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A protocol for the salvage and quarantine of unionid mussels from zebra mussel-infested waters.

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ABSTRACT: In 1995, a quarantine facility was assembled on Middle Island, of the USFWS Ohio River Islands National Wildlife Refuge, that could hold several thousand unionids salvaged from the zebra mussel-infested Ohio River. The facility was supplied with well water and equipped with fourteen, 500 L tanks and aerated by a 0.5 hp regenerative blower. Twenty-seven hundred unionids of 6 species were collected in 1995, scrubbed to remove zebra mussels, wrapped in wet burlap, and transported 1-3 h in ice-cooled containers to the quarantine facility. Unionids were fed 10 L of a dense algal suspension 3 times weekly. Unionids were quarantined for a minimum of 30 d, reinspected for zebra mussels, and then relocated to pond refugia if uninfested. Ninety-seven percent of unionids survived the summer 30 d quarantine. Suggestions for salvage and quarantine of unionids include: (1) collect unionids when glycogen reserves are high and during cool months when metabolism is low, (2) keep unionids cool during handling and transport, (3) check for zebra mussels every 7 d to shorten the quarantine period to <30 d, and (4) feed unionids twice daily to maintain their condition.

Keywords: Ohio River, salvage, protocol, transportation, quarantine.

Over 60 mussel species (Unionidae) are at risk of extinction in the U. S., and another 12 species support a declining commercial harvest of shells for the cultured pearl industry in Asia (Williams *et al.* 1993). Habitat degradation, toxicological effects of chemical pollution from municipal, agricultural, and industrial effluents, and the destruction of mussel beds are responsible for the observed decline in unionid populations. In addition, the invasion of the zebra mussel (*Dreissena polymorpha*) has the potential to wipe out many unionid populations (Neves 1993). For example, the lower Ohio River and its native unionid fauna have become heavily infested with zebra mussels to the extent that several states fear the eventual loss of many unionid beds and likely entire populations from the river (Chaffee 1993).

Extreme longevity, an unusual reproductive cycle, high juvenile mortality, and a sedentary lifestyle make unionids highly susceptible to perturbations (McMahon 1991). Because of these life history traits, unionids do not rapidly recover once populations have been depleted. One suggestion for perpetuating populations of unionids heavily infested by zebra mussels has been to transfer some of these species to temporary refugia (free of zebra mussels) and determine whether controlled propagation is a feasible alternative to sustain populations (Cope and Waller 1995). If captive animals can serve as broodstock, then a source of glochidia for induced host-fish infestations and juvenile culture will be available for stock enhancement of natural populations and for re-establishing endangered species. The immediate threat posed by zebra mussels in the Ohio River led biologists at Virginia Tech, United States Geological Survey (USGS), United States Fish and Wildlife Service (USFWS), Ohio Biological Survey (OBS), and West Virginia Department of Natural Resources (WVDNR) to establish as a management priority the salvage of Ohio River unionids. As a consequence, a quarantine facility, was assembled in 1995 on Middle Island, Ohio River Islands National Wildlife Refuge (ORINWR) in St Mary's, West Virginia. Salvaged unionids from the zebra mussel-infested Ohio River were quarantined at this facility prior to being relocated to experimental refugia. The long-term goal of this research is to determine the feasibility of using ponds as refugia for maintaining broodstock and for preserving species from possible extinction. The objectives of this study were: 1) to develop a protocol for the collection and quarantine of unionids that prevented the spread of zebra mussels into uninfested refugia, 2) to construct a facility that housed up to 10,000 unionids, 3) to conduct a literature review of water quality parameters and make recommendations for holding unionids in captivity, and 4) to develop on-site algae cultures for feeding unionids. Information on the effect of nutritive stress (starvation) on unionid energy reserves and maintaining condition of

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unionids in quarantine facilities was recently provided by Patterson *et al.* (1997) and Patterson (1998). We provide suggestions for improving each phase of the salvage and quarantine process, and a summary of recommendations for the salvage and care of quarantined unionids is provided in concluding paragraphs.

Protocol for Quarantine

Acquisition and handling of unionids

Beginning in July of 1995 and again in September and October, 1995, a total of 2,693 mussels of six species (630 *Amblema plicata plicata,* 731 *Quadrula pustulosa pustulosa,* 600 *Elliptio crassidens,* 481 *Pleurobema cordatum,* 137 *Obliquaria reflexa,* and 114 *Potamilus alatus*) were collected from the Ohio River. These unionids were targeted because they were big-river species found in zebra mussel-infested waters, and could be collected in large numbers for experimental tests designed to determine the feasibility of using ponds as refugia for unionids threatened by the invading zebra mussel. Information recorded at the collection sites included: method of collection (scuba or brail), river mile, water temperature, substrate type, and depth.

