North American Journal of Aquaculture 63:144–155, 2001

Design and Evaluation of Recirculating Water Systems for Maintenance and Propagation of Freshwater Mussels

WILLIAM F. HENLEY,* LORA L. ZIMMERMAN, AND RICHARD J. NEVES

Virginia Cooperative Fish and Wildlife Research Unit,[1]
U. S. Geological Survey,
Department of Fisheries and Wildlife Sciences,
Virginia Polytechnic Institute and State University,
Blacksburg, Virginia 24061 USA

MARK R. KIDD

Virginia Tech Aquaculture Center,
Department of Fisheries and Wildlife Sciences,
Virginia Polytechnic Institute and State University,
Blacksburg, Virginia 24061 USA

Abstract.—A complete system for culturing, rearing, and holding juvenile and adult freshwater mussels (Unionidae) was designed and evaluated for use at hatcheries and research facilities. The system includes air delivery, water conditioning and delivery, algal culturing and automated algal feed delivery, and air-driven water-recirculating units. These systems are appropriate for holding and conditioning adult mussels for spawning, as well as for the grow-out phase of juvenile rearing. In a 41-d trial without water changes in 350-L water-recirculating units, we used 200 adult eastern elliptios *Elliptio complanata* (average biomass = 1.36 g dry weight/L); mean values (mg/L) during the trial were 0.02 for NH_3, 0.01 for NO_2, and 2.7 for NO_3. An 8-L minirecirculating water unit (MRU) also was designed and evaluated for initial culturing of newly metamorphosed juvenile mussels. Survival of juvenile wavy-rayed lampmussels *Lampsilis fasciola* cultured in the MRU was 27.2% at 2 weeks, but this survival was negatively affected by the presence of a predacious flatworm *Macrostomum tuba*. Survival of the juvenile lampmussels through 14 weeks grow out in the 350-L trough recirculating water unit was 58.8%. We recommend this overall system of holding and rearing adult and juvenile freshwater mussels for use at hatcheries and research facilities because it is effective, low in cost, and easily maintained.

Various methods have been used to promote growth and survival of adult and juvenile freshwater mussels in captivity, including cages, ponds, raceways, tanks, and water-recirculating systems (Coker et al. 1921; Gatenby et al. 1996; Dunn and Layzer 1997; O'Beirn et al. 1998). The effectiveness of these methods has varied among studies. Lefevre and Curtis (1910) used aquaria to rear juvenile freshwater mussels but were not successful at maintaining survival for more than 4 weeks. Coker et al. (1921) reported mixed results for various methods of holding and rearing several species of adult and juvenile mussels in Iowa. These methods included use of floating crates in rivers and ponds, crates with substrate, concrete and earthen ponds, lakes, wooden and sheet metal troughs, concrete tanks, and aquaria supplied with water from a pond. In experiments using these methods, the highest rates of growth and survival of adult and juvenile mussels were reported to occur in ponds, and in troughs and aquaria that were supplied with pond water. Coker et al. reported a survival rate of 32% for adult Lake Pepin mucket *Lampsilis luteola* (= *L. siliquoidea*) after about 1 year.

More recently, Dunn and Layzer (1997) evaluated survival of 20 species of adult mussels that were held at various locations. In these experiments, which lasted up to 24 months, mussels were held in floating pocket nets in hatchery and farm ponds and a lake embayment and were allowed to burrow into sand and gravel substrate in a hatchery raceway. Survival of mussel species held in the farm pond, hatchery raceway, and lake ranged from 85% to 100%, but survival of mussel species in the hatchery pond ranged from 0% to 89%. They attributed low survival of mussel species at the hatchery pond to some untested water quality factor, possibly related to untreated discharge from the hatchery to the pond or extreme water temperature fluctuations during summer. Farris et al. (1999) reported 53.2–58.5% mean 2-year survival

* Corresponding author: whenley@vt.edu

[1] The Unit is supported jointly by the U. S. Geological Survey, Virginia Department of Game and Inland Fisheries, Virginia Polytechnic Institute and State University, and Wildlife Management Institute.

Received February 16, 2000; accepted September 8, 2000

rates for 29 species of adult freshwater mussels held in a spring-fed pond at an Arkansas hatchery. Gatenby (2000) reported a cumulative mortality of six species of freshwater mussels held in suspended cages in hatchery ponds in West Virginia to be 95% after 3 years and hypothesized that this low survival was due possibly to a lack of burrowing substratum and extreme summer temperatures. To determine possible contamination by zebra mussels *Dreissena polymorpha,* Layzer et al. (1999) used a closed water-recirculating system (including tanks, sump, and an unidentified biofilter) to quarantine three species of freshwater mussels (purple wartyback *Cyclonaias tuberculata,* monkeyface *Quadrula metanevra,* and pimpleback *Q. pustulosa*); for the 30-d quarantine preceding transplantation, survival exceeded 83%. MacMillan et al. (1994) reported 22-month survival of 74.8–98.9% for juveniles of the bay scallop *Argopecten irradians,* blue mussel *Mytilus edulis,* eastern oyster *Crassostrea virginica,* European oyster *Ostrea edulis,* softshell *Mya arenaria,* and northern quahog *Mercenaria mercenaria.* Buchanan et al. (1998) reported a low mortality (6.0%) of adult eastern oysters in a closed water-recirculating system after 8 weeks and reported maturation of the gonads of these animals during the trial.

O'Beirn et al. (1998) showed an overall survival of juvenile rainbow mussels *Villosa iris* of 26.8% after 22 weeks using water-recirculating trough units. These units were driven by electrical water pumps and located in a greenhouse. Gatenby et al. (1996) used aerated glass culture dishes to rear rainbow juveniles in different sediment types, and showed survival that ranged from 2.7 to 66.5% at 45 d. Hanlon (2000) compared survival of juvenile wavy-rayed lampmussels *Lampsilis fasciola* in hatchery raceways with survival of juveniles in pump-driven water-recirculating troughs (similar design to those of O'Beirn et al. 1998). Juveniles stocked into raceways in June showed greater 30-d survival (41%) than those stocked in September (14%) and March (4%). Survival of juveniles reared in pump-driven trough water-recirculating units was 89% through 30 d.

