ABSTRACT

In this study, we observed rocks with different types of zebra mussel influence in a freshwater lake and lab setting to determine whether benthic algal concentrations are greater on rocks are with the presence of both live and dead zebra mussels compared to rocks without any zebra mussel influence, and whether live zebra mussels promote greater algal growth than dead shells. We set up experimental plots containing rocks with live zebra mussels, with no zebra mussels, and with dead zebra mussel shells in both the littoral zone of Douglas Lake and in a stream trough in Lakeside Lab at the University of Michigan Biological Station. After 11 days, chlorophyll a analysis was conducted to determine benthic algal concentrations. There was no significant difference between benthic algal concentrations on rocks with live or dead zebra mussels and rocks without zebra mussels in the lab, but these same values were significant in the field study. The mean amount of chlorophyll a on rocks with live or dead zebra mussels was 1.3 μg/square cm, while there were 0 μg/square cm of chlorophyll a on rocks without zebra mussels. There was no significant difference between benthic algal concentrations on rocks with live zebra mussels and rocks with dead zebra mussel shells in either the lab or field study. From the results of this study, we concluded that the presence of live or dead zebra mussel shells results in greater benthic algal growth than areas without zebra mussels. We also concluded that the presence of live zebra mussels does not promote more benthic algal growth than dead zebra mussel shells.
INTRODUCTION

When two species interact very closely, over time strong selective pressures usually result in each species developing a response to the other, be it negative or positive. Therefore, it is very rare to find examples of interacting organisms in which one organism is affected by the interaction while the other remains completely unaffected. Interactions of this rare type are called commensalisms, and because these commensal relationships are rare, they can be very important finds in the scientific community, as any insight gained by studying known commensalisms can provide important answers about how and why organisms develop a response to each other, and also why they do not.

The zebra mussel (*Dreissena polymorpha*), an invasive species initially brought to North America in ballast water from the Caspian Sea in the 1980s, has come to dominate freshwater
ecosystems surrounding the Great Lakes due to its extremely high reproduction and filtering rates (Cecala et al. 2008). As a species invasive to the area, these mussels have been viewed as ecosystem engineers with largely negative effects on freshwater systems; as extremely efficient filter feeders, they remove an enormous amount of seston from the waters they inhabit, increasing the depth to which sunlight can penetrate, and this increase in sunlight penetration in turn leads to huge increases in benthic primary production, including benthic algal production (Cecala et al. 2008, Stewart et al. 1998). Zebra mussels have also been noted to excrete nutrients key to benthic algal production, including phosphorous and nitrogen (Ozersky et al. 2009); since phosphorous is a limiting resource in many inland lakes (Boegman et al. 2008), this zebra mussel phosphorous excretion further increases the benthic algae populations in invaded waters (Davies and Hecky 2005), which can lead to lake eutrophication (Cecala et al. 2008). Zebra mussels are also known to have negative effects on freshwater organisms in the ecosystems they inhabit; these invasive mussels out-compete native gastropod, chironomid, and caddisfly species for space on the limited amount of rocky substrate that is available (Stewart et al. 1998), reduce survival of native mussels by completely encrusting them and using them as substrate which reduces their mobility and subsequent ability to reach food (Ricciardi et al. 1998), and directly outcompete the native mussel species for food because they are much more efficient filter-feeders than are the natives (Ricciardi et al. 1998, Kurdziel 2009).

Because zebra mussels are invaders, many studies have focused on the negative impacts of their introduced presence in the Great Lakes and other freshwater systems, but positive effects have been shown in many studies (Greenwood et al. 2001). A positive relationship was discovered between zebra mussels and snails (Lithasia obovata) in which the snails received nutrients from zebra mussel excretions while there was no effect observed on the zebra mussels
(Greenwood et al. 2001). This is a possible example of a commensal relationship involving zebra mussels. Another study involving zebra mussels concluded that areas with zebra mussel populations had higher concentrations of organic matter than did areas without, suggesting that the invasive mussels could be benefiting some of the native species by increasing their population numbers (Stewart et al. 1998). However, this study did not conclude whether the higher concentrations of organic matter were correlated with the presence of actively filtering mussels, the presence of non-filtering dead zebra mussels shells, or both; the shells of the mussels themselves have been shown to provide a refuge for living organisms, making the true determination of the cause of increased organic matter important (Stewart et al. 1998).

