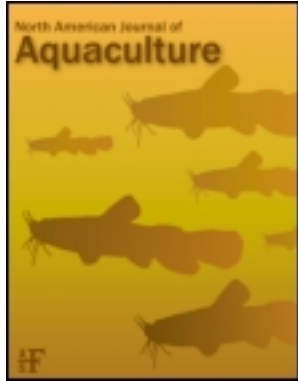


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Performance of a Recirculating Aquaculture System Utilizing an Algal Turf Scrubber for Scaled-Up Captive Rearing of Freshwater Mussels (*Bivalvia: Unionidae*)

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NOTE

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Abstract

To develop a system supporting rapid growth of juvenile freshwater mussels, a recirculating aquaculture system was designed and built at the Laboratory for Conservation Aquaculture and Aquatic Ecology, Virginia Polytechnic Institute and State University. The system included a rectangular trough tank, a sump (biofilter), a pump, a microalgae drip feeder, and an air delivery system. An algal turf scrubber (ATS) was evaluated for its potential to maintain and improve water quality within the system. Growth and survival rates of juvenile rainbow mussels *Villosa iris* after 90 d were compared between system units with and without ATSS. Flow rate through the culture units was approximately 23.3 L/min. Results showed no statistically significant differences between the growth and survival rates of juvenile mussels reared in systems with ATSS and those reared in systems without ATSS. Ammonia and nitrite levels were low and did not differ among treatments. However, systems with ATSS exhibited significantly lower levels of nitrate and phosphate than systems without ATSS. Our results show that freshwater mussel culture systems can be scaled up to increase production and that the use of ATSS may help to maintain

water quality in recirculating aquaculture systems during long-term culture of freshwater mussels.

North America contains the greatest diversity of freshwater mussels in the world—nearly 300 species (Williams et al. 1993; Neves 1999). However, about 70% (213) of the North American species are listed as endangered, threatened, or of special concern, and nearly 35 of these species are considered extinct (Williams et al. 1993; Neves 1999). Causes of population decline include habitat loss and destruction from impoundment of rivers, excessive sedimentation, water pollution, dredging, and other anthropogenic factors that affect the natural structure and function of free-flowing rivers (Neves et al. 1997; Neves 1999; Jones et al. 2005). Conservation of freshwater mussels has become a priority in the United States, and conservation measures include the propagation and culture of endangered mussel species in order to augment existing populations and

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reintroduce mussels into historical sites of occurrence (Jones et al. 2005).

Approaches for improving the survival and growth of cultured juvenile and adult mussels have included rearing in cages, ponds, raceways, and tanks (Gatenby et al. 1996; Dunn and Layzer 1997; Farris et al. 1999; Hanlon and Neves 2006). More recently, recirculating aquaculture systems (RASs) have been used to rear freshwater mussels (O'Beirn et al. 1998; Layzer et al. 1999; Henley et al. 2001; Kovitvadhi et al. 2006, 2008). Culture units for producing freshwater mussels tend to be small (on the order of 30 × 30 cm, with a volume of a few liters), thus limiting the number of mussels that can be produced. Up-scaling of the culture vessels could allow for increased mussel production and the establishment of flow regimes that mimic those of rivers, presenting advantages for providing food to filter-feeding mussels. However, scaling up may also lead to water quality issues (e.g., ammonia or nitrite accumulation) or other technical problems. In this study, we designed and evaluated a relatively large RAS to culture freshwater mussels and we assessed the utility of an algal turf scrubber (ATS) to help maintain water quality in the RAS. An ATS utilizes filamentous algae to take up excess nutrients, such as nitrate and phosphate, which tend to accumulate in aquatic systems (Adey et al. 1993, 1996). As the algae grow, they assimilate nutrients, such as inorganic nitrate, inorganic phosphate, nitrite, ammonia, and ammonium, thereby improving water quality (Veraart et al. 2008). Thus, the purpose of our study was to evaluate water quality in scaled-up RASs with and without ATSs and to assess the survival and growth rates of freshwater mussels reared in these systems.