From this literature, it is evident that constraints associated with closed water-recirculating systems for freshwater mussels have not been identified. Survival of adult and juvenile freshwater mussels in various captive holding and rearing environments is affected by species differences, type of system used (e.g., ponds, raceways, and water-recirculating units), and time of year that juveniles are held. The variation in results among different studies may be related to inadequate control of environmental conditions, water quality, and inconsistency of food availability to filtering mussels. Another factor that affects survival of juvenile mussels is the mortality bottleneck that occurs during early stages of development, which occurs from approximately 4–8 weeks of age (R. J. Neves, Virginia Cooperative Fish and Wildlife Research Unit, unpublished data). During this period, mortality increases for unknown reasons, but it may be related to such factors as mortality of unfit offspring, food quality and quantity, and unsuitable environmental conditions in captivity. It could also be related to the mode of food presentation during the pedal-feeding and filter-feeding stages of juvenile development (Yeager et al. 1994; Gatenby et al. 1996). Aquaculture system designs for juvenile freshwater mussels that are not guided by the feeding modes of juveniles in these distinct stages may unknowingly contribute to mortality.

In this paper, we present the design of different types of closed water-recirculating units used for holding, conditioning, propagating, and rearing adult and juvenile freshwater mussels, and we evaluate water quality and survival to determine the effectiveness of these units. Several practical considerations influenced the initial design phase of these systems. Our goals were to develop prototype systems, including air and water supply, algal culture and distribution, and aquaculture water-recirculating units, that could be easily maintained with a low probability of breakdown and that would be appropriate for research and production purposes. We evaluated these systems for potential future use at federal and state hatcheries and therefore designed the systems to accommodate constraints at these facilities, such as limited space, limited funding for equipment and personnel. These systems were also designed to be easily modified for future research on nutritional and environmental requirements of juvenile and adult mussels, as well as other invertebrates.

Methods

Water-recirculating units.—Two basic designs of air-driven water-recirculating systems are in use at the Virginia Tech Aquaculture Center, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. One type consists of an interconnected polyethylene feed trough, a polyethylene drum, and polyvinyl chloride (PVC) airlift and return tubes (Figure 1). These systems primarily are used for holding adult mussels and for the grow

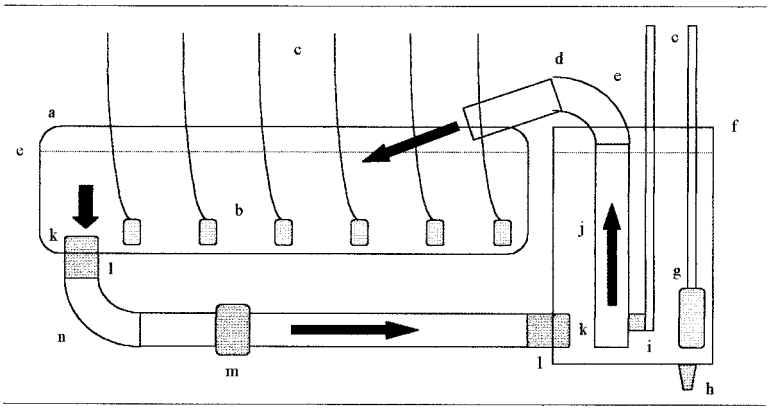

FIGURE 1.—Side-view schematic of the 350-, 200-, and 145-L recirculating trough units; a = trough, b = 5.1-cm air stones, c = 0.95-cm silicone air lines, d = 3.8-cm PVC head piece of airlift, e = 3.8-cm 90° long-sweep elbow, f = drum, g = 22.9-cm air stones, h = 1.9-cm spigot valve for draining the system, i = 1.9 male national pipe thread × barb air injector, j = 3.8-cm PVC tail piece of airlift, k = 5.1-cm bulkhead fittings, l = 5.1-cm female national pipe thread × slip connectors, m = 5.1-cm slip × slip threaded union, and n = 5.1-cm 90° long-sweep elbow.

out of juvenile mussels. There are three variations of this design, but all contain troughs, drums, return tubes, and airlifts. The systems differ primarily in the volume of water contained in them because of the sizes of troughs and drums. The volumes of the three systems are 350, 200, and 145 L. In all of these systems, water enters one end of the troughs via PVC airlift tubes from the drums, travels the length of the troughs, and exits the troughs through PVC piping to return to the drums. In the troughs, a series of 10 air stones promote the suspension of algae in the water column. Also, two air stones are used in the drums to sustain suspension of algae. In all recirculating trough units, the airlifts are inserted through removable drum tops. The inside dimensions of the 350-L system are 3.12 × 0.60 × 0.25 m, those of the 200-L system are 1.40 × 0.60 × 0.25 m, and those of the 145-L system are 1.78 × 0.25 × 0.17 m. The troughs in the 350-L and 250-L systems were purchased from Southern States Cooperative, and the 145-L troughs were purchased from Zeebest Plastics of Canada, Inc. (Edmonton, Alberta). The usable bottom areas of all three troughs are 1.25, 0.48, and 0.34 m^2, respectively. The volume of the open-top drums (Laboratory Safety Supply, Inc., Janesville, Wisconsin) is 113.6 L (8B–18087) for the 350-L troughs. The same type of drum is used with the 200-L and 145-L systems, and its volume is 75.7 L (8B–18088).

Three wooden stands support the 350-L and 145-L trough water-recirculating units. Each stand consists of three levels that contain water-recirculating units. The top and middle levels each have two side-by-side 350-L units, and the bottom levels have four 145-L units, making a laboratory total of 12 of both the 350-L and 145-L units. (In the past, a 200-L recirculating unit was installed on the bottom layer of a stand.) The dimensions of the stands are 3.75 m long × 1.61 m wide × 2.25 m high, and the footprint of one stand is about 6.0 m^2.

