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FOOD ABUNDANCE MODULATES JUVENILE MUSSEL (UNIONIDAE) GROWTH RESPONSES TO THE ASIAN CLAM (*CORBICULA FLUMINEA*)

Drew Edward Jerry White
University of Kentucky, dwh236@g.uky.edu
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Drew Edward Jerry White, Student

Dr. Steven J. Price, Major Professor

Dr. Steven J. Price, Director of Graduate Studies

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in Forest and Natural Resource Sciences in the College of Agriculture, Food and Environment
at the University of Kentucky

By

Drew Edward Jerry White

Lexington, Kentucky

Director: Dr. Steven J. Price, Professor of Forestry and Natural Resources

Lexington, Kentucky

2020

ABSTRACT OF THESIS

FOOD ABUNDANCE MODULATES JUVENILE MUSSEL (UNIONIDAE) GROWTH RESPONSES TO THE ASIAN CLAM (*CORBICULA FLUMINEA*)

Interactions between the Asian Clam (*Corbicula fluminea*) and freshwater mussels (Unionidae) have been documented, but the effect of food abundance on these interactions is not well understood. I examined the role of food abundance in modulating the growth and survival responses of juvenile Cumberland Bean (*Venustaconcha troostensis*) to *Corbicula*. I ran a series of controlled experiments in which I tested the effect of *Corbicula* on growth and survival of juvenile freshwater mussels in multiple environmentally relevant conditions of food abundance. *Corbicula* had no effect on juvenile mussel survival, regardless of food abundance. However, juvenile mussel growth was significantly related to the interaction between *Corbicula* biomass and food abundance in which the effect of *Corbicula* was dependent on food abundance. *Corbicula* had no effect on juvenile mussel growth at high food abundance but had a significant and positive effect on juvenile mussel growth at low food abundance.

KEYWORDS: Asian Clam, Unionidae, bivalve interactions, juvenile mussel growth

Drew Edward Jerry White
(Name of Student)

04/11/2020
Date

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By
Drew Edward Jerry White

Dr. Steven J. Price
Director of Thesis

Dr. Steven J. Price
Director of Graduate Studies

04/11/2020
Date

DEDICATION

To the late Elizabeth Kempfer – a beloved mother with an everlasting love for her family and friends. You are dearly missed.

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The following thesis, while an individual work, benefited from the insights and direction of several people. First, my co-advisors, Dr. Steven J. Price and Dr. Wendell R. Haag, who conduct science at the highest level to which I aspire and are exceptional people. In addition, Dr. Monte McGregor, who was instrumental in providing equipment and space for my experiments as well as teaching me along the way. I am thankful to have worked with such fine scientists and people; I would not have changed a single thing. Second, Dr. Christopher Barton who served on my committee and challenged my thinking by providing thought-provoking questions and comments about my studies. Last, I thank the personnel at the Center for Mollusk Conservation including Andy McDonald, Adam Shephard, Meghan Owings, Travis Bailey, Travis Williams and Julianne Jacobs for providing laboratory equipment, space, technical assistance, and instruction.

In addition to the technical and instrumental assistance above, I received equally important assistance from my girlfriend and best friend of eight years, Jamie Kempfer. She provided me with unconditional support and assistance every step of the way. I could not have done this without her.

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INTRODUCTION

North American freshwater mussels are critically imperiled (Williams et al. 1993). Causes of these declines are unknown in many cases (Haag 2012). Culturing mussels in captivity has emerged as a conservation tool to augment existing mussel populations and reintroduce extirpated ones. Research on diet and culture conditions has made it possible to culture large numbers of mussels in captivity (Patterson et al. 2018). In addition to their conservation application, culture facilities represent an opportunity to experimentally evaluate potential causes of mussel declines. However, culture conditions must be environmentally relevant so that results can be applied to the wild. The ability to replicate wild conditions in the culture environment will improve methods for culturing mussels in captivity.

Methods to culture mussels have been around since the early 1900s (e.g. Coker et al. 1921), initially developed to supplement mussel populations experiencing declines from the pearl button industry (Haag 2012). Since then, the objectives and techniques of culturing mussels have changed (O'Beirn et al. 1998; Patterson et al. 2018). Culturing mussels is now considered a primary strategy for conserving imperiled mussel species, and several facilities are able to culture large numbers of mussels (Haag 2012; Patterson et al. 2018). Several factors affecting mussel survival and growth during culture have been examined, such as algal food size (Beck and Neves 2003), substratum characteristics (Jones et al. 2005), predator abundance (Zimmerman and Neves 2003), and seasonal viability of glochidia (Jones et al. 2005). However, the effects of food

abundance on growth and the extent to which hatchery growth reflects growth in the wild have not been examined.

Research addressing the effects of various diet mixtures and abundances on growth and survival of marine bivalves in culture has been extensive (e.g. Epifanio 1979a; Epifanio 1979b; Helm and Bourne 2004). Marine bivalves fed larger abundances of multi-species diets have higher growth than bivalves fed smaller abundances of single-species diets (e.g. Epifanio 1979a; Epifanio 1979b; Helm and Bourne 2004). These same dietary factors are likely equally important to freshwater mussel growth and survival in culture (Jones et al. 2005; Patterson et al. 2018). Better understanding these factors will aid our ability to replicate natural conditions in a hatchery environment and improve growth and survival of juvenile mussels in culture.

Replicating natural conditions and growth responses during culture is a challenge for facilities that culture mussels because mussel diets in the wild are poorly known. Single-species algal diets of *Neochloris oleoabundans* or *Nannochloropsis oculata* appear to be adequate for juvenile mussel growth and survival in captivity (Jones et al. 2005; Barnhart 2006). However, multi-species algal diets are likely necessary to provide juvenile mussels with optimal macronutrient levels (Monte McGregor, personal communication). Even though hatchery diets are adequate for supporting growth, there is little information about how hatchery diets and growth in captivity compare with those seen in the wild.

I evaluated the effect of food abundance on growth and survival of juvenile Cumberland Bean (*Venustaconcha troostensis*), and I compared experimental conditions and results with measurements from the wild. I had two primary objectives: 1) Develop

environmentally relevant food abundances representing a range of conditions seen in the wild (i.e., low, medium, and high food abundance), and 2) Assess juvenile mussel growth and survival in response to food abundance and how these responses compare with growth and survival in the wild.

METHODS

1.1.1 Study Species

My study species was the Cumberland Bean, a federally endangered species endemic to the Cumberland River system, Kentucky and Tennessee (Lane et al. 2016; Haag and Cicerello 2016). This species has been cultured successfully in captivity (Monte McGregor, personal communication) and survival and growth has been studied under captive conditions (Guyot 2005).

1.1.2 Juvenile Mussel Rearing

I cultured and conducted experiments using juvenile Cumberland Bean at the Center for Mollusk Conservation (CMC), Kentucky Department of Fish and Wildlife Resources, Frankfort, KY. I used larvae (glochidia) from brood stock collected in Sinking Creek, Laurel County, Kentucky. Glochidia of most mussel species require a fish host on which to metamorphose from the larval to the juvenile stage. I used Fantail Darter (*Etheostoma flabellare*) as a host because they produce robust metamorphosis of Cumberland Bean (Guyot 2005) and were readily available. I infected host fish with glochidia on 15 January 2019 by pipetting glochidia directly onto the gills of hosts. I held infested fish in recirculating Aquatic Habitats® (AHAB) systems (Figure 1.1) until metamorphosed juveniles were excysted from the host 13 February to 19 February 2019.

I cultured post-metamorphosed juveniles in a recirculating aquaculture system (RAS; Figure 1.2) for about 3 months. The system consisted of 14, 41.1 x 13.3 x 10.7 cm (about 6 L capacity; about 5.8 L actual volume) flow-through holding trays (hereafter trays) supplied with water and food from a 105.0 x 21.0 x 19.0 cm, (42 L capacity; about 32 L actual volume) mixing tank. The mixing tank received water pumped from a 416 L sump filled with 138 L of water, and algal food was gravity-fed to the mixing tank from a 13 L conical reservoir (hereafter feeding cone). Total system volume was 263 L. Each tray was continuously gravity-fed an algal suspension from the mixing tank via a 6.4 mm diameter silicone tube, and water overflowed the trays into the sump through a tube attached to a barbed fitting connected to a 13 mm bulkhead fitting near the top of each tray. Each tray was aerated with an air stone. The sump contained Bio Barrels (Pentair; Cary, North Carolina) to promote the colonization of bacteria that act as biological filters and degassers. Water from the sump was fed through a mini jet-pump connected to two pipes; one pipe fed water to the mixing tank while the other drained water incrementally by an automated electronic ball-valve. Water changes occurred incrementally and with a complete turnover of the water in the system every 24 hrs. I cleaned system components using acetic acid and water to remove any colonized organisms. Each tray had 50 ml of 150-250 μm heat sterilized sand substrate that was evenly distributed across the bottom.

Juvenile mussels were reared on a mixed-species diet consisting of 2 cultured freshwater algae species at CMC, *Chlorella sorokiniana* (hereafter CS) and *Phaeodactylum tricornutum* (hereafter PT); two commercially available marine algae, *Nannochloropsis* spp. (Nanno 3600, hereafter NA) and *Thalassiosira pseudonana* (TP 1800, hereafter TP); and a commercially available mixture of six marine microalgae

(Shellfish Diet 1800, hereafter SD) (all marine algae from Reed Mariculture Inc., Campbell, California). I reared mussels on a standard food ration used at the CMC, which represents the maximum food ration that can be delivered to the RAS without causing water quality problems (e.g., increased ammonia, M. McGregor, personal communication). This ration consisted of 0.8976 g CS (dry mass; 12 ml wet volume), 0.5808 g PT (12 ml wet volume), 0.4640 g NA (4 ml wet volume), 0.2200 g SD (2 ml wet volume), and 0.6016 g TP (g dry mass; 8 ml wet volume). This equated to a total food density of 0.0105 g algal dry mass/L system volume. Methods used to culture PT and CS followed Patterson et al. (2018). I measured each algal species or mixture and pipetted into a centrifuge bottle. I filled the bottle with cold water and spun in a centrifuge at 3000 rpms for 20 minutes. After centrifuging, I poured the supernatant off and the bottle was refilled with water and shaken. I poured algal suspensions into the feeding cone, and topped off the cone with cold water. Feeding cones were wrapped in reflective covering to prevent the colonization of green-blue algae and maintain a lower temperature. I added a 2 L bottle of ice tof the feeding cone after filling with the dilution to maintain algal food quality.

1.1.3 Experimental System

I constructed an experimental RAS based on a modification of the standard RAS used to rear juvenile mussels after metamorphosis (Figures 1.3 and 1.4). My experimental RAS had a smaller mixing tank (23 L capacity; 15 L actual volume) and sump (57 L capacity; 26 L actual volume) than the RAS used for rearing, and it consisted of only 8 trays. Total system volume of my experimental RAS was about 100 L (about 38% of the standard RAS). These modifications were made because all of the trays within a single

RAS are supplied with the same food abundance administered by the feeding cone. Consequently, I constructed three separate, smaller RASs that could each be supplied with a different food abundance. Mussels were placed in only two trays in each RAS during this experiment, and the other six trays were filled with water and operated in the same way as experimental trays.

I developed three experimental food abundances based on the standard CMC feeding ration (Table 1.1). My high food abundance was similar to the standard CMC ration (food density 1.3× standard ration). I established my medium and low food abundance as 50% and 25% of the high food abundance, respectively, to encompass a wide range of food abundances. I adjusted food abundances for the smaller volume of my system as follows. I dosed one of the RAS with a series of different food rations bracketed around 50% of the ration used in the standard RAS (to account for the approximately 50% lower volume of the experimental system). I collected 9, 50 ml subsamples from the RAS trays approximately 2-3 minutes after dosing with each ration so that food abundances in the system equilibrated. I measured total suspended solids (TSS, mg/L) photometrically in each subsample (Hach® Method 8006; https://www.hach.com/quick.search-quick.search.jsa?keywords=DOC316.53.01139), computed the mean TSS for each food abundance, and selected the food abundance for which TSS most closely matched TSS values in the standard RAS (TSS ≈ 10 mg/L). I used this food abundance to produce the high food abundance, and I produced the medium and low food abundances by diluting this abundance by 50% and 25%, respectively (Table 1.1). I processed and administered food abundances to each treatment using the methods described previously (see Juvenile Mussel Rearing). I

mixed each food abundance separately, and I used one centrifuge bottle for each of the treatments.

I characterized food abundances in the trays by examining fine particulate organic matter (FPOM; mg/L). Because the diet composition of mussels is not well known, I used FPOM as a proxy for food abundance. I collected triplicate 500–2000 ml water samples from each tray containing mussels on day 1, 9, and 21 of the experiment to examine FPOM values over time. I vacuum filtered each sample through a precombusted (550°C for 1 h), preweighed (nearest 0.001g) glass fiber filter (Millipore® glass-fiber filters; 0.7 micron; 47 mm diameter) and weighed the filters after drying at 104°C for 1 h to obtain TSS (mg/L filtered). I then combusted the filters at 550°C for 1 h in a muffle furnace with digital controls, reweighed them, and calculated FPOM as TSS – ash mass.

I used a single-factor design to evaluate how the food abundance affected two response variables: growth and survival. I randomly assigned each food treatment to one RAS. Prior to the experiment, I cleaned all components of the system with acetic acid, filled the system with water, and placed sand substrate in each tray as described for juvenile mussel rearing. Within each RAS, I placed ten haphazardly selected mussels in each of two randomly chosen trays; trays that did not receive mussels were allowed to run with water similar to trays with mussels. Prior to placing mussels in trays, I measured shell length (hereafter length) of each individual using Nikon© NIS-Elements D Version 3.2 imaging software. Mussels were about 3 months old and had a mean initial length (Li) of 2.5 ± 0.5 (SE) mm at the start of the experiment.

I ran the experiment for 21 d from 22 May 2019 to 11 June 2019. Water temperature in the trays ranged from 24.7 to 26.7°C during the experiment (mean = 25.9).

Water flow rate through the trays was maintained at 100 ml/min. Automated, incremental water changes occurred in each RAS at the rate of 1.5× the system volume/24 h. This was accomplished with electronic ball valves and timers which drained and replenished 13 L of water into and out of the sump every 2 hours (Figure 1.3). Every seven d, I removed mussels from the trays and measured them, cleaned the trays with acetic acid, refilled the trays with water and sand, and then replaced mussels in the trays. I cleaned mixing tanks and sumps with acetic acid every 10 d. I cleaned feeding cones daily and refilled them with the designated abundance of food. Cleaning the systems in this way helped reduce colonization by blue-green algae and other aquatic organisms. On the final day of the experiment I remeasured each mussel.

To monitor general water quality during the experiment, I measured pH, total ammonia (mg/L, as NH_3–N), temperature (°C), and dissolved oxygen (DO, % saturation) daily for the first 9 d of the experiment and every 1–4 d thereafter. I measured pH and ammonia in 50 ml water samples from experimental trays using an Accumet Basic AB15 Plus pH meter and the nitrogen, ammonia – salicylate method (Hach® Method 10031; https://www.hach.com/quick.search-quick.search.jsa?keywords=DOC316.53.01079), respectively. I measured temperature and DO directly in the trays using a handheld digital thermometer and a Milwaukee MW600 portable DO meter, respectively. Water quality generally was similar among food abundances and showed no evidence of adverse conditions at any time during the experiment (Table 1.2). There were no significant differences in pH or DO among food abundances ($F_{2,4\text{-}12}$ = 0.95–1.37, P = 0.40–0.26). Ammonia ($F_{2,7\text{-}8}$ = 4.87, P = 0.01) and temperature ($F_{2,10\text{-}11}$ = 3.60, P = 0.04) differed significantly among food abundances, but the magnitude of the differences were small.

1.1.4 Field Measurements

I measured FPOM at 14 stream sites in Kentucky to provide information about the environmental relevancy of my experimental food abundances (Table 1.3). I selected streams in three physiographic regions to evaluate food abundance across a range of productivity and general stream conditions. I collected one to six samples at each site from June to September, 2019. On each sampling date, I collected a single, 1 L water sample following Kentucky Division of Water methodology for sampling lotic systems (KDOW 2009). I measured FPOM in stream samples, following methods described previously.

I compared Cumberland Bean growth in my experiment with existing data about Cumberland Bean growth in the wild (W. Haag, S. Price, et al., unpublished data). That study placed juvenile Cumberland Bean in flow-through chambers (silos) at 17 sites in the Rockcastle River system from June to September, 2018. Mussels in that study averaged 4.6 mm shell length and 0.019 g wet mass at the time they were deployed in streams, but growth during the study varied among sites (see Results). All stream sites selected for that study had historical populations of Cumberland Bean but current population status varied.

DATA ANALYSIS

I expressed mussel growth in each tray as instantaneous growth [ln (mean final length/mean initial length)/experiment duration in d; hereafter, growth]. I calculated proportional survival in each tray as the number of surviving individuals in the tray/the initial number of mussels in the tray. I arcsine transformed survival for further analysis.

Growth and survival data (survival arcsine transformed) were normally distributed (Shapiro-Wilks test, growth: $W = 0.89$, $P = 0.51$; survival: $W = 0.92$, $P = 0.33$). Growth and survival data did not meet assumptions of homogeneity of variance (Levene's test, growth: $F = 2.56 \times 10^{31}$, $P < 0.001$; survival: $F = 1.76 \times 10^{30}$, $P < 0.001$). I evaluated the relationship between food abundance and two response variables, mussel growth and survival, using Welch's Heteroscedastic F-test with food abundance as a categorical variable (low, medium, and high); this procedure is robust to violations of homogeneity of variance. I analyzed each response variable separately, and used a 0.05 significance level (α) to determine significance. I did all analyses in RStudio version 3.5.1 (RStudio Team 2018).

RESULTS

1.1.5 RAS Experiment

FPOM was significantly different among food abundances (ANOVA; $F_{2,11\text{-}12} = 6.78$; $P < 0.01$; Figure 1.5). Mean FPOM was 2.97 mg/L ± 1.06 (SD; n = 16) in the high, 2.08 ± 0.79 (SD; n = 17) in the medium, and 1.66 ± 1.22 (SD; n = 16) mg/L in the low food abundance. FPOM in the high food abundance was significantly different than both the low ($P < 0.01$) and the medium ($P = 0.04$). FPOM in the low and medium food abundances were not significantly different ($P = 0.50$). This was likely due to high FPOM in two of the low food abundance samples that may have been due to local aggregation of algae in the samples.

Growth was not significantly different among food abundances (Welch's Heteroscedastic F-test; $F_{2,3} = 19.26$; $P = 0.06$; Figure 1.6). Mean growth was 0.0041/d ±

0.0003 (SD; n = 2) in the high food abundance, 0.0033 ± 0.0009 (SD; n = 2) in the medium, and 0.0011 ± 0.0004 (SD; n = 2) mm/d in the low. The lack of difference in growth among the food abundances was likely due to the similarity of growth between the medium and high food abundances.

Survival was high overall in the experiment (80% ± 14 (SD; n = 6)). Survival was not significantly different among food abundances (Welch's Heteroscedastic F-test; $F_{2,3} = 1.81$; $P = 0.38$; Figure 1.7). However, there was an apparent trend of decreasing survival with decreasing food abundance and the two highest survival values were observed in the high food abundances.

1.1.6 Environmental Relevance

FPOM in trays fell within the distribution of FPOM from the 14 stream sites (Figure 1.8). FPOM from the RAS experiment did not exceed the values on either the low or high extremes of FPOM from streams. The median stream FPOM was 1.57 mg/L, which fell between the median FPOM of the low and medium food abundances, and FPOM in the high food abundance was similar to the highest value observed in streams. The interquartile range (IQR) of FPOM in the wild overlapped all three of the treatment IQRs.

The distribution of growth from the RAS experiment fell within the distribution of growth in the wild (Figure 1.9). However, growth in the high food abundance was 50% or less than the highest growth rates seen in the wild. Median growth in the low food abundance (0.0011/d) was within the 1st quartile of growth in the wild. Median growth

in the medium (0.0033) and high (0.0041) food abundances fell within the 2nd and 3rd quartiles of growth in the wild, respectively.

DISCUSSION

My experimental food abundances were remarkably similar to food abundances in the wild when viewed in the context of FPOM. This suggests that these food rations are appropriate for juvenile mussel culture and for producing environmentally relevant conditions for laboratory experimental studies. However, gross food abundance, as measured by FPOM, does not reflect food quality, which likely differs substantially between captive and wild mussel diets. Mussels in the wild consume a diverse diet including phytoplankton, zooplankton, bacteria, rotifers, and detritus from both the sediment and seston (Yeager et al. 1994; Vaughn et al. 2008). Bacteria may be particularly important as a source of macronutrients (Nichols and Garling 2000; Christian et al. 2004).

