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INFLUENCE OF DIET ON SURVIVAL, GROWTH, AND PHYSIOLOGICAL CONDITION OF FINGERNAIL CLAMS *MUSCULIUM TRANSVERSUM*

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ABSTRACT The effects of diet and laboratory holding time on survival, growth, and physiological condition of fingernail clams *Musculium transversum* were evaluated in a 112-day study. The diets included a commercial oyster diet, a suspension of commercial rabbit pellets, a suspension of fine, organic-rich sediment, and a complete sediment renewal every 14 days. Sediment and clams were obtained from a relatively uncontaminated site in the Upper Mississippi River. The experimental design consisted of 18 370-mL beakers per diet, each containing 5 cm of surficial sediment and 15 clams. Survival of clams was measured daily in each unit. Three units from each diet were randomly removed on days 7, 14, 21, 28, 56, and 112, and clams were measured for shell length. Glycogen and cellulase activity were measured in composite samples (5 clams per sample) at each of the six time intervals. Cellulase activity did not vary among diets or with time. Survival, growth, and glycogen varied significantly among diets, and glycogen concentrations varied with time, regardless of diet. Clams exposed to the two sediment diets were 2.4 times more likely to survive than clams exposed to the commercial diets. Survival of clams in all diets exceeded 80% through day 21. Although clams maintained an acceptable survival rate for 21 days, their physiological condition was compromised much earlier, given that glycogen reserves were reduced by 14–54% after only 7 days. Thus, laboratory tests with fingernail clams should include physiological measures, in addition to survival, to ensure that clams are in suitable condition before and during testing.

KEY WORDS: Diet, *Musculium transversum*, survival, growth, biomarker

INTRODUCTION

Fingernail clams are an important component in the benthic invertebrate community of many large rivers and, in the Upper Mississippi River, have undergone periodic, pronounced declines in abundance in recent decades (Wilson et al. 1995). For example, densities in Pool 19 (near Keokuk, IA) averaged 32,000/m^2 in 1985 and progressively declined to 0 in 1990, and river-wide recovery has been slow. Toxicity of bulk sediment or pore water has been suggested as a factor contributing to the decline in fingernail clams in the river (Wilson et al. 1995). In particular, concentrations of un-ionized ammonia in sediment pore water from the Upper Mississippi River often exceed concentrations demonstrated to inhibit growth of fingernail clams in laboratory studies (Frazier et al. 1996). To assess these and other potential causes of the decline in abundance requires that clams be collected from the field, held in the laboratory, and tested through controlled experimentation. However, information on the relative condition of clams during long-term holding and its effect on the outcome of laboratory tests is lacking (Naimo et al. 2000).

The physiological condition of an organism is dependent upon its nutritional status (Lanno et al. 1989, Foster et al. 1993). Yet, the importance of nutrition as a factor modifying physiological condition has been largely overlooked. Data on how the condition of an organism responds to its nutritional status are critical for understanding the importance of diet as a variable in designing experimental studies with benthic organisms.

Recently, physiological indicators of condition such as glycogen concentration and cellulase activity have been used to assess the relative health of bivalve mollusks (Hemelraad et al. 1990, Haag et al. 1993, Farris et al. 1994, Naimo et al. 1998). Glycogen is the most readily available storage form of glucose in many animals, including freshwater mussels. As such, glycogen concentrations have been used successfully as an indicator of physiological condition in unionid mussels after exposure to contaminants (Hemelraad et al. 1990) and after infestation by zebra mussels (Haag et al. 1993). Similarly, cellulase activity is an indirect measure of feeding because it measures the rate of breakdown of complex sugars into simple molecules (Farris et al. 1988). Extensive use of cellulase activity in monitoring programs for molluscs has shown that responses at the biochemical level can be measured where pollutants or stress first exert their effect (Beeby 1993, Milam and Farris 1998). In these studies, the predictive capability of the enzyme assay has been compared with extensive testing of more traditional biological endpoints in toxicity assessments. Controlled laboratory and field exposures have provided evidence that reductions in enzyme activity are related to the eventual survival of the animal and to more subtle changes that occur in filtration, growth, and bioaccumulation rates (Farris et al. 1994, Milam and Farris 1998).

