Barnhart 2006

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Aquaculture 254 (2006) 227–233
Aquaculture
www.elsevier.com/locate/aqua-online

Buckets of muckets: A compact system for rearing juvenile freshwater mussels

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Received 27 April 2005; received in revised form 17 August 2005; accepted 19 August 2005

Abstract

A novel system was developed for the culture of juvenile freshwater mussels (Unionidae). The system can be replicated economically to provide statistical power for experimental investigations of culture conditions. Two nested buckets partition a water volume of 18 l into upper and lower compartments. Water moves from the lower to the upper compartment via a small submersible pump, and returns to the lower compartment through screen-capped chambers containing the juveniles. Each bucket system includes 7 chambers, each of which can accommodate 2000 juveniles (14,000 total). Newly transformed juvenile unionids of 8 species were held in these systems for 9 to 12 wk and continuously drip-fed a monoculture of *Neochloris oleoabundans*. Survival rates were generally higher than those previously reported for newly metamorphosed unionids and exceeded 95% over 2 mo for *Lampsilis siliquoidea* and *L. reeveiana*. Mean growth rates varied among 5 species from 4.2 to 12.5 μm/d at 22 °C. These growth rates are within the range previously reported for lampsiline juveniles in recirculating systems. The bucket rearing system may be particularly useful for conducting studies of water quality and feeding regimes that require replication to account for container effects. It is also useful for short-term culture of juveniles to be used in toxicity testing.

Keywords: Unionidae; Juvenile; Mussel; Culture; Propagation

1. Introduction

Freshwater mussels of the family Unionidae concern conservationists because many species are threatened with extinction (Strayer et al., 2004). In another context, some species have achieved great economic significance in freshwater pearl culture (Dan and Ruobo, 2002). The life cycle of unionids is remarkable. The females brood eggs that develop into a larval stage, the glochidium, which is briefly parasitic on particular species of fish. The juvenile stage that develops from the glochidium is less than 0.4 mm long and lives interstitially in benthic habitats (Neves and Widlak, 1987; Yeager and Cherry, 1994). Juveniles feed on microscopic particles of algae, bacteria, and particulate organic material which they obtain by ciliary feeding mechanisms (Yeager and Cherry, 1994; Silverman et al., 1997).

Over the past decade, efforts to propagate and culture unionids have expanded. However, few studies have tested the effects of factors such as temperature, water quality, food type, or food availability on juvenile growth and survival (Gatenby et al., 1996, 1997; O'Beirn et al., 1998; Beck and Neves, 2003). Such studies are complicated by the need to replicate holding systems and water conditions for treatment groups. Flow can be provided in recirculating raceways (O'Beirn et al., 1998; Henley et al., 2001), but these

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doi:10.1016/j.aquaculture.2005.08.028

are bulky and contain a relatively enormous volume of water compared to the biomass of the juveniles. Given the small size of juvenile mussels, a suitably designed recirculating system can maintain thousands of individuals in only a few liters of water. Such a system can be replicated in a reasonable space and thereby provide statistical power for comparisons among treatment groups.

The small size of juvenile mussels presents difficulties in handling and confining them in flowing water. Newly metamorphosed juvenile unionids generally range between about 200 and 300 μm in length, depending on species (Surber, 1912). These juveniles are easily suspended by currents, such that they can be lost from open containers in flowing water. In addition to drift, juvenile mussels are quite mobile and can crawl up the sides of containers. Not surprisingly, losses in grow-out studies are sometimes attributed to emigration as well as death (Zimmerman, 2003).

Several studies have reported that providing a substrate of silt, in which juveniles can burrow, improves growth and survival. Silt is thought to serve as a source of food as well as a substrate (e.g. Hudson and Isom, 1984; Gatenby et al., 1996; Rogers, 1999; Mummert, 2001; Zimmerman, 2003). However, the presence of silt further complicates maintenance, observation and handling, and might encourage growth of potentially harmful organisms in the culture system, such as ciliate protists and turbellarian flatworms.

