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REVIEW OF TECHNIQUES TO PREVENT INTRODUCTION OF ZEBRA MUSSELS (*DREISSENA POLYMORPHA*) DURING NATIVE MUSSEL (UNIONOIDEA) CONSERVATION ACTIVITIES

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ABSTRACT Because of the declines in diversity and abundance of native freshwater mussels (superfamily Unionoidea), and the potential decimation of populations of native mussels resulting from the rapid spread of the exotic zebra mussel *Dreissena polymorpha*, management options to eliminate or reduce the threat of the zebra mussel are needed. Relocating native mussels to refugia (artificial and natural) has been proposed to mitigate the threat of zebra mussels to native species. Relocation of native mussels to refugia such as fish hatchery facilities or natural habitats within their historic range, which are unlikely to be infested by zebra mussels, necessitates that protocols be developed to prevent the inadvertent introduction of zebra mussels. Several recent studies have developed such protocols, and have assessed their effectiveness on the health and survival of native mussels during subsequent relocation to various refugia. The purpose of this project is to synthesize and evaluate the current protocols and to develop a set of procedures that resource managers and researchers should consider before conducting conservation activities in zebra mussel infested waters. We found that the existing protocols have many common points of concern, such as facility modification and suitability, zebra mussel risk assessment and management procedures, and health and disease management procedures. These conservation protocols may have broad applicability to other situations and locations. A summary and evaluation of the information in these main areas, along with recommended guidelines, are presented in this article.

KEY WORDS: relocation, Unionidae, *Dreissena polymorpha*, conservation, refugia

INTRODUCTION

Native freshwater mussels of the families *Margaritiferidae* and Unionidae (superfamily Unionoidea) are one of the most rapidly declining faunal groups in North America. About 67% of the nearly 300 native species found in North America are considered vulnerable to extinction or already extinct (Bogan 1993, Williams et al. 1993). The decline of native mussel populations in North America has occurred steadily since the mid 1800s and has been attributed to overharvest, construction of dams and impoundments, sedimentation, navigation, pollution, and habitat degradation (Fuller 1974, Bogan 1993, Naimo 1995, Brim Box & Mossa 1999, Vaughn & Taylor 1999). An additional recent threat to the native fauna has come from the introduction of the zebra mussel *Dreissena polymorpha*. This species colonizes native mussels and impedes their movement, reduces the ability to feed and eliminate wastes, and competes for food and space (Mackie 1991, Schloesser et al. 1996, Strayer 1999).

Because of the declines in diversity and abundance of native mussels and the rapid and severe impacts of zebra mussels on native mussels (Gillis & Mackie 1994, Nalepa et al. 1996), a national strategy for the conservation of native freshwater mussels was developed to provide a framework for preventing further population declines and species extinction (National Native Mussel Conservation Committee 1998). This document identified a number of conservation needs and outlined goals, strategies, and tasks to address these needs. Listed among these was the recommendation to develop management options for eliminating or reducing the threat of zebra mussels to native mussels. These options included relocating native mussels to artificial and natural refugia. Although many mussel relocations have had poor success (e.g., Cope & Waller 1995), recent studies conducted with improved techniques, experimental design, and monitoring programs, have been successful (Dunn et al. 2000, Cope et al. 2003). Thus, with the increased likelihood of successful relocation efforts, and the continued range expansion and adverse effects of zebra mussels on native mussel populations, any relocation done to conserve native mussels necessitates that protocols be developed to prevent the inadvertent introduction of zebra mussels.

Several recent studies have developed protocols to ensure that zebra mussels would not be inadvertently introduced during native mussel conservation activities and have assessed the health and survival of native mussels during subsequent relocation (Patterson et al. 1997, Patterson et al. 1999, Gatenby et al. 2000, Nichols et al. 2000, Hallac & Marsden 2001, Newton et al. 2001). The purpose of this project was to synthesize and evaluate the current protocols and to develop a set of procedures that resource managers and researchers should consider before conducting native mussel conservation activities in zebra mussel infested waters.

