

FINAL REPORT

LAKE ERIE PROTECTION FUND SG 3196

Interactions of Burrowing Mayflies and Zebra Mussels, and Effects on Sediment Oxygenation

Kenneth A. Krieger, Ph.D., Project Director
17 July 1998

Burrowing mayflies (two species of *Hexagenia*) were once an important benthic component of the food web of western Lake Erie. However, severe pollution in the first half of the twentieth century resulted in the disappearance of these native insects from most of the lake, and only isolated populations survived in some areas near the lake shore and in coastal wetlands and tributaries. Beginning in the early 1990s, *Hexagenia* began to return to the western basin of Lake Erie, probably as the result of improved water and sediment conditions (Krieger *et al.* 1996). Their recolonization of soft sediments of the lake bottom began at the same time when zebra mussels and quagga mussels (*Dreissena* spp.) were invading large areas of the sediment surface.

Hypotheses and Objectives

We established the following hypotheses:

1. That the success of recolonization by the burrowing mayflies, which construct U-shaped burrows with openings at the sediment surface, is adversely impacted by an overlying layer of *Dreissena* shells.
2. That accumulations of *Dreissena* shells on top of the sediment reduce sediment oxygenation and thereby create less hospitable conditions for the native benthic fauna.
3. That *Hexagenia* nymph burrows provide a major mechanism of oxygen transfer to the sediment beneath the shells.

The purpose of this small grant was to test the above hypotheses. The objectives were as follows:

1. Examine the impact of *Hexagenia* colonization on sediment oxidation-reduction (redox) conditions, which are closely tied to the amount of dissolved oxygen (O₂) present. We did this by measuring redox potentials of sediment under different experimental conditions.
2. Examine the impact of *Dreissena* shell deposition on *Hexagenia* survival and growth, redox conditions, and the benthic invertebrate community. This was to be done by measuring survival and growth of nymphs under layers of *Dreissena* shells of varied thicknesses.

Objectives 1 and 2 also included observation of the effects of *Hexagenia* colonization on the composition of the benthic invertebrate community in the absence of *Dreissena* shells, and the impact of *Dreissena* shells on the composition of

the benthic invertebrate community. With the amount of funding available, this part of the project was not accomplished. However, the experimental design for Objective 2, with support from field observations, was adapted directly from the original proposal (LEPF-08-94) and was incorporated as a major part of an Ohio Sea Grant project (Project R/ER-36 under Ohio Sea Grant NA46RG0482) which is now underway. Thus, this LEPF project provided the springboard for the later, more elaborate study.

Methods

1. Effects of *Hexagenia* on sediment redox conditions. The redox potential of water and sediment indicates the extent of oxygenation and the oxidation state of many chemicals. In general, as the amount of oxygen declines, the redox potential falls (Horne and Goldman 1994). The methods used for the redox study are explained in detail in the Methods section of a manuscript currently being drafted; that section of the manuscript is appended to this report as Appendix A. Briefly, platinum redox microelectrodes were specially constructed that permitted us to measure redox potentials at specific points within the water column and beneath the sediment in laboratory aquaria. The electrodes were tested for reproducibility of readings. Uniform sediments were placed in several aquaria, and a 2-cm layer of *Dreissena* shells was placed on top of the sediment in some of the aquaria. *Hexagenia* nymphs were added to some of the aquaria. After a period of time to allow for establishment of the mayfly nymphs and relatively stable redox conditions, redox potentials were measured in the water and sediments under a variety of combinations of nymphs and shells.

2. Impact of shells on *Hexagenia* survival and growth. A battery of 18 aquaria was established which contained sediments and three densities (none, low, high) of *Dreissena* shells as a layer on the sediment surface. The aquaria received a constant supply of fresh water from Rock Creek on the Heidelberg College campus. The experiment was begun by adding mayfly nymphs at either low or high density to each aquarium. The experiment was conducted for approximately six months, after which the sediments in each aquarium were sieved in order to remove the nymphs.

