BACTERIA AS A DIRECT FOOD SOURCE FOR ZEBRA MUSSELS

A Report of the 1996 Tibor T. Polgar Fellowship Program

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ABSTRACT

The ability of zebra mussels (*Dreissena polymorpha*) to use bacteria as a sole nutrient source was studied by examining the changes in dry weight, lipid amount, and O_2 uptake after starvation for three months and feeding for one month. *Pseudomonas sp.*, a river water isolate, was used as a food source. Dry weight decreased from 1.16±0.09 (*N*=8) mg/mm shell length to 0.84±0.09 (*N*=12) after one month and to 0.59±0.04 (*N*=7) after three months of starvation but increased to 0.83±0.11(*N*=3) after being fed. Values for lipid amount decreased from 0.058±0.003 (*N*=7) mg/mm shell length to 0.043±0.004 (*N*=4) to 0.025±0.005 (*N*=4) after three months of starvation. An insignificant increase in lipid amount after feeding was observed. O_2 uptake values followed the same trend decreasing from 2.35±0.02 (*N*=21) µl O_2/hr/mm shell length to 1.32±0.1(*N*=20) µl O_2/hr/mm shell length after one month of starvation, and to 0.78±0.1(*N*=7) µl O_2/hr/mm after three months of starvation and the O_2 uptake increased to 1.89±0.14 (*N*=3) µl O_2/hr/mm after feeding. These studies support the conclusion that zebra mussels are able to use bacteria as a carbon source. Clearance rates of different size bacteria by *D. polymorpha* was also examined. The clearance rates are size dependent, however, the zebra mussels showed a significant clearance of even the small river water bacteria.

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INTRODUCTION

Dreissena polymorpha (Bivalvia: Dreissenidae, the zebra mussel) have spread throughout Europe and are found in British (Morton 1971), Russian (Mikheev 1967; Shevtsova and Kharchenko 1981; Kharchenko and Lyashenko 1986), and Polish lakes (Stanczykowska 1975). *D. polymorpha* (Bivalvia: Dreissenidae, the zebra mussel) was first discovered in North America in 1985 or 1986 and spread to Lake St. Claire by 1988 (Mackie, Gibbons et al. 1989). Further spread has continued through the Hudson River Basin and the Mississippi River Basin. Populations were first discovered in the Hudson River estuary in May 1991 (Strayer et al. 1996). By the year 2000, it has been projected that all the North American bodies of water that can support *D. polymorpha* populations will support them (Ludyanskiy et al. 1993).

Oxygen and food are obtained by *D. polymorpha* through filtration. Seston is indiscriminately removed and then the particles are sorted. Some of the material is ingested and the remaining is expelled as pseudofeces which accumulates on the bottom with the feces. The result is a net removal of particles from the water column with deposition on the bottom (Reederş et al. 1989).

The interaction of bacterial populations with the zebra mussel populations has not been investigated. The extent to which the mussel's accumulation of matter on the bottom, either as pseudofeces or feces, affects the bacterial populations is not known. Nor is it known the extent to which zebra mussels can use bacteria in the water column as a source of food. It has been shown that zebra mussels can trap and ingest bacteria (Silverman et al. 1995). During this study we examined the ability of starved zebra mussels to restore their energy reserves of lipids and total biomass when fed bacteria.

MATERIALS AND METHODS

Animals

Zebra mussels (range 14 -21 mm length) were collected from the rock substrate from a site just below the Federal Dam on the Hudson River in Troy. The animals were then kept in artificial pond water (APW; 0.5 NaCl; 0.4 $CaCl_2$; 0.2 $NaHCO_3$, 0.05 KCl; 0.2 $MgSO_4$ in mM) at 13° C.

Dry weight determination

The weight of each animal was determined by removing the animal from its shell, drying it in an oven overnight at a temperature of 105°C, and weighing the dried specimen. For each experiment, the average weight of 10 mussels was taken and divided by the average shell length.

