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Effects of food abundance on juvenile freshwater mussel survival and growth in aquaculture, and comparison with growth in streams

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ABSTRACT

Captive propagation has become an important tool in the conservation of imperiled freshwater mussels. Previous studies provide conflicting results about the effects of food abundance on survival and growth of juvenile mussels in aquaculture, and the extent to which growth in the hatchery reflects growth in the wild is unknown. We evaluated the effects of abundance of an algal-based diet on survival and growth of juvenile Cumberland Bean (*Venustaconcha troostensis*) in a recirculating aquaculture system. We compared food abundance (as fine particulate organic matter, FPOM) in our experimental food rations with FPOM in 14 streams to assess the similarity of hatchery and natural food sources. We compared growth in our experiments with previously published growth estimates of Cumberland Bean at 17 stream sites. Growth in the hatchery increased linearly with increasing food abundance from 3.5 to 27.6 mg/L algal dry mass (about 111,000–1,147,225 cells/mL), and mussel size after two weeks increased 2.9% for every doubling of algal dry mass within the range of our experimental rations. We observed no negative effects of increasing food abundance on survival, and ammonia concentrations remained below chronic effect concentrations in all treatments, even in food rations much higher than recommended by previous studies. FPOM in our experiments spanned a similar range of values as FPOM in streams. Growth in our experiments was similar to growth in streams, but our experimental temperatures were higher than in streams. When the probable effect of temperature was accounted for, growth in most experimental food rations was substantially lower than expected in streams despite similar FPOM. This suggests that food quality or other conditions are more favorable for mussel growth in the wild.

1. Introduction

Captive propagation of freshwater mussels (order Unionida) has emerged as a powerful tool for restoring populations of these imperiled animals (Patterson et al., 2018; Strayer et al., 2019). Large-scale mussel production was facilitated by the development of hatchery diets capable of supporting survival and growth, which was an obstacle to previous efforts (Gatenby et al., 1997). Hatchery diets typically include mixtures of cultured live algae and commercially available concentrates of dead algal cells (Mair, 2018; Kovitvadhi et al., 2008). The effects on juvenile mussels of several aspects of hatchery diets have been evaluated, including algal species composition, cell size and density, and additives such as sediment, and findings of these studies have improved mussel production (e.g., Gatenby et al., 1996; Jones et al., 2005; Hua et al., 2013).

Food abundance is an important factor in developing optimal hatchery diets. Inadequate food obviously is detrimental to bivalve performance, and higher abundance is expected to result in increased growth. However, excessive feeding can adversely affect growth due to decreased clearance rates and increased energetic costs of food sorting and pseudofeces production (Riisgård et al., 2011). Excessive feeding also can result in higher mortality due to increased ammonia concentrations or other adverse water quality conditions (Mair, 2018), and it increases costs for hatchery facilities (Gatenby et al., 2013). Studies of the effects of food abundance on juvenile mussel growth or clearance rate have provided conflicting results despite evaluating similar ranges of cell densities (~30,000–175,000 cells/mL): some showed decreased growth with increasing algal concentrations (Mair, 2013), while others found no difference in growth among food levels (Hua et al., 2013) or increased clearance rates with increasing food (Gatenby et al., 2013).

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Although hatchery diets are capable of supporting survival and growth, it is widely recognized that natural water sources enhance juvenile mussel performance (Mair, 2018; Gum et al., 2011). Enhanced performance is attributed to a greater diversity of food items in natural water sources, but mussel diets in the wild are poorly known. Furthermore, previous studies have evaluated hatchery diets solely with the aim of maximizing growth in the hatchery, and none have evaluated how growth on hatchery diets compares with growth in the wild. Hatchery diets that resemble natural diets and produce growth rates similar to those in the wild may lessen hatchery selection and result in mussels that are better adapted to conditions in recipient streams (see Geist et al., 2021). In addition to its value for mussel restoration, captive propagation is important for providing test animals for toxicological or other experimental studies (e.g., Wang et al., 2007; Haag et al., 2019). These studies require information about hatchery diets that can produce environmentally relevant mussel growth.

Our poor understanding of mussel diets in the wild makes it difficult to compare them to hatchery diets. Mussels are reported to ingest a wide variety of fine particulate organic matter (FPOM) about 2–40 μm in size, including phytoplankton, small zooplankton, bacteria, fungal spores, and detritus (reviewed by Strayer, 2008). Juvenile mussels use pedal feeding extensively but are thought to switch to suspension feeding at about 2 mm shell length when gill ctenidia become well-developed (Gatenby et al., 1996; Shartum et al., 2017). Strong particle size selectivity has been demonstrated in the laboratory, but selectivity can vary widely among species and habitats (Nichols and Garling, 2000; Beck and Neves, 2003; Atkinson et al., 2011), and no consensus has emerged about mussel diets or nutritional requirements. Given our poor understanding of mussel diets, hatchery managers need a readily measured proxy for food abundance. Total organic carbon (TOC) in stream water was one of the most important variables for predicting juvenile mussel growth in the wild (Haag et al., 2019). FPOM is closely related to TOC and is more easily measured. Chlorophyll a has been used as a proxy for mussel food availability, but FPOM better reflects the broad potential diet of mussels, which includes many non-autotrophic sources (see Nichols and Garling, 2000; Christian et al., 2004).

We conducted a series of experiments to evaluate the effects of abundance of an algal-based diet on survival and growth of juvenile Cumberland Bean (*Venustaconcha troostensis*) in a recirculating aquaculture system. We compared food abundance (as fine particulate organic matter, FPOM) in our experimental food rations with FPOM measured in 14 streams in a variety of physiographic and ecological contexts to assess the similarity of hatchery and natural food sources. We compared growth rates in our experiments with growth estimates in the wild previously reported at 17 stream sites within the range of Cumberland Bean. We discuss the value of our results for captive mussel propagation and for understanding mussel diets in the wild.