The second type of water-recirculating design in our laboratory is used for rearing newly transformed juvenile mussels and is termed a mini-recirculating unit (MRU; Figure 2). The MRU is primarily used for newly metamorphosed juveniles that are younger than 10 weeks of age. The design of the MRU is similar to the design of the three types of recirculating water trough units described above, but it is filled to contain only 8.0 L of water. The MRU consists of two interconnected, tapered cylindrical polypropylene containers (Rubbermaid Commercial Products, Inc., Winchester, Virginia). One container serves as a juvenile rearing tank, the other as a reservoir. Water enters the juvenile rearing tank via an airlift through silicone tubing from the reservoir and exits the tank through a standpipe located at the center of the bottom of the rearing tank. Water returns to the reservoir through silicone tubing. The water entering the juvenile rearing tank is directed by silicone tubing to create a circular flow in the tank to promote suspension of algae. The water in the reservoir is highly agitated with two air stones to suspend algae. The useable area of the container of a MRU

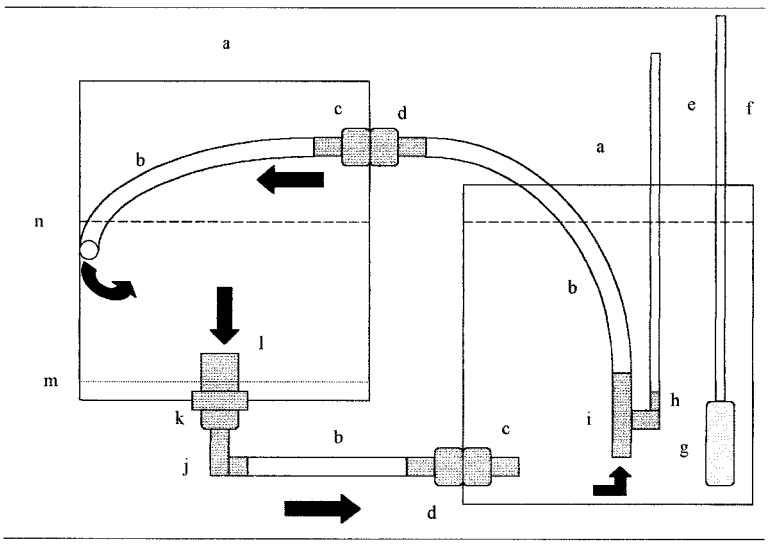

FIGURE 2.—Side-view schematic of the minirecirculating unit (MRU) for culturing juvenile freshwater mussels. System volume is 8.0 L; a = 6.0-L polyethylene containers, b = 1.3-cm silicone water tubes, c = 1.3-cm female national pipe thread × 1.3-cm barb fitting, d = 1.3-cm male national pipe thread × 1.3-cm barb fitting, e = 0.95-cm silicone air tubes, f = 0.64-cm silicone air tubes, g = 5.1-cm air stones, h = 1.3-cm × 0.95-cm connector-reducer, i = 1.3-cm tee barb fitting, j = 90° 1.3-cm female national pipe thread × 1.3-cm barb fitting, k = 1.3-cm bulkhead fitting, l = 1.3-cm male national pipe thread × 1.3-cm slip adapter, m = substrate level, and n = water level.

that serves as a juvenile rearing tank is 248.3 cm^2, and the footprint of one entire MRU is about 0.1 m^2.

At our laboratory, the MRU and 350-L trough units are used for juvenile culture and grow out. When juveniles are first transformed, they are transferred to the MRU for culture. After 10 weeks, juveniles then are transferred to a PVC tray in the 350-L trough unit for grow out. The MRU was designed to allow some algal settling to accommodate juveniles that are in the pedal-feeding stage of development. The grow-out tray in the 350-L recirculating unit is used for juveniles that have shifted to the filter-feeding stage of development. The dimensions of the PVC tray are 0.2 m × 1.2 m × 20 mm, and the useable bottom area is approximately 0.25 m^2. This grow-out tray is filled with approximately 10 mm of course sand and silt substrate. Juveniles are grown in this grow-out tray until they reach stocking size for translocation to regional streams and rivers. Experiments are underway to determine appropriate juvenile sizes for optimal survival after stocking.

Air distribution.—Air is delivered to the 145 and 350-L recirculating units from an outdoor 1.86-kW Sweetwater air blower (Aquatic Ecosystems, Inc., Apopka, Florida) via 3.8-cm PVC pipes. After entering the laboratory, the air pipes are suspended from the ceiling, follow the perimeter of the room, and also trisect it. In other words, the PVC piping forms a distribution grid at the ceiling, so that all areas of the laboratory are provided with air service. The junctions of the grid contain 3.8-cm PVC ball valves, so that sections of the air service piping can be turned off and repaired, without terminating service to the entire laboratory. At about every 0.25-m of the air service grid, 1.9-cm PVC ball valves point downward via 3.8 × 3.8 × 1.9-cm PVC tee connectors. From these PVC valves, threaded 1.9 × 0.95-cm barbed polyethylene nipples have been installed so that air service is provided to the individual trough units through 0.95-cm inside diameter silicon tubes. The MRUs are in a separate room that is serviced by a 0.75-kW Sweetwater air blower. Air service to the individual MRU is routed through a ceiling-suspended 2.5-cm PVC distribution grid similar to the one described above.

Water conditioning and distribution.—The mean values of water hardness for town and well water at our laboratory were 120 mg/L and 370 mg/L as $CaCO_3$, respectively. Town and well waters are mixed at a 1:1 ratio in two interconnected 2,270-L water-conditioning tanks. With this mixture, we

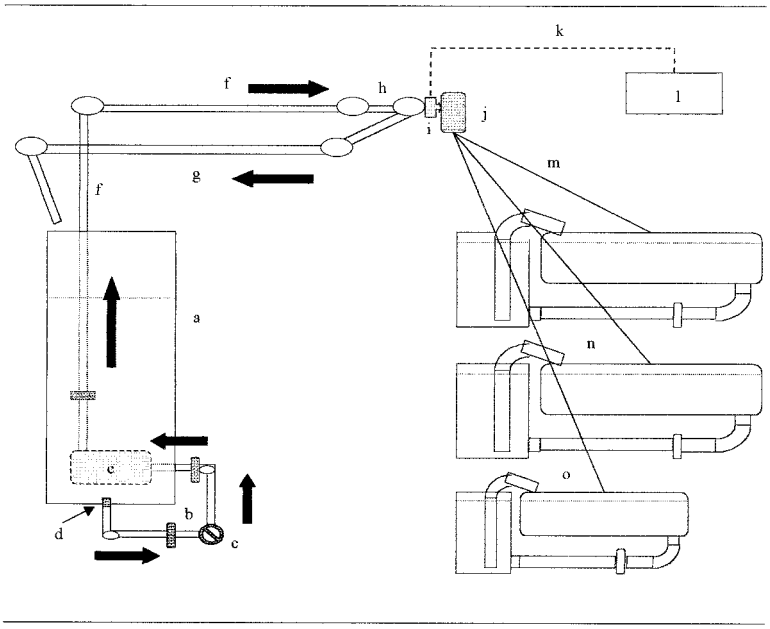

FIGURE 3.—Side-view schematic of the algal distribution system for automated feeding. Each of three stands has four 350-L and four 145-L trough recirculating units; a = 300-L Kalwall algae tubes, b = 2.5-cm PVC slip × slip threaded unions, c = 2.5-cm ball valves, d = 2.5-cm bulkhead fittings, e = 0.25-kW magnetic drive pump, f = 2.5-cm PVC output piping, g = 2.5-cm PVC return piping, h = 2.5-cm PVC rectangular circulating piping, i = solenoid valves, j = 5.1-cm PVC algal distribution manifold, k = electrical wires from timers to solenoid valves, l = repeat cycle timers, m = 0.95-cm inside diameter silicone algal distribution tubing, n = 350-L trough units, and o = 145-L trough units.

