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Acute physiological effects of zebra mussel (*Dreissena polymorpha*) infestation on two unionid mussels, *Actinonaias ligamentina* and *Amblema plicata*

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Abstract: Our laboratory studies of the physiological effects of zebra mussel (*Dreissena polymorpha*) infestation on the freshwater mussels *Actinonaias ligamentina* and *Amblema plicata* (Unionidae) show that (*i*) zebra mussel infestation causes stress and symptoms of starvation in unionid mussels, (*ii*) unionid species are affected unequally, and (*iii*) symptoms of starvation are greater when initial condition is low. Nutritive stress in infested unionid mussels was indicated by shifts to lower metabolic rates, more protein-based metabolism (lower O:N ratios), and compensatory increases in grazing rates. Starvation may be the result of local food depletion and (or) increased metabolic cost. *Actinonaias ligamentina* (subfamily Lampsilinae) was more sensitive to infestation than *Amblema plicata* (subfamily Ambleminae), as indicated especially by changes in oxygen uptake rate and grazing rate. The effects of infestation were greater in mussels that were already in low condition. Our results indicate that the decline in diversity of unionid mussels since the introduction of zebra mussels is due to species-specific rates of starvation.

Résumé : Nos études de laboratoire sur les effets physiologiques de l'invasion des moules zébrées (*Dreissena polymorpha*) sur les mulettes *Actinonaias ligamentina* et *Amblema plicata* (Unionidae) montrent que (*i*) l'infestation par la moule zébrée cause du stress et des symptômes d'inanition chez les unionidés, (*ii*) les espèces d'unionidés sont affectées de façon inégale et (*iii*) les symptômes d'inanition sont plus prononcés quand l'état de départ est faible. Le stress alimentaire chez les mollusques unionidés affectés se traduit par une baisse des taux métaboliques, un métabolisme davantage basé sur les protéines (rapports O:N plus bas) et une augmentation compensatoire des taux de broutage. L'inanition peut résulter d'une raréfaction locale des ressources alimentaires et (ou) d'un accroissement du coût métabolique. *Actinonaias ligamentina* (sous-famille Lampsilinae) était plus sensible à l'infestation que *Amblema plicata* (sous-famille Ambleminae), ce que révèlent particulièrement les changements dans le taux d'assimilation de l'oxygène et le taux de broutage. Les effets de l'infestation étaient plus manifestes chez les mollusques dont l'état était faible au départ. Nos résultats indiquent que la baisse de la diversité chez les mollusques unionidés depuis l'introduction des moules zébrées est due à des taux d'inanition spécifiques aux espèces.
[Traduit par la Rédaction]

Introduction

Freshwater mussels (family Unionidae) are distributed worldwide and reach their greatest diversity in North America (Williams et al. 1993). These partially infaunal bivalves are ecologically important in both riverine and lotic habitats. As filter feeders, they control seston and act as a trophic link to higher predators (Vannote et al. 1980). Unionid mussels are among the most imperiled groups of animals in the world. Their abundance and diversity have declined dramatically in the last 30 years. There are currently 297 species and subspecies of unionid mussels in North America. Of those, 42 species are federally listed as endangered or threatened, and 69 are being considered for protection. Twenty-one species are presumed extinct (Fish and Wildlife Service 1991; Bogan 1993; Williams et al. 1993). Habitat destruction (including increased siltation, pollution, and river modification, loss of fish hosts, commercial exploitation, and introduced species) are the primary causes of their decline (Bogan 1993). Freshwater mussels live in specific habitat types and are sensitive to many environmental changes.

The recent introduction of the zebra mussel (*Dreissena polymorpha*) to North American freshwater systems poses another threat to already stressed populations of native mussels. Zebra mussel are epifaunal bivalves that colonize all hard substrates, including unionid mussels, by secreting adhesive byssal threads. Having evolved without fouling organisms, unionid mussels have no mechanisms for dealing with epibionts. The decline of unionid mussel populations following the introduction of zebra mussel has been well documented. As early as 1938, Sebestyen suggested that *D. polymorpha* was responsible for the near disappearance of unionid mussels from Lake Balaton, Hungary. A dramatic decline in the abundance of unionid mussels in Lake St. Clair has been observed since the invasion of zebra mussel (Hunter and Bailey 1992; Gillis and Mackie 1994; Nalepa 1994). Schloesser and Nalepa

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(1994) reported 100% mortality of unionid mussels at sites in Lake Erie because of zebra mussel infestation. The relative amount of stress caused by zebra mussel may be species specific. For example, members of the subfamilies Anodontinae and Lampsilinae (long-term brooders) are more sensitive to zebra mussel infestation than are Ambleminae (short-term brooders) (Haag et al. 1993). This suggests that zebra mussel introduction could drastically alter unionid community structure and general biodiversity by affecting the fitness of community members unequally.

