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SUBLETHAL EFFECTS OF CADMIUM ON PHYSIOLOGICAL RESPONSES IN THE POCKETBOOK MUSSEL, *LAMPSILIS VENTRICOSA*

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Abstract—Recent studies indicate that the density and diversity of freshwater mussels are declining in many large river systems, possibly from low-level chemical contamination. Exposure of *Lampsilis ventricosa* (Barnes, 1823) to 0, 22, 111, and 305 μg/L of cadmium for 28 d in a proportional diluter resulted in a significant decrease ($p \leq 0.05$) in respiration rate as cadmium concentration increased. Although variations in cadmium concentrations did not significantly affect food clearance rates or ammonia excretion rates, mussels exposed to 305 μg cadmium per liter showed a decrease in ammonia excretion rates and a decrease in food clearance rates over the 28-d study. Assimilation efficiencies increased during the test in all treatments. Oxygen-to-nitrogen ratios were significantly increased in mussels exposed to either 111 or 305 μg cadmium per liter by day 28. Tissue condition index (TCI) values were significantly lower in mussels in the toxicity test than those in a field sample. The significant change in respiration rate after only a 28-d exposure to cadmium suggests that freshwater mussels may be sensitive indicators of sublethal contaminant exposure. However, the large variability in other physiological responses indicates that the study of contaminant effects requires careful selection of appropriate physiological indicators.

Keywords—Cadmium Freshwater mussels Physiology Energetics

INTRODUCTION

Freshwater mussels are large, long-lived benthic invertebrates that obtain food by filter feeding and are consequently exposed to contaminants that are dissolved in water, associated with suspended particles, and deposited in bottom sediments. Not surprisingly, freshwater mussels bioaccumulate heavy metals [1–3] and pesticides [4–6] more readily than many other aquatic organisms.

Sediments in most temperate rivers are contaminated with heavy metals from point and nonpoint sources. In rivers impounded by locks and dams, such as the upper Mississippi, backwater and main channel border habitats are depositional sites for fine-grained sediments and associated contaminants. These same sites provide important habitats for many species of freshwater mussels. Cadmium, which is discharged into many large river systems from municipal and industrial sources, is toxic to some aquatic organisms at aqueous concentrations as low as 0.8 μg/L [7]. In the upper Mississippi River, for example, surficial sediments are contaminated with cadmium and other heavy metals, even though discharge of cadmium into the river has been reduced in the past two decades [8].

Studies of the effects of cadmium on freshwater mussels have focused on uptake and distribution [3,9–11], lethality [12], detoxification mechanisms [13,14], and sublethal effects [15–17]. However, toxicity tests have largely involved the Asiatic clam *Corbicula* spp. Few acute toxicity studies have been conducted with mussels in the family Unionidae, which contains most freshwater bivalve species in North America.

In some large river systems, toxic contaminants may be contributing to the decline of certain mussel populations. Dissolved contaminant levels in many rivers are low, presumably below levels

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known to have acute effects. Yet subtle biological effects on mussels and other filter-feeding organisms may have occurred in response to continuous low-level contaminant exposure. Changes in the physiological condition of mussels may be an early indication of contaminant stress because physiological measurements represent an integration of both biochemical and cytological effects.

Several physiological responses have been used to evaluate the effects of contaminants on marine bivalves [18–20]. Respiration rate, food clearance rate, ammonia excretion rate, and food assimilation efficiency can be quantified and incorporated into a bioenergetics model known as scope for growth [21]. This model estimates an organism's instantaneous energy budget and quantifies the available energy for growth and reproduction. We applied some of these physiological techniques to freshwater mussels to determine the sublethal effects of cadmium. The objective of our study was to quantify the physiological responses of adult pocketbook mussels, *Lampsilis ventricosa* (Barnes, 1823) exposed to sublethal concentrations of cadmium. We selected *L. ventricosa* for study because it is abundant in the upper Mississippi River and its life history has been partially documented [22].

