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Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants

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Abstract. Freshwater mussels (superfamily Unionoidea) are in serious global decline and in urgent need of protection and conservation. The declines have been attributed to a wide array of human activities resulting in pollution and water-quality degradation, and habitat destruction and alteration. Linkages among poor water quality, pollutant sources, and mussel decline in rivers and streams have been associated with results of laboratory-based tests of specific pollutants. However, uncertainties remain about the relationship of laboratory data to actual contaminant exposure routes for various mussel species, life stages, and in the habitats occupied during these exposures. We evaluated the pathways of exposure to environmental pollutants for all 4 life stages (free glochidia, encysted glochidia, juveniles, adults) of unionoidean mussels and found that each life stage has both common and unique characteristics that contribute to observed differences in exposure and sensitivity. Free glochidia typically are exposed only briefly (e.g., seconds to days) through surface water, whereas adults sustain exposure over years to decades through surface water, pore water, sediment, and diet. Juveniles live largely burrowed in the sediment for the first 0 to 4 y of life. Thus, sediment, pore water, and diet are the predominant exposure routes for this life stage, but surface water also might contribute to exposure during certain periods and environmental conditions. The obligate parasitic stage (encysted glochidia stage) on a host fish might be exposed from surface water while partially encysted or from toxicants in host-fish tissue while fully encysted. Laboratory methods for testing for acute and chronic exposures in water have advanced, and toxicant-specific information has increased in recent years. However, additional research is needed to understand interactions of life history, habitat, and long-term exposure to contaminants through water, pore water, sediment, and diet so that the risks of environmental exposures can be properly assessed and managed.

Key words: freshwater mussel, habitat, pollution, Unionidae, water quality.

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Freshwater mussels (superfamily Unionoidea) are in serious global decline and urgently need protection and conservation (Bogan 1993, Williams et al. 1993, Lydeard et al. 2004, Augspurger et al. 2007). Nearly 70% of the 300 species in North America are endangered, threatened, of special concern, or already extinct (Williams et al. 1993, Williams and Neves 1995). The declines in the abundance and diversity of North American mussels have been attributed to a wide array of human activities that cause pollution and water-quality degradation, and habitat destruction and alteration; however, the specific reasons for the declines, unless site specific and catastrophic, generally are unknown (Strayer et al. 2004). Most of the main current threats are presumed to be chronic, low-level, and pervasive stressors, such as industrial and agricultural point- and nonpoint-source water pollution, sedimentation, construction of dams, and introduction of exotic species, among others (Strayer et al. 2004). The vast number of potential factors contributing to the declines makes it difficult to determine the key chemical contaminants or set of environmental conditions to target in a regulatory framework for protection and conservation of this imperiled fauna. However, the current consensus is that water pollution and physical habitat are important areas of investigation (Richter et al. 1997, Augspurger et al. 2007).

The associations between poor water quality, pollutant sources, and mussel declines were first detected in field studies (Ortmann 1909, Starrett 1971, Horne and McIntosh 1979). These field observations led to laboratory-based testing of specific pollutants. This research suggested that the early life stages of freshwater mussels were among the most sensitive of aquatic organisms tested to some chemicals, such as Cu and NH_3 (Jacobson et al. 1997, Augspurger et al. 2003, March et al. 2007), but not to others, such as some pesticides and solvents (Keller 1993, Keller and Ruessler 1997, Milam et al. 2005). Thus, evidence of mussel sensitivity to some chemicals was available, but the complex life-history characteristics of freshwater mussels, i.e., the existence of several forms of early life stages (e.g., glochidia and juveniles), and the lack of proven testing protocols prevented routine use of this information to establish chemical-specific water-quality criteria (Ingersoll et al. 2007). In recent years, ways have been found to evaluate the reliability of laboratory toxicity testing protocols for early life stages of mussels. Most important, an internationally approved standard guide for conducting laboratory water-only toxicity tests with early life stages of freshwater mussels has been published (ASTM 2006). Additional toxicological information on the relative sensitivity of early life stages of mussels to a variety of toxicants such as Cu, NH_3, Cl, and current-use pesticides has been generated with the American Society for Testing and Materials (ASTM) standard guide (Bringolf et al. 2007a, b, c, March et al. 2007, Newton and Bartsch 2007, Wang et al. 2007a, b, c). Use of the ASTM standard guide for toxicity testing with early life stages of freshwater mussels has shown that reliable data can be generated from mussel toxicity tests (Wang et al. 2007a).

However, uncertainties remain about how to apply laboratory data (e.g., 1- to 28-d exposures measuring survival or growth of mussels) to actual routes of contaminant exposure for various mussel species, life stages, and their habitats in nature. Therefore, available information on freshwater mussel life stages must be evaluated and synthesized, and potential contaminant exposure routes and toxicity endpoints must be placed in a life-history and habitat context. The objectives of our paper are to assess potential contaminant exposure sources, uptake routes, exposure durations, and relative sensitivities of the 4 major life stages of freshwater mussels.

