Corbicula fluminea Effects on Survival, Growth, and Drift of Juvenile Lampsilis siliquoidea in Laboratory Exposures

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CORBICULA FLUMINEA EFFECTS ON SURVIVAL, GROWTH, AND DRIFT OF JUVENILE LAMPSILIS SILIQUOIDEA IN LABORATORY EXPOSURES

A Master’s Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Allison Nicole Sieja

July 2021
**ABSTRACT**

The Asian clam, *Corbicula fluminea*, is an invasive species that is abundant and often co-occurs with native freshwater mussels. *Corbicula* is widely suspected of having negative effects on native mussels, but few studies have empirically tested this hypothesis. I used laboratory experiments to evaluate how adult *Corbicula* affected the survivorship, growth, and drift of juvenile *Lampsilis siliquoidea*. Survival and growth of newly metamorphosed mussels were tested in downwelling flow-through chambers with glass-bead substrate. Treatments were control (no clams), small adult clams, or large adult clams. After 28 days, large clams slightly but significantly reduced the number of juveniles recovered from the substrate. The proportion recovered alive did not change, suggesting that the missing juveniles were eaten and the shells digested. Small clams did not affect juvenile recovery or survival. Mussels grew nearly threefold in length over 4 weeks. *Corbicula* had small and inconsistent effects on growth. Mussels exposed to large clams grew 3% larger than the control group at the end of the 4 weeks. In contrast, mussels exposed to small clams grew 3% less than the control group. Tests on drift were carried out in small raceways with glass bead substrate. Drifting behavior (wash-out) of 2-week (0.5 mm) and 6-week-old (1.5 mm) mussels was tested with and without *Corbicula* over 3 days. Mussel drift increased with increasing clam density: 19%, 33% and 47% of 2-week-old mussels drifted at 0, 500 or 2000 clams/m², respectively. Drift of 6-week-old mussels increased only at the highest clam density, with 42% of mussels displaced, versus 5% and 8% in the control and low-density treatments. Interactions between *Corbicula* and native juvenile mussels could help explain why mussel populations continue to decline across the United States.

**KEYWORDS**: freshwater mussel, *Corbicula fluminea*, invasive species, juvenile, conservation, survival, growth, drift
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In the interest of academic freedom and the principle of free speech, approval of this thesis indicates the format is acceptable and meets the academic criteria for the discipline as determined by the faculty that constitute the thesis committee. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.
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INTRODUCTION

The United States has the highest diversity of freshwater mussels (Order Unionoida) in the world, with roughly 300 species (Carter et al., 2011; Williams et al., 2017; Graf & Cummings, 2021). Many of these species are highly imperiled with over 70% of species listed as needing some sort of conservation protection, and 10% of species are classified as extinct (Williams et al., 1993, Lydeard et al., 2004). These declines are attributed to several causes including habitat degradation and destruction, siltation, channelization, loss of host fish, climate change, and the introduction of invasive species of bivalves and fish (Williams et al., 1993; Strayer, 1999a; Ferreira-Rodríguez et al., 2019).

The loss of freshwater mussels (hereafter “mussels”) is significant because of the many benefits they provide (Strayer, 2017). These include direct economic value for their shells, which are used in commercial pearl production (Anthony & Downing, 2001; Haag, 2012; Strayer, 2017). Mussels also provide a variety of ecosystem services through their effects on the environment and other species. Mussels are filter feeders capable of filtering large volumes of water, removing bacteria, detritus, and algae from the water column. The food is either ingested or rejected as pseudofeces (particles filtered and aggregated but not ingested), making it available to eat for consumers, thus linking the water column to the benthos (Vaughn & Hakenkamp, 2001; Spooner & Vaughn, 2006, Atkinson et al., 2011; Vaughn, 2018). Mussels themselves are also food sources for some fish, turtles, birds, and small fur bearing mammals, and their shells can provide habitat for bacteria, algae, macroinvertebrates, crustaceans, and small fish (Spooner & Vaughn, 2006; Haag, 2012).
Besides contributing ecosystem services, mussels also provide intrinsic value (existence or amenity value) to stream ecosystems (Strayer, 2017). The freshwater mussel life cycle is unique among bivalves because its larvae, known as glochidia, must complete an obligate parasitic stage upon a fish. Mussels will either passively broadcast their glochidia into the water column or actively attract a fish by displaying a mantle lure or releasing conglutinates, small packages of glochidia that resemble fish prey items. Once a glochidium is attached to a fish’s gills or fins, it will be encapsulated by the fish’s tissue. The glochidium will remain encapsulated for days to months depending on species and on water temperature. During this time, the glochidium will metamorphose into a juvenile mussel, and once the metamorphosis is complete, the juvenile mussel will fall off the fish. Many of these mussel-fish host relationships are species specific so that if a mussel does not infest the correct species of fish, the glochidia will not transform into juvenile mussels (reviewed in Barnhart et al., 2008). Newly metamorphosed juveniles are microscopically small, 200-300 microns in length. However, they grow quickly, and most species reach adult size within a few years.

Mussels can live for decades or even centuries and are often found in dense aggregations called mussel beds (Haag, 2012). Mussel beds are patchily distributed and usually separated by large areas where no or very few mussels occur (Strayer, 2008, Atkinson & Vaughn, 2015). It is thought that mussel beds occur in areas with decreased shear stress, high levels of dissolved oxygen, and stable substrate to provide protection from scouring or dislodgment, but the exact requirements for mussel bed formation are still poorly known (Strayer, 1999b; Steuer et al., 2008; Allen & Vaughn, 2010; Daraio et al., 2010).

