Laboratory Measures of Filtration by Freshwater Mussels: An Activity to Introduce Biology Students to an Increasingly Threatened Group of Organisms

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Abstract: Many aquatic organisms survive by filter feeding from the surrounding water and capturing food particles. We developed a laboratory exercise that allows students to measure the effects of filtering by fresh water mussels on water turbidity. Mussels were acquired from Wards Scientific and exposed to a solution of baker's yeast. Over a period of one to two hours, students measured changes in water clarity using miniature Secchi discs. The exercise has been used in a freshwater biology class at a state university. This exercise allows students to make hypotheses, gather data, and explore interactions between living organisms and their environment. Many North American species of freshwater mussels are threatened or endangered because of habitat changes and the introduction of exotic mussels. Therefore, students are also able to examine the potential effects of biodiversity loss in aquatic environments.

Key words: freshwater mussel, macroinvertebrate, filter feeding, ecology

INTRODUCTION

Filter feeding organisms are a component of most aquatic ecosystems. The introduction of Dreissenid mussels, like the zebra mussels, into North American waters has elevated prominence of these organisms and their role in aquatic ecosystems. In spite of this increased awareness, little emphasis has been made in educational curriculum to demonstrate the potential impacts of filter feeders on aquatic ecosystems.

Lake Erie, one of the Great Lakes of North America, is the eleventh largest freshwater lake in the world. With a maximum depth of 64 meters and a length of 388 kilometers, it contains more than 484 cubic kilometers of water (Great Lakes Information Network, 2012). Lake Erie is the warmest of the Great Lakes and biologically the most productive. In the 1980s, Lake Erie was invaded by the zebra mussel, *Dreissena polymorpha* (Berkman et al., 1998; USGS, 2008). This species is native to Russia and is relatively small, reaching a maximum of about 5 centimeters in length. In the United States, it reproduces rapidly and reaches densities of 70,000 per square meter. These mussels live and grow by filter feeding by which they pass water through their gills and collect food particles from the water.

Today, it is estimated that zebra mussels filter the entire water volume of Lake Erie in less than one month. This filtration has removed many of the photosynthetic algal cells (phytoplankton), the aquatic producers that form the base of aquatic food chains, normally found in the lake. In the past twenty years, the zebra mussel has increased water clarity by up to 600 percent as a result of filtering out phytoplankton, (UW Seagrant Inst., 2005; USGS, 2009).

From this example, it is clear that filter feeding by aquatic organisms can have a large impact on aquatic food webs. A number of aquatic organisms, including microscopic rotifers, caddisfly larvae, paddlefish, and freshwater mussels rely on filter feeding to obtain energy. Among these filter feeding organisms, freshwater mussels live in the substrate of many freshwater streams and rivers, quietly filtering large volumes of water for most of their long lives, which in some cases can exceed 100 years (Bauer, 1992). As they filter the water, they extract nutrients and other suspended particles, changing the properties of the water around them. Because they are long lived, stationary, and sensitive to changes in the water quality, mussels are commonly used as bioindicators of water ecosystem health (Angelo et al., 2007; Jovic et al., 2011).

This laboratory exercise, developed and tested in a senior-level freshwater biology class at a state university, allows students to study live mussels and examine changes in water clarity as a result of their feeding behavior. It allows students to create hypotheses and collect quantitative data concerning filtration rates and can easily be used with both majors and non-majors as a laboratory exercise.

Background

Mussel Anatomy

Freshwater mussels have a two-part shell that is hinged on the posterior side (Cummings and Mayer, 1992) giving them the name "bivalves." Shell color is variable and generally ranges from yellow-green to black with green lines called rays that run

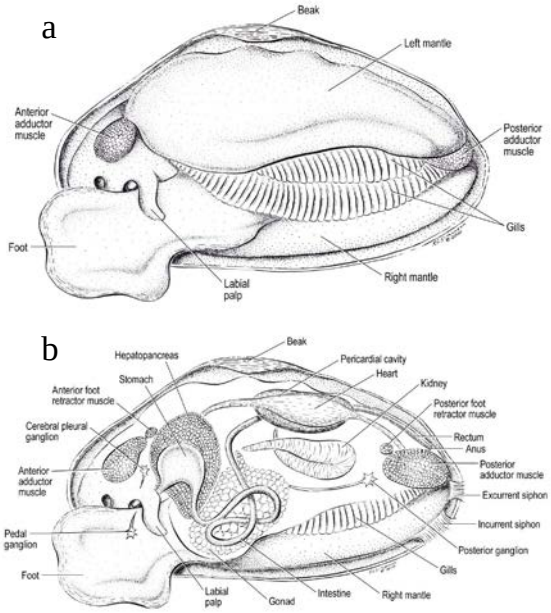

Fig. 1. Diagrams of mussel anatomy. a. Surface layers of the typical mussel. b. Internal structures of the typical mussel. Illustration by Rick L. Simonson (www.RLSimonson.com; used with permission).

