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ORIGINAL PAPER

Effects of the invasive bivalve, *Corbicula fluminea*, on juvenile freshwater mussel survival and growth in laboratory experiments

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Abstract *Corbicula fluminea* is a widespread aquatic invasive species that has major ecological effects. *Corbicula* is a proposed factor in global freshwater mussel declines, but its effects on mussels are poorly understood. We conducted five laboratory experiments to assess effects of *Corbicula* on juvenile mussel survival and growth and the modulating effects of food abundance, temperature, and the type of co-occurring bivalve. *Corbicula* had no effect on 60-day-old mussel survival. In two experiments, growth was *higher* in the presence of *Corbicula*; higher growth occurred only at low food in one experiment but not the other. Access to *Corbicula* pseudofeces did not produce higher growth, suggesting that mussels fed on suspended material. In another experiment, 60-day-old mussel growth was lower in the presence of *Corbicula*, but only at high food and warm temperature (29 °C). In contrast, 1-day-old juvenile survival was 50–70% lower in the presence of *Corbicula*, but neither food nor temperature modulated that effect. Growth was lower in the presence of *Corbicula*, but mainly at low food, suggesting food competition. In some cases, *Corbicula* had stronger effects than a similar biomass of adult native mussels, but in other cases their effects on juvenile mussels were similar. However, *Corbicula* removed most suspended food from experimental chambers 2–4h after feeding (at low and high food, respectively), but native bivalves removed substantial amounts of food only in the low food treatment. The strong negative effects of *Corbicula* on 1-day-old juveniles suggest that it may be an important factor in mussel declines.

Keywords Invasive species · Competition · Aquatic ecosystems · Ammonia · Unionida · Conservation

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Introduction

Corbicula fluminea (Order Venerida, hereafter "*Corbicula*") is one of the most widespread aquatic invasive species in the world and can have a wide range of ecological effects (Sousa et al. 2008). *Corbicula* is native to eastern Asia, arrived in western North America in the 1920–1930s and colonized much of the eastern USA by the 1970s, and it colonized much

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of South America, Europe, and North Africa in the last 20 years (Crespo et al. 2015). Dense populations of *Corbicula* can filter a substantial percentage of a stream's water and remove much of the seston (Cohen et al. 1984; Lauritsen 1986; Leff et al. 1990), resulting in major ecosystem changes. For example, *Corbicula* filtered daily about one-third of the volume of the Potomac River, USA, which was associated with a tripling of water clarity, reappearance of submerged aquatic vegetation, and other changes (Phelps 1994). Similarly, estimated turnover time in the Broad River, USA, rose from 5 to > 1200h after mass mortality of *Corbicula* (McDowell et al. 2017).

Freshwater mussels (Order Unionida, hereafter "mussels") once dominated benthic biomass in many streams worldwide, but they have declined dramatically and are now one of the most imperiled animal groups on Earth (Aldridge et al. 2022). Causes of mussel declines are unknown in many cases, but *Corbicula* is a potential factor because the timing of its arrival coincides closely with the advent of native mussel declines in the USA and Europe (Haag 2019). Relatively few studies have examined potential effects of *Corbicula* on native mussels, and most older studies are anecdotal or equivocal (Strayer 1999). A few recent studies provide support for negative effects of *Corbicula* on mussels (e.g., Yeager et al. 2000; Ferreira-Rodríguez et al. 2018; Haag et al. 2021), but no comprehensive experimental studies have been conducted.

Proposed mechanisms by which *Corbicula* could negatively affect native mussels include exploitative competition for food, interference competition (ingestion of mussel sperm, larvae, and juveniles; habitat disturbance by burrowing), water quality degradation from periodic, mass *Corbicula* die-offs or *Corbicula* waste excretion, and *Corbicula* as a vector of disease (Strayer 1999; Haag 2019; Modesto et al. 2019). Food competition is a plausible mechanism because *Corbicula* reaches high abundance (> 1000/m^2; Hakenkamp and Palmer 1999; Sousa et al. 2011), it has a higher mass-specific filtering rate than native mussels (Leff et al. 1990), and food competition is considered the mechanism for mussel declines after invasion by *Dreissena polymorpha*, another bivalve with high abundance and efficient filtering (Baker and Levinton 2003; Strayer and Malcom 2018).

Several factors could modulate the competitive effects of *Corbicula* or other bivalves on native mussels. First, food competition could be more intense where food is scarce than in habitats with abundant food resources (Haag 2019). Second, competition could be more important at warmer temperatures because mussel metabolic rates and energy needs are higher (Ganser et al. 2015). Finally, hypotheses about competition with *Corbicula* must consider that native mussels can occur in high-density beds (> 50/m^2), within which there is also potential for food competition. Native mussel density typically is much lower than *Corbicula* density, but biomass may be comparable because of native mussels' larger body size. However, even when they occur at equal biomass, *Corbicula* may represent greater potential for food competition than native mussels because of its higher filtering rate.

Another factor that may modulate the effects of *Corbicula* is native mussel life stage. Juvenile mussels can be more sensitive than adults to stressors such as contaminants and high temperature (e.g., Augspurger et al. 2003; Fogelman et al. 2023). Mussel larvae (glochidia) are obligate parasites that undergo anatomical metamorphosis on fish hosts. Glochidia and newly-metamorphosed juveniles can be particularly sensitive to stressors (Keller et al. 2007; Fogelman et al. 2023). Newly-metamorphosed juveniles could be more sensitive to food limitation than older juveniles if their growth rate is higher or their energy stores are limited until filter feeding mechanisms develop (Douda 2015; Schartum et al. 2017).

We conducted a series of five laboratory experiments to assess the effects of *Corbicula* on juvenile mussel (60- and 1-day-old) survival and growth and how those effects are modulated by food abundance, temperature, and the type of co-occurring bivalve (*Corbicula* or native mussels). We tested the following hypotheses:

Hypothesis 1 *Corbicula* negatively affects juvenile mussel survival (Hypothesis 1a) and growth (1b) (Experiments 1–5).

Hypothesis 2 *Corbicula* has a greater negative effect on mussel survival (2a) and growth (2b) when food is limiting, which may indicate exploitative competition (Experiments 1–5).

Hypothesis 3 *Corbicula* has a greater negative effect on mussel survival (3a) and growth (3b) at warmer temperatures, which may indicate environmental modulation of competitive effects (Experiments 3 and 5).

Hypothesis 4 *Corbicula* has a greater negative effect on mussel survival (4a) and growth (4b) than a similar biomass of adult native mussels, which may indicate different competitive effects of invasive and native bivalves (Experiments 3–5).

Hypothesis 5 Access to deposited food sources (*Corbicula* feces and pseudofeces) results in higher mussel growth, which may represent facilitation (Experiment 2).

Methods

Experimental systems

We cultured juvenile mussels and conducted all experiments at the Center for Mollusk Conservation (CMC), Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky, USA. We used two experimental systems, both housed in a greenhouse at CMC (see Supplemental Information). We conducted Experiments 1 and 2 in a recirculating aquaculture system (RAS), as described by White et al. (2022). Briefly, our RAS consisted of eight, 41.1 × 13.3 × 10.7 cm trays (6 L capacity; 5.8 L actual volume) continuously gravity fed with water and food from a 23-L (15 L actual volume) mixing tank. Water overflowed from the top of the trays though a bulkhead fitting and tubing into a 56-L sump. The sump contained biological filter media (Bio Barrels, Pentair, Cary, North Carolina, USA) to promote bacterial growth. Water was pumped from the sump to the mixing tank, and food was gravity fed to the mixing tank from a 13-L feeding cone. Each tray was aerated with an air stone and received the food mixture from the mixing tank via tubing and a ball valve, and the valve for each tray was calibrated to provide a 100 mL/min flow rate through the tray. The system was filled with water purified by reverse osmosis then mixed with well water to alkalinity of 60–80 mg $CaCO_3$/L and pH of 8.1. We used only four of the eight trays, and the other four trays were allowed to run but had no bivalves. We used three RAS for Experiment 1 and four RAS for Experiment 2. Before each experiment, we cleaned system components with acetic acid and water then placed into each tray 50 mL of 150–250 µm heat-sterilized sand distributed evenly across the bottom.

The RAS has two disadvantages. First, because water from all trays in the system originates from a common mixing tank, flows into a common sump, and is recirculated, treatments applied to any given tray are not limited to that tray. For example, despite establishing different levels of *Corbicula* abundance among trays, its effect can be system-wide based on the total number of *Corbicula*/system volume (White et al. 2022), and it is impossible to manipulate food abundance in individual trays. This necessitates a separate RAS and sump for each level of *Corbicula* or food abundance or combination of those factors (see "The experiments"). Second, even within a single RAS devoted to a single treatment combination (e.g., high food, low *Corbicula*), there is a potential for all trays to be influenced similarly by conditions in the sump, although biological filtration and the high volume of the sump minimize these effects. We acknowledge this lack of independence, but in practice, the magnitude of variation in mussel growth and ammonia levels among trays in a single RAS was similar to that seen among fully independent trays in the static system (see subsequent), indicating that they served as informative replicates.

We overcame the limitations of the RAS for Experiments 3, 4, and 5 by constructing a static system in which each tray was supplied with food and water individually. The trays, water source, aeration, and sand substrate in this system were identical to those in the RAS, but the static system had no mixing tank or sump. We placed 20 biological filter media (Bio-barrels or Bio-balls, Pentair, Cary, North Carolina, USA) in each tray and carried out a full, manual water change once daily. Additionally, every eight hours, an automated ball valve delivered via gravity feed 1.9 L of water over eight minutes (equivalent to a one-third water change), with excess water overflowing through a bulkhead fitting and tubing into a floor drain. We fed mussels by manually introducing the full, specified food ration (see subsequent) once/day, after the manual water change was complete.