Unionids were collected using SCUBA at river miles 170, 176, 238, and 292.5 in 1995 (zebra mussel density ranged from 0 to 200 m^{-2}). All unionids were examined for zebra mussels. They were hand-scrubbed on site with plastic-bristled brushes, scrubbing pads, and scraped of any attached zebra mussels and byssal threads. Shell-damaged unionids (broken hinges, crevices in the ventral margin) could contain veligers or juveniles that escaped initial inspection. Special care was taken with these specimens, and broken-shelled individuals were avoided to reduce the likelihood of infestation in the quarantine facility. Unionids were kept in large mesh-bags in a shaded area of the river to keep specimens cool until they could be cleaned, tagged, and measured. They were transferred to 20 L buckets when scrubbed and tagged. Water temperature was monitored, and buckets of unionids were frequently iced to keep temperatures less than 28 °C similar to July river temperatures. The water also was renewed to replenish oxygen. Unionids were tagged (Hallprint Pty. Ltd., South Australia, Australia) in order to follow survival and condition of individuals over time. A small area of shell was scrubbed with an abrasive pad, and acetone was applied by cotton swab over this area to prepare a dry surface for the glue to set. A very small drop of "super or crazy glue" was placed on the shell. The tag was applied with some pressure for 2 min. to ensure the glue had set. The unionid remained in air (8 min.) until the glue had dried. Total emersion time during the tagging process was approximately 20 min. Zebra mussel-free water was not available at our remote collecting sites. Scrubbed unionids were returned to a shaded area of the river (in mesh bags) until transportation to the quarantine site. Placing unionids in the river was a convenient holding method prior to transport, but could allow reinfestation by zebra mussel veligers present in the river, depending on the time of year. If possible, scrubbed unionids should be held and transported in fish trucks with aerated, clean (zebra mussel-free) water to avoid re-infestation of unionids, and reduce stress (see Transportation). In autumn, unionids were scrubbed on site; water temperature in the buckets was below 20 °C, similar to ambient conditions in the river. Scrubbed unionids were held in the river until transfer, and then tagged while in quarantine.

Transportation

The physiological effects of transportation to the laboratory and subsequent acclimation to aquaria or other holding facilities are relatively unknown in freshwater bivalves. It is known that in unionids experiencing respiratory or metabolic acidosis as the result of emersion or certain environmental pollutants, $CaCO_3$ reserves are dissolved to buffer protons, and calcium levels in the haemolymph increase (Byrne and McMahon 1991). The effects of emersion, anoxia, and hypoxia differs among bivalves (Dietz 1979, Chen 1998). Many unionids may experience short periods of emersion due to receding waters in drought conditions or post-flood conditions. These species may be better adapted to certain environmental stresses. Dietz (1979) reported that handling of *Ligumia subrostrata* altered the ionic concentrations in unionid body fluids, indicating stress. However, he also showed that *L. subrostrata* survived over 40 d in moist air but survived only 5-7 d in anoxic water. Analyses of the body fluids showed that the high body fluid solute concentration was due to water loss and not a build-up of metabolic products. Pekkarinen and Souranta (1995) found that *Anodonta anatina*, typically a lacustrine species, experienced stress from 15-20 min. waiting time in a bucket of river water in which they were partially emersed before transport to the laboratory. Further storage and transport in river water resulted in increased glucose and calcium concentrations in the body fluids (haemolymph and extrapallial). Calcium levels decreased to near normal levels after 2 wks, but were still elevated after 2 months acclimation in the laboratory. The authors suggested that the degree of normalization in calcium levels depended on the season and stage of the reproductive cycle. Englund and Pynnonen (1995) showed that *A. anatina* transported for 5 h without water under summer temperatures (20 °C) showed higher increases in haemolymph calcium concentrations than unionids transported in water at 20 °C and unionids transported at 1 °C with ice (cold but moist air). In all transferred unionids, the haemolymph solute concentration was significantly higher than that measured in

the field site. Unionids transferred in moist air with ice were assumed to have lowered their metabolism, which reduced the effect of transportation stress. After 17 d in the laboratory, these calcium levels were at or below normal, and unionids acclimated in sediment recovered from the transfer faster than those without sediment.

