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Zebra Mussel Feeding Relationships and Their Implications for Native Unionids

Abstract

Human impacts have altered many Great Lakes ecosystems. The human-induced appearance and spread of invasive zebra mussels (*Dreissena polymorpha*) has accompanied a remarkable decline in the population sizes of North American unionid mussel species, most of which are now endangered. Despite conclusive evidence that zebra mussels kill native unionid mussels in the areas they invade, the mechanism by which they do so is uncertain. Studies suggest that zebra mussels may kill unionid mussels by latching onto their shells and then out-competing them for food resources. To model the effects of zebra mussel infestation and food resource competition on scarce, endangered unionid mussels, we examined the ^{13}C and ^{15}N isotope compositions of zebra mussels found attached to other zebra mussels (i.e. "parasite" zebra mussels) and zebra mussels found with other zebra mussels attached to them (i.e. "host" zebra mussels) in Douglas Lake, Cheboygan County, Michigan. Results indicated that attached zebra mussels do not, in fact, out-compete their "hosts" for food. Tentatively, this also suggests zebra that mussels do not out-compete unionids for food.

Introduction

Since their first North American appearance in 1988 in Lake St. Clair, Michigan, invasive zebra mussels (*Dreissena polymorpha*) have spread to freshwater bodies throughout the Great Lakes region, the Mississippi River drainage, and parts of the southwestern United States (Benson and Raikow 2007). Native to the Black, Caspian, and Azov Seas of Central Asia (Benson and Raikow 2007), zebra mussels have caused tens of billions of dollars in damage to North American water pipes, ships and boats, water processing facilities, hydroelectric power plants, and harbor infrastructure, among other things (O'Neill 1995). They have also disturbed aquatic food webs throughout the eastern U.S. and Canada by depositing feces and particulate matter on sediment (Mitchell *et al.* 1996), decreasing phytoplankton abundance (Stanczykowska *et al.* 1975), and

increasing benthic production by directing nutrients and energy from the pelagia to the benthos (Walz 1979).

Zebra mussels have played a particularly significant role in the decline of North American unionid mussel species, 72% of which are now "extinct, endangered, threatened, or of special concern" (Great Lakes Science Center 2004). Zebra mussels possess byssal threads that allow them to quickly latch on to hard substrates, including unionid mussel shells (Schloesser *et al.* 1996). Relative to unionid mussels, zebra mussels are small, hardy, and quick to sexually mature and reproduce. These physical characteristics and life history traits enable zebra mussels to infest living unionid mussels at densities as high as 11,550 zebra mussels per unionid mussel (Schloesser *et al.* 1996). Previous research suggests a strong correlation between zebra mussel infestation and unionid mortality: Gillis and Mackie (1994) and Nalepa (1994) observed near total unionid mortality in Lake St. Clair within five years of zebra mussels' colonization of the lake, and Schloesser and Nalepa (1994) observed total unionid extirpation in western Lake Erie by September 1990, approximately two years after humans first observed zebra mussels in Lake Erie. In their study, Schloesser and Nalepa (1994) found dead unionid mussels carrying as many as 14,393 zebra mussels.

Despite indications that heavy zebra mussel infestations lead to near total unionid mortality (Schloesser *et al.* 1996), the mechanism by which zebra mussels kill their unionid "hosts" remains uncertain (Schloesser *et al.* 1996). Researchers have posited several explanations for zebra mussel-induced unionid mortality. These include (1) zebra mussels' tendency to restrict unionid valve (i.e. shell) movement and cause shell deformities, (2) their tendency to out-compete unionid mussels for food resources in the

ecosystems they invade, and (3) their tendency to impair unionid mussels' movement by weighing them down (Schloesser *et al.* 1996).

Contemporary studies of aquatic food webs (Mitchell *et al.* 1996; Kling *et al.* 1992) frequently employ stable isotope analysis, a technique for determining the diets and trophic levels of aquatic animals from the ratios of carbon-13 (^{13}C) to carbon-12 (^{12}C) and nitrogen-15 (^{15}N) and nitrogen-14 (^{14}N) in their tissues (Michener and Schell 1994). In this study, we used isotopic analysis in an attempt to determine whether zebra mussels out-compete unionid mussels for food resources – especially phytoplankton – in Douglas Lake, as suggested above in posited mechanism (2).