Food rations

In all experiments, we fed mussels a diet consisting of two live freshwater algae species cultured at CMC, *Chlorella sorokiniana* (CS) and *Phaeodactylum tricornutum* (PT); two commercially available concentrates of dead marine algae, *Nannochloropsis* spp. (Nanno 3600, NA) and *Thalassiosira pseudonana* (TP 1800, TP); and a commercially available mixture of six dead marine microalgae (Shellfish Diet 1800, SD) (all marine algae from Reed Mariculture Inc., Campbell, California). The diet consisted of the following proportions of each species based on dry mass: CS, 0.325; PT, 0.210; NA, 0.168; TP, 0.218; SD, 0.080. We used the same diet and proportions in all experiments, but the amount of food provided to mussels varied among experiments and treatments.

We used two food abundance levels, low and high, in all experiments, but these levels differed among experiments. In Experiments 1 and 2, we chose these food levels to represent the range of suspended fine particulate organic matter (FPOM) seen in Kentucky streams during the summer growing season (White et al. 2022). The high level consisted of 9.26 mg total algal dry mass/L/day, which corresponded to mean suspended FPOM of 3.96 mg/L and mean algal density of 359,833 cells/L. The low level was 47% of the high level (4.36 mg total algal dry mass/L/day), which corresponded to mean FPOM of 1.00 mg/L and mean algal density of 149,000 cells/L. In a previous study in RAS, juvenile mussel growth was positively related to food abundance, but food rations similar to our high and low levels produced environmentally relevant growth similar to that observed in the wild (White et al. 2022).

In Experiments 1 and 2, *Corbicula* substantially reduced suspended algal abundance even in the high food treatment (mean FPOM at day 4: no *Corbicula* treatment = 3.96 mg/L; high *Corbicula* treatment = 1.56). Consequently, we modified our food abundance levels for Experiments 3, 4, and 5 by increasing the high food level to lessen the potential for food limitation at that level and to increase the difference between the high and low levels. For these experiments, the high level consisted of 16.97 mg total algal dry mass/L/day, and the low level was 29% of the high level (4.89 mg/L/day). Similar levels of food abundance produced environmentally relevant growth (White et al. 2022).

Experimental animals

We cultured juvenile mussels following White et al. (2022). Briefly, we harvested mature glochidia from wild gravid female mussels and inoculated host fishes appropriate for each mussel species by anaesthetizing fishes and pipetting glochidia onto their gills. We held inoculated fishes in a recirculating aquarium system at 19–23 °C, and juvenile mussels metamorphosed and dropped off fishes three to four weeks after inoculation. After metamorphosis, we reared mussels in an RAS similar to that described previously and on the same diet described previously but consisting of 10.6 mg total algal dry mass/L/day (416,000 cells/L, see White et al. 2022). We tested responses of juveniles of three native mussel species in the experiments based on availability, and we cultured each species on the following host fishes: *Lampsilis fasciola* and *L. siliquoidea* (*Micropterus nigricans*, Centrarchidae); *Venustaconcha troostensis* (*Etheostoma virgatum*, Percidae). Prior to each experiment, we haphazardly selected 12–35 juvenile mussels to be placed into each experimental tray. We measured blotted wet mass (with shell) and shell length (maximum anterior to posterior dimension) of all 60-day-old juveniles, but newly-transformed mussels were too small to weigh, and we measured only their length. After placing mussels in the trays, we allowed them to acclimate to experimental conditions for 24h before adding *Corbicula* or other bivalves.

We collected wild *Corbicula* from North Elkhorn Creek, Scott County, Kentucky, acclimated them to laboratory conditions for a minimum of 6 days, and held them in a 416-L recirculating tank with a sump and biological filtration. We fed *Corbicula* the same food ration used for rearing juvenile mussels. We collected additional *Corbicula* as needed, but we held *Corbicula* in the laboratory a minimum of 6 days prior to using them in experiments. Over the course of the experiments, individual *Corbicula* averaged 4.1 g blotted wet mass (with shell) and 21.3 mm shell length.

In Experiment 1, we established three levels of *Corbicula* abundance: none, low, and high. Our target *Corbicula* levels for the low and high treatments were 100 and 1000/m^2, respectively, which are typical densities in the wild (Hakenkamp and Palmer 1999; McDowell et al. 2017; Haag et al. 2021). Actual densities varied among experiments because

we standardized *Corbicula* abundance for each treatment level based on estimated dry tissue mass, and *Corbicula* size varied slightly over the course of the experiments. We standardized by tissue mass to ensure similar levels of filter feeding within treatments (filtering rate of *Corbicula* and other bivalves is a strong function of body mass, Leff et al. 1990), and to account for differences in shell mass among bivalve species (see next paragraph). We estimated *Corbicula* dry tissue mass (without shell) based on shell length (mm) as log dry mass = 3.028(log shell length) + 5.133 (W.R. Haag, unpublished data). Based on mean *Corbicula* length at the beginning of Experiment 1 (20.8 mm), estimated dry mass of 5 individuals (representing 100/m^2 in a tray) was 0.3 g (16.6 g wet mass, with shell) in the low treatment, and estimated mass of 48 individuals (representing 1000/m^2) was 3.4 g (166.3 g wet mass) in the high treatment. For all subsequent experiments, we used only two levels of *Corbicula* abundance: none and high. We calculated the number of *Corbicula* required to produce 3.4 g tissue dry mass in the high treatment based on the mean size of *Corbicula* available at the time. The number of *Corbicula*/tray in the high treatment averaged 42 (range = 38–48) across experiments.

To test Hypothesis 4, in Experiments 3, 4, and 5 we included another bivalve treatment consisting of a tissue biomass of native mussels equivalent to that of the high *Corbicula* treatment (about 3.4 g tissue dry mass). For these experiments, we used adult *Lampsilis hydiana* from Obion Creek, Hickman County, Kentucky, USA. We estimated the number of individuals required to achieve the desired tissue mass using length-mass relationships for *L. straminea*, which is closely related and has similar shell morphology (Atkinson et al. 2020). Mean length of *L. hydiana* in each experiment was 60.0–63.4 mm, and achieving an estimated dry tissue mass of approximately 3.4 g/tray required 2–3 individuals/tray, which corresponded to mussel densities of 42.1–58.0/m^2. These are densities characteristic of native mussel beds in the eastern USA (Haag 2012).

General experimental procedures

We prepared food rations daily and delivered the specified amount to each RAS or individual tray in the static system based on system or tray volume. We measured temperature, pH, dissolved oxygen (DO, % saturation and mg/L), and total ammonia (mg N/L) throughout Experiments 1, 3, and 5. In Experiment 1, we measured water chemistry daily for the first nine days of the experiment and every 1–4 days thereafter. In experiments 3 and 5, we measured water chemistry in all trays at the beginning and end of the experiment and daily in half the trays on an alternating basis. We measured pH and ammonia in 50 mL water samples from each tray using a portable pH meter and the nitrogen, ammonia-salicylate method (Hach Method 10,031; https://cdn.hach.com/7FYZVWYB/at/r4vm8sw5hv7nhx8z6wxn98fk/DR_4000_Nitrogen-Ammonia_HR_TNT_Method_10031.pdf, accessed July 8, 2025), respectively. We measured temperature and DO directly in the trays using a handheld digital thermometer and portable DO meter, respectively. In Experiments 3 and 5, we recorded water temperature at 15 min intervals in half of the trays in each treatment combination using IBWetland temperature loggers (Alpha-Mach Inc., Sainte-Julie, Quebec, Canada). In addition to total ammonia, we estimated the concentration of un-ionized ammonia (the form toxic to aquatic organisms) on each sampling date based on temperature and pH (Emerson et al. 1975). We could not locate water chemistry data for Experiment 2, and only temperature data were available. In Experiment 4, we recorded total ammonia and temperature as described previously but only sporadically (average of three measurements/tray during the experiment), but we could not locate pH or DO data. For Experiment 4, we estimated un-ionized ammonia concentrations based on the overall mean temperature in the experiment and the grand mean of pH across all other experiments (8.1).

In Experiment 4, we measured the rate at which bivalves (juvenile mussels, *Corbicula*, or adult mussels) removed food from the water. We took a 10 mL water sample from each tray 15 min after feeding, every hour subsequently up to 7h, and a final sample at 22h, prior to the next feeding. We took care to sample only suspended material and not disturb deposited material. We measured turbidity as a proxy for food abundance because algal cells were the primary suspended material in the trays (White et al. 2022).

Corbicula mortality occurred throughout the experiments (mean = 4.5 individuals/day ± 1.6 SD, based on 10 days observation during Experiment 3). We removed dead *Corbicula* as soon as they were observed and added similarly sized live *Corbicula* to

maintain target treatment levels. There was no mortality of *Lampsilis hydiana* during any experiment.

The experiments

We conducted the five experiments sequentially, in the following order: 1, 2, 4, 3, 5 (see each experiment for specific times), based on the availability of experimental animals. We describe each experiment in an order reflecting the life stage of experimental animals (i.e., Experiments 1–3, 60-day-old mussels; Experiments 4 and 5, 1-day-old newly-metamorphosed mussels. See Supplemental Information for a diagrammatic representation of each experiment.

Experiment 1: Effects of Corbicula *and food abundance on growth of 60-day-old mussels*—We conducted this experiment at a single, ambient temperature in three RAS in two separate runs, which were required due to limitations of the RAS. Considered together, both runs constitute a 2 × 3 full factorial experiment with 24 trays and $n = 4$ for each treatment combination (Table 1). In the first run, all three RAS received the high food level, and in the second run, all three RAS received the low food level (see "Food rations"). In both runs, we randomly assigned each RAS to one of three *Corbicula* abundance levels: none, low, and high (see "Experimental animals").