RESULTS AND DISCUSSION

Almost all of the recent native mussel salvage and relocation projects have used some type of quarantine to prevent the incidental introduction of zebra mussels. The exceptions are those studies intended to remove zebra mussels from fouled native mussels and replace them back to their original location (e.g., Schloesser 1996, Hallac & Marsden 2000). By necessity, most of the quarantine protocols have been location and facility specific. For example, Gatenby et al. (2000) reviewed procedures for relocating native mussels from the Ohio River. Likewise, Newton et al. (2001) developed a specific set of procedures for relocating native mussels from the Mississippi River to artificial ponds and to fish hatchery facilities. However, these and other protocols developed for specific studies have many common points of concern, such as

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TABLE 1.

Summary of collection and quarantine-related conditions and procedures, and recommended guidelines for preventing introduction of zebra mussels during native mussel conservation activities.

Condition or Procedure	Reference: Gatenby et al. (2000)	Reference: Newton et al. (2001)	Recommended Guidelines
Collection setting			
Time of collection	July, September, October 1995	May 1995	Early spring, before zebra mussel spawning begins (water temperatures <15°C) or mid to late fall when natives have greater energy reserves and juvenile zebra mussels are visible (>2–5 mm shell length)
Species of native mussels	*Amblema plicata, Quadrula pustulosa, Elliptio crassidens, Pleurobema cordatum, Obliquaria reflexa, Potamilus alatus*	*Amblema plicata, Fusconaia flava, Leptodea fragilis, Obliquaria reflexa, Quadrula quadrula*	
No. of native mussels	2700	768	
Native mussels analyzed for disease and pathogens before relocation	No	Yes	If possible
Air temperature (°C)		6–18	Early spring or late fall temperatures; minimize differences between air and water temperature
Water temperature (°C)	20–28	11–14	Early spring or late fall temperatures; minimize differences between air and water temperature
Mechanism for removing zebra mussels from native mussels	Hand scrubbed with plastic-bristled brushes	Hand scrubbed with plastic-bristled brushes under ×2 magnification	Hand scrub with plastic-bristled brushes under magnification
Method for holding scrubbed native mussels at collection site	Mesh bags in river*	Hatchery truck with aerated well water	Hold in zebra mussel-free water after scrubbing
Emersion time (min) during collection and processing	20	5	Keep to minimum, but <20
Transportation to quarantine facility	Between moist burlap in coolers with ice (no direct contact of mussels and ice)	Between moist burlap in coolers with ice (no direct contact of mussels and ice)	Between moist burlap in coolers with ice in plastic bags for transport durations <12 h; no direct contact of mussels and ice bags
Quarantine facility			
Type	Above-ground tanks, 14–500 L	Pond (0.04 ha), mussels held in 8-2720 L mesh bags	
Mussel density (no./m^2)	150–250	39–159	Keep to minimum, but <150
Water source	Well water	Well water	Well water
Water temperature (°C)	2–28	13–27	<28
Dissolved oxygen (mg/L)	6–14	6–20	>6
pH	7.2–8.5	7.8–10.6	6.5–9.0
Potassium (mg/L)	1.6	2.6	<4
Alkalinity (mg $CaCO_3$/L)	90	110–160	>15
Hardness (mg $CaCO_3$/L)	90	180–200	>50
Total ammonia nitrogen (mg/L)	≤1.0	0.03–0.2	<1.0
Unionized ammonia (μg/L)	2–66	2–20	<25
Total residual chlorine (μg/L)			<17
Nutrition/feeding	≥1 × 10^6 cells/mL three times per week in quarantine; relocation ponds were fertilized with a nitrogen:phosphorous (N:P) ratio of 10:1 (1.0 mg/L N, 0.1 mg/L P) with NH_4NO_3 and $NaHPO_4$ salts	8.3 g/m^3 of 10:10:10 N:P:K fertilizer added to quarantine pond 2 weeks prior to adding unionids; relocation ponds were not fertilized	1 × 10^5 cells/mL or 4.0 mg dry wt./L twice daily or 2.0–5.0 × 10^4 cells/mL or 1.9 mg dry wt./L on a continuous basis (Gatenby 2000, 2002); suitable algal species include *Neochloris oleoabundans, Bracteacoccus grandis,* and *Phaeodactylum tricornutum*