Lipid extraction

Total lipid was determined using the gravimetric method of Barnes and Blackstock (1973). A portion of dried tissue (5 animals) was ground in a mortar and pestle, weighed and homogenized with 5 mls of a 2:1 (v/v) chloroform-methanol mixture using a Teflon grinder. The homogenate was filtered through a Whatman No. 1 filter and the grinder was rinsed with 5 mls of solvent to bring the final volume to 10 mls. The filtrate was purified by shaking in a separatory funnel with 4 mls of 0.9% aqueous sodium chloride. This mixture was allowed to separate overnight at 6°C, with the lower lipid containing phase being drained into a tarred beaker. Beaker solvent contents were evaporated before final weighing of the lipid extract.

Oxygen uptake

Determination of oxygen uptake rate (V_{O2}) was done with a YSI Model 5300 Biological Oxygen Monitor connected to a Macintosh Quadra 800 using the MacLab A/D converter. The oxygen probe was inserted into a sampling chamber (22mm X 38mm) in

series with the animal chamber (22mm X 90mm). The total volume of the chambers and connecting tubes was 71 mls. Bath water was circulated between the sampling chamber and the animal chamber at a rate of 6.9 ml/sec using a Cole-Palmer Master flex circulator. The animal chamber was submerged in a water bath to maintain the bath water temperature at 13°C. Prior to each run, the bath water was saturated with air by bubbling air through a container of APW maintained at 13°C in a water bath.

Saturated APW was used to fill the chambers and tubing without any animals to calibrate the system to 100% air saturation. The system was then run for 10 minutes to determine a baseline. The circulator was stopped and 10 zebra mussels were added to the animal chamber. The circulator was started and the animal chamber was covered to minimize disturbance to the animals. During the 20 minute measurement period P_{O2} in the chamber dropped in a linear fashion as the mussels respired oxygen. The oxygen consumption rates were calculated using linear regression analyses on the drop in P_{O2} during the last 10 minutes of the experiment. The rate of O_2 depletion minus the baseline rate times the total mls of O_2 gives the rate of O_2 loss in µl/sec (V_{O2}, microliters of oxygen consumed per hour at STP).

Animals that were starved showed a significant decrease in V_{O2} compared to controls when the V_{O2} was expressed per mm length of the shell (Figs. 1&2). While most values for metabolic rate in the literature are expressed per mg dry wt, we chose to express are results per mm shell length, because while there was a significant expected weight loss during the three month starvation there was no reason to believe that there would be a significant change in mussel shell length during that period.

Clearance rate

Experiments were conducted at a constant temperature of 13°C using four different types of bacteria: *Bacillus sp., Pseudomonas sp., Escherichia coli,* and the bacteria in river

water. Care was taken in culturing bacteria to prevent clumping and to ensure monocultures were obtained for each type of bacteria. A sample of the bacterial stock solution was added to 1 liter of APW to give a final concentration in the range of 10^5 to 10^6 cells/ml. The river water sample contained cells at 10^4 cells/ml. The APW water bacteria solution was well mixed and divided into two beakers. A 1 ml sample was taken from each beaker and 10 mussels were placed in one of the beakers, leaving the other mussel-free as a control. A 1 ml sample was taken from each beaker every 15 minutes for 1 hour. The samples were then diluted with 3 mls of sterile distilled water.

In the case of the experiment using river water bacteria, water from the Hudson River was collected at the same site as the mussels were collected. Two beakers, each with 500 mls of river water were set up and 10 mussels placed in one of them. A 5 ml sample was taken from each beaker every 15 minutes but was not diluted. The samples were stained using the DAPI epifluorescent staining method (Kepner and Pratt 1994). The cells in the samples were then counted under UV excitation microscope and the concentrations were calculated. Since there were different size particles in the river water, counts on only the smallest size bacteria were made to maintain uniformity. The clearance rate (CR) was calculated by the following relationship:

$$CR = \frac{V}{nt}\left[\left(\ln c_o - \ln c_t\right) - \left(\ln c_o^{'} - \ln c_t^{'}\right)\right]$$

where V is the total volume in the container, n is the number of active mussels, t is the time at which the sample was taken, c_o is the concentration at time zero, c_t is the concentration at time t, and $c^{'}$ indicates control values. The final clearance rates were normalized to shell length.

