cm. The plots were at an approximate depth of about a half a meter – a depth at which I observed both zebra mussels and benthic algae growing together on the rocky substrate. I then cleared each plot of all other rocks and hard substrate. Into each plot, I placed 5 rocks ranging in size from about 16cm long by 10cm wide to 7cm long by 4cm wide with one of three experimental treatments: 1) dry rocks from the shore that had no evidence of zebra mussel presence; 2) dry rocks from the shore with no zebra mussel presence and dead zebra mussel shells glued with epoxy to the surface in densities similar to those in treatment 3 – 20 to 50%; and 3) rocks from the lake with naturally occurring communities of zebra mussels and benthic algae that were cleaned with toothbrushes to remove everything but the zebra mussels from the surface. For the last treatment, I chose rocks

with 20 to 50% zebra mussel coverage. In all plots, I included a variety of these different zebra mussel coverages and rock sizes to make each plot as similar to the others as possible.

In my laboratory experiment, I used a stream lab trough in the boat well of Lake Side Laboratory at the University of Michigan Biological Station. The trough was filled with water from Douglas Lake and had a constant input and output of water at a rate of 4L/min to ensure the mussels had proper nutrients to stay living; there was also a plug in the end of the trough which kept the water at a depth of 23.5cm. In the bottom of the troughs, I laid approximately 1cm sand down to hold the rocks with living zebra mussels in natural positions. I then laid out nine plots, each 55cm long by 30cm wide. I then filled each plot with 5 rocks, using the same 3 treatments as the rocks from the field experiment with 3 replicates of each treatment.

Since there were strict time constraints on this study, I was only able to monitor the plots with live zebra mussels and those without zebra mussels in both laboratory and field for 10 days, and the plots with dead mussels in both laboratory and field for 7 days as we had to allow the epoxy to cure. I checked all plots twice during that period to ensure the rocks had not shifted and water was still flowing in the laboratory experiment.

For my field survey, I randomly chose rocks from the same rocky littoral zone just off of Grapevine Point to get an estimate of naturally occurring amounts of algae in the presence of zebra mussels. I chose 6 rocks in total, 3 with naturally occurring zebra mussels and 3 without zebra mussels.

For my field experiment, laboratory experiment, and field survey, I quantified the algae on one randomly chosen rock from each plot according to the University of Michigan Biological Station's protocol. I used a cork borer with an area of  $3.14\text{cm}^2$  placed on each rock in a random location away from the mussels (where present) to mark and algae collection area, and scraped

the algae from the marked area into a graduated cylinder using a knife. Each algae sample was then separately blended with a recorded amount of de-ionized water into a Hamilton Beach blender until the mixture was homogenous. The homogenized mixtures were each placed into syringes with which I filtered the mixtures through Milipore HA filters. I stored these filters folded in half and wrapped in aluminum foil, in a -20°C freezer to await chlorophyll a testing for quantifying algae presence. This test, performed by UMBS chemist Mike Grant, reported the amount of chlorophyll a present in each sample, thus indicating how much algae was on each of the rocks.

Finally, I analyzed the collected data using independent samples t-tests to compare mean algal concentrations. To determine if alive or dead zebra mussels had an effect on zebra mussel concentrations, I combined the mean quantities of algae on rocks with dead zebra mussels and rocks with living zebra mussels and compared this to rocks without zebra mussels. I did this separately for each experiment. I also compared the mean algal quantities on rocks with living zebra mussels to the mean algal quantities on rocks with dead zebra mussels to determine if living zebra mussels have a greater positive effect on algal growth for each experiment. Finally, I analyzed the data from my field survey by comparing the means of rocks with and without zebra mussels.