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TABLE 1.

continued

Condition or Procedure	Reference: Gatenby et al. (2000)	Reference: Newton et al. (2001)	Recommended Guidelines
Days in quarantine	Minimum of 30, but up to 120; re-inspected under 4× magnification	35; re-inspected under 2× magnification	Minimum of 30; re-inspect under magnification
Disinfection of equipment and supplies	Chlorine solution of 25 mg/L Dessication for up to 4 d	Not specified	Chlorine solution of 25–250 mg/L, depending on type of material; dessication in warm dry air for 3–5 d
Monitoring			
Temperature, dissolved oxygen, and pH	Twice daily	Daily	At least daily
All other water quality variables	Daily to weekly	Daily to weekly	Daily to weekly
Disease and mortality	Not specified	Not specified	At least weekly

* All native mussels were rinsed with a high pressure hose before being placed into the quarantine facility.

facility modification and suitability, zebra mussel risk assessment and management procedures, and native mussel health and disease management procedures, that may have broad applicability to other situations and locations.

Facility-Specific Concerns and Procedures

The availability of aquatic facilities for long-term captive care of freshwater mussels is limited. Thus, most of the salvage and quarantine facilities have involved the short-term use of state and US Government owned fish hatchery ponds and raceways or similar research aquaculture facilities (Dunn & Layzer 1997, Pinder et al. 1999, Gatenby 2000, Newton et al. 2001). The main facility concerns have focused on the type of rearing or holding system (e.g., ponds, raceways, or above-ground tanks capable of housing hundreds to thousands of mussels), the facility's proximity to the source of relocated mussels (to reduce transportation time and handling stress), on-site water quality for maintenance of mussel health, and production of an algal-based food supply. The objectives of any given conservation project will likely dictate the type of facility or holding system used and any modifications that may be required. Nonetheless, whether used for short-term quarantine or for long-term captive care, all facilities should be able to provide space for isolation and quarantine, water quality characteristics to meet requirements for shell growth and metabolic processes, and food quantity and quality to support growth and reproduction (Table 1).

Specific isolation and containment modifications are probably necessary at most facilities to control and contain source water inflow and potentially contaminated outflow. For example, the outflow of water from quarantine units may need to be passed through filtration or disinfectant treatments to remove or kill potential zebra mussels before the water is discharged through normal routes. Containment procedures commonly used at facilities conducting zebra mussel research have included filtration of outflow water through small mesh bags (100 μm or smaller), chlorine treatment tanks (250 mg/L for 1 h), and sand filtration units (J. J. Rach, U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, WI, pers. com.). Additional facility precautions may include the capping of all exterior drains to prevent the release of potentially contaminated water from the affected areas and the development of a flood risk assessment, if the facility is within a designated floodplain.