METHODS

Construction of recirculating aquaculture systems and algal turf scrubbers.—Recirculating aquaculture systems for rearing freshwater mussels were developed at the Laboratory for Conservation Aquaculture and Aquatic Ecology, Virginia Polytechnic Institute and State University (Virginia Tech). The RAS design (Figure 1) included (1) a plastic stock-watering trough that was utilized as the container for substrate and cultured mussels, (2) a sump that also served as a biofilter, (3) a pump,

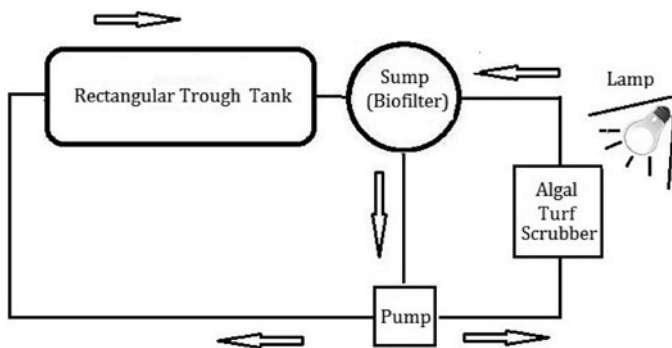


FIGURE 1. Schematic diagram of the recirculating aquaculture system used in the study of juvenile freshwater mussel growth and survival.

(4) a microalgae drip feeder (1-L volume), (5) an air delivery system, and (6) an ATS. The mussel culture trough was made of polyethylene and was 300 cm long, 68 cm wide, and 27 cm deep along the midline; the trough held 330–373 L of water at a depth of 16–18 cm. A magnetically driven pump (Model NH-100PX-X; Pan World Co., Ltd.) generated water flow in the RAS. The tank water volume was exchanged approximately four times per hour (once every 15 min) via a total system flow of 23.3 L/min. Water velocity at the surface along the center line of the trough was 0.77 m/s. Fine sand (<2 mm in diameter) and limestone gravel (<4 mm in diameter) were mixed and used as substrate for the mussels; substrate was placed evenly throughout the trough to a depth of 4–5 cm. Water was recirculated through the trough and sump by using a 3.08-cm polyvinyl chloride (PVC) pipe (1-in schedule-40 PVC) and other plastic tubing. Plastic biomedica (Dynamic Aqua Science, Inc., Laguna Beach, California) was added into the sump tank so that it would function as a biofilter. The nitrification function of the biofilter was not established before the experiment. The ATS was made from plastic mesh (60 cm long × 60 cm wide; mesh size = 1.3 × 1.3 cm) and received recirculated water through a bypass pipe (Figure 2). During the experiment, six RASs were used: three with ATSs (treatment) and three without ATSs (control). Aeration in the system was provided by a Sweetwater regenerative blower (Aquatic Eco-Systems, Inc., Apopka, Florida) and was delivered through PVC pipes, flexible tubing, and an air diffuser. All systems were located in a greenhouse and received natural light—no shade cloth was used. A 60-W lamp was used for nighttime illumination of systems with ATSs.

Food and feeding.—Mussels were fed a commercial algal mix (1:3 ratio) consisting of Nanno 3600 (*Nannochloropsis*) at a concentration of 0.02 mL/L and Shellfish Diet 1800 (*Isochrysis* sp., *Pavlova* sp., *Thalassiosira weissflogii*, and *Tetraselmis* sp.; Reed Mariculture, Inc., Campbell, California) at 0.007 mL/L. The feed densities were approximately 136,000 cells/mL for Nanno 3600 and 14,000 cells/mL for Shellfish Diet 1800. The algal diet was delivered into the system over each 24-h period by using a 1-L drip bottle mounted over the sump. Fresh algal mix was placed in each drip bottle daily at 0900 hours. Each RAS contained a 1:1 mix of pond water and well water, 50% of which was replaced each week.