Maintaining adequate flow in culture systems is essential, because juvenile unionids are small enough to occupy the diffusive boundary layer. The diffusive boundary layer is a benthic zone closely adjacent to surfaces, where friction reduces water movement to the point that diffusion, rather than convection, becomes the dominant mode of solute transport. Factors such as dissolved oxygen, ammonia, and food concentration in the boundary layer can differ substantially from those in adjacent flowing water (Boudreau, 2001). Investigation of the effects of these factors should therefore be carried out in a system designed to minimize stagnant zones and maintain uniform flow and water quality.

The bucket recirculating system described in this paper partitions a water volume of 18 l into lower and upper compartments. A small submersible pump moves water from the lower to the upper compartment. The water then returns to the lower compartment through a set of flow-through chambers (downwellers) that contain the juveniles. The design was tested by rearing juveniles of 8 unionid species for periods up to 12 wk and quantifying growth and survival.

2. Materials and methods

2.1. Chambers

The flow-through chambers for containing juveniles were constructed from 5.1 cm (2 in.) diameter Schedule 40 polyvinyl chloride (PVC) plumbing pipe and couplings. Nylon screen (Nitex®, 125 μm or larger mesh) was placed over a 4.4 cm length of pipe and press-fit tightly into a coupling, forming a filter cup. Pairs of filter cups were press-fit loosely together to form chambers that contained the juveniles (Fig. 1D). These chambers could be opened by separating the two filter cups, allowing access to the juveniles. Each chamber was positioned vertically in the recirculating system so that the juveniles rested on the screen of the lower cup. Water flowed downward through the chambers (Fig. 1).

2.2. Bucket recirculating systems

Each system consisted of two nested plastic buckets (HDPE, Encore Plastics, Sandusky, Ohio) (Fig. 1). The

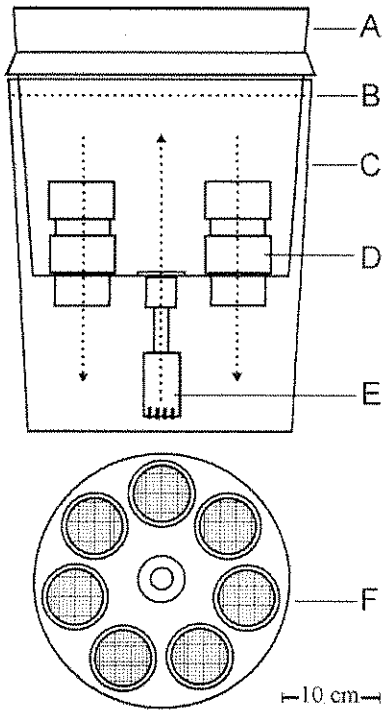

Fig. 1. Bucket rearing system. Dotted arrows indicate the direction of water flow. A. Upper bucket. B. Dotted line indicates water level. C. Lower bucket. D. Chamber (two nested filter cups). E. Power head pump attached to bulkhead through upper bucket. F. View from above upper bucket showing position of chambers and central bulkhead fitting.

capacity of the lower bucket was 18.9 l (5 gal) and that of the upper bucket was 13.2 l (3.5 gal). The upper bucket had a shoulder below the rim which rested on the rim of the lower bucket, so that the floor of the upper bucket rested about 16 cm above the floor of the lower bucket. Seven holes were drilled in the floor of the upper bucket with a 6 cm (2 ⅜ in.) hole saw. The bases of the chambers were inserted into these holes. It was important that the chambers fit the openings closely, so that the only path for water to return from the upper to the lower compartment was through the chambers. A 1.9 cm (¾-in.) bulkhead fitting was mounted in the center of the floor of the upper bucket. The bulkhead fitting was attached via adaptors to the outlet of a small "power-head" submersible aquarium pump (Aquarium Systems® Mini-jet model MN-404). The pump circulated water from the lower compartment to the upper compartment. Nominal flow rate for this model pump is 400 l (106 gal) per hour.