The type of facility selected, however, may influence the relative success of the conservation project. Success could depend on its use only as a short-term quarantine facility for subsequent relocation to a natural or artificial system, or its use for long-term captive care. For example, Newton et al. (2001) relocated five species of native mussels (1,392 mussels total) from the Upper Mississippi River to a fish hatchery pond after 35 d of quarantine in an artificial pond (81% of mussels survived during quarantine). Mussel survival in the hatchery pond averaged 80% after 1 y, but only 35% 3 y after relocation. Of the mussels in a handling-control treatment that were placed back into the Mississippi River after quarantine, survival was 80% after 1 y and 75% after 3.3 y. The authors attributed the differences in survival between the hatchery pond and riverine relocated mussels to inadequate nutritional resources in the pond. This study illustrates the potential utility of natural or managed refugia over artificial refugia for long-term conservation (Nichols et al. 2000, Cope et al. 2003). Gatenby (2000) observed similar decreases in survival of six large river species relocated to pond refugia after a 30-d quarantine in above-ground tanks. Mean survival of native mussels during quarantine was 97%. Mean survival after 1 y in the ponds ranged between 82 and 93%, depending on species. Despite an abundance of a suitable algal food supply and adequate water quality conditions in the ponds, however, the survival of relocated mussels decreased to 44% after 2 y and to 5% after 3 y. Gatenby (2000) attributed the mortality to high water temperatures in July and August during years 2 and 3 of that study. Large river species of mussels relocated (with no quarantine period) to fish hatchery raceways with flowing water and sediment also showed high survival (95%) after 1 y (Dunn & Layzer 1997), but their long-term (3–5 y) success in this type of system is unknown.

The relocation of native mussels after quarantine to natural refugia or raceway systems supplied by natural river water will likely have greater success for long-term preservation of the mussels than retention in artificial pond refugia for two key reasons: water temperature and food quality. These two components are critical to the livelihood of any aquatic organism. Rapid fluctuations in temperature, unnaturally high temperatures, and inadequate food supplies are known to cause stress in aquatic organ-

isms, and can lead to mortality (Bayne et al. 1973). Thus, temperature, food quality, and food quantity will also be key components to the success of native mussel captive care programs.

Zebra Mussel Risk Assessment and Management Procedures

Because the threat of zebra mussels to native mussels has been the primary causal factor for initiating most mussel conservation activities, special precautions have been necessarily incorporated into the collection and handling protocols where native mussels are relocated. These precautions taken during collection, transport, processing, and quarantine of native mussels are of utmost importance. Only the careful collection and handling of native mussels from zebra mussel-infested waters will ensure that hatchery fish, native mussels, and other aquatic species in the ecosystem are protected from the incidental introduction of zebra mussels.

In situations where there is uncertainty in the co-existence of zebra mussel populations in the watershed, the most prudent and conservative approach is to treat all native mussels as if they originated from zebra mussel-infested waters. A review of zebra mussel range distribution and population dynamics in the particular river basin is also warranted. Particular items of interest include, the nearest known reproducing population of zebra mussels to the native mussel collection site, the relative density and potential spawning periods of zebra mussels at that site, and the likelihood of an undetected presence at the native mussel collection site (e.g., lack of an active monitoring program).

The optimum time for collection of native mussels for a given conservation project is largely unknown. Conservation projects, however, should strive to select periods that reduce the stress associated with handling as much as possible. Potential criteria include choosing a period that coincides with the absence of zebra mussel larvae in the water column, minimizes the temperature differential between air and water, and does not interrupt the reproductive cycle for most of the species being relocated. Zebra mussel contamination can be minimized by collecting native mussels during early spring or late fall periods when zebra mussel larvae are likely not present in the water column (e.g., water temperatures <15°C, Mackie 1991) or when the settled juveniles are of a sufficient size to be easily seen (e.g., 2–5 mm in shell length), respectively. Freshwater mussels are categorized as either long-term (bradytictic) or short-term (tachytictic) brooders. Long-term brooders, like many species of lampsilines and anodontines, become gravid in late summer, retain the developing glochidia in the gill marsupia throughout winter, and spawn in early spring (McMahon & Bogan 2001). In contrast, short-term brooders, like many species of amblemines, become gravid in early spring and spawn in late summer (McMahon & Bogan 2001).