Results

1. Effects of *Hexagenia* on sediment redox conditions. The details of the results will be presented in the manuscript that is in preparation. The figures from that manuscript are included here with brief explanations.

a. Before measuring redox conditions in the aquaria containing combinations of nymphs and mussel shells, it was necessary to test the time to stability and the reproducibility of the four electrodes that were constructed for the study. Figure 1 shows that the electrodes were relatively stable following about 20 minutes in a pH 7 buffer solution and almost immediately after immersion in a pH 10 buffer solution. The pH of the water in our aquaria was presumed to be between 7.0 and about 8.5 based on readings taken in similar aquaria containing sediments. Electrode 4 (Figure 1) was selected for all experimental readings in order to avoid loss of precision that would result from the use of multiple electrodes.

b. The value of the redox potential indicates roughly the amount of free oxygen (O₂) present in the water overlying the sediment, or in the sediment itself. When we measured the redox potential in water above the two burrow holes of individual *Hexagenia* nymphs (Figure 2), we found relatively high redox potentials (~100 mv)

above one hole at 1.0 cm, and 0.5 cm, as well as at the opening (0.0 cm) and inside the burrow (-0.5 cm). Above the second hole of each burrow at 1.0 cm, the redox potential was either identical to that above the other hole, or somewhat lower (Figure 2), but was much lower (<0 mv) at the burrow opening and within the burrow at -0.5 cm. Therefore, it appeared that the openings with consistently high redox potentials above and within them were the incurrent openings, into which oxygenated water was flowing. The two holes with low redox potentials probably were the excurrent openings of the burrow, from which oxygen-depleted water was exiting.

c. To determine whether *Hexagenia* nymphs measurably affect the movement of oxygen into the sediment surrounding and between the burrow holes, straight-line transects were established between the paired burrow holes of individual nymphs, and redox potentials were measured at depths in the sediment of 1, 2, 3 and 4 cm at intervals of 1.0 cm between the holes (Figure 3). The actual depth of each burrow in the sediment between the holes was not known, but it was assumed that the redox potential would indicate whether or not the electrode had penetrated the burrow. The right-hand parts of the graphs in Figure 3, particularly Fig. 3B, show slight increases in the redox potential at the lower depths, suggesting that the burrows may have been penetrated.

As Figure 3 shows, the redox potentials were progressively lower with increasing depth in the sediment at most locations. Unlike the more-oxygenated conditions found within incurrent as opposed to excurrent burrow holes (Figure 2), the sediment redox potentials showed little if any increase in close proximity to either burrow hole of a pair. These results agree with field observations in October 1997 of a burrow which split lengthwise in a sample of sediment collected near Station 5B in western Lake Erie (Krieger *et al.* 1996). The burrow was well-oxygenated, as disclosed by a lining of light-brown sediment. The lining was sharply demarcated about 2 mm beyond the burrow from the typical slate-gray sediment indicative of anoxic conditions. Thus, it appears that the oxygenated water flowing through the burrow has little or no influence on the redox conditions of the sediments beyond about 2 mm. None of our sediment measurements were closer than 10 mm to the burrow holes, although, as mentioned above, the burrows may have been penetrated by our electrodes within the sediments.

d. The effect on redox conditions of a layer of *Dreissena* shells covering the sediment was also investigated. Figure 4 shows the redox potentials at depths of 1 cm and 4 cm measured across four transects in an aquarium containing a single *Hexagenia* nymph whose burrow was in an unknown location beneath the shells. The burrow apparently was not encountered along the transects, which yielded very similar readings. When compared to Figure 3, Figure 4 shows that a layer of *Dreissena* shells had no apparent impact on redox conditions at various depths in the sediment. The redox potentials at 1 cm and 4 cm were essentially the same in sediments with and without a layer of shells.

e. Redox conditions were compared between an aquarium containing no *Hexagenia* nymphs and an aquarium containing eight nymphs. In each aquarium four transects (two at right angles to the other two) were established and eight redox readings were measured at 1-cm intervals along each transect, irrespective of the locations of the burrow holes. Figure 5 shows that, at each level in the sediment, the redox values were higher (indicating more oxygenation) in the aquarium containing the eight nymphs than in the one without nymphs, and that the values at each depth (except 1 cm) were more variable in the sediment with eight nymphs. Both higher redox potentials and greater spatial variability would be expected in sediments that

are mixed and partially aerated by burrowing organisms than in sediments where that does not occur. These results should be accepted with caution, however, in that we did not replicate aquaria receiving each treatment and so cannot rule out variability between the sediments in each aquarium as the cause of the difference in our results.