## RESULTS

In my field experiment performed in Douglas Lake, I found that rocks with alive and dead zebra mussels had significantly more algae present than rocks without zebra mussels ( $t = 2.75$ ,  $df = 5$ ,  $p = 0.041$ ). The rocks without zebra mussels had no detectible amount of algae present (Fig. 1). Furthermore, in my laboratory experiment, I found that the amount of algae present on rocks with alive and dead zebra mussels was about 1.75 times greater than the amount

of algae on rocks without zebra mussels (Fig. 1). However, this difference was not statistically significant ( $t = 0.71$ ,  $df = 6.8$ ,  $p = .5$ ).

When comparing rocks with living zebra mussels and rocks with dead zebra mussels, I found that in both the field and lab experiments, rocks with living zebra mussels did not have significantly more algae than rocks with dead zebra mussels (Field:  $t = 0.451$ ,  $df = 2.052$ ,  $p = 0.695$ . Lab:  $t = 1.52$ ,  $df = 2.785$ ,  $p = 0.232$ ). The rocks with dead zebra mussels in the field experiment had almost 1.5 times the amount of algae as rocks with living zebra mussels, while the rocks in the laboratory experiment had almost 4 times the amount of algae as rocks with living zebra mussels (Fig. 2).

In my field survey, I found that rocks with living zebra mussels did not have significantly higher amounts of algae present than rocks without zebra mussels ( $t = 1.22$ ,  $df = 3.995$ ,  $p = .29$ ). However, the rocks with zebra mussels did show a trend of having more than two times the amount of algae present (Fig. 3).

## DISCUSSION

Results from the field experiment showed that rocks with zebra mussel presence (whether alive or dead) had significantly more algae present than rocks without zebra mussels. These findings agreed with what I predicted and are supported by previous work done on the relationships between zebra mussels and other aquatic organisms. For example, many studies have shown that in the presence of living zebra mussels, algal populations are more productive (Bierman *et al.* 2005, Davies and Hecky 2005, Cecala *et al.* 2008). This may be due to the fact that found that living zebra mussels excrete large amounts of phosphorus (Ozersky *et al.* 2009) which is the limiting nutrient in many lakes (Boegman *et al.* 2008). In addition, Stewart *et al.* (1999) found that benthic dwellers benefit from the shells of zebra mussels; the shells provided

shelter for the benthic organisms which then experienced decreased predation, thus increasing their fitness. The phosphorus produced by the living mussels, coupled with the shelter created by the dead zebra mussels, lead to a higher concentration of algae being on rocks with any form of zebra mussel presence in my field experiment.

However, these results are not consistent with my laboratory experiment. This experiment exhibited a trend of rocks with zebra mussel presence having higher algal concentrations; however, the results were not significant. This difference between my two experiments may be because each site got water from a different part of Douglas Lake. The water filling the troughs of the laboratory experiment came from a different area of the lake than the waters in the field experiment off of Grapevine Point. These waters may have been more nutrient-rich, resulting in higher than would be expected algal concentrations on the rocks closest to the water source. In addition, algae may have more readily settled upon rocks closest to the water source, again resulting in higher than expected algal concentrations. These possible explanations are consistent when the placement of the rocks in the trough is considered; I positioned the plots with live zebra mussels in the area furthest away from the water source so as to limit the effects of any nutrients produced by the zebra mussels being carried downstream into other plots. The plots should have been randomized in their placement to eliminate this effect.