Dreissenids have the potential to affect unionid mussels both directly, by actual attachment, and indirectly, through competition for food and nutrients (Hebert et al. 1989; Mackie 1991). In this study, we tested the following hypotheses: (*i*) zebra mussel infestation of unionid mussels causes starvation, (*ii*) unionid mussels in the subfamily Lampsilinae will show greater symptoms of starvation than those in the subfamily Ambleminae, and (*iii*) symptoms of starvation will be greater in unionid mussels that are in low condition. Physiological state was assessed by measuring metabolic rates, O:N ratios, and grazing rates.

Materials and methods

Mussel collection and maintenance

We completed two experiments with each of two species of unionid mussels in fall and spring. In the first two experiments we examined the effects of zebra mussel infestation on *Actinonaias ligamentina*, a long-term brooder in the subfamily Lamsilinae. In the third and fourth experiments, we examined the responses of the short-term brooder, *Amblema plicata* (subfamily Ambleminae).

Specimens of *Actinonaias ligamentina* were collected by SCUBA from the St. Croix River at Wild River State Park, Minnesota, in September 1993 and May 1994. *Amblema plicata* were collected from the St. Croix River at Lakeland, Minnesota, in November 1994 and May 1995. Specimens of *Actinonaias ligamentina* used in the experiments were between 47 and 130 g total wet mass; *Amblema plicata* were between 56 and 315 g. Zebra mussel, *D. polymorpha,* were collected by benthic grab or SCUBA from Lake Michigan at Oak Creek, Wisconsin, in November 1993 and June 1994. Subsequent groups of zebra mussel were collected by SCUBA from Lake Michigan at Milwaukee, Wisconsin, in November 1994 and May 1995. The Milwaukee zebra mussel were larger than the Oak Creek mussel.

To infest experimental unionid mussels with zebra mussel, the unionids were inserted into fine gravel in aquaria, in their natural infaunal position. A layer of zebra mussel was placed on top of them and allowed to attach to the unionids for 48 h. Control unionid mussels were also inserted into gravel in aquaria but were not covered by zebra mussel. Infested specimens of *Actinonaias ligamentina* had an average of 67 ± 3 (mean ± SE) zebra mussel attached, equal to 12.7 ± 0.72 g wet mass. *Actinonaias ligamentina* specimens, therefore, carried 18 ± 1.3% of their own mass (wet), or 14 ± 1.1% by dry mass. Specimens of *Amblema plicata* had an average of 28 ± 1 zebra mussel attached, equal to 28.2 ± 1.0 g wet mass. *Amblema plicata* specimens, therefore, carried 19 ± 0.8% of their own mass (wet), or 19 ± 1.5% by dry mass. The mean numbers of zebra mussel per unionid observed in the field range from less than 10 to over 600 individuals (Lewandowski 1976; Gillis and Mackie 1994). Following infestation, mussels were maintained in Frigid Units Living Streams at 17 ± 1°C, approximating early fall and late spring temperatures in the St. Croix River. The recirculating streams held 500 L of water and had screen and charcoal filtering. Each stream contained 10 cm of substrate, with proportions of cobble, gravel, and sand approximating that of the St. Croix River. Control and infested unionid mussels were held in separate streams.

The mussels were fed one of two diets during an experiment: (*i*) dehydrated *Chlorella* sp. (Algae-Feast, The Earthrise Co., Callpatria, Calif.) (15% lipid, 54% protein, 16% carbohydrate, and 12% inorganic material) or (*ii*) a mixture of preserved diatoms, primarily *Thalassiosira pseudonana* and *Skeletonema* sp. (Diet C, Coast Seafoods, Co., Quilcene, Wash.) (10% lipid, 32% protein, 14% carbohydrate, and 45% inorganic material). *Actinonaias ligamentina* collected in the fall were fed the *Chlorella* diet, while specimens collected in the spring were fed the diatom diet. *Amblema plicata* collected in the fall were fed the diatom diet, while those collected in the spring were fed the *Chlorella* diet. We considered the *Chlorella* diet to be the poorer of the two diets for two reasons. First, bivalves fed diets of *Chlorella* sp., freeze-dried foods, or unialgal cultures grow poorly (Walne 1970; Millican and Helm 1994). Second, we observed that the dried *Chlorella* diet tended to form clumps and quickly settled out of the water column, making it unavailable to the mussels. Therefore, mussels on the diatom diet were fed approximately 1.5% of their dry tissue mass per day, while mussels on the *Chlorella* diet were fed more, at approximately 2.2% of their dry tissue mass per day.