Furthermore, other studies have shown that areas with high densities of live zebra mussels have much higher rates of benthic algal production (Bierman et al. 2005, Davies and Hecky 2005). Altogether, these studies provide possible support for a commensal relationship existing between zebra mussels and benthic algae.

The purpose of this study then was to determine whether or not a commensal relationship occurs between zebra mussels and benthic algal communities in an inland lake ecosystem. Because zebra mussel shells have been known to provide refuge for benthic organisms (Stewart et al. 1999), and because the presence of live zebra mussels has been associated with higher algal productivity (Bierman et al. 2005, Davies and Hecky 2005), we predict that areas containing rocks covered with live or dead zebra mussels will have higher benthic algal concentrations than areas which lack zebra mussels. Also, because phosphorous is a known limiting-resource in many inland lakes (Boegman et al. 2008) and live zebra mussel excretions contain substantial concentrations of phosphorous (Ozersky et al. 2009), we predict that benthic algal concentrations will be highest in areas that contain live zebra mussels.
METHODS

Field Study

We established nine 55 cm by 30 cm plots at a depth of one half meter in a rocky littoral zone along the central shore (referred to as Grapevine Point) of Douglas Lake in Pellston, Michigan that was already host to both zebra mussel and algae communities. We removed all rocks and other hard substrate from each plot. To establish three plots without zebra mussel influence, we placed five dry rocks found along the shore that had no traces of zebra mussels nor algae into each plot. Into three more plots, we placed five rocks that were naturally covered with zebra mussels but which we had scraped clean of all algae and other organisms. Into the final three plots, we placed five rocks originally found along the shore that had no traces of zebra mussels nor algae and to which we had glued dead zebra mussel shells; we had attached the shells using epoxy to clean, dry rocks and we had placed the zebra mussels in patterns that mimicked the naturally zebra mussel-covered rocks in the previously described plots. The rocks placed in each plot were all similarly sized – the smallest rocks measured 7 cm by 4 cm, and the largest rocks measured 16 cm by 10 cm.

Laboratory experiment

We marked off nine 55 cm by 30 cm plots in the boatwell stream trough at the University of Michigan Biological Station’s Lakeside Laboratory in Pellston, Michigan, and in these we duplicated the lake plot arrangements using rocks from Douglas Lake and the surrounding shoreline such that three plots contained rocks with no zebra mussels, three plots contained rocks with live zebra mussels, and the last three plots contained rocks with dead zebra mussel shells. We covered the bottom of all of the plots with a 2 cm layer of sandy substrate to mimic the actual lake bottom. We filled the entire arrangement of plots in the stream container with water
from Douglas Lake to a depth of 23.5 cm, and we established a constant input from and output to Douglas Lake of water using a pump system so as to provide food and nutrients for these zebra mussels and algae, similar to what those in the field study were receiving; we kept the input flow of water to a minimum rate of 66.67 milliliters/second so as not to produce a current in the boatwell stream trough.

We monitored both the field and lab plots at 3 to 4 day intervals for 11 days to make sure that there were no disturbances to the plots. We placed the rocks with dead zebra mussel shells into their respective plots three days later than we had placed the rocks of the other treatments into their respective plots due to the necessity of allowing the epoxy to dry. After 10 days, we performed chlorophyll a tests on the algae in each plot, excluding the plots that contained rocks with dead zebra mussel shells, using the Lakeside laboratory protocols: using a cork borer, we scraped a 3.14 square cm area from one rock randomly chosen from each of the plots in both the lab and lake settings (a total of 18 rocks), selecting the most centered area on the top of each rock where the borer would fit between the zebra mussels or shells, we added each scraping to a known volume of water, blended this mixture in a kitchen blender until it was homogenized, and filtered a known volume of each mixture through a milipore HA filter using a syringe, recording each of the two known volumes for each sample. We then submitted these filters, folded in half and wrapped in tinfoil, and the recorded known volumes to Lakeside Lab for chlorophyll a analysis, and we received our results μg of chlorophyll a per area sampled. After 11 days (the 7th day since the rocks holding dead zebra mussel shells had been added to the plots), we harvested algae from the rocks holding dead zebra mussel shells and processed and submitted them for chlorophyll a analysis as previously described.
Field survey