Mussel growth responded positively to increased food abundance, as expected. Interestingly, mean growth in the medium food abundance was not different than mean growth in the high food abundance, despite the fact that the medium food abundance received half the food ration as the high. The lack of an overall difference in growth among food levels was likely caused by the similarity in growth between the medium and high food abundances. This similarity in growth between the medium and high food abundances suggests that these growth rates represent near-maximum rates for my culture conditions, and it is difficult to increase food abundance without causing water quality problems. However, growth in the medium and high food abundances was 50% or less

than maximum growth rates seen in the wild, despite similar levels of FPOM. This discrepancy highlights the probable large differences in food quality between wild diets and captive diets. Captive diets based strictly on algae likely do not provide the full range of nutrients required to support maximal growth similar to that seen in the wild.

My experimental food abundances all were adequate to support high survival for 21 d. The apparent trend of lower survival with decreasing food abundance suggests that survival may be compromised at lower food abundances over longer culture periods, and mussels in the low food abundance had little to no food in their guts. Longer experiments are necessary to more fully evaluate the effects of food abundance on survival because bivalves can survive and grow on lipid or carbohydrate energy reserves, depending on life-stage, in food-limited conditions (Holland and Spenser 1973; Lasee 1991).

I showed that it is possible to reproduce environmentally relevant food abundances and growth rates in a hatchery environment. Nevertheless, hatchery growth rates do not completely mimic those seen in the wild. More research is needed to identify specific components of wild mussel diets that contribute to higher growth in the wild. Future studies also should examine potentially more sensitive indicators of juvenile mussel fitness, such as fatty acid profiles, glycogen, and other biomarkers, and how they are affected by food abundance and quality.

Table 1.1. Mussel feeding rations for three experimental food abundances. Values are g dry mass (ml wet volume). Algal types are as follows: CS = *Chlorella sorokiniana*; PT = *Phaeodactylum tricornutum*; NA = *Nannochloropsis* spp.; TP = *Thalassiosira pseudonana*; SD = Shellfish Diet; see text for details about algal types. Total food density is g algal dry mass/L system volume, based on system volume of 100 L.

	Experimental Food Rations		
Algal type	Low	Medium	High
CS	0.1120 (1.5)	0.2244 (3.0)	0.4488 (6.0)
PT	0.0726 (1.5)	0.1452 (3.0)	0.2904 (6.0)
NA	0.0580 (0.5)	0.1160 (1.0)	0.2320 (2.0)
SD	0.0275 (0.25)	0.0550 (0.5)	0.1100 (1.0)
TP	0.0752 (1.0)	0.1504 (2.0)	0.3008 (4.0)
Total food density (g/L)	0.0035	0.0070	0.0138

Table 1.2. Water quality parameters during the experiment. Values are means (range). Sample size (N) refers to the number of measurements in each tray.

Food abundance	pH (N = 8–9)	NH_3-N (mg/L) (N = 6–7)	Temperature (°C) (N = 7–8)	DO (% saturation) (N = 4–6)
High 1	8.42 (8.27–8.55)	0.04 (0.00–0.06)	26.2 (25.7–26.7)	88 (84–93)
High 2	8.40 (8.27–8.54)	0.03 (0.01–0.06)	26.0 (25.5–26.7)	90 (78–100)
Medium 1	8.45 (8.39–8.54)	0.03 (0.00–0.06)	25.5 (24.7–26.5)	86 (78–99)
Medium 2	8.43 (8.31–8.54)	0.04 (0.01–0.06)	25.8 (24.7–26.6)	87 (79–98)
Low 1	8.45 (8.30–8.55)	0.01 (0.00–0.03)	25.6 (25.0–26.4)	91 (90–93)
Low 2	8.45 (8.37–8.60)	0.02 (0.00–0.05)	26.0 (25.1–26.4)	90 (83–97)

Table 1.3. Kentucky streams sampled for FPOM (mg/L).

Stream	KY Physiographic Province	Avg. FPOM (mg/L)	*SD*	*N*
Buck Creek	Highland Rim	1.43	0.76	3
Little South Fork	Cumberland Plateau; Highland Rim	0.72	0.25	6
Horse Lick Creek	Cumberland Plateau	0.70	0.41	3
Eagle Creek	Bluegrass	2.42	0.35	2
South Fork Kentucky River	Cumberland Plateau	1.69	0.66	3
Slate Creek	Bluegrass	3.12	2.37	2
Drakes Creek	Highland Rim	1.88	0.65	3
North Elkhorn Creek	Bluegrass	2.22		1
Green River	Highland Rim	1.72	0.36	3
Little River	Highland Rim	1.88		1
Red River (W. Kentucky)	Highland Rim	2.14	1.13	3
Red River (E. Kentucky)	Cumberland Plateau; Bluegrass	1.43	0.61	2
Redbird River	Cumberland Plateau	0.95	0.07	2
Rockcastle River	Cumberland Plateau	2.33		1

Figure 1.1. AHAB system used to hold Fantail Darter infested with glochidia.

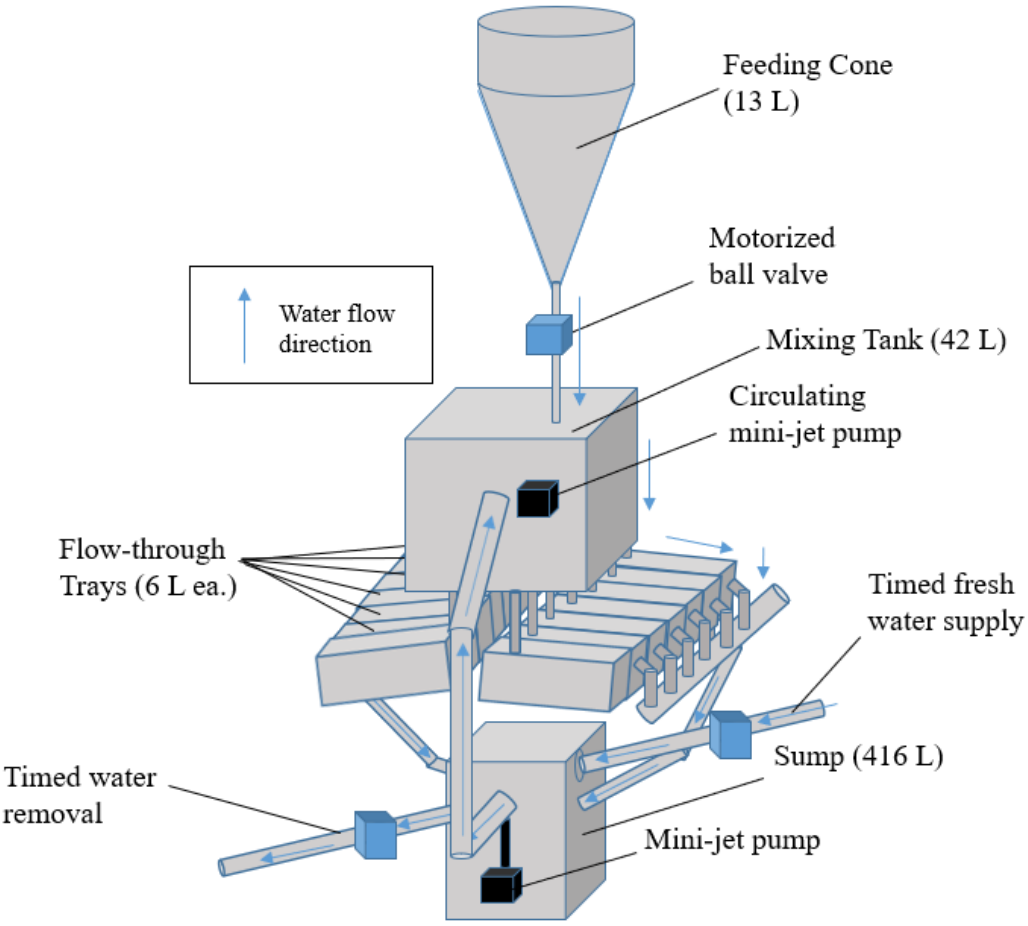

Figure 1.2. Design of the RAS used to rear juvenile mussels.

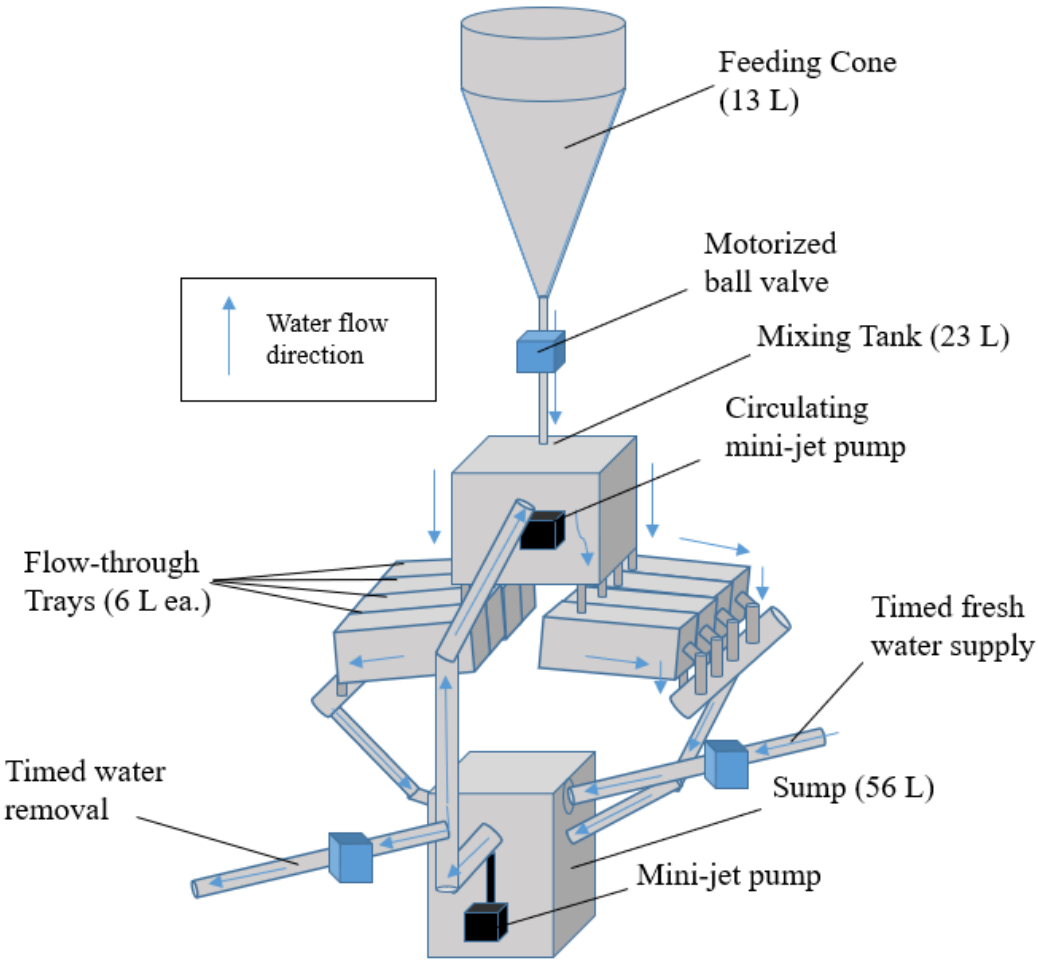

Figure 1.3. Design of the experimental RAS.

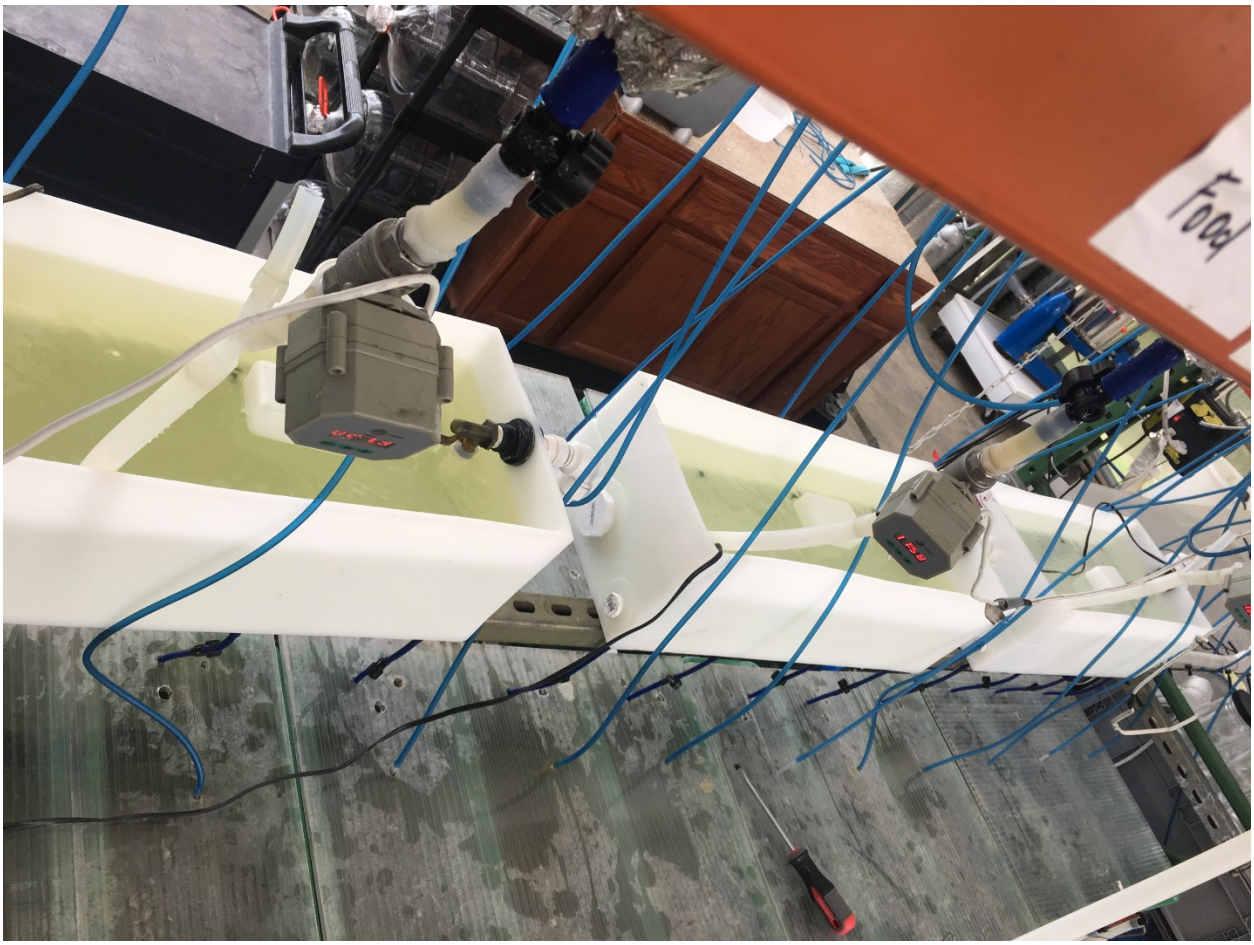

Figure 1.4. Overhead view of the three RASs used in the experiment. The white tanks are mixing tanks supplied with water from a sump and algal food from a feeding cone above via a tube connected to it. Note decreasing intensity of green hue in high food abundance (left) to low food abundance (right).

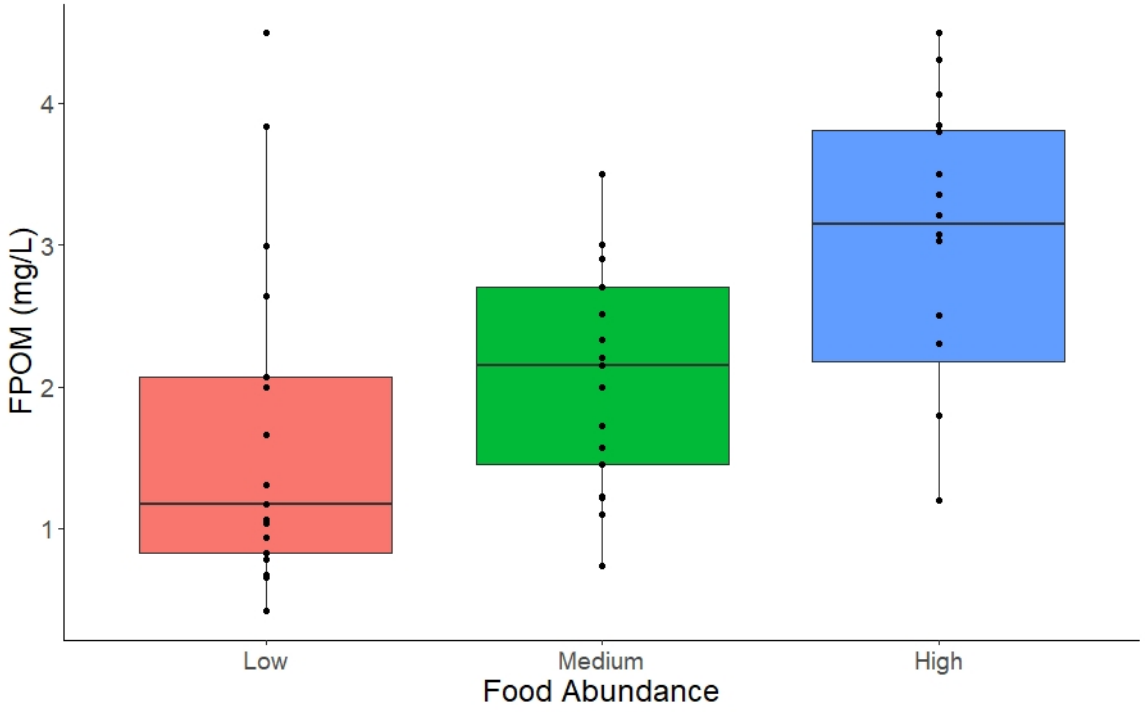

Figure 1.5. Fine particulate organic matter (FPOM) in three experimental mussel food abundances. Data points are individual FPOM measurements taken throughout the experiment (low, N = 17; medium, N = 17; high, N = 16).

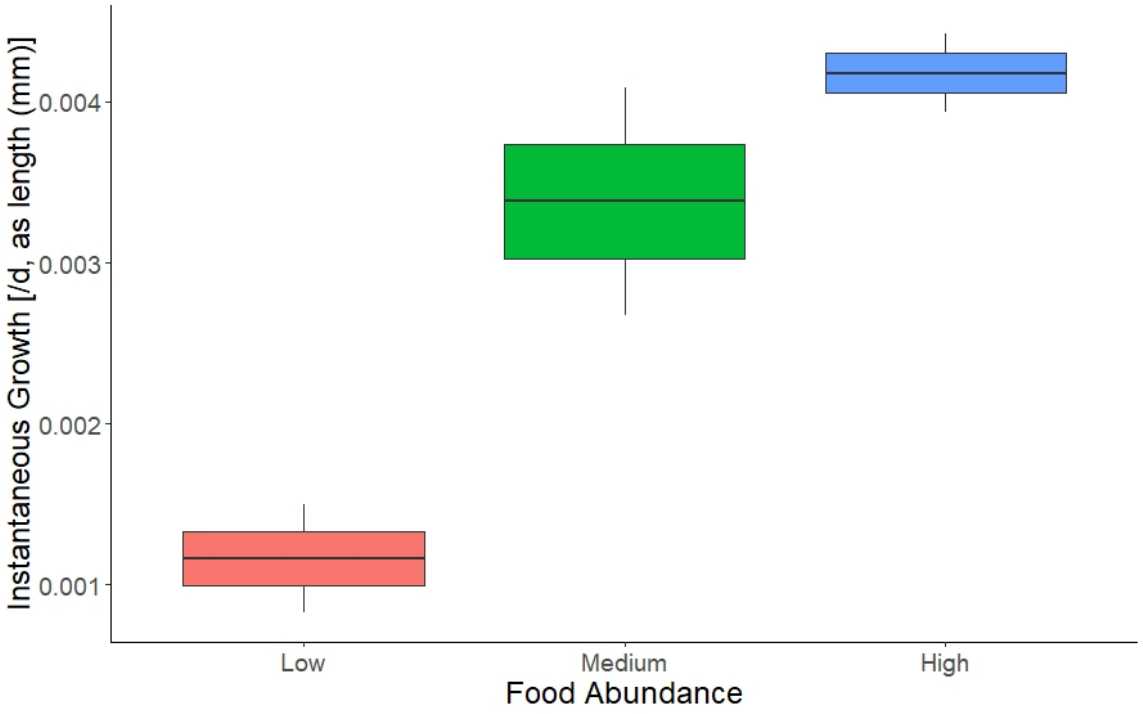

Figure 1.6. Instantaneous growth [/d, as length (mm)] in three experimental mussel food abundances.