Physiological measurements

In the first experiment, with *Actinonaias ligamentina* collected in the fall, physiological measurements were made at 1, 2, and 3 months following initial infestation. In subsequent experiments, physiological measurements were made at 1, 2, and 4 months following initial infestation. Oxygen uptake, ammonia excretion, and grazing rates were measured for 10–15 control and 10–15 infested unionid mussels, from which zebra mussel had been carefully removed. Water from the streams was filtered (0.45 μm) for use in the experiments. Metabolic chambers consisted of acrylic containers with total volumes of 1400 mL. Temperature was maintained at 18°C (similar to holding temperature) by immersing the chambers in a temperature controlled water bath containing submersible magnetic stirrers. Water in the metabolic chambers was stirred with magnetic bars throughout all experiments. Mussels were scrubbed before experiments and individuals were placed on grid platforms in the chambers.

Rates of oxygen uptake by individual unionids were measured as the oxygen depletion in the metabolic chambers. Oxygen concentrations were measured with YSI oxygen probes (5331) held in Lucite plungers, inserted into the metabolic chambers and connected to YSI biological oxygen monitors (5300). The oxygen probes were calibrated daily with air-saturated water (100% of air saturation). Ten metabolic chambers and two control chambers, without mussels, were run concurrently. Oxygen concentration in each chamber was recorded every 30 min for up to 4 h. Measurements were stopped if the oxygen concentration in a chamber decreased to 50% of air saturation. Although we did not determine critical oxygen concentrations for these species, our data indicated that above oxygen concentrations of 50% of air saturation, metabolic rates were independent of oxygen availability. Air saturation values reached average lows of 75 and 69% in the *Actinonaias ligamentina* and *Amblema plicata* experiments, respectively. Oxygen uptake rates were corrected for the volume of water displaced by the mussels, barometric pressure, and any changes in oxygen concentration in the control chambers.

Nitrogen excretion, measured as the accumulation of ammonia and urea during the oxygen uptake experiments, was measured using an Orion ammonia electrode (model 95-12) connected to an Orion bench-top pH–ISE meter (model 920A). Two 50-mL water samples were drawn from the metabolic chambers immediately upon ending the oxygen uptake measurements. Any urea in the samples was converted to ammonia by adding 3 mg of urease powder (Fisher Scientific, Fair Lawn, N.J.), according to Orion Research Inc. (1990). Samples were stirred during measurements. Four standard curves were produced each day by adding known quantities of Orion ammonia standard (ammonia chloride, 1000 ppm as N) to samples from the control chambers. Ammonia excretion rates were calculated using the

standard curves and corrected for the ammonia concentrations in the control chambers. Atomic ratios of O:N were calculated from the oxygen uptake and nitrogen excretion rates. O:N ratios have been used as a measure of physiological stress (Bayne and Widdows 1978; Widdows 1978).

Grazing rates of individual unionid mussels, measured as the decrease in light absorbance of an algal suspension, were obtained with a Brinkman probe colorimeter (PC 800) equipped with a 450-nm filter, fiber optic light guide, and stainless steel probe tip (5-cm light path) (see Hornbach et al. 1991). The colorimeter was calibrated before each use with distilled water (0% absorbance). Individual unionid mussels were placed in the metabolic chambers in 1000 mL of water. Ten metabolic chambers and one control chamber were run concurrently. Diatom diet was added to the chambers to an initial concentration of 11.6 ± 1.2 mg dry mass·L^{-1} (6.4 mg ash-free dry mass). This concentration approximates the mean total suspended solids observed in the St. Croix River (D.J. Hornbach, unpublished data; U.S. Geological Survey data retrieved through STORET). Light absorbance of each chamber was recorded every 15 min for 90 min. A standard curve was produced each day. Known quantities of diatom diet were added to six 1-L volumes of water, the absorbance was measured, and the water was filtered onto preweighed Whatman glass microfiber filters (934-AH). The filters were then dried (100°C for 24 h) and weighed. Grazing rates were calculated according to Coughlan (1969) and Sprung (1984) using the standard curve and corrected for absorbance changes in the control chamber.

Unionid mussels were wet weighed and measured for height, length, and width. Upon completion of the monthly physiological measurements, the tissues were removed from the shells and the presence or absence of brooded glochidia in the gills was noted. Visceral dry mass of each mussel was determined by oven drying (100°C for 24 h) or by freeze drying to constant mass. Freeze-dried tissues did not lose additional mass if they were oven dried. Unionid mussel shells were dried for several days at room temperature and weighed.

Condition index, a commonly used measure of nutritive status in marine bivalves, was calculated according to Crosby and Gale (1990): condition index = (dry tissue mass (g) × 1000)/internal shell cavity capacity (g), where internal shell cavity capacity (g) = total wet mass (g) – dry shell mass (g).