We examined survival, growth, and physiological condition in clams provided different food sources in a 112-day laboratory study. Our specific objective was to evaluate the effect of diet on the survival, growth, and physiological condition of fingernail

clams *Musculium transversum* (Say 1829). Furthermore, because we were interested in the transferability of these data to standardized tests with benthic invertebrates, we examined differences in survival, growth, and physiological condition between clams fed two commercially available diets (easily reproducible, but a non-indigenous diet) and two diets containing sediment (not as reproducible, but more indigenous).

MATERIALS AND METHODS

Experimental Design

We obtained about 600 fingernail clams with a Ponar dredge from Pool 13 of the Upper Mississippi River for use in the laboratory test. During collection, clams were placed in ice chests containing sediment and water from the river. The water in the ice chests was aerated and its dissolved oxygen content was measured at 30-min intervals to maintain concentrations above 60% of saturation. To obtain an estimate of the physiological condition of clams at this point in time, we obtained an additional 15 clams, placed them on dry ice in the field, and stored them at −84 °C in the laboratory before analysis of glycogen concentration and cellulase activity.

The uppermost 5 cm of sediment from a single sampling site in Pool 7 of the Upper Mississippi River (Lake Onalaska, river mile 704.5) that contained an abundant fingernail clam population was obtained with a van Veen dredge. Sediment was placed into 4-L glass jars, held on ice, transported to the laboratory, and stored in a refrigerator for no more than 5 days before the start of the test. Three subsamples of homogenized sediment (each 20–25 g wet weight) were analyzed to describe textural composition (Guy 1969, Plumb 1981) and volatile matter content (American Public Health Association et al. 1992). Sediments averaged (mean ± 1 standard error [SE]) 4 ± 0.2% sand, 54 ± 2.4% silt, 42 ± 1.8% clay, and 7.8 ± 0.9% volatile matter.

The experimental unit was a 370-mL beaker. All experimental units were placed into one of two 900-L water baths (3 m length × 0.8 m width × 0.4 m height). Each water bath was partitioned lengthwise with Plexiglas to provide four compartments, one for each diet. Eighteen experimental units were randomly allocated into each compartment. A temperature of 17 ± 2 °C was maintained with submersible quartz heaters. About 24 h before the addition of clams, 184–188 g of surficial sediment (about 4–5 cm) and 200 mL of well water from the Upper Midwest Environmental Sciences Center were added to each experimental unit. On day 0, we randomly allocated 15 clams, each measuring 4–6 mm in shell length, into each experimental unit.

We measured the temperature, pH, and dissolved oxygen of the overlying water every Monday, Wednesday, and Friday in five randomly selected experimental units in each diet. Because fingernail clams are particularly sensitive to un-ionized ammonia (Hickey and Vickers 1994), we measured concentrations of total and un-ionized ammonia in three randomly selected experimental units every 14 days (Frazier et al. 1996). On days 7, 14, 21, 28, 56, and 112, clams from three randomly selected experimental units from each diet were sieved from test sediments, counted, recorded as dead or alive, measured for shell length to the nearest 0.1 mm, and stored at −84°C for later analysis of glycogen concentrations and cellulase activity. Glycogen concentrations (Naimo et al. 1998) and cellulase activity (Farris et al. 1988) were measured on composite samples containing five individuals from each experimental unit. Glycogen concentrations were reported as mg/g wet weight, and cellulase activity was expressed as a product (exocellulase activity times endocellulase activity in [units/g dry weight]^2). One unit of the enzyme is defined as the amount of enzyme required to liberate 1 mg of reducing sugar equivalent to that of glucose per hour with carboxymethylcellulose as a substrate.