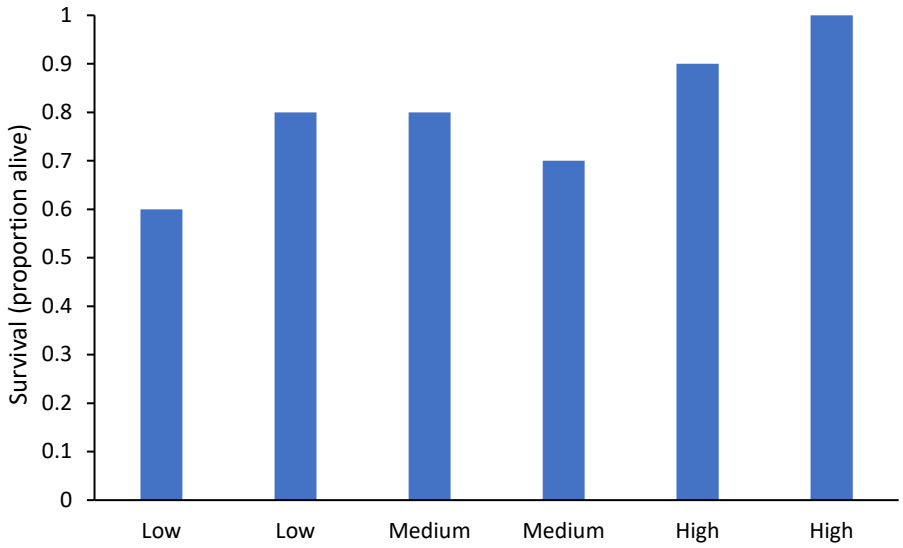

Figure 1.7. Juvenile mussel survival from each replicate within each of three levels of experimental mussel food abundance.

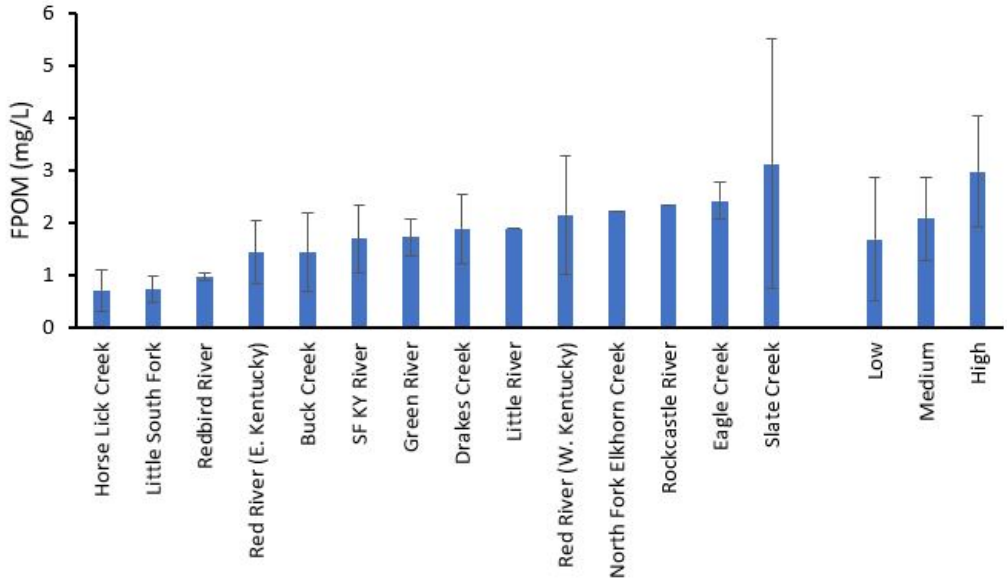

Figure 1.8. Mean fine particulate organic matter (FPOM) in 14 Kentucky streams and three experimental mussel food abundances. Error bars are SD.

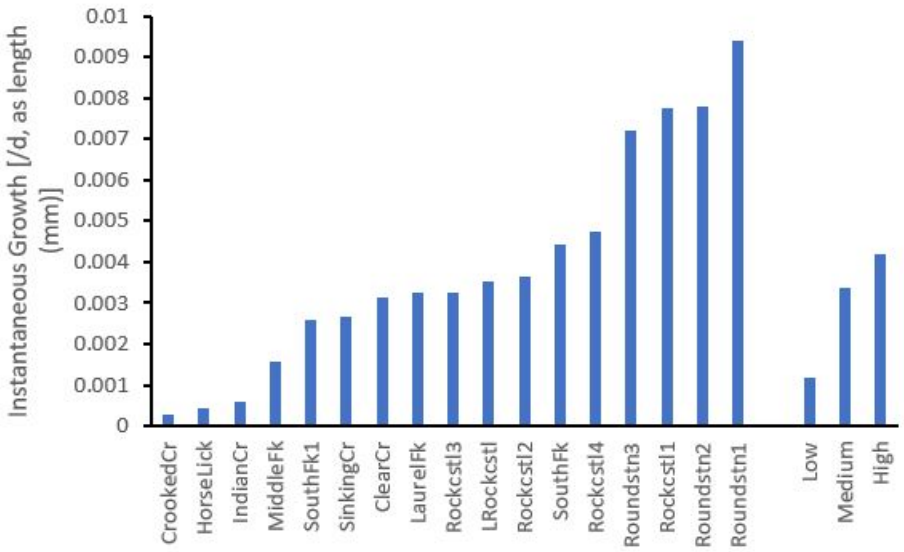

Figure 1.9. Mussel instantaneous growth [/d, as length (mm)] in 17 Kentucky streams and three experimental mussel food abundances.

CHAPTER 2. THE EFFECT OF FOOD ABUNDANCE AND *CORBICULA* BIOMASS ON GROWTH AND SURVIVAL OF JUVENILE MUSSELS IN A RECIRCULATING AQUACULTURE SYSTEM, PART I.

INTRODUCTION

Freshwater mussels are one of the most imperiled taxonomic groups in North America. Habitat destruction from dams initially drove population declines in the early 20th century (Haag 2012). Mussels began again to decline precipitously in the 1960s resulting in the widespread decline of mussel populations throughout the southeastern United States (Haag 2012). However, recent mussel extinctions and widespread population declines are difficult to link to any one cause because they are occurring in the absence of obvious impacts (Haag 2012; Haag 2019). These declines are characterized by a cessation of recruitment, which results in populations dominated by older individuals (Haag 2012). This finding suggests the cause of recent declines particularly affects juvenile mussels. The invasive Asian Clam (*Corbicula fluminea*) is a potential factor in enigmatic declines, but its effects on native mussels are not well studied (Haag 2019).

Another invasive bivalve, the Zebra Mussel (*Dreissena polymorpha*), is documented as a primary factor in mussel declines in some areas. Bivalves are filter feeders and have a diverse diet, including phytoplankton, zooplankton, bacteria, rotifers, and detritus (Vaughn et al. 2008). Zebra Mussels have higher mass-specific filtration rates than native mussels and can reach extremely high densities (e.g., 30,000/m^2; Griffiths et al. 1991; Strayer 1999). Consequently, Zebra Mussels can reduce food abundance to 10-20% of pre-invasion levels, resulting in strong food competition with native mussels and cessation of recruitment (Caraco et al. 1997; Strayer and Smith 1996;

Strayer 1999). The introduction and spread of Zebra Mussels in the upper Midwest nearly eliminated native mussel assemblages in much of the region due largely to food competition (Strayer 1999). However, Zebra Mussels have not successfully colonized most of the southeastern U.S., which eliminates them as a potential factor in enigmatic mussel declines in that region.

The Asian Clam, *Corbicula fluminea* (hereafter *Corbicula*), was introduced into North America by the 1930s and spread across much of the U.S. by the 1970s (Crespo et al. 2015). Similar to Zebra Mussels, *Corbicula* can reach extremely high densities (10,000/m^2, Gardner et al. 1976) and can reduce plankton abundance by 40–60% in streams (Cohen et al. 1984). Negative effects of *Corbicula* on native mussels have been shown in some cases, but these results differ according to context. Growth and survival of 1–3 d old mussels in the laboratory was sharply reduced at *Corbicula* densities of 625/m^2, and mortality was 100% at densities >1250/m^2 (Yeager et al. 1999). Another laboratory study showed lower feces production by adult mussels (a proxy for food acquisition) in a single high *Corbicula* treatment combination, but *Corbicula* interacted with other experimental factors and other high *Corbicula* treatments did not show reduced growth (Ferreira-Rodríguez and Pardo 2017). In a related field study, growth and energy stores of adult mussels were unaffected by *Corbicula* except at high density (2000/m^2; Ferreira-Rodríguez et al. 2018), and other field studies have failed to find unequivocal evidence of negative effects of *Corbicula* on adult mussels (Belanger et al. 1990; Leff et al. 1990). Both *Corbicula* and juvenile mussels rely heavily on pedal feeding and siphoning water from interstitial spaces (Yeager et al. 1994; Hakenkamp and Palmer 1999). Thus, juvenile mussels may be particularly vulnerable to competition with *Corbicula* for food

and space. However, further investigation is needed to fully understand the relationship between *Corbicula* and juvenile mussels.

The objectives of this experiment were to determine the effect of (1) food abundance, (2) *Corbicula* biomass, and (3) the interaction of food abundance and *Corbicula* biomass on the growth and survival of juvenile freshwater mussels. Specifically, I tested the following hypotheses:

(1) Increased food abundance has a positive effect on growth and survival of juvenile Cumberland Bean.

(2) Increased *Corbicula* biomass has a negative effect on growth and survival of juvenile Cumberland Bean

(3) The interaction between *Corbicula* biomass and food abundance has a significant effect on growth and survival of juvenile Cumberland Bean such that the strength of competition by *Corbicula* increases with decreasing food abundance.

METHODS

2.1.1 Juvenile Mussel Rearing

I cultured and conducted experiments using juvenile Cumberland Bean at the Center for Mollusk Conservation, Kentucky Department of Fish and Wildlife Resources, Frankfort, KY. I used larvae (glochidia) from brood stock collected in Sinking Creek, Laurel County, Kentucky. Glochidia of most mussel species require a fish host on which to metamorphose from the larval to the juvenile stage. I used Fantail Darter (*Etheostoma*

flabellare) as a host because they produce robust metamorphosis of Cumberland Bean (Guyot 2005) and were readily available. I infected host fish with glochidia on 15 January 2020 by pipetting glochidia from a petri dish directly onto the gills of hosts. I held infested fish in recirculating Aquatic Habitats® (AHAB) systems (Figure 2.1) until metamorphosed juveniles were produced from 13 February to 19 February 2019.

I cultured post-metamorphosed juveniles in a recirculating aquaculture system (RAS; Figure 2.2) for about 5 months. The system consisted of 14, 41.1 x 13.3 x 10.7 cm (about 6 L capacity; about 5.8 L actual volume) flow-through holding trays (hereafter trays) supplied with water and food from a 105.0 x 21.0 x 19.0 cm, (42 L capacity; about 32 L actual volume) mixing tank. The mixing tank received water pumped from a 416 L sump filled with 138 L of water, and algal food was gravity-fed to the mixing tank from a 13 L conical reservoir (hereafter feeding cone). Total system volume was 263 L. Each tray was continuously gravity-fed an algal suspension from the mixing tank via a 6.4 mm diameter silicone tube, and water overflowed the trays into the sump through a tube attached to a barbed fitting connected to a 13 mm bulkhead fitting near the top of each tray. Each tray was aerated with an air stone. The sump contained Bio Barrels (Pentair; Cary, North Carolina) to promote the colonization of bacteria that act as biological filters and degassers. Water from the sump was fed through a mini jet-pump connected to two pipes; one pipe fed water to the mixing tank while the other drained water incrementally by an automated electronic ball-valve. Automated, incremental water changes occurred in each RAS at the rate of 1.0× the system volume/24 h. This was accomplished with electronic ball valves and timers that allowed water to flow from the sump into a drain and fresh water to replenish the sump volume, both at regular intervals (Figure 2.3). I

cleaned system components every 7 d using acetic acid and water to remove any colonized organisms. Each tray had 50 ml of 150-250 μm sand substrate that was evenly distributed across the bottom.

Juvenile mussels were reared on a mixed-species diet consisting of 2 cultured freshwater algae species at CMC, *Chlorella sorokiniana* (hereafter CS) and *Phaeodactylum tricornutum* (hereafter PT); two commercially available marine algae, *Nannochloropsis* spp. (Nanno 3600, hereafter NA) and *Thalassiosira pseudonana* (TP 1800, hereafter TP); and a commercially available mixture of six marine microalgae (Shellfish Diet 1800, hereafter SD) (all marine algae from Reed Mariculture Inc., Campbell, California).). I reared mussels on the standard rearing ration used at the CMC, which represents the maximum food abundance that can be delivered to the RAS without causing water quality problems (e.g., increased ammonia, M. McGregor, personal communication). This ration consisted of 0.8976 g CS (dry mass; 12 ml wet volume), 0.5808 g PT (12 ml wet volume), 0.4640 g NA (4 ml wet volume), 0.2200 g SD (2 ml wet volume), and 0.6016 g TP (g dry mass; 8 ml wet volume). This equated to a total food density of 0.0105 g algal dry mass/L system volume. Methods used to culture PT and CS followed Patterson et al. (2018). I measured each algal species or mixture and pipetted it into a centrifuge bottle. I filled the bottle with cold water and spun in a centrifuge at 3000 rpms for 20 minutes. After centrifuging, I poured the supernatant off and the bottle was refilled with water and shaken. I poured algal suspensions into the feeding cone, and the cone was topped off with cold water. Feeding cones were wrapped in reflective covering to prevent the colonization of green-blue algae and maintain a

lower temperature. A 2 L bottle of ice was added to the feeding cone after filling with the dilution to maintain algal food quality.

2.1.2 Experimental System

I constructed an experimental apparatus based on a modification of the standard RAS used to rear juvenile mussels after metamorphosis (Figure 2.3). My experimental RAS had a smaller mixing tank (23 L capacity; 15 L actual volume) and sump (57 L capacity; 26 L actual volume) than the RAS used for rearing, and it consisted of only 8 trays. Total system volume of my experimental RAS was about 100 L (about 38% of the standard RAS). These modifications were made because all of the trays within a single RAS are supplied with the same food ration administered by the feeding cone. Consequently, I constructed three separate, smaller RASs that could each be supplied with a different food ration.

I developed three experimental levels of food abundance based on the feeding rations used in the previous experiment (see Chapter 1, Juvenile Mussel Rearing). Food levels in that experiment were based on the standard CMC feeding ration. My high food level was 2× the quantity of the ration used in the high food level in that experiment. I established my medium and low food levels as 50% and 18% of the high food level, respectively (Table 2.1). I established these food rations to encompass a wider range of food abundance than Experiment 1. I doubled the ration of the high food level from Experiment 1 for the high food level in this experiment to better replicate growth from high growth streams. I also increased the quantity of the ration in the low food level from the low food level in Experiment 1 because mussels in that level appeared to have little to no food in their guts. I processed and administered food rations to each treatment using

the methods described previously (see Juvenile Mussel Rearing). I mixed each food ration separately, and I used one centrifuge bottle for each of the treatments.

I collected about 1,100 *Corbicula* from North Elkhorn Creek at Robinson Dam, Scott County, Kentucky (38.211950, -84.626419) on 31 June 2019. Individual Corbicula averaged 20.8 mm ± 4.2 (SD; shell length; n = 239) and 3.50 g ± 1.67 (SD; blotted wet mass, including shell; n = 91). I transported *Corbicula* to the CMC in aerated 45 L coolers filled with river water. I acclimated *Corbicula* to laboratory water over about 3 hours by exchanging about 4 L of river water with 4 L of laboratory water every 20–30 minutes until the river water was completely exchanged with laboratory water. After acclimation, I housed *Corbicula* in a 416 L flow-through tank (actual water volume = 138 L) with a flow rate that resulted in complete water changes every 6 hours. I fed *Corbicula* the same food ration fed to juvenile mussels during rearing (see Juvenile Mussel Rearing). *Corbicula* were held for 6 days before the experiment started. *Corbicula* experienced substantial mortality during holding and during the experiment. An additional 700 individuals were collected at the same location on 18 July 2019 and acclimated as described previously.

I chose four treatment levels of *Corbicula* biomass to be placed in experimental trays: 0, 3.7, 32.0, and 186.5 g/tray (blotted wet mass). These levels corresponded to 0, 1, 8 and 50 individuals/tray and about 0, 21, 145 and 910 individuals/m^2, based on the average mass of one *Corbicula*. I chose these levels because they represent a range of frequently reported *Corbicula* densities in the wild (e.g. Gardner et al. 1976; Stites et al. 1995; Miller and Payne 1998; Karatayev et al. 2003; Sousa et al. 2008). *Corbicula*

densities in the wild higher than 1,000 /m^2 are uncommon and may not be sustainable for long periods (Gardner et al. 1976).

I used a full-factorial design to evaluate how food abundance and *Corbicula* biomass affected two response variables: growth and survival. I randomly assigned each food level to one RAS. I randomly assigned each *Corbicula* biomass to two trays within each RAS and placed the specified number of *Corbicula* in each tray. Prior to the experiment, I cleaned all components of the system with acetic acid, filled the system with water, and placed sand substrate in each tray as described for juvenile mussel rearing. Within each RAS, I placed twenty haphazardly selected native mussels in each experimental tray. Prior to placing mussels in trays, I measured shell length (hereafter length) of each individual using Nikon© NIS-Elements D Version 3.2 imaging software. Mussels were about 5 months old and had a mean initial length (L_i) of 4.4 ± 0.4 (SD) mm at the start of the experiment.

I ran the experiment for 17 d from 5 July 2019 to 22 July 2019. Water temperature in the trays ranged from 25.6 to 28.7°C during the experiment (mean = 27.7°C). Water flow rate through the trays was 100 ml/min. Automated, incremental water changes occurred in each RAS at the rate of 1.5× the system volume/24 h. This was accomplished with electronic ball valves and timers which drained and replenished 13 L of water into and out of the sump every 2 hours (Figure 2.3). Every seven d, I removed mussels from the trays and measured them, cleaned the trays with acetic acid, refilled the trays with water and sand, and then replaced mussels in the trays. I cleaned mixing tanks and sumps with acetic acid every 10 d. I cleaned feeding cones daily and refilled them with the designated food ration. Cleaning the systems in this way helped

reduce colonization by blue-green algae and other aquatic organisms. On the final day of the experiment I remeasured each mussel.

I characterized food abundance in the trays during the experiment by examining fine particulate organic matter (FPOM; mg/L). Because the diet composition of mussels is not well known, I used FPOM as a proxy for food abundance. I collected 500–2000 ml water samples from each tray containing mussels on day 6 of the experiment. I vacuum filtered each sample through a precombusted (550°C for 1 h), preweighed (nearest 0.001g) glass fiber filter (Millipore® glass-fiber filters; 0.7 micron; 47 mm diameter) and weighed the filters after drying at 104°C for 1 h to obtain TSS (mg/L filtered). I then combusted the filters at 550°C for 1 h, reweighed them, and calculated FPOM as TSS – ash mass.

To monitor general water quality during the experiment, I measured pH, total ammonia (NH_3–N, mg/L), temperature (°C), and dissolved oxygen (DO, % saturation and mg/L) daily for the first 9 d of the experiment and every 1–2 d thereafter. I measured pH and ammonia in 50 ml water samples from experimental trays using an Accumet Basic AB15 Plus pH meter and the nitrogen, ammonia – salicylate method (Hach® Method 10031; https://www.hach.com/quick.search-quick.search.jsa?keywords=DOC316.53.01079), respectively. I measured temperature and DO directly in the trays using a handheld digital thermometer and a Milwaukee MW600 portable DO meter, respectively. Water quality showed no evidence of adverse effects during most of the experiment (Table 2.2). Ammonia reached relatively high levels (>0.1 mg/L) in the first day of the experiment but subsequently stabilized to lower levels for the remainder of the experiment. There were no significant differences in DO

(% saturation) and DO (mg/L) ($F_{11,45}$ = 1.27–1.78; P = 0.09–0.28). Ammonia ($F_{11,39}$ = 2.32; P = 0.03), pH ($F_{11,40}$ = 4.59; P < 0.001), and temperature ($F_{11,45}$ = 3.59; P < 0.01) differed significantly among treatments. Ammonia generally was higher, and pH was lower in treatments with lower food abundance, and temperature was higher in treatments with higher *Corbicula* biomass; these patterns probably are explained by higher biological activity in those treatments (Figures 2.10–2.12). However, the magnitude of these differences was small, and I did not consider them further in data analysis.