2. Impact of shells on *Hexagenia* survival and growth. The outcome of the experiment with the battery of aquaria was presented in the Interim Report dated 29 January 1997. The experiment demonstrated that *Hexagenia* can survive beneath a relatively thick (~2 cm) layer of *Dreissena* shells and thus did not support the hypothesis that there is a negative impact of the shells on survival.

As pointed out in the Interim Report, because nymphs were able to migrate from one aquarium to another, the experimental results were not conclusive, and it was desirable to run the experiment again with a modified set-up. However, by the time the initial experiment ended, the new Sea Grant project had been approved which has permitted an expanded set of experiments that address the interactions of *Dreissena* and *Hexagenia*. This set of experiments is currently underway at the F. T. Stone Laboratory of Ohio State University at Put-in-Bay, Ohio, and forms the basis for the thesis of Mr. Ken Freeman, a master's student under the direction of Dr. David Berg at Miami University of Ohio. As part of the Sea Grant project, field study of the associations between the nymphs and mussels is near completion at the Water Quality Laboratory of Heidelberg College. We will convey the results of the Sea Grant project to the Lake Erie Office as an outgrowth of this Small Grant from the Lake Erie Protection Fund.

Conclusions

Our data indicate that individual *Hexagenia* nymphs are ineffective at increasing the oxygenation of lake sediments beyond a few mm from the burrow. They further indicate, however, that groups of nymphs in a small area may be effective in slightly increasing the redox potential of sediments. A surface layer of *Dreissena* shells had no measurable impact on the redox condition of sediments at depths of 1 cm or more. Additional experimentation is needed to confirm these initial results.

Benefits

An important outgrowth of this LEPF Small Grant has been further elaboration of the experimental objectives into a major project funded by the Ohio Sea Grant College Program which has provided funding and a thesis project for a graduate student (Mr. Ken Freeman) from Miami University (Ohio). Two undergraduates at Heidelberg College (Mr. Brian Villalon and Mr. David Dariano) received training in ecological and experimental methods through their participation in this project. This project has added to our understanding of the ecology of *Hexagenia* in western Lake Erie.

Dissemination

We will definitely present the findings of our redox experiments at the 1999 meeting of the Ohio Academy of Science; the presentation most likely will be made by Mr. David Dariano, who constructed the electrodes and performed the experimental work. A manuscript is in preparation which we plan to submit this fall to the *Ohio Journal of Science*. The methodologies used and the results of the

experiments will be discussed with students in the limnology and water pollution biology classes at Heidelberg College.

References (including Appendix A)

Bohn, H. L. 1971. Redox potentials. *Soil Science* 112:39-45.

Cogger, C. G., P. E. Kennedy, and D. Carlson. 1992. Seasonally saturated soils in the Puget Lowland II. Measuring and interpreting redox potentials. *Soil Science* 154:50-58.

Faulkner, S. P., W. H. Patrick, Jr., and R. P. Gambrell. 1989. Field techniques for measuring wetland soil parameters. *Soil Sci. Soc. Am. J.* 53:883-890.

Horne, A. J., and C. R. Goldman. 1994. *Limnology*. 2nd Ed. McGraw-Hill, Inc., New York. 576 pp.

Krieger, K. A., D. W. Schloesser, B. A. Manny, C. E. Trisler, S. E. Heady, J. J. H. Ciborowski, and K. M. Muth. 1996. Recovery of burrowing mayflies (Ephemeroptera: Ephemeridae: *Hexagenia*) in western Lake Erie. *J. Great Lakes Res.* 22:254-263.