Diet and Ration

Clams were fed one of four diets daily; two were commercially available diets, and two were formulated with sediments from the Upper Mississippi River (sediment diets). The commercial diets included an oyster diet, which was a mixture of two marine diatoms (50% *Thalassiosira pseudoana* and 50% *Skeletonema sp.*) fed at a rate of about 7μL/clam/day (8–10 × 10^9 cells/mL; Pacific Oyster Diet B, Coast Seafood Company, Quilcene, WA). The second commercial diet was a suspension of Kaytee® rabbit feed, with pellets made largely from alfalfa, fed at a rate of 2.5 mg/clam/day. The two sediment diets contained organic-rich sediments from relatively uncontaminated areas in the river and were the same sediment used as the substrate in all experimental units. One was a suspension of fine sediment fed at a rate of 2.5 mg/clam/day, and the other was a complete sediment renewal every 14 days.

The oyster diet, rabbit pellet diet, and suspended sediment diet were prepared about 2 days before the start of the experiment. The oyster diet comes in liquid form and was kept refrigerated. The rabbit pellet and the suspended sediment diets were prepared by blending 38 g of rabbit pellets or sediment with 400 mL of well water in a commercial blender for 5 min. The contents of the blender were transferred into a 1,000-mL volumetric flask and filled to the meniscus with well water. This process was repeated until we obtained 32 140-mL bottles of each diet. Once a week, one bottle of food for each diet was removed from a −20°C freezer and placed into a refrigerator; the quantity of food in each bottle was sufficient to feed all clams receiving those diets for 1 wk. Clams in the sediment-renewal diet were sieved from test sediments every 14 days, and another aliquot of sediment was replaced into each experimental unit. Sediments for this diet were the same sediments that were obtained at the start of the test, stored in a refrigerator until needed.

Statistical Analyses

Survival of clams was assessed by daily counts of dead shells on the sediment surface. In addition, at the six time intervals in which clams from three beakers were removed for physiological measurements, we also made direct mortality estimates; these data allowed us to check the accuracy of the daily mortality counts. Because these two estimates agreed more than 90% of the time, analyses of survival rate were performed on daily survival counts. We used the Cox proportional hazards model to determine whether survival rates of clams varied among diets (Cox 1972). To test for differences in survival between the commercial and sediment diets, we used the Wald test of equality (Parmar and Machin 1995).

We analyzed growth, glycogen concentrations, and cellulase activity with analysis of covariance (ANCOVA), with time in the laboratory as the covariate. Because most clams did not survive after day 56, statistical analyses were only conducted until day 56. Orthogonal contrasts were used to compare differences in growth and physiological condition between the commercial and sediment diets when the ANCOVA was significant. We did not record the shell length of each clam on day 0; instead, we ensured that all

clams ranged from 4 to 6 mm in length. Because shell length did not differ among diets at day 7 ($P = 0.21$), subsequent analyses were performed on shell length measures from day 7 through day 56. A type I error α of 0.05 was used to reject all null hypotheses.

RESULTS

The quality of the overlying test water was similar among diets (Fig. 1). For example, grand means (averaged over all diets and time periods) ranged from 15.4°C to 15.7°C for temperature, 8.2 to 8.3 for pH, and 9.7 to 9.8 mg/L for dissolved oxygen. Concentrations of total (range, 0.03–0.13 mg/L) and un-ionized (0.002–0.008 mg/L) ammonia were well below concentrations that adversely affect fingernail clams in laboratory exposures (Sparks and Sandusky 1981).

The survival rate of fingernail clams varied significantly among diets ($P = 0.0001$). Survival rates were lowest in clams fed the oyster diet, whereas survival was highest in clams receiving the sediment-renewal treatment (Fig. 2). For example, survival averaged 44% in the oyster diet, 66% in the rabbit-pellet diet, 73% in the suspended-sediment diet, and 84% in the sediment-renewal diet at day 56. By day 112, only 6% of the clams in the sediment-renewal treatment were alive, and none survived in the other three dietary treatments.