2. Methods

2.1. Study species and captive propagation

The Cumberland Bean is endemic to the Cumberland River system, Kentucky and Tennessee, USA, but it has disappeared from most of its historical range and is listed as Endangered under the US Endangered Species Act (Lane et al., 2016; Haag and Cicerello, 2016). We propagated juvenile Cumberland Bean and conducted all experiments at the Center for Mollusk Conservation (CMC), Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky. We collected brood stock of Cumberland Bean from Sinking Creek, Laurel County, Kentucky (Rockcastle River system). Larvae (glochidia) of most mussel species, including Cumberland Bean, require a fish host on which to metamorphose from the larval to the juvenile stage. We used Fantail Darter (*Etheostoma flabellare*) as a host because it produces robust metamorphosis of Cumberland Bean (Guyot, 2005).

We harvested glochidia from brood stock on January 15, 2019, by flushing the gills with a syringe filled with sterile water. We produced juvenile mussels by artificially inoculating fishes with glochidia. We inoculated fishes by anaesthetizing them and pipetting glochidia onto their gills. We held inoculated fishes in a recirculating aquarium system at 19 to 23 °C, and juvenile mussels metamorphosed three to four weeks after inoculation. Prior to using them in experiments, we reared juveniles for three to five months at 24 to 26 °C in 6-L trays within a recirculating aquaculture system with biological and mechanical filtration; the system was identical to the experimental system (Section 2.2). Juveniles were fed a mixture of commercial and cultured algae (Section 2.3).

2.2. Experimental system

We constructed three separate recirculating aquaculture systems (RAS), one for each of three different food rations (Fig. 1). Our systems were similar to previously described RAS for freshwater mussels (Kovitvadhi et al., 2008). Each of our RAS consisted of 8, 41.1 × 13.3 × 10.7 cm (6 L capacity; 5.8 L actual volume) flow-through trays supplied with water and food from a 23 L (15 L actual volume) mixing tank. The mixing tank received water pumped from a 56 L sump filled with 26 L of water, and algal food was gravity-fed to the mixing tank from a 13 L feeding cone. Each RAS had a total system volume of about 100 L. Each tray was aerated with an air stone and continuously gravity-fed an algal suspension from the mixing tank via a valve and silicone tubing. Water overflowed from the top of the trays though a bulkhead and tubing into the sump. The sump contained Bio Barrels (Pentair; Cary, North Carolina) to promote colonization by bacteria that act as biological filters. A jet pump in the sump was connected to two pipes; one pipe returned water continually to the mixing tank while the other discharged water to a floor drain at 2-h increments controlled by a timer and electronic ball valve. Water removed through the ball valve was replenished automatically by conditioned well water, resulting in a turnover rate of about 1.5× system volume every 24 h. Flow rate through each tray was maintained at 100 mL/min. Before the experiments, we cleaned system components with acetic acid and water then placed into each tray 50 mL of 150–250 μm heat-sterilized sand distributed evenly across the bottom. Prior to placing mussels in the trays, we measured shell length (maximum anterior-posterior dimension, nearest 0.1 mm) of each individual under a binocular microscope with imaging software.

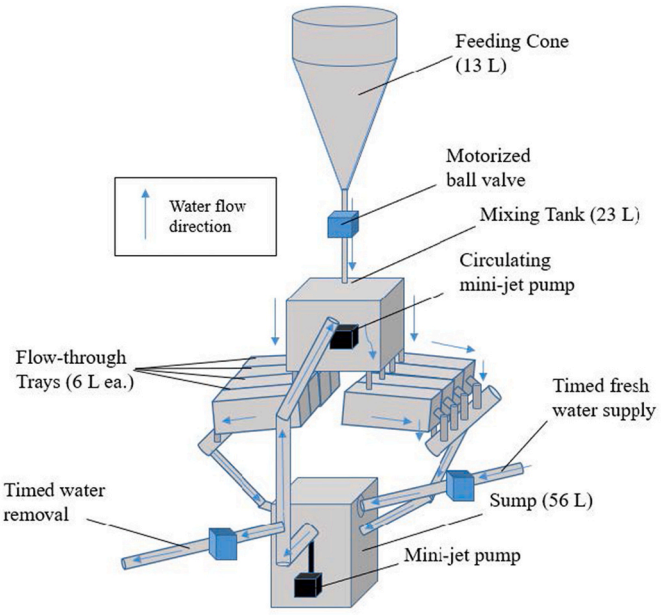

Fig. 1. Schematic of the recirculating aquaculture system (RAS).

During the experiments, we removed all mussels from the trays every seven days, cleaned trays with acetic acid and water, refilled the trays with water and sand, then replaced mussels in the same tray. We cleaned feeding cones with acetic acid and water once daily, prior to refilling the cones with the algal ration, and we cleaned the mixing tanks and sumps once at about the midpoint of each experiment. Cleaning of system components was meant to reduce colonization by cyanobacteria and other organisms. Water temperature in the trays ranged from 25.7 to 27.1 °C. At the end of each experiment, we calculated survival in each tray as the proportion of live individuals. We measured length of all surviving mussels and expressed growth as instantaneous growth [per day: ln(final length in mm/initial length in mm)/number of days; Ricker, 1975]. Instantaneous growth is the exponential factor by which length is predicted to increase each day; we used this measure instead of raw growth because it can be more easily compared with other studies (by accounting for differences in initial size and study duration) and it has better statistical properties.

We characterized food abundance in the trays by measuring fine particulate organic matter (FPOM). We used FPOM to provide a generalized measure of energy availability and to allow comparison with food abundance in streams; mussel diets in the wild are poorly understood but probably encompass a wide range of organic matter in addition to algal cells (e.g., Nichols and Garling, 2000). For Experiment 1, we collected triplicate 500–2000 mL water samples from each tray containing mussels on day 1 and 9 of the experiment. Standard deviations for the triplicate samples averaged <20% of the mean, so we took only a single sample/sample date in subsequent experiments. We vacuum filtered each sample through a pre-combusted (550 °C for 1 h), preweighed (nearest 0.001 g) 0.7 μm glass fiber filter (47 mm diameter) and weighed the filters after drying at 104 °C for 1 h to obtain total suspended solids (TSS, mg/L). We then combusted the filters at 550 °C for 1 h, reweighed them, and calculated FPOM as TSS – ash (inorganic) mass. We also estimated algal cell density in each food abundance ration. We did not measure cell density directly; rather, we estimated cell density in our rations based on a regression of cell density on algal dry mass derived in a separate study using the same algal mixture [cell density = 43,027(algal dry mass) – 40,320; White, 2020].