Data distributions were tested for normality using Shapiro–Wilk W tests and visually inspected using normal quantile plots. Condition index and O:N ratios were arcsin transformed (Zar 1984). Oxygen uptake, ammonia excretion, grazing rates, and dry mass were log transformed (Zar 1984). Analyses of covariance were performed to test the null hypotheses that there were no effects of treatment (infested versus control), collection season (fall versus spring), or interaction on the physiological measures of condition index, oxygen uptake, ammonia excretion, O:N ratio, or grazing rate. Dry mass per individual and days of infestation (30, 60, 90 or 120) were the covariables. If interactions were significant, Student–Newman–Keuls (S–N–K) multiple comparison tests were used to identify differences between specific treatment and season combinations. For months in which sufficient brooding mussels were found, an analysis of covariance (dry mass per individual as covariable) was conducted to test the null hypothesis that there was no effect of reproductive state on the physiological measures. Data are reported as the back-transformed least squares means and standard errors of the means. Statistical analyses were conducted using JMP version 3 software (SAS Institute Inc. 1994).

Results

For both *Actinonaias ligamentina* and *Amblema plicata*, fall-collected specimens had lower condition indices than did spring-collected specimens ($F_{[1,134]} = 54.9$, $P \leq 0.0001$; $F_{[1,166]} = 6.8$, $P = 0.01$) (Fig. 1A). *Actinonaias ligamentina* had a lower condition index than *Amblema plicata* (Fig. 1A). Mass-specific rates of oxygen uptake, ammonia excretion, and grazing were higher in *Actinonaias ligamentina* than in *Amblema plicata* (Figs. 1B–1E). The condition index of *Actinonaias ligamentina* did not change ($F_{[1,134]} = 1.3$, $P = 0.26$) over the course of the experiment regardless of treatment, collection season, or diet. In *Amblema plicata*, condition index decreased (20%) over the course of the experiment ($F_{[1,166]} = 46.9$, $P \leq 0.0001$); however, the decline was similar regardless of treatment, collection season, or diet.

For *Actinonaias ligamentina*, there were significant interactions between treatment and collection season in rates of ammonia excretion ($F_{[1,133]} = 5.0$, $P = 0.03$) and grazing ($F_{[1,132]} = 7.1$, $P = 0.009$) (Figs. 1C and 1E). Unionids infested by zebra mussel or collected in the fall had lower ammonia excretion rates (S–N–K, $P \leq 0.0001$) than controls collected in the spring (Fig. 1C). Mussels collected in the fall and infested by zebra mussel had greater grazing rates (S–N–K, $P \leq 0.0001$) than did the other three treatment–season combinations (Fig. 1E). Both treatment and collection season had significant effects on the oxygen uptake rates of *Actinonaias ligamentina* (Fig. 1B). Unionids infested with zebra mussel had lower oxygen uptake rates ($F_{[1,129]} = 14.7$, $P = 0.0002$) than control mussels. Oxygen uptake was lower ($F_{[1,129]} = 20.9$, $P \leq 0.0001$) in mussels collected in the fall than in those collected in the spring. Only O:N ratios were not significantly affected by treatment, collection season, or their interaction (Fig. 1D).