Experimental design and analyses.—In total, six RASs were used (three with ATSs and three without ATSs). Juvenile rainbow mussels *Villosa iris* (~8 months old; average shell length = 17.3 mm) were reared for 13 weeks during the experiment. Mussels were produced at the Freshwater Mollusk Conservation Center, Virginia Tech. Three-thousand mussels were randomly assigned to troughs (500 mussels/trough); initial stocking density was 245 mussels/m². Thirty of the mussels in each trough were tagged (Hallprint, Ltd., Hindmarsh Valley, South Australia) on the shell surface. Tagged mussels were sampled and measured for length once per week to monitor growth.

Water quality.—Data on ammonia, nitrite, nitrate, phosphate, conductivity, salinity, temperature, pH, and dissolved oxygen

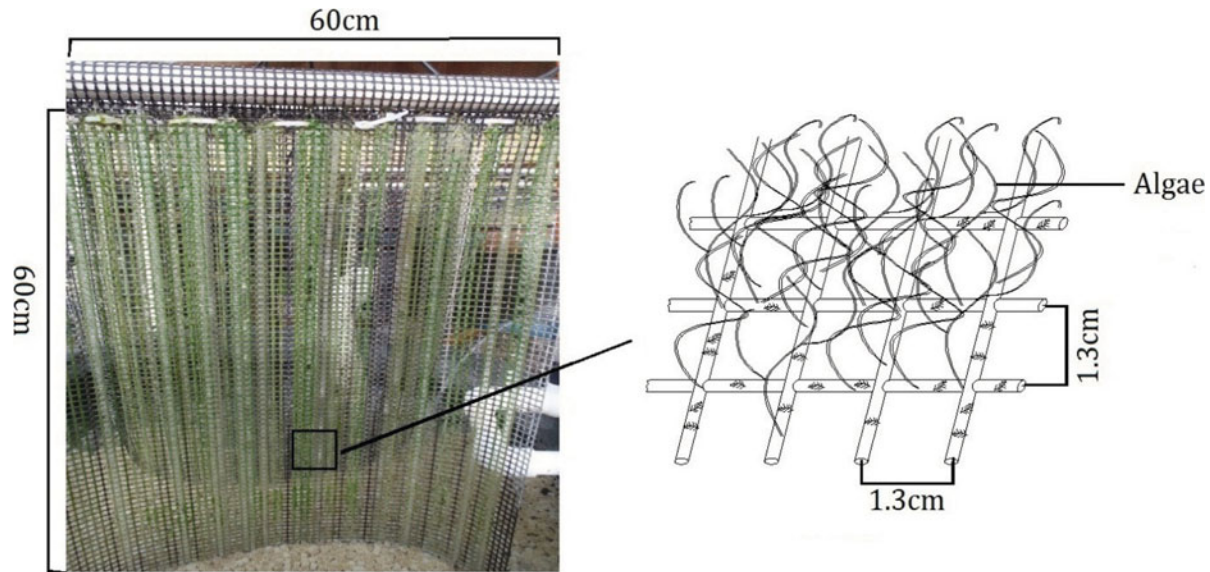


FIGURE 2. Algal turf scrubber with growing algae; the turf scrubber was evaluated for its ability to maintain water quality in recirculating aquaculture systems used for rearing juvenile freshwater mussels. [Figure available in color online.]

were collected from each RAS every other day. Ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, and phosphate were determined using a Hach DR2400 spectrophotometer (Hach Company, Loveland, Colorado). Temperature and dissolved oxygen were measured with a YSI Model 550A dissolved oxygen meter (YSI, Inc., Yellow Springs, Ohio). Conductivity was determined by use of a YSI Professional Plus conductivity meter. Salinity was measured with a salinometer (Model TDS-4TM; HM Digital, Inc., Korea), and pH was determined with a pH meter (Thermo Electron Corp., Waltham, Massachusetts).