We randomly picked three rocks covered with zebra mussels and three rocks without zebra mussels from the rocky, littoral zone of Douglas Lake near Grapevine Point. We harvested algae from each of the rocks and processed and submitted them for chlorophyll a analysis as previously described.

We analyzed the collected data using independent samples t-tests to compare benthic algal concentrations in areas with live and dead zebra mussels to areas without zebra mussels. We also used independent samples t-tests to compare benthic algal concentrations in areas with live zebra mussels to areas with dead zebra mussel shells, and also to compare areas with live zebra mussels to areas with no zebra mussels in the field survey.

RESULTS

Overall, benthic algal concentrations on rocks were significantly higher in lake plots with live or dead zebra mussel presence than lake plots without zebra mussels (t = 2.75, df = 5, p = .041). In the lake there was no benthic algal presence on rocks without any zebra mussel influence, and much higher algal production on rocks with zebra mussel influence (Fig. 1).

Benthic algal concentrations were not significantly different in the lab experiment when comparing rocks with live and dead zebra mussels to rocks without any zebra mussel presence (t = .71, df = 6.8, p = .5). However, benthic algal concentrations were nearly three times greater on rocks with live and dead zebra mussels than rocks with no zebra mussels in the lab (Fig. 1).

Benthic algal concentrations on rocks with live zebra mussels were not significantly higher than benthic algal concentrations on rocks with dead zebra mussel shells in the lake study (t = .451, df = 2.052, p = .695). There was little difference between benthic algal concentrations on rocks with dead zebra mussel shells and rocks with live zebra mussels in the lake study (Fig.
2). Also, benthic algal concentrations on rocks with live zebra mussels were not significantly higher than benthic algal concentrations on rocks with dead zebra mussel shells in the lab study (t = 1.521, df = 2.785, p = .232). In the lab, however, benthic algal concentrations on rocks with dead zebra mussels were nearly four times higher than rocks with live zebra mussels (Fig. 2).

There was no significant difference between benthic algal concentrations on rocks with live zebra mussels and rocks without zebra mussels in the lake survey (t = 1.223, df = 3.995, p = .289). However, benthic algal concentrations on rocks with live zebra mussels were more than two times greater than benthic algal concentrations on rocks without zebra mussels (Fig. 3).

DISCUSSION

Our original hypothesis that areas containing rocks covered with live or dead zebra mussels would have higher benthic algal concentrations on rocks than areas which lack zebra mussels is supported by our results from the lake study, which showed that benthic algal concentrations were higher in plots that had live or dead zebra mussels compared to plots without any zebra mussel influence. Because the presence of live and dead zebra mussel shells promoted more benthic algal growth than rocks with no shells, it can be reasoned that live and dead zebra mussels on rocks have some positive effect on algae growth. This statement is supported by a previous study involving zebra mussels and algae, which concluded that zebra mussel shells provide a refuge effect for benthic dwellers to be protected by water movement and other organisms (Stewart et al. 1999). The dead zebra mussel shells in this experiment must have provided the same refuge effect for algae in Douglas Lake and offered them protection in some form, either from wave disturbance or predation. This statement is also supported by an earlier experiment involving zebra mussels that concluded that phosphorous excreted from live
zebra mussels promoted gastropod growth (Greenwood et al. 2001). The live zebra mussels in the lab must have provided nutrients key to algal growth not found on rocks with no zebra mussel presence.