We conducted each run for 14 days; run 1 began on July 26, 2019, and run 2 began on August 12, 2019. The test species was *V. troostensis*, which were 60 days old (mean individual mass and length = 0.011 g ± 0.002 SD and 3.8 mm ± 0.3 SD) at the beginning of run 1, and 77 days old (0.027 g ± 0.005, 4.9 mm ± 0.3 SD) at the beginning of run 2. We placed 20 V. *troostensis* in each tray. Mean temperature during the first and second run was 24.9 °C ± 1.4 SD, and 25.9 ± 0.9, respectively.

Experiment 2: Effects of Corbicula*, food abundance, and access to deposited food sources (*Corbicula *feces and pseudofeces) on growth of 60-day-old mussels*— In Experiment 1, we found an unexpected *positive* relationship between *Corbicula* abundance and mussel growth (see Results). Experiment 2 was designed to test a possible explanation for that relationship, in addition to the effects of *Corbicula* and food. This was a 2 × 2 × 2 full factorial experiment with 16 trays and $n = 2$ for each treatment combination conducted at single, ambient temperature in four RAS simultaneously in a single run (Table 1). We randomly assigned two RAS to the high food level and two RAS to the low food level. We randomly assigned each of the two RAS in each food level to one of two *Corbicula* abundance levels: none and high. Of the eight trays in each *Corbicula* level, we randomly assigned half to an "isolated" treatment and the other half to a "non-isolated" treatment. In the isolated treatment, we placed juvenile mussels in a 10 cm-diameter glass Petri dish with a layer of sand similar to that added to trays, and we elevated the dish above the bottom of the tray at about half the tray depth. This treatment was meant to isolate mussels from *Corbicula* feces and psuedofeces that accumulated on the tray bottoms. In the non-isolated treatment, we placed mussels directly in the tray as in all other experiments.

We conducted this experiment for 14 days beginning on June 29, 2020. The test species was *L. siliquoidea*, which were 60 days old (mean individual mass and length = 0.011 g ± 0.002 SD and 3.8 mm ± 0.3 SD) at the beginning of the experiment. We placed 20 *L. siliquoidea* in each tray. Mean temperature during the experiment was 26.1 °C ± 1.2 SD.

Experiment 3: Effects of Corbicula*, adult native bivalves, food abundance, and temperature on growth of 60-day-old mussels*—This was a 2 × 2 × 3 full factorial experiment conducted at two manipulated temperatures in an array of 36 static trays with $n = 3$ for each treatment combination (Table 1). We placed six trays in a water bath in each of six, 56 × 120 × 18 cm plastic crates; water level in each bath was about 2.5 cm below the top of the trays to prevent exchange of water. We randomly assigned three crates to a "warm" treatment (target temperature = 28 °C) and the other three to a "cool" treatment (target temperature = 22 °C), and we maintained temperatures in each crate with a combination of heating mats, submersible aquarium heaters, and inline chillers. This range encompasses mean summer temperatures typically experienced by mussels in the eastern USA, and it is above the minimum temperature for growth of many species (~ 20 °C) but below temperatures at which heat stress begins to occur (> 30 °C; Carey et al. 2013; Haag et al. 2019; Fogelman et al. 2023). In each crate, we randomly assigned three trays to the high food level and three to the low food level. We then randomly assigned the trays in each food level to one of three bivalve treatments: no bivalves, high *Corbicula*, and native mussels.

Table 1 Analysis of variance results for Experiments 1–3 evaluating the effects of food, temperature, access to deposited food sources (isolation), and co-occurring bivalves on 60-day-old juvenile mussel growth

Source of variation	df	*F*	*P*
Experiment 1 (*Venustaconcha troostensis*)			
Error	18	–	–
Food (two levels: low, high)	**1**	**357.2**	**< 0.001**
Corbicula (three levels: none, low, high)	**2**	**7.23**	**0.005**
Food × *Corbicula*	**2**	**18.32**	**< 0.001**
Contrasts			
***Corbicula* at low food**	**2**	**374.38**	**< 0.001**
Corbicula at high food	2	2.57	0.137
Experiment 2 (*Lampsilis siliquoidea*)			
Error	8	–	–
Food (two levels: low, high)	**1**	**156.8**	**< 0.001**
Corbicula (two levels: none, high)	**1**	**127.1**	**< 0.001**
Isolation (two levels: not isolated, isolated)	1	0.19	0.671
Food × *Corbicula*	**1**	**33.86**	**< 0.001**
Food × isolation	1	0.01	0.931
Corbicula × isolation	1	1.81	0.216
Food × *Corbicula* × isolation	1	1.63	0.238
Contrasts			
Effect of *Corbicula* at low food	**1**	**13.91**	**0.005**
Effect of *Corbicula* at high food	**1**	**136.58**	**< 0.001**
Experiment 3 (*Lampsilis fasciola*)			
Error	24	–	–
Food (two levels: low, high)	**1**	**53.10**	**< 0.001**
Temperature (two levels: 22, 29 °C)	**1**	**47.23**	**< 0.001**
Bivalve (three levels: none, *Corbicula*, native)	**2**	**27.86**	**< 0.001**
Food × temperature	**1**	**25.79**	**< 0.001**
Food × bivalve	2	0.74	0.487
Temperature × bivalve	**2**	**3.97**	**0.032**
Food × temperature × bivalve	2	0.75	0.482
Contrasts			
Effect of temperature, no bivalve vs. native bivalve	**1**	**6.02**	**0.021**
Effect of temperature, no bivalve vs. *Corbicula*	**1**	**6.20**	**0.020**
Effect of temperature, native bivalve vs. *Corbicula*	1	0.00	0.971
Effect of temperature, high vs. low food	**1**	**26.30**	**< 0.001**

Test species are given after the experiment number. Bolded model factors are significant ($\alpha = 0.05$). See text for description of treatment levels

We conducted this experiment for 14 days beginning on July 16, 2020. The test species was *L. fasciola*, which were 60 days old (mean individual mass and length = 0.114 g ± 0.014 SD and 10.2 mm ± 0.4 SD) at the beginning of the experiment. We placed 12 *L. fasciola* in each tray. Mean temperature in trays ranged from 28.0 °C ± 0.9 SD to 29.1 ± 1.4 in the warm treatment and from 21.1 ± 0.8 to 22.1 ± 1.1 in the cool treatment, but mean temperature did not differ among crates within each treatment (cool, $F_{2,6} = 1.64$, $P = 0.271$, mean = 21.6 °C; warm, $F_{2,6} = 2.77$, $P = 0.141$, mean = 28.5).

Experiment 4: Effects of Corbicula*, adult native bivalves, and food abundance on survival and growth of newly-metamorphosed mussels*—This was a 2 × 3 full factorial experiment conducted at a single ambient temperature in an array of 12 static trays with $n = 2$ for each treatment combination (Table 2). We randomly assigned half of the trays to the high food level and the other half to the low food level. In each food level, we randomly assigned two trays each to

Table 2 Analysis of variance results for Experiments 4 and 5 evaluating the effects of food, temperature, and co-occurring bivalves on survival and growth of 1-day-old newly-metamorphosed mussels

Source of variation	df	*F*	*P*
Experiment 4, survival (*Venustaconcha troostensis*)			
Error	6	–	–
Food (two levels: low, high)	1	4.42	0.080
Bivalve (three levels: none, *Corbicula*, native)	**2**	**18.40**	**0.003**
Food × bivalve	2	3.07	0.121
Experiment 4, growth			
Error	5	–	–
Food	1	1.52	0.273
Bivalve	2	4.38	0.080
Food × bivalve	**2**	**6.92**	**0.036**
Contrasts			
Effect of bivalve at low food	**2**	**10.59**	**0.016**
Effect of bivalve at high food	2	0.27	0.775
No bivalve vs. native bivalve at low food	**1**	**17.56**	**0.026**
No bivalve vs. *Corbicula* at low food	1	11.89	0.055
Native bivalve vs. *Corbicula* at low food	1	0.00	1.000
Experiment 5, survival (*Lampsilis siliquoidea*)			
Error	24	–	–
Food (two levels: low, high)	1	0.96	0.337
Temperature (two levels: 22, 28 °C)	**1**	**66.83**	**< 0.001**
Bivalve (three levels: none, *Corbicula*, native)	**2**	**18.14**	**< 0.001**
Food × temperature	1	0.02	0.895
Food × bivalve	2	1.50	0.242
Temperature × bivalve	**2**	**6.02**	**0.007**
Food × temperature × bivalve	2	0.51	0.604
Contrasts			
Effect of bivalve at 22 °C	**2**	**11.54**	**< 0.001**
Effect of bivalve at 28 °C	**2**	**12.62**	**< 0.001**
Effect of temperature, no bivalve vs. native bivalve	**1**	**10.16**	**0.004**
Effect of temperature, no bivalve vs. *Corbicula*	1	0.17	0.685
Effect of temperature, native bivalve vs. *Corbicula*	**1**	**7.72**	**0.010**
Experiment 5, growth, 22 °C treatment only			
Error	11	–	–
Food	1	0.60	0.454
Bivalve	**2**	**15.92**	**< 0.001**
Food × bivalve	**2**	**4.53**	**0.037**

Test species are given after the experiment number. All results are based on Type I sums of squares except for growth in experiments 4 and 5, which are based on Type III sums of squares to account for an unbalanced design due to complete mortality in some trays. In Experiment 5, we evaluated the response of growth only for the 22 °C treatment because of nearly complete mortality in the native bivalve and *Corbicula* treatments at 28°. Bolded model factors are significant ($\alpha = 0.05$). See text for description of treatment levels

one of three bivalve treatments: no bivalves, high *Corbicula*, and native mussels.

