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

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#### 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 1 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.

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#### 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 intersti-

tially 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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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  $\mu$ m 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

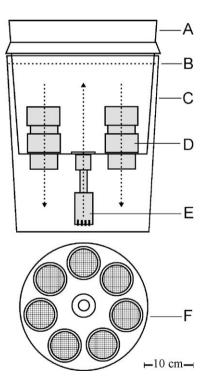


Fig. 1. Bucket rearing system. Dotted arrows indicate the direction of water flow. A. Upper bucket. B. Dotted line indicates water level. C. Lower bucket. D. Chamber (two nested filter cups). E. Power head pump attached to bulkhead through upper bucket. F. View from above upper bucket showing position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

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