Hypoglycemia indicates stress in fish (Heath 1995), but the exact mechanisms leading to hypoglycemia in unionids are not completely understood (Chen 1998). Most physiologists assume the increase in glucose results from the mobilization of glycogen to meet energy requirements. The ability to tolerate hypoxia and the anaerobic metabolic capacity of bivalves is related to their glycogen levels (Hochachka 1982). The condition (amount of glycogen) of the unionid at the time of transportation, therefore, also may influence the extent of the physiological stress experienced in transfer and acclimation to a laboratory setting. In addition, some bivalves can reduce their metabolism in response to emersion (Wang and Widdows 1993). Pora *et al.* (1969) showed that oysters held in moist air at 15 °C for 23.5 h, with a daily 30 min bath in hypercalcic seawater (Ca content double that of seawater), lowered their metabolic rate which increased survival compared to oysters held in water and starved over 2 wk. Finally, Waller *et al.* (1995) reported that unionids exposed to the atmosphere for 4 h (and less) had greater survival 4-5 months in the river post-handling than those exposed to the atmosphere for 8 h. Thus, transportation in moist air for short periods (< 4 h) may not adversely affect unionids if glycogen reserves are high. In 1995, we transported unionids out of water for 1-3 h. They were covered in moist burlap/towels. Ice was placed on top of the wet burlap (not directly on unionids) to keep unionids cool and lower metabolic activity. The ice was presumed to be chlorinated and was kept in plastic bags. Waller *et al.* (1995) also showed that unionids handled in October, when temperatures were cooler had greater survival than those handled in June. Collecting unionids when metabolic activity is lowest and conditon is high, therefore, is recommended to minimize stress. The condition of mussels depends on its reproductive status and metabolic activity, and likely varies among species. Some long-term brooders may abort glochidia if collected in autumn; however, gravid *Villosa iris* will hold glochidia in the laboratory for months if temperatures are kept between 12°C-16 °C (C. Gatenby, personal observation). Further research to better understand the long-term effects of handling and transfer stress is needed for species being considered for relocation.

Quarantine facility

The quarantine facility was assembled in June 1995. Groundwater seeping from the Ohio River into a well provided the water source. The facility consisted of fourteen 500 L fiberglass tanks, with insides epoxy-painted or coated (donated by the Aquatic Ecology Lab, Leetown Science Center (LSC), USGS, and the Bowden National Fish Hatchery, USFWS). Because bacterial contamination is often a problem in hatcheries, all donated equipment was washed carefully with a mild, biodegradable detergent and freshwater. An airline was plumbed to each tank and upwellers were added to circulate the water (Figs. 1 and 2). Upwellers conveniently fitted into the drain-hole (inside tanks), which stablilized the upwelling pipe inside the tank. A 0.5 Hp regenerative blower (Sweetwater Model S-31, Aquatic EcoSystems Inc., Apoka, FL) delivered enough air to aerate all 14 tanks. An air cooling line, 1.5 m in length made from 3.75 cm galvanized pipe, was added to dissipate the heat generated from the blower because air temperature from the blower exceeded 40 °C. All tanks were plumbed to a common drain line with a gravity flow discharge; drain lines from each tank to the common line were fitted with ball valves. Only Schedule 40 polyvinylchloride (PVC) piping was used.

Preparation for quarantine

Because unionids were held in the river prior to transport, they were rinsed with a high pressure hose before being placed in the quarantine facility. After 30 d, each unionid was hand-inspected with a 4X magnifying glass and direct light. In November 1995, zebra mussels were found after the 30 d quarantine. These unionids repeated an additional 30-90 d in quarantine. This probably reduced their energy reserves, because the facility did not have adequate amounts of algae for feeding unionids in late autumn (Patterson *et al.* 1997). We recommend the following reinspection procedures to avoid additional time in quarantine. Unionids should be reinspected and rescrubbed for zebra mussels that may have attached to unionids held in the river awaiting transportation. Careful inspection of the umbo area, crenulations of sculptured shells, and the ventral margin where new shell material is produced is advised. This is extremely time-consuming, and the condition of mussels could be adversely affected if held out of water for too long, held without food, or at elevated temperatures. Therefore, mussels should be rinsed and placed in clean tanks containing aerated water and food. Subsequently, mussels can be hand-inspected, rerinsed with a high pressure hose, and placed in a quarantine tank. All tanks containing potentially contaminated unionids should be sterilized with 25 mg L^{-1} chlorine and a mild detergent prior to reuse.