perpendicular to the long axis of the shell found on the shell in some species. The interior lining of the shell (nacre) is usually white; however, the nacre of some species of freshwater mussels is highly iridescent and nearly purple. The exterior of the shell can be smooth as it is in the plain pocketbook mussel, *Lampsilis cardium*, or the shell can be bumpy as found in the pimpleback mussel, *Quadrula pustulosa* (USFWS, 2006; Cummings and Mayer, 1992). Shell shape ranges from elongate to round with many variations within and between species (USFWS, 2006). Freshwater mussels range in size from a few centimeters to 30 centimeters across the longest axis (Cummings and Mayer, 1992).

Freshwater mussels have strong adductor and retractor muscles (Figure 1a) that work together to open and close the shell. When threatened, the mussel will tighten its retractor muscles sealing itself inside the hard shell. Most predatory organisms are not strong enough to overcome the mussel's defense and pry the shell open. When the danger has passed, the mussel will extend a muscular foot that allows it to move slowly until it reaches an appropriate area of the substrate (Figure 1a). The mussel then extends its foot into the substrate, orients itself so that the hinge is dorsal and buries itself in the substrate so that it is anchored. If habitat conditions are sufficient, the mussel may not have to move for the rest of its life (Utterback, 1916).

Mussel Feeding

During feeding, the majority of freshwater mussels draw water through the incurrent siphon which is at the posterior end of the shell (Figure 1b). The water passes through the gills in a U-shaped tube and then exits through the anal siphon at the anterior end of the shell. The gills of the mussel produce mucus that traps food particles. The food particles are then transported by ciliary action to the mouth where the food is consumed. In addition to capturing food, the gills conduct gas exchange with the surrounding water. Thus, the mussels feed and respire almost constantly. Their long sedentary lives and constant exposure to the water make freshwater mussels highly sensitive biological indicators of changes in water quality, including reductions in dissolved oxygen and the accumulation of metals and toxins (Strayer et al., 2004).

Awareness of Freshwater Mussels

Historically 297 different species of freshwater mussels were native to North America (Williams et al., 1993). Nineteen of these species are currently listed as extinct or no longer occurring in nature, 62 species are federally listed as endangered, and 130 species are in need of conservation efforts. Thus, approximately 70 percent of the mussel species native to North America are now either extinct or threatened (Williams et al., 1993, Strayer et al. 2004).

Despite declines, freshwater mussels can be found in many streams, rivers, ponds, and lakes throughout the United States. In addition, conservation efforts have increased rapidly due to range expansion of invasive freshwater mussels, providing recent distribution maps and popular literature for many U.S. states (USGS, 2009). These resources can support informed classroom discussions of mussel lifecycle, ecology, and niche.

Measures of Water Quality

Many methods are used to assess water quality including chemical tests and biological integrity indices. One common measure of water quality measurement is turbidity, or the measure of water clarity caused by suspended solids. This is an important measure because murky water with little light penetration can indicate high levels of nutrients which may cause an algal bloom. The algal cells produce oxygen through photosynthesis during daylight hours if sunlight can reach them; however, at night or if the water becomes too clouded, the algal cells' respiration will be greater than photosynthesis , removing oxygen from the water and potentially leading to the death of aquatic organisms in the system.