DATA ANALYSIS

I expressed mussel growth in each tray as instantaneous growth [ln (mean final length/mean initial length)/experiment duration in d; hereafter, growth]. I calculated proportional survival in each tray as the number of surviving individuals in the tray/the initial number of mussels in the tray. I arcsine transformed survival for further analysis. Growth and survival data (survival arcsine transformed) were not normally distributed and variance was heterogeneous (Shapiro-Wilk test, growth: W = 0.874, P = 0.006; survival: W = 0.533, P <0.0001; Levene's test, growth: F = 1.6 x 10^{30}, P < 0.0001; survival: F = 1.7 x 10^{30}, P <0.0001). I evaluated the relationship between two factors, *Corbicula* biomass and food abundance, and two response variables, mussel growth and survival, using a two-way ANOVA including both effects terms (*Corbicula* and food) and the interaction term (*Corbicula* × food). I analyzed each response variable separately, and I used a significance level (α) of 0.05 to determine significance. I also evaluated the relationship between two factors, *Corbicula* biomass and food abundance, and one response variable, FPOM, using a two-way ANOVA including both main effects

(*Corbicula* and food) and the interaction term (*Corbicula* × food). I did all analyses in RStudio version 3.5.1 (RStudio Team 2018).

RESULTS

Mussel growth was significantly related to food abundance, but *Corbicula* biomass and the interaction term were not significant (Table 2.3; Figures 2.4-2.6). Growth increased with increasing food abundance, and all pairwise comparisons were significantly different (Table 2.4; Figure 2.4). Growth was highest in the high food level (mean = 0.0128/d, as length (mm) ± 0.0039 SD; n =8) and lowest in the low food level (mean = 0.0020 ± 0.0004 SD; n =8).

Survival was high overall in the experiment (98% ± 3 SD; n = 24). None of the factors were significant in explaining differences in survival in the experiment (Table 2.5, Figures 2.7-2.9). There were no significant pairwise differences in survival among levels of *Corbicula* biomass (Table 2.6).

FPOM was significantly related to food abundance, but *Corbicula* biomass and the interaction term were not significant (Table 2.7, Figure 2.13). Mean FPOM was highest in the high food level (mean = 3.67 ± 0.71 SD; n = 8).

DISCUSSION

I evaluated the effect of food abundance, *Corbicula* biomass, and the interaction on growth and survival of juvenile Cumberland Bean. Food abundance was a significant factor in explaining differences in growth among treatments, as expected. Growth was

surprisingly high despite the presence of *Corbicula* in the systems, and overall, growth was much higher in this experiment than in Experiment 1.

Food abundance was not related to survival, and overall, survival was high in the experiment. This suggests that these food levels were appropriate for juvenile mussel culture and provided adequate food for survival even in the presence of *Corbicula*.

Negative effects of *Corbicula* on mussel growth were not detected in this study. I expected greater *Corbicula* biomass to negatively affect juvenile mussel growth, especially in the low food abundance treatment, but this effect was not seen. This result was surprising because in a previous study, growth of newly transformed Rainbow Mussel (*Villosa iris*) was 90% of controls at *Corbicula* densities >1250/m^2 (Yeager et al. 1999). In my study, the high *Corbicula* level corresponded to about 910/m^2, which is similar to the density in the Yeager et al. (1999) study.

One possible explanation for the lack of *Corbicula* effects in my study could be that Cumberland Bean is less affected by *Corbicula* than other species. For example, Cumberland Bean may be able to use undigested or uneaten food from *Corbicula* pseudofeces as a food source to a greater extent than other native mussel species, which may negate competition for food in suspension. However, this aspect of Cumberland Bean feeding is unknown, and it is unlikely that mussel species differ substantially in this regard. Coprophagy (the eating of feces) is known in marine bivalves (e.g. Blue Mussel, *Mytilus edulis*) as well other marine invertebrates (see Frankenberg and Smith 1967), but little is known about this behavior in freshwater mussels. Previous studies showed that *Corbicula* can have a negative effect on carbohydrate content, growth, survival, and fecal production of two unrelated mussel species, *Villosa iris* and *Unio delphinus* (Yeager et al.

1999; Feirrera-Rodríguez and Pardo 2017; Feirrera-Rodríguez et al. 2018). Furthermore, enigmatic mussel declines are characterized by fauna-wide collapse, suggesting that the cause of the decline affects all of mussel species in a stream (Haag 2019). *Corbicula* is not likely the cause of enigmatic declines if its effects differ among mussel species.

A more likely explanation for the lack *Corbicula* effects relates to the recirculating design of my RAS. Because all trays in the RAS (regardless of *Corbicula* biomass) circulated through a common sump, mixing in the sump and redistribution of water throughout the system probably created similar food abundance in all trays. This is supported by the FPOM values measured in trays; FPOM was similar among trays in each food level regardless of *Corbicula* biomass in each tray. *Corbicula* may have reduced food availability, but this reduction appears to have been system-wide, rather than in individual trays with high *Corbicula* density. Consequently, my system design was not able to directly address the effects of *Corbicula* on mussel growth or survival.

I expected greater *Corbicula* biomass to negatively affect juvenile mussel survival, especially in lower food abundance treatments, similar to the Yeager et al. (1999) study (see Introduction). High mortality in that study was attributed to direct ingestion of juveniles by *Corbicula*, but lower growth also suggests food competition. *Corbicula* densities in my high treatment (910/m^2) were similar to the densities used in the Yeager et al. (1999) study. However, mussels in that study were only 1–3 d old and <0.5 mm in size, making them significantly smaller than the mussels used in this experiment. Further, the volume of water in the experimental system of the Yeager et al. (1999) study was 0.001 L which may have caused an unrealistically large effect on juvenile mussels from *Corbicula*. The lack of mortality due to ingestion in my study was

not surprising because my mussels were too large to be ingested by *Corbicula* and a much greater volume of water was used in my experimental system.

My study is the first to experimentally examine the combined effects of *Corbicula* and food abundance on juvenile mussel growth and survival. The lack of *Corbicula* effects has several possible explanations. First, *Corbicula* may not compete for food with juvenile Cumberland Bean. Second, *Corbicula* densities higher than 910/m^2 may be required to induce competitive interactions with Cumberland Bean. This contrasts with the negative *Corbicula* effects shown by Yeager et al. (1999), but it is supported by Ferreira-Rodríguez and Pardo (2017), who found negative effects of *Corbicula* only at densities of 2000/m^2. It is more likely that the lack of *Corbicula* effects in my study was an artifact of the system design, but the system did not allow these various explanations to be evaluated conclusively. The deficiency of my system design was unanticipated, but it provides useful information for design of more appropriate experimental systems. Future studies of *Corbicula*-native mussel interactions should be conducted in a system in which food concentrations can be maintained independently among *Corbicula* treatments.

Table 2.1. Mussel feeding rations for three levels of experimental food abundance. Values are g dry mass (ml wet volume). Algal types are as follows: CS = *Chlorella sorokiniana*; PT = *Phaeodactylum tricornutum*; NA = *Nannochloropsis* spp.; TP = *Thalassiosira pseudonana*; SD = Shellfish Diet; see text for details about algal types. Total food density is g algal dry mass/L system volume, based on system volume of 100 L.

	Experimental Food Rations		
Algal type	Low	Medium	High
CS	0.1700 (2.125)	0.4488 (6.0)	0.8976 (12.0)
PT	0.1100 (2.125)	0.2904 (6.0)	0.5808 (12.0)
NA	0.0900 (0.75)	0.2320 (2.0)	0.4640 (4.0)
SD	0.0400 (0.375)	0.1100 (1.0)	0.2200 (2.0)
TP	0.1100 (1.5)	0.3008 (4.0)	0.6016 (8.0)
Total food density (g/L)	0.0052	0.0138	0.0276

Table 2.2. Water quality parameters during the experiment. Values are means (range). Sample size (N) refers to the number of measurements in each treatment combination.

Treatment (Food, *Corbicula*)	pH (N = 3–7)	NH_3-N (mg/L) (N = 3–7)	Temperature (°C) (N = 3–7)	DO (% saturation) (N = 3–7)	DO (mg/L) (N = 3–7)
Low, Control	8.42 (8.38–8.48)	0.04 (0.02–0.06)	26.0 (25.6–26.6)	99 (99–100)	8.0 (7.9–8.2)
Low, Low	8.47 (8.43–8.54)	0.03 (0.00–0.03)	26.9 (26.3–27.4)	99 (97–100)	8.1 (7.9–8.3)
Low, Medium	8.50 (8.48–8.53)	0.04 (0.02–0.07)	27.7 (27.3–28.0)	99 (98–100)	8.0 (7.9–8.1)
Low, High	8.45 (8.35–8.50)	0.05 (0.02–0.08)	27.6 (26.4–28.7)	99 (96–100)	8.0 (7.9–8.2)
Medium, Control	8.38 (8.34–8.45)	0.01 (0.06–0.14)	25.9 (25.6–26.6)	99 (97–100)	8.2 (8.1–8.2)
Medium, Low	8.43 (8.39–8.49)	0.09 (0.05–0.13)	26.8 (26.0–27.4)	100 (99–100)	8.2 (8.1–8.3)
Medium, Medium	8.41 (8.36–8.45)	0.06 (0.04–0.08)	27.6 (27.3–27.9)	99 (98–100)	8.1 (8.0–8.1)
Medium, High	8.42 (8.36–8.45)	0.07 (0.03–0.11)	27.5 (26.3– 28.6)	99 (94–100)	8.0 (7.7–8.2)
High, Control	8.30 (8.24–8.41)	0.08 (0.06–0.09)	26.0 (25.7–26.6)	98 (97–100)	8.1 (8.1–8.2)
High, Low	8.39 (8.38–8.40)	0.09 (0.05–0.13)	26.9 (26.3–27.3)	99 (96–100)	8.2 (7.9–8.3)
High, Medium	8.35 (8.28–8.44)	0.06 (0.03–0.09)	27.6 (27.4–27.7)	100 (99–100)	8.1 (8.1–8.2)
High, High	8.35 (8.29–8.41)	0.09 (0.05–0.16)	27.5 (26.0–28.5)	97 (95–100)	7.9 (7.7–8.2)

Table 2.3. Results of a two factor ANOVA model evaluating the effects of food abundance and *Corbicula* biomass on mussel growth.

Factor	Sum of Squares	*F*	*P*	df
Food	0.00038	81.1	<0.0001	2
Corbicula	0.00000	0.8	0.51	3
Food × *Corbicula*	0.00000	0.1	0.98	6
Total	0.00042	-	-	23

Table 2.4. Results of Tukey HSD tests evaluating the pairwise comparisons in mussel growth among *Corbicula* levels.

Contrast	Estimate	SE	*P*	df
High – Low	0.009	0.008	<0.001	12
High – Medium	0.007	0.008	<0.001	12
Low – Medium	-0.002	0.008	0.026	12

Table 2.5. Results of a two factor ANOVA model evaluating the effects of food abundance and *Corbicula* biomass on mussel survival.

Factor	Sum of Squares	*F*	*P*	df
Food	0.02134	1.2	0.34	2
Corbicula	0.07684	2.9	0.08	3
Food × *Corbicula*	0.08158	1.5	0.25	6
Total	0.28701	-	-	23

Table 2.6. Results of Tukey HSD tests evaluating the pairwise comparisons in mussel survival among *Corbicula* levels.

Contrast	Estimate	SE	*P*	df
Control – High	0.0376	0.0546	0.8995	12
Control – Low	-0.0912	0.0546	0.3791	12
Control – Medium	-0.0912	0.0546	0.3791	12
High – Low	-0.1288	0.0546	0.1387	12
High – Medium	-0.1288	0.0546	0.1387	12
Low – Medium	0.0000	0.0546	1.0000	12

Table 2.7. Results of a two factor ANOVA model evaluating the effects of food abundance and *Corbicula* biomass on FPOM.

Factor	Sum of Squares	*F*	*P*	df
Food	40.63	26.6	<0.0001	2
Corbicula	3.98	1.3	0.21	3
Food × *Corbicula*	9.81	1.6	0.12	6
Total	63.59	-	-	23

Figure 2.1. AHAB system used to hold fantail darter infested with glochidia.

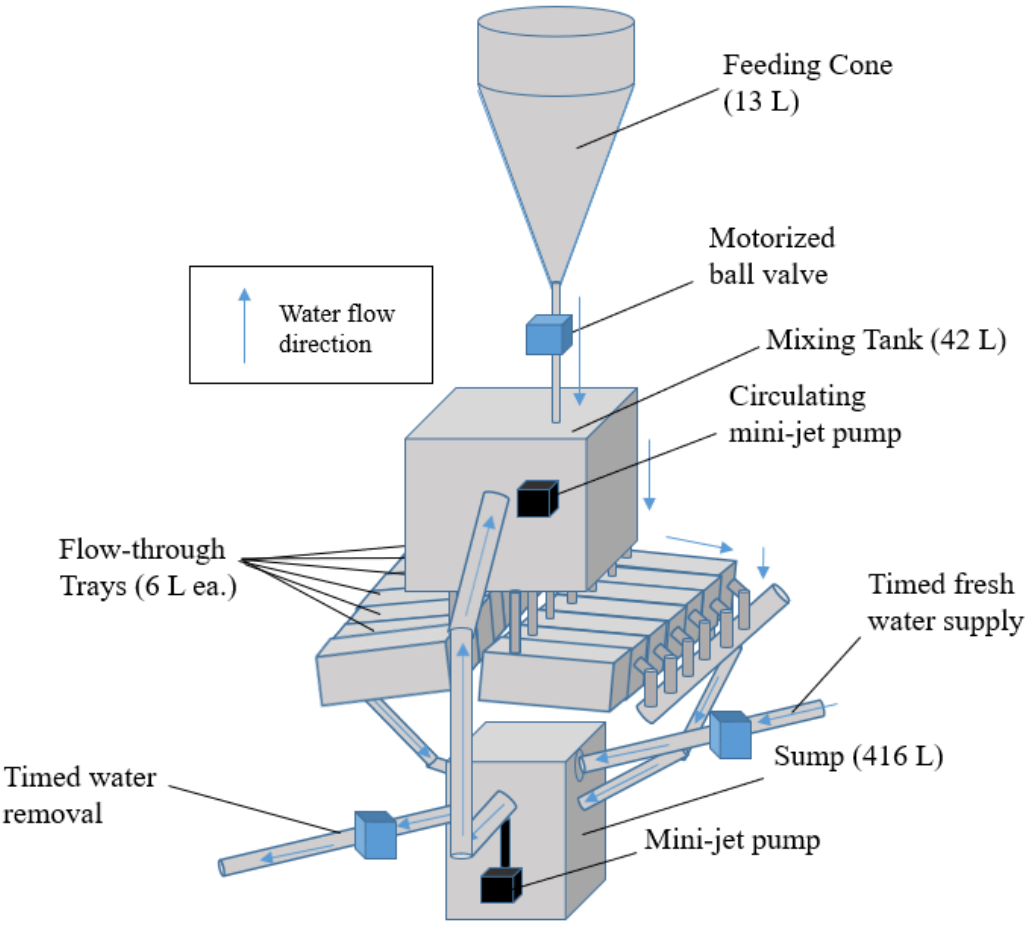

Figure 2.2. Design of RAS used during juvenile mussel rearing.

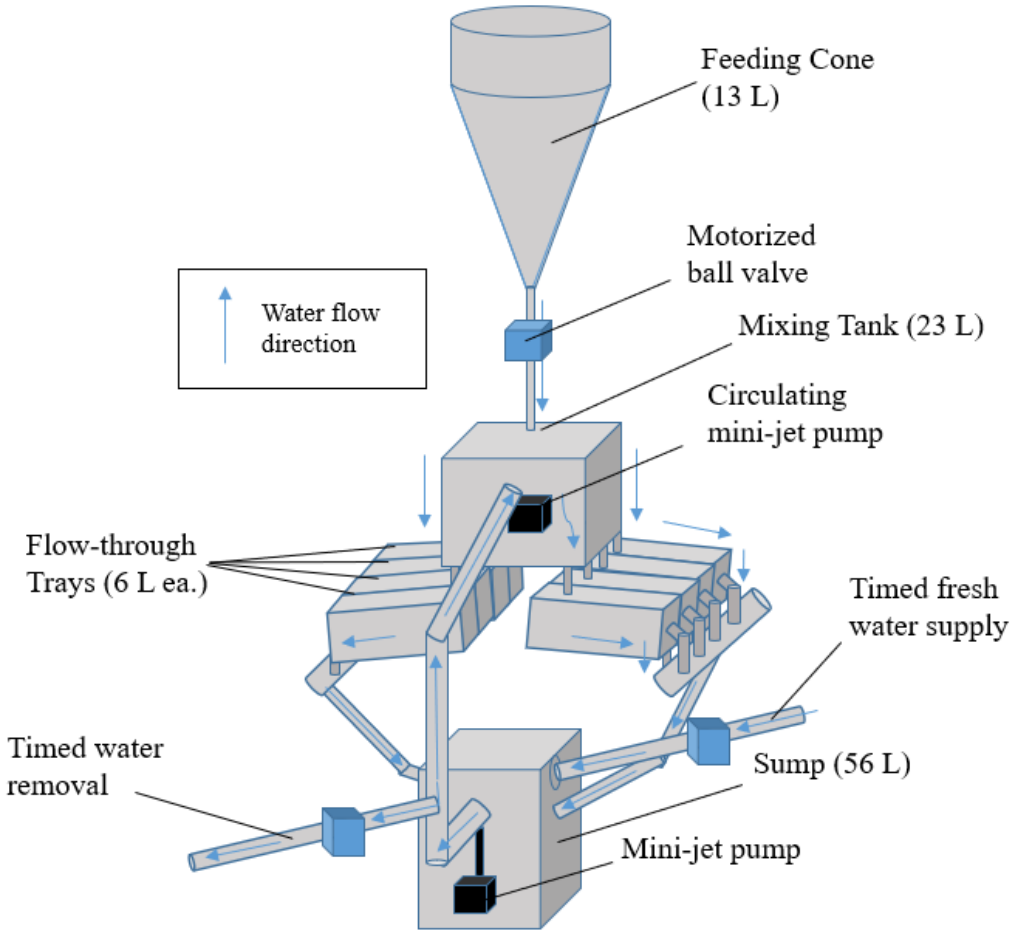

Figure 2.3. Design of the experimental RAS apparatus.

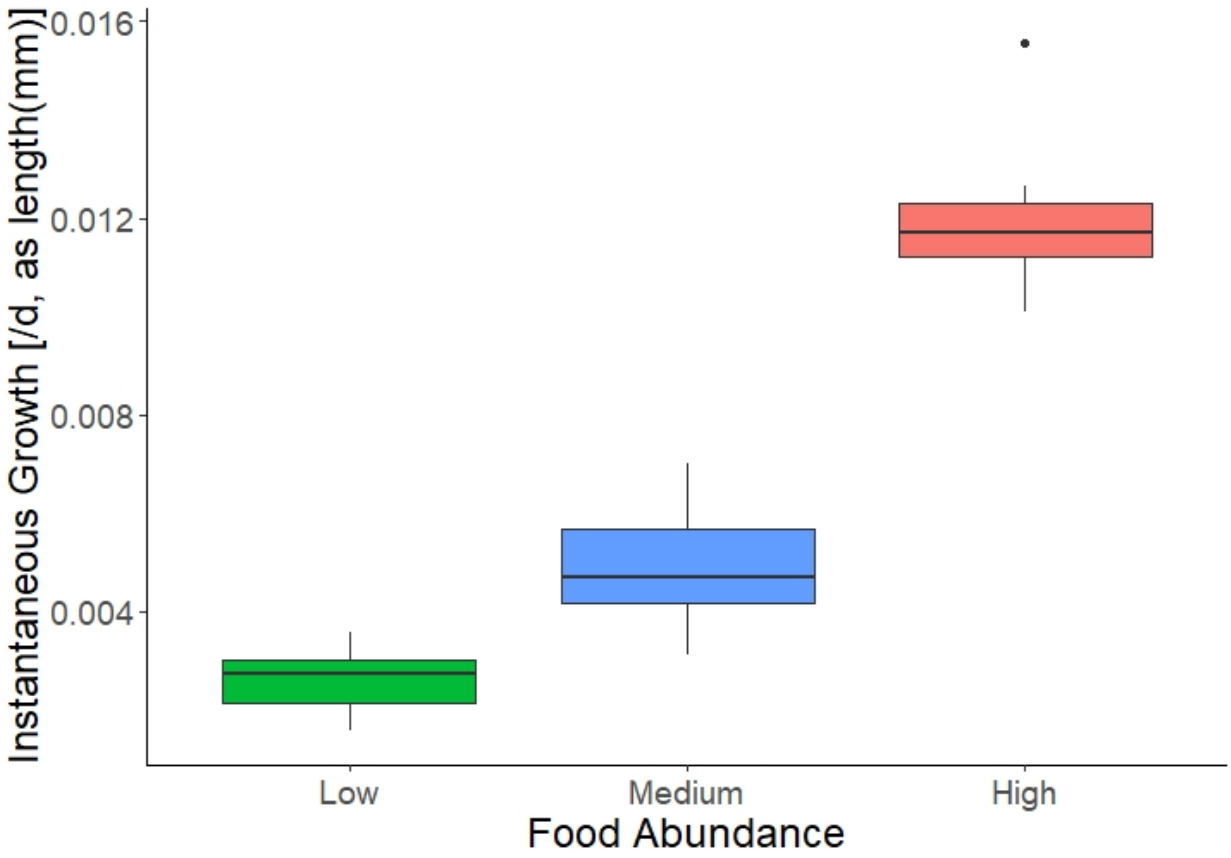

Figure 2.4. Instantaneous growth [/d, as length (mm)] response in each of the three levels of experimental mussel food abundance.