Survival was significantly greater in clams provided the sediment diets, relative to the commercial diets ($P = 0.0001$). After 56 days in the laboratory, for example, survival of clams fed the sediment diets averaged 79%, whereas survival averaged 55% in clams fed the commercial diets. However, there was little difference in survival of clams among diets early in the test; survival of clams in all diets exceeded 80% through 21 days of exposure. A unique feature of the proportional hazards model is the ability to calculate a risk ratio, or the estimated hazard of surviving in one diet versus another. For example, clams provided the oyster diet were 1.9 times more likely to die than clams fed rabbit pellets (Table 1). Additionally, clams fed the oyster diet were almost 5 times more likely to die than clams in the sediment-renewal treatment. Furthermore, clams fed the commercial diets were 2.4 times more likely to die than clams fed the two sediment diets.

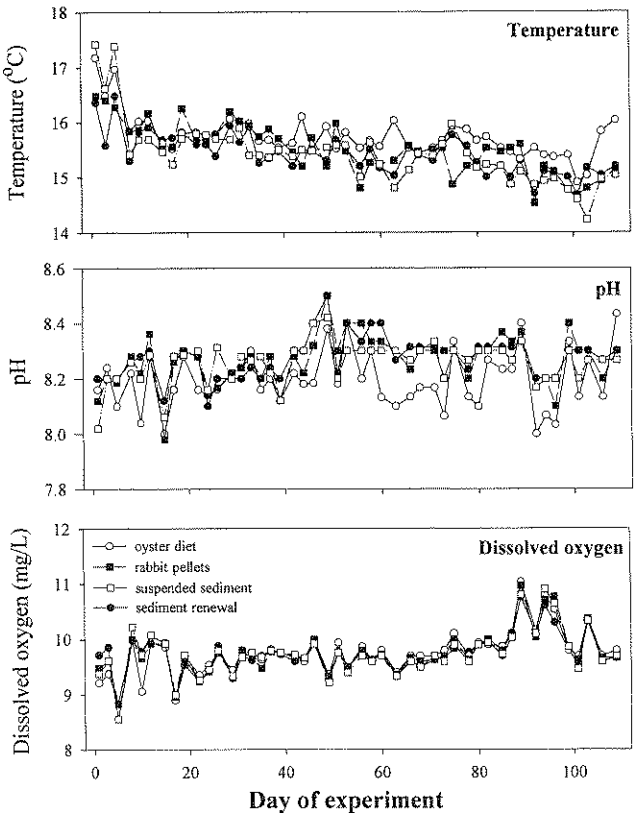

Figure 1. Mean temperature, pH, and dissolved oxygen in overlying test water from five randomly selected experimental units containing fingernail clams *Musculium transversum* fed one of four diets daily for 112 days.

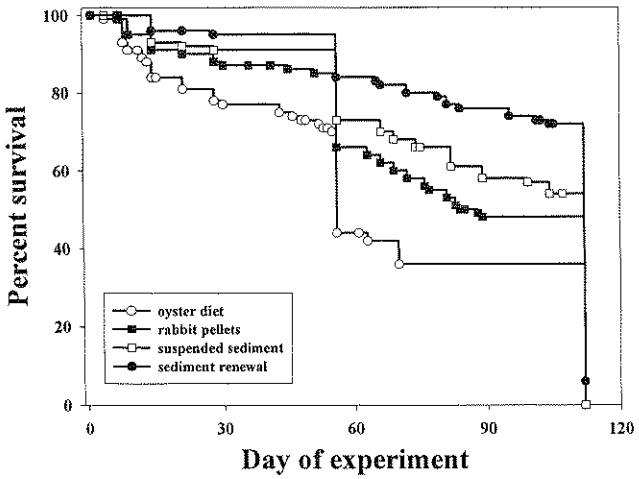

Figure 2. Survival of fingernail clams *Musculium transversum* fed one of four diets in a 112-day laboratory test.