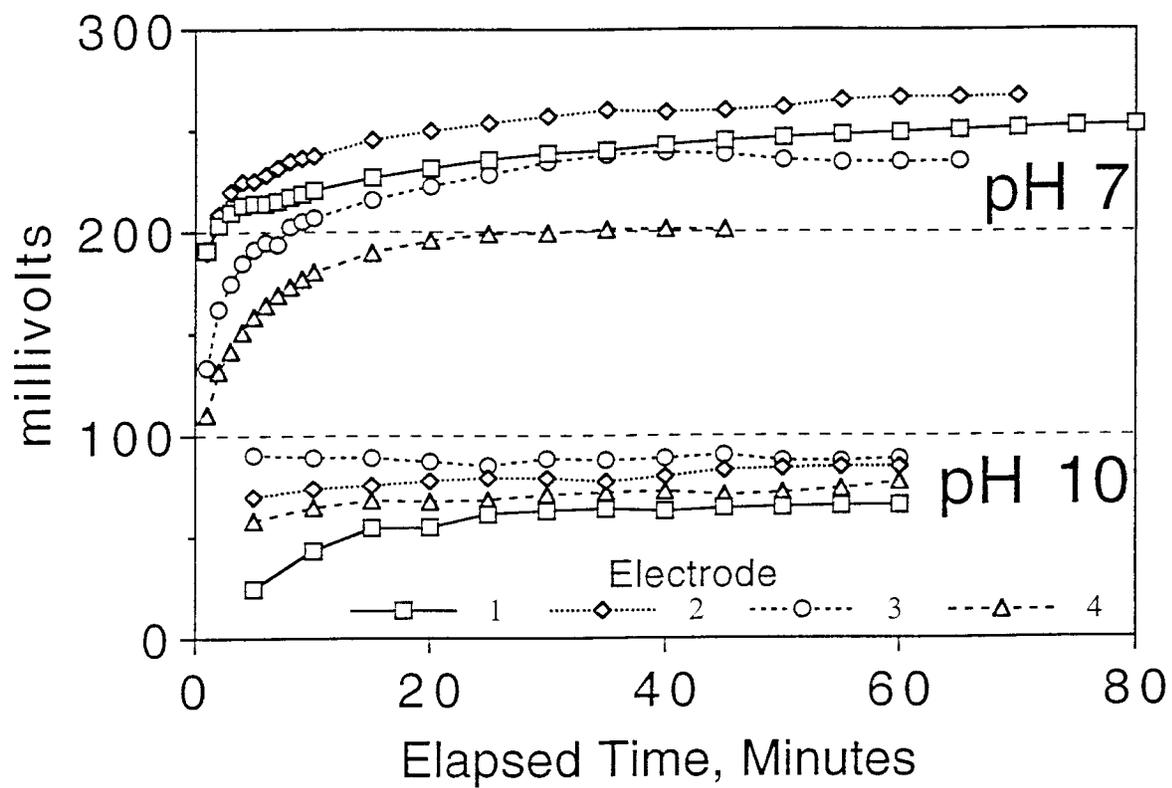


Figure 1. Time to stabilization of redox electrodes in pH 7 and pH 10 buffers. Temperature of pH 7 buffer was 23.3-24.2°C and of pH 10 buffer was 22.1-22.9°C.

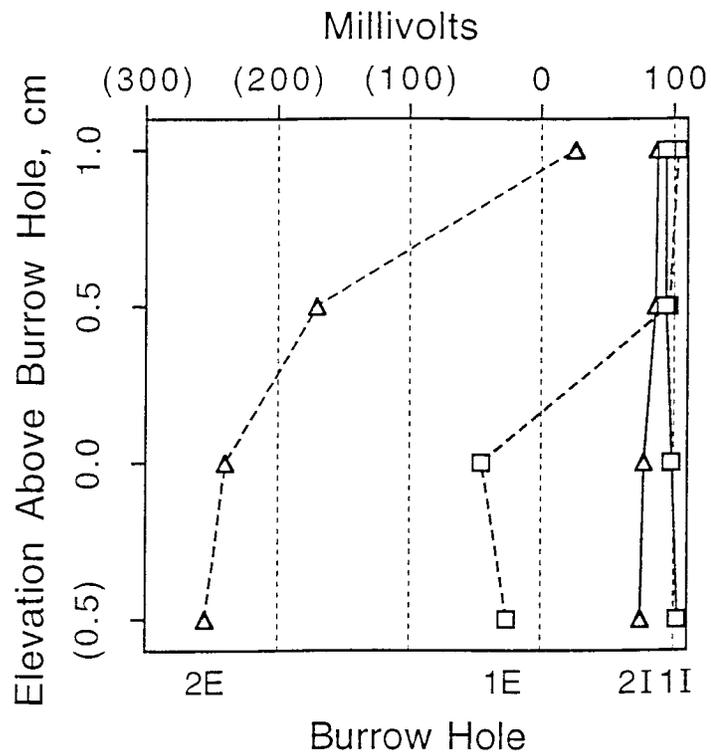


Figure 2. Redox readings above and within presumed incurrent (I) and excurrent (E) burrow holes in two separate aquaria, each containing a single *Hexagenia* nymph.