While adult mussel requirements are poorly understood, even less information is known about the habitat requirements for juveniles. Few studies have investigated the habitat of early
juveniles. Neves and Widlak (1987) found juvenile mussel occurrence in a Virginian head water stream to be patchily distributed, with the greatest number juvenile mussels (aged 3 years or less) in riffles and runs with some juveniles found behind boulders and along stream banks. Juvenile occurrence was also correlated with fingernail clam occurrence (Neves & Widlak, 1987). In Oklahoma streams, juvenile mussels were found in riffles and runs, usually attached to gravel by a byssus thread (Isely, 1911). Small juvenile mussels will also produce byssus threads for semi-permanent attachment to help prevent them from drifting downstream (Neves & Widlak, 1987; Bradley, 2011).

While adult mussels may spend their time divided between being buried in the interstitium and siphoning at the sediment/water interface, juvenile mussels may spend their time entirely in the interstitium (Balfour & Smock, 1995; Strayer et al., 2004; Cope et al., 2008). For example, juvenile Pearl Mussel (*Margaritifera margaritifera*) spend at least 5 years buried and require well-aerated, stable sediment so that interstitial water movement brings food and oxygen while carrying out waste (Buddensiek et al., 1993; Geist, 1999a,b; Geist & Auerswald, 2007). The depth of burrowing may be related to water movement. In static, laboratory exposures, 95% of juvenile mussels (up to 20 weeks post-transformation) of 6 North American species burrowed shallowly into sediment within 15 minutes of placement, and 91% of juveniles were recovered from a sediment depth of 3.4 mm or less (Kemble et al., 2020). Like byssus attachment, burrowing might help to prevent displacement and drift downstream.

**Corbicula fluminea**

The Asian clam *Corbicula fluminea* (hereafter, *Corbicula* or clams) was first introduced to the United States in Washington State in the late 1930s (Burch, 1944; McMahon, 1982;
Benson & Williams, 2021). Since then, it has spread across the continental United States through human-mediated and natural dispersal, often occurring together with native mussels in both lotic and lentic habitats (McMahon, 1982, Sinclair & Ingram, 1961, Prezent & Chalermwat, 1984; Benson & Williams, 2021). Unlike mussels, *Corbicula* have relatively short life spans, usually between 2-4 years, and rapidly grow to reach a maximum length of 50 mm (Hornbach, 1992; McMahon & Bogan, 2001). Anthropogenic disturbance makes it easier for *Corbicula* to become established, thus threatening the majority of river systems found within the United States (Strayer, 1999a). In some systems, *Corbicula* can constitute a large portion of the benthos, with reports of 2000 or more clams per square meter (Hornbach, 1992; Poff et al., 1993; McMahon & Bogan, 2001; Sousa et al., 2008). In 2010, Lake Tahoe biologists reported *Corbicula* densities reaching over 10,000 individuals per square meter (Wittman et al., 2012). *Corbicula* can be found in all substrates but generally prefer smaller-grained substrates, such as sand (Schmidlin & Baur, 2007; Turek & Hoellein, 2015).

The reproduction of *Corbicula* differs from that of unionid mussels. *Corbicula fluminea* is hermaphroditic. It reproduces asexually via androgenesis in which the offspring are paternal nuclear clones (Pigneur et al., 2012). Like mussels, *Corbicula* embryos are brooded in the adult demibranchs, but they develop directly and do not require a fish host. Reproduction occurs mainly in the fall and spring, associated with temperature changes (Hornbach, 1992; McMahon & Bogan, 2001). *Corbicula* can reach sexual maturity within six months and have high fecundity which helps them disperse and colonize new areas (McMahon & Bogan, 2001; Sousa et al., 2008).

Dense aggregations of *Corbicula* can alter nutrient availability and turbidity by filter feeding. In different situations these effects may be considered beneficial or harmful. For
example, after the invasion of *Corbicula* in an estuary near Washington D.C, decreased turbidity and increased light levels allowed submerged aquatic vegetation to increase. Nuisance algae populations decreased while fish and bird populations increased (Phelps, 1994). In contrast, the invasion of *Corbicula* in Lake Tahoe, California created nutrient hotspots through the accumulation of clam biodeposits. These hotspots led to growth of filamentous algal species and bacteria (Forrest et al., 2012; Wittman et al., 2012).

*Corbicula* is an effective suspension feeder capable of capturing particles as small as 80 nanometers with high efficiency (Silverman et al., 1997; Faust et al., 2009; Vaughn, 2018). Mass-specific filtration rates of *Corbicula* are comparable to similar size mussels, and are relatively high compared with adult mussels, because of body size and scaling effects (Silverman et al., 1997; Pletta, 2013). In addition to filter feeding, *Corbicula* may deposit feed from the substrate by picking up food items using their foot which can reduce organic matter found in the substrate (Hakenkamp & Palmer, 1999; Vaughn & Hakenkamp, 2001). Alternatively, *Corbicula* may increase available nutrients through the production of feces and pseudofeces (Novais et al., 2017). Smaller *Corbicula* are more efficient feeders and better bioturbators because they have smaller body mass to volume ratio, are more active, and may experience less mechanical resistance when moving than larger adult mussels (Crespo et al., 2018).