MATERIALS AND METHODS

Mussel collection

Fifty male and 50 female *L. ventricosa* (same as *L. cardium* [23]) were collected from pool 7 of the upper Mississippi River (river mile 704.5–710.5) on July 21, 1989. Males ranged in length from 96 to 109 mm and in height from 72 to 89 mm. Females ranged in length from 88 to 107 mm and in height from 71 to 92 mm. Average bottom water temperature at all collection sites was 23°C. Mussels were transported in wet burlap on ice to a laboratory in Ames, Iowa, and placed in 57-L flow-through aquaria containing dechlorinated tap water without substrate at a temperature of 8°C. Water temperature was increased 1°C/d to the experimental temperature of 20°C. Each aquarium received 1.44 g of Microfeast Plus L-10® (Zeigler, Gardners, PA) aquatic food every third day during the acclimation period (maximum of 17 d) and the experiments. To minimize disturbance of the mussels during acclimation and testing, aquaria were covered with black plastic.

Exposure system

Mussels were exposed to cadmium in a proportional diluter system with reconstituted Ames, Iowa, tap water. The reconstituted tap water (hereafter referred to as dilution water) was made by continuously adding a 52-g/L solution of sodium bicarbonate (flow rate 6.9 ml/min) and a 1.2 N solution of hydrochloric acid (flow rate 0.26 ml/min) by separate peristaltic pumps to simulate the water quality of upper Mississippi River water at Dresbach, Minnesota. The dilution water (mean ± SE, $n = 18$) had a temperature of 20.5 ± 1.3°C, dissolved oxygen of 8.3 ± 0.3 mg/L, pH of 8.1 ± 0.4, alkalinity of 159 ± 6.2 mg/L as $CaCO_3$, hardness of 165 ± 3.4 mg/L as $CaCO_3$, and conductivity of 653 ± 12.1 μmhos/cm. Analysis of tap water before the study with graphite furnace AAS for metals revealed (mean ± SE, $n = 3$) Na, 17 ± 2.1 mg/L; Pb, <10 μg/L; Cr, <10 μg/L; Cd, <1 μg/L; Hg, <2 μg/L; Zn, 0.6 ± 0.3 mg/L; As, <10 μg/L; Se, <10 μg/L; Cu, <10 μg/L; Fe, <0.5 mg/L; and Al, 38 ± 1.6 μg/L.

A proportional diluter delivered cadmium (as $CdCl_2$) to a series of eight 57-L glass aquaria. Target cadmium concentrations were chosen from a preliminary toxicity test that determined the lower toxicity limit of cadmium to adult *L. ventricosa* over 28 d. Measured mean (±SE, $n = 12$) cadmium concentrations during the study were as follows: 0 (<5 μg/L detection limit), 22.1 ± 2.5, 23.1 ± 3.5, 107.2 ± 3.7, 115.4 ± 3.4, 304.7 ± 1.2, and 305.6 ± 5.5 μg/L, with two replicates per treatment. The controls were below the detection limit by flame AAS, but the graphite furnace method indicated that cadmium levels were below 1 μg/L in the tap water.

Once cadmium levels in the test aquaria stabilized, 10 mussels were randomly chosen, numbered with a Moto-tool® (Dremel, Racine, WI) tool, and placed into each treatment tank (20 mussels per treatment). If a mussel died during the test, it was removed and replaced with a new mussel from a holding tank. Replacement mussels were used to maintain similar biomass among tanks but were not used in any of the physiological measurements. Because the physiological responses of all 80 mussels could not be measured in 1 d, the start of the test was staggered; two of the eight tanks were randomly chosen and started on four different days.

Respiration rate, ammonia excretion rate, and food clearance rate were measured and assimilation efficiency was calculated for each mussel at day 0 (before cadmium exposure), day 14, and day 28. The first three rates were determined in an environmental chamber at a temperature of 20°C under subdued lighting. On exposure day 28, all mussels were separated into shell and tissue to obtain the dry weight for each component. Estimates of res-

piration, ammonia excretion, and food clearance rates were expressed on the dry tissue weight of the mussels at day 28.