When the air temperature of the ice-cooled containers was more than 5 °C colder than the quarantine tank water, the mussels were placed in cool well water and gradually warmed to ambient temperature. A temperature change of 5 °C is lethal to most fish (Romaire, 1985), and acclima-

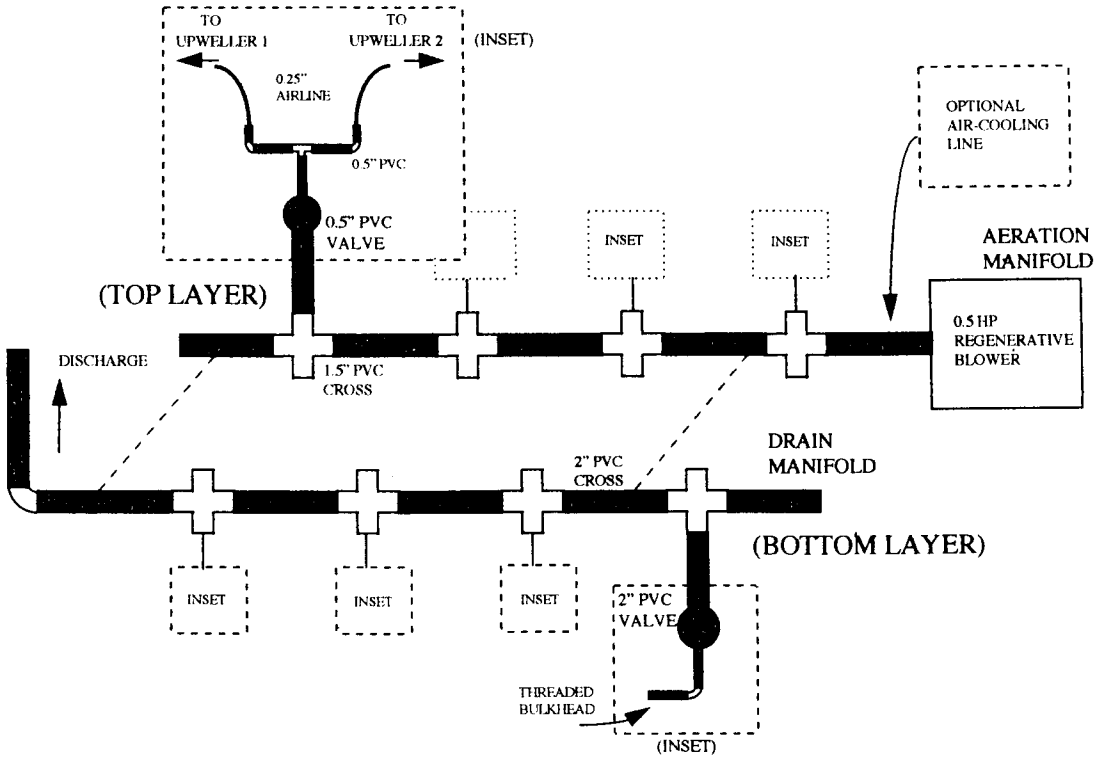

Figure 1. Middle Island quarantine facility: tank design with aeration and drain manifolds.

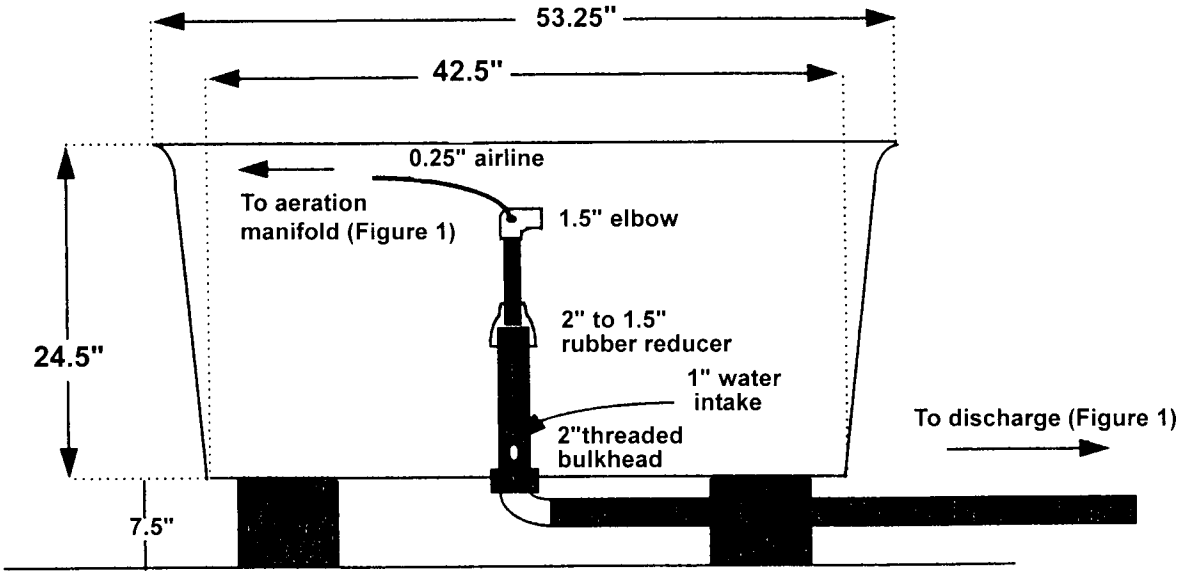

Figure 2. Tank (500 L) with removable upwelling tube.