Because *Corbicula* can alter nutrient availability and physical habitat, *Corbicula* can also influence stream communities. In field and lab experiments, increasing densities of *Corbicula* reduced the abundance of bacteria and some protists, perhaps through predation (Hakenkamp et al., 2001). In contrast, other field experiments found *Corbicula* increased bacterial diversity and fungal biomass, perhaps through nutrients provided by biodeposits (Novais et al., 2016). Further field studies have illustrated that live and dead *Corbicula* increase the abundance of crustaceans,
gastropods, some fishes and aquatic insects in freshwater and estuarine ecosystems (Werner & Rothhaupt, 2007; Ilarri et al., 2012; Ilarri et al., 2014; Novais et al., 2015). Like mussel shells, accumulated *Corbicula* shells may provide an ecosystem service by providing habitat for aquatic insects and other invertebrates and protection from predators (Ilarri et al., 2015). However, because of their small and uniform size, compared to the variety of native bivalve shells, *Corbicula* shells produce a relatively homogenous and less complex habitat. That may reduce the density of macroinvertebrate assemblages found on shells but not necessarily reduce functional diversity of the macroinvertebrate community (Ilarri et al., 2018).

Besides influencing stream communities, *Corbicula* also affects activities within human sectors. *Corbicula* shells are small enough to be transported by moderate water movement. Although the clams do not attach to surfaces, the drifting shells can accumulate and obstruct the intake screens of water pipes of hydropower and nuclear power plants (Eng, 1979; Isom et al., 1986; Phillips et al., 2005; Haubrock et al., 2021). Control and mitigation costs are estimated to cost stakeholders within the United States billions of dollars per year (McMahon, 1982; Pimental et al., 2005; Karatayev et al., 2007; Haubrock et al., 2021).

**Mussel and Corbicula Interactions**

Many populations of mussels are declining enigmatically in seemingly pristine habitat. These declines are characterized by a collapse of mussel fauna, a lack of recruitment, and no conclusive cause of the decline has been identified (Haag, 2019). *Corbicula* are suspected of causing declines in mussel abundance in river systems across the United States (Strayer, 1999a; Haag, 2019). Vaughn and Spooner (2006) noted scale-dependent associations between *Corbicula* and mussels within river systems in the Ouachita Highlands. At a 0.25 m² patch scale, in well-
established mussel beds, where mussel density is high, *Corbicula* abundance was low. Away from mussel beds, *Corbicula* density increased as mussel density decreased. However, at a stream reach scale, this relationship was no longer obvious. Furthermore, an experimental study documented that high densities of *Corbicula* (2000 clams/m²) inhibited growth and reduced the physiological condition of adult *Unio delphinus* mussels in the River Minho (Ferreira-Rodríguez et al., 2018).

Several possible mechanisms have been suggested for *Corbicula* impact on native mussels. One of these mechanisms is toxification of interstitial water during mass mortality events. Compared to mussels, *Corbicula* are more sensitive to temperature and water level fluctuations that decrease levels of dissolved oxygen. Hypoxic events can cause mass mortality (Werner & Rothhaupt, 2007; Ilarri et al., 2011). Mass *Corbicula* die-offs increase ammonia levels in the water column and interstitial spaces to toxic levels that can induce stress or mortality in mussels (Cherry et al., 2005; Cooper et al., 2005). Other direct effects of *Corbicula* on native mussels have been suggested. These mechanisms include mechanical disturbance (Fuller & Richardson 1977), food competition (Haag, 2019), and predation on glochidia and post-metamorphic juvenile mussels (Yeager et al., 1999).

Juvenile mussels may be especially vulnerable to *Corbicula* because of their small size and interstitial habitat. Native mussel larvae and newly metamorphosed juveniles are generally less than 300 microns in size. In laboratory experiments, high densities of *Corbicula* (>1250 clams/m²) caused mortality in glochidia and in newly metamorphosed juveniles, possibly through ingestion or crushing the shells (Yeager et al., 1999; Modesto et al., 2019). High densities of *Corbicula* may also reduce juvenile growth by competing for food in interstitial water. Field studies using caged three-month-old mussels found a negative correlation between
the presence of *Corbicula* and mussel growth, although the mussels were not in direct contact with the clams (Haag et al., 2020). Another potential effect is drift. Preliminary laboratory studies indicated that *Corbicula* displaced newly metamorphosed juveniles, causing them to drift downstream (Yeager et al., 1999). Given these multiple lines of evidence, there is clearly a need for further study of how *Corbicula* affect the survivorship and growth of juvenile native mussels.

**Objectives**

The purpose of this study was to evaluate interactions between *Corbicula* and juvenile mussels by housing them together in laboratory experiments. My first experiment assessed how two different size classes of *Corbicula* affected survivorship and growth of newly metamorphosed juveniles over the course of 28 days. I hypothesized that both size classes of clams would reduce the survivorship and growth of juvenile mussels. In my second experiment, I evaluated how three different densities of *Corbicula* affected the drifting behavior of two age classes of native juvenile mussels. I hypothesized that high densities of *Corbicula* would increase the drift of juvenile mussels and that older juveniles would be less susceptible to displacement.
METHODS

Bivalve Collection

All work was conducted under IACUC protocols 17-032 and 2020-13 (Appendices A, B). *Corbicula* were collected from the James River in Greene County, Missouri (37.105, -93.329) throughout 2019 and 2020. Adult clams were collected and were transported back to Missouri State University (MSU) and kept in a recirculating 60-gallon aquarium system in screen-bottom containers with upwelling flow. The system was fed hourly with algae (Reed Mariculture *Thalassiosira pseudonana*, Shellfish Diet, and *Nannochloropsis*) to maintain 1-2 nL of cell volume per mL of water. The system received semi-weekly water changes with 50% of the water refreshed with 50 micron filtered river water. Clams experienced seasonal mortality in the spring and fall. However, ammonia levels within the system remained at a safe level as indicated by a colorimetric indicator (SeaChem Ammonia Alert). When a clam died while in holding, it was removed from the system. *Corbicula* were acclimated to the system for at least a week before any experiments began.