attempted to approximate the hardness of water sampled from regional streams in southwestern Virginia, such as the North Fork Holston River and Clinch River, where freshwater mussels commonly are collected. The range of hardness values of these rivers is typically 150–200 mg/L of $CaCO_3$. Water in the conditioning tanks is dechlorinated with sodium thiosulfate in an application ratio of 2.0 mg of sodium thiosulfate to 1.0 mg of chlorine. Water in this conditioning system is recirculated through a Lifegard Ultraviolet Sterilizer (Rainbow-Lifegard Aquarium Products, El Monte, California; model QL-40) via a Teel Jet 0.37-kW water pump (Dayton Electric Manufacturing Co., Niles, Illinois; model 9K679). In addition, water is pumped to destinations in the laboratory through garden hoses. We segregate hoses for pumping clean water and wastewater to minimize potential disease transmission among mussel holding units.

Algal culture and distribution.—Algal rations are fed to mussels in the holding troughs through an algal recirculating system (Figures 3, 4). Algae are cultured in two sets of paired 300-L Kalwall tubes (Kalwalls Aquacenter, Leland, Mississippi), which are fertilized with Kent Pro-Culture F2 Algae Culture Formula (parts A and B; Kent Marine, Inc., Marietta, Georgia). The Kalwall tubes are adjacent to a transparent wall so that natural light is provided for culturing algae. For supplemental lighting, we use wide spectrum fluorescent grow-lights. Algae are delivered to the troughs from one set of Kalwall tubes, and algae are cultured in the other. The Kalwall sets are separated and opened by PVC ball valves.

From the Kalwall tubes, algae are recirculated through a PVC piping system that is suspended from the laboratory ceiling over the holding troughs (Figures 3, 4). Algae are pumped to this water-recirculating system from the Kalwall tubes with a 0.25-kW magnetic pump (Iwaki Co., LTD, Tokyo, Japan; model MD-100 RLT). From the overhead portion of this system, algae are rationed to the individual troughs through 24-V, 60- Hz, 0.32-cm solenoid valves (Dayton Electric Manufacturing Co., Niles, Illinois; Grainger number 6 × 542) through PVC algal distribution manifolds. From these distribution manifolds, algae are routed to the troughs through silicone tubing. The sole-

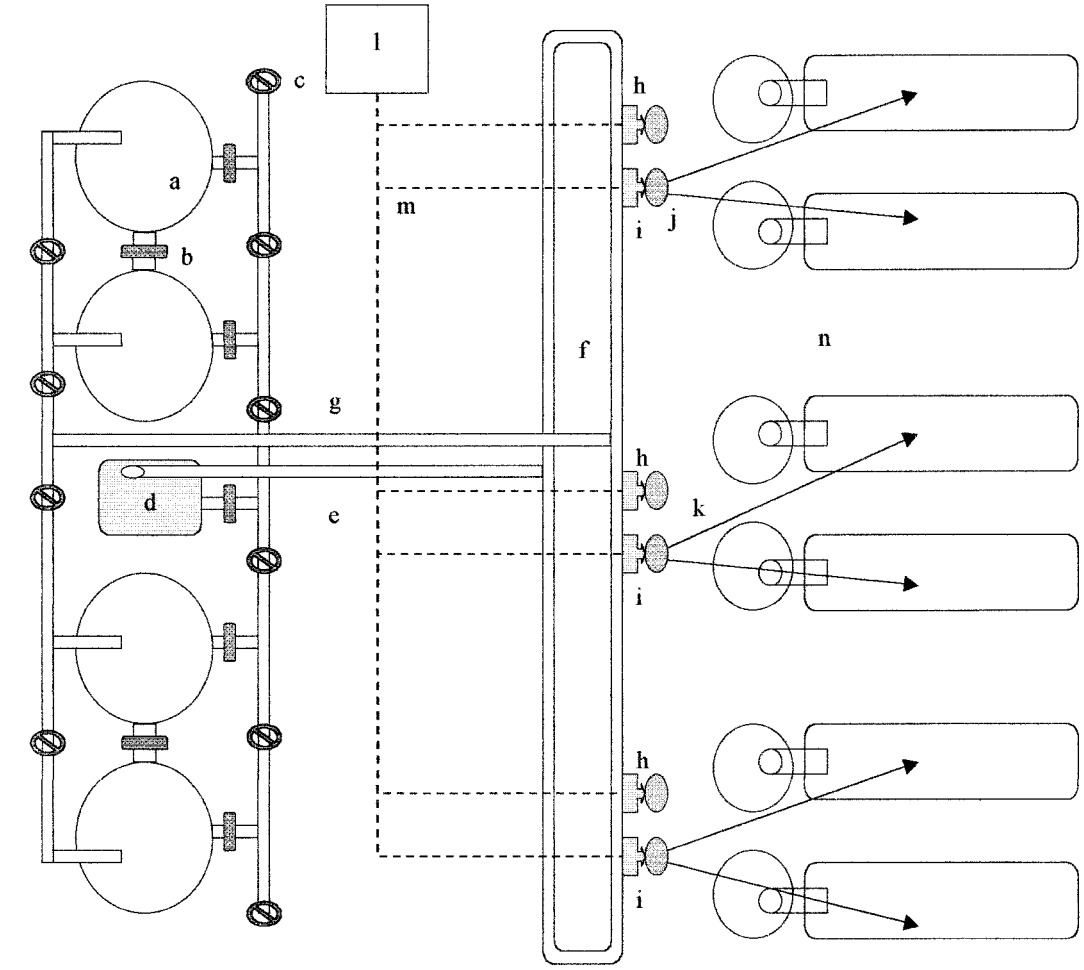

FIGURE 4.—Top-view schematic of the algal distribution system for automated feeding; a = 300-L Kalwall algae tubes, b = 2.5-cm PVC slip × slip threaded unions, c = 2.5-cm PVC ball valves, d = 0.25-kW magnetic drive pump, e = 2.5-cm PVC output piping, f = 2.5-cm PVC rectangular circulating piping, g = 2.5-cm PVC return piping, h = solenoid valves for 145-L trough units, i = solenoid valves for 350-L trough units, j = 5.1-m PVC algal distribution manifolds, k = 0.95-cm inside diameter silicone algal distribution tubing, l = repeat cycle timers, m = electrical wires from timers to solenoid valves, and n = stands with four 350-L trough units and four 145-L units per stand.