Metal analysis

Cadmium concentrations in the dilution water were analyzed weekly. Water samples were taken from each tank, acidified with Baker® instra-analyzed nitric acid (J.T. Baker, Phillipsburg, NJ) (16 N) to pH <2, and analyzed by direct aspiration into an AA-flame ionization (FI) spectrophotometer (Instrumentation Laboratories, Wilmington, MA, AA/AE spectrophotometer, model 251). Every sampling period, each tank was sampled in triplicate and three samples were randomly spiked; the mean percentage of recovery of the spiked samples was 97.7% ($n = 12$).

Physiological measurements

Because *L. ventricosa* is principally an ammonotelic organism (excretes primarily ammonia), excretion rates were determined by measuring the total ammonia nitrogen (TAN) excreted with methods of Aldridge et al. [24]. Each mussel was isolated in a 1-L beaker containing 0.7 L of dilution water for 1 h, and the TAN concentration of the dilution water was then measured with an Orion (Boston, MA) ammonia electrode (model 95-12) and an Orion specific ion meter (model 407A). The excretion rate for each mussel was determined by subtracting the TAN concentration of the dilution water without mussels from the TAN concentration of dilution water with mussels and was reported as milligrams of TAN per hour per gram dry weight.

Respiration rates were monitored in respirometers made from 1.5-L glass canning jars; the snap-on lids were modified to allow insertion of a dissolved oxygen probe (YSI® (Yellow Springs, OH) model 5739 connected to a YSI model 51-B dissolved oxygen meter). The respirometers were placed in a water bath at 20°C. The bottom of each respirometer contained a stir bar under a perforated plastic platform that was operated by a magnetic stir plate positioned under the water bath. Each mussel was cleaned with a toothbrush to remove attached growth that could have contributed to oxygen consumption and placed into the respirometer. After a mussel began siphoning water, oxygen concentration was measured every 10 min until the percentage of saturation fell below 65%. Respiration rates were recorded as milligrams of oxygen per hour per gram dry weight.

The food clearance rate of a mussel is defined as the volume of water cleared of particles per unit time [25]. Bayne et al. [21] found that short-term food clearance rates were similar in both static and flow-through tests. Consequently, each mussel was isolated in a 1-L beaker containing 0.7 L of dilution water and 30 mg/L of Microfeast Plus L-10 aquatic food. After 15 min, three 30-ml samples were removed from each beaker, and the remaining particle concentration was measured by a Hach® (Loveland, CO) ratio turbidimeter (model 18900). Determination of liters of water cleared per hour was made by comparing particle concentrations in beakers with and without mussels.

The efficiency of food absorption by the digestive system was calculated as the proportion of organic matter in the food to the proportion of organic matter in the feces. The organic content of both food and feces was defined as the ratio of ash-free dry weight (muffle sample at 550°C for 4 h) to dry weight (dry sample at 100°C for 48 h). The mean (± SE) ratio of 20 Microfeast Plus L-10 samples was 0.883 ± 0.001. Because this measurement is a ratio, quantitative collection of mussel feces was not necessary. A system was developed that delivered a constant food supply for 18 h into multiple containers (without cadmium), with food inflow at the bottom and outflow at the top. After 18 h, feces (including pseudofeces) were collected, and most of the water was removed from the sample with capillary tubes. Because mussel feces consist of particles enclosed in a mucilaginous sheath, they were easily distinguished from uneaten food. The feces sample was dried, weighed, muffled, and reweighed. The percent assimilated (e) was estimated by Conover's [26] formula:

$$e = (F - E)/(1 - E)F$$

where F = ash-free dry weight:dry-weight ratio of food and E = ash-free dry weight:dry weight ratio of feces.

Tissue condition index (TCI) often is used as a measure of physiological condition in bivalves [27,28]. The index was determined by dividing the tissue dry mass by shell dry mass and multiplying the quotient by 100. Values for test animals were compared to a sample of 33 *L. ventricosa* that was collected from pool 7 of the upper Mississippi River and processed immediately.