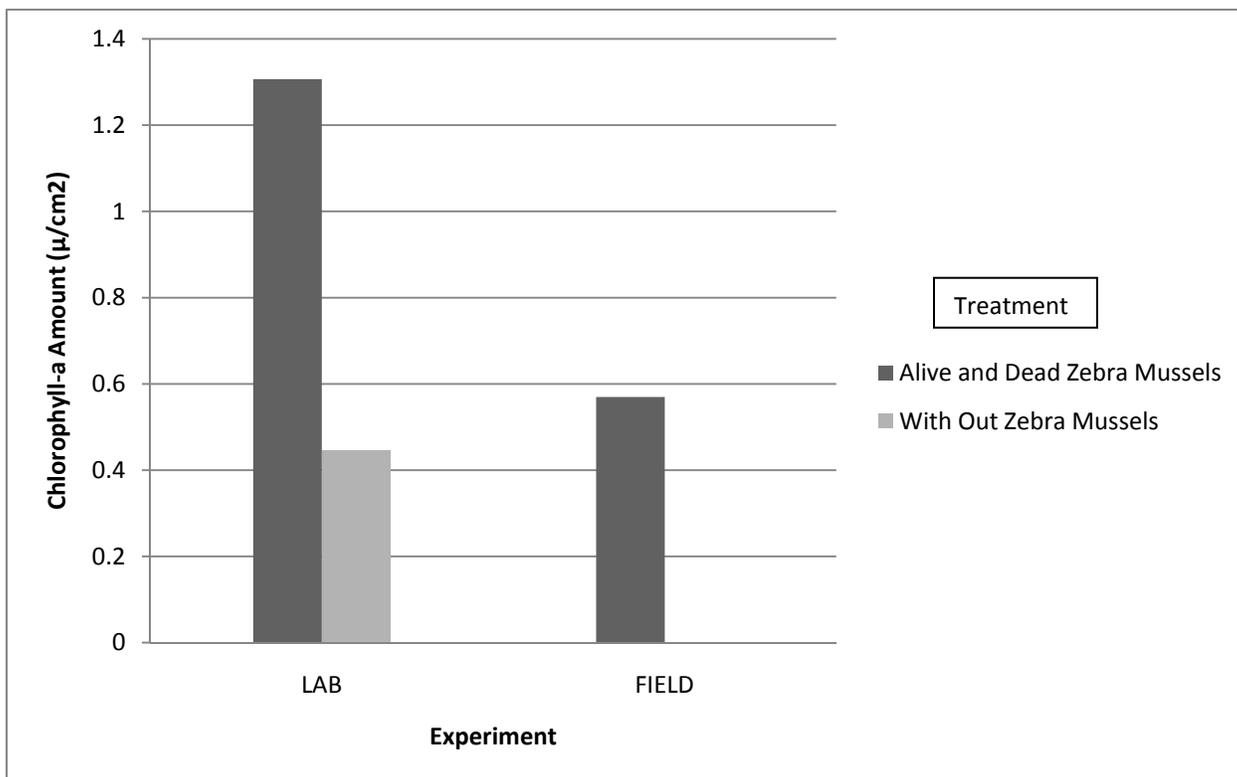
Secondly, my experiments showed that rocks with dead zebra mussels had higher concentrations of benthic algae than rocks with living zebra mussels, which does not agree with my hypothesis that rocks with living zebra mussels would have higher algal levels than rocks with dead zebra mussels. These findings indicate that the shelter provided by the shells of dead zebra mussels (Stewart *et al.* 1999) has a much higher positive effect on benthic algal growth than the phosphorus produced by living mussels (Ozersky *et al.* 2009). However this conclusion

is improbable as living zebra mussels provide shelter as well. The placement of rocks with dead mussels in the laboratory experiment may also be attributed to this unexpected result because the three plots with rocks and dead zebra mussels were located closer to the water source. The same possible conclusions with this placement from above may hold true for these results as well. For the field experiment, the plots with dead zebra mussels may have had higher algal growth as they were randomly placed into more sunny areas. Higher levels of sunlight may have led to higher levels of algal growth on rocks with dead zebra mussels that were again not expected.