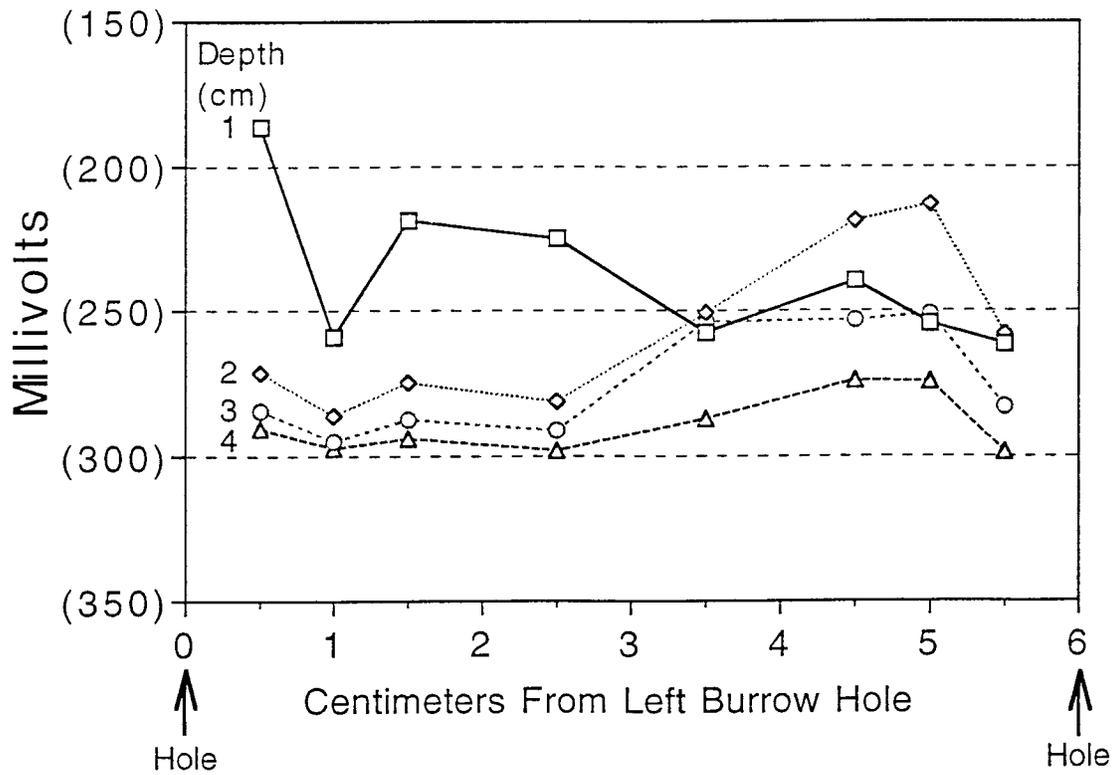
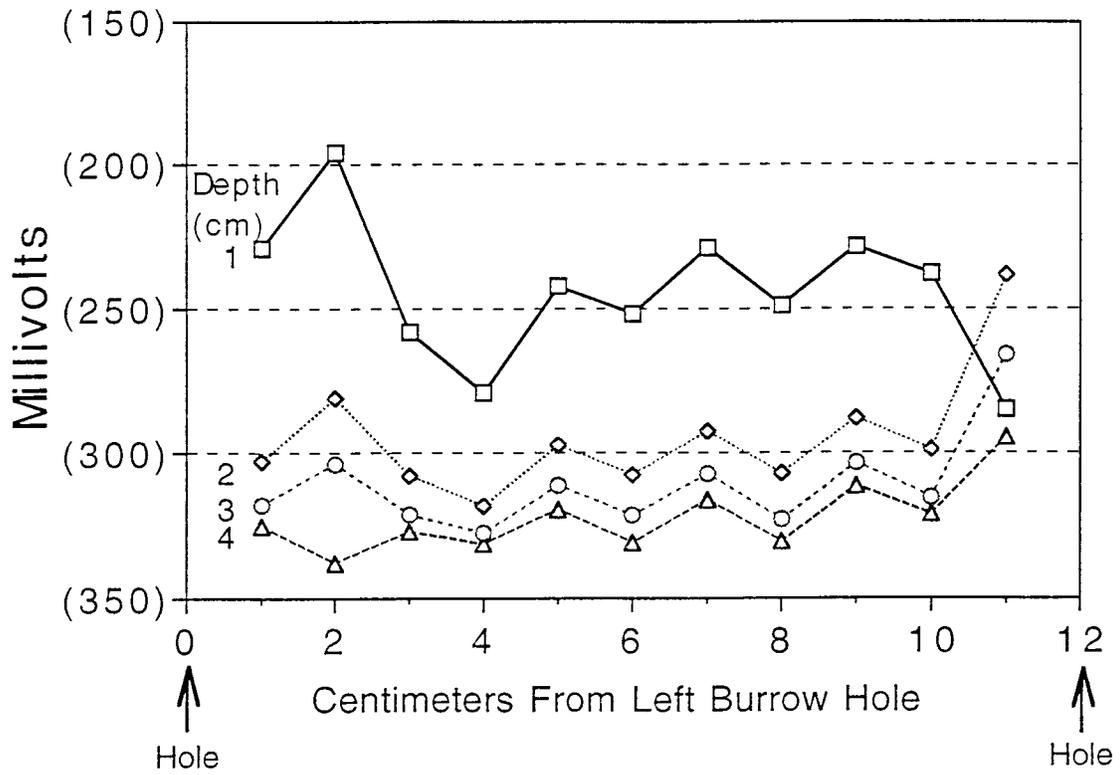