The final test used to assess the physiological condition of a mussel after cadmium exposure was the oxygen-to-nitrogen (O:N) ratio. This ratio of moles of oxygen consumed to moles of nitrogen excreted is an index of protein use in metabolism [29–31]. In a study of freshwater mussels, Aldridge

et al. [24] reported O:N ratios of <20 indicated catabolism based on proteins, and O:N ratios >100 indicated catabolism based on lipids and carbohydrates.

Statistical analyses

The physiological responses of the organisms represented a split-plot experimental design with each experimental unit (treatment tanks) measured repeatedly over time [32,33]. Because time is a factor that cannot be randomized, a repeated measure analysis was used, allowing incorporation of data from several time periods without assuming that sampling dates were independent of each other. Another assumption of this analysis was that errors for differences between tanks receiving the same treatment should be larger than errors made measuring the same tank on different occasions. Differences among cadmium treatments were tested with the variance of the treatment tanks nested within cadmium as the main-plot error term (F with 3,4 *d.f.*). The subplot contained the variance of time by treatment tanks within cadmium as the error term for testing either the effects of time (F with 2,8 *d.f.*) or the time by cadmium effects (F with 6,8 *d.f.*). All analysis of variance (ANOVA) tests were done with the general linear models (GLM) procedure in the Statistical Analysis System (SAS®) [34]. Null hypotheses were rejected at $p \leq 0.05$. Data are expressed as mean plus or minus one standard error.

RESULTS

Except for food assimilation efficiency and O:N ratio, the physiological responses of the mussels exposed to increasing cadmium concentrations did not change significantly over time. Hence, we averaged the responses of individuals from days 14 and 28 when plotting respiration rates, food clearance rates, and ammonia-nitrogen excretion rates; day 0 measurements were not included in the average because they represented the baseline physiological rate for a specific cadmium treatment (Table 1). Physiological responses were not significantly different among mussels in replicate tanks at a specific cadmium concentration (Table 2).

Respiration rates were significantly different in mussels exposed to either 22, 111, or 305 μg cadmium per liter from those in unexposed mussels; mussels exposed to 305 μg cadmium per liter had respiration rates that were significantly different from the rates of mussels in all other treatments.

Table 1. Mean (±SE) respiration, ammonia excretion, and food clearance rates; assimilation efficiency; and oxygen-to-nitrogen ratio in *Lampsilis ventricosa* exposed to different cadmium concentrations

	Exposure concentration			
	Control	22 μg Cd/L	111 μg Cd/L	305 μg Cd/L
Respiration rate (mg O_2/h/g dry wt.)				
day 0	0.496 ± 0.050	0.357 ± 0.044	0.462 ± 0.066	0.367 ± 0.042
day 14	0.568 ± 0.065	0.567 ± 0.066	0.575 ± 0.059	0.405 ± 0.053
day 28	0.558 ± 0.067	0.438 ± 0.052	0.440 ± 0.070	0.358 ± 0.085
Ammonia-nitrogen excretion rate (mg NH_3-nitrogen/h/g dry wt.)				
day 0	0.016 ± 0.004	0.013 ± 0.001	0.013 ± 0.002	0.022 ± 0.004
day 14	0.017 ± 0.004	0.017 ± 0.001	0.013 ± 0.002	0.016 ± 0.002
day 28	0.014 ± 0.002	0.016 ± 0.003	0.006 ± 0.001	0.004 ± 0.002
Food clearance rate (L H_2O/h/g dry wt.)				
day 0	0.017 ± 0.002	0.029 ± 0.003	0.020 ± 0.004	0.030 ± 0.003
day 14	0.033 ± 0.007	0.025 ± 0.003	0.016 ± 0.003	0.025 ± 0.003
day 28	0.032 ± 0.003	0.025 ± 0.003	0.032 ± 0.004	0.010 ± 0.003
Assimilation efficiency (% assimilation)				
day 0	−43.7 ± 10.0	20.9 ± 9.5	−16.6 ± 11.2	−39.6 ± 14.9
day 14	−5.2 ± 13.6	23.5 ± 7.1	−2.0 ± 14.4	0.2 ± 15.6
day 28	31.6 ± 15.5	46.6 ± 5.7	56.3 ± 7.2	17.9 ± 8.3
Oxygen:nitrogen ratio (moles oxygen consumed to moles nitrogen excreted)				
day 0	77.6 ± 23.7	29.0 ± 6.3	51.5 ± 14.0	24.7 ± 5.9
day 14	45.7 ± 7.7	31.4 ± 4.8	47.7 ± 7.2	28.6 ± 5.1
day 28	45.3 ± 6.0	39.3 ± 12.9	85.6 ± 23.9	125.5 ± 35.0