The shell length of fingernail clams also varied significantly among diets ($P = 0.02$). Clams receiving the sediment-renewal treatment were significantly larger than clams in the other three dietary treatments. For example, clams in the sediment-renewal

TABLE 1.

Estimated probability values, risk ratios, and upper and lower 95% confidence limits from the survival rate analysis in fingernail clams fed four different diets in a 112-day laboratory experiment.

Contrast	*P* Value	Risk Ratio	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Oyster diet, suspended sediment	0.0001	2.6	1.9	3.5
Rabbit pellets, suspended sediment	0.1124	1.3	0.9	1.9
Sediment renewal, suspended sediment	0.0120	0.5	0.3	0.9
Oyster diet, rabbit pellets	0.0001	1.9	1.9	2.0
Oyster diet, sediment renewal	0.0001	4.8	4.0	5.8
Rabbit pellets, sediment renewal	0.0002	2.4	2.2	2.8
Commercial diets, sediment diets	0.0001	2.4	1.9	3.2

The risk ratio is the estimated hazard of surviving in one diet *versus* another diet; for example, clams fed the oyster diet were 2.6 times more likely to die than clams fed the suspended-sediment diet.

treatment averaged 4.8 mm in length over the 56-day duration, whereas clams in the other three dietary treatments ranged from 4.3 to 4.4 mm. Furthermore, the size of clams did not differ between clams provided the commercial and sediment diets (P = 0.50), nor did shell length vary with time in the laboratory (P = 0.23; Fig. 3a). At day 7, clams ranged in length from 4.2 to 4.8 mm and at day 56, they ranged in length from 4.5 to 4.8 mm.

Glycogen concentrations in clams varied significantly among diets (P = 0.049; Fig. 3b). In particular, glycogen concentrations differed between the commercial and sediment diets (P = 0.02). For example, mean glycogen concentration was 3.5 mg/g in clams fed the oyster diet and 4.1 mg/g in clams fed the rabbit pellets. In contrast, glycogen concentrations averaged 2.8 mg/g in the suspended-sediment diet and 3.0 mg/g in the sediment-renewal treatment. However, glycogen concentrations declined significantly with time in the laboratory, regardless of diet (P = 0.0001). For example, glycogen concentrations in clams in the sediment-renewal treatment averaged 4.6 mg/g at day 7 and had declined to only 2.2 mg/g by day 56. Moreover, because there was no diet*time interaction (P = 0.49), the response of glycogen with time was similar among diets. For reference, glycogen concentrations averaged 5.4 ± 0.5 (SE) mg/g in clams when they were removed from the Mississippi River.

