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REGULAR ARTICLE

SURVIVAL AND GROWTH OF JUVENILE MUSSELS IN AN OUTDOOR POND AFTER 28-DAY LABORATORY EXPOSURE TO AQUEOUS ZINC

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ABSTRACT

The extent to which effects seen in chronic toxicity studies in the laboratory affect mussel fitness later in life is poorly known. We examined juvenile Fatmucket (*Lampsilis siliquoidea*) survival and growth for 56 d following exposure to Zn in a laboratory bioassay. We conducted a 28-d chronic toxicity bioassay with 6-wk-old juvenile mussels exposed to a control and two Zn treatments (120 and 240 µg/L). We then transferred surviving mussels into a grow-out pond and monitored their survival and growth for 56 d. Survival and shell length were lower in both Zn treatments than in the control after the 28-d toxicity bioassay. After the 56-d grow-out period, survival did not differ among treatments, but shell length was lower in the 240-µg/L treatment than in the control and mass was lower in both Zn treatments than in the control. Mussel length was lower throughout the experiment in both Zn treatments than in the control, but there was weak support for a difference in the slopes, suggesting that Zn-exposed mussels may fall farther behind in size over time. Persistence of reduced size following Zn exposure could result in delayed sexual maturation and lowered fecundity, which could have long-term population-level effects.

KEY WORDS: freshwater mussel, metal toxicity, risk assessment, growth, recovery

INTRODUCTION

Freshwater mussels (order Unionida) are considered at risk of decline in part because of exposure to environmental contaminants (FMCS 2016). In toxicological studies, mussels are among the most sensitive freshwater species to a variety of contaminants, including ammonia, metals, and road salts (Wang et al. 2007, 2017; Gillis 2011). Resource agencies have prioritized mussel conservation (USFWS 2023) and included them as receptors within ecological risk assessment and natural resource damage assessment and restoration injury assessment (Besser et al. 2009, 2015, 2018). Assessing ecological risk or injury within these frameworks requires data on the toxicological effects of the chemical of concern in controlled, spiked laboratory waters and sediment or, alternatively, by directly measuring the toxicity of field-collected contaminated water or sediment.

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Standardized laboratory toxicity bioassays determine toxicity thresholds (e.g., 20% effect concentration) by using endpoints such as survival, growth, or reproduction tested under feeding, temperature, and other conditions that optimize control survival (ASTM International 2019). Duration of exposure, life stage, and endpoints all influence the outcomes of the bioassays. The sensitivity of organisms may increase with increasing exposure duration, and juveniles are considered the most sensitive mussel life stage (Wang et al. 2010). Chronic toxicity studies show that mussel growth is a more sensitive endpoint than survival in 28-d exposures to several toxicants (Wang et al. 2018, 2010, 2020). Consequently, the standard laboratory bioassay method for mussel chronic toxicity bioassays stipulates measurement of juvenile growth over a 28-d exposure (ASTM International 2019).

The extent to which sublethal effects seen in chronic toxicity studies in the laboratory affect mussel fitness later in life is poorly known (Newton and Cope 2007). Growth reduction

from 84-d exposures to NaCl, Ni, and Zn was similar or greater in magnitude than in standard 28-d exposures (Wang et al. 2018, 2020). Mussel fecundity is primarily a function of body size, and a 10% reduction in length is predicted to result in a 19–44% reduction in fecundity (Hanson et al. 1989; Haag and Staton 2003). For other invertebrates, statistical or modeled relationships between laboratory bioassay endpoints and population responses have been derived based on field experiments (Moore et al. 2019). However, uncertainty remains about how growth reduction from standard 28-d exposures affects survival, growth, age at maturity, fecundity, and other fitness components over longer time periods.

Quantitative relationships between reduced growth in bioassays and long-term population outcomes are necessary to extrapolate laboratory-derived data to contaminant risk or injury of mussels in natural systems. We examined juvenile Fatmucket (*Lampsilis siliquoidea*) survival and growth for 56 d following exposure to Zn. We conducted a 28-d laboratory chronic Zn toxicity bioassay with juvenile mussels with endpoints of survival and length following standard methods (ASTM International 2019). At the end of the 28-d bioassay, we transferred surviving mussels into a grow-out pond and monitored their survival and growth for an additional 56 d. We chose Fatmucket because it is commonly used in aquatic toxicity testing, its growth rates in culture are well studied, and it is a useful surrogate species for inferring the sensitivity of other mussel species to a wide variety of toxicants with different modes of action (Raimondo et al. 2016; Wang et al. 2017). We used Zn as a model toxicant because mussels, including Fatmucket, are highly sensitive to Zn in acute and chronic exposures and exhibit significant reductions in growth during bioassays (Wang et al. 2020).