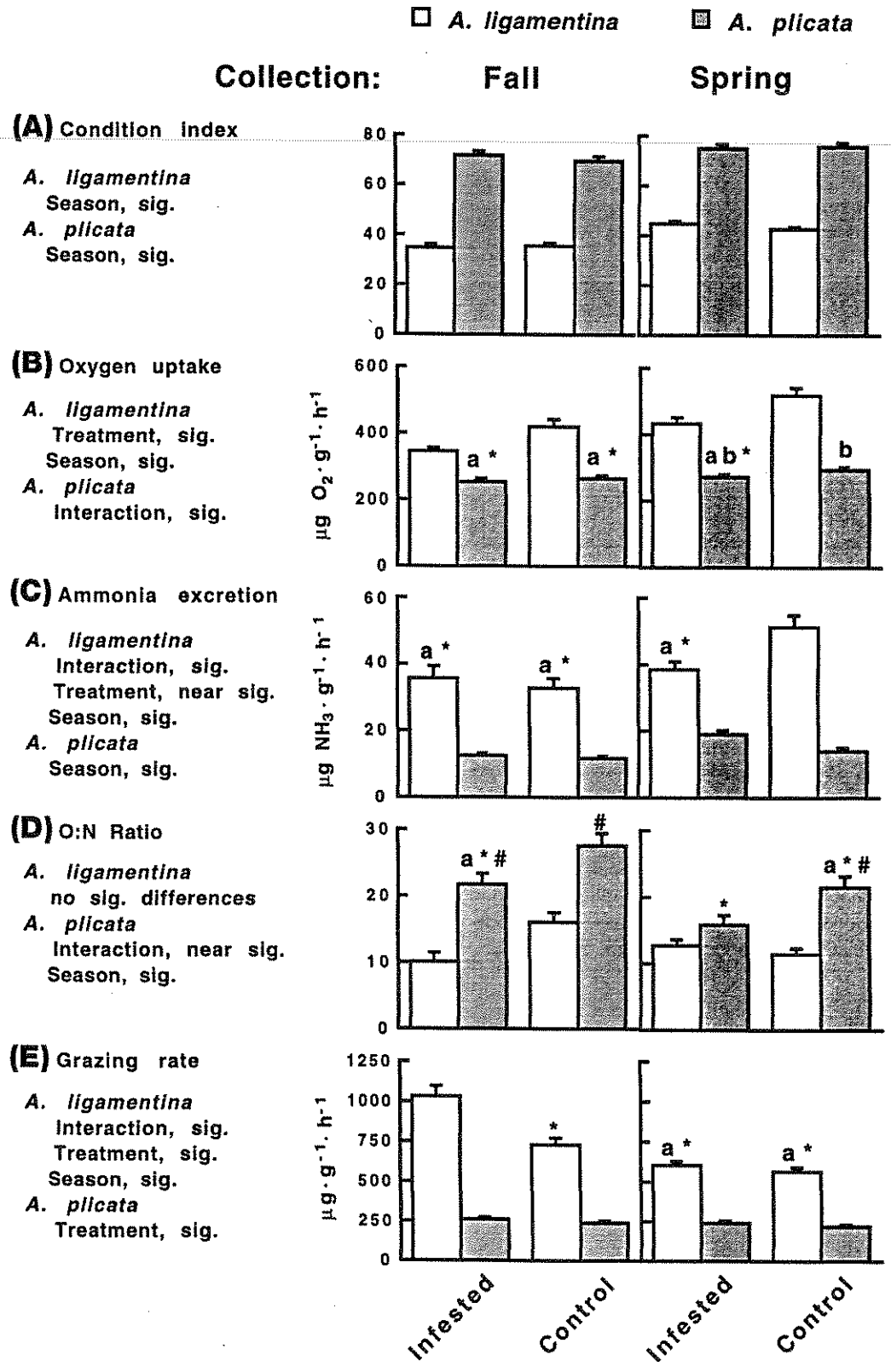
Two of 10 control specimens and 1 of 10 infested specimens of *Actinonaias ligamentina* were brooding glochidia during the day 30 measurements of the fall experiment. One of 10 control specimens and 5 of 9 infested specimens were brooding during the day 60 measurements of the fall experiment. The number of infested specimens brooding in the day 60 measurements was sufficient to test the effects of brooding on physiology. Brooding had no effect on condition index, oxygen uptake, ammonia excretion, O:N ratio, or grazing rate.

No specimens of *Amblema plicata* were found to be brooding glochidia during the experiments. For *Amblema plicata*, there was a significant interaction between treatment and collection season in rate of oxygen uptake ($F_{[1,171]} = 12.5$, $P = 0.0005$) (Fig. 1B). Oxygen uptake was lower (S–N–K, $P = 0.008$) in infested mussels and those collected in the fall than in control mussels collected in the spring. The interaction between treatment and collection season was nearly significant for O:N ratios ($F_{[1,164]} = 3.7$, $P = 0.06$) (Fig. 1D); mussels infested by zebra mussel and (or) collected in the spring had lower O:N ratios than control mussels collected in the fall. Treatment also had a significant effect on the grazing rates of *Amblema plicata* (Fig 1E). Unionids infested with zebra mussel had greater grazing rates ($F_{[1,168]} = 5.9$, $P = 0.02$) than control mussels. Collection season also had a significant effect on ammonia excretion rates (Fig 1C). Ammonia excretion rates were lower ($F_{[1,173]} = 26.5$, $P \leq 0.0001$) in mussels collected in the fall than in those collected in the spring.

Discussion

Our study shows that (*i*) zebra mussel infestation causes stress and symptoms of starvation in unionid mussels, (*ii*) unionid

Fig. 1. *Actinonaias ligamentina* (open columns) and *Amblema plicata* (filled columns): (A) condition index, (B) oxygen uptake rates, (C) ammonia excretion rates, (D) O:N ratio, and (E) grazing rates of control mussels and mussels infested by the zebra mussel, *Dreissena polymorpha*. Mussels were collected in either the fall or spring. Sig., significant differences between treatments (control or infested), collection seasons (fall or spring), or their interaction. For those parameters in which there was a signficant interaction between treatment and collection season, columns that are not significantly different are indicated. Letters (*a* or *b*) indicate pairs of columns within a species that are not significantly different. Symbols (* or #) indicate triplets of columns within a species that are not significantly different (mean ± SE; some SEs are too small to be shown, n = 131–136 for *Actinonaias ligamentina*, n = 166–175 for *Amblema plicata*).



mussel species are affected unequally, and (*iii*) symptoms of starvation are greater when initial condition is low. Of the two mussels examined, *Actinonaias ligamentina* was the most sensitive to infestation as indicated especially by changes in oxygen uptake rate and grazing rate. This supports the report by Haag et al. (1993) that mussels in the subfamily Lampsilinae are more affected by zebra mussel infestation than are those in the subfamily Ambleminae. Following infestation, both species had lower metabolism and greater grazing rates.