For ease of explanation, this paper refers to zebra mussels found attached to other zebra mussels as "parasites" and zebra mussels found with other zebra mussels attached as "hosts", even though this is not a true parasite–host interaction. In light of zebra mussels and unionid mussels' similar filter feeding apparatuses and mutual reliance on phytoplankton as a primary source of food, we hypothesized that "parasite" zebra mussels deprive their "hosts" of food and nutrients – especially phytoplankton – leaving the "hosts" only less-preferred food sources. From this we predicted that the isotopic signatures of the "parasite" and "host" zebra mussels would differ. This would have meant that the "parasite" zebra mussels forced the "host" zebra mussels to shift either their trophic levels or food sources by out-competing them for preferred food resources. We believe our experiment aided scientific understanding of the mechanism by which invasive zebra mussels kill native mussels.

Methods and materials

We analyzed the ^{13}C and ^{15}N isotopic signatures of zebra mussel flesh, a technique that has proved powerful and accurate for examining trophic relationships in freshwater food webs (Mitchell *et al.* 1996). We did not directly compares these signatures to those of unionid mussel flesh, due to live unionid mussels' scarcity in Douglas Lake, the illegality of collecting many unionid species in Michigan, and the difficulty of distinguishing particular unionid species from one another (Great Lakes Science Center 2004). Instead, we used the isotopic signature of zebra mussels found covered with other zebra mussels as a proxy for the isotopic signature of unionids found covered with zebra mussels. To model the conditions in which zebra mussels that affix themselves to unionid mussels live and eat, we collected and separately analyzed (1) large (18-25 mm) "host" zebra mussels (i.e. zebra mussels found with other zebra mussels attached), (2) large "non-host" zebra mussels found with no zebra mussels attached, (3) small (10-17 mm) "parasite" zebra mussels (i.e. zebra mussels found attached to other zebra mussels), and (4) small "non-parasite" zebra mussels found on abiotic substrates. (In our experience, virtually none of the zebra mussels smaller than 18 mm held other zebra mussels on their shells.) We then compared the isotopic signatures of these different varieties of mussel flesh to one another and to the isotopic signatures of two potential food sources: phytoplankton from lake water and detritus from the bottom of Douglas Lake.

We collected zebra mussels, lake water, and detritus samples at a depth of 1.5 meters near Douglas Lake's Grapevine Point on June 2, 2007 and at 0.5 meters near Douglas Lake's Sedge Point on June 2, 2007 (Figure 1). Using chisels, we removed and separated into buckets (1) zebra mussels found on logs, rocks, and other non-living

substrates and (2) zebra mussel "clusters" consisting of zebra mussels attached to one another. We used a Van Dorn depth sampler to collect lake water samples at the depths and locations at which we harvested the mussels. At these locations, we also collected lake-bottom detritus samples by hand.

Upon returning from each collection, we removed roughly 200 zebra mussels from their substrates, sorted them into four separate aerated clean water tanks in accordance with their sizes, their substrates, and their roles in the "parasitic" attachment relationships (in other words, their placement either on top of or beneath other zebra mussels). Upon returning from our collections, we left the mussels in the aerated tanks to cleanse their digestive tracts of wastes for 24 hours. We removed the zebra mussels from their shells using razor blades and forceps, placed them in a -80ºC freezer for one hour, and freeze-dried them for 24 hours. We used mortars and pestles to pulverized the dried flesh, producing eight different five milligram samples of powdered zebra mussel flesh – four samples from each test site – and submitted the samples to Mike Grant, a chemist at the University of Michigan Biological Station, for analysis in a Thermo Finnigan Delta XP stable isotope mass spectrometer (error 0.000 for nitrogen and 0.287 for carbon).