Starvation experiments

Mussels were taken from the river and immediately placed in containers of APW at 13°C and not provided with any nutrient source. The APW bath was changed approximately once every week. The animals were starved from one to three months before use. *Pseudomonas sp.* was used for bacterial feeding. Again, care was taken in culturing the bacteria to prevent clumping and to ensure monocultures were obtained. Feeding was done using a group of 10 animals in each beaker. They were randomly chosen and placed in a beaker with 500 mls of APW. The mussels were fed at three concentrations: 10^6, 10^8 and 10^9 cells/ml. Animals at 10^6 and 10^8 cells/ml were fed every Monday, Wednesday and Friday and would clear the beaker by the next feeding. Those at 10^9 cells/ml were fed every Monday and would also clear the beaker by the next feeding. The baths were changed to fresh APW at each feeding. The results are expressed as the total cells that were presented to each group: 0.05×10^{11} cells for the mussels fed at a concentration of 10^6 cells/ml, 5.5×10^{11} cells at 10^8 and 20×10^{11} cells for the mussels fed at 10^9 cells/ml.

Statistical Analysis

Data sets were analyzed using one way analysis of variance and students t test. Results were considered significant at the $P<0.05$ Data are presented as mean ± standard error of the mean (N=number of experiments).

RESULTS

Dry weight determinations

The initial dry weight of the mussels was 1.16±0.09 (N=8) mg/mm shell length which decreased to 0.84±0.09 (N=12) mg/mm shell length after one month of starvation and to 0.59±0.04 (N=7) after three months of starvation. When fed *Pseudomonas sp.*

bacteria at a concentration of 10^9 cells/ml for one month the dry weight increased to 0.83±(*N*=3) mg/mm shell length (Fig. 1). The dry weight increased as the amount of available biomass increased. At 5.5 and 20 X 10^{11} bacterial cells there was a significant increase (P<0.05) in dry weight (Fig. 2).

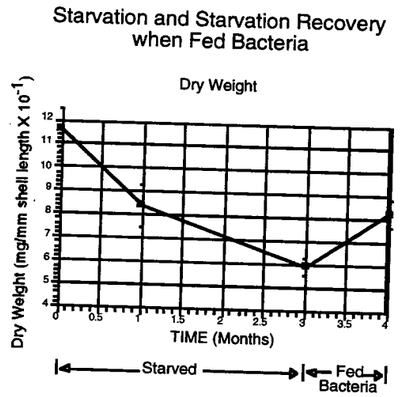

Figure 1. Effect of starvation of zebra mussels on dry weight and recovery when fed an estuarine bacterial isolate (*Pseudomonas sp.*). The number of experiments, was N = 8, 12, 7 and 3 at 0, 1, 3 and 4 months respectively.

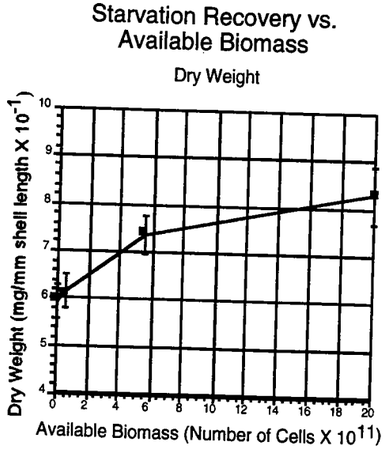

Figure 2. Zebra mussels starved for four months and fed bacteria (*Pseudomonas sp.*) at a concentration of 10^6, 10^8 or 10^9 cells/ml. The number of experiments, using 10 animals for each experiment, was N = 7, 4, 4, and 3 at 0, 0.055, 5.5 and 20 X 10^{11} cells respectively.