We conducted this experiment for 14 days beginning on June 16, 2020. The test species was *V. troostensis*, which were 1 day old (mean individual length = 0.279 mm ± 0.022 SD, mass not determined). We placed 28–58 V. *troostensis* in each tray. Mean temperature during the experiment was 25.9 °C ± 1.1 SD.

Experiment 5: Effects of Corbicula*, adult native bivalves, food abundance, and temperature on survival and growth of newly-metamorphosed mussels*—This was a 2 × 2 × 3 full factorial experiment identical to Experiment 3, except that we used newly-metamorphosed (1 day old) juveniles instead

of 60-day-old juveniles (Table 2). We conducted this experiment for 21 days beginning on August 3, 2020. The test species was *L. siliquoidea* (mean individual length = 0.280 mm ± 0.010 SD, mass not determined). We placed 35 *L. siliquoidea* in each tray. Mean temperature in trays ranged from 28.0 °C ± 0.7 SD to 28.8 ± 0.9 in the warm treatment and from 21.8 ± 0.4 to 22.8 ± 0.7 in the cool treatment, but mean temperature did not differ among crates within each treatment (cool, $F_{2,6} = 3.87$, $P = 0.083$, mean = 22.2 °C; warm, $F_{2,6} = 3.16$, $P = 0.115$, mean = 28.4).

Data analysis

We estimated proportional survival in each tray as the number of live individuals recovered at the end of the experiment/the number of juveniles placed in the tray at the beginning of the experiment. We used arcsine-square root transformation of survival for all statistical analyses. We expressed growth as instantaneous growth (/day: ln[final size/initial size]/deployment period in days, Ricker 1975; hereafter, "growth"). Instantaneous growth is the exponential factor by which mass is predicted to increase each day; we used this measure instead of raw growth because it can be more easily compared among experiments (by accounting for differences in initial mussel size and study duration) and it has better statistical properties. In Experiments 1–3, we calculated growth based on mass (as g) and shell length (as mm), but we performed all statistical analyses based on mass. In Experiments 4 and 5, we expressed growth of newly-metamorphosed mussels only as length. Data for all experiments satisfied assumptions of normality and homogeneity of variance. We analyzed data from each experiment separately. We evaluated differences in survival, growth, and ammonia concentration among treatments using ANOVA with all interactions and all experimental factors (food, temperature, bivalve, isolation) as fixed class variables. For ANOVA models with significant interactions that precluded interpretation of experiment-wide main effects, we used the SLICE and CONTRAST procedures in SAS (version 9.4, SAS Institute, Cary, NC, USA) to test specific hypotheses about treatment effects. The three-way interaction was not significant for any model (Experiments 2, 3, and 5), and results did not change after omitting that interaction term from the models. We report ANOVA results based on the full models, but all contrasts are based on the models without the three-way interaction term. Within each experiment, we evaluated relationships between growth or survival and mean ammonia concentration in each tray using linear regression.

Results

Growth of 60-day-old juvenile mussels was similar overall across Experiments 1–3 despite varying experimental conditions (grand means ± SD, /d, as g: Experiment 1 = 0.017 ± 0.012; Experiment 2 = 0.010 ± 0.010; Experiment 3 = 0.015 ± 0.007). These values are similar to 90-day-old juvenile mussel growth reported in the wild in Kentucky streams representing a wide range of ecological conditions (grand means ± SD: Haag et al. 2019 = 0.021 ± 0.013, 23 streams; Haag et al. 2021 = 0.015 ± 0.009, 17 streams). Growth based on length also was similar across Experiments 1–3 (grand means ± SD, /d, as mm: Experiment 1 = 0.006 ± 0.003; Experiment 2 = 0.003 ± 0.004; Experiment 3 = 0.005 ± 0.002). Growth of newly-metamorphosed mussels in Experiments 4 and 5 (/d, as mm: Experiment 4 = 0.038 ± 0.009; Experiment 5 = 0.032 ± 0.012) was higher than growth of 60-day-old juveniles.

Experiment 1: Effects of *Corbicula* and food abundance on growth of 60-day-old mussels

Survival was 100% in all trays. Food abundance, *Corbicula* abundance, and the food × *Corbicula* interaction all were significant factors in explaining mussel growth (Table 1, Fig. 1A). Growth was higher in the high food treatment than in the low food treatment at all three levels of *Corbicula* abundance. Contrasts showed that growth did not differ among *Corbicula* levels in the high food treatment (Tukey's HSD, $\alpha = 0.05$). Growth differed among all three *Corbicula* levels in the low food treatment, and growth was highest in the high *Corbicula* treatment.

Mean values for pH and DO were similar across all treatment combinations (ranges: pH = 8.2–8.3; DO, % = 96.0–99.8; DO, mg/L = 8.0–8.4; Table S1). Mean total ammonia in treatment combinations ranged from 0.03–0.07 mg/L, and mean un-ionized ammonia was 2.5–5.6 µg/L. Both *Corbicula* abundance and food

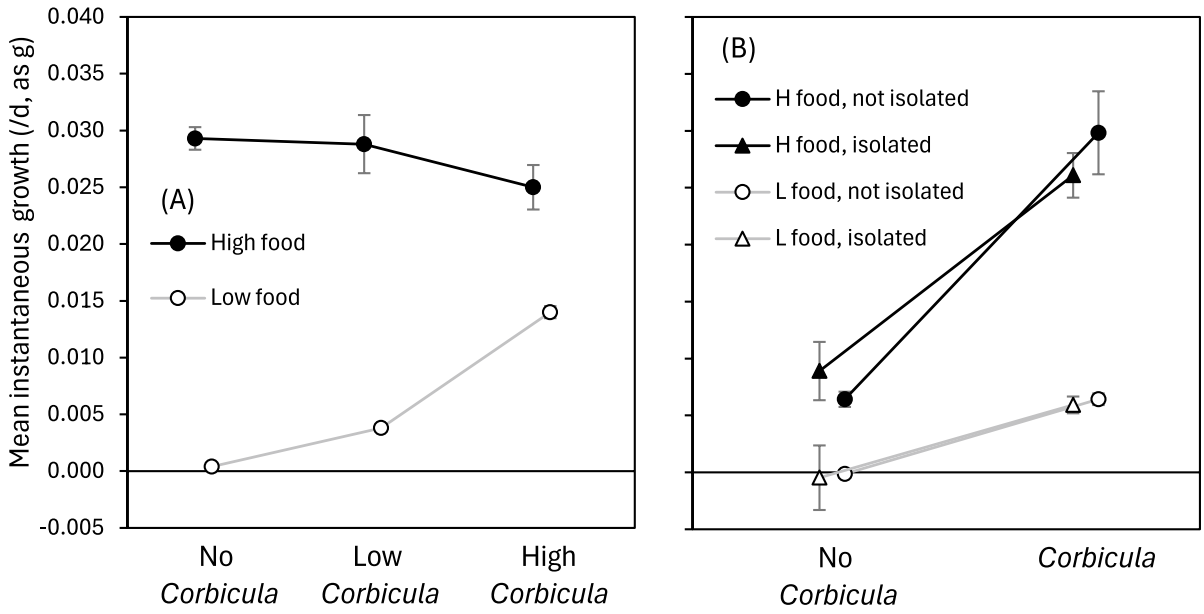

Fig. 1 Effects of food abundance (H = high, L = low) and *Corbicula fluminea* on growth of 60-day-old mussels in **A** Experiment 1 (*Venustaconcha troostensis*) and **B** Experiment 2 (*Lampsilis siliquoidea*). In Experiment 1, low *Corbicula* represents ~ 100/m^2 and high *Corbicula* ~ 1000/m^2. In Experiment 2, *Corbicula* ~ 1000/m^2, and "isolated" means that juveniles had access to suspended food but not to potential deposited food sources (*Corbicula* feces and pseudofeces); not isolated mussels had access to both food sources. Symbols are mean values in each treatment combination, and error bars are SE. Both experiments were conducted at a single, ambient temperature (Experiment 1: 24.9–25.9 °C; Experiment 2: 26.1 °C)

were significant factors in explaining variation in total ammonia, but the food × *Corbicula* interaction was not significant (Fig. 2; Table S2). Total ammonia was slightly higher in both *Corbicula* treatments than in the no bivalve treatment (least square means, high = 0.05 mg/L; low = 0.05; no bivalve = 0.03), and higher in the high food treatment than at low food (high = 0.05 mg/L; low = 0.04). Growth was not related to total or un-ionized ammonia ($P = 0.157$, 0.527, respectively).

Experiment 2: Effects of *Corbicula*, food abundance, and access to deposited food sources (*Corbicula* feces and pseudofeces) on growth of 60-day-old mussels

Survival was 100% in all trays. Food abundance, *Corbicula* abundance, and the food × *Corbicula* interaction were significant factors in explaining mussel growth (Table 1, Fig. 1B). Access to deposited food sources (isolation) was not a significant factor, and all other interaction terms were non-significant. Contrasts showed that growth was higher in the presence of *Corbicula* than in its absence at both food levels, but *Corbicula* had a greater effect on growth at the high food level (difference, *Corbicula*–none = 0.020/d, as g) than at the low food level (difference = 0.007).

Experiment 3: Effects of *Corbicula*, adult native bivalves, food abundance, and temperature on growth of 60-day-old mussels.