2.3. Food and feeding

The unicellular green alga *Neochloris oleoabundans* Chantanachat and Bold was cultured and provided as food. This species was previously identified as a suitable food for juvenile unionids (Gatenby et al., 1997; O'Beirn et al., 1998). Stock cultures were obtained from the University of Texas (UTEX Culture Collection of Algae, accession number 1185). The growth medium was autoclaved tap water fertilized with a commercial nutrient mix (Kent ProCulture® F2, Aquatic Ecosystems, Apopka FL). The alga was grown in 100-ml flasks and in 1.5-l glass jugs. The 100-ml flasks were inoculated from a stock culture on agar or serially from other 100-ml cultures. Each 1.5-l culture was inoculated with a 100-ml culture. Each jug was aerated via a glass pipette inserted through a rubber stopper. The air was filtered (0.5 μm) to remove contaminant spores of other microorganisms.

Four 1.5-l algae cultures were prepared weekly and harvested after 4–5 d of growth. The algae were separated from the culture medium by centrifuging at about 1500 RCF for 10 min. The cells were then resuspended in water to achieve a concentration of about $20 \cdot 10^6$ cells·ml^{-1}. Algae were refrigerated after resuspension and generally used within 1 wk of harvest.

The bucket systems were fed from 500-ml drip bags, similar to those used to deliver intravenous solutions. Each bag was filled daily with 400 ml of water from the system and 100 ml of algae suspension (total $20 \cdot 10^8$ cells). Flow through the drip line was controlled with a length of fine polyethylene tubing and was adjusted to approximately 500 ml·d^{-1}. Cell concentration in each system was checked with a hemocytometer weekly and remained at about 10–15,000 cells·ml^{-1}. The bags were washed out weekly with hot water and the drip lines rinsed with bleach to prevent them from becoming blocked.

2.4. Water and cleaning

The chambers were moved into clean buckets and new water weekly. River water (James River, Greene County MO) was used in order to provide a natural community of microorganisms. The water was collected one day before use and was filtered to remove particles larger than 30 μm. The screens in each chamber were cleaned at least weekly by spraying with water from a modified garden sprayer. When replacing a chamber in the system, care was taken to spill trapped air from underneath the chamber, because trapped air would block water flow. Air inside submerged chambers was removed by drawing it out through the upper screen with a large rubber-bulb syringe (cooking baster). Water temperature was checked daily and remained at 22–23 °C.

2.5. Juvenile mussels

Juvenile mussels were propagated on host fish for population augmentation of rare species or during the course of research on host relationships and toxicology studies. From several hundred to several thousand juveniles of each species were collected and placed in the systems within 2 d of excystment from the host fish. Four bucket systems (each with 7 chambers) were used and all were treated similarly throughout the course of the tests.

Growth rates of 5 species were checked at 10 to 20 d intervals. Each chamber was opened and the juveniles were rinsed into a 3-in. (7.6 cm) plastic Petri dish. The group of juveniles was then photographed several times under a compound microscope with a digital camera. A stage micrometer was photographed to provide scale. The photographs were later analyzed using ImageJ image analysis software (NIH, 2004). The maximum shell length of each suitably oriented individual was measured. An average sample of 72 individuals was measured in each group. Survivorship was determined at the end of the study by complete counts of each group of juveniles using Bolgorov plankton counting cells and a dissecting microscope. Dead individuals were recognized as empty shells.

3. Results

A total of 18,800 juveniles of 8 species were included in the study. The juveniles were counted and survivorship was determined only once during the study, at which time the age of the cohorts varied from 6 to 12 wk (Table 1). Survival varied among species and among chambers but exceeded 75% in 20 of 23 groups. Survival of *Lampsilis siliquoidea* and *L. reeveiana* exceeded 95% at 2 mo.

Growth was determined for 5 of the 8 species over periods of up to 12 wk. In each species, the increase of shell length over time was linear after about 2 wk of age. Growth rates ranged among species from 4.7 to 12.2 $\mu m \cdot d^{-1}$ at 22 to 23 °C (Table 2). Growth was fastest in *L. reeveiana*, and slowest in *L. abrupta*.

Growth varied greatly among individuals within cohorts, so that some individuals were twice as large as others after 84 d (Figs. 2 and 3). Coefficient of variation of shell length increased with size from about 7.5% at 400 μm length to 18.7% at 1000 μm, indicating sustained differences in growth rates among individuals (pooled data from all species and cohorts).