Figure 3. Redox potential between the burrow holes of two *Hexagenia* nymphs in different aquaria at depths in the sediment of 1, 2, 3, and 4 cm.

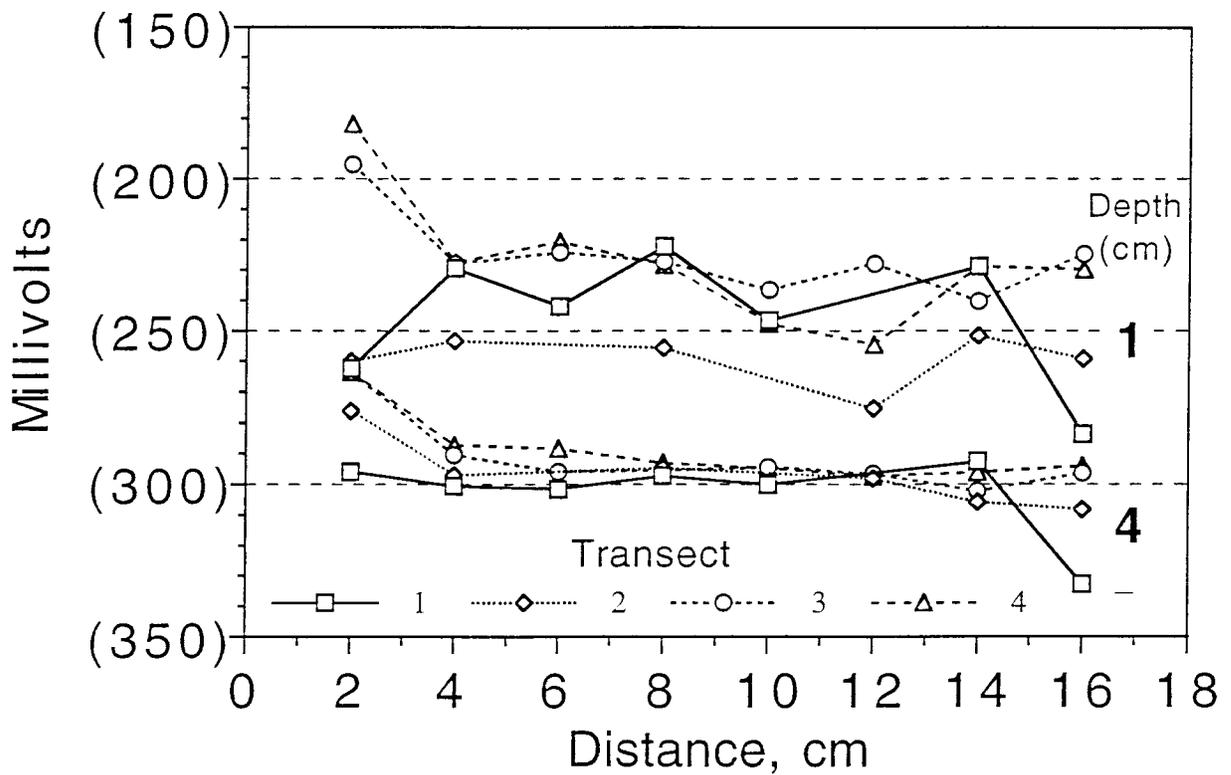


Figure 4. Sediment redox potential along four transects at depths of 1 cm and 4 cm across an aquarium containing one *Hexagenia* nymph in an unknown location beneath a layer of zebra mussel shells.

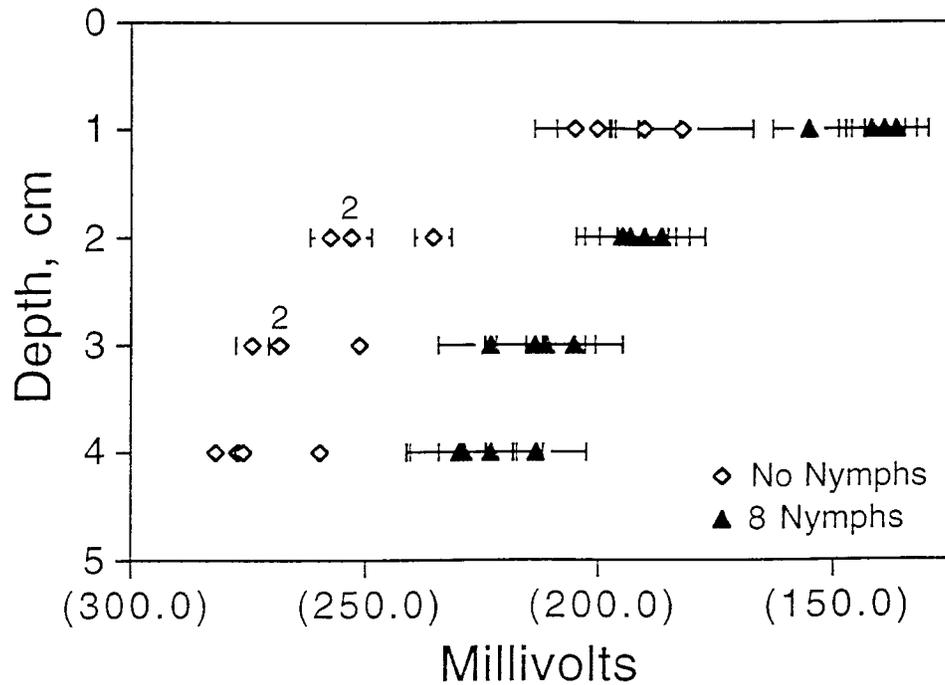


Figure 5. Sediment redox potentials (mean + 1 S.E.) measured along four transects at four depths each in an aquarium containing no *Hexagenia* nymphs (top) and another containing eight nymphs. Measurements were taken at eight one-cm intervals along each transect. Where error bars are absent, the standard error was <3.5 mv. The numbers above diamonds indicate two means with the same value. Two measurements in the water 1 cm above the sediment with no nymphs were -62.9 mv and -67.5 mv.

APPENDIX A.

PARTIAL DRAFT OF MANUSCRIPT IN PREPARATION

Redox Potentials in Water and Sediment as an Indicator of the Impact of Burrowing Mayflies (*Hexagenia* spp.) and Zebra and Quagga Mussels (*Dreissena* spp.) on Sediment Reduction-Oxidation Conditions

David J. Dariano and Kenneth A. Krieger
Water Quality Laboratory, Heidelberg College, Tiffin, OH 44883

Methods

Aquaria were constructed of 3 mm thick plate glass divided into 3 chambers. Each chamber, or aquarium, was 17.5 cm x 17.5 cm x 20.0 cm, with an overall length of each tank 54 cm. Dried sediment originally obtained from the Portage River near Bowling Green, OH, for an earlier study was rehydrated with reverse osmosis (RO) water (100 μ S/cm) and was homogenized to a batter consistency. Approximately 1.5 L of sediment was added to each aquarium, providing a depth of about 8 cm. Approximately 2.0 L of RO water was carefully added to each aquarium, which was permitted to stand undisturbed until clear.