Survival was 100% in all trays. All factors were significant in explaining mussel growth, except the food × bivalve interaction and the 3-way interaction (Table 1, Fig. 3). The lack of a food × bivalve interaction showed that food did not modulate the response to bivalve treatment. Least square mean growth was higher in the high food treatment than in low food in all bivalve treatments, and growth in the no bivalve/high food treatment was higher than all other treatment combinations. The temperature × bivalve interaction showed that bivalve treatment affected the response to temperature. Contrasts and least square

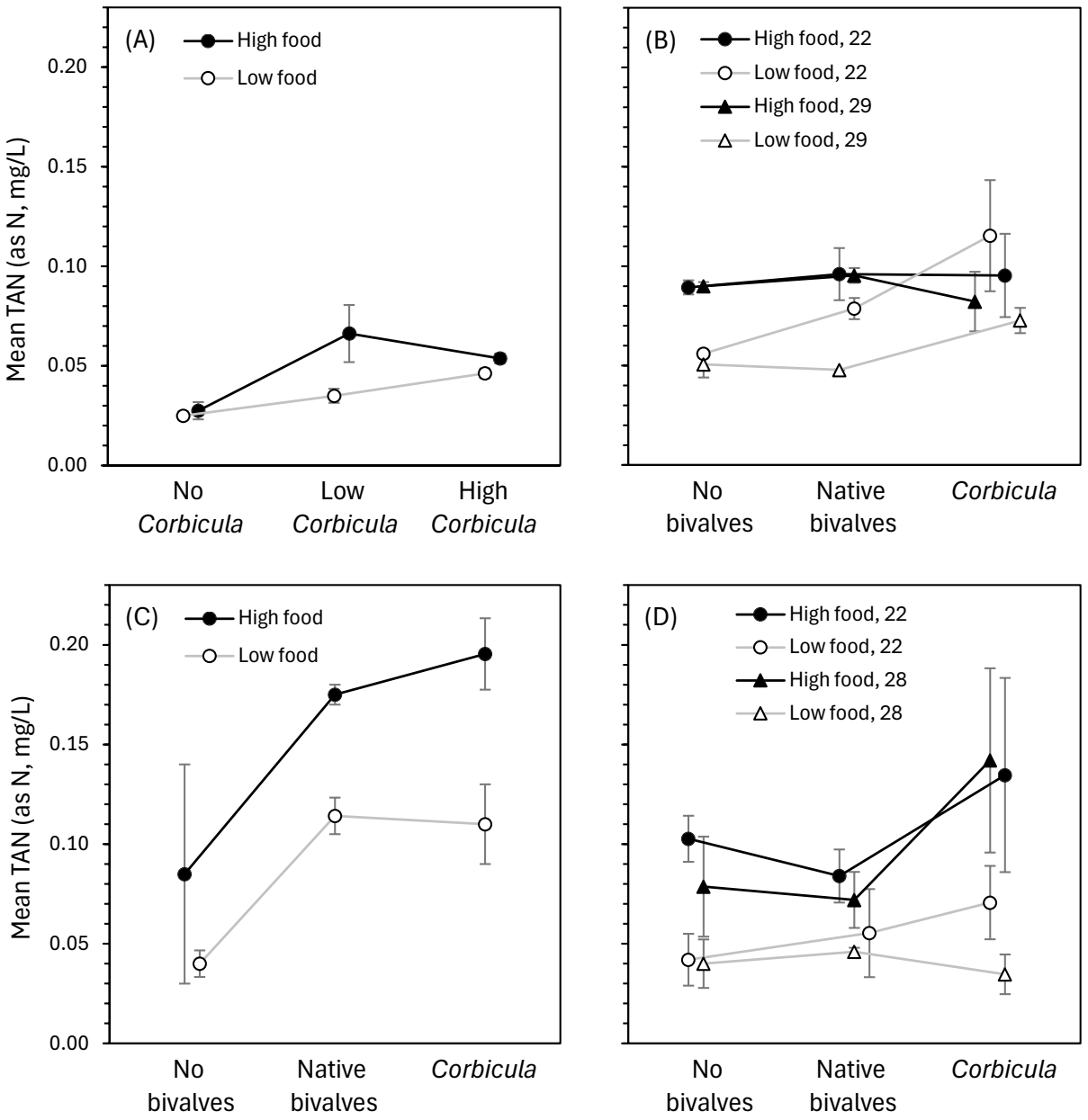

Fig. 2 Effects of food abundance, temperature, and bivalve presence (*Corbicula fluminea* or adult native mussels) on total ammonia (TAN) concentration in **A** Experiment 1, **B** Experiment 3, **C** Experiment 4, and **D** Experiment 5. Temperature is °C, symbols are mean values in each treatment combination, and error bars are SE

means showed that temperature affected growth only in the no bivalve treatment, and growth in the no bivalve/warm treatment was higher than in all other treatment combinations. The food × temperature interaction showed that temperature affected the response to food. Contrasts and least square means showed that mussel growth was higher in the high food treatment than in low food only in the warm treatment (ls mean growth, high = 0.024, low = 0.013), but growth did not differ between food treatments at the cool temperature (high = 0.013, low = 0.011).

Mean values for pH and DO were similar across all treatment combinations (ranges: pH = 8.1–8.2; DO, % = 83.6–88.7; DO, mg/L = 7.0–7.3; Table S1). Total

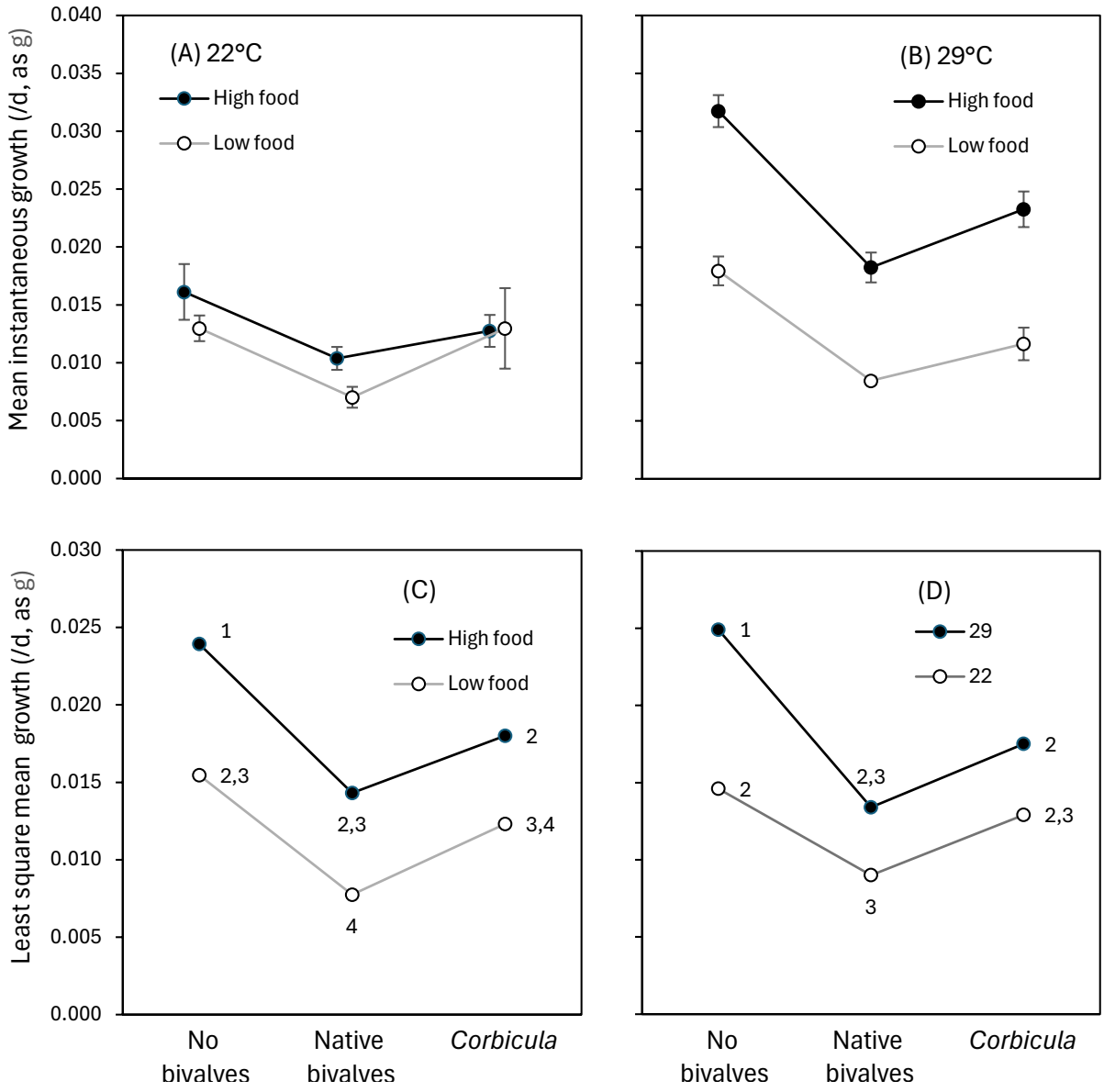

Fig. 3 Effects of food abundance, temperature, and bivalve presence (*Corbicula fluminea* or adult native mussels) on growth of 60-day-old mussels (*Lampsilis fasciola*) in Experiment 3. **A** Effects of food and bivalve treatments at 22 °C. **B** Effects of food and bivalve at 29 °C. **C** Experiment-wide least-square mean growth as a function of food abundance and bivalve. **D** Experiment-wide least-square mean growth as a function of temperature and bivalve. *Corbicula* treatments represent ~ 1000/m^2, and native bivalve treatments are a biomass equivalent to the *Corbicula* treatment (see text). Symbols are mean values in each treatment combination, and error bars are SE. On panels **C** and **D**, values with the same number are not significantly different based on Tukey post-hoc multiple comparisons ($\alpha = 0.05$)

ammonia was 0.05–0.12 mg/L. Mean un-ionized ammonia was 3.6–5.9 µg/L in most treatment combinations except the no bivalve and native bivalve treatments at high food and warm temperature (7.9–8.4 µg/L). Food abundance, temperature, and all two-way interaction terms were significant factors in explaining variation in total ammonia (Fig. 2; Table S2). Bivalve treatment and the three-way interaction were not significant. Ammonia appeared to be explained mainly by food abundance,

with generally higher levels in high food treatments. However, although the bivalve main effect was not significant, the food × bivalve interaction suggested that food affected the response of ammonia to the bivalve treatment, with higher levels in the presence of *Corbicula* only at low food. Ammonia was similar in all treatments involving *Corbicula*, but it varied in other treatment combinations. Growth was not related to total ammonia ($P = 0.167$) but was positively related to un-ionized ammonia ($P < 0.001$, $R^2 = 0.44$).