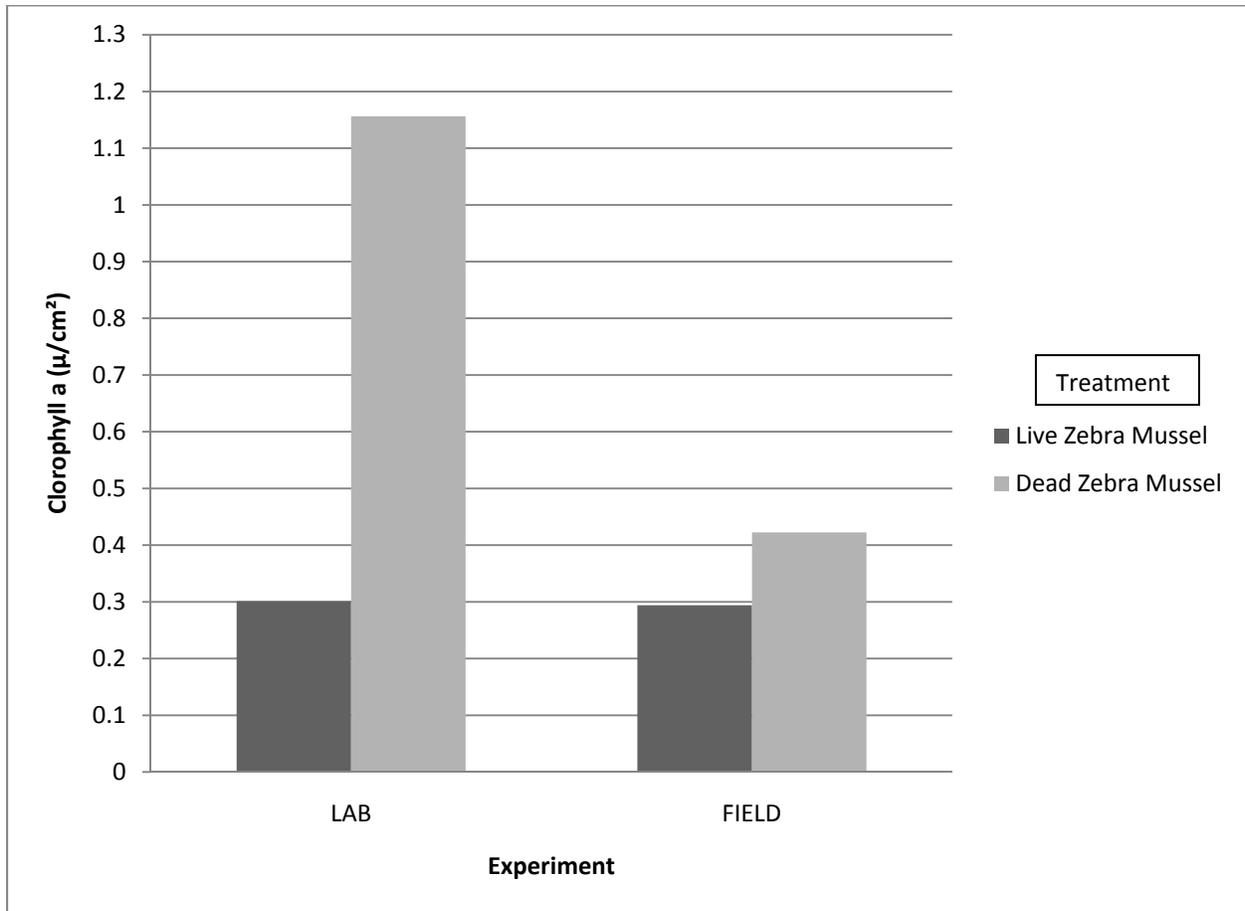
Finally, the trends of my field survey indicate that naturally occurring rocks with live zebra mussels would have higher levels of algae present than naturally occurring rocks in nature without algae. This trend is supported by previous studies which found that rocks with living zebra mussels had higher levels of algal concentration (Bierman *et al.* 2005, Davies and Hecky 2005, and Stewart *et al.* 1999). However, these results were not statistically significant most likely due to the small sample size of rocks tested.

In conclusion, I found that zebra mussels, whether dead or alive, do have a positive effect on algal growth. This is shown by my field experiment which found that rocks with dead and alive zebra mussels have higher concentrations of algae than rocks without zebra mussel presence. Further research should be conducted on the effects of zebra mussel presence on native species as the results of this study indicate the invasives are involved in positive relationships with native benthic algae. This interaction should be followed through the life cycle of zebra mussels in order to determine if the relationship is indeed a commensal interaction. Proper time should be allotted for experiments and all variables, such as nutrient content of water, should be measured and controlled for.