METHODS

Juvenile Culture

We collected female Fatmucket brooding mature larvae (glochidia) from the Bourbeuse River, Gasconade County, Missouri, USA, and held them at the U.S. Geological Survey (USGS) Columbia Environmental Research Center (CERC), Columbia, Missouri. We held adult female Fatmucket at 10–12°C (to prevent release of glochidia) in a 600-L recirculating tank with pond water (hardness 260 mg/L as $CaCO_3$, alkalinity 180 mg/L as $CaCO_3$, pH 7.8) at a flow rate of approximately 2 L/min. We fed mussels twice daily approximately 20 mL of a commercial nonviable microalgal concentrate (*Nannochloropsis*, Nanno 3600™) and 20 mL of a mixture of six microalgae (Shellfish Diet 1800™; both from Reed Mariculture, Campbell, CA, USA).

We removed approximately equal numbers of glochidia from each of three adult mussels by gently flushing the mussel marsupium with a syringe filled with culture water. We pooled the glochidia and inoculated them on laboratory-reared Largemouth Bass (*Micropterus salmoides*), which we maintained at 22°C in a recirculating, flow-through water system composed of Zebrafish tanks (Aquaneering Incorporated, San Diego, CA, USA) and designed to collect transformed juvenile mussels. We collected newly metamorphosed juveniles during the peak drop-off days (14–20 d after inoculation) and cultured them at 23°C in 300-mL lipless beakers with sand substrate and well water (hardness ca. 300 mg/L as $CaCO_3$) diluted with deionized water to a hardness of approximately 100 mg/L. Beakers had a 2.5-cm hole in the side covered with 50-mesh (279-μm-width opening) stainless steel screen to allow for water to overflow during renewal. We used an automated flow-through proportional diluter, typically used in toxicology studies, to deliver water and food every hour (Kunz et al. 2020). During culture, we replaced the sand and inspected the mussels weekly; we aimed for a relatively uniform juvenile size by discarding mussels that failed to grow and were noticeably smaller than other mussels (Barnhart 2006; Kunz et al. 2020).

Twenty-Eight-Day Chronic Toxicity Bioassay

Using our cultured juvenile Fatmuckets, we conducted a standard 28-d toxicity bioassay with three treatments (control water and 120 and 240 μg Zn/L) and eight replicates per treatment. We selected the Zn exposure concentrations based on a previous study with Fatmucket in which survival was high in all treatments, but growth was lower at the two high treatments and the 20% effect level was 66 μg Zn/L (Wang et al. 2020). For context, the 120-μg Zn/L level is approximate to the hardness adjusted chronic water quality criteria for Zn (122–127 μg Zn/L at 104–110 mg/L $CaCO_3$ hardness), which is intended to be protective of 95% of aquatic life (USEPA 1980). For each replicate exposure chamber, we placed 10 mussels (2.42 ± 1.6 mm, mean ± SD) and approximately 5 mL of silica sand into a 300-mL lipless glass culture beaker with 200 mL of water. We prepared silica sand (<500 μm; Granusil #5020, Unimin Corporation, New Canaan, CT, USA) by washing it in a container overnight with flow-through well water, rinsing it with deionized water for 5 min, and holding it in control water for 24 h before placing it in the beakers.

We used an intermittent proportional diluter to renew exposure water, maintain desired Zn concentrations, and deliver food throughout the bioassay. We prepared stock solutions of ZnCl (>98% purity; Sigma-Aldrich, St. Louis, MO, USA), and 125 mL of solution was delivered to each replicate beaker via a syringe pump (Hamilton, Reno, NV, USA), with each cycle of the diluter (once per hour, 15 times/d). Each day, we prepared a stock algal food mixture consisting of 1 mL of Nanno 3600 and 2 mL of Shellfish Diet 1800 (Reed Mariculture\) in 1.8 L of water (algal concentration ~510 nL cell volume/mL), maintained in aerated containers at <12°C in a cooler with ice packs (Wang et al. 2018). We provided 2 mL of the algal mixture per hour to each replicate beaker by using a peristaltic pump (Masterflex L/S model 07522-20 with 7535-08 multichannel head, Cole-Parmer Instrument Company, Vernon Hills, IL, USA) calibrated to automatically deliver the volume to each of six mixing cells in the diluter following each diluter