We monitored water quality conditions during the experiments by measuring 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. We measured pH and ammonia in 50 mL water samples from experimental trays using a portable pH meter and the nitrogen, ammonia-salicylate method (Hach Method 10,031; https://www.hach.com/quick.search-quick.search.jsa?keywords=DOC316.53.01079, accessed March 3, 2021), respectively. We measured temperature and DO directly in the trays using a handheld digital thermometer and portable DO meter, respectively.

2.3. Food rations

We developed a series of experimental food rations based on the standard diet and feeding ration used at the CMC (see subsequent and Tables 1 and 2). All rations were based on a diet containing two live freshwater algae cultured at CMC, *Chlorella sorokiniana* and *Phaeodactylum tricornutum*; two commercially available concentrates of dead marine algae, *Nannochloropsis* spp. (Nanno 3600) and *Thalassiosira pseudonana* (TP 1800); and a commercially available mixture of six dead marine microalgae (Shellfish Diet 1800) (all marine algae from Reed Mariculture Inc., Campbell, California). The standard ration used to rear juvenile mussels at CMC is 10.6 mg algal dry mass/L (about 416,000 cells/L), which is considered the highest ration that can be fed without causing water quality problems (M. McGregor, unpublished data). We prepared a batch of each food ration once daily, placed the mixture in the feeding cone, and topped off the feeding cone with cold water. Flow from the feeding cone was calibrated to deliver the entire 13-L volume of the cone over the next 24 h.

Table 1
Feeding rations for Experiment 1. Values are g dry mass (mL wet volume).

Algal type	Food abundance ration		
	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)
Estimated cell density (cells/mL)	111,000	261,000	553,000
Algal dry mass (mg/L)	3.5	7.0	13.8

Algal types are: CS = *Chlorella sorokiniana*; PT = *Phaeodactylum tricornutum*; NA = *Nannochloropsis* spp.; TP = *Thalassiosira pseudonana*; SD = Shellfish Diet; see text for details about algal types. See text for details about estimation of cell density. Algal dry mass is based on total system volume of 100 L.

Table 2
Feeding rations for Experiment 3. Values are g dry mass (mL wet volume).

Algal type	Food abundance ration		
	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 (0.750)	0.2320 (2.0)	0.4640 (4.0)
SD	0.0400 (0.375)	0.1100 (1.0)	0.2200 (2.0)
TP	0.1100 (1.500)	0.3088 (4.0)	0.6016 (8.0)
Estimated cell density (cells/mL)	183,420	553,453	1,147,225
Algal dry mass (mg/L)	5.2	13.8	27.6

Algal types are: CS = *Chlorella sorokiniana*; PT = *Phaeodactylum tricornutum*; NA = *Nannochloropsis* spp.; TP = *Thalassiosira pseudonana*; SD = Shellfish Diet; see text for details about algal types. See text for details about estimation of cell density. Algal dry mass is based on total system volume of 100 L.

2.4. Experiment 1

We established three food abundance rations as follows: our high food ration was 1.3× the standard CMC ration, and our medium and low food abundance rations were 50% and 25% of the high ration, respectively (Table 1). We randomly assigned each RAS to one of the three food rations. We placed ten haphazardly selected juvenile mussels in each of two randomly selected trays in each RAS (six total experimental trays); trays that did not receive mussels were operated in the same way as experimental trays. At the beginning of the experiment, mussels were about 3 months old and mean length was 2.5 mm ± 0.5 SD. We ran the experiment for 21 days from May 22 to June 11, 2019.

2.5. Experiment 2

Because we observed no negative effects of the high food ration on survival or growth in Experiment 1 (see Results), we conducted a second experiment to test a higher food ration. This experiment had a single food ration, which was 1.5× the high food ration for Experiment 1. The formulation for this ration was: CS = 0.6732 g dry mass (9.0 mL wet volume); PT = 0.4356 (9.0); NA = 0.348 (3.0); SD = 0.165 (1.5); TP = 0.4512 (6.0; see Tables 1 and 2 for abbreviations). Algal dry mass adjusted for system volume was 20.7 mg/L, and estimated cell density was 829,500 cells/L. We used surviving mussels from Experiment 1 in this experiment because the number of available juveniles was limited. We mixed mussels from all trays in Experiment 1 and haphazardly assigned six mussels to each of the eight trays in one RAS. Mussels were about four months old and averaged 2.6 mm ± 0.5 SD at the beginning

of this experiment. We ran the experiment for 14 days from June 12 to June 26, 2019.

2.6. Experiment 3

Experiment 3 was originally conceived to test effects of invasive Asian Clams (*Corbicula fluminea*) on juvenile mussel growth by placing different densities of *Corbicula* in different trays within a RAS. However, we were unable to test those effects because *Corbicula* did not reduce food abundance in individual trays within a RAS due to the flow-through nature of the system and common sump for all trays. Instead, *Corbicula* appeared to affect food abundance throughout the entire RAS. We do not know the extent to which *Corbicula* affected food abundance in the systems, but we were able to measure food abundance after this effect, and *Corbicula* abundance in all three RASs was identical. Furthermore, *Corbicula* did not appear to appreciably reduce food abundance within each RAS due to their overall low abundance relative to total RAS volume (see subsequent and Discussion). For these reasons, we report the results of this experiment to provide additional information about the effects of food abundance on mussel growth.

We established three food abundance rations to represent a wider range of food abundance than Experiment 1 (Table 2). We established our high food ration as 2× the high ration for Experiment 1 because we observed no negative effects of the high food ration on survival or growth in Experiments 1 or 2 (see Results). Our medium and low rations were 50% and 18% of the Experiment 2 high ration, respectively; these rations were 2.0× and 1.5× higher than the medium and low rations in Experiment 1, respectively. We randomly assigned each RAS to one of the three food rations. We placed 20 haphazardly selected juvenile mussels in each of the eight trays in each RAS (eight trays at each food ration, 24 trays total). Mussels were from the same cohort as Experiment 1, but they had not been used in previous experiments. At the beginning of the experiment, mussels were about 5 months old and mean length was 4.4 mm ± 0.4 SD.