Brooding *Lampsilis siliquoidea* originated from the Bourbeuse River and were obtained from the USGS Columbia Environmental Research Center located in Columbia, Missouri. Glochidia from these females were propagated on host fish at MSU to provide newly metamorphosed juveniles for experimentation. Glochidia were extracted by flushing the marsupial gills with filtered, cold river water. Largemouth bass (*Micropterus salmoides*) were then infected with glochidia in a water bath containing 1-1.2 L of water per fish at a concentration of roughly 4000 glochidia per L of water. Inoculated fish were then placed in individual holding tanks in a recirculating aquarium system modified for recovery of juveniles
(see Patterson et al. 2018). Fish were fed frozen bloodworms (chironomids; San Francisco Bay Brand Bloodworms) for 9 days. Thereafter the fish were starved to minimize feces production and the recovery filters were checked daily for dropped juvenile mussels. Juveniles from the peak day of drop were counted and sorted into twelve sets of 212 mussels and twelve sets of 87 (total 3588). Experiments were begun within 24 hours of collection.

Survival and Growth Experiments

Aquarium Assembly. The growth and survival experiments were carried out in a 75 L (20 gal) aquarium with a false floor arranged to draw water downwelling through the chambers, similar to an under gravel filter (Figure 1). The false floor was a shallow inverted tray constructed of Schedule 80 polyvinyl chloride (PVC) sheet 72.4 cm (28.5 inch) long by 26.7 cm (10.5 inch) wide with a 3.2 cm tall perimeter (Figure 1). Two sizes of cylindrical chambers (3.5 and 5 cm PVC, inside diameter) were seated in couplings glued over corresponding holes in the floor (Figure 1). Each chamber was comprised of two nested cups bounded by 150 μm Nitex® nylon screening (Figure 2). Two “powerhead” submersible aquarium pumps (Aquarium Systems® Minijet model MN-404) were also mounted to draw water from underneath the floor and circulate water above the floor. This arrangement drew water downward through each of the cylindrical chambers. Based on dimensions, flow through the chambers was approximately 0.5 cm/s. Substrate was 1-2 mm diameter glass beads (MO SCI Specialty Products, L.L.C.). The volume of substrate provided was proportional to the sizes of the clams, and the number of mussels was proportional to the volume of substrate. Each large chamber contained 71 mL of substrate, one large adult clam, and 212 juvenile mussels (3 mussels/mL substrate) (Figure 2).
Each small chamber contained 29 mL of substrate, one small adult clam and 87 *L. siliquoidea* juveniles (3/mL substrate). Controls had substrate and juveniles but no clams.

**Water and Feeding.** The system received filtered (1 μm) river water weekly (James River, Greene Co., MO). Temperature of the system was recorded semi-hourly (HOBO Pendant®, Onset Products) and averaged 22-23 C. The system was fed the previously mentioned algae mixture hourly via a peristaltic pump (Kangaroo™ Joey Enteral Feeding Pump). The food volume was adjusted to maintain approximately 1-2 nL of cell volume per ml of water. Cell volume was determined semiweekly using a coulometer cell counter (Beckman Coulter MS-4). Conductivity, pH, and dissolved oxygen were assessed weekly using Hach probes while the unionized ammonia level was assessed continuously using a colorimetric indicator (Seachem Ammonia Alert).

**Exposure to Clams.** Seventy-two hours before the start of a trial, six large adult clams averaging 33.8 mm (± 2.1 mm standard deviation [SD]) and six small adult clams measuring 17.7 mm (± 2.0 mm SD) were transferred from the fed holding system to an aerated cooler without food. The potential disappearance of juveniles was first assessed after 24 hours, and the initial fasting period was used to ensure that the clams would feed during the entire trial.

The entire 4-week experiment was repeated four times. In each experiment, the chambers were randomly assigned into 6 blocks, each consisting of two small chambers, one control and experimental, and two large chambers, one control and experimental. Blocks were randomly assigned to be exposed to clams for either 1 day, 2 days, 7 days, 14 days, 21 days or 28 days. The 28 d exposure period was chosen because this is a typical period for chronic laboratory toxicology studies. In order to recover juveniles, each chamber was opened and its contents rinsed on nylon screening over a large watch glass, so that juveniles could fall through but not
the substrate. The substrate was gently sprayed with river water and the filtrate examined repeatedly until no juveniles were collected two times in a row. Clams were preserved in 95% ethanol.

Underneath a dissecting microscope, juveniles were counted to assess recovery and survival. Survival was evaluated by counting the number of recovered live, dead, and half shells present. Mussels were considered dead if shells were empty or if there was a lack of foot movement for over five minutes and no heartbeat detected. Half shells were considered to be $\frac{1}{2}$ of a dead individual, i.e. if 5 halves were recovered, 3 dead were recorded.

In order to assess growth, juveniles were transferred to a 5 cm petri dish. Juveniles were swirled to the center of the petri dish and then photographed using an Olympus TG-5 camera. The camera was supported directly on the lid of the dish, and a hole was cut in the lid to improve the image. The maximum linear dimension of each shell image was then measured using Image J software (Schneider et al., 2012). Shells were oriented haphazardly in each image due to mussel movement, and the recorded lengths are less than or equal to the true maximum length of each shell.