noid valves are connected to a repeat-cycle timer (Omron Corp., Kyoto, Japan; model H3CR), so that the number of feeding events per day may be selected and to control the period that the solenoid valves remain open. In the timer housing box, power is transformed and then routed to the repeat cycle timer with a 120–24-V transformer (Steveco, Inc., St. Louis, Missouri; Grainger number 3TZ69). With this timer system, the desired number of algal cells per feeding event, and therefore cells per hour, are provided to mussels in the troughs. Timer settings for the solenoid valves are selected based on desired feed rations and algal cell concentrations in the Kalwall tubes. Each solenoid valve is connected to an electrical switch that allows service to each solenoid valve to be turned on and off.

On a daily basis, we estimate algal cell concentrations by measuring fluorescence (Turner Designs, Sunnyvale, California; model 45F00–05) of chlorophyll *a* in algal samples of *Scenedesmus* sp. from the Kalwall tubes. This method of cell density estimation is based on a regression procedure ($r^2 = 0.973$, $P < 0.0001$) of hemocytometer cell

counts versus fluorescence of chlorophyll *a* (Seppälä and Balode 1998). After measuring fluorescence, we use a macro program (developed by the lead author in Minitab12; Minitab, Inc., State College, Pennsylvania) to determine the solenoid settings in seconds, and thereby the daily algal ration to the troughs. This program determines timer settings by considering the algal cell concentration in the Kalwall tubes (cell/mL), the number of mussels held in a trough, an estimate for the volume of water the mussels filter per hour (mL/h), the desired feed rate (cells/mL), the required cell input to the trough (cells/mL/h), and the desired number of feeding events/h. The 350-L and 145-L troughs are associated with independently timed solenoid valves so that separate ration amounts can be distributed to these units simultaneously, and each solenoid valve can be turned on and off by the aforementioned switches. Each stand of four 350-L and four 145-L trough units is associated with two overhead, timed solenoid valves, one each for the 350-L and 145-L units. The 350-L and 145-L units are connected to separate timers, so that separate rations can be delivered to the two sizes of recirculating trough units. The juveniles in the MRU are not supplied with algal rations in an automated manner; they are batch fed on a daily basis using an algal rate of *Scenedesmus* sp. at 13,000 cells/mL (2.09 mg dry weight; all dry weights determined at 100°C for 24 h; Buchanan et al. 1998). At this algal ration, some of the algae settle to the substrate in the MRU for the pedal-feeding juveniles.

System evaluations.—To investigate the relationship between the holding capacity (mussels/m^2) of the 350-L trough units and water quality, we used 200 adult eastern elliptios *Elliptio complanata*, equaling a density of 160 mussels/m^2 (average biomass = 1.36 g dry weight/L) in one trough. We chose this number of mussels because it exceeded the maximum in-trough density that would be used during normal captive holding of freshwater mussels. The mean length of the mussels used in this experiment was 68.9 mm (SD = 11.1). From June 1997 to August 1997 (41 d), water quality measurements were taken for dissolved oxygen (DO, mg/L), pH, alkalinity (Mg^{++} and Ca^{++}, mg/L), hardness ($CaCO_3$, mg/L), NH^3 (mg/L), NO_2 (mg/L), and NO_3 (mg/L). These measurements were obtained from one treatment trough (with mussels) and one control trough (no mussels), while supplying an algal feed rate of 30,000 cells/mL (2.26 mg dry weight) of *Scenedesmus* sp. to both troughs. During the experimental period, water was not changed in the treatment and control troughs. Because of the small sample size used in this observational experiment, a statistical analysis of the data was not conducted.

During another experiment between October 1997 and February 1998 (5 months), we tracked these same water quality parameters in nine 350-L troughs stocked with 45 adult eastern elliptios (36 mussels/m^2; average biomass = 0.32 g dry weight/L). Thus, 405 adults were used in this experiment; their mean length was 71.3 mm (SD = 6.4). Every 2 weeks during this experimental period, the units were drained, scrubbed with brush and water, and refilled. Mussels were fed *Scenedesmus* sp. at a rate of 30,000 cells/mL during this experiment. We conducted these observations to examine water quality changes over time while normal hygienic procedures were conducted. The water quality tests were conducted using a spectrophotometer (Hach Co., Loveland, Colorado; model DR/2000), and flow rates (L/min) were measured with a doppler flowmeter (Cole-Parmer, Vernon Hills, Illinois; model HFM-1). To analyze these data, we used a two-sample *t*-test for detection of differences among treatment and control means.

To measure the degree to which algae remained in suspension in the 350-L units, we measured algal concentrations (previously described fluorescence method) in eight of these units every 8 h for 48 h (7 sampling events). These observations occurred in July 1999. Four of these trough units contained 40 adult eastern elliptios (32 mussels/m^2; average biomass = 0.27 g dry tissue/L), and the other four troughs served as controls having no mussels. The mean length of the mussels used in this experiment was 68.1 mm (SD = 10.8). Thus, with this experiment we examined the settling of algae over time in these troughs and the effects of filtering of mussels on algal concentrations. During this period, the automated feeding system delivered algae at a rate of 30,000 cells/mL in the water-recirculating units. Above this density of algae, pseudofeces production occurs, suggesting wasteful feeding (W. F. Henley, unpublished data). At each 8-h sample, we also estimated concentrations of algae in the Kalwall culture tubes using the fluorescence method. With these estimations of algal concentrations in the Kalwall tubes, we adjusted the timers connected to the solenoid valves to ensure a delivery close to the desired feed rate of 30,000 cells/mL over the duration of the experiment.

Mean algal concentrations in the treatment and

control groups were compared using a two-sample *t*-test. The expected algal cell concentrations in the control units were compared to the observed concentrations, and the differences in these values were attributed to algal settling. The expected algal cell concentrations for the control units were calculated by estimating the number of algae cells delivered to the units during the experimental period. The mean volume of algae delivered from the solenoid valves to the troughs, as well as the mean volume delivered via the silicone feed lines to the troughs, also were compared statistically using a two-sample *t*-test. The predictive relationship between the timer settings of the solenoid delivery system and the volume output of the silicone feed lines was investigated with linear regression analysis.