We chose four levels of *Corbicula* biomass (blotted wet mass, including shell) to be placed in trays: control (0.0 g/tray), low (3.7 g), medium (32.0 g), and high (186.5 g); these levels corresponded to 0, 1, 8, and 50 individual *Corbicula*, respectively, based on the average mass of a single individual. We randomly assigned two trays within each RAS to each of the four *Corbicula* levels. Based on total RAS volume, overall *Corbicula* density was 4.4 g/L and 1.2 individuals/L in each RAS. This design resulted in a 3 × 4 full-factorial experiment with two replicates of each treatment combination. We ran the experiment for 17 days from July 5 to July 22, 2019.

2.7. Field measurements

We measured FPOM in 14 streams in Kentucky to provide information about the environmental relevancy of our experimental food rations. The streams encompassed a wide range of environmental conditions in three physiographic regions: the Appalachian Plateaus physiographic province, and the Highland Rim and Bluegrass sections of the Interior Low Plateaus physiographic province (see Supplemental Information). Streams in the Bluegrass region are warm, highly productive, and well-buffered, those in the Appalachians region are cooler, less productive, and less well-buffered, and those in the Highland Rim are intermediate between the two other regions (see Haag et al., 2019). We collected 1 L water samples from each stream on one to three dates from July to September 2019 following Kentucky Division of Water methodology (KDOW (Kentucky Division of Water), 2009). At the time of collection, we pre-filtered water samples across a 50 μm filter to remove larger material that is not likely used as food by mussels (see Section 1). In the laboratory, we measured FPOM in each sample following methods described previously.

We compared growth in our experiment with growth of Cumberland Bean in the wild at 17 sites in the Rockcastle River system, as measured by a previous study (Haag et al., 2021). Haag et al. (2021) measured growth of juvenile Cumberland Bean placed in streams in flow-through chambers (silos) for 84 days (average, June to August 2018). Mussels used in that study were captively propagated as described previously and were 4 months old and 4.6 mm (mean, ± 0.6 SD) at the time of deployment in streams. Haag et al. (2021) reported growth as instantaneous growth based on mass (/day, as g), but we used their data to calculate instantaneous growth based on shell length as described previously.

3. Results

3.1. Experiment 1

FPOM in experimental trays differed significantly among food abundance treatments (single-factor ANOVA: $F_{2,3} = 86.59$, $P = 0.002$). FPOM was significantly higher in the high food ration than in the medium and low rations, but those two rations did not differ from each other (Table 3). All three food rations were almost entirely organic; organic content averaged 100.0% (± 0.2 SD) and ranged from 99.4 to 100.0% in all trays.

Mussel survival did not differ significantly among food treatments (single-factor ANOVA: $F_{2,3} = 3.70$, $P = 0.155$, arsine-transformed proportion survival). However, raw values of survival were highest in the high food ration (90 and 100%), followed by the medium (70% and 80%) and low (60% and 80%) rations.

Mussel growth differed significantly among treatments (single-factor ANOVA: $F_{2,3} = 10.94$, $P = 0.042$). Growth was significantly higher in the high food ration than in the low ration, but growth in the medium ration did not differ significantly from growth in the high or low rations (Table 3).

Ammonia differed significantly among treatments (single-factor ANOVA: $F_{2,3} = 12.00$, $P = 0.037$). Ammonia was significantly higher in the high food ration than in the low ration, but ammonia in the medium ration did not differ significantly from the high or low rations (Table 4). Despite higher ammonia in the high food ration, ammonia was low in all trays and the maximum observed value was 0.060 mg/L. Growth was significantly and positively correlated with ammonia concentration ($r = 0.897$, $P = 0.016$, $N = 6$).

There was a marginally significant difference in pH among treatments (single-factor ANOVA: $F_{2,3} = 9.37$, $P = 0.051$). Mean pH values were highest in the low and medium rations, but differences were small and no post-hoc comparisons among means were significant (Table 4).

Table 3
Food abundance and mussel growth in three experiments. Algal dry mass is based on total system volume of 100 L. FPOM is fine particulate organic matter measured in experimental trays. Growth is instantaneous growth.

Experiment	Food ration	Algal dry mass (mg/L)	FPOM (mg/L)	Growth (/d, as mm)
1	Low	3.5	0.951 ± 0.056[a]	0.0012 ± 0.0003[a]
1	Medium	7.0	1.615 ± 0.245[a]	0.0034 ± 0.0007[a,b]
1	High	13.8	3.670 ± 0.081[b]	0.0042 ± 0.0002[b]
2	High	20.7	3.555 ± 0.459	0.0077 ± 0.0022
3	Low	5.2	0.978 ± 0.128[a]	0.0026 ± 0.0002[a]
3	Medium	13.8	3.054 ± 0.175[b]	0.0049 ± 0.0005[b]
3	High	27.6	4.111 ± 0.602[b]	0.0120 ± 0.0006[c]

Values within a column and within an experiment with the same superscripted letter are not significantly different (Tukey's HSD, $P < 0.05$). Values are means ± SE.

Table 4
Water quality in three experiments.

Experiment	Food ration	pH	NH_3 (mg/L, as N)	Temperature (°C)	DO (% saturation)
1	Low	8.45 ± 0.02	0.013 ± 0.005[a]	25.8 ± 0.1	90.8 ± 1.2
1	Medium	8.44 ± 0.02	0.033 ± 0.006[a,b]	25.7 ± 0.2	86.7 ± 2.5
1	High	8.41 ± 0.02	0.034 ± 0.005[b]	26.1 ± 0.1	89.3 ± 2.4
2	High	8.17 ± 0.02	0.043 ± 0.007	26.0 ± 0.0	85.3 ± 2.0
3	Low	8.46 ± 0.01[a]	0.043 ± 0.004[a]	27.1 ± 0.2	98.9 ± 0.3
3	Medium	8.41 ± 0.04[b]	0.078 ± 0.009[b]	27.1 ± 0.2	99.0 ± 0.4
3	High	8.35 ± 0.02[c]	0.082 ± 0.009[b]	27.1 ± 0.2	98.3 ± 0.5

Values within a column and within an experiment with the same superscripted letter are not significantly different (Tukey's HSD, $P < 0.05$). Comparisons for pH in experiment 3 are based on least square means adjusted for the effect of *Corbicula* (see text). Values with no superscripted letters are not significantly different. Values are means ± SE.