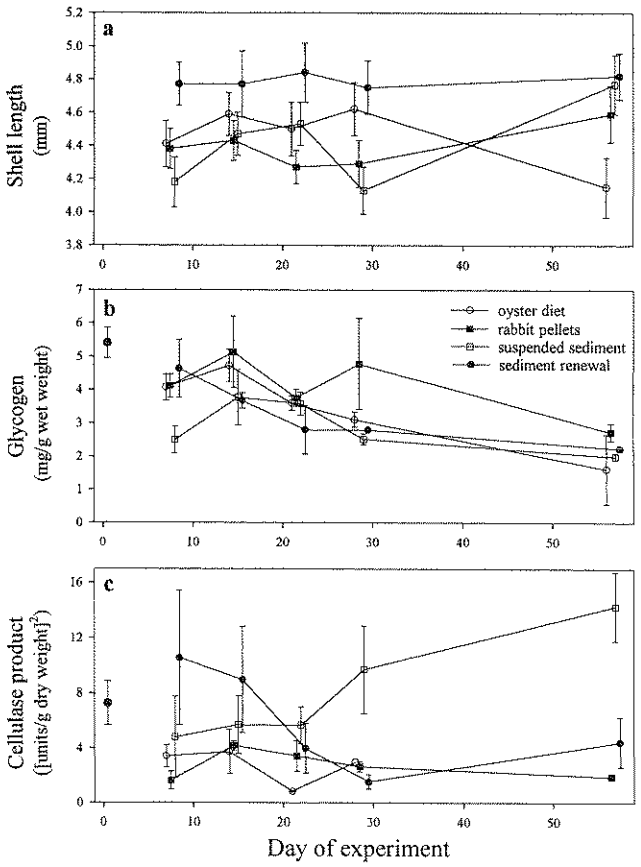

Figure 3. Mean (a) growth, (b) glycogen concentrations, and (c) cellulase activity in fingernail clams *Musculium transversum* fed one of four diets in a 112-day laboratory test. Glycogen (mg/g wet weight) and cellulase activity ([units/g dry weight]^2) were measured on a composite of five clams from each of three experimental units sampled on days 7, 14, 21, 28, and 56. Data point at day 0 is the mean (±1 SE) glycogen and cellulase in clams at the time they were collected from the Upper Mississippi River.

In contrast, cellulase activity did not vary among diets (P = 0.12) nor with time held in the laboratory (P = 0.32; Fig. 3c). Cellulase activity, averaged over the 56-day exposure, ranged from 0.8 to 5.3 (units/g dry weight^2) in the oyster diet, 0.8 to 4.8 in the rabbit pellets, 1.1 to 14.7 in the suspended sediment, and 0.6 to 19.8 in the sediment renewal. Likewise, cellulase activity remained similar throughout exposure (averaged over all diets) and ranged from 1.6 to 10.5 at day 7 and from 1.8 to 14.3 at day 56. The lack of significant diet or time effects was presumably due to the large variance in cellulase activity among replicates. The coefficient of variation (CV) usually averaged well over 50%, likely obscuring any diet or time effects. For reference, cellulase activity averaged 7.3 ± 1.6 (SE) in clams when collected from the Mississippi River.

DISCUSSION

Survival of fingernail clams was greater in treatments containing sediment from the Upper Mississippi River than in treatments with commercial diets. A similar observation was made by Gatenby et al. (1996) with juvenile *Villosa iris*. In a 45-day laboratory experiment, juvenile mussels reared on sediment and algae had significantly higher survival (67%) than juveniles reared without sediment and fed only algae (22%). Although several investigators have observed higher survival rates in molluscs in experiments with sediment, relative to no sediment (Gatenby et al. 1996, Naimo et al. 2000, present study), the mechanism(s) contributing to this are largely unknown. It has been hypothesized that the addition of a food source, along with fine sediments and their associated resident bacteria, may enhance digestion in molluscs (Crosby et al. 1990, Naimo et al. 2000). However, the addition of bacteria common to riverine systems did not improve survival or enhance growth in laboratory studies with juvenile *Villosa iris* (Gatenby et al. 1996). Naimo et al. (2000) hypothesized that physical contact with sediment may enhance the survival of fingernail clams relative to exposures without direct sediment contact. They observed that *Musculium transversum* were twice as likely to survive when provided with direct sediment contact, suggesting that clams received nutritional benefit from sediment contact by feeding directly on indigenous, sediment-associated food sources.

Although survival of fingernail clams differed substantially among diets after 112 days, survival exceeded 80% through day 21 in all diets. In standardized toxicity tests with benthic invertebrates, 21–28 days is a standard test duration (American Society for Testing and Materials 1992), and tests are generally considered unacceptable if survival of control animals is less than 80%. Thus, in short-term standardized tests with fingernail clams, excessive mortality in control organisms would not invalidate test results.