Lipid extraction

Some of the loss in dry weight is due to a loss in lipid. The lipid content of animals taken from the river was 0.058±0.003 (*N*=7) mg/mm shell length. Animals starved one month had their lipid content drop to 0.043±0.004 (*N*=4) and after three months of starvation it decreased to 0.025±0.005 (*N*=4) (Fig. 3). When the mussels were fed *Pseudomonas sp.* for one month at a concentration of 10^9 cells/ml and at 10^8 cells/ml there was a significant increase in the lipid content (Fig. 4). When fed bacteria at a concentration of 10^6 cells/ml there was an increase but with the small numbers used, it was not significant.

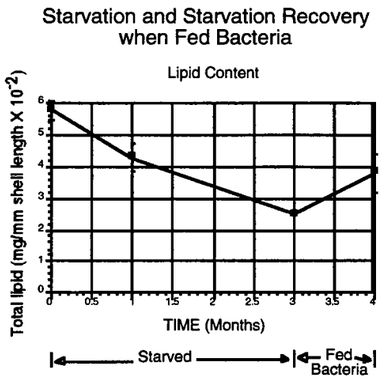

Figure 3. Effect of starvation of zebra mussels on lipid content and recovery when fed an estuarine bacterial isolate (*Pseudomonas sp.*). The number of experiments, using 5 animals each, was N = 7, 4, 4, and 3 at 0, 1, 3 and 4 months respectively.

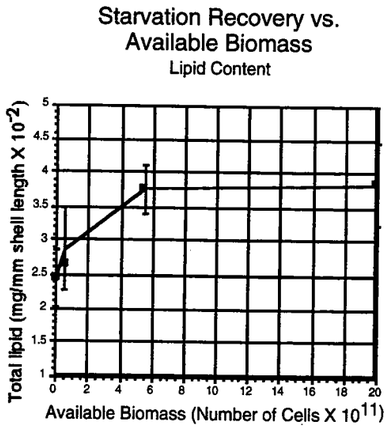

Figure 4. Zebra mussels starved for four months and fed bacteria (*Pseudomonas sp.*) at a concentration of 10^6, 10^8 or 10^9 cells/ml. The number of experiments, using 10 animals each, was N = 6, 4, 4, and 3 at 0, 0.055, 5.5 and 20 X 10^{11} cells respectively.

Metabolic rate

The metabolic rate under laboratory conditions shows the same trends as the change in biomass of the zebra mussels as indicated by the dry weight and lipid content when expressed as mg/mm shell length. There was a significant decrease from 2.35±0.02 (*N*=21) µl O_2/hr/mm shell length to 1.32±0.1 (*N*=20) µl O_2/hr/mm shell length and 0.78±0.1(*N*=7) µl O_2/hr/mm shell length after one and three months of starvation respectively (Fig. 5). Similarly, when the zebra mussels were fed bacteria at a concentration of 10^8 and 10^9 cells/ml, there was a significant increase in the O_2 uptake. At 10^6 there was an increase but it was not significant (Fig. 6).

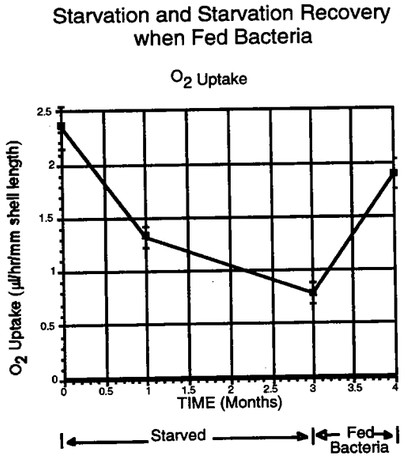

Figure 5. Effect of starvation of zebra mussels on O_2 Uptake, and recovery when fed an estuarine bacterial isolate (*Pseudomonas sp.*). The number of experiments, using 10 animals each, was N = 21, 20, 7 and 3 at 0, 1, 3 and 4 months respectively.