Newton et al. (2001) collected native mussels in early spring when water temperatures ranged between 11 and 14°C, a period before zebra mussel spawning, which generally occurs when water temperatures reach 15 to 17°C (between May and June), in northern temperate regions of the United States and Canada (Mackie 1991). The collection of native mussels in early spring also has an added potential benefit of reduced energetic stresses associated with handling because of the cooler water temperatures (Jokela 1996, Newton et al. 2001). For example, glycogen concentrations in *Amblema plicata* were highest between May and July and dropped precipitously thereafter—a pattern that closely paralleled reproduction in this short-term brooder (Monroe & Newton 2001). Similarly, Jokela et al. (1993) observed that glycogen concentrations decreased substantially between July and October in *Anodonta piscinalis*, a long-term brooder. Furthermore, Jokela (1996) suggested that transplanting females before fertilization or during the early development of the brood had no detectable effect on reproductive output.

Data on energetic reserves in marine bivalves contradict the recently reported data in freshwater bivalves. In the marine environment, it has been suggested that mussels collected in fall may be able to better withstand handling stress because of their higher energy reserves and because their metabolism is slowed by the cooler water temperatures (Bayne et al. 1973). For example, by mid to late fall, the marine species *Mytilus edulis* and *M. trossulus* had accumulated abundant carbohydrate energy reserves (Hawkins & Bayne 1985, Kreeger 1993, Kreeger et al. 1995). The differences between marine and freshwater species may be caused by differing reproductive strategies. Results from a recent study with native freshwater mussels, however, suggest that some species of native mussels may build up their energy reserves in fall (Gatenby 2002). Obviously, this is an area where additional research is needed.

When native mussels are collected from multiple sites in a watershed with a known or suspected gradient in zebra mussel density, working from the least infested site to the most infested site will reduce potential zebra mussel contamination of boats and other equipment. Optimally, boats used to collect or deploy native mussels in zebra mussel infested areas should be cleaned (before and after) by a high-pressure hot-water wash and diver wet suits, supplies, and equipment (e.g., ropes, buckets, etc.) used in the study should be disinfected with a mild solution of chlorine bleach (25 mg/L) or air dried (3–5 d) before use (Gatenby et al. 2000).

If the quarantine or relocation facility is also an operational fish hatchery or aquaculture center, precautionary measures to protect endemic wild species and cultured fish species should be considered. Before entrance into the facility, a subsample of native mussels should be obtained from the collection site and submitted to a United States Fish and Wildlife Service, National Fish Health Center (Newton et al. 2001) or similar laboratory, to assess potential disease and pathogen presence (see section later on native mussel health and disease management procedures).

After screening for diseases and pathogens, collection of native mussels should proceed with procedures to minimize contamination from adult and larval zebra mussels. These include scrubbing individual native mussels with plastic bristled brushes, visual inspection of all exterior surfaces of the shell with magnifying lenses, and holding cleaned natives in zebra mussel-free water (Table 1). Care should be taken during scrubbing and inspection to avoid overlooking small zebra mussels that may be attached in crevices, in areas of shell erosion (native mussels with severely eroded or damaged valves should be discarded), or along the hinge line (Gatenby et al. 2000, Newton et al. 2001). Only personnel experienced in mussel biology should conduct the inspections to ensure accuracy and efficiency of these procedures.

During collection and processing of native mussels, emersion (exposure to air) and thermal stress should be kept to a minimum. Recent studies have shown that handling mussels over a range of emersion air temperatures (15–35°C) and emersion durations (15–60 min) did not acutely impair survival, behavior, or biochemical composition (Bartsch et al. 2000, Greseth et al. 2003). A minimal emersion time (<20 min), however, is generally recommended from recent efforts (Table 1). Moreover, water temperature and

dissolved oxygen concentrations in the holding vessels during collection should be measured frequently (at least once per hour) and maintained at or near (±2°C) the ambient stream conditions at the time of collection with non-chlorinated ice and external aeration, if possible (Gatenby et al. 2000).