To prepare the phytoplankton samples, we used syringes to filter one liter of each of the lake water samples through glass fiber filters precombusted at 550ºC. To prepare the detritus samples, we removed sticks, stones, and other hard objects larger than one centimeter in length or diameter from the samples we collected. We then freeze-dried the filters and detritus samples for 24 hours, pulverized them with mortars and pestles, and submitted them for analysis in the mass spectrometer. This isotopic analysis yielded the ^{13}C and ^{15}N isotopic ratios of our zebra mussel, detritus, and phytoplankton samples.

Methods of analysis

We used a paired t-test to compare the $\delta^{13}C(‰)$ values of our samples. $\delta^{13}C(‰)$ denotes the mean change in the ratios of ^{13}C to ^{12}C (in parts per thousand) for our samples relative to that for Vienna Pee Dee Belemnite (VPDB) – a kind of fossilized shell now used internationally as one of carbon's isotopic ratio standards (Kendall 2006) – and is calculated as follows (Mitchell *et al.* 2006):

$$\delta^{13}C(‰) = \left(\frac{\left(^{13}C/^{12}C\right)sample}{\left(^{13}C/^{12}C\right)VPDB} - 1 \right) \times 10^3$$

We then used a paired t-test to compare the $\delta^{15}N(‰)$ values of our samples. $\delta^{15}N(‰)$ denotes the mean change in the ratios of ^{15}N to ^{14}N (in parts per thousand) for our samples to that for atmospheric nitrogen, nitrogen's isotopic ratio standard, and is calculated as follows (Mitchell *et al.* 2006):

$$\delta^{15}N(‰) = \left(\frac{(^{15}N/^{14}N)sample}{(^{15}N/^{14}N)atmosphere} - 1 \right) \times 10^3$$

To test the prediction that "parasite" and "host" zebra mussels differ in their feeding preferences we compared the ^{13}C and ^{15}N compositions of "host" zebra mussels to the isotopic compositions of "parasite" zebra mussels using a t-test paired by both collection site and role in the "parasitic" attachment relationship (i.e. either "parasite" or "host").

If we had found significant differences, we would have regarded them as having either a different food source or a different trophic level. If differences had existed in the $\delta^{13}C(‰)$ values of the zebra mussel flesh types, we would have concluded that the composition of the zebra mussels' food sources were different. If differences had existed in the $\delta^{15}N(‰)$ values of the zebra mussel flesh types, we would have concluded that the

zebra mussels' trophic levels were different. If no significant differences had existed, we would have regarded trophic levels and composition to be practically the same.

We plotted $\delta^{13}C(‰)$ against $\delta^{15}N(‰)$ values for the eight zebra mussel flesh samples, two detritus samples, and two phytoplankton samples described above in a graph depicting the samples' $\delta^{13}C(‰)$ values against their $\delta^{15}N(‰)$ values. We included the isotopic signatures of detritus and phytoplankton in this graph so as to determine whether our zebra mussels' isotopic signatures were in the correct ^{13}C and ^{15}N ratio ranges (relative to the studies of other zebra mussel researchers). This enabled us to visually place the zebra mussels' isotopic signatures – and thus their food sources – along ^{13}C and ^{15}N gradients. All of these analyses allowed us to better understand the differences between our samples' isotopic signatures.

Results

For the hypothesized relationship between the feeding habits of "host" zebra mussels *and* "parasite" zebra mussels, there was no significant difference in either ^{13}C or ^{15}N composition (p = .314, df = 1, T = 1.862 for $\delta^{13}C(‰)$; p = 0.344, df = 1, T = 1.667 for $\delta^{15}N(‰)$), as seen in Table 1. This lack of a significant difference can be observed in Figure 2, which reveals that the isotopic signatures of all zebra mussel samples (including "parasite" and "host" samples) were very similar in both ^{13}C and ^{15}N composition.

Discussion

The lack of a difference in feeding habits between large "host" zebra mussels and small "parasite" zebra mussels suggests that they occupy the same feeding niche and trophic level. This suggestion does not support our hypothesis that "parasite" zebra mussels kill their "host" mussels by out-competing them for food. From this we

tentatively deduce that zebra mussels kill unionid mussels by one of the two other mechanisms Schloesser *et al.* (1996) propose: (1) by restricting unionid valve movement and causing shell deformities through attachment, or (2) by weighing unionid mussels down until they can no longer move.