Figure 1. Floating upweller system (FLUPSY) used to hold juvenile mussels in the grow-out pond. (Left) Interior of FLUPSY with cover removed showing polyvinyl chloride holding chamber and water pump. (Right) FLUPSYs deployed in the grow-out pond. Inset shows anchor and line used to hold FLUPSYs in place. Photographs by J. Kunz, U.S. Geological Survey.

cycle (Kunz et al. 2020). We conducted the bioassay at 23°C in a temperature-controlled water bath and ambient laboratory light (~500 lux) with 16:8-h light:dark photoperiod.

We measured water quality variables (dissolved oxygen, pH, conductivity, hardness, alkalinity, and ammonia) in each treatment weekly. We measured dissolved oxygen with an HQ30d meter, pH with an HQ440d meter, and conductivity with an HQ40d meter with a CDC401 probe (Hach, Loveland, CO, USA). We measured hardness and alkalinity using the colorimetric burette method (ASTM International 2016, 2017). We measured total ammonia as nitrogen in water by using the titration method (ASTM International 2021). We collected water samples at the beginning and end of the bioassay to confirm Zn concentrations in all three treatments. Zn concentrations were measured by the USGS-CERC Environmental Chemistry Branch by using an inductively coupled plasma mass spectrometry (ICP-MS; NexION 2000 spectrometer, PerkinElmer, Waltham, MA, USA) following U.S. Environmental Protection Agency method 6020B (USEPA 2014). Before analysis, samples were filtered using a 0.45-µm polyethylsulfone membrane (Whatman Puradisc PES, GE Healthcare Bio-Sciences, Chicago, IL, USA) and preserved by adding house-distilled nitric acid to a final concentration of 2% (v/v). Chemical analysis followed established quality management system procedures including laboratory reference control samples, analysis duplicates, and analysis spikes. Percent recovery of spiked samples was 101.2%. Two National Institutes of Standards and Technology laboratory control samples (1640 and 1643) were used to confirm the accuracy of the ICP-MS calibration and were within 3% of the target values. The limit of detection and limit of quantitation was 0.1 and 1 µg/L, respectively.

We replaced bioassay beakers and sand at 14 d. We first rinsed mussels from each replicate beaker into a 200-mL glass dish with the exposure water for survival determination. We classified mussels with empty or gaping shells containing decomposed tissue as dead and removed them from the beakers. We transferred surviving mussels to a new beaker and sand with fresh solution. After 28 d, we removed and counted surviving mussels in each beaker. We measured shell length of each surviving mussel to the nearest 0.001 mm by using digital images captured with an SMZ 1270 stereo microscope and NIS Elements imaging software (Nikon Industries Inc., Melville, NY, USA).

Post-toxicity Bioassay Grow-Out in a Pond

We concluded the bioassay on August 23, 2019. We immediately transferred surviving mussels into a pond on August 23, 2019 (day 1), and monitored their survival and growth for 56-d grow-out until October 18, 2019. The pond was 290 m^2 and approximately 1 m in depth. It received well water at a rate of approximately 5 L/min via a 7.6-cm inlet pipe, and water exited the pond via an outlet weir at the end opposite the inlet. We transferred mussels from each beaker into separate holding chambers that were placed inside a six floating upweller systems (FLUPSY; Fig. 1); the FLUPSY is frequently used to rear bivalves from the juvenile-to-adult stage (Mair 2018). Our FLUPSY was 40 × 60 cm and 25 cm in depth and constructed of high-density polyethylene with foam on the upper edge for floatation. We drilled 11.4-cm-diameter holes in the bottom of each FLUPSY to accommodate four holding chambers. We fabricated the holding chambers from a 10-cm-diameter polyvinyl chloride pipe 16.5 cm in height with 1-mm mesh Nitex screen on top and bottom caps. We placed a model 7 magnetic drive utility pump (Danner Pondmaster, Islandia, NY, USA) in each FLUPSY to create an upward flow through the holding chambers. Before placing mussels in ponds, we acclimated them for approximately 1 h by gradually adding pond water to the holding chambers. We randomly assigned holding chambers across six FLUPSY systems.