Life Stage Assessment

The life history of most unionoids includes several critical periods, such as sperm release by adult males into the water column, uptake of sperm by siphoning females, fertilization of ova, release of viable larvae (glochidia) from females, and attachment of glochidia to a suitable host fish (encysted) for transformation to a free-living juvenile mussel (McMahon and Bogan 2001, Watters 2007). Water quality, sediment quality, health of host fish, and diet (of life stages that feed) all have the potential to influence survival of each of these life stages and subsequent reproduction and recruitment.

Adult mussels

Adult freshwater mussels are long-lived organisms that burrow in the sediments of rivers, streams, and lakes. Adults typically burrow to the depth of the posterior edge of the shell, but might burrow completely beneath the sediment surface, depending on water temperature, flow, day length, and reproductive activity (Balfour and Smock 1995, Amyot and Downing 1997, Watters et al. 2001, Schwalb and Pusch 2007). Burrowing activities and patterns might greatly influence the exposure routes and subsequent uptake of contaminants by adults. For example, the historical view was that adult mussels were primarily suspension-feeders positioned at or above the sediment surface, and thus, most of their contaminant exposure was from surface water (Naimo 1995). However,

TABLE 1. Routes, location, and duration of potential contaminant exposure for the 4 main life stages of unionoid mussels.

Life stage	Exposure route	Exposure location	Exposure duration
Adult	Surface water, sediment, pore water, diet	Partially burrowed, burrowed	Years to decades
Glochidia (brooded and free)	Surface water	Marsupial gill	Weeks to months
		Water	Seconds to days
Glochidia (encysted)	Surface water	Host fish (partially encysted)	Hours to days
	Host fish tissue	Host fish (fully encysted)	Weeks to months
Juvenile (up to 2–4 y old)	Sediment, pore water, diet, surface water	Burrowed, partially burrowed	Months to years

mussels can obtain up to 80% of their food by deposit feeding (Raikow and Hamilton 2001), siphoning in food from the sediment and its interstitial (pore) water, and pedal-feeding directly from the sediment (Yeager et al. 1994, Vaughn and Hakenkamp 2001). Therefore, contaminants in sediment and sediment pore water are potentially important sources of exposure during the 50 to 74% of time that adult mussels can be completely burrowed beneath the sediment surface (Amyot and Downing 1997, Watters et al. 2001, Schwalb and Pusch 2007). Moreover, the mussel diet is varied and contains such items as detritus, zooplankton, and bacteria, as well as algae (Nichols and Garling 2000, Gustafson 2002, Christian et al. 2004). Contaminants that are on or in food items and ingested by mussels represent a potentially substantial source of exposure. The primary routes of exposure to contaminants for adult mussels are surface water, sediment, pore water, and diet, and adults can be exposed when either partially or completely burrowed in the substrate (Table 1).

The general consensus that adults are less sensitive than glochidia and early juveniles (Ingersoll et al. 2007, Keller et al. 2007, Van Hassel and Farris 2007) might be overstated because many of the past studies with adults were short-term laboratory tests conducted with relatively high toxicant concentrations that were not environmentally relevant (Naimo 1995). Adult mussels have the ability to detect toxicants in the water and they close their valves to avoid exposure (Naimo 1995, Van Hassel and Farris 2007). Thus, accurate measures of toxicity are difficult to obtain in acute toxicity tests on adult mussels. However, the toxicant avoidance response might be less problematic in chronic, low-level exposures (e.g., the type of exposure most common in nature; Strayer et al. 2004), which could yield more realistic estimates of toxicity.

For example, we recently exposed *Lampsilis siliquoidea* (Barnes, 1823) to environmentally relevant Cd concentrations (0.0, 0.2, 1.0, and 5.0 μg/L) while simultaneously measuring O_2 consumption. Briefly, 6 mussels were randomly assigned to each of 4 respirometry chambers in an N-CON Systems® respirometer (N-CON Systems, Crawford, Georgia). This approach allowed continuous monitoring of O_2 consumption at each Cd exposure concentration over 24-h periods between water and toxicant renewals.

Lampsilis siliquoidea detected the toxicant, closed their valves, and ceased respiration in the highest Cd treatment (the only test concentration greater than the current US Environmental Protection Agency, National Aquatic Life Water Quality criterion recommendation of 2.0 μg/L for acute exposures at a hardness of 100 mg/L $CaCO_3$; USEPA 2007) (RBB and DBB, unpublished data; Fig. 1). However, the toxicant avoidance response was sustained only for the first 24 h of the test, after which metabolic requirements presumably forced the mussels to open their valves, respire, and become exposed (Fig. 1). The toxicant avoidance response in mussels at 5.0 μg Cd/L in this test was further verified by measuring ^{109}Cd in test mussels and exposure water, before and after exposure, using a Perkin Elmer Wallac Wizard™ 1480 automatic gamma counter (Perkin Elmer, Shelton, Connecticut). These analyses showed that mussels did not accumulate Cd (Fig. 2) or reduce the activity of ^{109}Cd in the exposure water on day 1 (data not shown). However, beginning on day 2, mussels had linear uptake that was lower than uptake in the 0.2 and 1.0 μg/L treatments (Fig. 2).