Neither temperature nor DO differed significantly among treatments (single-factor ANOVA: temperature, $F_{2,3} = 3.29$, $P = 0.175$; DO, $F_{2,3} = 7.06$, $P = 0.073$, arcsine-transformed % DO).

3.2. Experiment 2

FPOM averaged 3.555 mg/L, and organic content averaged 100.0% (± 0.0 SD). Survival averaged 95.1% (range = 83.3–100.0%). Mean mussel growth was 0.0077/day (as mm) ± 0.0022 SE. Mean values of pH and DO were slightly lower, and ammonia was slightly higher, than in the high food ration for experiment 1; temperature was similar to experiment 1 (Table 4)

3.3. Experiment 3

FPOM in experimental trays differed significantly among food abundance treatments (two-factor ANOVA: $F_{2,12} = 25.59$, $P < 0.001$), but it was not related to *Corbicula* abundance ($F_{3,12} = 1.74$, $P = 0.213$) or the food ×*Corbicula* interaction ($F_{6,\ 12} = 2.14$, $P = 0.123$). A reduced model including only food abundance also was highly significant ($F_{2,21} = 18.58$, $P < 0.001$). FPOM did not differ significantly between the high and medium food rations, but FPOM was significantly higher in both than in the low ration (Table 3). Organic content averaged 100.0% (± 1.0 SD) and ranged from 97.8 to 100.0% in all trays.

Mussel survival was high in all trays (mean = 98.5%, range = 90–100%) and did not differ significantly due to food (two-factor ANOVA: $F_{2,\ 12} = 1.18$, $P = 0.340$), *Corbicula* ($F_{3,12} = 2.84$, $P = 0.082$), or the food ×*Corbicula* interaction ($F_{6,\ 12} = 1.53$, $P = 0.250$). Despite the marginal significance of *Corbicula*, mean survival was similarly high in all *Corbicula* treatments (97.5, 100, 100, and 96.7% in the control, low, medium, and high *Corbicula* treatments, respectively).

Mussel growth differed significantly among food abundance treatments (two-factor ANOVA: $F_{2,12} = 81.10$, $P < 0.001$), but it was not related to *Corbicula* abundance ($F_{3,12} = 0.81$, $P = 0.511$) or the food ×*Corbicula* interaction ($F_{6,\ 12} = 0.17$, $P = 0.980$). A reduced model including only food abundance also was highly significant ($F_{2,21} = 110.18$, $P < 0.001$). Growth differed significantly among all three food rations and was highest in the high food ration, followed by the medium ration, and lowest in the low food ration (Table 3).

Ammonia differed significantly among food abundance treatments (two-factor ANOVA: $F_{2,12} = 10.14$, $P = 0.003$), but it was not related to *Corbicula* abundance ($F_{3,12} = 1.86$, $P = 0.190$) or the food ×*Corbicula* interaction ($F_{6,\ 12} = 1.49$, $P = 0.263$). A reduced model including only food abundance also was significant ($F_{2,21} = 8.04$, $P = 0.003$). Ammonia was significantly lower in the low food ration than in the high or medium ration, and ammonia did not differ between those two rations (Table 4). Growth was significantly and positively correlated with ammonia concentration ($r = 0.424$, $P = 0.039$, $N = 24$).

pH differed significantly among food abundance and *Corbicula* treatments (two-factor ANOVA: food, $F_{2,12} = 24.01$, $P < 0.001$; *Corbicula*, $F_{3,12} = 6.30$, $P = 0.008$), but mean squares indicated a stronger effect of food; the food ×*Corbicula* interaction was not significant ($F_{6,\ 12} = 0.52$, $P = 0.780$). When adjusted for the effect of *Corbicula*, pH differed among all three food treatments and declined with increasing food abundance (Table 4). Least square mean pH adjusted for the effect of food differed among *Corbicula* treatments by a maximum of 0.07; only two of six pairwise comparisons were significant, and these differences did not correspond to increasing or decreasing abundance of *Corbicula*. Temperature did not differ among food treatments, and the food ×*Corbicula* interaction was not significant (two-factor ANOVA: food, $F_{2,12} = 0.05$, $P = 0.948$; interaction, $F_{6,12} = 0.07$, $P = 0.998$), but *Corbicula* was a significant factor ($F_{3,12} = 56.55$, $P < 0.001$). Mean temperature was lowest in the control *Corbicula* treatment (25.9 °C), highest in the medium treatment (27.6 °C), and five of six pairwise comparisons were significant. DO did not differ among food treatments, and the food ×*Corbicula* interaction was not significant (two-factor ANOVA: food, $F_{2,12} = 0.14$, $P = 0.874$; interaction, $F_{6,12} = 1.13$, $P = 0.402$), but *Corbicula* was a marginally significant factor ($F_{3,12} = 3.34$, $P = 0.056$). However, no post-hoc comparisons among means were significant.

3.4. Combined results from all experiments

We examined all pairwise correlations between algal dry mass, FPOM, ammonia, and growth (Table 5); we did not examine correlations involving pH, temperature, or DO because these factors did not differ consistently or strongly among food treatments. FPOM was positively correlated with algal dry mass, and algal dry mass explained 74.1% of the variation in FPOM (Fig. 2). Growth was positively correlated with algal dry mass and FPOM, but algal dry mass explained a higher percentage of variation in growth (95.1 vs. 60.3%, Fig. 2, Table 5). Correlations between ammonia and all other variables were positive but only marginally significant. Standard partial coefficients for multiple

Table 5
Correlations and multiple regression results for factors related to mussel growth in three experiments based on mean values for food treatment levels in each experiment ($N = 7$ observations for each variable).

Correlations

	Algal dry mass	FPOM	Ammonia	Growth
Algal dry mass	–	0.861, 0.013	0.679, 0.094	0.975, <0.001
FPOM	–	–	0.662, 0.105	0.777, 0.040
Ammonia	–	–	–	0.700, 0.080

Multiple regression

Variable	df	Partial coefficients	*P*	Standard partial coefficients
Intercept	1	0.0002	0.860	0.000
Algal dry mass	1	0.4719	0.010	1.137
FPOM	1	−0.0008	0.250	−0.276
Ammonia	1	0.0148	0.471	0.111

Cell entries for correlations are Pearson correlation coefficients, followed by *P*-values.