$n = 20$.

Table 2. Effects of cadmium exposure on the respiration, ammonia excretion, and food clearance rates; assimilation efficiency; and oxygen-to-nitrogen ratio in *Lampsilis ventricosa*

	p value				
Statistical question	RESP[a]	TAN[b]	CLEAR[c]	ASSIM[d]	O:N[e]
Were there treatment differences?	0.022	0.824	0.749	0.182	0.396
Were there differences between the two tanks at each treatment level?	0.921	0.078	0.072	0.099	0.063
Were there differences over time?	0.207	0.052	0.980	0.003	0.003

Null hypotheses rejected at $p \leq 0.05$.
[a]RESP = respiration rate (mg oxygen per hour per gram dry weight).
[b]TAN = ammonia excretion rate (mg NH_3-nitrogen per hour per gram dry weight).
[c]CLEAR = clearance rate (liters of water per hour per gram dry weight).
[d]ASSIM = assimilation efficiency (percent assimilated).
[e]O:N = moles oxygen consumed to moles nitrogen excreted.

Mean respiration rates decreased from 0.563 ± 0.046 mg oxygen per hour per gram dry weight in control mussels to 0.382 ± 0.045 mg oxygen per hour per gram dry weight in mussels exposed to 305 μg cadmium per liter (Fig. 1a).

Ammonia excretion rates were 34% lower in mussels exposed to 111 μg cadmium per liter and 25% lower in mussels exposed to 305 μg cadmium per liter than those in controls (Fig. 1b), but the differences were not significant (Table 2). Ammonia excretion rates in controls were similar over the 28-d exposure period (Table 1). The TAN excretion rates in mussels exposed to 305 μg cadmium per liter fell from a mean of 0.022 mg NH_3-nitrogen per hour per gram dry weight at day 0 to a mean of 0.004 mg NH_3-nitrogen per hour per gram dry weight by day 28 (Table 1). However, the change was not significant over time (Table 2, $p = 0.052$).

Although food clearance rate measurements tended to exhibit a dose-response relation, with food clearance rates decreasing with increasing cadmium concentration, variances were high and no statistical differences existed (Fig. 1c). The average clearance rate in mussels exposed to 305 μg cadmium per liter was 0.018 ± 0.002 L water per hour per gram dry weight compared with the rate of 0.033 L water per hour per gram dry weight in controls (Table 1). Mussels exposed to cadmium exhibited greater mucus production than that of controls during measurements of ammonia excretion and food clearance.

Determination of TCI required disposing of the mussel and, therefore, was calculated only on day 28 for all treatments. The TCI values of all mussels remained similar over all cadmium exposure levels and did not exhibit a dose-response relation (Fig. 1d). However, the TCI values of all mussels used in the toxicity test were significantly lower than the mean TCI (7.91 ± 0.37) of *L. ventricosa* from the field.

Food assimilation efficiency was the most variable physiological response in this study. Assimilation efficiencies in most mussels were negative on day 0, which indicated a higher percentage of organic matter in the feces than in the food (Fig. 2a). Food assimilation efficiencies increased significantly over time, indicating that the percentage of organic matter in the feces declined (Table 2).