Depending on the proximity of the native mussel collection site to the quarantine facility (a transport time generally <12 h), mussels should be transported in coolers covered with moist burlap and kept cool (within ±2°C of the water collection temperature, if possible) with ice in plastic bags without direct contact of ice bags and mussels (Gatenby et al. 2000, Newton et al. 2001, Cope et al. 2003). This method is advantageous over the use of water-filled, aerated tanks (Chen et al. 2001) because of the reduced need for costly and cumbersome trucks and equipment and of minimizing potential problems associated with maintaining stable dissolved oxygen concentrations in water during transport.

At the quarantine facility, native mussels have generally been held for a minimum of 30–35 d (Gatenby et al. 2000, Newton et al. 2001) to allow any small or previously undetected zebra mussels to become visually apparent on re-inspection. The 30–35 d quarantine period is based on reported zebra mussel growth rates of 0.06–0.15 mm/d (Mackie 1991, Martel 1995, Chase & Bailey 1999), which would allow a newly settled zebra mussel to reach a visible shell length of about 2–5 mm during quarantine. During this time, basic water quality measurements (e.g., temperature, dissolved oxygen, and pH) should be taken at least daily. Other water chemistry variables such as alkalinity, hardness, potassium, total ammonia nitrogen (TAN), and unionized ammonia should be measured at least weekly to ensure that water quality conditions for minimum life requirements are met (Table 1). In addition, mussels in quarantine should be monitored at least weekly for disease (see section below on native mussel health and disease management procedures) and mortality.

Isolation of native mussels from other aquatic species, their contact water, nets, or other equipment at the quarantine facility is necessary to protect organismal health and the physical facility. These concerns can largely be addressed by applying standard best practices for maintaining fish health. Disinfection of equipment and supplies for native mussel quarantine should be guided by National Fish Health Policy and Procedures, Part 713, sections FW1 and FW 3 (USFWS 1995); chlorine (200–250 mg/L for 1 h), sodium or potassium salts (saturated solutions) or other chemical treatments (e.g., benzalkonium chloride at 100 mg/L for 3 h) and desiccation (3–5 d) have been successfully used or recommended (Reid et al. 1993, Waller et al. 1996, Gatenby et al. 2000).

After the minimum quarantine period (30–35 d), individual mussels are thoroughly re-inspected by hand with magnifying lenses to evaluate the presence of zebra mussels. If zebra mussels are not found, the mussels are deemed zebra mussel-free and can be relocated elsewhere (e.g., to natural or artificial systems or to other facilities for long-term captive care). Because no zebra mussels were found after quarantine in the study of Newton et al. (2001), the mussels were subsequently relocated to fish hatchery ponds. In contrast, Gatenby et al. (2000) found zebra mussels on initial re-inspection and consequently held native mussels in quarantine for additional 30 d intervals each time zebra mussels were found, up to a total of 120 d. Because of declines in mussel health and condition over time during quarantine (Patterson et al. 1997, Newton et al. 2001), Gatenby et al. (2000) recommended re-inspection of mussels at 7 d intervals after the initial 30 d period when zebra mussels are found, and to hold them only for 30 additional days after the last zebra mussel is found, to shorten the overall quarantine time. However, the added stress of handling native mussels more frequently must be weighed against the probability of earlier detection of zebra mussels.

Additionally, native mussels could be treated with chemical disinfectants. Certainly, the benefit of this type of treatment must be weighed against the risk of added stress and reduced fitness in the native mussels, but a study by Waller and Fisher (1998) found that limited application of specific chemicals (e.g., 20,000 mg NaCl/L for 6 h) may be feasible for certain tolerant native species. They cautioned, however, that chemical disinfectants cannot guarantee the elimination of all zebra mussels from native mussel shells and stated that pre-treatment or multiple treatment (e.g., once per week) of native mussels and their holding tanks may be most valuable for reducing the time held in quarantine. Many fish hatchery and aquaculture facilities may already be using various chemical treatments (Waller et al. 1996, Edwards et al. 2000, Edwards et al. 2002) or hazard analysis protocols such as the Aquatic Nuisance Species-Hazard Analysis Critical Control Point (ANS-HACCP) approach (Gunderson & Kinnunen 2001) to prevent the spread of zebra mussels and other aquatic nuisance species during their activities, which may be adapted to the collection, transport, and quarantine of native mussels.