To enhance reducing conditions, liquified horse manure was blended into the sediment. One hundred grams of manure was gradually added to 750 mL RO water in a beaker on a magnetic stirrer. The volume then was increased to 1.0 L. This mixture was stirred for 30 min and was strained through a 0.600-mm mesh sieve. The strained material was compressed to remove excessive liquid, which was returned to the solution. RO water was added until the overall volume was once again returned to 1.0 L. 150.0 mL of the liquified manure was mixed into the sediment of all aquaria. This sediment-manure mixture was permitted to stand for 48 h before water was added to the aquaria.

Zebra mussel (*Dreissena polymorpha*) valves were collected from the beach at Crane Creek Wildlife Area, OH. Valves were washed over a 2.0 mm sieve to remove sand and detritus. They were spread on a steel sheet to a thickness of 3 cm and were put in a drying oven at 100°C for 1 h. The valves were mixed and returned to the drying oven hourly for a total drying time of 3 h. Material other than *Dreissena* valves was removed by hand.

The redox electrodes (Figure X) were constructed by adapting the method of Faulkner *et al.* (1989). Copper tubing (1.2 mm ID) was cut to a length of 30.5 cm. Platinum wire (0.75 mm diam., 10.0 cm long) was inserted into the Cu tubing. The cavity was filled with silver solder. The Cu tubing was insulated with plastic shrink tubing. The Cu-Pt joint was sealed back to the insulation with epoxy and was permitted to cure for 24 h. The tip of each electrode was filed to expose 2.0 mm of Pt beyond the epoxy seal. On the opposite end of the electrode, 3.0 cm of Cu tubing was left exposed for attachment of an alligator clip, which was Ag soldered to a 16 gauge braided Cu wire attached to a BNC connector. This created the flexibility to switch to different electrodes. To know the depth of the electrode tip in the sediment, 2 mm-wide model airplane pin striping was applied at 1.0 cm intervals along its length. The tape was sealed with 3 coats of clear lacquer for protection from water and sediment.

During the experiment the electrode was supported by a bracket made of 6 mm thick Plexiglas®. The bracket was built into an angle configuration with the horizontal leg 6.3 cm wide and the vertical leg 5.0 cm wide. The overall length of the bracket was 40.0 cm. Two strips of 1.9 cm wide and 0.7 cm thick closed foam weather strip was applied to the vertical leg of the bracket. A third piece of Plexiglas was cut 5.0 cm wide and the weather strip was applied in the same manner as the vertical leg of the bracket. This last piece of Plexiglas was marked into 1.0 cm units to aid in accurate transects. Two holes were drilled through the vertical leg and third Plexiglas piece to permit insertion of a bolt to create a compression clamp on the electrode. This configuration held the electrode very rigid throughout the experiment. Two more holes were drilled into the horizontal leg of the angle which permitted the entire bracket to slide over two ring stands on opposite sides of the tank. The bracket itself was supported by the sides of the tank. The ring stands added stability to the probe bracket by preventing lateral movement.

We used an Accumet® Calomel KCl reference electrode and an Accumet Model 20 pH/conductivity meter. The electrodes were calibrated using pH 7.00 and pH 10.00 buffer solutions (Fisher Scientific Co.). Earlier research has suggested that redox potentials are pH dependent (Bohn 1971). The use of pH 7.00 and pH 10.00 buffers provided reliable standards to determine any differences in mv readings between the electrodes as well as error associated with mv drift and time required for the probes to stabilize (Bohn 1971, Faulkner *et al.* 1989, Cogger *et al.* 1992).

Immediately prior to experimentation, grease, dirt, and oxidized Pt were removed by dipping the Pt tip in a dilute detergent solution followed by a distilled water rinse. The opposite end of the probe was abraded with steel wool until shiny and dry wiped with a Kimwipe.