The oxygen-to-nitrogen ratios (O:N) of all mussels were not significantly different at day 0 and day 14, but by day 28, those mussels exposed to either 111 or 305 μg cadmium per liter had O:N ratios that were significantly greater than O:N ratios in unexposed mussels (Fig. 2b). Over the course of the study, O:N ratios decreased in control mussels but increased in mussels exposed to either 111 or 305 μg cadmium per liter.

DISCUSSION

The physiological characteristics of *L. ventricosa* were often highly variable, masking detection of sublethal cadmium effects at an acceptable statistical level. Changes in the least variable parameter, respiration, were significant in response to various cadmium concentrations. In contrast, neither food clearance nor ammonia-nitrogen excretion rates changed significantly in response to various cadmium concentrations.

The mean respiration rate in mussels exposed to 305 μg cadmium per liter in this study (0.382 ± 0.045 mg oxygen per hour per gram dry weight) was similar to the mean respiration rates in three unionid species (*Quadrula pustulosa, Fusconaia cerina*, and *Pleurobema beadleanum*) after exposure

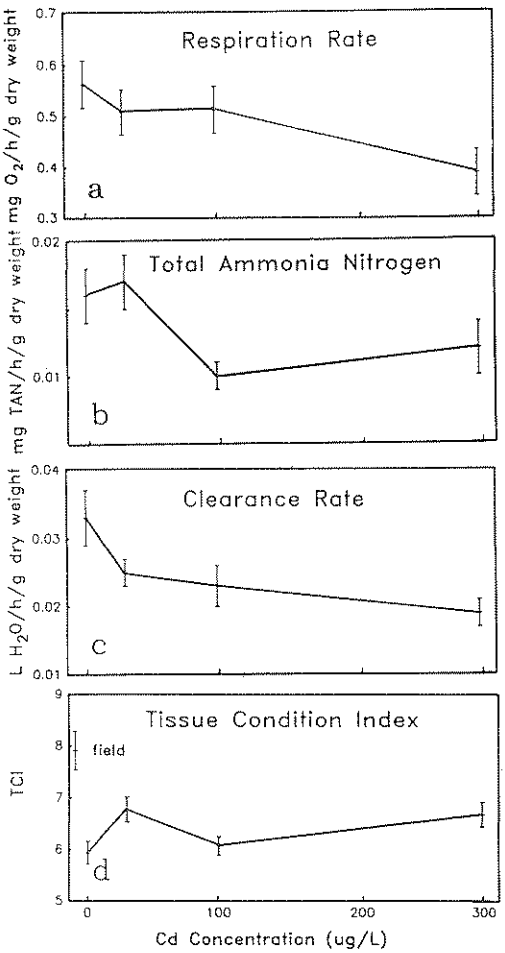

Fig. 1. Mean (±SE) combined respiration rate (**a**), ammonia excretion rate (**b**), and food clearance rate (**c**) of *Lampsilis ventricosa* from days 14 and 28 of cadmium exposure. Mean (±SE) tissue condition index (TCI) (**d**) based on tissue dry weight on day 28 of cadmium exposure; field samples represent 33 *Lampsilis ventricosa* collected from the river and processed immediately for TCI measurements. TCI = (tissue dry weight/shell dry weight) × 100.

to infrequent turbulence and turbidity [24]. The reduced respiration rate of cadmium-exposed mussels could have resulted from the large production of mucus on the gill surfaces. The mucus could have reduced the efficiency of gas exchange across the gills and resulted in lower rates of oxygen uptake. In the absence of enough available oxygen, mussels could respond by either increasing oxygen extraction efficiency or decreasing metabolic rate. Because a reduction in oxygen uptake often indicates a lower metabolic rate for aerobic organisms [35], mussels exposed to cadmium probably responded by decreasing metabolic rates rather than by increasing oxygen extraction efficiency.