Several mechanisms have been suggested that may reduce fitness of unionids colonized by zebra mussels, eventually resulting in mortality. Researchers have proposed that zebra mussel infestation may (*i*) impair locomotion, burrowing, and balance; (*ii*) interfere with valve closure, increasing the risk of predation and parasitism; (*iii*) hinder valve opening, impairing feeding, reproduction, respiration, and excretion; (*iv*) smother siphons, obstructing respiration and feeding; (*v*) strip inhalant water of food, reducing food intake; (*vi*) cause shell deformities, interfering with normal growth; and (*vii*) generate toxic metabolic wastes (Schloesser and Kovalak 1991; Mackie 1991). Our physiological studies indicate that starvation, owing to reduced food intake and (or) increased metabolic costs, is the major mechanism that reduces fitness and eventually causes mortality of unionid mussels infested with zebra mussel.

Ricciardi et al. (1996) reported a decline in unionid mussel condition corresponding with an increase in zebra mussel infestation in the St. Lawrence River. Condition index is proportional to energy stores and is therefore an indication of the health of a bivalve (Bayne et al. 1985). Reductions in the condition of bivalves have been reported as the result of parasitism (Barber et al. 1988), competition for food and space (Côté et al. 1994), turbidity (Mason and Nell 1995), and exposure to metals (Naimo et al. 1992; Couillard et al. 1995). In our short-term experiments, we found that zebra mussel infestation did not affect the condition index of either *Actinonaias ligamentina* or *Amblema plicata*. However, in both species, condition index was higher if mussels were collected in the spring, regardless of the diet subsequently fed to them. This indicates that in these two species of unionid mussel, energy stores are greater in the late spring than they are in the fall.

The condition index of *Actinonaias ligamentina* did not change during the experiments, regardless of which diet was fed to them. This suggests that the *Chlorella* diet and the diatom diet provide equally adequate nutrition for this species. The condition index of *Amblema plicata*, however, decreased during the experiments, but the decrease was the same with both diets. This indicates, again, that the two diets are equally nutritious but that we did not feed *Amblema plicata* enough of either diet.

Metabolic rates of bivalves are influenced by stress and starvation. Stress caused by parasitism (Bierbaum and Shumway 1988; Holopainen and Penttinen 1993) or exposure to toxic metals (Naimo et al. 1992) results in decreased metabolic rates. Starvation or poor diets also lower the metabolism of both freshwater and marine bivalves (Lomte and Nagabhushanam 1971; Bayne 1973; Grant and Cranford 1991). We found that, either alone or in interaction with initial condition, zebra mussel infestation significantly reduced the oxygen uptake rates of both of the mussel species that we examined. In our study, zebra mussels were removed prior to oxygen uptake measurements, so the decline in oxygen uptake rate was not an instantaneous response to infestation. Instead, the reduction in oxygen uptake clearly shows a degradation in nutritional status and a shift to a conservative metabolic strategy. Specimens of *Amblema plicata* that were already in poor condition (collected in the fall) may have been metabolizing near their basal rate and unable to decrease their metabolism further. Oxygen uptake rates indicate that zebra mussel infestation causes stress and (or) starvation, that *Actinonaias ligamentina* is more sensitive to infestation than *Amblema plicata*, and that the degree of response to zebra mussel can depend on the initial condition of the unionid mussel.

Bivalves exhibit a variety of shifts in protein turnover and thus ammonia excretion rates in response to stress and starvation. Marine bivalves increase protein degradation and thus excretion rates in response to stress (Grant and Thorpe 1991), although the initial condition of the bivalve may influence this response. Bayne (1973) found that marine mussels collected in summer (high carbohydrate reserves) decreased ammonia excretion rates in response to nutritive stress whereas mussels collected in winter (low carbohydrates) increased ammonia excretion. Freshwater mussels lower their excretion rates in response to stressors such as metals and turbulence (Aldridge et al. 1987; Naimo et al. 1992). We observed a species-specific response to zebra mussel infestation in the rates of ammonia excretion by unionid mussels. Ammonia excretion rates again indicate that *Actinonaias ligamentina* is more sensitive to zebra mussel infestation than *Amblema plicata*. The variations in response to infestation may reflect species-specific and (or) seasonal differences in tissue chemical composition. The relationships between protein intake, synthesis, catabolism, and deposition in bivalves are not clearly understood.