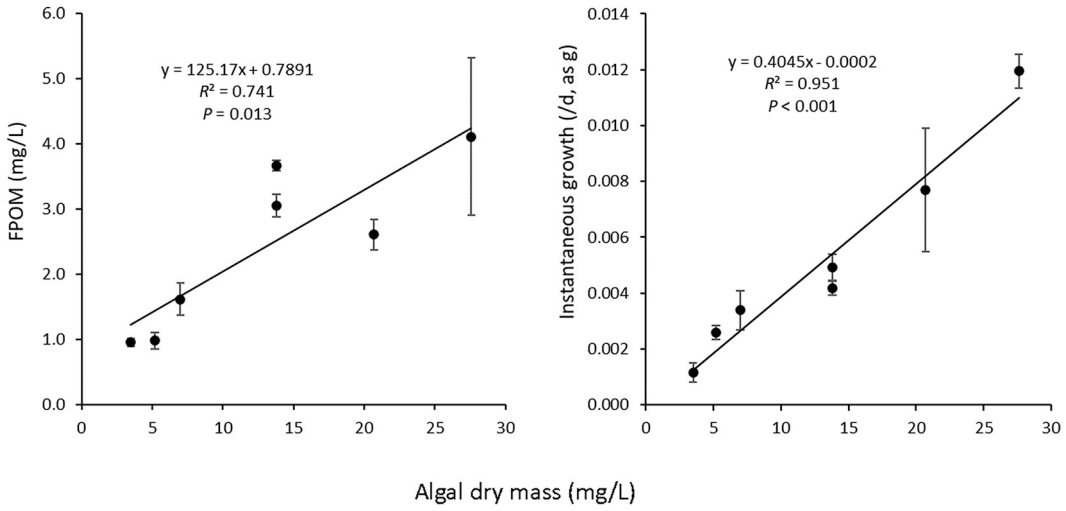

Fig. 2. Relationships between algal dry mass, FPOM, and mussel growth in three experiments. Data points represent mean values for each algal dry mass level in each experiment. Error bars are SE.

regression of the relationship between growth (dependent variable) and algal dry mass, FPOM, and ammonia (independent variables) indicated that algal dry mass was the most important factor in explaining growth (Table 5), and stepwise multiple regression indicated that adding other factors did not significantly improve explanatory power (*P*-to-retain = 0.15). The regression equation including only algal dry mass predicted that, within the range of food densities we used, final juvenile mussel size after two weeks should increase 2.9% for every doubling of algal dry mass (Table 6).

3.5. Food abundance and growth in streams

FPOM in 14 streams spanned a similar range as that seen in our experimental food rations (Fig. 3). FPOM averaged 1.723 g/L ± 0.706 SD (range = 0.704–3.122) in streams and 2.427 ± 1.274 SD (range = 0.952–4.111) in our experiments. FPOM in our low food rations was similar to FPOM in less productive, Appalachian streams (e.g., Horse Lick Creek, Little South Fork, Redbird River), and FPOM in our high food rations only slightly exceeded FPOM in productive Bluegrass streams (Eagle Creek, Slate Creek). Unlike our experimental food rations, suspended material in streams was dominated by inorganic matter (Fig. 3). Suspended material was composed of an average of 20.8% organic matter (± 12.0 SD, range = 9.5–55.2). FPOM was positively related to inorganic matter (linear regression: $R^2 = 0.560$, $P = 0.002$, slope = 0.895, intercept = 0.082).

Survival of Cumberland Bean at 17 sites in the Rockcastle River system over 84 days was high (94.5% ± 5.1 SE, Haag et al., 2021), similar to survival over 14–21 days in our experiments. Growth in streams was similar to growth in our experiments (Fig. 4). Growth averaged 0.0040/d (as mm) ± 0.0007 SE (range = 0.0004–0.0095) in streams and 0.0051 ± 0.0014 SE (range = 0.0012–0.0120) in our experiments. Growth in our low food treatments was higher than the lowest growth observed in streams (Crooked Creek, Horse Lick Creek),

Table 6
Juvenile mussel growth in different food rations predicted from the equation *Instantaneous growth* = 0.4045*algal dry mass* – 0.0002 (see Fig. 2). Predicted final shell length is based on initial size of 3.2 mm (the mean initial size in our experiments) after two weeks and was calculated as *Final length* (*mm*) = ($L_i e^{gd}$), where L_i is initial length, *e* is the base of natural logarithms, *g* is instantaneous growth (/d, as mm), and *d* is the number of days.

Algal dry mass (g/L)	Predicted instantaneous growth (/d, as mm)	Predicted final shell length (mm)	Predicted length increase (mm)
0.005	0.0018	3.28	0.08
0.010	0.0038	3.38	0.18
0.015	0.0059	3.47	0.27
0.020	0.0079	3.57	0.37
0.025	0.0099	3.68	0.48
0.030	0.0119	3.78	0.58

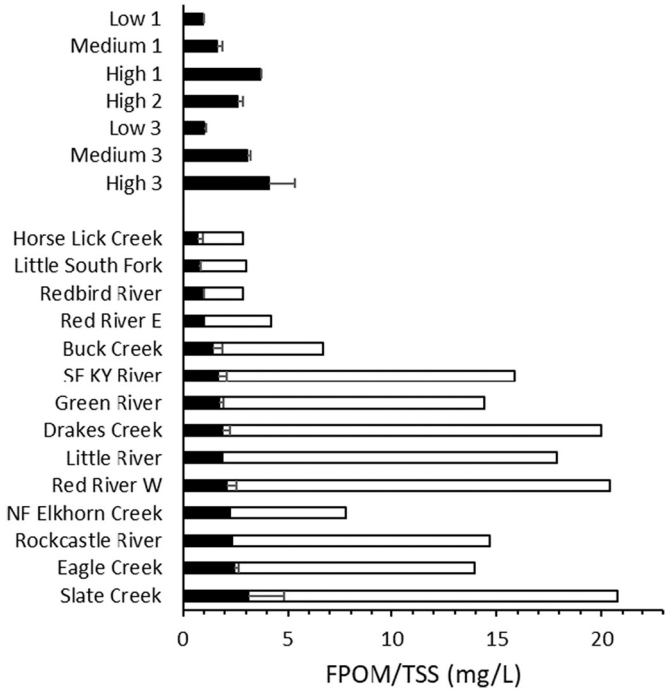

Fig. 3. FPOM and total suspended sediment (TSS) in experimental food rations and in 14 streams in Kentucky, USA. Labels for experimental food rations are followed by the experiment number (e.g., Low 1 is the low food treatment in experiment 1). Red River E (east) is in eastern Kentucky, and Red River W (west) is in western Kentucky. SF KY River is the South Fork Kentucky River, and NF Elkhorn Creek is North Fork Elkhorn Creek (see Supplemental Information for details about study streams). Solid portions of bars are FPOM and open portions are inorganic matter; the total value of each bar represents TSS. Error bars are SE and are shown only for FPOM. Only one sample was available for Red River E, Little River, NF Elkhorn Creek, and Rockcastle River. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