tion of 2 °C per hour is recommended (G. Libey, Aquaculture Center, Virginia Tech, personal communication). Shumway (1996) reported that the rate of change in water temperature had a greater effect on survival and physiology of oysters than absolute temperature. The temperature of the water from the well at the facility was sometimes 13 °C. The tanks were filled 2 d previous to collecting the unionids, so that river temperatures were similar. Acclimation of unionids entering the quarantine facility was rarely necessary.

The ORINWR quarantine facility is located on a zebra mussel-infested portion of the Ohio River, but it is supplied with clean well-water. Potentially contaminated water, therefore, was drained on site without concern for further spread of zebra mussels into an uninfested watershed. Brushes, buckets, tanks and miscellaneous equipment were sterilized with a concentrated chlorine solution (25 mg L^{-1}). The insides of the tanks also were scrubbed clean with a chlorine solution, and the equipment was allowed to dry for up to 4 d before reuse (Brown and Gratzek 1980).

Quarantine water quality

Little information exists on the water quality requirements of unionids. Imlay (1973) reported that potassium (K) > 4 mg L^{-1} was lethal to unionids. Alkalinity below 15 mg L^{-1}, hardness less than 47 mg L^{-1}, and pH < 6.1, have been reported tp inhibit unionid growth (Matteson 1955, Clarke and Berg 1959, Harman 1970). Grantham (1969) found no mussels alive where DO occasionally dropped below 3 mg L^{-1}. Adults and juveniles of several unspecified unionid "riffle species" required 2.5 mg L^{-1} dissolved oxygen (DO) for survival at summer temperatures, but required at least 6 mg L^{-1} DO for normal growth (Imlay 1971). Mouthon (1996) reported that DO less than 7 mg L^{-1} had a limiting effect on freshwater molluscs in water of pH 8.1-8.2. Chen (1998) showed that the responses to low DO varied between unionid species.

A water chemistry analysis indicated that the pH (7.0), hardness and alkalinity (90 mg L^{-1}), and potassium levels (1.6 mg L^{-1}) in the well water of our quarantine facility were suitable for holding unionids. We set a minimum threshold of 7.0 mg L^{-1} DO for maintaining unionids. Dissolved oxygen was measured twice daily (morning and late afternoon), and ranged from 6.0 - 14.0 mg L^{-1} during the quarantine periods. Freshwater was added to the tanks every 2 d, and dissolved oxygen increased with aeration.

Temperature tolerances vary among species of unionids; specifically, 29 °C was lethal for most *Anodontoides ferussacianus* tested, whereas most *Pyganodon (Anodonta) grandis* and *Lampsilis radiata luteola* survived (Salbenblatt and Edgar 1964). *Anodontoides ferussacianus* is typically found in lotic environments, whereas the other two species can be found in a variety of habitats from riverine pools, reservoirs, and lakes to fast flowing streams (Bright *et al.* 1990). Lake-adapted and river-adapted species will likely have different temperature tolerances. Bayne *et al.* (1973) measured the scope for growth of the marine mussel, *Mytilus edulis*, at various temperatures and distingished between a "zone of tolerance" (Fry 1947) within which the effects of temperature were minimal, and a zone of stress within which temperature had a deleterious effect on growth. Between 10 °C-20 °C, scope for growth was constant; between 20 °C-25 °C, scope for growth was impaired, signalling effects of stress. We set the upper threshold temperature for holding unionids at 28 °C (similar to river temperatures in July).

Temperature was monitored twice daily (morning and afternoon). The summer temperatures in the quarantine facility ranged from 13 °C-27.5 °C. The temperatures in autumn ranged from 2 °C-18 °C. The greatest temperature change occurred in early November when the temperature dropped from 14.5 °C to 2.0 °C in 2 d. We saw no evidence of temperature shock from this change. However, we know little about the effect of cold temperatures on unionids. On December 8, 1995, the water temperatures dropped to freezing and several hundred unionids died because ice formed at the bottom.