Water turbidity can be measured through a number of methods. The simplest and least expensive method relies on a Secchi disc (Preisendorfer, 1986). This weighted disc is 20 cm in diameter and is divided into black and white quadrants (example in Figure 2). The water turbidity is determined by lowering the Secchi disc on a rope or tape which is marked with measurement increments. The disc is lowered into the water column until it disappears from sight. This depth is

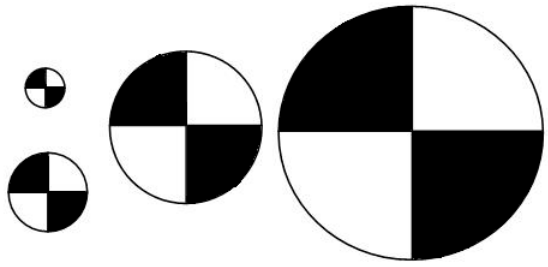

Fig. 2. Mini-Secchi discs. Discs pictured are 10 mm, 15 mm, 35 mm, and 55 mm in diameter. Students should use the smallest possible disc for the experiment. Photo-reduction can also be used to adjust disc size as needed.

recorded. Then the disc is raised until it reappears. This depth is also recorded. The two measurements are averaged; this is the Secchi disc transparency measure (Wetzel and Likens, 2000), which is a standard water turbidity measure.

In this laboratory activity, miniature Secchi discs (Figure 2) are used to measure water turbidity to determine if the mussels are filtering the water and reducing water turbidity. Relative to control conditions in which mussels are not present, filtration of water by the mussels increased the water clarity by reducing the turbidity and allowed the Secchi disc to be seen over greater distances.

MATERIALS AND METHODS

Pre-laboratory Preparation

First, instructors need to obtain freshwater mussels. We purchased mussels from Ward Scientific. At the time of this work, 10 live mussel specimens of assorted species could be purchased from Ward Scientific for approximately $23.00. Specimens obtained were generally small (Wards offer mussels ranging from 1.5-4 inches). Multiple mussels of 1.5 inches were placed into each aquarium.

If funding is not available to purchase freshwater mussels, they can be found in local ponds, streams, or rivers. State and Federal agencies must be consulted prior to field collection, not only to acquire the proper permits, but also to comply with state and federal laws and to avoid collection of any threatened species. Live mussels are often found in water less than one meter deep with their white hinge structure pointing up. If the water is relatively clear, mussels can be found by wading in the water and searching for the white structures. Mussels can be found associated with gravel, mud, and sand bottoms. Often, empty shells can be found on land or in the water and can be used to target areas in which to find live ones.

Housing for the mussels should be prepared ahead of time. The aquaria used in our study were 9.5 L (2.5 gallon); however, depending on the size of the mussels, different aquaria or plastic containers may be appropriate. Approximately 5 cm of sand was placed in each aquarium to serve as substrate for the mussels. Water from the mussels' natural environment or tap water was then added until the aquaria were approximately two-thirds full. Tap water should not be used without conditioning to remove chlorine and other chemicals added during municipal water treatment.

The aquaria need to be aerated to ensure that dissolved oxygen is available to the mussels. We accomplished aeration with Whisper aquarium pumps and a single airstone per aquarium. If the exercise is to be performed within 2 to 3 days of obtaining the mussels, food will not need to be added to the aquaria; however, if the mussels are obtained well in advance of the laboratory, food in the form of phytoplankton or single-celled algae should be placed in the aquaria. Phytoplankton can be found at pet supply stores. At the time of this work, Petco provided Two Little Fishies PhytoPlan Advanced Phytoplankton Diet for $16.00.

In these experiments, yeast was used as the turbidity-producing agent. It was chosen because mussels can filter single celled organisms effectively and baker's yeast can be purchased from local grocery stores. One gram of baker's yeast was dissolved in 250 mL of pond water for each experimental aquarium. The suspension was added to all aquaria except Control 2(C2), and then the water was stirred with a large glass stir rod to ensure thorough mixing (Figure 3).

Miniature secchi discs were used to measure changes in turbidity. For our study, the discs were made using Microsoft Publisher. Four circles with diameters of 5mm, 10mm, 20mm, and 40mm were created (Figure 2). The circles were separated into four quadrants and two of the quadrants located diagonally from one another were colored black. These circles were then laminated and secured to small wooden dowels with a staple. The miniature secchi discs are inexpensive and simple to make. Standard 30 cm rulers were used to make the secchi measurements.