Experiment 4: Effects of *Corbicula*, adult native bivalves, and food abundance on survival and growth of newly-metamorphosed mussels.

Survival was uniformly high in no bivalve and native bivalve treatments (all observations ≥ 0.70), but survival was lower and more variable in *Corbicula* treatments, including one tray in the low food/*Corbicula* treatment combination with 0.00 survival (0.45–0.62 in other trays; Fig. 4). Bivalve was a significant factor in explaining mussel survival, and neither food nor the bivalve × food interaction were significant (Table 2). Survival did not differ between the no bivalve and native bivalve treatments (Tukey's HSD, $\alpha = 0.05$; means: no bivalve = 0.85, native bivalve = 0.76), but both were significantly higher than the *Corbicula* treatment (mean = 0.41). Data scatter suggested a more severe effect of *Corbicula* in the low food treatment, but variance was high in the low food/*Corbicula* treatment due to complete mortality in one tray.

Neither main effect (food or bivalve) were significant factors in explaining mussel growth, but the food × bivalve interaction was significant (Table 2, Fig. 4). Contrasts and least square means showed that there was no effect of bivalve on growth at high food abundance, but there was a strong effect of bivalve at low food. At low food, growth in the no bivalve treatment was significantly higher than in the native bivalve treatment, and growth did not differ between the native bivalve and *Corbicula* treatments. The difference between growth in the no bivalve and *Corbicula* treatments at low food was only marginally significant, probably because of low power due to the lack of a growth estimate in one tray in the low food/*Corbicula* treatment combination that experienced complete mortality.

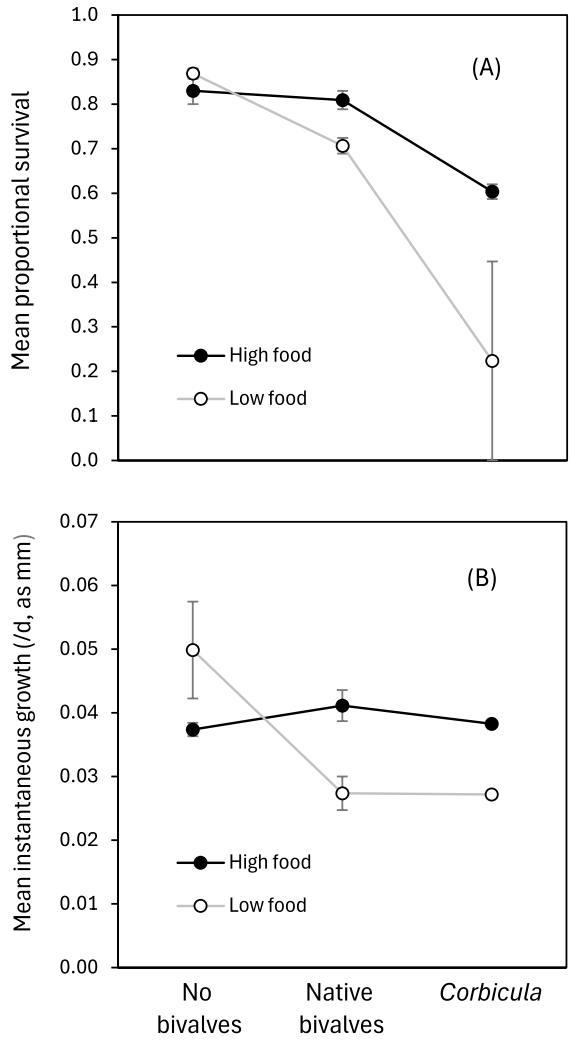

Fig. 4 Effects of food abundance and bivalve presence (*Corbicula fluminea* or adult native mussels) on **A** survival and **B** growth of newly-metamorphosed (1-day-old) mussels (*Venustaconcha troostensis*) in Experiment 4. *Corbicula* treatments represent ~ 1000/m^2, and native bivalve treatments are a biomass equivalent to the *Corbicula* treatment (see text). Symbols are mean values in each treatment combination, and error bars are SE. This experiment was conducted at a single, ambient temperature (25.9 °C)

Total ammonia was 0.04–0.20 mg/L, and un-ionized ammonia was 2.8–13.9 µg/L (Fig. 2; Table S1). Food abundance and bivalve both were significant factors in explaining variation in total ammonia, but the interaction term was not significant (Table S2). Ammonia was highest in and did not differ between the *Corbicula* and native bivalve

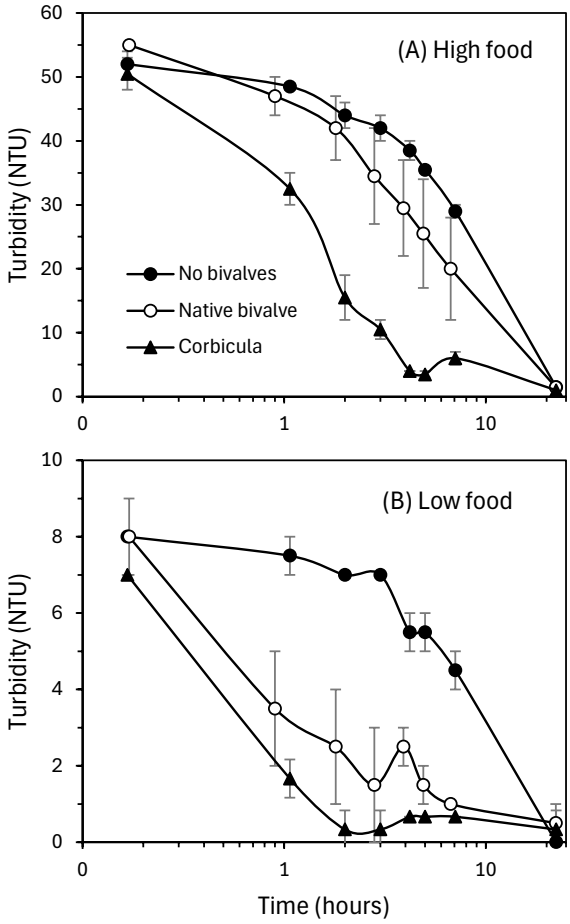

Fig. 5 Effects of bivalves on food abundance (as turbidity) over 23 h at two food levels in Experiment 4. *Corbicula* treatments represent ~ 1000/m^2, and native bivalve treatments are a biomass equivalent to the *Corbicula* treatment (see text). Symbols are mean values in each treatment combination, and error bars are SE. Note the log scale of the *x*-axis

treatments (least square means = 0.15 mg/L in both treatments) and both were significantly higher than in the no bivalve treatment (0.06 mg/L; Tukey's HSD, $\alpha = 0.05$). Ammonia was higher in the high food treatment than in low food (least square means = 0.15 and 0.09, respectively). Neither survival nor growth were related to total ammonia ($P = 0.475$, 0.594, respectively) or un-ionized ammonia ($P = 0.476$, 0.591, respectively).

Corbicula removed most food from trays by 4h after feeding in the high food treatment and by 2h in the low food treatment (Fig. 5). In the no bivalve treatments, juvenile mussels removed food slowly, and turbidity was reduced by < 50% at 7h after feeding. The effect of adult native mussels depended on food abundance. At high food, native mussels removed food slowly, and the turbidity attenuation curve was similar to that of the no bivalve treatment. At low food, native mussels removed food more rapidly, similar to *Corbicula*.

Experiment 5: Effects of *Corbicula*, adult native bivalves, food abundance, and temperature on survival and growth of newly-metamorphosed mussels.

Survival was lower in this experiment than in Experiment 4, particularly at 28 °C; survival was 0.00 in all *Corbicula* trays at 28 °C and mean survival was ≤ 0.16 in both *Corbicula* treatments at 22 °C (Fig. 6). Temperature, bivalve, and the temperature × bivalve interaction were significant factors in explaining mussel survival (Table 2). Neither food nor any other interaction terms were significant. The temperature × bivalve interaction showed that temperature affected the response of survival to the bivalve treatment. Contrasts showed that there was a significant effect of bivalve at both temperatures, and the effect of temperature in the native bivalve treatment differed from that in the no bivalve and *Corbicula* treatments, in which temperature had similar effects. Least square means showed that at 22 °C, survival was highest in the no bivalve and native bivalve treatments and did not differ between those two treatments, and survival was lowest in the *Corbicula* treatment. At 28 °C, survival also was highest in the no bivalve treatment, but it was higher than the native bivalve treatment, which did not differ from the *Corbicula* treatment. Survival did not differ between 22 and 28 °C in the no bivalve treatment, but survival was significantly lower at 28° than at 22° in the native bivalve and *Corbicula* treatments.