Unionized ammonia (NH_3) is toxic to most aquatic organisms, but the ammonium ion (NH_4^+) is relatively nontoxic except at extremely high concentrations (Downing and Merkens 1955). Aquatic invetebrates are more sensitive to NH_3 than vertebrates, and the trend in sensitivity to NH_3 seems to be aquatic insects < molluscs < fish (Arthur *et al.* 1987, Hickey and Vickers 1994). Temperature and pH regulate the proportion of total ammonia that occurs in the unionized form in freshwater, yet the pH has the greatest influence. As temperature and pH increase, there is proportionately more toxic ammonia (Emerson *et al.* 1975). At pH 7 and 26 °C, the percentage of TAN in unionized form is usually 0.60 %; whereas, at pH 8 and 26 °C more than 5% is unionized. This level is lower than the previously mentioned NH_3 levels (0.09-0.33 mg L^{-1}) reported to affect survival and growth in several molluscs. Therefore, we set a conservative upper threshold for NH_3 at 0.025 mg L^{-1}. Where temperatures are not lower than 5°C and pH values not higher than 8.0, the European Inland Fisheries Advisory Commission recommends a concentration less than 0.025 mg L^{-1} NH_3 in salmonid waters (Solbe 1988). Toxic levels of NH_3 for short-term exposure reported for pond fish were between 0.6 - 2.0 mg L^{-1}, and sublethal levels occurred at 0.1 - 0.3 mg L^{-1} (Boyd 1979). Zische and Arthur (1987)

reported that the lowest NH_3 levels affecting survival, growth, and reproduction in *Musculium transversum* were 0.09 - 0.16 mg L^{-1}. At pH 7.8 - 8.0, temperature 25°C, and total ammonia nitrogen (TAN) levels of 5 mg L^{-1}, unionized ammonia (NH_3) levels of 0.27 mg L^{-1} were reported lethal to unionids after 7 d exposure (Horne and McIntosh 1979). More recently, Hickey and Vickers (1994) reported acute toxicity in *Sphaerium novaezelandiae* after 96 h exposure to NH_3 levels of 0.33 mg L^{-1}. Goudreau *et al.* (1993) reported 50% mortality in glochidia of *Villosa iris* exposed to 0.284 mg L^{-1} of unionized ammonia. The summer temperatures in the quarantine facility ranged from 13°C - 27.5°C and pH ranged 7.2 - 8.0. If TAN reached 1.0 mg L^{-1} in the quarantine tanks, NH_3 would range from 0.002 - 0.066 mg L^{-1}. We measured TAN daily using a freshwater ammonia kit (Mydor, Ft. Lauderdale, Florida). We changed the tank water whenever TAN reached 1.0 mg L^{-1}, temperature reached 27.5° C, or every 2 d to avoid potential accumulation of ammonia and other metabolites. TAN reached 1.0 mg L^{-1} twice in the summer in 2 tanks containing a few dead unionids, and the water was changed immediately. The temperatures in autumn ranged from 2°C - 18°C, and pH ranged from 8.1 - 8.5. By adopting a protocol of changing the water every 2 d, TAN was rarely over 0.25 mg L^{-1}, with the exception of the previously mentioned 2 days in summer, and therefore, NH_3 levels did not exceed 0.025 mg L^{-1}.

If a quarantine facility is supplied by chlorinated city water, dechlorination is necessary. Chlorine is extremely toxic to aquatic species at concentrations as low as 0.1 mg L^{-1} (Boyd 1982), and the amount of total residual chlorine depends on pH (Romaire 1985). The oyster, *C. virginica* exhibited 50 % mortality after 48 h exposure to 0.026 mg L^{-1} total chlorine residuals (Roberts and Gleeson 1978). Glochidia of *V. iris* showed 50% mortality after 24 h exposure to 0.084 mg L^{-1} monochloramines (a chlorine residual) (Goudreau *et al.* 1993). Because our quarantine facility was supplied with well water, dechlorination was not necessary. Depending on the volume, water can be dechlorinated with one to several days aeration (Spotte 1979).

Algae culture

Lighting inside the quarantine building was not sufficient for indoor algal culture. Thus, in 1995 one epoxy-painted 500 L tank was set outside to take advantage of sunlight. Nitrogen (N), phosphorus (P), and light are usually the most limiting factors to algae growth. An N:P ratio of at least 10:1 will lead to a eutrophic system with dense algal populations (Golterman 1975). Two tablespoons of plant fertilizer (Stern's Liquid Miracle Gro, Port Washington, NY) provided adequate N and P to stimulate an algal boom in our outside tank. Our objective was to provide the algae with at least 200 µg L^{-1} N and 20 µg L^{-1} P. Commercial fertilizers generally contain high levels of potassium which can be toxic to unionids (Imlay 1973). The algal cell density reached 1 x 10^6 cells ml^{-1} within 5 d. The outdoor tanks provided enough algae throughout the summer to feed 2700 mussels three times weekly; algal production was not sufficient during the cool autumn months. Therefore, algae were collected from local ponds, and grown indoors in glass aquaria to feed unionids in autumn.