Data analyses.—Statistical analyses were performed using JMP version 9 for Windows. Mussel growth was analyzed with repeated-measures multivariate ANOVA (MANOVA) at a significance level α of 0.05. Survival rate of the mussels and water quality in the RASs were analyzed using one-way ANOVA.

RESULTS AND DISCUSSION

Scaling up of freshwater mussel production units can increase the mussel numbers and biomass produced. In the present study, we designed and demonstrated the suitability of an RAS for the grow-out of freshwater mussels, and we obtained excellent survival and growth of juvenile rainbow mussels. Furthermore, suitable water quality was maintained in our RASs, especially in systems that were equipped with ATSSs.

Water Quality

Temperature, dissolved oxygen, pH, ammonia, and nitrite did not differ significantly ($P > 0.05$) between RASs with ATSSs and those without ATSSs (Table 1). At the laboratory site, high pH (~ 8.0) and conductivity ($\sim 420 \mu\text{S}/\text{cm}$) are characteristic

of the well water, which is drawn from a karst aquifer. After the first 3 weeks of the study, ammonia concentrations in both types of system were less than $0.04 \text{ mg}/\text{L}$ (Figure 3), which is considered safe for freshwater mussels (Layzer et al. 1999). It took approximately 25–33 d for the biofilters and ATSSs to become biologically functional—that is, fully capable of processing ammonia, nitrite, and nitrate. For example, ammonia and nitrite levels were maintained below 0.05 and $0.01 \text{ mg}/\text{L}$ after 1 month in RASs with ATSSs and in those without ATSSs, respectively (Figure 3). Therefore, our data demonstrate that the RAS and biofilter together were sufficient for nitrification of ammonia and nitrite to nitrate. However, the RASs with ATSSs were much better at eliminating nitrate, with concentrations

TABLE 1. Water quality in recirculating aquaculture systems (RASs) with algal turf scrubbers (ATSSs) and in those without ATSSs over the 13-week study period (mean \pm SE). Within a row, values with different lowercase letters are significantly different ($P < 0.05$).

Variable	RASs with ATSSs	RASs without ATSSs
Temperature ($^{\circ}\text{C}$)	22.1 \pm 1.60 z	22.6 \pm 1.24 z
DO (mg/L)	8.40 \pm 1.61 z	8.51 \pm 1.74 z
pH	8.72 \pm 0.30 z	8.69 \pm 0.31 z
Conductivity ($\mu\text{S}/\text{cm}$)	437.1 \pm 69.1 z	403.1 \pm 45.8 y
Ammonia (mg/L)	0.034 \pm 0.02 z	0.029 \pm 0.03 z
Nitrite (mg/L)	0.028 \pm 0.06 z	0.056 \pm 0.112 z
Nitrate (mg/L)	0.46 \pm 0.28 z	1.82 \pm 1.07 y
Phosphate (mg/L)	0.71 \pm 0.32 z	1.2 \pm 0.54 y