**Drift Experiments**

**Study Animals.** *Corbicula* collection and propagation methods used were previously described above. After metamorphosis, juveniles were placed in a recirculating bucket system, a common mussel culture system (Barnhart, 2005), for either 1-2 weeks or 6-7 weeks depending on the experiment. Juveniles aged 1-2 weeks old measured $529 \pm 61\mu$m (SD) and 6–7-week-old juveniles measured $1548 \pm 345\mu$m (SD). The bucket rearing system was fed hourly with the previously mentioned algae mixture to maintain 1-2 nL of cell volume per mL of water.
**Flume assembly.** Drift was assessed in a system hereafter called a flume (Figure 3). The flume consisted of a shallow tray of PVC sheet that was partitioned into 9 parallel lanes and a header box that delivered water into the lanes (Figure 3 gives details). The water flowing down the lanes exited into filter cups to recover any juvenile mussels that drifted. The flume was placed over a 40-gal reservoir filled with 10-μm filtered James River water. Flow to the flume from the reservoir was driven by a small pond pump (330-GPH Smartpond Submersible Pond Pump, Lowes). Water exiting the flume from the header box and the filters fell back into the reservoir to create a recirculating system. The flume was carefully leveled and water was delivered to the header box from a manifold to achieve uniform flow in each lane, which was tested volumetrically.

Before the start of each trial, substrate was added to each lane. The substrate was 1-2 mm glass beads (MO SCI Specialty Products, L.L.C.) for 1-2 week-old juvenile trials or 2 mm glass beads (Walter Stern) for 6-7 week-old juvenile trials. The substrate was lightly tamped with a tool to create a level surface. Each 6.5 cm-wide lane had 2.5 cm depth of bead substrate and 2.5 cm of water flowing over the beads. Based on this cross-section (16 cm²) and measured flow rates (22 cm³/s), flow speed in the section over the beads was approximately 1.4 cm/s.

**Exposure to Clams.** At the beginning of each trial, the flow was interrupted by removing the standpipe in the header box and 10 juvenile mussels were randomly placed into each lane. Juveniles were given 20 minutes to burrow into the substrate (Kemble et al., 2020). After 20 minutes, the standpipe was replaced to reestablish water flow, and clams were placed on their side on top of the substrate. Clam density treatments were randomly assigned to lanes. Adult clams measured 24.7 ± 4.91 mm (SD) in trials with 6–7-week-old juveniles and 23.5 ± 5.56 mm (SD) in trials with 1–2-week-old juvenile mussels. Clam density treatments were either
a control treatment of 0 clams (0 clams/square meter), a low-density treatment of 4 clams (500 clams/square meter), or a high-density treatment of 14 clams (2000 clams/square meter). These densities were chosen as they are ecologically relevant densities that mussels encounter. Each treatment was replicated three times in each experiment. Each experiment lasted for 72 hours. Experiments involving 6–7-week-old juvenile mussels were run twice while those involving 1–2-week-old juveniles were run five times. Between experiments, substrate was cleaned and rinsed with RO water, and the reservoir received a 50% water change using 25 µm filtered river water. Clams were not reused in trials. Ammonia levels were constantly monitored within the reservoir using a colorimetric indicator (SeaChem Ammonia Alert). Temperature was monitored daily and measured 20.3 ± 0.8°C (SD) in trials with 6-7-week-old juveniles and 19.5 ± 0.5°C (SD) in trials with 1-2-week-old juveniles.

To see if any juveniles were resuspended and drifted out of the lanes, the contents of each filter cup were sprayed into a dish and checked for juveniles at 1, 2, 4, 8, 24, 48, and 72 hours post-initial clam exposure. Recovered juveniles were counted and assessing for survivorship by checking for foot movement. At the end of each trial, the substrate was siphoned out in thirds and rinsed over a 2 mm screen so that any remaining juveniles could fall through but not substrate. The substrate was then sprayed until no additional juveniles were recovered. The number of juveniles displaced from the lanes and total number of juveniles recovered from the system (juveniles collected from filters and sediment) were recorded. Juveniles were placed back into culture after they were recovered.

Data Analysis
All data were analyzed using R v.4.0.3 (R Development Core Team). To analyze how presence of *Corbicula* affected survivorship and growth of juvenile mussels, I fit generalized linear mixed effects models (GLMM) using the `glmmTMB` package (Brooks et al., 2017) specifying type II Wald chi-squared ($\chi^2$)-tests via the `Anova` function from the `car` package (Fox, 2015). I evaluated survivorship and growth of mussels exposed to large and small clams separately. Model assumptions were checked using the `DHARMa` package (Hartig, 2020).

Survivorship was assessed in two phases. I first determined how the presence of either the large or small clam affected the recovery of juveniles from chambers using GLMMs. The response variable was proportion of juveniles recovered, and the explanatory variable was ‘Treatment’ (the presence or absence of *Corbicula*) with ‘Day’ included as a covariate. I included ‘Trial’ as a random effects variable as ‘Day’ within ‘Trial’ would not converge. I then assessed survivorship of recovered juveniles using GLMMs to determine if surviving juveniles suffered from excess mortality from the presence of the clam. The response variable was survival of the recovered juveniles, and the explanatory variable was ‘Treatment’ (the presence or absence of *Corbicula*) with ‘Day’ included as a covariate. I included ‘Day’ within ‘Trial’ as a random effects variable. Two data points were excluded from survivorship analyses because chamber screens broke during the recovery process, and two additional outlier data points were excluded to fit model assumptions.

To assess how *Corbicula* affected mussel growth, I ran separate GLMMs for each size class of clam tested. Length data was natural log transformed to fit model assumptions. The response variable was ln(length), and the explanatory variable was ‘Treatment’ (the presence or absence of *Corbicula*) with ‘Day’ included as a covariate. I included ‘Day’ within ‘Trial’ as a random effect.
To analyze the effect of clam density upon the resuspension of juveniles from the system, I fit GLMMs with a Poisson distribution for each age class tested. To analyze the effect of clam density upon the total recovery of juveniles, I fit GLMMs with a Gaussian distribution for each age class tested. For each model, density treatment was treated as an explanatory variable and block was treated as a random effect. When significant effects were found, I ran a Tukey’s post hoc test using LS means function in the car package to determine which pairs were significant.
RESULTS

Survival and Growth Experiments

Trials were conducted 4 times, in August and October 2019 and again in August and October 2020. Large clams reduced the number of juveniles that were recovered (Figure 4; GLMM: $\chi^2_1 = 7.01, p < 0.01$) but did not increase mortality among those that were recovered (Figure 5; GLMM: $\chi^2_1 = 0.23, p = 0.62$). In other words, the missing juveniles were not recovered as dead shells. The presence of small clams did not affect the proportion of juveniles recovered from chambers (Figure 6: $\chi^2_1 = 2.13, p = 0.14$) nor did it cause excess mortality in surviving juveniles (Figure 7: $\chi^2_1 = 0.23, p = 0.63$).