Atomic O:N ratios provide a more accurate index of metabolic substrate than ammonia excretion alone. O:N ratios are a measure of the balance between the breakdown of proteins and the catabolism of carbohydrates and lipids. Carbohydrate, specifically glycogen, is the major energy storage material in bivalve molluscs (Gabbott 1975). Low O:N ratios (<30) in marine bivalves reflect a high reliance on protein catabolism and are associated with severe stress and starvation (Bayne and Widdows 1978; Widdows 1978). Freshwater mussels, however, appear to have low O:N ratios, ranging from 13 to 78, even under favorable conditions (Aldridge et al. 1987; Fujikura et al. 1988; Naimo et al. 1992). These low O:N ratios are attributed to the breakdown of dietary rather than body protein and may be the result of low rates of protein deposition and growth. The unionid mussels that we studied also had low O:N ratios. Control *Actinonaias ligamentina* had an average O:N ratio of 14, while control *Amblema plicata* had an average O:N ratio of 27. These ratios are similar to those for mussels in which measurements were made immediately upon collection from the field (S.M. Baker and D.J. Hornbach, unpublished data).

As with ammonia excretion rates, bivalves exhibit a variety of shifts in O:N ratios in response to stressors. Many marine bivalves show decreases in O:N ratios when stressed or starved, which are attributed to catabolization of stored protein (Widdows 1978; Grant and Thorpe 1991). The effects of stress on O:N may depend on initial levels of reserves, however. Marine mussels collected in winter or spring (low carbohydrate reserves) decrease O:N ratios when nutritively stressed,

while mussels collected in summer or fall (high carbohydrate reserves) increase the O:N ratio in response to stress (Bayne 1973). The mussels collected in winter or spring begin using stored proteins whereas those collected in summer or fall use stored carbohydrates. Other studies, however, show that whatever the initial level of reserves, starvation results in a rapid reduction in carbohydrates and lipids (high O:N) followed by a balanced use of all body components (lower O:N) (Ansell and Sivadas 1973). In contrast to marine bivalves, freshwater bivalves increase O:N ratios when stressed or starved (Aldridge et al 1987; Naimo et al. 1992). These increases in O:N ratio are attributed to a shift in catabolism to body stores other than protein, such as carbohydrates and lipids. We observed that specimens of *Actinonaias ligamentina* collected in the fall and infested with zebra mussel tended to have lower O:N ratios than control mussels, while those collected in the spring when condition was higher did not. The lower O:N ratios in fall-collected infested *Actinonaias ligamentina* were primarily the result of a relative decrease in oxygen uptake. In *Amblema plicata*, O:N ratios tended to be lower in specimens infested by zebra mussel and were significantly lower in specimens collected in the spring. The low O:N ratios in *Amblema plicata* were primarily the result of relative increases in ammonia excretion. Our O:N data indicate that zebra mussel infestation causes unionids to shift to a more protein-based metabolism, suggesting that food is limiting and (or) metabolic costs are increased, under infestation conditions. The effects of zebra mussel infestation on O:N ratios are species specific, depending on initial condition in *Actinonaias ligamentina*.

Both marine and freshwater bivalves decrease food clearance rates when they are stressed by parasitism (Mane 1975; Bierbaum and Shumway 1988), pollution (Bayne et al. 1979), or high sediment or seston load (Widdows et al. 1979; Aldridge et al. 1987; Grant and Thorpe 1991). Marine bivalves subsisting on a suboptimal diet, however, compensate by increasing filtration rates (Bayne et al. 1993; Iglesias et al. 1992), and starving bivalves boost filtration rates when food is finally supplied (Thompson and Bayne 1972; Bayne et al. 1973; Widdows 1973). Information on the grazing rate response of freshwater mussels to starvation is lacking. In the present study, grazing rates of infested mussels of both species were significantly greater than those of controls. In *Actinonaias ligamentina*, grazing rates were significantly greater in infested mussels collected in the fall when condition was low. In *Amblema plicata*, grazing rates were significantly greater in infested mussels, and fall grazing rates tended to be greater than spring grazing rates. It appears that the infested unionid mussels in our study, especially those in low condition, were experiencing nutritive stress, and when provided with food in the feeding experiments, they compensated by increasing their grazing to above normal rates.