Experiments

Depending on time available and student knowledge, a discussion with students should be conducted prior to setting up the experiment. Students should be asked about what affects water turbidity. Students may talk about nutrient loading, increase in phytoplankton, and water mixing effects on turbidity. An emphasis should be made on filter feeding and the significant reduction in turbidity that can result. Students should also define the controls that they will need for an experiment. At a minimum, students should be introduced to the concept of experimental versus control conditions in an experiment and should develop hypotheses to be tested by the experiment. For more advanced science students, instructors can teach students about sample

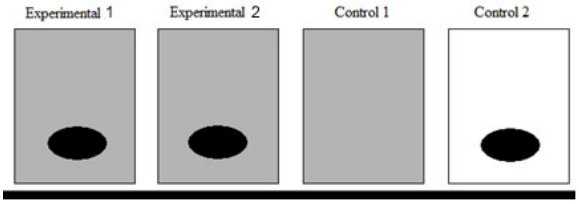

Fig. 3. Experiment design. The two experimental aquaria (E1 and E2) and one control aquarium (C1) contain the yeast suspension (gray fill). The other control aquarium (C2) contains no yeast. The experimental aquaria (E1 and E2) and the control 2 aquarium (C2) contain mussels (black ovals).

size, measurement and statistical analysis as part of the experimental design.

In this experiment, mussel filtration with 2 replicates (E1 and E2) was compared to two control aquaria (C1 and C2) (Figure 3). Control aquarium C1 received yeast but did not contain mussels. This allows students to measure if the yeast settles out of the water, making the water less turbid, or if the yeast replicate in the tanks, making the water more turbid. The second control tank, C2, contained a mussel but did not have any yeast. Because mussels are filtering the water and potentially expelling waste products, C2 should be measured as a negative control.

At the start of the experiment 250 ml of yeast suspension was added to C1, E1 and E2. To ensure that the yeast stay suspended in the water column, before taking a turbidity reading, all aquaria were stirred for 30 seconds with a stir rod, being careful not to disturb the substrate or the mussel if present. The turbidity of the water was then measured with the mini-Secchi discs. Although all four discs were tested, the smallest visible disc (5mm) was used for the 9.5 L (2.5 gallon) tanks in this experiment (Figure 2). Rulers were placed on the top of the aquaria, running parallel to the longest side of the aquaria, to allow students to measure their distances (Figure 4). The Secchi discs were placed in the aquarium by holding onto one end of the dowel and lowering the disc into the water column facing the short end of the aquarium perpendicular to the ruler. To determine a turbidity measurement, the dowel was moved along the longest side of the aquaria while looking through the short end of the aquarium until the disc was no longer visible. This distance was noted as the distance at which the disc disappeared. The disc was then moved back toward the viewer until it was visible again and that distance was noted. The Secchi disc transparency measure was determined by averaging the distance at which the disc disappeared with the distance when the disc reappeared.

Turbidity measurements were taken every 15 minutes thereafter in each aquarium, C1, C2, E1, and E2, for a period of 90 minutes using the same stirring procedure prior to conducting measures. At the conclusion of the experiment, mussels were immediately removed from the aquaria and placed into fresh water. Note that mussels may die if left in the nutrient rich aquaria with the yeast.

Data analysis was performed on Microsoft Excel. Students input all Secchi disc transparency measures for each aquarium, C1, C2, E1, and E2. An experimental mean for each time period was found using the data from the replicates E1 and E2. Students created a scatter plot and tested the data with a linear regression for changes in water clarity over time.

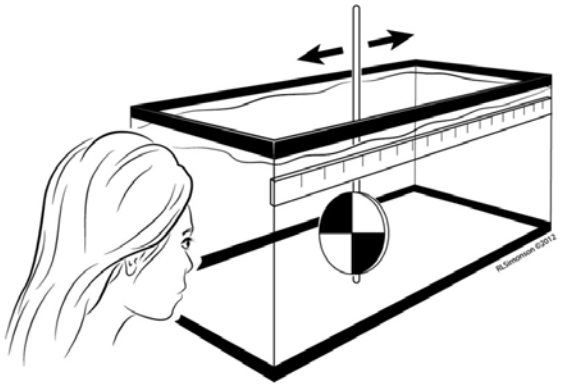

Fig. 4. Diagram of experimental set up. Ruler is placed near the top of the tank. The Secchi disk is placed on the end of the dowel. One student can look through the narrow end of the tank at the Secchi disk to judge the point at which they can no longer see the Secchi disk, while another student moves the dowel. A third student uses the ruler to mark the point at which the dowel appears to the observer in the tank. This procedure is then repeated as the dowel moves the Secchi disk back into view and the second measurement marked. The students should then average the measurement to figure the Secchi disk transparency measure.