Regardless of chamber or clam size, mussels grew almost three-fold in length during the 28-day trials (Figure 8; large chamber, large clams: GLMM: $\chi^2_1 = 400.79, p < 0.001$; Figure 9; small chamber, small clams: GLMM: $\chi^2_1 = 307.39, p < 0.001$). In total, 6,261 mussels were measured, including 2133 mussels in large control chambers, 2011 mussels in large experimental chambers, 1091 mussels in small control chambers, and 1026 mussels in small experimental chambers. Relative to controls (629 ± SE 4.3 μm), mussels exposed to large clams were 3% longer (648 ± SE 4.6 μm), while mussels exposed to small clams were 2.8% shorter (633 ± SE 5.9 μm) relative to the control (651 ± SE 6.7 μm) (Table 1; GLMM: $\chi^2_1 = 7.07, p = 0.02$; Table 2; GLMM: $\chi^2_1 = 7.07, p < 0.01$).

Drift Experiments
Trials were conducted in October-November 2020. Lane discharge ranged from 20.8-22.4 mL/s in trials using 1–2-week-old juveniles and from 21.8-23.6 mL/s in trials using 6–7-week-old juveniles. Fifteen trials were conducted using 1–2-week-old juveniles, and 6 trials were conducted using 6–7-week-old juveniles. In almost all experiments, at least one clam died and was promptly replaced within 24 hours with another live individual of similar size. Ammonia levels did not spike during the experiments. In drift experiments involving 1–2-week-old juveniles, clam density did not affect the recovery of juveniles from lanes (GLMM: $\chi^2_2 = 3.75, p = 0.15$). As clam density increased, the percent of displaced 1–2-week-old juveniles also significantly increased (Figure 10; GLMM: $\chi^2_2 = 17.09, p < 0.001$), with 18.6%, 32.7% and, 46.7% being displaced from the control, low-clam density, and high-density treatments. Clam density did not affect the recovery of 6–7-week-old juveniles (GLMM: $\chi^2_2 = 3.41, p = 0.18$). Only the high-density treatment of clams resuspended a significant proportion of 6–7-week-old juveniles, with 41.7% of juveniles displaced versus 5.0% and 8.3% displaced from the control and low-clam density treatments (Figure 11; GLMM: $\chi^2_2 = 20.15, p < 0.001$).
DISCUSSION

In the present study, I demonstrated that under laboratory conditions, the invasive clam, *Corbicula fluminea*, had negative impacts on survivorship, growth, and drift of native juvenile *L. siliquoidea*. While many studies have suggested that *Corbicula* could be capable of harming juvenile mussels, this study is among the first to assess *Corbicula* effects upon young juvenile mussels. Furthermore, this study is one of the first to assess the long-term (28 day) effects of *Corbicula* on newly metamorphosed juveniles.

Large adult clams (33.8 ± 2.1 mm) but not small clams (17.7 ± 2.0 mm) reduced the number of juveniles recovered from chambers in five of the six exposure points, yet the percent of dead mussels (empty shells) did not increase. This result suggests that the missing juveniles were most likely ingested and digested by clams rather than killed by mucus entanglement or mechanical disturbance, and that smaller clams had smaller gape and were unable to swallow even the smallest juveniles. While small clams were not able to ingest mussels, in the wild, they may indirectly reduce the survival of juveniles through bioturbation, which may bring juveniles closer to clams that have a large enough gape to filter out juveniles out of the environment.

Yeager et al. (1999) documented shell fragments within the feces of 25 mm *Corbicula* and documented juvenile mussels entangled with mucus at the pedal gape of *Corbicula*. However, if *Corbicula* are ingesting mussels, juveniles are only vulnerable for a short period of time because they grow rapidly in the first month post-metamorphosis. Presumably, they soon exceed the maximum size that can be ingested by *Corbicula*. Based on previous work and this study, it seems likely that *Corbicula* can ingest post-metamorphic mussels (250-300 microns) if the clams are larger than about 20 mm.
Adult mussels have life expectancies of years to decades, and each female produces tens of thousands to millions of larvae annually (Haag, 2012). Larval mortality is extremely high because of the low probability of encountering a suitable fish host (Lefevre & Curtis, 1912; Jansen et al., 2001; Haag, 2012). Mortality of the post-metamorphic juveniles is presumably also high. Their small size renders them vulnerable to a wide variety of predators, and they are presumably unable to locomote more than a few centimeters to find suitable habitat. While there are no studies regarding the survival rates of newly metamorphosed recruits in the wild, in hatchery facilities, the survival of juvenile mussels within the first month post-metamorphosis is extremely variable, ranging from <1% to 90% survival (Hanlon & Neves, 2006; Patterson et al., 2018; M. C. Barnhart, pers. comm.). Consumption by Corbicula or other unrecognized micropredators might help explain why enigmatic declines are characterized by a lack of recruitment.