Nutritive stress in infested unionid mussels was indicated by shifts to lower metabolic rates, more protein-based metabolism, and compensatory increases in grazing rates. Several mechanisms working simultaneously may have caused the observed symptoms of starvation. Zebra mussel infestation may cause starvation by increasing the metabolic costs of balance, valve movement, respiration, and excretion. Further, zebra mussel infestation may decrease energy intake as a result of food removal from the inhalant current of the unionid mussel. Zebra mussel attached to individual *Actinonaias ligamentina* and *Amblema plicata* filtered an average of 121 and 130 $mL \cdot h^{-1}$ (approximately 7 and 2 mg dry algae·g dry $mass^{-1} \cdot h^{-1}$), respectively (S.M. Baker and D.J. Hornbach, unpublished data). Specimens of *Actinonaias ligamentina* and *Amblema plicata* filtered an average of 105 and 74 $mL \cdot h^{-1}$ (approximately 0.8 and 0.3 mg dry algae·g dry $mass^{-1} \cdot h^{-1}$), respectively. Therefore, inhalant water could have been turned over 1.1–1.8 times by encrusting zebra mussel before entering the unionid mussel, effectively removing all seston. In addition, Silverman et al. (1995) suggested that zebra mussel are more efficient filter feeders than unionids. Even if food availability was in excess in the water column, food could be limiting in the benthic boundary layer and a depleted feeding zone could develop around the unionid mussel (Wildish and Kristmanson 1984; Fréchette and Bourget 1985; Muschenheim and Newell 1992). Our study indicates that starvation, whether the result of local food depletion or increased metabolic costs, is the underlying cause of the reported decline in energy stores and fitness of infested unionid mussels (Hebert et al. 1991; Haag et al. 1993; Ricciardi et al. 1996) and of the mortalities observed in the Great Lakes (Hunter and Bailey 1992; Gillis and Mackie 1994; Nalepa 1994; Schloesser and Nalepa 1994).

We observed symptoms of starvation despite the short experiment duration (≤4 months) and low infestation load, compared with loads reported for unionid mussels at sites in Lake St. Clair (Mackie 1991; Gillis and Mackie 1994) and western Lake Erie (Schloesser and Kovalak 1991; Schloesser and Nalepa 1994). The infestation loads in this study were similar to those observed in European lakes (Lewandowski 1976), Lake St. Clair sites (Hebert et al. 1989), the upper Mississippi River system (Tucker et al. 1993), and the St. Lawrence River (Ricciardi et al. 1995). Declines in unionid mussel populations have been associated with mean infestations of as few as 10 zebra mussel per unionid mussel (Ricciardi et al. 1996). Even low infestation rates can cause starvation and eventual mortality.

Populations of unionid mussels have suffered species-specific mortality, and thus a decline in diversity, because of zebra mussel infestation. Shell shape and (or) reproductive strategy may be a factor in mortality. Haag et al. (1993) found that, in Lake Erie, there has been a greater reduction in unionid mussels belonging to the subfamilies Anodontinae and Lampsilinae than in those belonging to the subfamily Ambleminae. The Anodontinae and Lampsilinae have light shells and brood their larvae for long periods of time, while the Ambleminae are heavy shelled and brood for shorter periods of time. Thin-shelled, flattened species such as *Potamilus alatus* and *Leptodea fragilis* (both subfamily Lampsilinae) have declined by over 93% in Lake St. Clair. The thicker shelled *Elliptio dilatata* (subfamily Ambleminae) has declined by only 25% since the introduction of the zebra mussel (Gillis and Mackie 1994).

Unionid mussel mortality following infestation is generally attributed to a decline in physiological fitness. Laboratory-infested *Lampsilis radiata* (subfamily Lampsilinae) and *Amblema plicata* have significantly lower energy stores (glycogen content) and greater stress levels (lower inherent cellulase activity) than unionid mussels from which zebra mussel have been removed 4 months previously (Haag et al. 1993). Field-collected specimens of *Lampsilis radiata* also

have reduced glycogen stores and cellulase activity, while field-collected *Amblema plicata* show no reduction in biochemical fitness (Haag et al. 1993). Hebert et al. (1991) reported that the lipid contents of heavily infested unionid mussels are 50% that of uninfested mussels. To date, little else is known about how zebra mussel infestation affects the physiology of unionid mussels.

In summary, we have shown that zebra mussel infestation of unionids causes stress because of starvation and that stress is greater when the condition of the mussels is already low. We also found that unionids in different subfamilies are affected by zebra mussel infestation to varying degrees and that the decline in diversity of unionid mussels since the introduction of zebra mussel may be due to species-specific rates of starvation. The diversity and geographic distribution of unionid mussels will continue to decrease as areas are invaded by zebra mussel. We predict that zebra mussel related mortalities of unionids will be greater in regions where unionids are already stressed by other environmental or anthropogenic factors. Even in bodies of water where zebra mussel infestation is not great enough to be lethal, there may be long-term changes in population density and diversity because of species-specific metabolic stress leading to reduced fecundity.

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