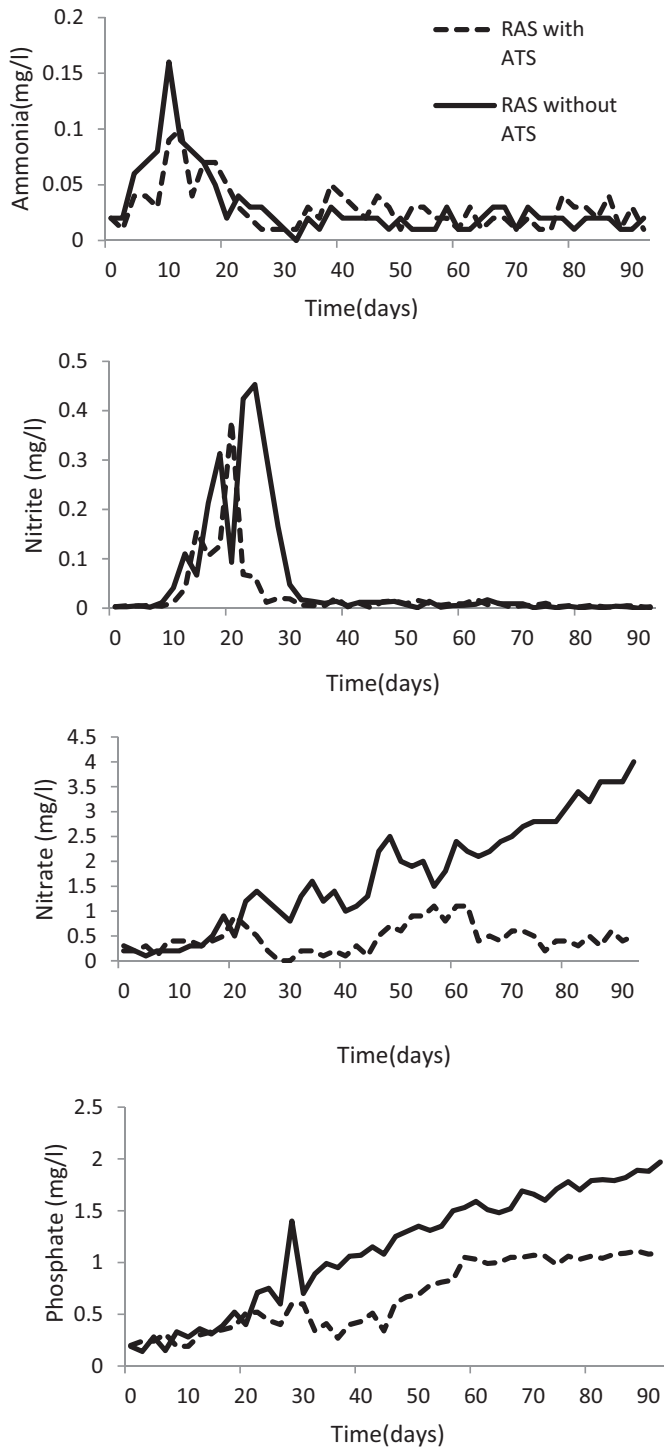


FIGURE 3. Concentrations of ammonia, nitrite, nitrate, and phosphate over the 13-week study period in recirculating aquaculture systems (RASs) equipped with algal turf scrubbers (ATSS) and in those without ATSSs.

remaining less than 1 mg/L over the course of the study; in contrast, nitrate increased from 0.2 to 4 mg/L in RASs without ATSSs (Figure 3). From day 26 to the end of the experiment, nitrate was significantly greater ($P < 0.05$) in RASs without ATSSs,

whereas nitrate in the RASs with ATSSs did not increase. Further, phosphate was also significantly lower ($P < 0.05$) in RASs with ATSSs, increasing from 0.2 to 1.09 mg/L; in RASs without ATSSs, phosphate increased from 0.19 to 1.97 mg/L. Thus, the ATSSs effectively utilized filamentous algae (including species of *Eunotia* and *Melosira*) to absorb both nitrate and phosphate as nutrient sources.

Our results are broadly convergent with those of earlier studies. Algal turf scrubbers have been used successfully to treat multiple types of pollution, including agriculture runoff, excess nutrient accumulation in lakes, and manure effluents (Adey et al. 1993, 1996; Craggs et al. 1996; Mulbry et al. 2008). This was accomplished simply by allowing native algae to grow attached to a screen in a shallow, flowing-water system and then regularly cropping the algae from the screens to permanently remove sequestered nutrients and promote continued algal growth (Adey et al. 1993).

Results from our study showed that water quality variables were maintained below known effect levels for mussels. At a temperature of 25°C and a pH of 8, the acute and chronic criteria for total ammonia nitrogen concentration are 2.9 and 0.26 mg/L, respectively, for freshwater mussels (USEPA 2009). In a study of juvenile fatmucket mussels *Lampsilis siliquoidea*, Myers-Kinzie (1998) reported 48-h LC50 values (concentration lethal to 50% of test organisms) of 0.09 mg/L for ammonia and 0.19 mg/L for nitrite. We found no references on the toxicity of nitrate to adult or juvenile freshwater mussels, but MacMillan et al. (1994) reported that for marine bivalves, nitrite should not exceed 0.01 mg/L and nitrate should not exceed 19.16 mg/L.