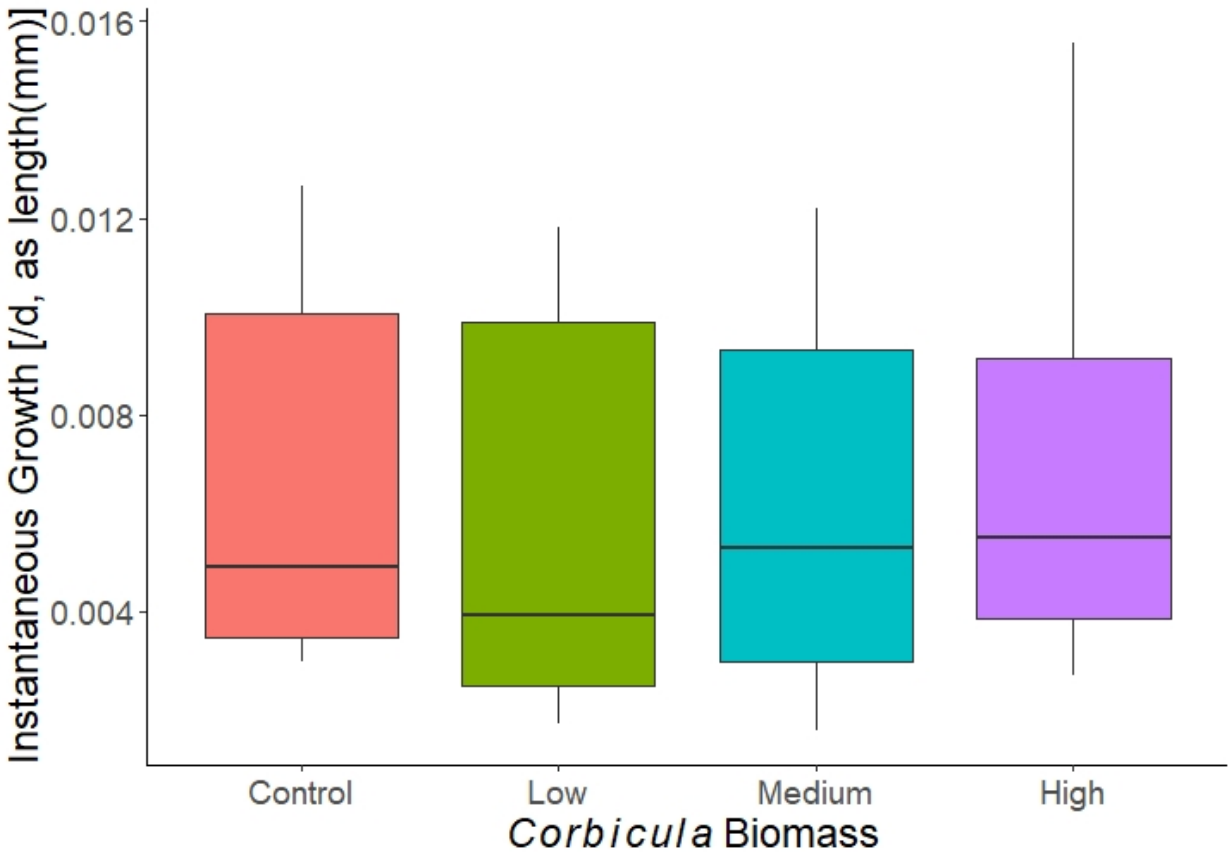

Figure 2.5. Instantaneous growth [/d, as length (mm)] in each of the four levels of *Corbicula* biomass.

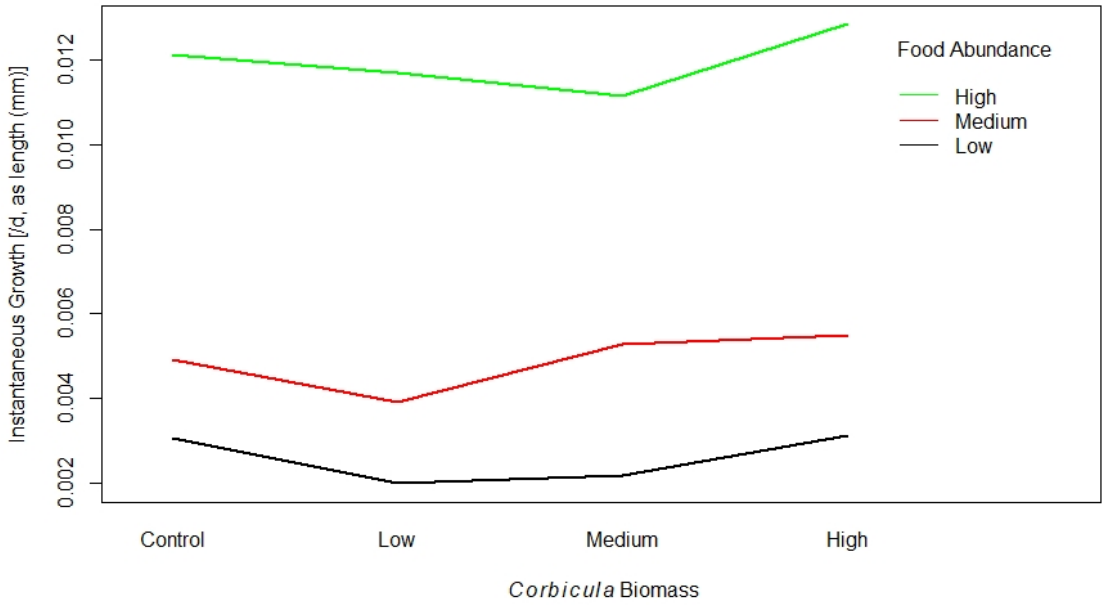

Figure 2.6. Mean instantaneous growth [/d, as length (mm)] in relation to food abundance and *Corbicula* biomass.

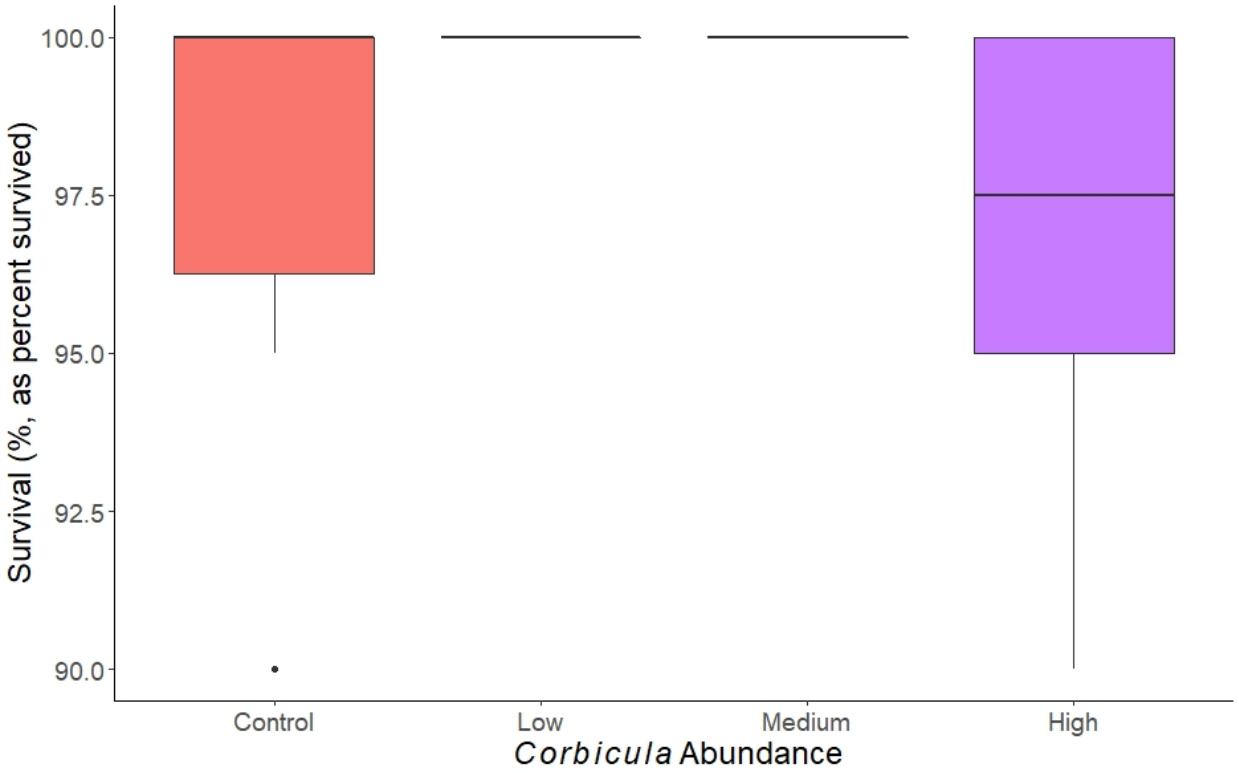

Figure 2.7. Survival (%, as percent survived) in each of the four levels of *Corbicula* biomass.

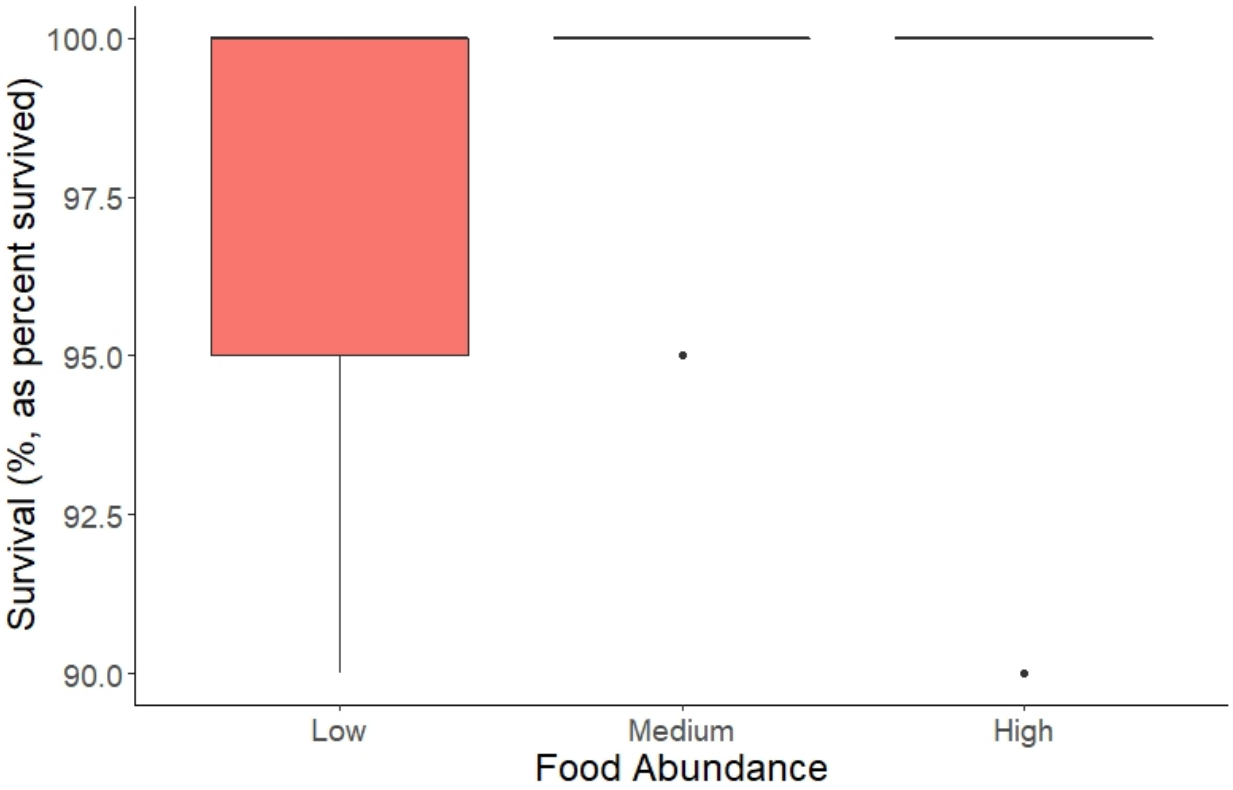

Figure 2.8. Survival (%, as percent survived) in each of the three levels of experimental mussel food abundance.

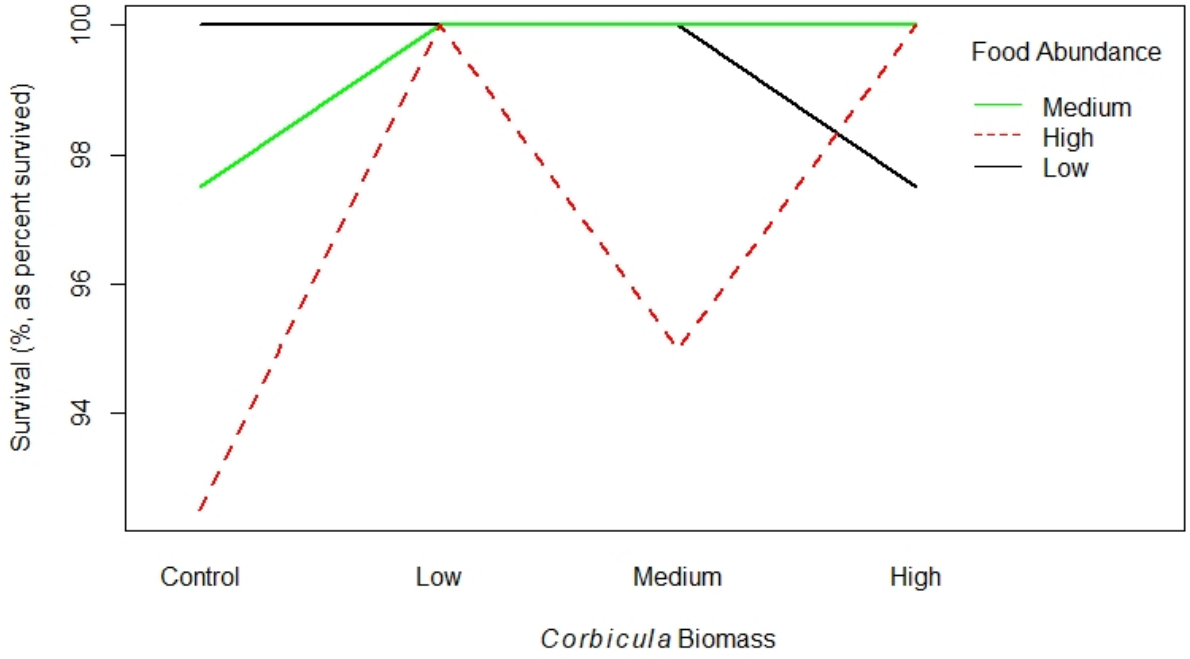

Figure 2.9. Mean survival (%, as percent survived) in relation to food abundance and *Corbicula* biomass.

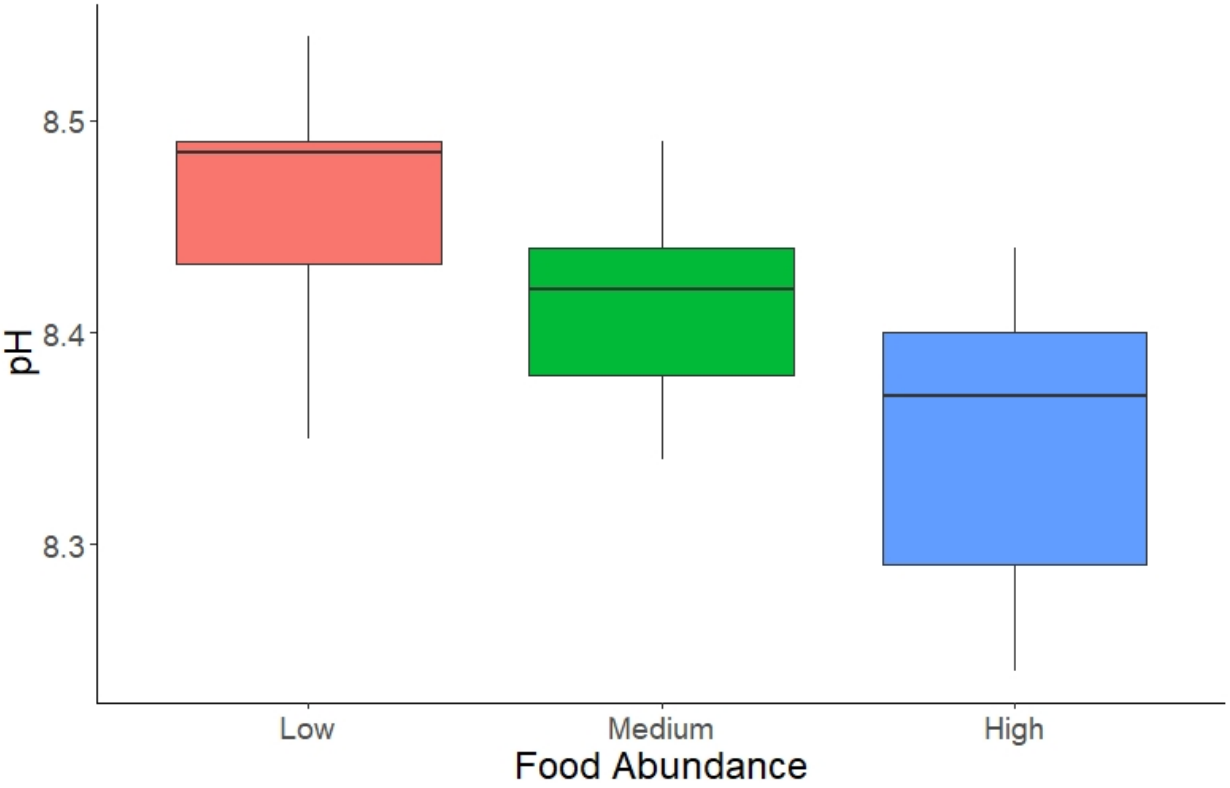

Figure 2.10. pH in each of the three levels of experimental mussel food abundance.

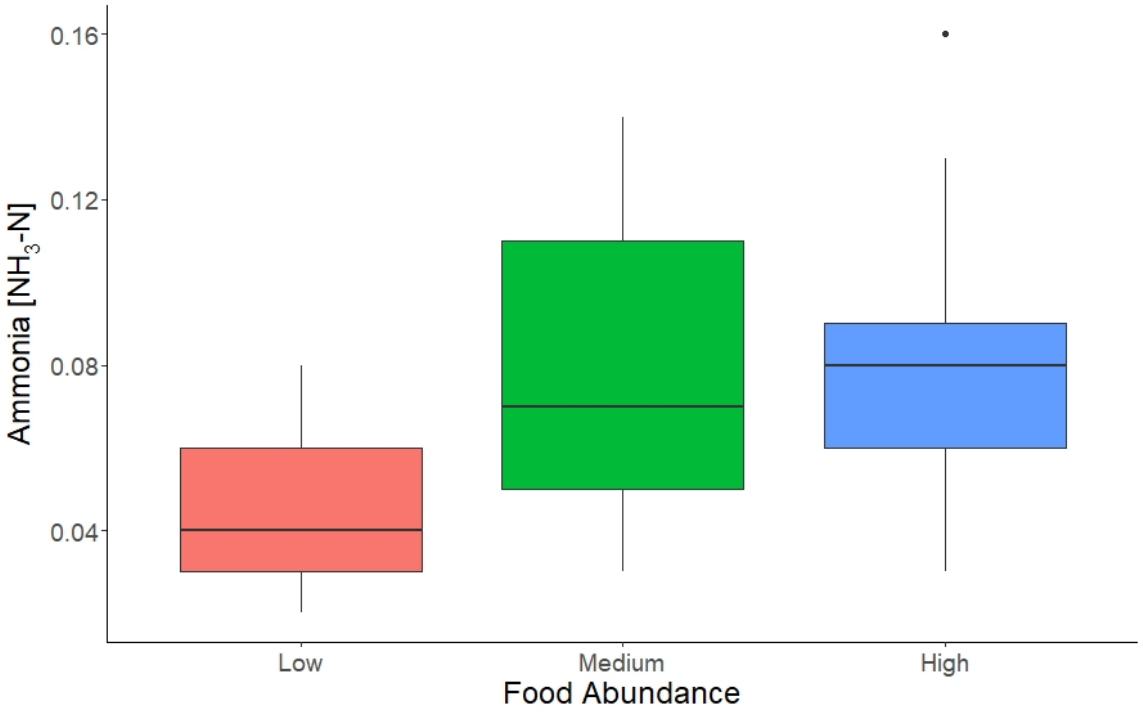

Figure 2.11. Ammonia (mg/L, as NH_3-N) in each of the three levels of experimental mussel food abundance.