Our experiment suffered from a number of procedural flaws. Our reading of Mitchell *et al.* (1996) – who examined ^{13}C and ^{15}N signatures of zebra mussels and their food sources in Oneida Lake, New York – indicates that the isotopic signatures of our zebra mussel and detritus samples were skewed, with both kinds of samples showing higher ^{13}C concentrations than those of the zebra mussel and detritus samples in Mitchell *et al.* (1996). Skewing in our zebra mussel samples may have resulted from contamination of the samples with bits of mussel shell, which contain considerably higher concentrations of ^{13}C isotopes than do zebra mussel flesh (personal communication, Mike Grant, June 13, 2007). Skewing in our detritus samples may have resulted from contamination during the freeze drying process. While the samples were freeze drying, the glass vials for both detritus samples broke, causing parts of the samples to mix and also complicating their removal. When we removed the broken detritus vials from the freeze dryer, the vials fell on the floor and broke further. We salvaged as much of each sample as we thought usable for the mass spectrometer analysis, but this probably led to some mixing of the samples and, hence, some distortion of the data.

Especially important to note is our experiment's low number of replicates (N = 2), which occurred due to a lack of collecting and processing time for the samples. This very small number of replicates renders even our statistically significant results inconclusive.

If we were to conduct this experiment again, we would change our procedures in several ways to ensure better accuracy and precision in our results. First and foremost, we would include more replicates in our experiment. Small sample size was our study's principal drawback, since it prevented us from drawing firm conclusions from our statistical test. Second, we would take the time to select a unionid mussel-rich collection site and to learn to distinguish between native mussel species – not all of which are endangered – which would enable us to collect both zebra mussels and native unionid mussels. A direct comparison of the feeding habits of zebra mussels and unionid mussels would have enabled us to make firmer conclusions about food resource competition between zebra mussels and unionid mussels than our proxy study of "parasite" and "host" zebra mussels has. Finally, we would incorporate a hydrochloric acid (HCl) fumigation step into our processing of the zebra mussel flesh samples. In such an HCl fumigation, we would place mussels in an HCl bath for 24 hours, causing the calcium carbonate ($CaCO_3$) in the mussels' shells to bleed off entirely in the form of CO_2 and leaving only the flesh of the zebra mussels. This step would likely eliminate skewing toward higher ^{13}C contents in the isotopic ratios of our zebra mussel samples, as discussed above.

In determining that zebra mussels do not out-compete individuals of their own species for food, we arrived at the tentative conclusion that zebra mussels harm native mussels in a manner other than out-competing them for food. This supports previously posited notions (Schloesser *et al.* 1996) that zebra mussels kill native mussels by physically restricting their movement, an example of interference competition (rather than exploitation competition for food resources). Understanding the direct interactions between invasive zebra mussels and native mussels may in time lead to improvements in

management strategies and a better comprehension of the complex aquatic food webs zebra mussels have already completely changed.

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Figure 1. We collected zebra mussels at site one, near Grapevine Point and the University of Michigan Biological Station, on May 26, 2007, at a depth of 1.5 m. We collected zebra mussels at site two, near Sedge Point, on June 2, 2007, at a depth of 0.5 m. All collecting took place in Douglas Lake, Cheboygan County, Michigan.

Paired T-Test of Comparing isotopic ratios	*p*	df
$\delta^{13}C(‰)$ Large Zebra Mussels (ZM) with Small ZM attached $\delta^{13}C(‰)$ Small ZM attached to Large ZM	.314	1
$\delta^{15}N(‰)$ Large ZM with Small ZM attached $\delta^{15}N(‰)$ Small ZM attached to Large ZM	.344	1

Table 1. Compared ^{13}C and ^{15}N isotopic ratios of large "host" zebra mussels and small "parasite" zebra mussels