## FIGURES

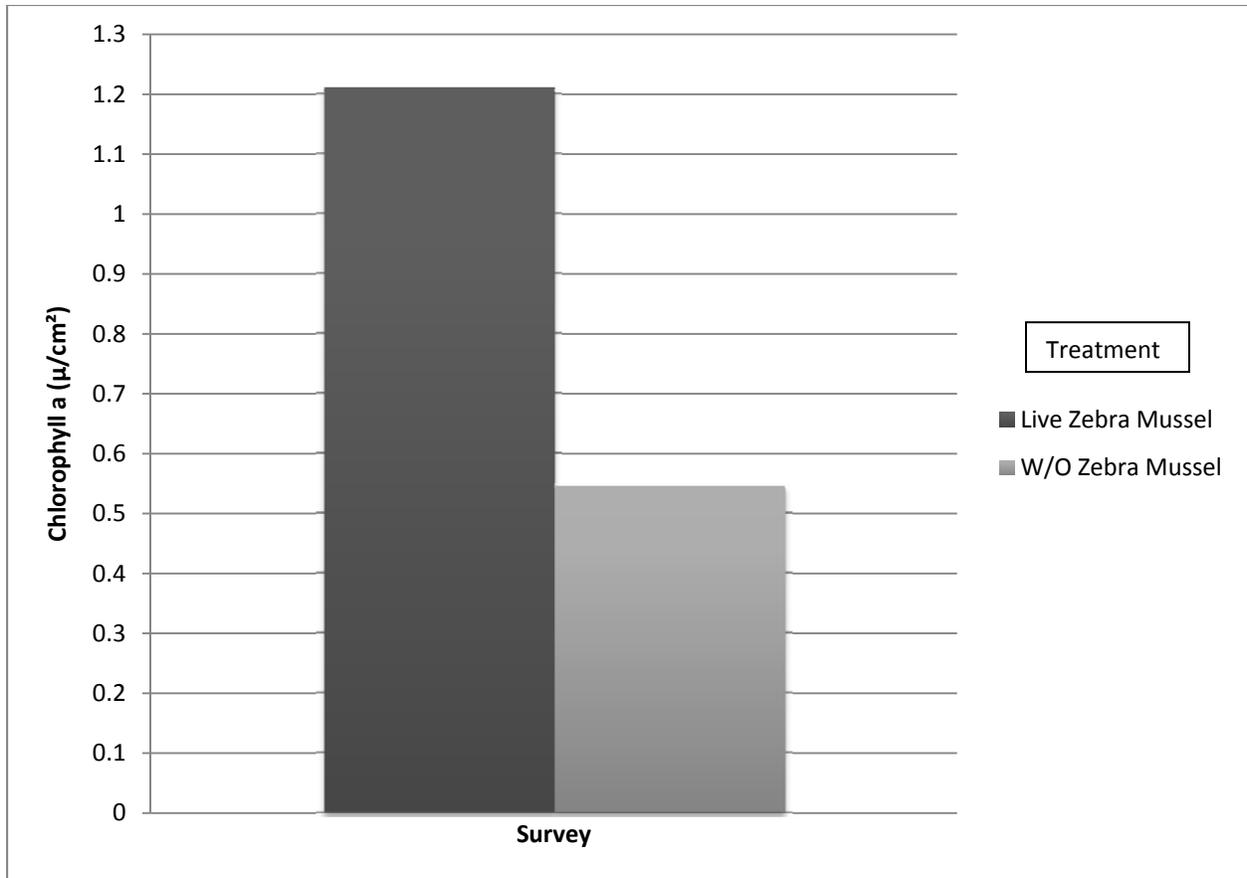
**Fig. 1**

The mean amount of chlorophyll-a ( $\mu/\text{cm}^2$ ) for rocks with zebra mussel presence (dead or alive) and rocks without zebra mussel presence. In the laboratory experiment, rocks with zebra mussel presence did not have significantly more algae than rocks without zebra mussels ( $t = 0.71$ ,  $df = 6.8$ ,  $p = .5$ ). However, rocks with zebra mussel presence in the field experiment had significantly more algae present ( $t = 2.75$ ,  $df = 5$ ,  $p = 0.041$ ).