To evaluate survival of juvenile mussels in the MRU and the grow-out tray in the 350-L trough, we stocked newly transformed wavy-rayed lampmussels in six of the MRUs. Each MRU was stocked with 125 juveniles (0.5 juveniles/cm^2 of substrate). The rates of survival at 2 and 4 weeks were measured by counting juveniles from each MRU with a dissecting microscope. Also, 65 wavy-rayed lampmussels juveniles, 6 weeks old, were placed in one grow-out tray to assess the appropriateness of this method for promoting survival of older juveniles. We enumerated survivors at 14 weeks of age to assess their survival. During these studies, all juveniles were fed *Scenedesmus* sp. at a rate of 13,000 cells/mL (2.09 mg dry weight). Because no treatment versus control comparisons were conducted in this observational experiment, a statistical comparison was not conducted.

Results and Discussion

Water Quality

The mean flow rates for the 350-L ($N = 12$), 200-L ($N = 1$), 145-L ($N = 8$), and 8-L ($N = 6$) units were 25.2, 25.2, 12.6, and 0.52 L/min, respectively. No appreciable variation was detected within the different designs. The times required for the water volume to recirculate (turnover rate = min/cycle) through the 350, 200, 145, and 8-L units were 13.9, 7.9, 11.5, and 15.4 min, respectively. We found no published research reports on the flow requirements of freshwater mussels. This is an area of research that needs to be pursued vigorously before intensive large-scale holding and conditioning of mussel broodstocks can be undertaken. Many endangered and threatened freshwater mussels are found in riffle habitats with higher velocities. One solution to the possible need for creating higher velocities in the recirculating units described here is to use multiple airlift tubes from larger drums. Also, airlift tubes of greater diameter could be installed. We believe that the development and evaluation of artificial, recirculating riffles is essential to the future success of captive breeding efforts with fast-flow species, such as endangered species of *Epioblasma*.

In the 41-d study of troughs with 200 mussels and without mussels, DO, pH, alkalinity, hardness, and NH_3 were not significantly different ($P > 0.06$), and no mortality was observed in the treatment troughs. The mean values for NO_2 and NO_3 were significantly greater in water samples from the trough with mussels versus those of the control trough ($P < 0.0002$ and $P < 0.01$, respectively). The mean of NH_3 in the treatment trough was only 0.02 mg/L (SD = 0.01); thus, it appeared that the total combined surface area of the trough, drum, and piping was sufficient for nitrification of NH_3 to NO_3. The mean values for NO_2 and NO_3 from the treatment trough were 0.01 mg/L (SD = 0.005) and 2.7 mg/L (SD = 0.7), respectively, and the mean of total ammonia nitrogen (TAN) was 0.10 mg/L (SD = 0.07). The fraction of unionized ammonia (NH_3) was 20% of the mean TAN value; this value was relatively high because of the high pH value (mean = 8.7, SD = 0.01). Despite this, the mean NH_3 value of 0.02 mg/L was well below the water quality criterion (see below). The mean values of other water quality measurements were $CaCO_3$ = 262.6 mg/L (SD = 43.3) , temperature = 18.4°C (SD = 1.8) , DO = 7.8 mg/l (SD = 0.6) , and percentage saturation DO = 83.4% (SD = 4.5).

Water quality criteria for adult and juvenile freshwater mussels are limited; therefore, some guidelines can be extrapolated from criteria for other aquatic organisms. Peterson (1987) found that no freshwater mussels were collected from lakes and streams in New Brunswick and Nova Scotia with pH values lower than 6.0. Hunter (1990) reported significantly lower growth rates and gross fecundity of freshwater pulmonate snails *Planorbella trivolvis* maintained in low pH treatments (4.6 and 4.7) than those maintained at pH values of 7.2 and 7.4. Goudreau et al. (1993) reported a 24-h EC50 of 0.237 mg/L for rainbow juveniles tested with NH_3 and an LC50 of 0.284 mg/L. Hickey and Martin (1999) found a no-observed-effect (NOEC) concentration value for the fingernail clam *Sphaerium novaezelandiae* tested

with of 0.97 mg/L total ammonia. Myers-Kinzie (1998) reported 48-h LC50 values for juvenile fatmuckets *L. siliquoidea* of 0.09 mg/L for NH_3 and 0.19 mg/L for NO_2. No references were found on the toxicity of NO_3 to adult or juvenile freshwater mussels, but Rubin and Elmaraghy (1977) recorded a 96-h LC50 for guppy fry *Poecilia reticulata* of 180–200 mg/L. Also, Westin (1974) reported a 96-h LC50 for rainbow trout *Oncorhynchus mykiss* of 1,360 mg/L of NO_3. For reference, the U.S. Environmental Protection Agency national drinking water standards (maximum contaminant level, MCL) are 10.0 mg/L for nitrate and 1.0 mg/L for nitrite (Van der Leeden et al. 1990). Our results from the study with nine 350-L troughs, each stocked with 45 eastern elliptios, showed that all water quality variables stayed well within these levels of determined effects.

The water quality values measured during our experiments compare favorably with data from other experiments using freshwater and marine bivalves in closed water-recirculating systems. Layzer et al. (1999) reported maximum total ammonia nitrogen (TAN) levels of less than 0.25 mg/L during a quarantine period for unionids. During experiments with marine bivalve juveniles, MacMillan et al. (1994) reported maximum levels of less than 0.01 mg/L for NO_2 and less than 19.16 mg/L for NO_3. In experiments with adult eastern oysters, Buchanan et al. (1998) observed maximum NO_2 and NO_3 values of 1.9 and 4.2 mg/L, respectively; they also observed DO concentrations of 5.6–7.8 mg/L. By comparison, we observed mean values of NO_2, NO_3, and DO of 0.01, 2.7, and 7.8 mg/L, respectively. We conclude from our results that acceptable water quality was maintained for eastern elliptios over the duration of the experiment. For the 41-d test period, carrying capacity exceeded 160 mussels/m^2; and for the mussel density tested, a biofilter was not required to maintain acceptable water quality. Although only eastern elliptios were tested in these trials, we believe that these water-recirculating units can maintain water chemistry values within acceptable limits for most species of freshwater mussels, although further testing is required.