Native Mussel Health and Disease Management Procedures

Although little is known about the diseases of native freshwater mussels, recent studies have shown the potential for pathogen transmission among native mussels and fish (Starliper et al. 1998, Starliper & Morrison 2000). The primary concern for fish hatchery or aquaculture facilities that contain native mussels is the potential for transmission of disease and pathogens between host mussels and hatchery fish. Transmissions from hatchery fish to mussels and from mussel to mussel are also important vectors to control for maintaining mussel health. Therefore, a pathogen and disease monitoring plan for native mussels, similar to that commonly used for hatchery-reared fish, should be considered. Hatchery personnel are routinely trained in fish health protocols and record keeping; these procedures could easily be adapted for monitoring mussel health. The United States Government standards and protocols currently exist for a disease control and classification system for coldwater fish (salmonid) pathogens—similar guidelines for warmwater fish or native mussels do not exist (USFWS 1995). Revisions to the United States Fish and Wildlife Service, Fish Health Policies and Procedures are currently underway to include warmwater fish and other aquatic organisms (Richard Nelson, United States Fish and Wildlife Service, La Crosse Fish Health Center, Onalaska, WI, pers. com.). Until those changes are implemented, however, native mussels may only be screened in the near term for reportable coldwater pathogens and diseases. On a positive note, a recent study evaluating the effect of depuration on the transmission of the bacterial fish pathogen *Aeromonas salmonicida* (the causative agent of fish furunculosis) between the unionid *Amblema plicata* and two strains of Arctic char *Salvelinus alpinus* found that the minimum 30-d quarantine of native mussels recommended for preventing the spread of zebra mussels was sufficient for depuration of the fish pathogen and eliminating transmission of the disease (Starliper 2001). Therefore, when adequate safeguards and standard best practices for fish health are used in combination with a 30-d quarantine, disease and pathogen transmission risks should be minimal. Native mussels held in quarantine should be

screened before being placed in the quarantine facility and monitored monthly throughout the duration of their captive care to document disease and pathogen incidence and history. More research and policy development is needed in this area to ensure protection of fish and native mussels.

Maintaining the physiologic condition of native mussels during quarantine is difficult because diet and nutritional requirements are poorly understood. Although the specific time course for changes in biochemical indices of mussels caused by quarantine is unknown, recent studies have shown that substantial decreases in glycogen concentrations occur in as little as 7–35 d after quarantine. For example, Patterson et al. (1997) found that glycogen concentrations in mantle tissue in *Amblema plicata* and *Quadrula pustulosa* dropped significantly after 7 d in quarantine and by day 30, concentrations had declined to only 15–31% of that measured in wild-caught specimens. Likewise, glycogen concentrations in foot tissue of *A. plicata* decreased 44% from 279 ± 191 mg/g dry weight at day 0 to 178 ± 105 mg/g dry weight after 35 days in quarantine (Newton et al. 2001).