RESULTS AND DISCUSSION

In this study, the experimental aquaria, E1 and E2, had a mean Secchi disc transparency measure of 3.5 cm immediately following the addition of the yeast suspension, and a Secchi disc transparency measure of 5.0 cm after 90 minutes (Table 1). Mussels reduced the turbidity in the experimental aquaria by 32%. Thus, water clarity, due to filter feeding, increased within a 90 minute period.

The turbidity readings for both controls demonstrated smaller changes over time. At zero minutes, the yeast only control, C1, had a Secchi disc transparency measure of 3.7 cm. After 90 minutes it had increased to 4.1 cm (Table 1). This indicated a 10% increase in water clarity. These data suggest that yeast numbers may change slightly even if mussels are not present so having this control is important for understanding if mussels are responsible for the increased water clarity. Students can hypothesize possible causes for the change in water clarity even if mussels are not present. Students may suggest that the yeast settled out of solution, that the conditions killed the yeast, or that

Time (minutes)	E-1 (cm)	E-2 (cm)	Experimental Average (cm)	C-1 (cm)	C-2 (cm)
0	3.4	3.4	3.5	3.7	18.1
15	3.5	3.4	3.5	3.7	18.1
30	4.0	4.6	4.3	3.8	18.0
45	4.1	4.7	4.4	3.7	18.0
60	4.4	4.8	4.6	4.0	18.1
75	4.7	4.9	4.8	4.0	18.0
90	4.9	5.0	5.0	4.1	18.0

Table 1. Representative Secchi disc transparency measures in cm reported over time. Experimental aquaria (E-1 and E-2) had three mussels each plus yeast, Control 1 (C-1) had only yeast added and control 2 (C-2) had only 3 mussels added.

other aquatic invertebrates were in the original pond water feeding on the yeast. Instructors may want to point out experimental variation and the concept of significance. Depending upon the level of the class, the instructor may want to have students collect more data that can be analyzed for statistical significance.

The mussel only control, C2, remained relatively constant. The Secchi disc transparency measure was 18 cm throughout the 90 minutes (Table 1). This is still an important control to have to introduce students to the concept of negative control.

Students were asked to graph their data as the percent change in Secchi disc transparency measures over time. This plot indicates an increase in water clarity produced by mussel filtration (Figure 5). After the graphs were made, students were asked to make comparisons of data they had collected and to draw conclusions about the experiment. Students were able to accept or reject their hypothesis and to offer possible reasons for why the experimental results occurred.

At the conclusion of the experiment, mussels must be removed from the experimental aquaria to keep them alive. This activity was completed several times, and after the first trial, mussels were kept in the experimental aquaria with the yeast suspension overnight instead of removing them. As a result all four mussels died. Mortality could have resulted from oxygen limitation due to microbial growth or clogging of the mussels' gills as a result of the extremely high concentration of yeast. To ensure mussel survival, it is important to remove them from the mussel suspension and to place them in new pond water after the trial is complete.

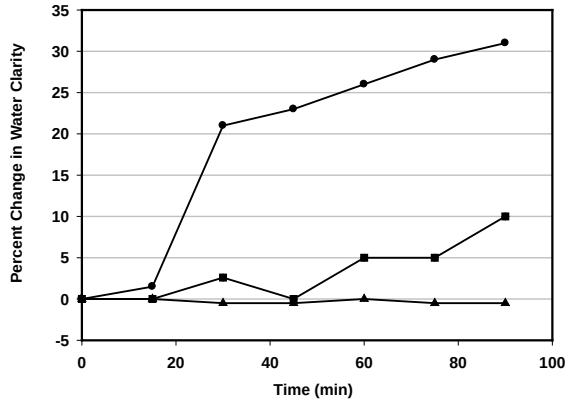

Fig. 5. Observed mean percent change in water clarity as compared to time 0. Experimental aquaria, E1 and E2, had mussels and yeast (circles), C1 aquarium had mussels only (triangles), and C 2 aquarium had yeast but no mussels (squares). Positive numbers indicate increasing clarity, while negative numbers indicate reduced clarity.

Depending on the goals of the laboratory exercise, experimental mussels can be sacrificed to allow students to conduct a dissection and learn about mussel organs (Figure 1). A number of laboratory guides for mussel dissection are available, as well as videos placed on YouTube. It should be noted that mussels purchased from a commercial facility should not be released into local waters.