In all treatments, mussels grew nearly three-fold in longest dimension in 28 days. As described in Methods, the longest dimension of haphazardly oriented individuals was measured rather than anatomical (anterior-posterior) length, because of the difficulty of orienting juveniles broadside. This approach was efficient and allowed me to measure a larger number of individuals, but it should be noted that average final measurements (625-650 microns) are smaller than maximum length. It should also be noted that shape changes during early growth: newly metamorphosed L. siliquoidea are initially taller (dorsal-ventral) than long (anterior-posterior) but grow faster in length than height. The relationship between maximum shell length and dry weight of 2- to 6-week-old Fatmucket (0.4 to 1.4 mm length) was log \( y = 2.71 \log x - 1.15 \) \((r^2 = 0.99, p<0.001)\), where \( y \) is dry weight in mg and \( x \) is maximum shell length in mm (Wang et al., 2019). Using this equation to approximate the dry mass, a 3-fold change in length represents a 19.6-fold increase in dry mass.
*Corbicula* caused small and inconsistent yet statistically significant effects on the growth of juvenile mussels. Over 28 days, small clams caused a 3% reduction in growth, while large clams caused a 3% increase in growth. Calculated as in the previous paragraph, 3% change in length corresponds to 8.3% change in dry mass. Reduced growth rate could leave juvenile mussels vulnerable to small predators (e.g. flatworms, crayfish) for longer periods of time. Possible mechanisms for clam effects on growth include disturbance by clam activity (higher in small clams) and biodeposits. Another laboratory study reported *Corbicula* had no effect on the growth of three-month old Cumberland Bean (*Venustaconcha troostensis*) at high food abundances, presumably because there was sufficient food for both species. However, at low food abundances *Corbicula* significantly increased the growth of mussels when compared to the control group, perhaps because the mussels were consuming *Corbicula* feces and pseudofeces or associated bacteria (White, 2020).

Biodeposited material (feces and pseudofeces) can increase the amount of organic matter and nutrient content within the substrate (Hakenkamp & Palmer, 1999). This material might increase the abundance of bacteria, another food source utilized by freshwater mussels (Nichols & Garling, 2000). Furthermore, biodeposits could act as a direct food source. Coprophagy, the consumption of feces, is documented in marine bivalves, such as the Blue Mussel (*Mytilus edulis*) (Frankenberg & Smith, 1967). Mussels in my study were fed at a standard rate that promotes growth in captive culture. The juvenile mussels could have supplemented their diet with *Corbicula* biodeposits, which may have negated any localized depletion of algal resources caused by *Corbicula* within the chambers. Upon visual inspection on exposure days, chambers with *Corbicula* had far less algal debris than control chambers. Presumably, a larger abundance of biodeposits was made available to mussels exposed to large clams since large clams produce a
greater amount of biodeposits than small clams due to their body size. Thus, the positive effect of large clams on mussel growth might be nutritional, while the negative effect of small clams might be the result of their greater activity. It is not clear whether *Corbicula* biodeposits would act as food for juvenile mussels in a natural setting. The trophic importance of suspension feeder biodeposits appears to be an interesting area for further study (Limm et al., 2011, Seibert et al., 2014).

It is likely that effects on growth are dependent on the density of *Corbicula*. Density within large chambers represented approximately 500 clams/m², and density in small chambers represented approximately 900 clams/m². A field study of *Corbicula* effects found that growth and energy stores of adult *Unio delphinus* mussels were reduced at a density of 2000 clams/m² but not at 1000 or less clams/m² (Ferreira-Rodríguez et al., 2018). It is possible that *Corbicula* densities in my experiments were below the level needed to cause direct negative effects on the growth of freshwater mussels. Nonetheless, the densities in my experiments are of densities typically reported in Midwestern surveys (Vaughn & Spooner, 2006; Angelo et al., 2007; Turek & Hoellein, 2015; Curtis et al., 2020; Sieja, unpublished data).

Results from my drift experiment showed that high densities of *Corbicula* could displace 1-2-week-old and 6-7-week-old juvenile *L. siliquoidea*. As *Corbicula* density increased, the percent of displaced 1-2-week-old mussels increased as well. However, only the highest *Corbicula* density (2000 clams/m²) resuspended a significant proportion of the larger 6-7-week-old juveniles. *Corbicula* may be breaking byssus threads used by juveniles for semi-permanent attachment. Older juveniles may have better foot adhesion and stronger byssus threads, which may make them less vulnerable to displacement by *Corbicula* at lower densities. My results with older juveniles are contradictory to Yeager et al. (1999) who found *Corbicula* resuspended 2-4
day old Rainbow mussel (*Villosa iris*) but not 2-3-week-old *V. iris*. However, Yeager et al. (1999) used differing numbers of juvenile mussels and *Corbicula* in their experiments that compared the drift of two different age classes, which could help explain the differences in the results between the studies. Because *Corbicula* increase juvenile resuspension and drifting rates, *Corbicula* could interfere with the settlement of juveniles into existing mussel beds and could impair the creation of new mussel beds.

The natural displacement rates of juvenile mussels in the wild are not known, and this study is one of the first to measure drift in mussels. Laboratory studies have documented that that newly metamorphosed juveniles have some control over their settling velocity once they drop off their fish host through active waving of their foot and shell opening (Schwalb & Ackermann, 2011). Bed roughness and flow velocities are other factors thought to influence settling velocity. In flume experiments, microspheres used as proxy for newly metamorphosed juveniles drifted farther with increasing flow velocities but consistently drifted less over a simulated mussel bed than over gravel, perhaps because of turbulence created by the presence of embedded mussels (Irmscher & Vaughn, 2018). In the field, during low flow summer conditions microspheres had limited dispersal, drifting less than 10 meters from a release point over a mussel bed (Irmscher & Vaughn, 2018).