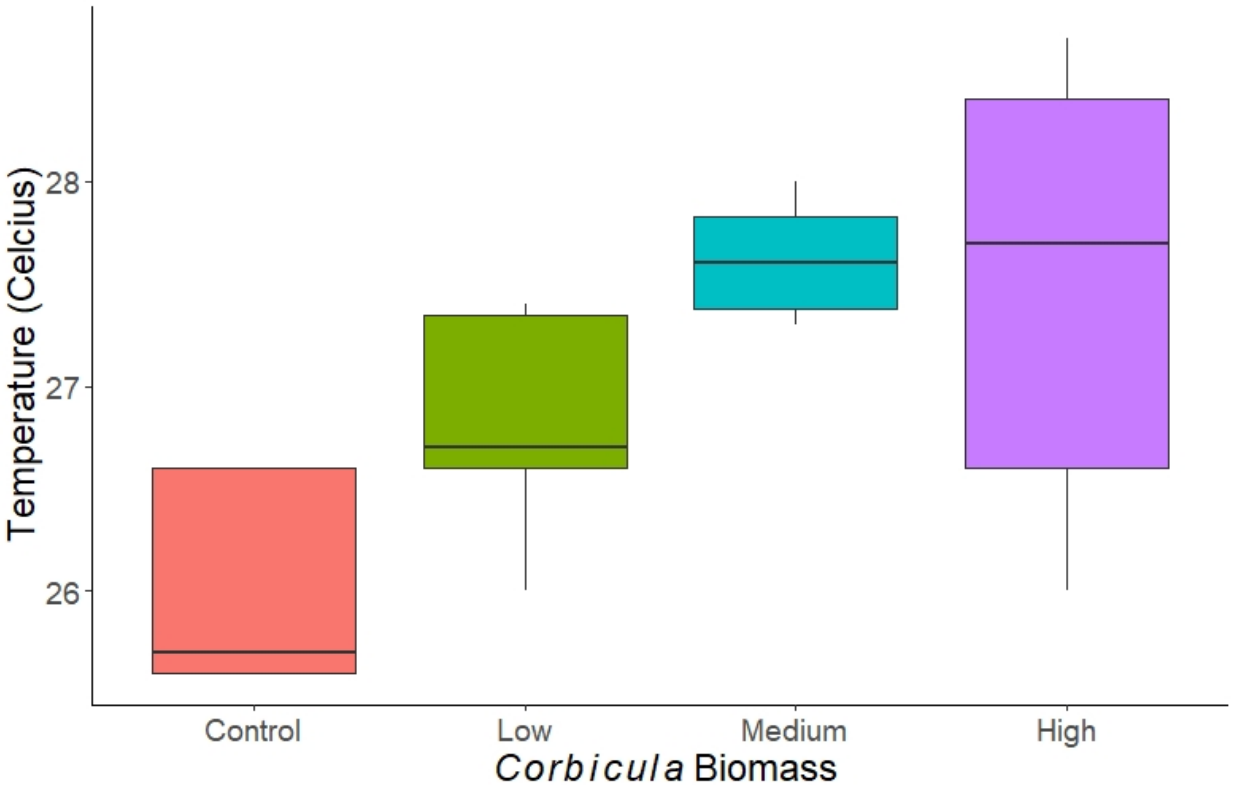

Figure 2.12. Water temperature in each of the four levels of *Corbicula* biomass.

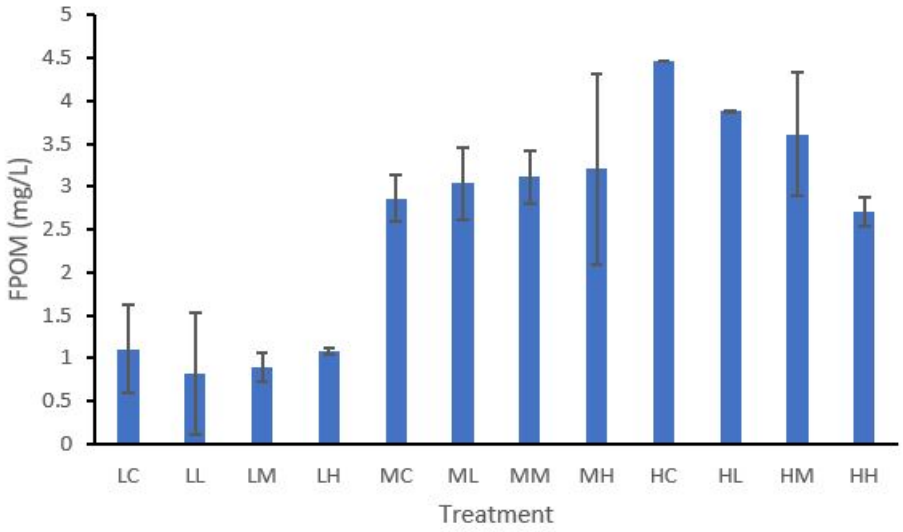

Figure 2.13. Mean fine particulate organic matter (FPOM) in relation to food abundance and *Corbicula* biomass. Each two letter code indicates a treatment combination: first letter is food treatment where L = low, M = medium, and H = high, and the second letter is *Corbicula* treatment where C = control, L = low, M = medium, and H = high. Error bars represent SD.

CHAPTER 3. THE EFFECT OF FOOD ABUNDANCE AND *CORBICULA* BIOMASS ON GROWTH AND SURVIVAL OF JUVENILE MUSSELS IN A RECIRCULATING AQUACULTURE SYSTEM, PART II.

INTRODUCTION

The previous chapter was an attempt to determine the effects of food abundance, *Corbicula* biomass, and the interaction of food abundance and *Corbicula* biomass on the growth and survival of juvenile freshwater mussels. However, the system design for the experiment reported in Chapter 2 did not allow food abundance to be maintained independently in individual *Corbicula* treatments. Therefore, I was able to evaluate only the effect of food abundance on mussel survival and growth, and I was not able to evaluate the effects of *Corbicula* or the *Corbicula* × food abundance interaction.

In this chapter, I modified the experimental design to allow food abundance to be maintained independently among *Corbicula* treatments. Due to space limitations in the laboratory, this necessitated conducting two successive experiments, one at high food abundance and another at low food abundance, each with three levels of *Corbicula* biomass. I used the same study species, the Cumberland Bean. As in the Chapter 2, I tested the following hypotheses:

(1) Increased food abundance has a positive effect on growth and survival of juvenile Cumberland Bean.

(2) Increased *Corbicula* biomass has a negative effect on growth and survival of juvenile Cumberland Bean.

(3) The interaction between *Corbicula* biomass and food abundance has a significant effect on growth and survival of juvenile Cumberland Bean such

that the strength of competition by *Corbicula* increases with decreasing food abundance.

METHODS

I conducted all aspects of these experiments following methods described in Chapter 2, with the following exceptions.

Instead of conducting a single experiment in which food abundance and *Corbicula* biomass were manipulated simultaneously as in Chapter 2, I conducted two successive experiments. This was necessary because only three experimental RAS were available. The first experiment was conducted under conditions of high food abundance with three levels of *Corbicula* biomass. The second experiment was conducted under conditions of low food abundance with the same three levels of *Corbicula* biomass. In each experiment, all three RAS received the same food ration, but each RAS received a different level of *Corbicula* biomass. This allowed me to evaluate the effect of *Corbicula* biomass at two different levels of food abundance. Both experiments were run for 14 d. The high food experiment was conducted from 26 July 2019 to 9 August 2019, and the low food experiment was conducted from 13 August 2019 to 27 August 2019.

I chose two experimental food abundances based on the feeding rations used in previous experiments (see Chapter 1 and Chapter 2; Table 3.1). The low food level in this experiment was 19% lower than the low food level in Chapter 2, and the high food level was 49% lower than the medium food level in Chapter 2. I chose these two food levels

because they represented the high and low extreme of food abundance in streams (see Chapter 1; Figure 3.1).

I chose three levels of *Corbicula* biomass, used as treatments in each RAS, to capture the range of *Corbicula* densities in the wild (e.g. Gardner et al. 1976; Stites et al. 1995; Miller and Payne 1998; Karatayev et al. 2003). Each RAS was randomly assigned one *Corbicula* treatment: 1) 0 g *Corbicula*, 2) 156.5 g *Corbicula*, or 3) 2,147 g *Corbicula* (blotted wet mass). These treatments corresponded to about 0, 73, and 1000 individuals/m^2, based on the average mass of one *Corbicula* (3.5 g). These treatments also corresponded summarily to the low and high *Corbicula* treatments in Chapter 2, and the medium treatment in this experiment was the approximate midpoint of the two medium treatments in Chapter 2. In the two RAS that received *Corbicula*, I distributed 75% of the specified number of *Corbicula* biomass equally among the 8 trays and placed the remaining 25% of *Corbicula* in the mixing tank. I placed *Corbicula* in the mixing tank to mimic any down-stream effect *Corbicula* may have on mussels in the wild. I distributed *Corbicula* biomass amongst the trays in this way because my previous experiment showed that *Corbicula* has a system-wide effect on food abundance (see Chapter 2; Discussion).

In the high food experiment, Cumberland Bean were about 3 months old and had a mean length (L_i) of 3.8 mm ± 0.8 (SD) and mean individual mass of (M_i) of 0.011 g ± 0.003 (SD) at the start of this experiment. In the low food experiment, Cumberland Bean were about 3.5 months old and had a mean length (L_i) of 4.9 mm ± 1.0 (SD) and mean individual mass of (M_i) of 0.027 g ± 0.005 (SD) at the start of this experiment.

I monitored water quality as described in Chapter 2. Physical conditions were similar in the two experiments. Mean water temperature was 24.9°C (range = 21.9 to 26.4°C) in the high food experiment and 25.9°C (range 24.6 to 27.1°C) in the low food experiment. Water quality showed no evidence of adverse effects during either experiment (Table 3.2; Table 3.3). There were no differences in pH, temperature, and DO among the *Corbicula* treatments (high food abundance, $F_{2,9\text{-}21}$ = <0.01–2.46; P = 0.11–0.99; low food abundance, $F_{2,27\text{-}33}$ = 0.04–1.62; P = 0.21–0.96). Ammonia was significantly higher in both experiments in the high *Corbicula* treatment than the other two treatments (high food, $F_{2,21}$ = 6.73; P < 0.001; low food, $F_{2,20}$ = 6.13; P < 0.01; Figures 3.2 and 3.3).

I characterized food abundance in experimental trays by examining Fine particulate organic matter (FPOM; mg/L) as described in Chapter 2. In this experiment, I also estimated algal cell density as an additional measure of food availability. To measure algal cell density, I collected 500 ml water samples from trays containing mussels during the experiments. I loaded two counting chambers of a hemocytometer (Fisher Scientific; Nuebauer Ruled; 0.1 mm depth) each with 10 µl subsamples from the 500 ml samples using a glass pipette. I used a light microscope at 10× magnification to count the number of algal cells in 5, 1 mm^2 grid cells located in each corner and in the center of each counting chamber. Within each grid cell, I counted algal cells in a top-to-bottom zig-zag pattern starting in the top left-hand corner. I estimated cell density (cells/ml) as: total cells counted from all 5 grid cells × 10,000/5 grid cells.

DATA ANALYSIS

I expressed mussel growth as instantaneous growth [ln (mean final mass/mean initial mass)/experiment duration in d; hereafter growth]. For subsequent analysis of growth, I used the mass of all mussels in each tray. I calculated proportional survival in each tray as the number of surviving individuals in the tray/the initial number of mussels in the tray. I arcsine transformed survival for further analysis.

3.1.1 Food Abundance

I evaluated the relationship between one factor, *Corbicula* biomass, and two response variables, FPOM and cell density, using a one-way ANOVA with *Corbicula* treatment as a categorical variable. I analyzed each response variable and each experiment separately.

3.1.2 Survival

Survival did not meet the assumption of normality in either food experiment (Shapiro-Wilk Test, high food, $W = 0.49$, $P < 0.001$; low food, $W = 0.32$, $P < 0.001$). Survival did not meet the assumption of homogeneity of variance in the high food experiment (Levene's Test, $F = 32.3$, $P < 0.001$) but did in the low food experiment (Levene's Test, $F = 1$, $P = 0.41$). I evaluated the relationship between *Corbicula* biomass and survival (arcsine transformed), using a Kruskal-Wallace Rank Sum Test with *Corbicula* treatment as a categorical variable. I used this procedure because it is robust to violations of normality. I analyzed survival from each experiment separately.

3.1.3 Growth

Growth met the assumption of normality in the high food experiment (Shapiro-Wilk Test, high food, $W = 0.90$, $P = 0.15$) but not in the low food experiment (Shapiro-Wilk Test, $W = 0.81$, $P = 0.01$). Growth met the assumption of homogeneity of variance in both experiments (Levene's Test, high food, $F = 0.41$, $P = 0.67$; low food, $F = 0.53$, $P = 0.61$). For analysis of growth in the high food experiment, I used a one-way weighted least squares (WLS) model to evaluate the relationship between one factor, *Corbicula* biomass, and one response variable, growth, in the high food experiment with *Corbicula* treatment as a categorical variable. I used a power transformation of variance covariance to reduce heteroskedasticity. I validated the WLS model by visually analyzing residual plots. I then used estimated marginal means produced by the WLS model to analyze pairwise differences in growth between *Corbicula* levels. For analysis of growth in the low food experiment, I evaluated the relationship between *Corbicula* biomass and growth, using a Kruskal-Wallis Rank Sum Test with *Corbicula* treatment as a categorical variable. I then evaluated pairwise differences in treatments using a Pairwise Wilcoxon Rank Sum Test which is robust to violations of the assumption of normality.

For further analysis of growth, I combined data from both experiments. I then used a weighted generalized least squares (WLS) regression including both the main effect terms (*Corbicula* and food) and the interaction term (*Corbicula* × food) to evaluate the relationship. I used this procedure because it is robust to non-constant variance. To use this procedure, I first analyzed residual autocorrelation in the data by using an ordinary least squares (OLS) regression which included both the main effects (*Corbicula* and food) and the interaction term (*Corbicula* × food) followed by a Durbin-Watson

Test. Residuals were not correlated (Durbin–Watson Test, lag = 1, $D–W = 2.22$, $P = 0.57$). I used an exponential transformation of variance covariance to reduce heteroskedasticity in the WLS regression. I validated the WLS model by visually analyzing residual plots.

I analyzed all response variables separately, and I used a 0.05 significance level (α) to determine significance. I used RStudio version 3.5.1 (RStudio Team 2018) for all analyses.

RESULTS

3.1.4 Food Abundance

Food abundance was negatively affected by *Corbicula* biomass in both experiments such that treatments with increased *Corbicula* had decreased FPOM (high food abundance, Figure 3.4; $F_{2,15} = 92.39$; $P < 0.001$; low food abundance, Figure 3.5; $F_{2,9} = 14$; $P = 0.002$) and cell density (high food abundance, Figure 3.6; $F_{2,33} = 135.4$; $P < 0.001$; low food abundance, Figure 3.7; $F_{2,25} = 31.4$; $P < 0.001$).

3.1.5 Survival

Cumberland Bean survival was high in both experiments (low food abundance, mean = 98.7 ± 0.0 SD, n = 12; high food abundance, mean = 99.6% ± 0.01 SD), and there were no differences in survival among *Corbicula* treatments in either experiment (Figures 3.8 and 3.9; high food abundance, $H = 4.4$; df = 2; $P = 0.11$; low food abundance, $H = 2$; df = 2; $P = 0.36$). The greatest variation in survival among trays occurred in the high

Corbicula treatment from the high food experiment in which two trays from this treatment had 90% and 95% survival, respectively. All other trays had 100% survival.

3.1.6 Growth

Corbicula was not significant in explaining differences among Cumberland Bean growth in the high food experiment (Figure 3.10; $F_{2,17} = 1.91$; $P = 0.10$), and growth was not significantly different for any of the pairwise comparisons (Figure 3.10; $P = 0.20$–0.96). Growth in the low food experiment was significantly different among the *Corbicula* levels, and growth was higher in treatments with greater *Corbicula* biomass (Figure 3.11; $H = 9.85$; df = 2, $P = 0.007$). The highest growth from the low food experiment was in the high *Corbicula* level (mean = 0.0133/d, as mm ± 0.0010 SD, n = 4).

The main effect terms (*Corbicula* and food) and the interaction term (*Corbicula* × food) all were significant in the two factor WLS model for growth (Table 3.4). This indicates that the relationship between growth and *Corbicula* biomass depends on food abundance such that *Corbicula* has a strong positive effect on mussel growth when food abundance is low but no effect when food abundance is high (Figure 3.12; Tables 3.5–3.6). Based on the fitted versus standardized residual plot, the residual variance was constant across the fitted values for growth indicating that the model was a good fit.

DISCUSSION

I found no evidence of negative effects of *Corbicula* on juvenile mussel survival. Survival was generally high in all treatments and was not related to *Corbicula* biomass or food abundance. High survival in this study was surprising because Yeager et al. (1999) found 100% mortality in treatments with >1250/m^2. However, the juvenile mussels in that study were only 1–3 d old and <0.5 mm in size, making them vulnerable to ingestion by *Corbicula*. The mussels in my experiment were > 2 mm which is probably too large for ingestion by *Corbicula*. Growth at the low food abundance level was substantially lower than at high food abundance, particularly for the *Corbicula* control group. This suggests that food was severely limited in this treatment, which may be expected to decrease long-term survival. Longer experiments are necessary to more fully evaluate potential effects of *Corbicula* on mussel survival, but I found no evidence of short-term negative effects.

I found only marginal evidence of negative effects of *Corbicula* on juvenile mussel growth, despite *Corbicula* having a negative effect on the amount of available food (i.e. FPOM and cell density). Growth was significantly related to *Corbicula* biomass, but the significant interaction term showed that the effect of *Corbicula* depended on food abundance. The interaction term made it difficult to evaluate the main effects of food abundance or *Corbicula* in the full model, but separate analysis of each experiment yielded surprising results. Overall, mean growth at high food abundance was nearly 5× higher than at low food abundance. At high food abundance, there was an apparent trend of slightly lower growth at medium and high *Corbicula* abundance, but this effect was not statistically significant. At low food abundance, *Corbicula* was a

significant factor, but the effect was positive, and mean growth in the high *Corbicula* treatment (1000/m^2) was nearly 3× higher than in the control treatment with no *Corbicula*. Furthermore, relative to the control treatment there was a detectable, positive effect of *Corbicula* on growth even at the relatively low *Corbicula* density represented by the medium *Corbicula* treatment (73/m^2).

The positive effect of *Corbicula* that I found is contrary to previous studies that show reduction of mussel growth with increasing *Corbicula* abundance. Yeager et al (1999) found a sharp decrease in newly transformed juvenile mussel growth with increasing *Corbicula* density, including a 10× lower growth rate relative to controls at 1250 *Corbicula*/m^2, which was similar to my high *Corbicula* treatment. The vastly different response seen by Yeager et al. (1999) could indicate that newly-transformed mussels are more vulnerable to food competition or other negative effects of *Corbicula*. However, the experimental chambers used by Yeager et al. (1999) contained only 0.001 L of water, which could have unrealistically increased the potential for food competition, and the potential confounding effects of high mortality in that study were not examined. Ferreira-Rodríguez et al. (2018) found that growth and energy stores of adult mussels were unaffected by *Corbicula* except at high density (2000/m^2). This density is twice my highest *Corbicula* density, and it is possible that higher densities are needed to induce food competition or other negative effects of *Corbicula*. However, my study is the first to show positive effects of *Corbicula* on mussel growth.