Algal Culture and Distribution

The algal volume did not differ significantly among the timed solenoid valves ($N = 3$, $P = 0.82$), and volume delivery from the silicone feed lines from the solenoid valves to the troughs was not different ($N = 12$, $P = 0.91$). In fact, timer settings were very predictive of volume output from the silicone feed lines ($r^2 = 0.95$, $P < 0.0001$; linear regression). Thus, the timer-solenoid delivery method provided a low-cost system of delivering consistent algal rates to freshwater mussels in the trough units. Daily labor to maintain these units was modest; one technician needed approximately 20 min/d to determine the daily algal cell concentrations in the Kalwall tubes using fluorescence and then to set the timers. On a quarterly basis, the solenoid valves should be inspected for wear and clogging, and with continual use, these valves probably should be replaced yearly.

The algal settling study in the 350-L troughs showed no significant differences ($P = 0.85$) in algal concentrations between treatments (with 40 mussels) and controls (without mussels). In fact, there were no significant differences among trough units regardless of treatment or control status ($P = 0.90$). Comparisons of mean algal concentrations of controls and expected concentrations at 48-h showed that approximately 35% of algal cells input to the control units settled out of suspension. The expected mean algal concentration in the control units after 48 h was approximately 50,157 cells/mL (2.47 mg dry weight), but the observed mean concentration in controls was 32,552 cells/mL (2.29 mg dry weight; samples collected from the tops of the water column in the troughs). The difference between observed and expected concentrations may have been smaller if the samples were collected from the bottoms of the troughs. Possible methods for decreasing the number of algal cells that settle out of the water columns in the troughs include the use of more and larger air stones in the troughs and drums, use of multiple airlifts from the drums, and increasing the inside diameter of the airlift tubes. If the algal supply is adequate at a facility, then the settling of algae in the troughs may not be a problem as long as desired algal concentrations in the troughs are achieved and maintained. However, more algal settling will increase the needed frequency for trough cleaning for hygienic purposes. We emphasize the need for further research to refine designs for keeping a higher percentage of an algal ration in suspension and thereby maximize availability to filter-feeding mussels.

Water-Recirculating Systems

Tests using juvenile wavy-rayed lampmussels showed that the MRU and the grow-out tray in the 350-L trough supported survival. Juveniles in the six MRUs showed mean growth over initial size of 44.0% (SD = 17.5) after 2 weeks and 62.0%

(24.0) after 4 weeks, and the respective mean survival rates were 29.9% (17.0) and 28.5% (16.1). These rates of survival and growth were lower than expected. However, in the sediments in which the juveniles were cultured we found a high density of the predatory flatworm *Macrostomum tuba.* These flatworms are confirmed predators of young bivalves (Sickel 1998) and were observed eating juvenile mussels, so we believe the low growth and survival rates were probably caused by these flatworms. When the juveniles grew large enough to prevent ingestion by the flatworms, survival rates increased. For example, from 2 to 4 weeks of age, the survival rates of the juveniles in the MRU increased to 87.9% (7.5). We believe that when ongoing research allays predation of juveniles by these flatworms, the MRU will be highly effective for the culturing of newly metamorphosed freshwater mussels.

We will be conducting experiments in the future to determine the optimal stocking density of juvenile mussels in the MRU; our experiments will begin with a density of 1,600 wavy-rayed lampmussels per MRU (6.4 juveniles/cm^2 of substrate). Although the results from the grow-out tray in the 350-L trough were inconclusive ($N = 1$), survival rates of juveniles grown to 14 weeks (from 6 weeks) were 80.0%. We believe that this survival rate is high enough to stimulate further use and testing of these grow-out trays.

The 350-, 200-, and 145-L troughs have been used in many ways since their installation in our laboratory. The 350-L units have been employed in various experiments with adult freshwater mussels, including investigations testing different algal diets and experiments on reproductive activity and hermaphroditism. To substantiate the effectiveness of the 350-L unit, a mortality rate of less than 2% was observed (Henley, unpublished data) during the course of diet tests with 405 eastern elliptios that spanned 14 months. Also, during experiments on reproduction using the 350-L troughs, we observed adult wavy-rayed lampmussel and rainbow females that became gravid in captivity. These mussels were collected when gravid, discharged their glochidia, and became gravid again the next reproductive period in the 350-L troughs. Thus, it appears that the 350-L units may be appropriate for use at hatcheries and other facilities when conditioning for spawning is the objective.

We also have used the 350-, 200-, and 145-L units for holding gravid adult mussels on a temporary basis before extracting their glochidia for use in host-fish identification experiments and juvenile production. These units provide a means of holding gravid mussels in a captive environment while supplying them with adequate water quality and food. However, we have observed that the length of time that females retain their glochidia in these units appears to be related to water temperature. Therefore, during warmer months, we prefer to hold gravid mussels in a temperature-controlled Living Stream (Frigid Systems, Inc., Toledo, Ohio) at temperatures below 15°C.

The suitability of the trough recirculating water units for long-term holding (>14 months) with adult freshwater mussels is untested. Although relatively hardy species, such as eastern elliptio, showed sustained survival in these units, it is important to substantiate the long-term utility of these units with more sensitive species. Feeding these species adequate and high-quality food rations is essential. *Scenedesmus* sp. has been cultured easily at our laboratory. In fact, this species eventually contaminated most of the algal monocultures that we have maintained. At other hatcheries and research facilities, other species of algae probably will invade and dominate algal cultures over time. We recommend that the appropriateness of locally predominant species of algae be tested at facilities that use these units for holding of freshwater mussels over various time periods. The maintenance of algal monocultures is labor intensive in open culture systems, such as the Kalwall recirculating system used in our laboratory. Whether predominant resident species or monocultures are used to feed freshwater mussels in these recirculating units, a high quality source of food is essential to support growth and survival. Another source of food for mussels could be natural algae supplied through pond water management. We envision a system where pond water could be circulated through the trough units and believe this method should provide adequate food for mussels on a sustainable basis.

Costs

The total estimated cost of the suite of systems for juvenile rearing and adult holding, including 16 MRU units, water, algae, and air distribution systems, and twelve 350-L and twelve 145-L troughs (three stands) is less than U S $20,000. This estimate also includes the cost of a dissecting microscope for counting juvenile mussels and a fluorometer for computing algal densities. The estimate does not include costs for space, construction labor, daily technical support, and the equip-

ment for fish host identification and juvenile transformation. Because the systems have few mechanical components that can malfunction and because the materials used in construction are very durable, the expected operating life of the systems is long. If the anticipated longevity of the systems is 10 years, then the amortized yearly cost of the systems is approximately $2,000. This amortized estimate does not include yearly replacement expenses for items such as air stones and solenoid valves.