We were able to evaluate the response of growth only for the 22 °C treatment because of nearly complete mortality in the native bivalve and *Corbicula* treatments at 29° (Table 2, Fig. 6D). Bivalve was a significant factor in explaining mussel growth. Food was not a significant factor. The food × bivalve interaction was significant, showing that the effect of bivalve on growth was greater at low food abundance than at high food, but we did not conduct contrasts for these data. Ignoring the interaction term, least square mean growth differed significantly between

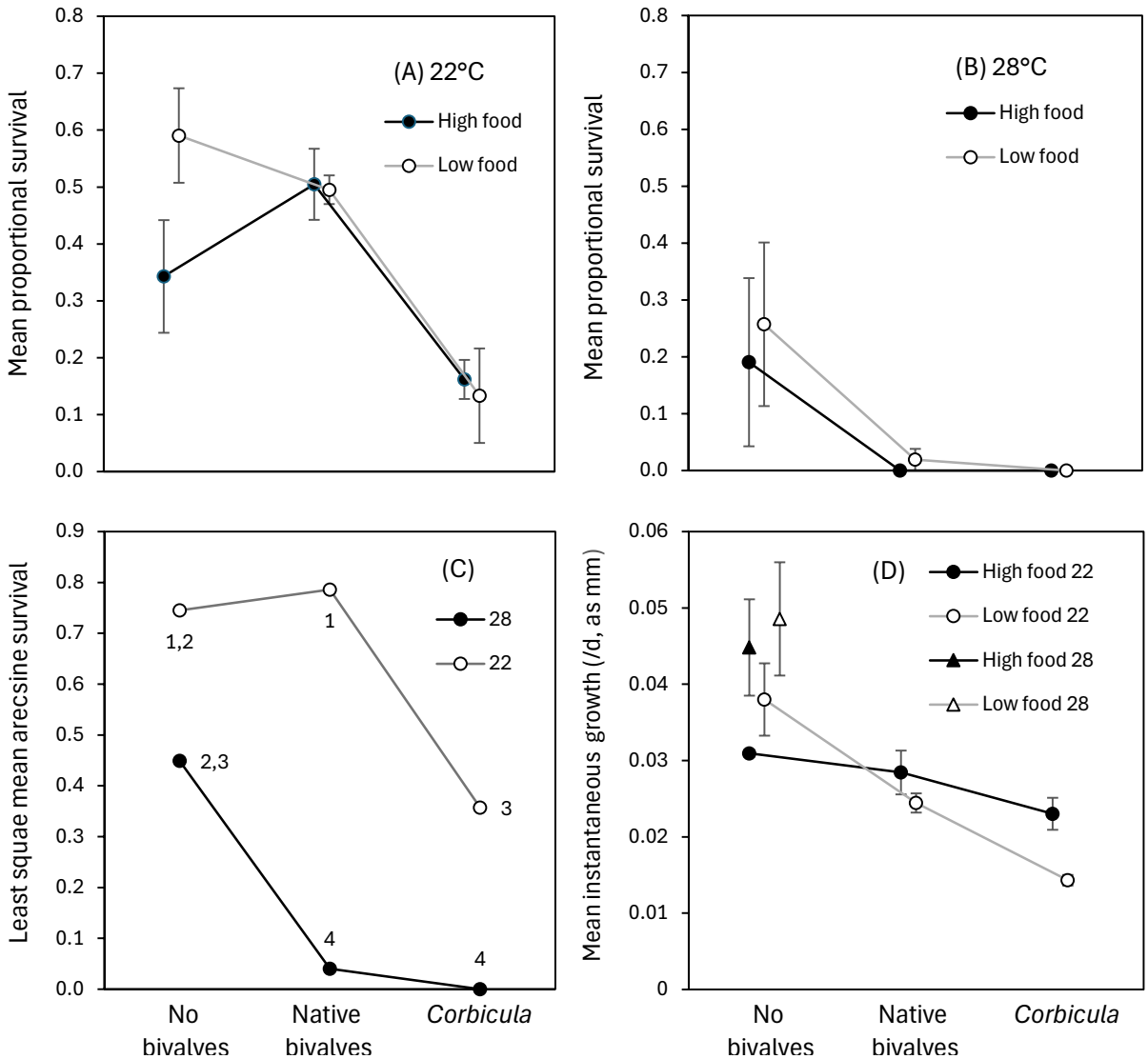

Fig. 6 Effects of food abundance, temperature, and bivalve presence (*Corbicula fluminea* or adult native mussels) on survival and growth of newly-metamorphosed (1-day-old) mussels (*Lampsilis siliquoidea*) in Experiment 5. **A** Effects of food and bivalve treatments on survival at 22 °C. **B** Effects of food and bivalve on survival at 28 °C. **C** Experiment-wide least-square mean survival as a function of temperature and bivalve. **D** Effects of food, temperature, and bivalve on growth. Growth estimates were not available for the native bivalve and *Corbicula* treatments at 28 °C due to high mortality. *Corbicula* treatments represent ~ 1000/m^2, and native bivalve treatments are a biomass equivalent to the *Corbicula* treatment (see text). Symbols are mean values in each treatment combination, and error bars are SE. On panel **C**, values with the same number are not significantly different based on Tukey post-hoc multiple comparisons ($\alpha = 0.05$)

the no bivalve and *Corbicula* treatments, but native bivalve did not differ from the other two bivalve treatments (Tukey's HSD, $\alpha = 0.05$; means: no bivalve = 0.034 (/day, as mm), native bivalve = 0.027, *Corbicula* = 0.020).

Mean values for pH and DO were similar across all treatment combinations (ranges: pH = 7.9–8.0; DO, % = 84.5–89.6; DO, mg/L = 7.0–7.4; Table S1). Total ammonia was 0.03–0.14 mg/L, and mean un-ionized ammonia was 1.9–6.2 µg/L. Food abundance was the only significant factor in explaining variation in

total ammonia (Fig. 2; Table S2). Total ammonia was higher in high food treatments than in low food (means = 0.10 and 0.05 mg/L, respectively). Survival was not related to total or un-ionized ammonia ($P = 0.565$, 0.237, respectively). At 22 °C, growth was not related to total or un-ionized ammonia ($P = 0.365$, 0.369, respectively).

Discussion

We found conflicting support for hypotheses that *Corbicula* negatively affects juvenile mussel survival and growth because responses differed between mussel life stages and between RAS and static systems (Table 3). Survival was 100% in all experiments involving 60-day-old juveniles, and Hypothesis 1a was not supported in those experiments. In contrast, *Corbicula* was associated with strongly decreased survival of newly-metamorphosed juveniles. Growth responses also differed between 60-day-old and newly metamorphosed juveniles, and growth responses of 60-day-old juveniles differed between RAS and static system experiments.

The most surprising result with 60-day-old juveniles was their *higher* growth in the presence of *Corbicula* in RAS in Experiments 1 and 2. Hypotheses 1b and 2b were not supported either in Experiment 1 or 2. In Experiment 1, *Corbicula* affected growth only at low food abundance, as predicted, but the effect (positive) was opposite our predictions. In Experiment 2, *Corbicula* affected growth in both food treatments, but the effect was positive and greatest at

Table 3 Results of laboratory experiments to test hypotheses that *Corbicula* has negative effects on juvenile mussel survival and growth

	60-day-old juveniles			Newly-metamorphosed juveniles	
	Experiment				
Hypothesis	1	2	3	4	5
1a: *Corbicula* negatively affects survival	Not supported	Not supported	Not supported	Supported	Supported
1b: *Corbicula* negatively affects growth	Not supported	Not supported	Partially supported	Supported	Supported
2a: *Corbicula* has a greater negative effect on survival when food is limiting	Not supported	Not supported	Not supported	Not supported	Not supported
2b: *Corbicula* has a greater negative effect on growth when food is limiting	Not supported	Not supported	Not supported	Supported	Supported
3a: *Corbicula* has a greater negative effect on survival at warmer temperatures	n/a	n/a	Not supported	n/a	Not supported
3b: *Corbicula* has a greater negative effect on growth at warmer temperatures	n/a	n/a	Supported	n/a	n/a[1]
4a: *Corbicula* has a greater negative effect on survival than native mussels	n/a	n/a	Not supported	Supported	Partially supported
4b: *Corbicula* has a greater negative effect on growth than native mussels	n/a	n/a	Not supported	Not supported	Partially supported
5: Access to *Corbicula* feces and pseudofeces results in higher mussel growth	n/a	Not supported	n/a	n/a	n/a

[1]The effect of temperature on growth could not be evaluated in this experiment because of high mortality at the higher temperature

"Not applicable" (n/a) indicates that the hypothesis was not tested in the experiment

high food abundance, both of which were opposite our predictions.

We do not know the cause of the strong, positive effect of *Corbicula* on mussel growth in the RAS. We hypothesized that *Corbicula* feces and psuedofeces represented a concentrated food source for juvenile mussels that fostered higher growth (Hypothesis 2). However, the lack of an effect of isolation in Experiment 2 did not support this hypothesis and suggests that mussels fed mainly on suspended material; alternatively, pseudofeces could represent a low-quality food source. Nitrogen excretion by *Corbicula* could have stimulated primary production in the RAS, resulting in increased food abundance for juvenile mussels. We cannot explain the difference in the modulating effect of food between Experiments 1 and 2.

Responses of 60-day-old juveniles in the static system of Experiment 3 differed markedly from responses in the RAS of Experiments 1 and 2. Notably, mussel growth was not higher in the presence of *Corbicula*. Unlike in Experiments 1 and 2, ammonia was not higher in *Corbicula* or native bivalve treatments; instead, ammonia was higher at high food abundance regardless of bivalve treatment. This result suggests that *Corbicula* or native bivalve excretion did not stimulate primary production or otherwise increase food abundance for mussels above that provided by the experimental food rations.

Experiment 3 provided partial support for Hypothesis 1b, but a negative effect of *Corbicula* on growth was seen only at high food, which is opposite our predictions and did not support Hypothesis 2b. Hypothesis 3b was supported by a negative effect of *Corbicula* on growth only at the warmer temperature. However, the temperature × bivalve interaction accounted for the least amount of variation of any significant factor in the model. The effects of native bivalves and *Corbicula* on growth were similar, which did not support Hypothesis 4b.