The mechanism by which *Corbicula* may positively influence mussel growth is unknown, but my results show that food abundance modulates this effect. The high mussel growth and lack of a *Corbicula* effect at high food abundance indicates that food

was not limiting in that treatment and sufficient food existed to support mussel growth regardless of *Corbicula* biomass. Conversely, the lower overall growth at low food abundance shows that food was sharply limited in this treatment. I was unable to measure *Corbicula* growth during the experiment because of high *Corbicula* mortality. Therefore, it is unknown if food also was limiting for *Corbicula*. Regardless, the presence of high *Corbicula* biomass appeared to facilitate higher mussel growth when food was limiting.

Facilitative feeding interactions are documented or proposed among other bivalves, including freshwater mussels and marine bivalves. Proposed mechanisms of facilitative feeding interactions include deposition of feces, which are fed upon by other organisms; resuspension of biodeposited food; and decreased metabolic costs due to increased resource availability (Vaughn and Spooner 2009). Biodeposition of feces and pseudofeces by bivalves, including *Corbicula*, can substantially increase organic matter and nutrient content in the sediment, which serves as a food source for other organisms (Jordan and Valiela 1982; Kautsky and Evans 1987; Hakenkamp and Palmer 1999). Furthermore, bivalve feces and pseudofeces often contain live, undigested algal cells (Vaughn et al. 2008). Feces and pseudofeces produced by *Corbicula* may have represented a direct food source, and this material may have stimulated increased abundance of bacteria, which can be an important component of mussel diets (Nichols and Garling 2000). I did not measure the filtration rate of *Corbicula* in my study, but *Corbicula* typically has higher mass-specific filtration than mussels (reviewed by Strayer 1999). Because of their larger size and high filtration rate, *Corbicula* may have efficiently concentrated a scarce food resource and released these nutrients in a form that was more readily available to the juvenile mussels than in the control treatment.

The extent to which my results are applicable to the wild is unclear. The flow rate through my experimental trays was not sufficient to mobilize and flush *Corbicula* feces and pseudofeces from the trays, and this material accumulated on the bottom in close proximity to the mussels. In streams, feces and pseudofeces probably are washed downstream at a greater rate, reducing their availability to mussels. In addition, a large number of other organisms in streams probably feed on bivalve feces and pseudofeces (e.g., fishes, crayfishes, aquatic insects), which may further reduce their availability to mussels.

Other factors may modulate the effects of *Corbicula* on mussels. Ferreira-Rodríguez and Pardo (2017) found that the presence of *Corbicula* negatively affected food acquisition of the mussel *Unio delphinus* at 20°C, but it had no effect at 24 or 28°C. *Corbicula* is more physiologically efficient at 18-25°C than at higher temperatures (Xiao et al. 2014), which may explain the temperature-dependent effects observed by Ferreira-Rodríguez and Pardo (2017). My experiment was conducted near the upper end of the optimal temperature range for *Corbicula* (~25°C). More efficient feeding and assimilation by *Corbicula* at lower temperatures could result in production of less nutrient-rich feces and pseudofeces, which, in turn, could increase the potential for food competition with mussels. The role of temperature in modulating effects of *Corbicula* is supported by the occurrence of enigmatic mussel declines primarily in streams with mean summer water temperature <24°C (Haag et al. 2019).

My study showed that food abundance modulates interactions between *Corbicula* and juvenile mussels. The lack of a negative effect on growth and survival of juvenile mussels under conditions of high food abundance was not surprising because suspended

food was adequate to support growth and survival regardless of *Corbicula* biomass. The findings from low food abundance were contrary to the expected juvenile mussel growth response to greater *Corbicula* biomass. This probably occurred as a result of mussels consuming deposited pseudofeces and feces produced by *Corbicula*; thus, future studies should evaluate a more realistic environment with regard to transport and availability of feces and pseudofeces. Future studies should also evaluate the role of temperature in modulating food interactions between *Corbicula* and freshwater mussels. The growth response of juvenile mussels to *Corbicula* may be context-dependent and strongly correlated with a set of physical factors, which includes food abundance and water temperature.

Table 3.1. Mussel feeding rations for each level of food abundance. Values are g dry mass (ml wet volume). Algal types are as follows: CS = *Chlorella sorokiniana*; PT = *Phaeodactylum tricornutum*; NA = *Nannochloropsis* spp.; TP = *Thalassiosira pseudonana*; SD = Shellfish Diet; see text for details about algal types. Total food density is g algal dry mass/L system volume, based on system volume of 100 L.

	Experimental Food Abundances	
Algal type	High Food abundance	Low Food abundance
CS	0.3007 (4.0)	0.1417 (1.6)
PT	0.1946 (4.0)	0.0917 (1.6)
NA	0.1554 (1.35)	0.0733 (0.535)
SD	0.0737 (0.68)	0.0347 (0.255)
TP	0.2015 (2.70)	0.0950 (1.10)
Food Density (g/L)	0.0093	0.0044

Table 3.2. Water quality parameters during the high food abundance experiment. Values are means (range). Sample size (N) refers to the number of measurements in each treatment combination.

Corbicula Treatment	pH (N = 8)	NH_3-N (mg/L) (N = 8)	Temperature (°C) (N = 8)	DO (% saturation) (N = 4)	DO (mg/L) (N = 4)
Control (0 g)	8.28 (8.08–8.48)	0.03 (0.00–0.06)	24.8 (21.9–26.4)	97 (96–97)	8.2 (8.1–8.3)
Medium (147 g)	8.29 (8.09–8.45)	0.03 (0.01–0.05)	24.9 (22.2–26.4)	99 (98–100)	8.4 (8.2–8.5)
High (2,157 g)	8.16 (8.04–8.30)	0.07 (0.03–0.14)	24.9 (22.0–26.4)	98 (95–100)	8.2 (7.9–8.4)

Table 3.3. Water quality parameters during the low food abundance experiment. Values are means (range). Sample size (N) refers to the number of measurements in each treatment combination.

Treatment	pH (N = 10)	NH_3-N (mg/L) (N = 8)	Temperature (°C) (N = 12)	DO (% saturation) (N = 12)	DO (mg/L) (N = 12)
Control (0 g)	8.23 (8.14–8.33)	0.02 (0.01–0.04)	25.8 (24.7–27.0)	96 (95–98)	8.0 (7.8–8.2)
Medium (147 g)	8.26 (8.18–8.33)	0.03 (0.02–0.05)	25.9 (24.6–27.1)	97 (95–100)	8.0 (7.8–8.3)
High (2,157 g)	8.21 (8.09–8.31)	0.05 (0.03–0.07)	25.9 (24.7–27.0)	96 (94–98)	8.0 (7.7–8.1)

Table 3.4. The relationship between two factors, food abundance and *Corbicula* biomass, and one response variable, growth, using a two factor weighted least squares model including both main effect terms (*Corbicula* and food) and the interaction term (*Corbicula* × food).

Factor	*F*	*P*	df
Intercept	469.0	<0.0001	1
Food	418.5	<0.0001	1
Corbicula	299.3	<0.0001	2
Corbicula × Food	12.4	<0.0001	2

Table 3.5. Results of pairwise contrasts for growth among the two food abundance levels within each of the three *Corbicula* biomass levels.

Corbicula Level	Contrast	Estimate	SE	df	*T* ratio	*P*
Control	High Food – Low Food	0.030	0.003	6.87	10.42	<0.0001
Medium	High Food – Low Food	0.021	0.002	11.11	11.96	<0.0001
High	High Food – Low Food	0.013	0.002	10.97	6.62	<0.0001

Table 3.6. Results of pairwise contrasts for growth among each of the three *Corbicula* biomass levels within each of the two food levels.

Food Level	Contrast	Estimate	SE	Df	*T* ratio	*P*
Low	Control *Corbicula* – High *Corbicula*	-0.013	0.001	15.32	22.82	<0.0001
Low	Control *Corbicula* – Medium *Corbicula*	-0.004	0.000	6.87	13.54	<0.0001
Low	High *Corbicula* – Medium *Corbicula*	0.009	0.001	14.97	15.59	<0.0001
High	Control *Corbicula* – High *Corbicula*	0.004	0.003	7.66	1.23	0.47
High	Control *Corbicula* – Medium *Corbicula*	0.005	0.003	7.72	1.48	0.35
High	High *Corbicula* – Medium *Corbicula*	0.001	0.003	10.39	0.29	0.95

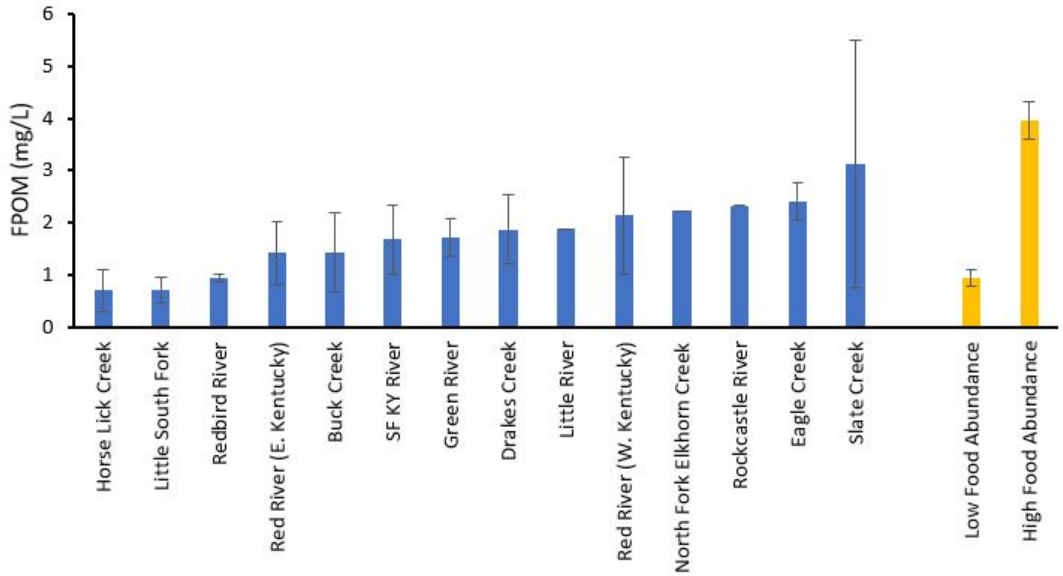

Figure 3.1. Mean fine particulate organic matter (FPOM) in 14 Kentucky streams and the two levels of experimental food abundance. Error bars are SD.

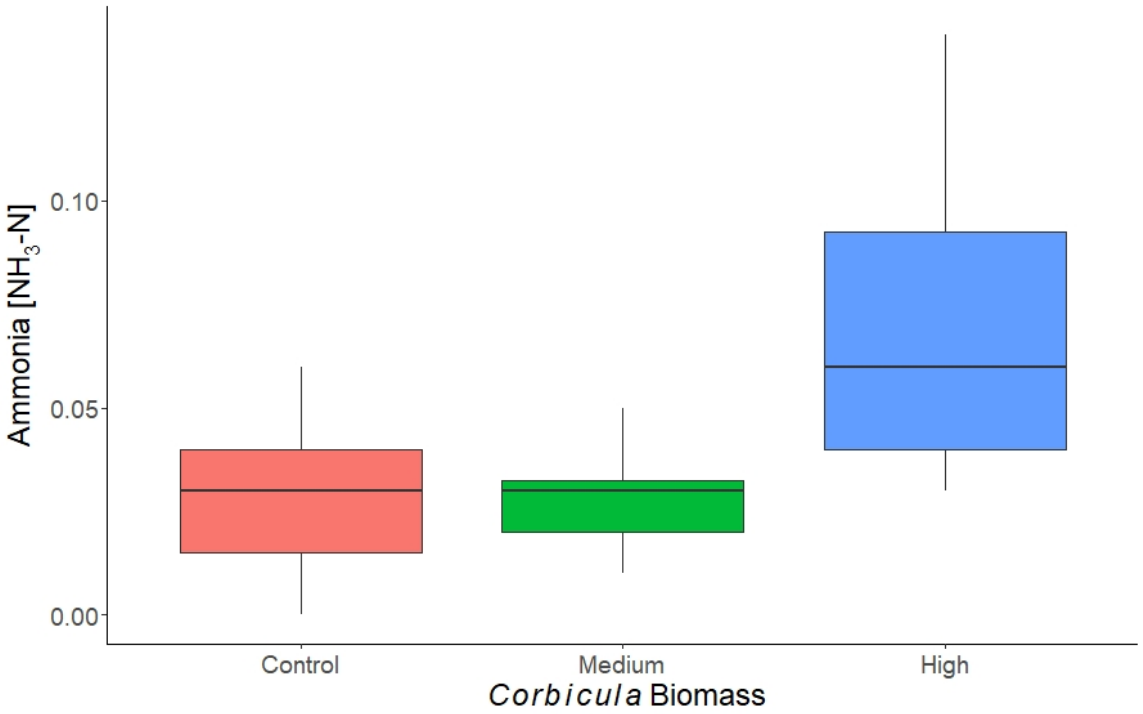

Figure 3.2. Ammonia (mg/L, as NH_3-N) in each of the three *Corbicula* biomass levels in high food conditions.

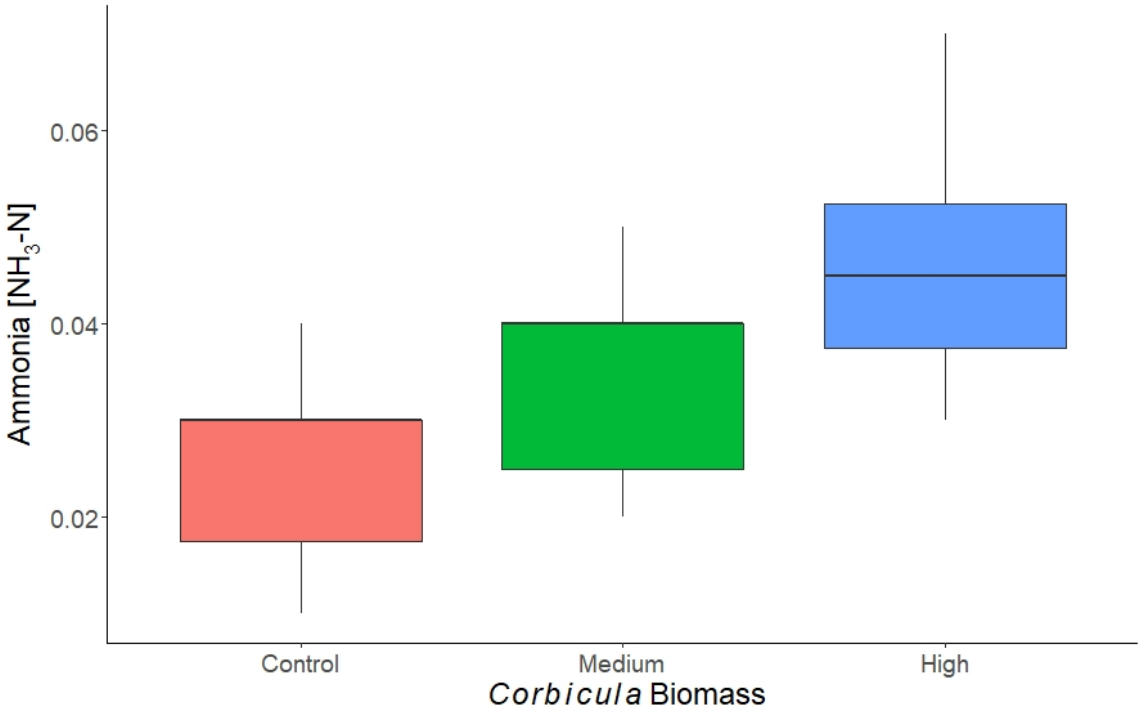

Figure 3.3. Ammonia (mg/L, as NH_3-N) in each of the three *Corbicula* biomass levels in low food conditions.

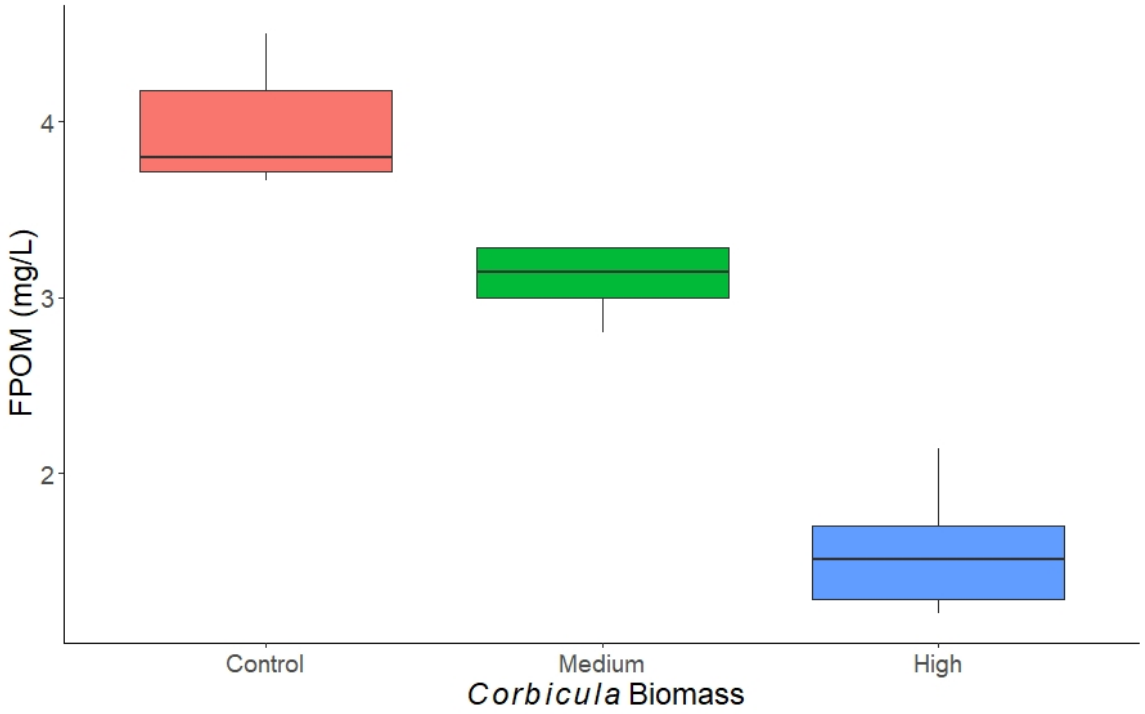

Figure 3.4 FPOM (mg/L) in each of the three *Corbicula* biomass levels in high food conditions.

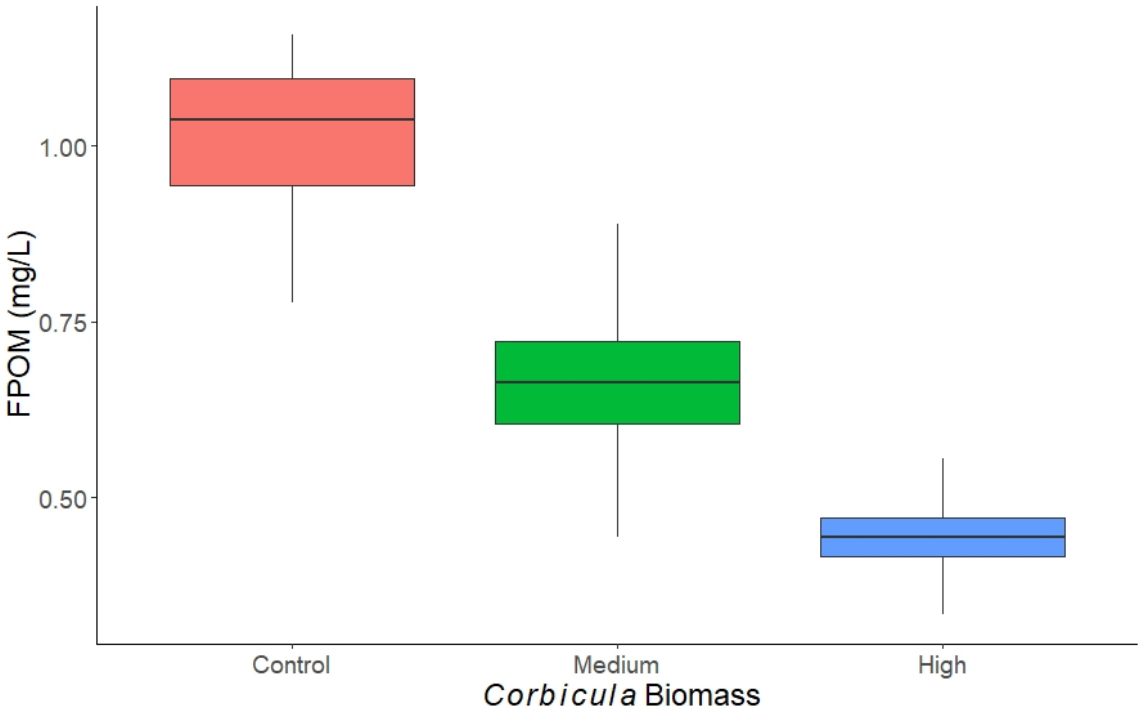

Figure 3.5 FPOM (mg/L) in each of the three *Corbicula* biomass levels in low food conditions.