We recorded water temperature in each FLUPSY every 30 min throughout the grow-out period by using three data

Table 1. Survival and length ($N = 8$/treatment) of juvenile Fatmucket (*Lampsilis siliquoidea*) in the 28-d Zn toxicity bioassay. Within a column, values (mean ± SD) with the same superscripted letter are not significantly different (Dunnett's test: $P < 0.05$).

Nominal Concentration (µg Zn/L)	Measured Concentration (µg/L)	Survival (%)	Length (mm)
Control	1.9 ± 0.1	95.0 ± 10.7[a]	4.4 ± 0.3[a]
120	147.0 ± 1.4	81.3 ± 11.3[b]	3.6 ± 0.3[b]
240	248.0 ± 2.1	81.3 ± 12.5[b]	3.2 ± 0.4[b]

loggers. We obtained light condition (solar radiance) and daylight hours from the University of Missouri South Farm weather station (38.906992°, −92.269976°), located approximately 755 m from the pond at CERC (University of Missouri 2023). We measured dissolved oxygen, pH, conductivity, hardness, alkalinity, and ammonia in each FLUPSY weekly, as described previously. Every 14 d, we checked the mussels for survival and photographed them for length measurements. We collected water samples from the pond every 14 d for measurement of metals, total nitrogen, total phosphorus, total particle volume, and dissolved organic carbon (DOC) as general indicators of diet quality in the pond water. We measured metals by ICP-MS following method 6020B, described above. We froze samples for total nitrogen and phosphorus, stored them for 4 mo, and analyzed nitrogen by derivative spectroscopy (APHA 2017a) and phosphorus by using the ascorbic acid method (APHA 2017b). We held samples for particle count at 4°C and measured total particle volume (size fraction = 2–20 µm) within 24 h by using a particle counter (Beckman Coulter, Indianapolis, IN, USA). We vacuum-filtered samples for DOC (0.45-µm PES), acidified them with 9 N high-purity sulfuric acid to pH 2 or lower, refrigerated them for <28 d, and measured DOC by high-temperature catalytic oxidation-nondispersive infrared spectroscopy by using a TOC-L analyzer (Shimadzu Scientific, Kyoto, Japan). At the end of the 56-d grow-out period, we collected mussels from each holding chamber to determine survival, length, and dry mass (shell and tissue, 60°C for 48 h).

RESULTS

Twenty-Eight-Day Chronic Toxicity Bioassay

Mean Zn concentration in the control, 120-µg/L Zn/L treatment, and 240-µg Zn/L treatment was 1.9, 147.0, and 248.0 µg/L, respectively (Table 1), representing 103–123% of nominal concentrations. Water quality conditions met performance criteria for standard toxicity bioassays (ASTM International 2019) and were as follows: pH, 8.0–8.4; alkalinity, 90–96 mg/L as $CaCO_3$; hardness, 104–110 mg/L as $CaCO_3$; conductivity, 263–269 µS/cm at 25°C; Ca 25–26 mg/L; Mg, 8.4–9.1 mg/L; K, 0.9–1.0 mg/L; Na, 9.2–10.0 mg/L; Cl, 9.7 mg/L; and SO_4, 21 mg/L. Ammonia concentrations ranged from 0.05 to 0.08 mg N/L.

Mean survival in the control treatment after 28 d was 95.0% (Table 1) and met test acceptability criterion of >80% survival (ASTM International 2019). Mean survival differed among treatments (analysis of variance [ANOVA]: $F_{2,21} = 3.82$, $P = 0.039$). Survival in both Zn treatments was 81.3%, significantly lower than in the control (Table 1). Mean shell length differed among treatments (ANOVA: $F_{2,21} = 29.55$, $P < 0.0001$). Mean shell length in the control treatment was 4.4 mm; mean length was 3.6 and 3.2 mm in the 120- and 240-µg Zn/L treatments, respectively; and length in both Zn treatments was significantly lower than in the control (Table 1).

Post-toxicity Bioassay Grow-Out in a Pond

Water quality conditions in the pond throughout the grow-out period were maintained within the range typically considered adequate for mussel culture (Fig. 2; Kunz et al. 2020). Mean temperature in the pond was 23°C (range, 12–28°C). Total nitrogen concentration (mean ± SD) was 470 µg/L, total phosphorus was 47.6 ± 21.1 µg/L, and values for both were lowest on day 56. Total particle volume (2–10 µm) was 19.3 μm^3/mL on day 1 and 6.1 μm^3/mL on day 56. DOC (mean ± SD) was 2.34 ± 0.52 mg/L. The apparent decline in nutrients and particles likely was due to the seasonal decline of solar radiance and temperature later in the study.