Bivalves commonly decrease food clearance rates when stressed [24,25,36]. A decrease in the metabolic activity of mussels exposed to cadmium may also reduce both the amount of water passing over the gills and the amount of food collected. Furthermore, if the gills are coated with mucus, their functional capacity to sort and transport food may be reduced. However, we observed neither a significant reduction in food clearance rates nor a significant decrease in TAN excretion rates after exposure to cadmium. We expected a decrease in TAN excretion rates as the mussels lowered their metabolic activity and began to use their extensive carbohydrate reserves. Aldridge et al. [24] reported significant decreases in nitrogen excretion rates in three species of freshwater mussels (*Q. pustulosa*, *F. cerina*, and *P. beadleanum*) exposed to frequent turbulence (7 min every 0.5 h) compared with infrequent turbulence (7 min every 3 h).

Assimilation efficiency measurements in freshwater mussels were highly variable in this experiment. We are not sure why most mussels exhibited negative assimilation efficiencies before cadmium exposure, but assimilation efficiencies significantly increased in all treatments by day 28. However, estimates of food clearance rates in mussels exposed to 305 μg cadmium per liter were only 31% of the rates in control mussels by day 28 and probably represented a substantial reduction in food intake. Therefore, the higher assimilation efficiencies in these mussels might have resulted from a breakdown of the large glycogen stores into simpler inorganic molecules that increased the percentage of inorganic material in the feces. Because a reduction in clearance rate is a common response to environmental stress [24,25,36] and because the measurement of food assimilation depends on an actively feeding mussel, the utility of the food assimilation efficiency measurement is questionable. Different methods, such as the use of radiotracers, provide more information on the fate of the organic portion of the food.

Any alteration in the use of carbohydrates and proteins as energy sources should be reflected in the O:N ratio. In *Mytilus edulis*, starvation resulted in a shift from catabolism of carbohydrates (high O:N ratio) to catabolism of proteins (low O:N ratio [31]). Although researchers suggest that low O:N ratios in marine organisms are indicative of a stressed condition [21,31], studies of freshwater molluscs do not support this suggestion. Oxygen-