Based on the poor physiologic condition of native mussels after quarantine shown by previous studies, it is critical to provide the best source of nutrition during quarantine. Previous studies have relied on an algal-based diet, either produced *in situ* by stimulating algal growth with fertilizers in ponds or cultured indoors on site and added directly to mussel holding tanks (Gatenby et al. 1997, Patterson et al. 1997, 1999, Gatenby 2000, Gatenby et al. 2000, Newton et al. 2001). A number of algae have been tested as food for juvenile and adult mussels (Gatenby et al. 1997, Gatenby 2000, Beck 2001). Recent biochemical analysis of three algae (*Neochloris oleoabundans*, *Bracteacoccus grandis*, and *Phaeodactylum tricornutum*) indicate that these could be nutritionally suitable for maintaining freshwater mussels in captivity (Gatenby et al. 2002). If mussels are to be quarantined or relocated to ponds, the following should be kept in mind: (1) standard commercial pond fertilizers should not be used to stimulate growth of algae; (2) the potassium levels in commercial fertilizers are toxic to freshwater mussels (Imlay1973); (3) the nitrogen:phosphorous ratio (N:P) of the standard 10:10:10 nitrogen:phosphorous:potassium (N:P:K) fertilizer will not promote suitable algae for mussels that typically require an N:P ratio of 10:1 (McCombie 1953); and (4) an unsuitable, or indigestible filamentous blue-green algal bloom will result when 10:10:10 N:P:K is used. Therefore, we recommend using the fertilizers indicated in Table 1, following Gatenby et al. (2000). Although feeding requirements for native mussels will likely depend on the species involved, temperature conditions, and metabolic activity, Gatenby et al. (2000) recommended that native mussels be fed 1×10^5 cells/mL or 4.0 mg dry weight/L twice daily (Table 1). This was a conservatively high recommendation based on initial feeding studies and assimilation efficiencies. This concentration resulted in the greatest assimilation of organic carbon, but a significant amount of this ration went unused by the animals (Gatenby 2000). More recent data indicate that a diet ration of $2.0–5.0 \times 10^4$ cells/mL or 1.9 mg dry weight/L per feeding chamber should maintain mussel condition during summer growth periods (Gatenby 2002). Particle concentrations should be monitored and not allowed to drop below 60% of this recommended ration. Feeding frequency will depend on the species and total biomass being held in captivity (Gatenby 2002). Thus, monitoring the particle concentration on a daily basis is necessary. Initially, particle concentration may need to be monitored two to three times daily until the manager is familiar with the particle depletion rate or clearance rate of the native mussels held in captivity.

CONCLUSIONS AND RECOMMENDATIONS

Native freshwater mussels should only be relocated from existing areas as a last resort (Cosgrove & Hastie 2001). Other options to relocation and salvage, such as periodic cleaning of zebra mussels from native mussels and replacement (Hallac & Marsden 2000, Hallac & Marsden 2001), and the use of natural or managed refugia (Nichols et al. 2000), should be considered as first alternatives where practical. For example, Hallac & Marsden (2000, 2001) suggested that periodic cleaning and replacement might be a viable option for conservation of native mussels, especially in areas where food is not limiting and where collection and cleaning are logistically feasible. If, however, freshwater mussel relocations are required to conserve localized populations from zebra mussels or other catastrophic events, the concerns and procedures described in this article should provide general guidance for developing plans to prevent the incidental introduction of zebra mussels during these activities and for maintaining the health of the native refugees while under captive care.

In addition, procedures for ensuring long-term viability of native mussel populations need to be considered throughout the planning and implementation process. For example, similarities in water quality, substratum characteristics, food, and necessary fish hosts among the systems are critical elements in a native mussel relocation strategy. Additional ecological and evolutionary concerns, such as retention of genetic diversity of the mussel populations, need to be carefully considered before relocating native mussels to natural refugia, especially if the mussels are to be relocated between river basins or between sub-basins of the same river system (Villella et al. 1998, Storfer 1999).

Because of costs and limited availability of facilities for quarantine and captive care of native mussels, the United States Fish and Wildlife Service and its resource conservation and management partners may wish to designate several facilities within regions of the United States that can accept, hold, and screen mussels for disease and pathogens. These facilities may include state or national fish hatcheries, research or aquaculture centers, and fish health centers.

To our knowledge, this synthesis represents the "state-of-the-science" for minimizing the incidental introduction of zebra mussels during native mussel conservation activities and for ensuring their short-term and long-term health and viability. Readers of this article should be cautioned that the information presented is only recommended guidelines and that future improvements to procedures will be made through research and policy development.

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