The results from the lab test, however, did not support our original hypothesis that rocks covered with live or dead zebra mussels would have higher benthic algal concentrations than rocks that lack zebra mussels. These results are inconsistent with previous studies that concluded that zebra mussels provide nutrients to other organisms (Davies and Hecky 2005) and also provide a refuge for benthic dwellers (Stewart et al. 1999). The most likely reason for this inconsistency is the time constraint involved with our experimental lab setup. Because less than two weeks were available to perform this experiment, there may not have been sufficient time in the lab setting for enough algae to grow on rocks with zebra mussel influence to result in significant results. Also, there was a constant inflow of water in the lab setting, and this small inflow could have produced a current high enough to wash the majority of algal populations down the trough before they were able to settle. Additionally, in the experimental setup plots with live and dead zebra mussels were located further away from the water input source than rocks with no zebra mussels, and large numbers of algae could have settled on the first rocks encountered in the trough and biased the results to have lower algal concentrations in areas further from the water source. Finally, the water constantly pumped into the experimental trough from Douglas Lake did not come from the same zone of the lake in which the lake study was performed, and there could have been different concentrations of algae in this water, resulting in benthic algal concentrations much different from those found in the lake.

Neither the lab nor the field supported our hypothesis that rocks with live zebra mussels would have higher benthic algal concentrations than rocks with dead zebra mussel shells. In
both studies algal concentrations were actually higher for rocks with dead zebra mussel shells. These results are consistent with a previous study involving zebra mussels and benthic organisms that concluded that zebra mussel shells provide a refuge that promote benthic organism growth (Stewart et al. 1999). However, live zebra mussels should still provide a refuge effect equal to that of dead shells, and this discrepancy may lie in experimental design. It can be reasoned that there was either something in the epoxy used to attach the dead shells to rocks that promotes algal growth, or there is something involved in the presence of live zebra mussels that actually inhibits algal growth. The lab study may also have been influenced by the fact that rocks with dead zebra mussels were located nearer to the water input than rocks with live zebra mussels; the algae released into the trough may have settled on the rocks with dead zebra mussel shells before ever reaching the rocks with live zebra mussels. Again, the time constraint may also have played a role in both the lab and lake studies. If more time were allowed for the experiment, more algae could settle on rocks with live zebra mussels. The difference between algal concentrations on rocks with dead zebra mussels and live zebra mussels could also have been magnified in the lab experiment because it was performed in a small space with a constant input of water, so that it was much more likely for algae to reach rocks in this experiment than in the lake test where there was an entire lake full of rocks to settle on.

In conclusion, rocks with either live or dead zebra mussel presence have higher benthic algal concentrations than rocks without any zebra mussel presence. This conclusion is supported by the results of our natural lake study, which indeed found that there was significantly more algal growth on rocks with a zebra mussel presence. Although these same results were not found in the lab setting, there were many factors that could have influenced the experimental setup to result in inaccurate findings. We also concluded that live zebra mussels did not promote more
benthic algal growth on rocks than dead zebra mussel shells, and this conclusion is supported by our findings from both the lake and lab study. Dead zebra mussel shells actually resulted in more algal growth than live zebra mussels. However, there are many factors involved in the experimental setup that could have resulted in inaccurate results, including the time constraint involved with our experiment, and more work should be done to determine if dead zebra mussels result in greater algal growth than live zebra mussels.
Fig 1: Benthic algal concentrations based on chlorophyll a (µg/square cm) presence on rocks with live or dead zebra mussels compared to rocks without zebra mussels in both the lab (p = .5) and lake (p = .041) settings.
Fig. 2: Benthic algal concentrations based on chlorophyll a (μg/square cm) presence on rocks with live zebra mussels compared to rocks with dead zebra mussel shells in both the lab (p = .232) and lake (p = .695) studies.
Fig. 3: Benthic algal concentrations based on chlorophyll a presence (μg/square cm) on rocks with live zebra mussels compared to rocks without zebra mussels in the lake survey (p = .289).