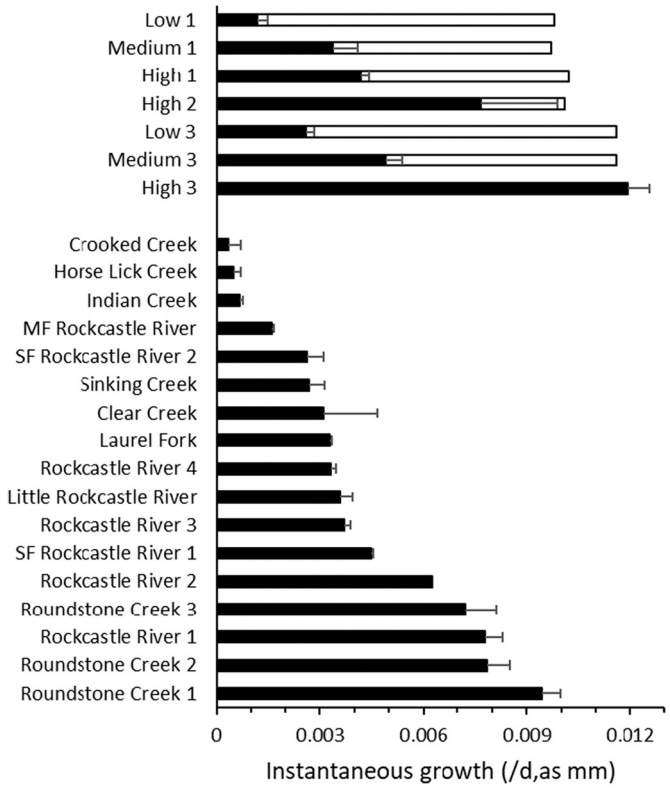

Fig. 4. Growth of juvenile Cumberland Bean in experimental food rations and in 17 streams in the Rockcastle River system, Kentucky, USA. Labels for experimental food rations are followed by the experiment number (e.g., Low 1 is the low food treatment in experiment 1). For experimental food rations, solid portions of bars are observed growth and open portions are growth predicted by accounting for the probable effect of temperature (see text). Error bars are SE. No estimates of error were available for Rockcastle River 2. See Haag et al. (2021) for details about study streams.

and growth in our highest food treatment (experiment 3) was slightly higher than the highest growth observed in streams (Roundstone Creek 1).

Although growth was generally similar in our experiments and in streams, temperatures in our experiments were substantially higher than stream temperatures (mean, experiments = 26.4 °C; wild = 22.2). Based on the relationship between growth in streams and stream temperature [growth = 0.0014(temperature) – 0.0263; Haag et al., 2021; Haag et al., unpublished data), predicted instantaneous growth at our mean experimental temperature was twice as high as mean observed growth across all experiments (predicted = 0.0107/d, as mm; observed = 0.0051). For individual treatments, predicted growth was within ±30% of observed growth only for experiment 2 (predicted = 0.0101, observed = 0.0077) and the high food ration in experiment 3 (predicted = 0.0116, observed = 0.0120); predicted growth at all other food rations was 2.4–8.5× higher than observed growth in our experiments (Fig. 4).

We had estimates of FPOM and mussel growth for only two sites in the Rockcastle River system (Horse Lick Creek and Rockcastle River 1; see Figs. 3 and 4). Based on our regression equation for growth as a function of FPOM [growth = 0.0022(FPOM) – 0.0003], predicted growth in Horse Lick Creek was about 2.5× higher than observed growth, and predicted growth at Rockcastle River 1 was about 40% lower than observed growth. However, 95% confidence limits for predicted and observed values overlapped at both sites (Horse Lick Creek: predicted = 0.0013 ± 0.0043, observed = 0.0005 ± 0.0005; Rockcastle River 1: predicted = 0.0049 ± 0.0024, observed = 0.0078 ± 0.0014).

4. Discussion

Algal dry mass was a remarkably good predictor of mussel growth, despite variation in experimental conditions and mussel age. *Corbicula* appeared to have little effect on mussel growth or food availability in experiment 3, probably due to their low system-wide abundance. The few studies that have experimentally evaluated the effects of *Corbicula* on native mussel growth found negative effects only at high *Corbicula* abundance (>600/m^2, Yeager et al., 2000; 2000/m^2, Ferreira-Rodríguez et al., 2018); these area-based estimates of abundance cannot be compared directly to our volume-based abundance. However, based on the volume of Yeager's test chambers, *Corbicula* abundance in the lowest abundance treatment in which they saw significant effects on mussel growth was 7.3 individuals/L, compared with 1.2/L in our experimental system [Yeager et al., 2000 found no significant effects of *Corbicula* on mussel growth at 3.6 individuals/L]. The best argument supporting the lack of effects of *Corbicula* in our study is that mean mussel growth and FPOM in the medium food ration in experiment 3 (growth = 0.0049/d; FPOM = 3.054 mg/L) were similar to values at the same food density in experiment 1 (high food ration) where *Corbicula* were absent (growth = 0.0042/d; FPOM = 3.374 g/L).