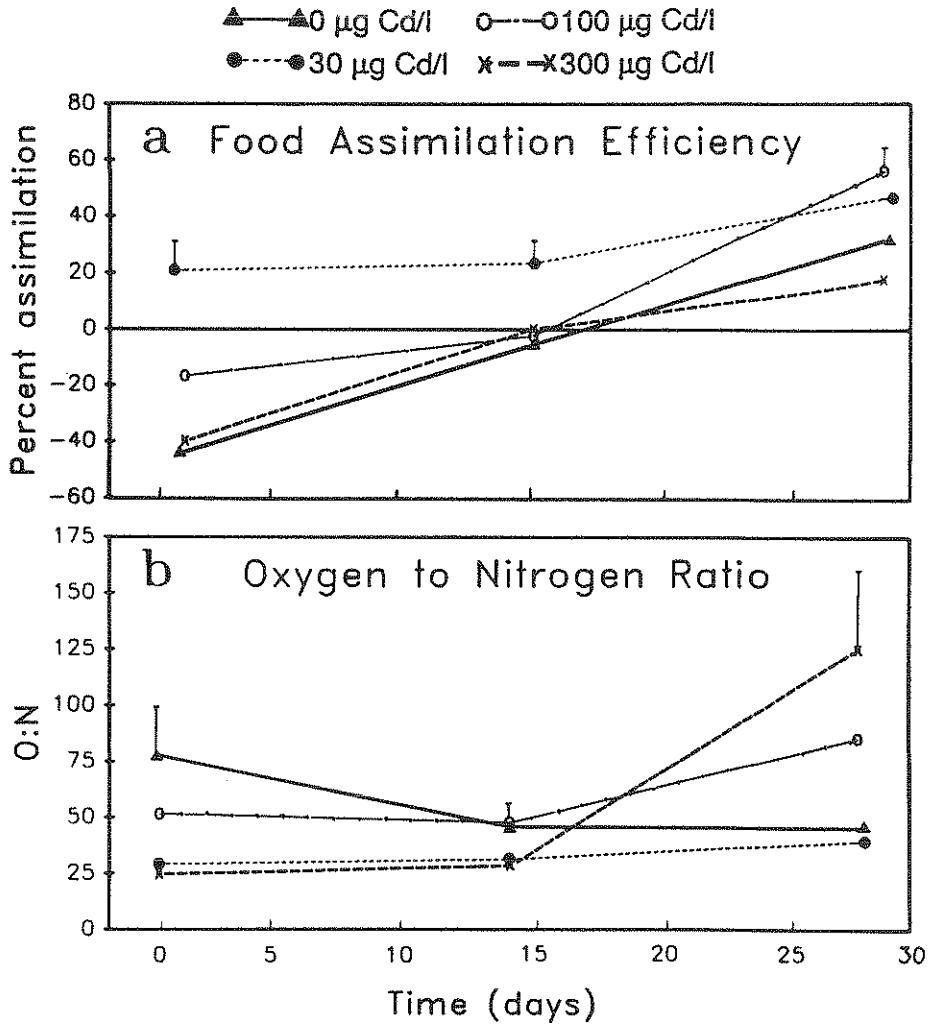

Fig. 2. Changes in (a) food assimilation efficiency and (b) O:N ratios over time at different cadmium concentrations. Means represent the average response of 20 mussels per treatment per time. Food assimilation efficiency measured as percent of food assimilated. O:N ratios measured as moles of oxygen consumed to moles of nitrogen excreted. Measured cadmium concentrations were 0, 22, 111, and 305 µg per liter.

to-nitrogen ratios in *Helisoma trivolvis* increased from 16.02 in control snails to 84.60 in unfed snails after 124 d [30]. Exposure of *Q. pustulosa* to turbidity resulted in a significantly greater mean O:N ratio of 233.5 compared with a mean O:N value of 17.2 in the reference individuals [24]. In the present study, O:N ratios also increased after the 28-d exposure to 305 µg cadmium per liter, indicating catabolism of carbohydrates. The large glycogen stores [37] in freshwater mussels were a likely source of this carbohydrate material.

Measurements of subtle physiological responses of freshwater mussels to a contaminant were problematic. Natural variability was high for most measures. A common technique of increasing statistical confidence is to increase either the number of replicates or the number of organisms per treatment. However, although adult freshwater mussels can be regionally abundant, once restrictions are placed on species, size, and sex, sufficient sample sizes can be limiting. Another problem in measuring the responses of mussels to contaminants is their tendency to avoid acute contaminant exposure by closing their valves [38–40]. Valve closing may not necessarily be a hindrance, as valve opening and closing could serve as a toxicity test end point.

The nutritional status of freshwater mussels has often been overlooked when assessing the effects of contaminant exposure on bioenergetics; information on the nutritional requirements of freshwater mussels is lacking. Even when food is present, freshwater mussels may use body reserves for many months without visible effects; it could be interpreted that they are being well fed. Tissue condition index is often used to assess the nutritional and reproductive status of an individual. The higher the TCI, the greater the proportion of tissue mass to shell mass. In the present study, exposure to cadmium did not affect TCI, but TCI values were lower in *L. ventricosa* in the laboratory than in *L. ventricosa* in the field. Feeding and maintenance of organisms such as freshwater mussels in a laboratory setting to maintain healthy test organisms are critical but, to date, have proven difficult.

Freshwater mussels in nature are likely exposed to an array of contaminants (including cadmium) that are far more chemically complex than the dissolved form tested in this study. However, the experimentation and methods development in the present study are fundamental to the ultimate analysis of realized environmental effects of contaminants on the freshwater mussel community. We have found that physiological criteria applied to test similar reactions to contaminants in other species (e.g., marine bivalves) prove highly variable in freshwater mussels. Further methods development or development of less variable, but still sensitive, measures will be necessary to proceed in evaluation of contaminant effects on freshwater mussels.

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