After 56-d grow-out, survival of mussels from the control treatment was 91%, survival was 79% and 80% for mussels from the 120- and 240-µg Zn/L treatments, respectively, but survival did not differ among treatments (ANOVA: $F_{2,21} = 2.23$, $P = 0.133$; Table 2). Mussels from all treatments grew 3.0–3.5 × in length and 27–35 × in mass during grow-out. Final mean shell length of mussels differed among treatments (ANOVA: $F_{2,21} = 7.13$, $P = 0.004$). Mean shell length in the 240-µg Zn/L treatment (10.9 mm) was significantly lower than in the control and 120-µg Zn/L treatments (13.7 and 12.7 mm, respectively), which did not differ from each other (Table 2). Final dry mass of mussels differed among treatments (ANOVA: $F_{2,21} = 16.58$, $P < 0.0001$). Mean mass in the 120- and 240-µg Zn/L treatments (0.78 and 0.63 g, respectively) did not differ from each other, but both values were significantly lower than in the control (1.41 g; Table 2).

Mussel length increased approximately linearly over time (analysis of covariance: time: $F_{1,114} = 1185.07$, $P < 0.0001$), and Zn concentration was a significant factor in predicting length (treatment: $F_{3,114} = 22.8$, $P = 0.0001$; Fig. 3). The time × treatment interaction was marginally significant ($F_{2,114}$, $P = 0.054$), and the estimated slope of the regression equation for the 240-µg Zn/L treatment was lower than for the other two treatments. However, 95% confidence intervals around the estimated slopes overlapped among all three treatments (slopes, 95% confidence intervals: control = 0.163, 0.093–0.234; 120 µg Zn/L = 0.167, 0.126–0.209; and

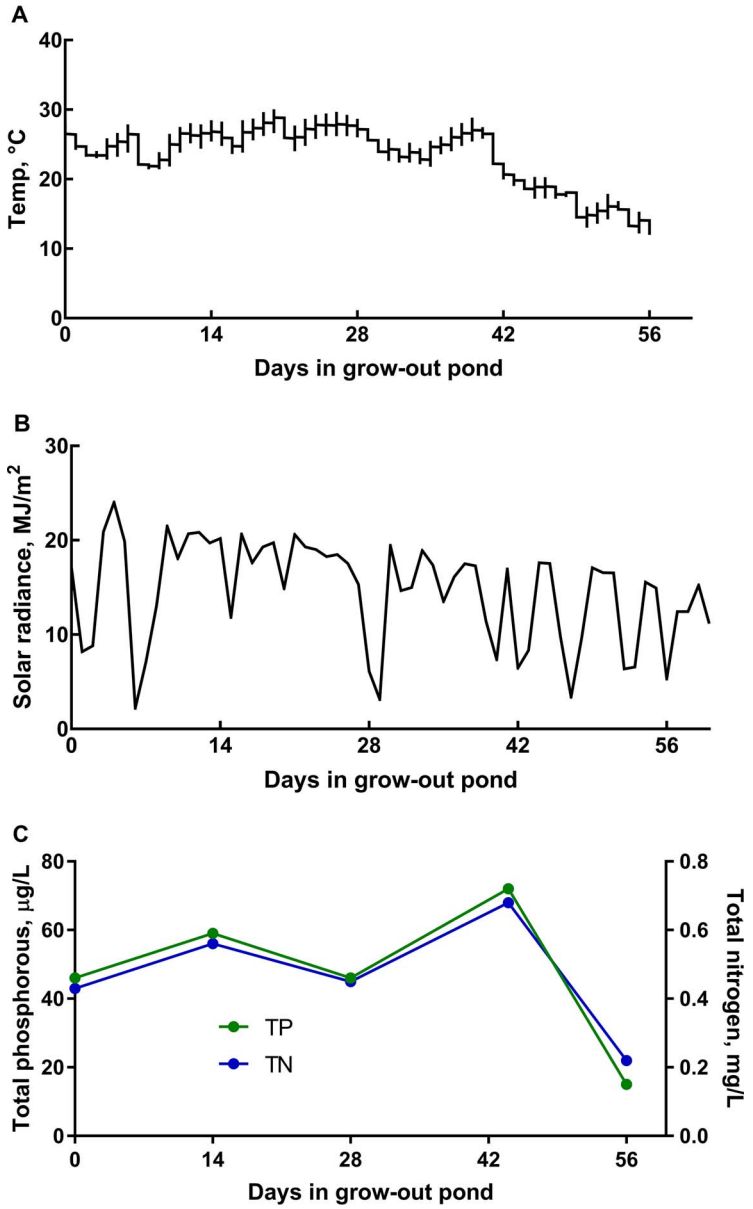

Figure 2. (A) Water temperature, (B) solar radiance, and (C) nutrients over 56 d in the grow-out pond.