Our relationship between algal dry mass and growth predicts that growth increases linearly across the range of food rations we used. Notably, we observed no negative effects of increasing food ration on mussel survival, growth, or water quality, despite using food rations considerably higher than recommended (e.g., 1.92 mg/L dry mass, Mair, 2013). We found no evidence of negative effects of ammonia within the range observed in our experiments (maximum single observed value = 0.16 mg/L; maximum mean in treatments = 0.08). The weak positive relationship between ammonia and growth is probably spurious and a function of slightly higher ammonia in higher food rations. Ammonia toxicity to Cumberland Bean has not been tested. However, ammonia concentrations in our experiment were well below chronic effect concentrations for other mussel species at pH 8.2 and 20 °C (0.20 to 0.67 mg/L; *Villosa iris*, *Lampsilis siliquoidea*, *L. fasciola*; Wang et al., 2007, 2011) and USEPA chronic water quality criteria for our experimental conditions (0.22–0.37 mg/L at pH 8.2–8.5 and 27 °C, USEPA (US Environmental Protection Agency), 2013).

The strong predictive relationship we found between algal dry mass and growth can provide guidance for hatchery managers seeking to maximize or achieve a desired level of growth. However, our results depart substantially from those of other studies. Growth of 2-week-old *Actinonaias ligamentina* and *Epioblasma rangiana* decreased with increasing food from 1.90 to 9.88 mg/L algal dry mass (30,000–140,000 cells/mL; Mair, 2013); these dry mass values correspond to our low to medium rations within which range we saw increased growth. In contrast, Hua et al. (2013) found no effect of food ration on growth of newly-metamorphosed *Villosa iris* despite using a similar range of food abundance (35,000–175,000 cells/mL) as Mair (2013). Some of the differences between studies may relate to ontogenetic differences in feeding mode; based on their size (>2.5 mm), mussels in our study are expected to have switched to suspension feeding, whereas smaller mussels used on other studies may have relied more heavily on pedal feeding (Shartum et al., 2017). Mussel survival reported by Hua et al. (2013) and Mair (2013) also was conspicuously lower (<50% by day 30) than in our study, where survival typically was >90%. Growth of *Chamberlainia hainesiana* was highest in the lowest of three rearing densities (500, 1500, and 3000/culture unit; Kovitvadhi and Kovitvadhi, 2013), which suggests that food competition can be an important factor in a hatchery environment. The architecture and operation of a hatchery system also probably have large effects on mussel performance. For example, water turnover rate, the potential for accumulation of organic matter, and frequency of system cleaning all can affect food availability. These differences among studies suggest that survival and growth are highly dependent on culture system, conditions, characteristics of the diet, and mussel age and species.

It is difficult to compare our results with most other studies of mussel food rations because those studies reported food abundance only as cell density (e.g., O'Beirn et al., 1998; Hua et al., 2013; Huang et al., 2013). Cell density can be an informative measure, but it is strongly influenced by the size of algal species in the diet. We computed regression equations of cell density on algal dry mass for three studies that reported both values for their diets (Bush, 2008; Mair, 2013; Gatenby et al., 2013). Slopes of these regressions differed significantly and by a large magnitude among studies (slopes = 13,604, 79,236, 147,059; ANCOVA, dry mass × study, $F_{2,6} = 14{,}272.2$, $P < 0.001$). Algal dry mass or FPOM better facilitate comparisons among studies because they more directly represent biomass in the food ration regardless of algal cell size.

FPOM probably provides a more accurate measure of actual food availability in the culture system. The error in our relationship between algal dry mass and FPOM indicates that there was some inconsistency in food delivery among individual trays, and food delivery also likely varied over time within trays. These sources of error are unaccounted for in our relationship between growth and algal dry mass, which probably explains why FPOM was a less precise predictor of growth. More frequent sampling of FPOM might yield a more precise predictive relationship for growth. However, measuring FPOM is time-consuming, and algal dry mass is a useful approximation of food availability for the purposes of developing hatchery diets.

FPOM is a far more practical measure for comparing hatchery diets with food abundance in the wild because algal dry mass cannot be measured easily in streams due to the presence of non-algal organic material. FPOM in our hatchery diets was similar to FPOM observed in streams spanning a wide range of environmental conditions. However, FPOM provides no information about the energetic and nutritional content of food sources.

Despite the overall similarity between growth in our experiments and in streams, growth in the experiments was generally much lower than predicted when differences in temperature were accounted for. Our experimental temperatures were beyond the range of observed stream temperatures used to develop the regression equation between growth and temperature (max mean stream temperature = 23.7 °C). Therefore, lower observed than predicted growth in our experiments could be due to our experimental temperatures exceeding the thermal optimum for Cumberland Bean. However, in a similar hatchery environment, highest growth of three mussel species that co-occur with and have similar distributions as Cumberland Bean (*Epioblasma brevidens*, *E. capsaeformis*, and *Lampsilis fasciola*) occurred between 26 and 28 °C (Carey et al., 2013).

Observed growth in our experiments was similar to predictions based on temperature only for our two highest food rations (experiment 2, mean FPOM = 3.555 mg/L; experiment 3, high ration, FPOM = 4.111 mg/L). FPOM of these food rations was considerably higher than mean FPOM observed in two streams in the Rockcastle River system (Horse Lick Creek = 0.704 mg/L; Rockcastle River 1 = 2.333). This suggests that food quality or other conditions are more favorable in the wild, and this supports the idea that natural water sources enhance juvenile mussel performance (Mair, 2018). In contrast to hatchery diets, which are typically dominated by algae, the composition of FPOM is highly variable in streams, and it is often dominated by microbial biomass or other carbon sources (Christian et al., 2004; Geist et al., 2005). In addition to a better understanding of mussel diets in the wild and nutritional requirements, a critical next step in refining hatchery diets is a comparison of wild and hatchery diets in terms of energy, protein, and fat content; fatty acid composition; and other biochemical characteristics (e.g., Bartsch et al., 2017).

Perhaps the least-known aspect of freshwater mussel ecology is their diet. A better understanding of mussel diets is needed not only to improve captive propagation methods, but to evaluate causes of mussel declines and a wide array of ecological questions (e.g., Vaughn et al., 2008; Haag et al., 2019). Despite differences between growth in our experiments and in streams, the responsiveness of mussels to changes in food abundance shows that experimental studies can provide valuable information for assessing mussel responses to environmental factors and for better understanding mussel diets in the wild.

Author statement

D.E.J. White: Conceptualization, Methodology, Investigation, Writing - original draft.

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M.A. McGregor: Methodology, Resources, Writing - review.

S.J. Price: Conceptualization, Supervision, Writing - review ... editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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