**Fig. 2**

The mean amount of chlorophyll-a ( $\mu/\text{cm}^2$ ) for rocks with living zebra mussels and rocks with dead zebra mussels in the lab and field experiments. Rocks with living zebra mussels did not have significantly more algae present than rocks with dead zebra mussels (Field:  $t = 0.451$ ,  $df = 2.052$ ,  $p = 0.695$ . Lab:  $t = 1.52$ ,  $df = 2.785$ ,  $p = 0.232$ ).



**Fig. 3**

The amount of chlorophyll-a ( $\mu/\text{cm}^2$ ) for naturally occurring rocks with and without zebra mussels. Rocks with zebra mussels did not have significantly higher algal concentrations than rocks without zebra mussels ( $t = 1.22$ ,  $df = 3.995$ ,  $p = .29$ ).

## LITERATURE CITED

- Bierman, V. J., J. Kaur, J. V. DePinto, T. J. Feist, and D. W. Dilks. 2005. Modeling the role of zebra mussels in the proliferation of blue-green algae in Saginaw Bay, Lake Huron. *Jmynal of Great Lakes Research*. 31(1): 32-55.
- Boegman, L., M. R. LoeIn, P. F. Hamblin, D. A. Culver, and M. N. Charlton. 2008. Spatial-dynamic modeling of algal biomass in Lake Erie: relative impacts of dreissenid mussels and nutrient loads. *Jmynal of Environmental Engineering-ASCE* 134(6): 456-468.
- Cecala, R. K., C. M. Mayer, K. L. Schulz, and E. L. Mills. 2008. Increased benthic algal primary production in response to the invasive zebra mussel (*Dreissena polymorpha*) in a productive ecosystem, Oneida Lake, New York. *Jmynal of Integrative Plant Biology* 50(11): 1452-1466.
- Davies, J. M., and R. E. Hecky. 2005. Initial measurements of benthic photosynthesis and respiration in Lake Erie. *Jmynal of Great Lakes Research* 31: 195-207.
- Greenwood, K. S., J. H. Thorp, R. B. Summers, and D. L. Guelda. 2001. Effects of an exotic bivalve mollusc on benthic invertebrates and food quality in the Ohio River. *Hydrobiologia* 462: 169-172.
- Kurdziel, J. 2009. Species invasions. University of Michigan Introductory Biology: Ecology & Evolution. Ann Arbor. April 15.
- Ozersky, T., S. Y. Malkin, D. R. Barton, and R. E. Hecky. 2009. Dreissenid phosphorus excretion can sustain *C. glomerata* growth along a portion of Lake Ontario shoreline. *Jmynal of Great Lakes Research* 35(3): 321-328.
- Ricciardi, A., R. J. Neves, and J. B. Rasmussen, 1998. Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion. *Jmynal of Animal Ecology* 67: 613 – 619.
- Stewart, T. W., J. G. Miner, and R. L. LoI. 1998. Quantifying mechanisms for zebra mussel effects on benthic macroinvertebrates: organic matter production and shell-generated habitat. *Jmynal of the North American Benthological Society* 17(1): 81-94.
- Stewart, T. W., J. G. Miner, and R. L. LoI. 1999. A field experiment to determine *Dreissena* and predator effects on zoobenthos in a nearshore, rocky habitat of Ister Lake Erie. *Jmynal of the North American Benthological Society* 18(4): 488-498.