240 µg Zn/L = 0.149, 0.0984–0.200). Consequently, we assumed homogeneity of slopes and omitted the interaction term to interpret main effects. Omitting the interaction term, time ($F_{1,116} = 1145.8$, $P < 0.0001$) and treatment ($F_{3,116} = 39.0$, $P < 0.0001$) remained significant factors in predicting mussel length. When time was accounted for, mean length differed among all three treatments (Tukey's post hoc test: $P < 0.001$ for all comparisons). Predicted lengths showed that, on any given day, mussels from the control treatment were 1.4 ± 0.24 mm (mean ± SE) longer than mussels from the 120-µg/L Zn treatment and 2.3 ± 0.24 mm longer than mussels from the 240-µg/L Zn treatment and mussels from the low Zn treatment were 0.9 ± 0.24 mm SE longer than those from the high Zn treatment.

DISCUSSION

Mussels exposed to even a low level of Zn (120 µg Zn/L) in our study were smaller than control mussels after a 28-d toxicity bioassay. The 18–26% reduction in our Zn treatments compared with the control was similar to the 25–35% reduction observed at the same concentrations in a previous study of chronic Zn toxicity with juvenile mussels (Wang et al. 2020). In our study, this reduced size persisted even after 56 d in a pond uncontaminated by Zn. Persistent stunting after brief exposure to stressors could result in long-term effects on a mussel population. For example, reduced growth can delay sexual maturation and reproduction for ≥1 y (Haag and Rypel 2011; Haag 2012), and reduced size can increase vulnerability to predators (Brondel 2010).

Table 2. Survival, length, and dry mass ($N = 8$ replicates) of juvenile Fatmucket (*Lampsilis siliquoidea*) after 56 d in the grow-out pond. Treatment group refers to conditions to which juvenile mussels were exposed previously in the 28-d toxicity bioassay. Within a column, values (mean ± SD) with the same superscripted letter are not significantly different (Dunnett's test: $P < 0.05$).

Treatment Group (µg Zn/L)	Survival (%)	Length (mm)	Dry Mass (g)
Control	91.3 ± 11.3	13.7 ± 1.8[a]	1.41 ± 0.44[a]
120	78.8 ± 15.5	12.4 ± 1.8[a]	0.78 ± 0.19[b]
240	80.0 ± 12.0	10.9 ± 0.57[b]	0.63 ± 0.13[b]

It is unclear whether exposure to Zn resulted in similar long-term effects among treatments. The slopes of the relationship between shell length and time were similar for the control and 120-µg Zn/L treatments, which suggests that mussels exposed to low levels of Zn grew at a similar rate as unexposed mussels after removal of the stressor. The slope of this relationship appeared to be slightly lower for the 240-µg Zn/L treatment, and the time × treatment interaction was marginally significant; however, confidence intervals around the slopes overlapped widely for all three treatments, potentially due to low precision of the slope estimates. A lower slope for the 240-µg Zn/L treatment would indicate that exposure to a higher concentration of Zn can reduce growth rates even after removal of the stressor, causing mussels to fall further behind in size over time. Such an effect could compound population-level effects, but additional work is needed to clarify this issue.

In ecological risk assessment and resource injury assessment, the effects of a contaminant in controlled laboratory studies must be related to its effects in natural systems. Data collected in the laboratory are limited in duration and complexity, whereas contaminant effects in natural systems may occur over longer time scales, changing exposure concentrations, and in the presence of other stressors. The effects of contaminant and habitat stressors on freshwater mussel growth in natural systems have been measured directly using in situ–caged mussels (Rogers et al. 2018; Haag et al. 2019; Pieri 2022). Relating laboratory bioassay data to such studies is challenging because of the difficulty of establishing relationships between survival and growth effects seen in the laboratory and factors that affect wild populations (Barnthouse and Stahl 2017). Studies that compare results of laboratory bioassays to results of invertebrate surveys, in situ exposures, or colonization of test sediments placed in natural systems can help translate responses seen in the laboratory to those seen in natural systems (Ingersoll et al. 2005; ASTM International 2018; Johnson 2018; Moore et al. 2019; Pieri 2022). Such studies found that endpoints derived from laboratory bioassays were protective of adverse biological effects observed in natural systems, but they did not always accurately predict biological effects observed in wild populations, particularly for sublethal endpoints such as reproduction (Crane et al. 2007). For example,