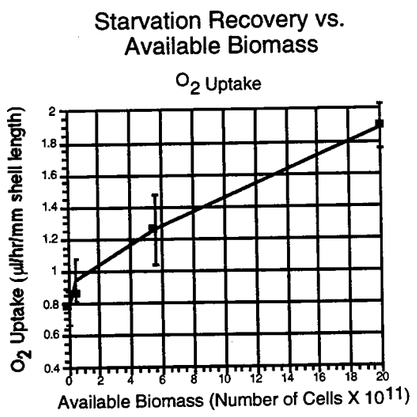

Figure 6. Zebra mussels starved for four months and fed bacteria (*Pseudomonas sp.*) at a concentration of 10^6, 10^8 or 10^9 cells/ml. The number of experiments, using 10 animals for each experiment, was N = 7, 4, 4 and 3 at 0, 0.055, 5.5 and 20 X 10^{11} cells respectively.

Clearance rates

When zebra mussels were fed bacteria of different sizes there was a decrease in clearance rate with decreasing size of the bacteria. However, even for the small river water bacteria there was a significant filtration of the bacteria from the river water (Fig. 7).

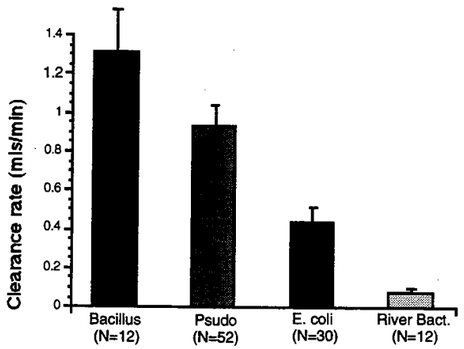

Figure 7. Clearance rates based on removal of different bacteria from 500 mls at a concentration between 10^5 and 10^6 cells/ml except river water which was at a concentration of 10^4 cells/ml.

DISCUSSION

There was a drop in V_{O2} (fig. 5) similar to that found for the decrease in dry weight with starvation. Sprung (1991) found no change in metabolic rate between post-spawning individuals and pre-spawning individuals even though post-spawning individuals can loose up to 30% of their body weight. Thus, it is most likely that the decrease in dry weight is due to a loss in body mass as a result of the decrease in carbon intake rather than a loss associated with an event such as spawning. This also suggests that the loss of lipid is due to a nutritional deficit. The significant increase in weight when fed

Pseudomonas sp. bacteria at concentrations of 10^8 and 10^9 cells/ml suggests that zebra mussels can use these bacteria as a nutrient source at these concentration levels. This is supported by the work of Silverman et al. (1995) who found that when zebra mussels were fed S^{35} labeled *E. coli* the S^{35} showed up in the mussel's proteins. They also found that the zebra mussels in comparison to marine mussels had a finer gill structure which would make it easier for them to filter out the small bacteria.

One concern of extrapolating these results to the environment is that bacteria in the laboratory are larger than those found in the environment. While we found that as the bacteria size decreased the filtration rate also decreased, there was a significant clearance by mussels of the small bacterial found in river water. Thus, bacteria may be a source of carbon, especially when other sources are scarce. It is interesting to note that in 1993 Strayer et al. (1996) estimated that the total assimilation of carbon by zebra mussel population in the Hudson River to be about 120 - 150 g C m^{-2} yr^{-1}, which was significantly larger that the estimated production of phytoplankton of 50 g C $m^{-2}yr^{-1}$. These authors suggest that the zebra mussels may have been feeding on bacteria and other detritus.

The metabolic rates (V_{O2}) observed for the controls (Figs. 1 & 2) are in the same range but higher than those determined for *Dreissena polymorpha* in Lake Erie (Alexander et al. 1994, McMahon 1996). Possibly, this is due to different populations having different metabolic rates in different habitats.

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