In 1997, three 250-L clear, fiberglass tanks (Aquatic Ecosystems Inc., Apoka, FL) with cool-white fluorescent lightning were set up to grow unialgal cultures for feeding quarantined unionids. The green alga, *Neochloris oleoabundans,* was grown in 20-liter clear carboys, and when this culture was between 10^6 and 10^7 cells ml^{-1}, a third of the volume was added to each of three, 250-liter algal tanks. Fritz F/2 Media (Aquatic EcoSystems Inc., Apoka, FL) for freshwater algae was used according to specifications by the manufacturer. All algal cultures were aerated. Fresh media and water were added to fill half these tanks. After 24 h, additional media and water were added to fill the tanks. These cultures reached 1 x 10^6 cells ml^{-1} within 4 d. High temperatures and insufficient lighting caused some indoor algal cultures to crash. Algae were grown indoors by innoculating with stock culture in exponential phase, maintaining culture temperatures at 15-22 °C, and directing 60-100 µE m^{-2} s^{-1} of cool white fluorescent light at each culture tank.

Holding and maintenance of unionids

In 1995, unionids were fed 10 L of a dark green culture of algae (cell density above 1 x 10^6 cells ml^{-1}) three times per week, following the recommendations of Patterson (1998). Unionids were stocked to maximum capacity in each of the tanks, which ranged 150-250 mussels per tank. Species were not mixed wherever possible, and unionids from different river locales were never mixed in order to avoid cross contamination in quarantine. In the autumn, each tank of unionids was fed weekly with 2 L of a dense (1077 c ml^{-1}) algal suspension.

In 1995, 96.5% of unionids survived the summer 30d quarantine. *Q. p. pustulosa* exhibited the greatest mortality (43 specimens), which occurred in one tank stocked with 55 *P. alatus* and 145 *Q. p. pustulosa*. Dissolved oxygen levels were low (2.0 mg L^{-1}) when compared to other tanks (6 - 7.2 mg L^{-1}). A loose belt on the compressor contributed to low DO levels for several hours; however, dissolved oxygen levels came back up to 7.0 mg L^{-1} after the belt was repaired. *Q. p. pustulosa* in the tank with *P. alatus* continued to die, and DO levels were still low (4 mg L^{-1}) . Once these species were separated, no *Q. p. pustulosa* died in quarantine. Specimens of *P. alatus* were very active in their tanks. They may have consumed more oxygen than *Q. p. pustulosa* to meet their metabolic requirements. We would not recommend mix-

ing other species with salvaged *P. alatus* in quarantine tanks until the oxygen demand for this species is determined. In addition, we recommend monitoring DO in 4 h intervals for the first day to ensure that oxygen levels in tanks fully stocked with unionids remains above 7 mg L^{-1}.

Glycogen levels in unionids after 7d starvation in quarantine were approximately 50% lower than in unionids from the river, and after 30 d, glycogen levels showed a 70-80% reduction (Patterson *et al.* 1997). Unionids fed twice daily at 1 x 10^5 cells ml^{-1} (4.0 mg dry wt L^{-1}) of algae for 30 d maintained glycogen levels equivalent to those in the river (Patterson 1998). Although less than 2% of the starved unionids died in quarantine (Patterson *et al.* 1997), survival in ponds after 1 yr was 70% compared to 90 % for unionids collected in 1995 and fed three times weekly (C. Gatenby, unpublished dissertation data). These preliminary results from the LSC ponds indicate that adequate food resources for quarantined animals are important to the survival and reproductive potential of unionids upon relocation to refugia. If resources are not available for rearing algae indoors, fertilizing outdoor ponds to maintain a dense population of algae is an alternative to indoor algae cultures. Because commercial fertilizers are high in potassium, we suggest fertilizing ponds as needed with a 10:1 ratio of N:P (McCombie 1953) using inorganic fertilizers that do not contain potassium. Feeding requirements will vary between species and will depend on metabolic activity at different temperatures. Until these feeding requirements for different species of unionids is known, we recommend unionids be fed twice daily at 4.0 mg dry wt L^{-1} (Patterson 1998). A suitable food source should be chosen that is digestible and nutritious to unionids (Gatenby *et al.* 1997, Patterson 1998).