Growth of fingernail clams in the laboratory was minimal over the 56-day duration. Clams in the sediment renewal treatment seemed to maintain their size, whereas shell growth in clams in the other diets was variable. Differences in shell growth in the sediment-renewal treatment, relative to the other diets, may be related to the volume of available food (i.e., sediment). Clams in the sediment renewal treatment received about 736 g of sediment over 56 days, whereas clams in the suspended-sediment and rabbit-pellet treatments received only 2.1 g of food over this duration. Although food quality as well as quantity are important, the magnitude of the difference in quantity may have contributed to differences in growth among diets. In addition, the magnitude of shell growth observed in our study (0.1–0.6 mm over 56 days) was sufficiently small such that variation in measurement of shell

length could be a major source of variation and uncertainty in this analysis. Thus, future studies should measure individually marked organisms and should use techniques appropriate for detecting small changes in size. The lack of shell growth in this experiment was not unexpected. For example, Gale (1977) observed that *Sphaerium transversum* maintained in the laboratory in chambers containing silt from the Mississippi River grew slowly, with a mean length increase of 1.3 mm after 33 days.

Glycogen concentrations have been used extensively in bivalves as an indicator of physiological health (Haag et al. 1993, Naimo et al. 1998); however, it is unclear how much glycogen is required for maintenance, growth, and reproduction. In the present experiment, we documented significant differences in glycogen concentrations among diets, particularly between the commercial diets and the sediment diets. However, the pattern in glycogen concentrations was such that glycogen was elevated in clams fed the commercial diets, relative to the sediment diets, in contrast to the patterns in survival. Two alternate hypotheses for the reduction in glycogen in the sediment diets include (1) clams were getting enough nourishment from the sediment for maintenance metabolism but were unable to store glycogen and (2) clams were not getting enough nourishment from the sediment and were catabolizing carbohydrate stores. Whichever the case, glycogen concentrations declined with time in all dietary treatments, suggesting that health was declining over this time period. Glycogen concentrations declined by 14–54% by day 7 and 50–70% by day 56, relative to concentrations in clams when they were taken from the river.

Some researchers have suggested that the benefit of addition of sediment to juvenile bivalve cultures is to provide resident bacteria to enhance enzymatic activity (Crosby et al. 1990). However, we did not observe any enhancement in cellulase activity between clams maintained in sediment and clams fed commercial diets. Cellulase activity in clams was highly variable (mean CV = 67%), making detection of dietary effects at an acceptable statistical level difficult. To our knowledge, measurement of cellulase activity has not been previously performed on fingernail clams; thus, further refinement of methods could reduce variation associated with this measure.

In conclusion, we observed significant differences in survival, shell growth, and glycogen concentrations of fingernail clams fed different diets, implying that some diets were better than others. However, the general negative slope of most response variables (survival, shell growth, and glycogen) suggests that clams were declining in health with time in the laboratory, regardless of diet. Therefore, a better diet is needed to maintain clams in a healthy state in the laboratory. Although clams maintained an acceptable survival rate for 21 days in the laboratory, their physiological condition was compromised much earlier. Thus, valid short-term toxicity tests with fingernail clams can be conducted in the laboratory, but their ability to predict toxicity to field populations is uncertain. Therefore, laboratory tests with clams should include a physiological measure, such as glycogen, in addition to survival to ensure that clams are in suitable condition before and during testing in laboratory studies.

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LITERATURE CITED

American Public Health Association, American Water Works Association and Water Environment Federation. 1992. Standard Methods for the Examination of Water and Wastewater, 18th ed. American Public Health Association, Washington, DC.

American Society for Testing and Materials (ASTM). 1992. Annual Book of ASTM Standards, vol. 11.04. Water and Environmental Technology. American Society for Testing and Materials, Philadelphia, PA.

Beeby, A. 1993. Toxic metal uptake and essential metal regulation in terrestrial invertebrates: a review. *In:* Metal Ecotoxicology: Concepts and Applications. Lewis Publishers, Chelsea, MI. 441 pp.

Cox, D. R. 1972. Regression models and life tables. *J. R. Stat. Soc. Ser. B* 34:187-220.

Crosby, M. P., R. I. E. Newell & C. J. Langdon. 1990. Bacterial mediation in the utilization of carbon and nitrogen from detrital complexes by *Crassostrea virginica*. *Limnol. Oceanogr.* 35:625–639.