Macroinvertebrates that enter the drift may do so multiple times and may drift distances varying between less than a meter to several meters, with some individuals drifting up to a kilometer, depending on flow, life cycle stage, and substrate (Brittain & Eikeland 1988; Lancaster et al., 1996; Elliott 2003; Fingerut et al., 2006). The presence of *Corbicula* could add an additional factor influencing drift and may increase the rate at which juvenile mussels experience drift, especially since *Corbicula* and juvenile mussels occupy similar microhabitat.
My results could help explain why mussel populations face a lack of recruitment and continue to decline enigmatically across the United States. Further research regarding Corbicula and juvenile mussel interactions is vital and is growing increasingly urgent. In the 2000s, a second Corbicula species, Corbicula largillieri, was introduced and has spread across the Midwest (Lee et al., 2005; Tiemann et al., 2017; Douglass et al., 2020). In 2015, a novel, third Corbicula species was discovered in the Illinois River and since its discovery, it has been documented in the Ohio, Tennessee, and Mississippi rivers (Tiemann et al., 2017; Douglass et al., 2020). To my knowledge, there have been no studies investigating how North American mussels interact with these other Corbicula species, and with these recent range expansions, it is increasingly likely that mussels will encounter at least one species of Corbicula. Further research should aim to assess the effects of these more recent invaders on juvenile and adult mussels. Given the widespread distribution of Corbicula, the effects of Corbicula should be taken into account by stakeholders when designing management acts for mussel conservation.
REFERENCES


Fuller, S. L. H., & J. W. Richardson, 1977. Amensalistic competition between *Corbicula manilensis* (Philippi), the Asiatic clam (Corbiculidae), and freshwater mussels.
(Unionidae) in the Savannah River of Georgia and South Carolina (Mollusca: Bivalvia).
ASB Bulletin 24: 52.


Table 1. Average lengths (μm) of mussels in large chambers throughout the growth experiment. Numbers within parentheses represent one standard error.

<table>
<thead>
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<th>Days Exposed</th>
<th>Untransformed Means</th>
<th>Transformed Means</th>
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<td>Clam Present</td>
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<tr>
<td>28</td>
<td>629.18 (4.252)</td>
<td>648.28 (4.658)</td>
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Table 2. Average lengths (μm) of mussels in small chambers throughout the growth experiment. Numbers within parentheses represent one standard error.

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Figure 1. Aquarium assembly for survival and growth experiments. (A) Lateral view without chambers inserted. (B) Dorsal view without chambers inserted. (C) Side view of aquarium with chambers inserted into the false bottom floor. (D) Dorsal view of aquarium with chambered inserted into the false bottom floor.
Figure 2. Chambers for survival and growth experiments. Nylon mesh (150 micron) was press-fit between sections of PVC pipe and couplings (A, C) to form filter cups. A pair of filter cups (A) was then nested to form a chamber (B). The lower cup of each chamber contained substrate, juveniles, and a clam.
Figure 3. Flume for drift experiments. Manifold (A) delivers water to header box (B). Header box contains a standpipe (C) to allow exit of water and control water level. Header box has openings (not visible) to admit water into each lane. Foam baffles (D) over each opening rectify flow into the lanes, which contain 2.5 cm-deep substrate (glass beads (E)). An outflow fitting (F) from each lane conducts water into the filter cups (G) for recovery of drifting juveniles. The flume was constructed of 1/8 in polyvinyl chloride (PVC) sheet. The header box measured 57.2 cm long by 7.6 cm wide by 10 cm deep. Filter cups consisted of 150 μm Nylon screening press fit between a 3.8 cm (inside diameter) coupling and a piece of pipe. The entire assembly was supported over a water reservoir and pump to maintain recirculating flow (see Methods).
Figure 4. The mean percent of juvenile mussels recovered from large chambers. Circles represent chambers without clams, and triangles represent chambers with clams. Error bars indicate ± one standard error.
Figure 5. The mean survival of recovered juveniles from large chambers. Circles represent chambers without clams, and triangles represent chambers with clams. Error bars indicate ± one standard error.
Figure 6. The mean percent of juvenile mussels recovered from small chambers. Circles represent chambers without clams, and triangles represent chambers with clams. Error bars indicate ± one standard error.
Figure 7. The mean survival of recovered juveniles from small chambers. Circles represent chambers without clams, and triangles represent chambers with clams. Error bars indicate ± one standard error.
Figure 8. The average natural log transformed lengths of mussels in large chambers. Circles represent chambers without clams, and triangles represent chambers with clams. Error bars indicate ± 95% confidence intervals.
Figure 9. The average natural log transformed lengths of mussels in small chambers. Circles represent chambers without clams, and triangles represent chambers with clams. Error bars indicate ± 95% confidence intervals.
Figure 10. The average percent of 1–2-week old juvenile mussels displaced from lanes. Different letters above bars indicate significant differences among treatment $p < 0.05$ according to Tukey’s post hoc test. Error bars indicate ± one standard error.
Figure 11. The average percent of 6–7-week old juvenile mussels displaced from lanes. Different letters above bars indicate significant differences among treatment $p < 0.05$ according to Tukey’s post hoc test. Error bars indicate ± one standard error.
APPENDICES

Appendix A. IACUC protocol 17-032 approval.
Appendix B. IACUC protocol 2020-13 approval.

September 8, 2020

RE: IACUC Protocol 2020-13

Hello.

Your IACUC protocol has been approved.

Access to your protocol is available through LabTracks.

Thank you and if you need anything in the future regarding this protocol please contact me either via email (janene.proctor@missouristate.edu) or at 417-836-8419.

Sincerely,

Janene Proctor