End of the quarantine period

After 30 d all unionids were hand-inspected with a lamp and magnifying glass, for zebra mussels that may have escaped the initial inspection. Once all unionids were examined and no zebra mussels were present, the unionids were relocated to their experimental refugia. Assuming we had removed all attached juveniles and adult zebra mussels during the intitial scrubbing and inspection, we presumed a 30 d quarantine period was sufficient time for hidden veligers to grow to a size visible with a magnifying glass. If any zebra mussels were found after 30 d, all unionids in the facility repeated another 30 d quarantine. In October, and again in November, zebra mussels were found. The unionids were quarantined an additional 30 d each time zebra mussels were found, for a total of 120 d in autumn. Patterson *et al.* (1997) showed that condition (glycogen reserves) of unionids is at great risk if they are starved over 7 d. All quarantine facilities, therefore, should have resources for feeding unionids throughout the year. A quick freeze in December also caused high mortality of unionids in quarantine, and high indoor temperatures caused algal crashes. Quarantine facilities should be insulated against high heat and extreme cold. In order to shorten the total time in quarantine, inspect unionids every 7 d and quarantine unionids for 30 d after the last zebra mussel is found.

Summary of Recommendations

Little information exists on the water quality and feeding requirements of unionids. Users of this report, therefore, should treat our recommendations as guidelines, and note that future research must be conducted to determine the specific tolerances and requirements of unionids proposed for relocation.

1) To minimize stress-related effects of handling, keep unionids cool. Consider salvaging unionids during cooler months when metabolism is low and energy reserves are high.
2) Keep unionids cool during handling, < 28 °C, and in a shaded area of the river prior to transport. Monitor temperature and DO in buckets with unionids waiting to be scrubbed, tagged, and transported. Maintain temperature < 28°C and DO above 6 mg L^{-1}.
3) Scrub mussels with plastic bristle brushes or scour-pads on site, but do not remove the protective outer periostracum. Remove all zebra mussels. Do not collect shell-damaged unionids that could sequester zebra mussels and later contaminate the quarantine facility.
4) Transportation of unionids for short distances (< 6 h) in air, wrapped in moist towelling, and cooled does not appear to adversely affect their condition. Enclose chlorinated-ice in plastic bags and avoid direct contact with the unionids. Transporting unionids in zebra mussel-free water, aerated, and supplied with algal food may further reduce stress.
5) Upon arrival at the quarantine facility, reinspect and rescrub unionids for zebra mussels that may have attached from the river while unionids awaited transportation. Rinse unionids with a high pressure hose and place them in clean tanks supplied with food. Then, hand-inspect each unionid, rinse again, and place in the appropriate quarantine tank.
6) Avoid cross-contamination between tanks of unionids. Sterilize all equipment and waste water with 25 mg L^{-1} chlorine, and allow equipment to dry for several days prior to reuse.
7) Hold unionids from different collecting sites in separate tanks (if possible) to avoid cross-contamination between individuals.
8) Stock tanks with a single species until dissolved oxygen requirements are known for the different species. Monitor DO in 4 h intervals for the first day to ensure oxygen levels remain above 6 mg L^{-1}.

9) Inspect unionids every 7 d, and quarantine for a total of 30 d after the last zebra mussel is found. This may help to reduce total quarantine time if zebra mussels are spotted early in the quarantine period.
10) Care in quarantine:
    a) Acclimate unionids to quarantine tank temperatures 2°C h^{-1}.
    b) Suggested water quality parameters for holding unionids:
        Upper threshold temperature = 28°C.
        DO (summer temperatures ≈ 24°C) > 6 mg L^{-1}.
        Potassium (K) < 4 mg L^{-1}.
        Alkalinity > 15 mg L^{-1}, hardness >50 mg L^{-1}, and pH > 6.5.
        Upper threshold unionized ammonia (NH_3)<0.025 mg L^{-1}.
        Total chlorine residuals < 0.026 mg L^{-1}. Depending on the volume, water can be dechlorinated using aeration for 24 h to several days.
    c) Feed unionids twice daily 1 x 10^5 cells ml^{-1} algae or 4.0 mg dry wt L^{-1} of food.

Research Needs

Future research on the protocol for salvage and quarantine of unionids should focus on water quality tolerances of unionids in tanks and ponds (T °C, DO, TAN, and pH), nutritional requirements for maintaining condition of unionids in captivity, and stress-related factors associated with transport, handling, and acclimation. Methods for efficiently removing zebra mussels (primarily how to flush or remove veligers) that would shorten the quarantine period and avoid additional stress are needed. The optimal stocking density per water volume also should be determined for holding and rearing unionids in captive environments.

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