Extensions

A number of extensions of this laboratory exercise are possible. For example, if the volume of water used in the experiment is known and the number of yeast cells is calculated, filtration rates can be determined (number of cells per unit volume through time). Alternatively, sub-samples of the experimental water can be collected and a hemocytometer can be used to estimate the number of yeast cells equivalent to a given Secchi disc transparency measure. Because freshwater mussels will filter any suspended material, the filtration of algal cells could be tested instead of yeast.

Outcomes of the laboratory exercise can be in the form of a formal laboratory write up or in the form of graphs or answers to questions. For example, students can be asked to predict water clarity after 3 hours based on regression equations generated from the experiment.

CONCLUSIONS

Freshwater mussels are common members of the benthic community in most freshwater ecosystems of North America, although as a group the majority of their species are threatened. This laboratory exercise offers biology students a chance to physically interact with and collect data on the filtration behavior of these mollusks. This exercise also gives students the opportunity to calculate the amount of water that these animals can filter and offers a logical extension

to any course that currently incorporates dissection of mussels. The laboratory preparation for the instructor is simple, and costs are low. In addition, this laboratory may be of particular interest to instructors in regions infested with zebra mussels *(Dreissena polymorpha)*, which have been shown to drastically change water quality in the Great Lakes (Holland, 1993).

ACKNOWLEDGEMENTS

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REFERENCES

ANGELO, R.T., CRIGAN, M.S., CHAMBERLAIN, D.L., STAHL, A.J., HASLOUER, S.G., AND C.A. GOODRICH. 2007. Residual effects of lead and zinc mining of freshwater mussels in the Spring River Basin (Kansas, Missouri, and Oklahoma, USA). *Science of the Total Environment*. 384:467-496.

BAUER, T. 1992. Variation in the life span and size of the freshwater pearl mussel. *Journal of Animal Ecology* 61:425-436.

BERKMAN, P.A., HALTUCH, M.A., TICHICH, E., GARTON, D.W., KENNEDY, G.W., GANNON, J.E., MACKEY, S.D., FULLER, J.A., AND D.L. LIEBENTHAL. 1998. Zebra mussels invade Lake Erie muds. *Nature*. 393:27-28.

CUMMINGS, K.S., AND C.A. MAYER. 1992. Field Guide to Freshwater Mussels of the Midwest. Illinois Natural History Survey Manual 5. 194p.

HOLLAND, R.E. 1993. Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island area, western Lake Erie. *Journal of Great Lakes Restoration*. 19: 617-624.

GREAT LAKES INFORMATION NETWORK. 2012. http://www.great-lakes.net. Accessed January, 2009.

JOVIC, M., STANKOVIC, A., SLAVKOVIC-BESKOSKI, L., TOMIC, I., DEGETTO, S., AND S. STANKOVIC. Mussels as a bio-indicator of the environmental quality of the coastal water of the Boka Kotorska Bay (Montenegro*). Journal of the Serbian Chemical Society*. 76:933-946.

PREISENDORFER, R.W. 1986. Secchi disc science: Visual optics of natural waters. *Limnology and Oceanography*. 31: 909-926.

STRAYER, D.L., DOWNING, J.A., HAAG, WENDELL, R., KING, T.L., LAYZER, J.B., NEWTON, T.J., AND S.J. NICHOLS. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience*. 54: 429-439.

UTTERBACK, W.I. 1916. The naiades of Missouri. *American Midland Naturalist*. 4: 41-53.

UNIVERSITY OF WISCONSIN SEAGRANT INSTITUTE. 2005. Http://seagrant.wisc.edu/zebramussels. Accessed January, 2009.

UNITED STATES FISH AND WILDLIFE SERVICE. 2006. Http://www.fws.gov/midwest/mussel/species.html. Accessed January, 2009.

U.S. GEOLOGICAL SURVEY. 2008. Http://www.glsc.usgs.gov/main.php?content=research_invasive_zebramussel&title=Invasive%20Invertebrates0&menu=research_invasive_invertebrates. Accessed January, 2009.

U.S. GEOLOGICAL SURVEY. 2009. http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel. Accessed May, 2012.

WETZEL, R.G., AND G.E. LIKENS. 2000. Limnological Analyses. 3rd ed. Springer-Verlag. 21p.

WILLIAMS, J.D., WARREN, M.L., CUMMINGS, K.S, HARRIS, J.L., AND R.J. NEVES. 1993. Conservation status of the freshwater mussels of the United States and Canada. *American Fisheries Society: Fisheries.* 18: 6-22.