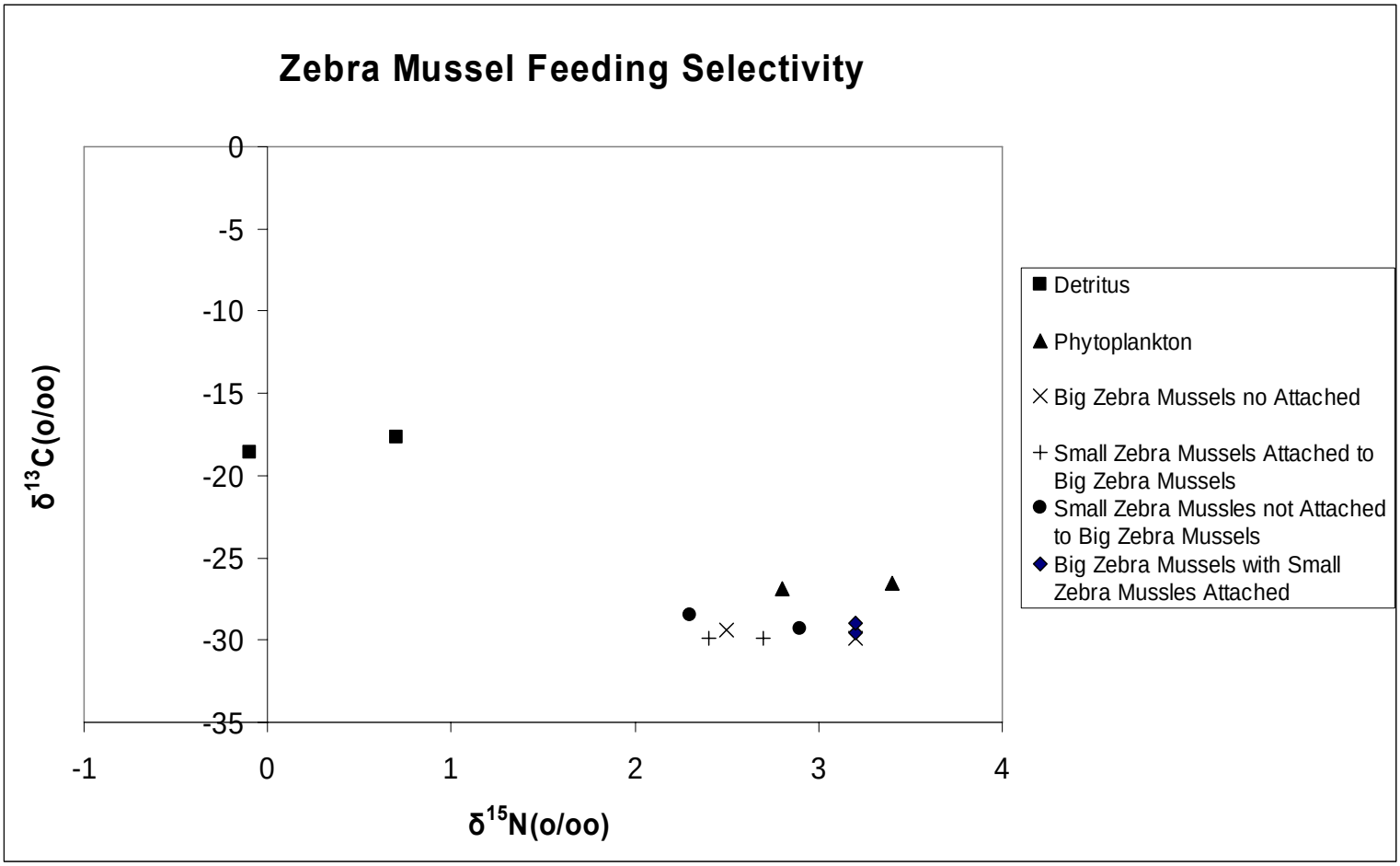

Figure 2. A comparison of the ^{13}C and ^{15}N isotopic ratios of zebra mussels, detritus, and phytoplankton from sites one and two. Zebra mussel isotopic signatures were very similar to one another in both $\delta^{13}C(‰)$ (indicating similarity in food source composition) and $\delta^{15}N(‰)$ (indicating similarity in trophic level).