Table 1
Survivorship of 8 species of juvenile unionids after 6 to 10 wk of growth in bucket systems

Species	Age (d)	*N* total	Percent survival
Epioblasma triquetra (snuffbox)	44	77	24.7
Lampsilis abrupta (pink mucket)	69	1285	84.1
"	69	800	76.0
"	70	1293	79.5
"	70	756	77.6
Lampsilis rafinesqueana (Neosho mucket)	47	690	86.8
"	47	802	87.0
"	47	1262	86.8
Lampsilis reeveiana (broken rays)	68	725	95.2
"	72	296	93.9
Lampsilis siliquoidea (fatmucket)	61	1307	96.1
"	47	1584	95.3
"	47	1684	94.8
"	61	1005	97.4
"	59	1248	97.0
"	48	1373	97.1
"	47	986	94.7
Leptodea leptodon (scaleshell)	84	70	37.1
Ligumia recta (black sandshell)	74	530	75.5
"	69	321	84.4
"	69	293	79.9
"	69	304	87.2
Villosa iris (rainbow mussel)	67	111	72.1

Juveniles were placed in the chambers within 1 to 2 d of excystment from host fish. Each group of juveniles was kept in a separate chamber in one of 4 separate bucket systems.

Table 2
Regressions of mean shell length (y, μm) on age (x, days post-excystment)

Species	*N* dates	Regression	R^2
Lampsilis reeveiana	5	$y=12.24x+200$	0.989
Lampsilis siliquoidea	5	$y=8.21x+370$	0.998
Ligumia recta (cohort #1)	6	$y=7.58x+332$	0.987
Ligumia recta (cohort #2)	5	$y=6.29x+386$	0.997
Lampsilis rafinesqueana	4	$y=6.24x+353$	0.999
Lampsilis abrupta	4	$y=4.70x+366$	0.999

Length was measured on 4 to 6 different dates between 2 and 10 to 12 wk post-excystment, with an average of 72 individuals measured on each date in each cohort. The slope of the regression is the growth rate in micrometers per day.

4. Discussion

The bucket recirculating system has several advantages for experimental investigations of growth and survival of juvenile mussels and perhaps other bivalves as well. Studies that test effects of variables such as temperature, water quality, food type, or food availability require replicated systems with separate water supplies to allow statistical comparisons of treatment groups and account for container effects. The bucket system can be replicated at low cost and a number of separate systems can be maintained on a laboratory bench. Recently, this system has been used to produce juvenile mussels for use in toxicity testing (ASTM, 2005).

Downwellers, in which water flows downward through a screen partition, are favored for the culture

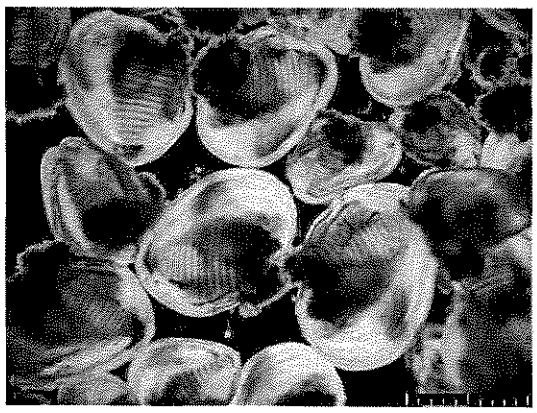

Fig. 2. Juvenile *Lampsilis reeveiana*, 84 d post-excystment. Masses on umbones are clumps of adherent bacteria and algae. Dark field illumination. Scale line at lower right = 1 mm.