In contrast to 60-day-old mussels, newly-metamorphosed mussels showed strong negative responses to *Corbicula*. Hypothesis 1a was supported both in Experiments 4 and 5 by lower overall survival in the presence of *Corbicula* than in the no bivalve treatment. Despite the suggestion of a greater negative effect of *Corbicula* at low food abundance in Experiment 4, there was no statistical support for Hypothesis 2a, probably because of the high variance in the low food/*Corbicula* treatment combination. Hypothesis 2a was not supported in Experiment 5, where there was no effect of food on survival. Hypothesis 1b was supported both in Experiments 4 and 5 by lower growth in the presence of *Corbicula* than in the no bivalve treatment, and Hypothesis 2b was supported because this effect was present only, or was stronger, at low food abundance.

Hypothesis 3a was not supported in Experiment 5. Temperature strongly affected survival, but the effect was similar in no bivalve and *Corbicula* treatments, and temperature modulated only the effects of native bivalves. We were unable to evaluate Hypothesis 3b in Experiment 5 because of high mortality. Hypothesis 4a was supported in Experiment 4 by lower survival in the presence of *Corbicula* than in the presence of native bivalves. In Experiment 5, Hypothesis 4a was supported at 22 °C but not at 28°, where *Corbicula* and native mussels had similar effects on survival. Hypothesis 4b was not supported in Experiment 4, where *Corbicula* and native bivalves had similar effects on growth. There was partial support for Hypothesis 4b in Experiment 5 because the effect of native bivalves on growth was intermediate between that of the no bivalve and *Corbicula* treatments.

There are several potential mechanistic explanations for the strong negative effects of *Corbicula* on newly-metamorphosed mussels that we observed. Yeager et al. (2000) evaluated the effects of *Corbicula* abundance on newly-metamorphosed mussel survival and growth over 10 days in 600 mL beakers at a single food and temperature level (25 °C). They observed ~ 80% mussel mortality at abundance equivalent to 313–625 *Corbicula*/m^2 and 100% mortality at ≥ 1250/m^2 (compared with ~ 0% mortality with no *Corbicula*), and they observed a sharp decrease in growth with increasing *Corbicula* abundance. They attributed mussel mortality to ingestion by *Corbicula* and entanglement in the mucus of *Corbicula* pseudofeces, based on videotaped observations; they did not propose an explanation for reduced growth. Other proposed explanations pertinent to our experimental conditions are food competition, ammonia toxicity (from *Corbicula* mortality or excretion), and *Corbicula* as a vector of disease (Strayer 1999; Haag 2019).

Ingestion by *Corbicula* and entanglement in pseudofeces could explain the high mussel mortality we saw in *Corbicula* treatments, and entanglement of survivors could have impaired feeding, resulting

in decreased growth. This explanation also could explain the lack of mortality of 60-day-old juveniles, which are too large to be ingested and may be less vulnerable to entanglement. However, the food × bivalve interaction in Experiments 4 and 5, showing that growth (and potentially survival) was affected by *Corbicula* mainly under conditions of food limitation, is not consistent with ingestion or entanglement and instead suggests food competition. The greater effects of *Corbicula* on newly-metamorphosed juveniles compared with older juveniles could be explained by the former's higher growth rate and lower energy reserves, which are more quickly depleted than those of older juveniles. It is also possible that a combination of these factors (ingestion, entanglement, and competition) result in low survival and growth. We cannot discount disease carried by *Corbicula* as a cause of mussel mortality or low growth because our experiments may have been too short to see the full effects of pathogens or parasites.

Ammonia toxicity is a major challenge in any aquaculture system, and mussels are highly sensitive to the un-ionized form (Augspurger et al. 2003; Wang et al. 2007). As in our study, excretion by *Corbicula* at high densities can result in elevated ammonia in experimental chambers (Modesto et al. 2019), but we also observed elevated ammonia in high food and native bivalve treatments. It is unlikely that ammonia toxicity influenced the results of any of our experiments. The maximum values we observed (total ammonia as N: 0.20 mg/L, Experiment 4, high food/*Corbicula* treatment; 0.14 mg/L, Experiment 5, high food/28 °C/*Corbicula* treatment; Table S1) were well below levels expected to negatively affect mussel survival or growth in long-term exposures (USEPA aquatic life criteria for chronic ammonia exposure, adjusted to experimental conditions of pH and temperature: Experiment 4 = 0.46 mg/L; Experiment 5 = 0.40; USEPA 2013). In a previous experimental study, survival of mussel glochidia over 48 h increased appreciably relative to controls only at ammonia concentrations ≥ 1.00 mg/L (Modesto et al. 2019). Neither survival nor growth were correlated with ammonia concentrations in any experiment, except Experiment 3, where growth was positively correlated with ammonia, reflecting higher growth in the high food treatment where ammonia was elevated.

One experimental factor that may have influenced our results was temperature in Experiment 5. The high temperature treatments in Experiments 3 and 5 (29 and 28 °C, respectively) were near LT50 (lethal temperature at which 50% mortality is expected) values for 60-day-old juvenile mussels (including one of our study species, *Lampsilis siliquoidea*) in 14-day chronic exposures (30.1–30.8 °C, Ganser et al. 2015). These are the only long-term exposure values available for mussels, and no values are available for newly-metamorphosed juveniles (see Fogelman et al. 2023). There was no evidence of negative effects of higher temperature on survival or growth of 60-day-old juveniles, but the much lower survival of newly metamorphosed juveniles at 28 °C in Experiment 5 may have been due in part to high temperature. Nevertheless, bivalve treatment had a strong effect in that experiment, suggesting that lower survival was a combined stress response to temperature and the presence of competing bivalves. Dissolved oxygen tended to be lower in static systems than in RAS (Table S1), but all values were ≥ 83% saturation and 7.0 mg/L, which are far above lethal levels for mussels (< 2.0 mg/L, Sparks and Strayer 1998; Hyvärinen et al. 2022), and DO was similar in all treatment combinations in all experiments.

All of the native mussel species we used are in the same tribe (Lampsilini) and have similar life histories; nevertheless, some of the differences in the results of our experiments could be explained by differences among mussel species in their responses to *Corbicula*. For example, growth of both *V. troostensis* and *L. siliquoidea* was higher growth in the presence of *Corbicula* in Experiments 1 and 2, respectively, but the different modulating effect of food in those experiments could reflect different species responses. However, results of other experiments differed markedly despite being conducted with the same species. For both *V. troostensis* (Experiments 1 and 4) and *L. siliquoidea* (Experiments 2 and 5), *Corbicula* had no effect on 60-day-old juvenile survival but was associated with increased mortality of newly-metamorphosed juveniles, indicating that these differences are attributable to life stage, not species. These differences also could be attributable to differences in the experimental system, but survival of 60-day-old juveniles in the static system of Experiment 3 was uniformly high, similar to that seen in the RAS in Experiments 1 and 2. In the wild, juvenile mussels of four species in the Lampsilini (including *V. troostensis* and *Lampsilis*) showed nearly identical growth

responses to stream conditions (Haag et al. 2021), suggesting that they can be considered interchangeable for the purposes of our experiments.

We were unable to conclusively evaluate the relative effects of *Corbicula* versus a similar biomass of adult native mussels. *Corbicula* and native mussels appeared to affect growth of 60-day-old juveniles similarly, and their relative effects on newly-metamorphosed juveniles varied among experiments. However, our evaluation of food attenuation in Experiment 4 showed that *Corbicula* can reduce food abundance to a greater extent than native bivalves under some conditions.

The environmentally relevant growth produced in the RAS and static systems and the low variance around survival and growth in most treatment combinations both indicate that our study provides informative, reproducible assessments of the effects of the experimental conditions on mussels. Nevertheless, it is difficult to assess the extent to which our experimental conditions and results apply to streams. For example, although *Corbicula* was associated with higher mussel growth in the 5.8-L trays of the RASs, we do not know if they can have the same effect in streams, and field studies have found a negative association between mussel growth and *Corbicula* abundance (Ferreira-Rodríguez et al. 2018; Haag et al. 2021). Similarly, it is unknown if the strong, negative effect of *Corbicula* on newly-metamorphosed mussel survival that we observed in the laboratory can be expected in the wild. The large differences in mussel growth responses in RAS versus static systems illustrate the difficulty of devising a relevant, experimental representation of a complex, natural system.

Despite uncertainties about the environmental relevance of our experiments, we found consistent evidence that *Corbicula* can have strong negative effects on newly-metamorphosed mussels, including dramatically increased mortality. Such effects could explain the lack of mussel recruitment seen in many streams. Our evidence for negative effects of *Corbicula* on older juvenile mussels was contradictory and equivocal, as was our evidence for assessing the functional equivalency of *Corbicula* and native bivalves as potential competitors with juvenile mussels. The lack of an effect of *Corbicula* on survival of 60-day-old juveniles could be because our experiments were too short to result in energy depletion sufficient to cause mortality. In Experiment 3, our finding of reduced growth only at the warmer temperature (when metabolic needs are higher) suggests that energy depletion and mortality could occur over longer time periods, and additional experiments are needed to evaluate this. Because of the ability of *Corbicula* to modify ecosystems and its rapidly expanding distribution, a better understanding of its effects on mussels is vital for conservation.

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Author contributions WRH and DEJW conceived the ideas, and WRH, AJI, MAM, DEJW, and SJP designed methodology; WRH, AJI, DEJW, and SJP collected the data; WRH analyzed the data and led writing of the manuscript; all authors contributed critically to the drafts and gave final approval for publication.

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Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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