The labor investment for daily maintenance and oversight of these systems is surprisingly small. We estimate that activities such as refilling and dechlorinating the water conditioning tanks, trough cleaning and water changes, cleaning the Kalwall tubes, culturing algae, determining algal cell concentrations in the Kalwall tubes, and setting the timers requires one technician approximately 4 h/d. Thus, the overall costs associated with the construction and maintenance of the systems presented here are relatively low, considering returns such as durability during long-term use, low probability of breakdown, low personnel costs, modest mortality rates in captivity, and low attributed cost per juvenile grown to adequate size for release.

The integrated air, water, feeding, and water-recirculating subsystems that we have presented form a complete system for holding and rearing adult and juvenile freshwater mussels in captivity. These subsystems are reliable, low cost, easily constructed, and require a small labor investment for maintenance. The reliability of these systems is emphasized; in the 3 years that we have been using them, no breakdowns have occurred. The designs of the troughs and MRU recirculating units are simple and can be modified to accommodate the flow requirements of various mussel species. Through modification, these systems allow for the flexible control of water flow, water quality, and feed distribution requirements for captive juvenile and adult mussels. They also can serve as low-cost independent replicates for experimental purposes and are well-suited for use at hatcheries and other propagation facilities.

Acknowledgments

We thank Braven Beaty, Jess Jones, Rachel Mair, and Rebecca Winterringer for their assistance in the construction of the aquaculture systems presented here. We thank Eric Hallerman, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, for his editorial review. This project was funded by the Virginia Cooperative Fish and Wildlife Research Unit and the Asheville Field Office of the U. S. Fish and Wildlife Service. Doug Smith, Zoology Museum, University of Massachusetts, identified the predatory flatworm. Use of particular products, brands, and suppliers does not imply endorsement by the U. S. government.

References

Buchanan, J. T., G. S. Roppolo, J. E. Supan, and T. R. Tiersch. 1998. Conditioning of eastern oysters in a closed recirculating system. Journal of Shellfish Research 17:1183–1189.

Coker, R. E., A. F. Shira, H. W. Clark, and A. D. Howard. 1921. Natural history and propagation of freshwater mussels. U.S. Bureau of Fisheries Bulletin 37:76–181.

Dunn, C. S., and J. B. Layzer. 1997. Evaluation of various holding facilities for maintaining freshwater mussels in captivity. Pages 205–213 *in* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo, editors. Conservation and Management of Freshwater Mussels II: Initiatives for the Future. Proceedings of the Upper Mississippi River Conservation Committee, St. Louis, Missouri. Publications Office of the Illinois Natural History Survey, Champaign.

Farris, J. L., C. D. Milam, and J. L. Harris. 1999. Zebra mussel impacts on freshwater mussels in Arkansas. Arkansas Game and Fish Commission, Nongame Aquatic Section, 1999 Summary Report, Bryant, Arkansas.

Gatenby, C. M. 2000. A study of holding, and feeding requirements for captive care of freshwater mussels (Bivalvia: Unionidae). Doctoral dissertation. Virginia Polytechnic Institute and State University, Blacksburg.

Gatenby, C. M., R. J. Neves, and B. C. Parker. 1996. Influence of sediment and algal food on cultured juvenile freshwater mussels. Journal of the North American Benthological Society 15:597–609.

Goudreau, S. E., R. J. Neves, and R. J. Sheehan. 1993. Effects of wastewater treatment plant on freshwater mollusks in the upper Clinch River, Virginia, USA. Hydrobiologia 252:211–230.

Hanlon, S. 2000. A comparison of reintroduction techniques for recovery of freshwater mussels (Bivalvia: Unionidae) Master's Thesis. Virginia Polytechnic Institute and State University, Blacksburg.

Hickey, C. W., and M. L. Martin. 1999. Chronic toxicity of ammonia to the freshwater bivalve *Sphaerium novaezelandiae*. Archives of Environmental Contamination and Toxicology 36:38–46.

Hunter, R. D. 1990. Effects of low pH and low calcium concentration on the pulmonate snail *Planorbella trivolvis*: a laboratory study. Canadian Journal of Zoology 68:1578–1583.

Layzer, J. B., L. M. Madison, J. R. Khym, and R. D. Quinn. 1999. Developing technology for long-term holding of mussels in captivity. 1998 Annual Report

to the U.S. Fish and Wildlife Service, Asheville, North Carolina.

Lefevre, G., and W. C. Curtis. 1910. Experiments in the artificial propagation of freshwater mussels. U.S. Bureau of Fisheries Bulletin 28:615–626.

MacMillan, R. J., R. J. Cawthron, S. K. Whyte, and P. R. Lyon. 1994. Design and maintenance of a closed artificial seawater system for long-term holding of bivalve shellfish. Aquacultural Engineering 13: 241–250.

Myers-Kinzie, M. L. 1998. Factors affecting survival and recruitment of unionid mussels in small midwestern streams. Doctoral dissertation. Purdue University, West Lafayette, Indiana.

O'Beirn, F. X., R. J. Neves, and M. B. Steg. 1998. Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system. American Malacological Bulletin 14:165–171.

Peterson, R. H. 1987. Influence of water pH on frequency of collection of certain invertebrates during lake and stream surveys in New Brunswick and Nova Scotia. Department of Fisheries and Oceans, Canadian Technical Report, Number 1523, St. Andrews, New Brunswick.

Rubin, A. J., and G. A. Elmaraghy. 1977. Studies on the toxicity of ammonia, nitrate, and their mixtures to guppy fry. Water Research 11:927–935.

Seppälä, J., and M. Balode. 1998. The use of spectral fluorescence methods to detect changes in the phytoplankton community. Hydrobiologia 363:207–217.

Sickel, J. B. 1998. Gluttonous feeding behavior in the rhabdocoel, *Macrostomum* sp., induced by larvae of the Asiatic clam, *Corbicula fluminea.* Journal of Freshwater Ecology 13:135–137.

Van der Leeden, F., F. L. Troise, D. K. Todd. 1990. The Water Encyclopedia, 2nd edition. Lewis Publishers, Inc, Chelsea, Michigan.

Westin, D. T. 1974. Nitrate and nitrite toxicity to salmonid fishes. Progressive Fish-Culturist 36:86–89.

Yeager, M., D. S. Cherry, and R. J. Neves. 1994. Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae). Journal of the North American Benthological Society 13:217–222.