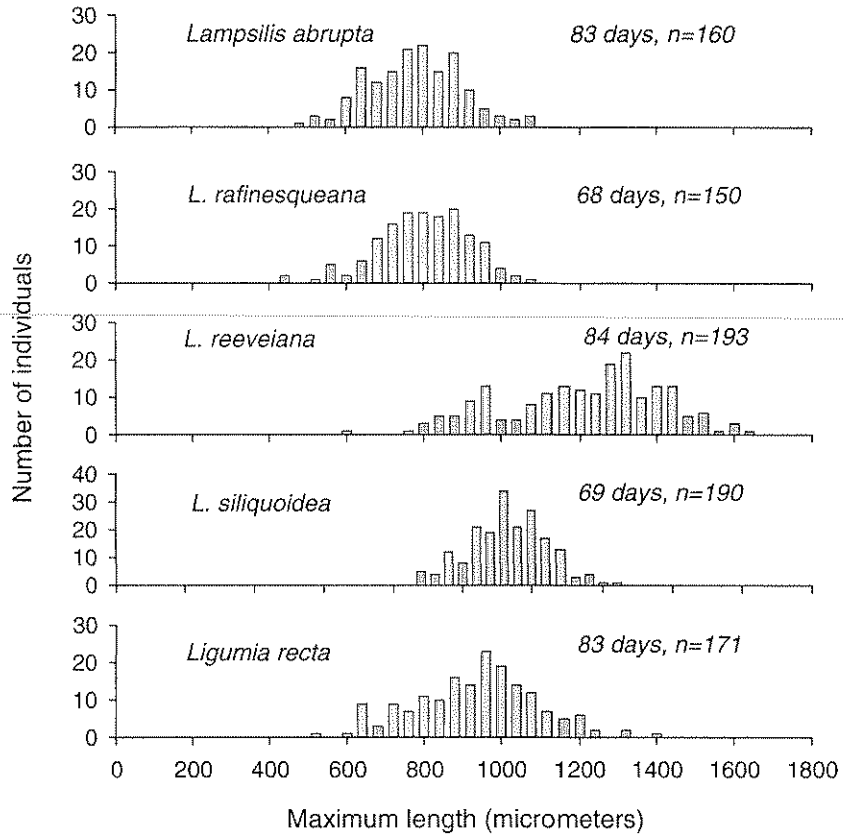

Fig. 3. Frequency plots of shell length of juvenile unionids after 9 to 12 wk of growth. The age of each cohort and the total number of individuals measured are indicated.

of young marine bivalves (e.g. Heffernan et al., 1988). The design largely eliminates the diffusive boundary layer and ensures that all individuals experience the same water quality. The two-piece chambers in this study are easy to manufacture, require no adhesives, and can be made with different mesh sizes to accommodate the size of the juveniles as they grow. Juvenile cohorts are easily contained, handled and examined, and up to seven cohorts can be kept separate within each system.

Most but not all of the species tested had high survivorship. The two species that did relatively poorly (*Epioblasma triquetra* and *Leptodea leptodon*) were both represented by single cohorts. Therefore, it is uncertain whether those species are unsuited to the system or whether those cohorts were in poor condition initially. The low mortality observed in the other species in this study contrasts with previous reports. Survival of captive juvenile unionids after 2 mo is usually below 50% and often below 20% (Gatenby et al., 1996; Rogers, 1999; Beck and Neves, 2003; Beaty and Neves, 2004). One problem frequently cited is predation by rhabdocoel flatworms (Delp, 2002; Zimmerman, 2003). Emigration (loss from culture containers) is also a common problem because early juveniles are highly mobile and small enough to easily be suspended by currents (Rogers, 1999; Zimmerman, 2003). The present system minimizes both of these problems by confining the juveniles and by excluding predators such as flatworms.

The growth rates observed among species in this study (4.7 to 12.2 $\mu m \cdot d^{-1}$, Table 2) are within the range previously reported for lampsiline juveniles in recirculating systems with cultured algae as food. For example, *Villosa iris* held in aerated dishes and fed various diet combinations of cultured algae and silt grew at rates of 3.9 to 10.4 $\mu m \cdot d^{-1}$ over 140 d (Gatenby et al., 1997). Juveniles of *Lampsilis fasciola* held in a recirculating system and fed cultured algae for 105 to 112 d grew to lengths of 1.1 to 2.1 mm (mean 1.7 mm) (Steg, 1998) or about 15 $\mu m \cdot d^{-1}$. Comparisons of mean growth rates may be complicated by the high mortality reported in other studies. If the slowest-growing individuals tend to die, then mean growth rates calculated at

the end of the study might be exaggerated in studies with high juvenile mortality.