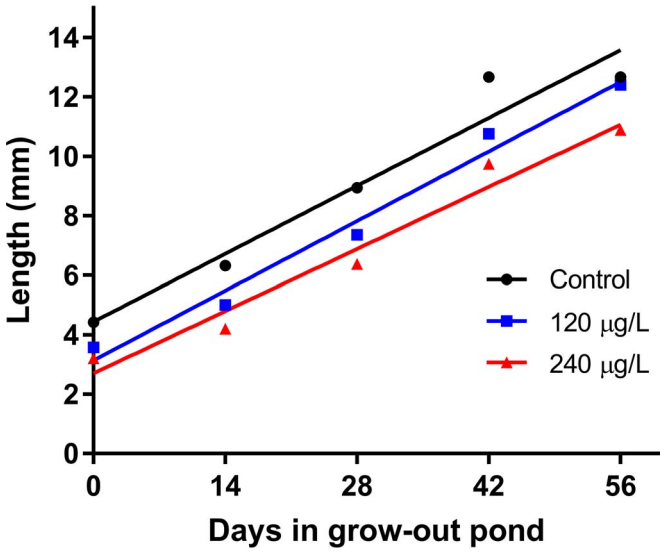

Figure 3. Mean length (mm) of juvenile Fatmucket (*Lampsilis siliquoidea*) over 56 d in the grow-out pond after previous exposure to three Zn treatments (control, 0 µg/L; 120 and 240 µg/L).

mussels exposed to environmental contaminants may experience sublethal effects including reduced energy stores, fecundity, and reproduction (Rajalekshmi and Mohandas 1993; Leonard et al. 2017).

The relationship between responses seen in the laboratory and in natural systems can be inferred by using data from laboratory studies to model longer term outcomes for a mussel population. Energetic and population models can be used to predict adverse biological effects on wild mussel populations based on effects seen in laboratory bioassays (Sherborne and Galic 2020; Accolla et al. 2021; Raimondo et al. 2021). For example, dynamic energy budget (DEB) models, which couple organism energy balance with toxicant exposure, have been used to predict the effect of toxicants on energy reserves and reproduction in aquatic organisms (Kooijman and Metz 1984). The DEB model has been used to interpret growth responses seen in laboratory toxicity bioassays, but it has not been applied to freshwater mussels. Bioenergetic models have been used to predict the energetic costs of environmental and toxicant stressors on marine mussels. For example, DEB modeling predicted reduced gamete production associated with reduced mussel biomass following exposure to oil production water and Zn particles (Muller et al. 2010, 2014). Similarly, environmental stressors such as variable salinity and hypoxia can incur significant energetic costs for marine mussels, a finding that can inform management decisions (Maar et al. 2015; Thomas et al. 2019; Lavaud et al. 2021). Sublethal growth endpoints, such as mass and length, from a toxicity bioassay can be coupled with energetic and population models to predict the consequences of contaminants on wild mussel populations (Widdows and Donkin 1991). In addition, individual-based energetic models can predict contaminant effects on threatened or endangered fishes, and such models

may be useful for assessing contaminant effects on endangered freshwater mussels (Petersen et al. 2008; Bartell et al. 2019).

We showed that brief exposure to Zn can have lasting effects on mussel size and perhaps growth. Longer term studies are needed to better understand the persistent effects of brief contaminant exposure and resulting long-term population effects. For example, our study ended near the onset of winter; longer studies would be valuable to determine whether reduced growth due to Zn exposure reduces energy stores needed to overwinter. Furthermore, longer studies can clarify whether persistent effects differ according to initial contaminant concentration or whether exposed mussels eventually catch up to unexposed mussels via compensatory growth or other mechanisms.

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DATA AVAILABILITY STATEMENT

Full data sets for the present study are available through a data repository in ScienceBase at https://doi.org/10.5066/P96RI2Z4.

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