The time required to turn over the entire water volume in a culture trough was 15 min given an approximate flow rate of 23.3 L/min for the trough. Although flow requirements for freshwater mussels are likely quite variable among species, many threatened and endangered freshwater mussels are found in riffle habitat, where water velocities are high. Higher flow velocities are likely required for many mussel species, emphasizing the need for further research on flow requirements for cultured freshwater mussels.

Growth and Survival

At the conclusion of the study, mean mussel length (\pm SE) was 22.5 ± 0.46 mm for RASs with ATSSs and 20.8 ± 0.29 mm for RASs without ATSSs. The growth data were analyzed using MANOVA; the results indicated a significant time \times treatment interaction effect ($P = 0.001$) on the mean length of rainbow mussels. Time refers to the time of culture in the RASs; treatment refers to the RASs with and without ATSSs. Mean growth of mussels in the two treatments overlapped for much of the experiment but began to diverge after week 10 (Figure 4), when growth became faster in the RASs with ATSSs than in those without ATSSs. This divergence in juvenile mussel growth may indicate (1) a chronic impact of excess nitrate or phosphate, despite the occurrence of both nutrients at relatively low levels in the RASs; or (2) differing levels of an unmeasured water quality variable.

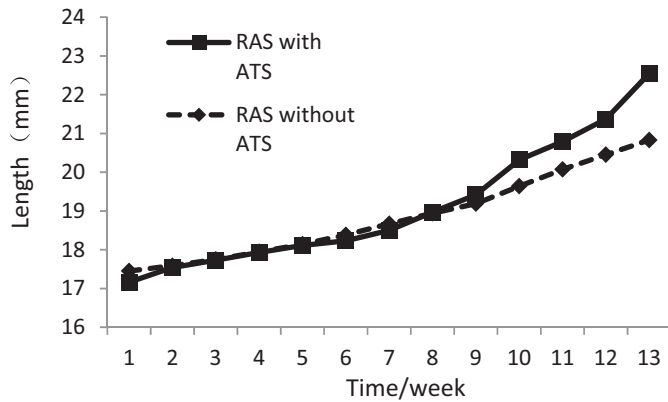


FIGURE 4. Mean growth curves (shell length, mm) for juvenile rainbow mussels that were reared in recirculating aquaculture systems (RASs) equipped with algal turf scrubbers (ATSs) and in RASs without ATSs.

The survival rate of juvenile mussels was 96.2% in RASs with ATSs and 96.8% in RASs without ATSs. Hence, no significant difference in survival was observed between RAS types ($P > 0.05$). Generally, survival rates of the juvenile mussels in our study were higher than or similar to those reported in other comparable studies. For example, O'Beirn et al. (1998) reported a survival rate of 26.8% for juvenile rainbow mussels after 22 weeks in a recirculating trough system. Gatenby et al. (1996) obtained survival rates ranging from 2.7% to 66.5% for juvenile rainbow mussels after 45 d in aerated glass culture dishes containing different types of sediment. Layzer et al. (1999) reared three species of freshwater mussel in a closed recirculating system, and survival rates were over 83%. The high survival rates observed in RASs with and without ATSs during our study may be attributable to (1) the maintenance of suitable water quality in each system type or (2) greater robustness of the older mussels we used.

We regard the results of this pilot-scale trial of relatively large culture vessels for producing freshwater mussels as promising. We recommend evaluation of such systems at higher biomass loadings and over longer time periods, which would allow more rigorous assessment of system design and biofilter and ATS capacity. Development of high-capacity systems will promote production of imperiled species at such a scale that augmentation or restoration of mussel populations of conservation interest can be realized.

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