Higher growth rates of lampsiline juveniles have been obtained using natural water and food supply from ponds or river water passing through ponds. Growth of *L. fasciola* and *V. iris* in flow-through systems with natural water and food was about 13 to 15 $\mu m \cdot d^{-1}$ (calculated from Mummert, 2001 and references therein, and Zimmerman, 2003). Comparisons of growth rates in natural water are complicated by temperature fluctuations of the water supply. *Villosa iris* in Clinch River water grew at 0.809 $\mu m \cdot °C^{-1}\ d^{-1}$ above 15 °C (Beaty and Neves, 2004). This relationship predicts growth of 16 $\mu m \cdot d^{-1}$ at 20 °C.

The growth rates of lampsilines observed in laboratory culture, regardless of food source, appear to be much slower than natural growth rates. Known-age juvenile *Lampsilis rafinesqueana* reached shell lengths of about 10 mm within their first growing season (roughly September to January) after release in the Verdigris River (M. C. Barnhart, unpublished observations). Likewise, *Lampsilis higginsi*, a close relative of pink mucket, reached 8 to 22 mm within 90 d after excystment from the host fish in the St. Croix River and in Lake Pepin (USFWS, 2002). These growth rates are 88 to 242 $\mu m \cdot d^{-1}$, or roughly 20–50 times faster than pink mucket growth observed in the present study.

The disparity between rates of natural growth and growth in culture indicates that one or more important limitations exist in culture. These limitations probably include inadequate diet. The present study supplied only one species of algae as food. Gatenby et al. (1997) obtained a 2-fold range of growth rates with different experimental diets. As noted above, provision of natural water and food enhances growth in culture. Several studies have investigated the significance of a layer of silt (i.e. fine sediment) in the culture of juvenile mussels and these studies have generally shown measurable benefit (e.g. Hudson and Isom, 1984; Gatenby et al., 1996, 1997; Rogers, 1999; Mummert, 2001; Zimmerman, 2003). Both nutritional and physical roles of silt have been suggested. That is, silt may be both a source of food and a medium in which juveniles can burrow and orient for effective feeding.

Another possible factor limiting growth in culture is energy expenditure. Unionids are sedentary filter feeders and are probably normally stationary. O'Beirn et al. (1998) found that juvenile *Lampsilis* and *Villosa* cultured in silt grew faster and also produced byssal thread attachments to the substrate and to each other, while those kept without substrate did not. Possibly juveniles cultured without substrate remain mobile rather than becoming sedentary, thereby expending energy and feeding less continuously. Disturbances caused by periodic handling for cleaning and measurement could also result in increased energy expenditure and slower growth in laboratory culture.

It is notable that the present study provided no silt, yet obtained superior survivorship and comparable or superior growth to many other studies in which silt was provided. The bucket system could be used to investigate the nutritional and physical roles of silt. The design of the chambers prohibits the provision of a layer of fine sediment, which would either pass through or occlude the mesh. However, suspended silt could be provided as a nutritional source, and a layer of coarser particles, such as glass beads or sand, could be provided as substrate without blocking the screens.

5. Conclusions

The bucket rearing system described herein is well-suited to the early culture of juvenile freshwater mussels and to investigations of factors affecting growth. Seven chambers, each containing 2000 juveniles, can be supported in a system having a volume of 18 l and the space requirement of a 5-gal bucket. The system can be replicated economically for use in experimental investigations of culture conditions, as well as for temporary holding and grow-out of propagated juveniles. Six of the eight species of lampsiline mussels tested exhibited survivorship considerably higher than that reported in previous studies, and exceeding 95% at 2 mo in two species. Growth rates were similar to previously reported results in other recirculating systems. Workers with facilities lacking extensive floor space and raceways may appreciate the ability to grow buckets of muckets on a lab bench or table top.

6. Note added in proof

Feeding a mixture of live *Neochloris* and commercial marine larviculture feeds at concentrations of ~50K cells/ml resulted in several-fold higher growth rates of unionids in this system (up to 40 microns/d).

Acknowledgements

I thank Zac Beussink, Bob Brown, Ben Dodd, Scott Faiman, Todd Fobian, Bri Kaiser, Andy Roberts, Charley Scott, and Steve McMurray for assistance. Chris Ingersoll, Paul Johnson, and Dick Neves made useful suggestions that improved the manuscript. Funding was

provided by the Missouri Department of Conservation and the U.S. Fish and Wildlife Service, Cooperative Endangered Species Conservation Fund.

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