Farris, J. L., J. H. Van Hassel, S. E. Belanger, D. S. Cherry & J. Cairns, Jr. 1988. Application of cellulolytic activity of Asiatic clams (*Corbicula* sp.) to in-stream monitoring of power plant effluents. *Environ. Toxicol. Chem.* 7:701–713.

Farris, J. L., J. L. Grudzien, S. E. Belanger, D. S. Cherry & J. Cairns, Jr. 1994. Molluscan cellulolytic activity responses to zinc exposure in laboratory and field stream comparisons. *Hydrobiologia* 287:161–178.

Foster, A. R., D. F. Houlihan & S. J. Hall. 1993. Effects of nutritional regime on correlates of growth rate in juvenile Atlantic cod (*Gadus morhua*): comparison of morphological and biochemical measurements. *Can. J. Fish. Aquat. Sci.* 50:502–512.

Frazier, B. E., T. J. Naimo & M. B. Sandheinrich. 1996. Temporal and vertical distribution of total ammonia nitrogen and un-ionized ammonia nitrogen in sediment pore water from the upper Mississippi River. *Environ. Toxicol. Chem.* 15:92–99.

Gale, W. F. 1977. Growth of the fingernail clam, *Sphaerium transversum* (Say) in field and laboratory experiments. *Nautilus* 91:8–12.

Gatenby, C. M., R. J. Neves & B. C. Parker. 1996. Influence of sediment and algal food on cultured juvenile freshwater mussels. *J. N. Am. Benthol. Soc.* 15:597–609.

Guy, H. P. 1969. Laboratory Theory and Methods for Sediment Analysis: Techniques of Water-Resources Investigations of the United States Geological Survey, book 5, chapter C1, Washington, D.C.

Haag, W. R., D. J. Berg, D. W. Garton & J. L. Farris. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. *Can. J. Fish. Aquat. Sci.* 50:13–19.

Hemelraad, J., D. A. Holwerda, H. J. Herwig & D. I. Zandee. 1990. Effects of cadmium in freshwater clams. III. Interaction with energy metabolism in *Anodonta cygnea*. *Arch. Environ. Contam. Toxicol.* 19:699–703.

Hickey, C. W. & M. L. Vickers. 1994. Toxicity of ammonia to nine native New Zealand freshwater invertebrate species. *Arch. Environ. Contam. Toxicol.* 26:292-298.

Lanno, R. P., B. E. Hickie & D. G. Dixon. 1989. Feeding and nutritional considerations in aquatic toxicology. *Hydrobiologia* 188/189:525–531.

Milam, C. D. & J. L. Farris. 1998. Risk identification associated with iron-dominated mine discharge and their effect upon freshwater bivalves. *Environ. Toxicol. Chem.* 17:1611–1619.

Naimo, T. J., W. G. Cope & M. R. Bartsch. 2000. Sediment-contact and survival of fingernail clams: implications for conducting short-term laboratory tests. *Environ. Toxicol.* 15:23–27.

Naimo, T. J., E. D. Damschen, R. G. Rada & E. M. Monroe. 1998. Nonlethal evaluation of the physiological health of unionid mussels: methods for biopsy and glycogen analysis. *J. N. Am. Benthol. Soc.* 17:121–128.

Parmer, M. K. B. & D. Machin. 1995. Survival Analysis: A Practical Approach. John Wiley and Sons, New York, 255 pp.

Plumb, R. H. Jr. 1981. Procedures for Handling and Chemical Analysis of Sediment and Water Samples. Technical Report EPA/CE-81-1. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Sparks, R. E. & M. J. Sandusky. 1981. Identification of factors responsible for decreased production of fish food organisms in the Illinois and Mississippi Rivers. Final report project No. 3-291-R, Illinois Natural History Survey, River Research Laboratory, Havana, IL.

Wilson, D. M., T. J. Naimo, J. G. Wiener, R. V. Anderson, M. B. Sandheinrich & R. E. Sparks. 1995. Declining populations of the fingernail clam *Musculium transversum* in the Upper Mississippi River. *Hydrobiologia* 304:209–220.