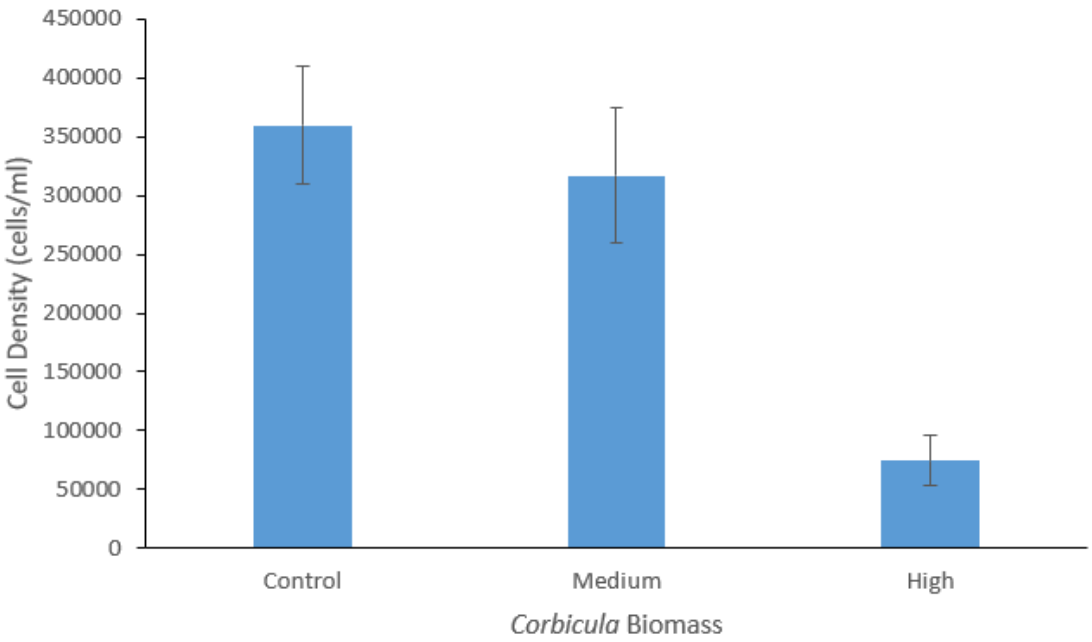

Figure 3.6 Cell density (cells/ml) in each of the three *Corbicula* biomass levels in high food conditions. Error bars represent SD.

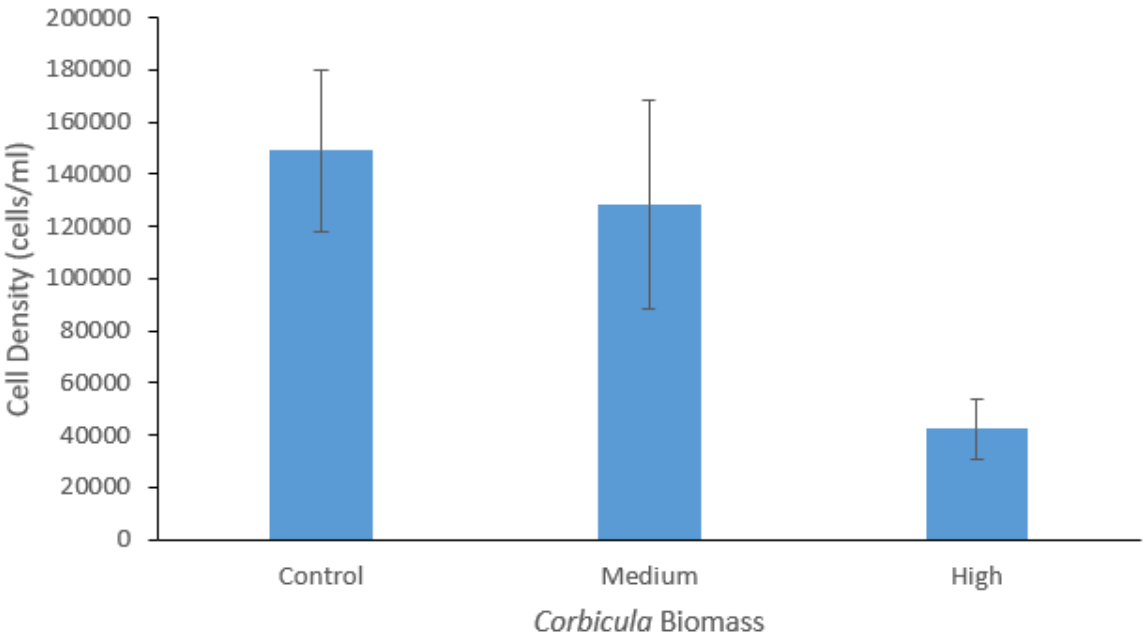

Figure 3.7 Cell density (cells/ml) in each of the three *Corbicula* biomass levels in low food conditions. Error bars represent SD.

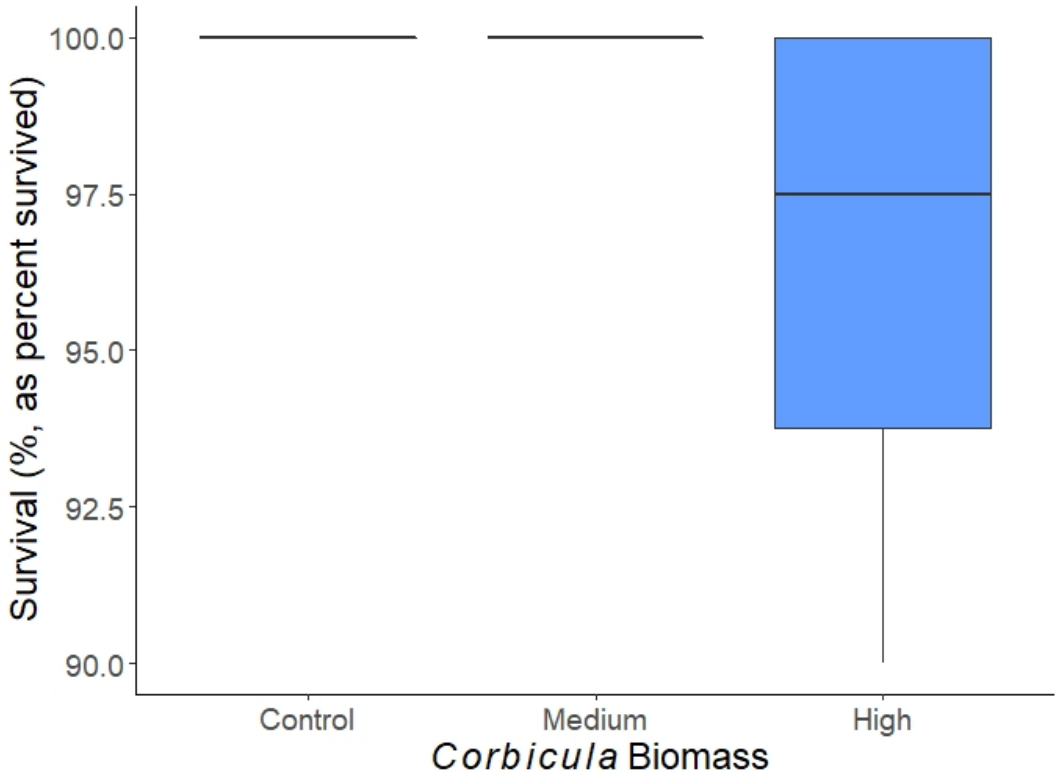

Figure 3.8 Survival (%, as percent survived) in each of the three *Corbicula* biomass levels in high food conditions.

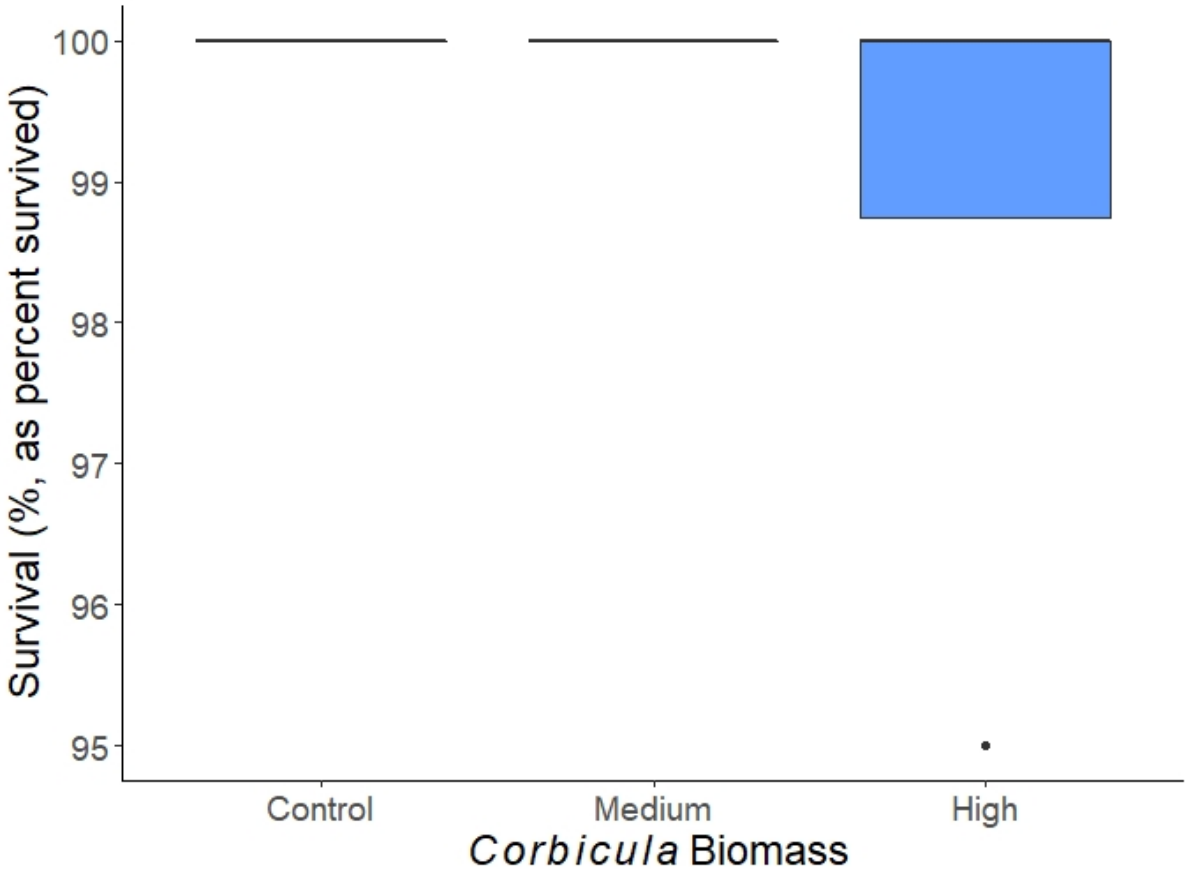

Figure 3.9 Survival (%, as percent survived) in each of the three *Corbicula* biomass levels in low food conditions.

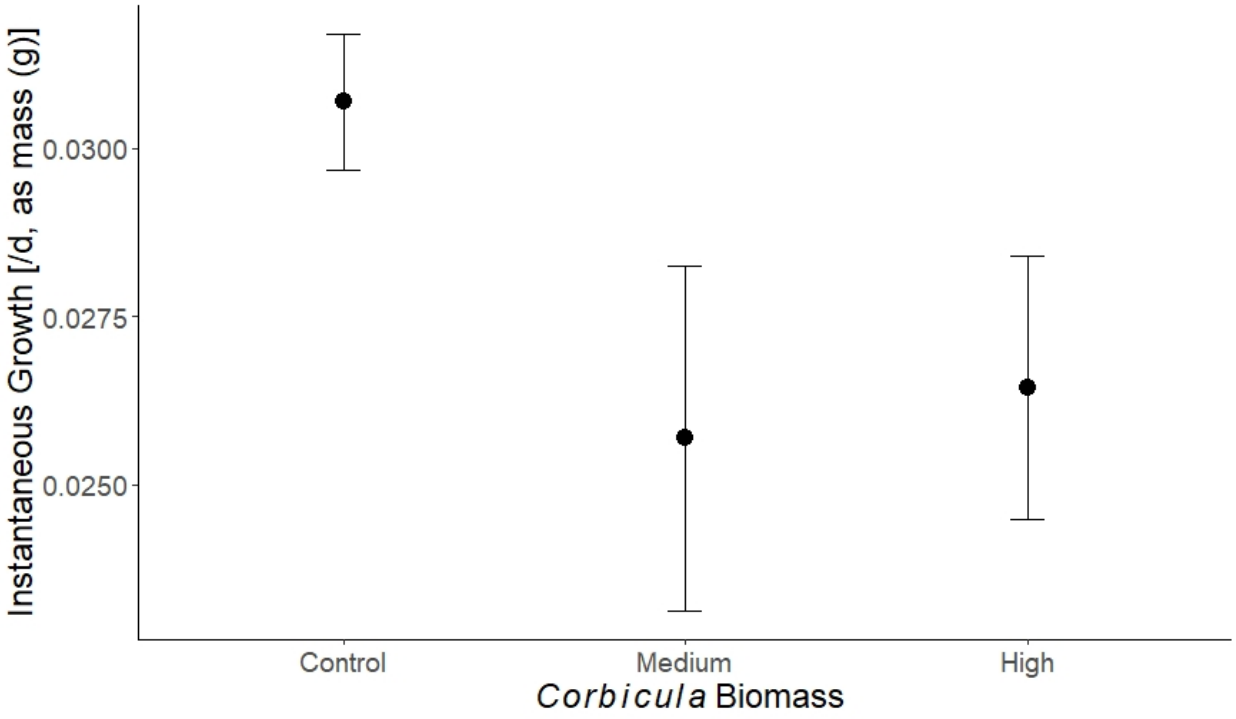

Figure 3.10 Mean instantaneous mussel growth [/d, as mass (g)] in each of the three *Corbicula* biomass levels in high food conditions. Error bars represent SE.

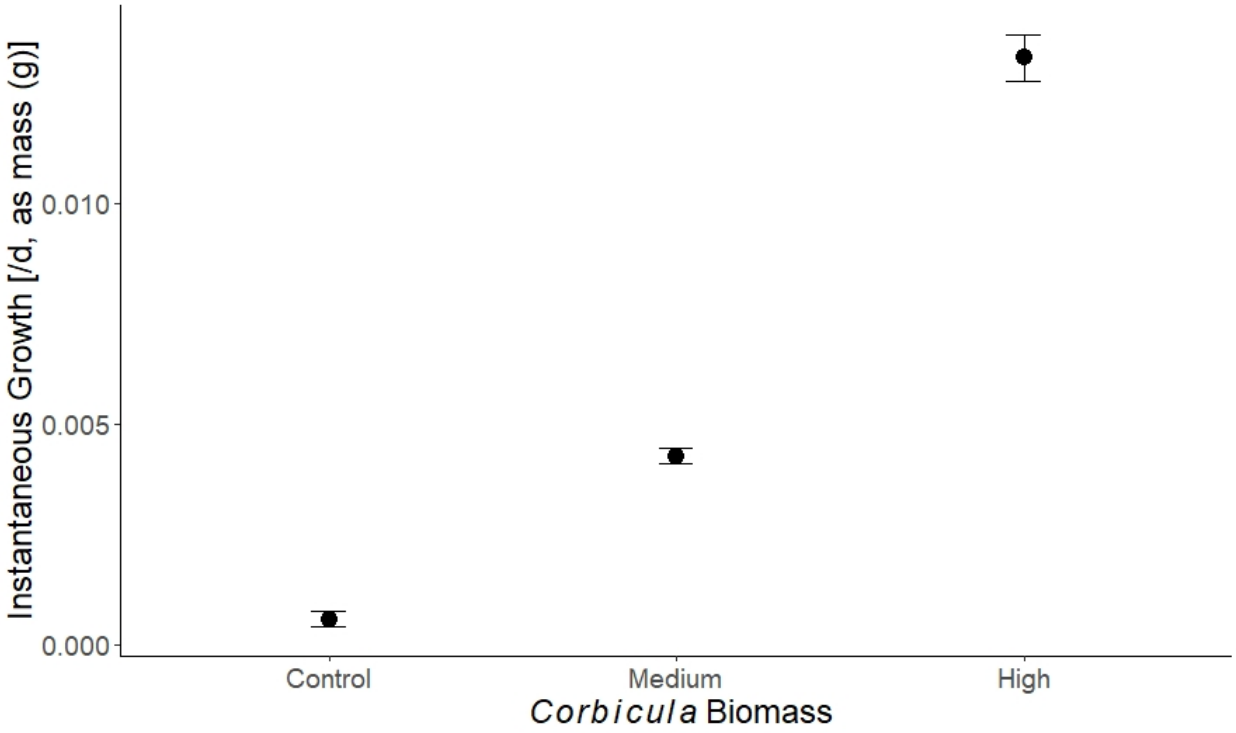

Figure 3.11 Instantaneous growth [/d, as mass (g)] response in each of the three *Corbicula* biomass levels in low food conditions. Error bars represent SE.

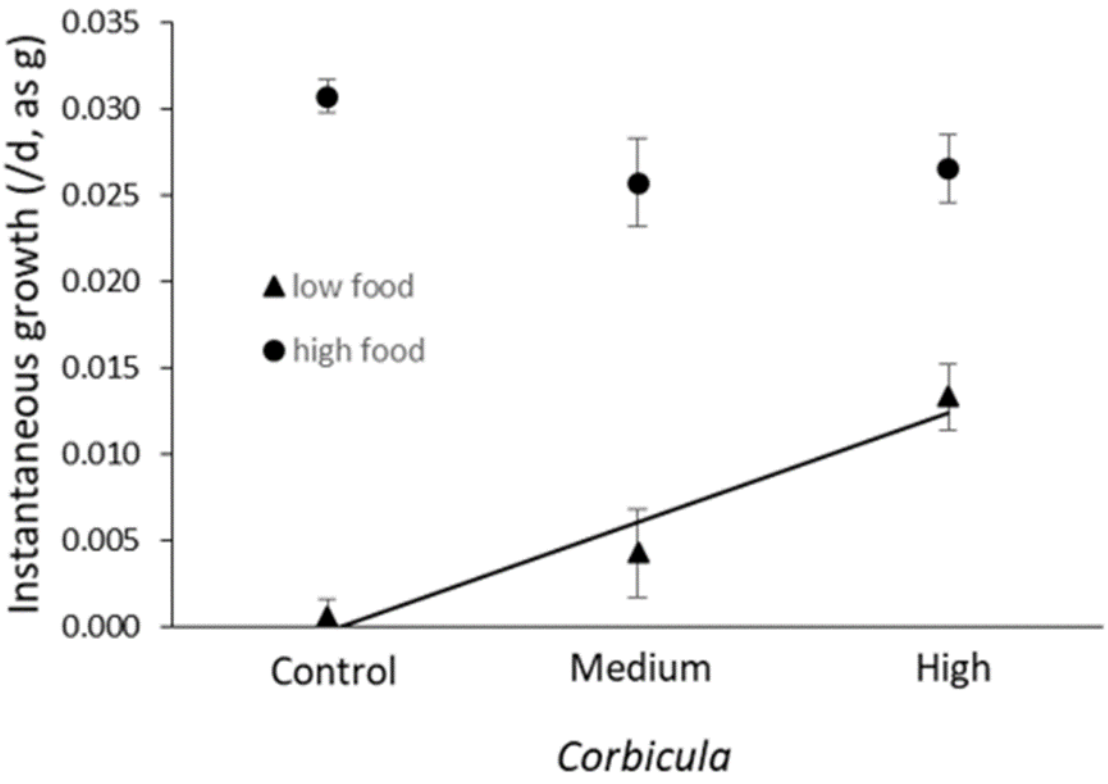

Figure 3.12 Interaction plot showing the effects of food abundance and *Corbicula* biomass on mean instantaneous mussel growth [/d, as mass (g)]. Error bars represent SE.

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VITA

I attended the University of Missouri-Columbia, receiving my Bachelor of Science in Biology. After my undergraduate studies, I worked as a resource technician and subsequently a resource assistant in the Resource Science division of the Missouri Department of Conservation in Columbia, Missouri.

Drew White