These results demonstrate that adult mussel toxicity and relative sensitivity might not be assessed accurately in acute tests with high concentrations, and that

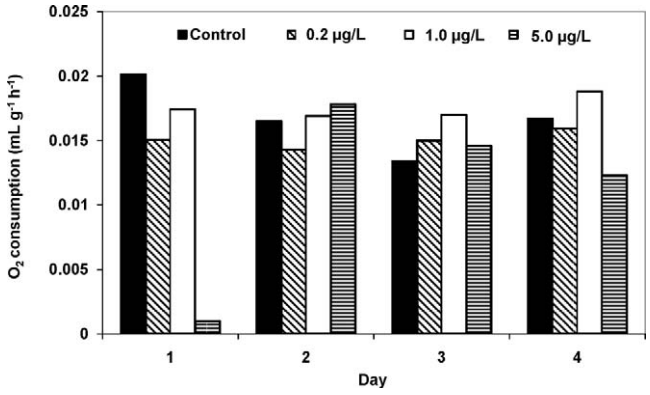

FIG. 1. Total consumption of O_2 by adult *Lampsilis siliquoidea* exposed to Cd for 4 d ($n = 6$ mussels/treatment) in a laboratory respirometry study.

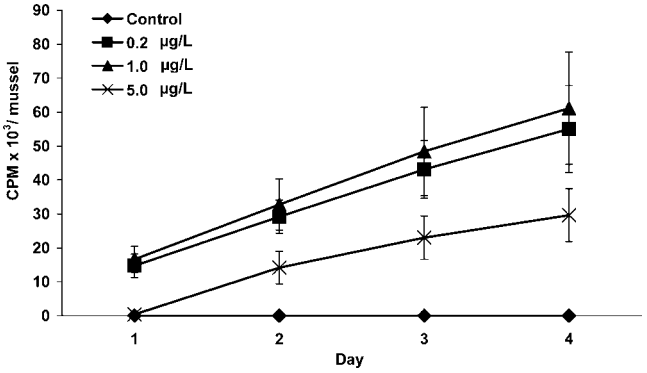

FIG. 2. Mean (±1 SD) accumulation of ^{109}Cd, expressed as counts/min (CPM), by adult *Lampsilis siliquoidea* exposed to Cd for 4 d ($n = 6$ mussels/treatment) in a laboratory respirometry study.

when avoidance behavior can no longer be sustained for physiological reasons, subsequent exposure to and uptake of toxicants might be lower at high than at low toxicant concentrations because uptake is affected by prolonged or periodic toxicant avoidance responses. These results agree with the published literature, which indicates that toxicity estimates based on chronic low-level exposure of adults are similar to estimates for glochidia and juveniles for some toxicants. For example, the toxicity of Cu (LC50 at a hardness of 185 mg/L $CaCO_3$) to 2 species of adult mussels in 28-d flow-through tests ranged from 4.5 to 69 µg/L (Keller et al. 2007) and were similar to acute tests with 9 species of glochidia (24-h static tests, mean: 39 µg/L, range: 10–100 µg/L) and 7 species of juveniles (96-h static-renewal tests, mean: 30 µg/L, range: 6.8–60 µg/L) (Wang et al. 2007c). The duration of any toxicant avoidance response by adult mussels is likely to vary with respect to several key variables, such as species, age, shell thickness and gape, properties of the toxicant, and temperature, and this topic is in need of additional research. Until such research can be completed, tests with glochidia and juveniles will be valuable for protecting adults.

Glochidia (brooded and free)

Most unionoid mussels are dioecious, and glochidial larvae are produced as a result of internal fertilization of ova by sperm in the gills of the female (Watters 2007). The developing embryos are brooded in the gills and develop into mature glochidia for eventual release and attachment on a suitable host fish for transformation into juveniles. Waterborne toxicants have the potential to affect this process adversely through direct action on sperm viability, although almost nothing is known of the potential mechanisms and consequences of this type of exposure. Male mussels release spermatozoa into the water in spherical aggregates called spermatozeugmata (sperm spheres; Waller and Lasee 1997, Ishibashi et al. 2000). Spermatozeugmata have limited propulsion and movement from flagella oriented outward from the sphere. Ishibashi et al. (2000) found that spermatozoa embedded in the spermatozeugmatum were active for at least 48 h after being discharged, although individual spermatozoa lost their motility within minutes in fresh water after dissolution of the sphere. They postulated that spermatozoa might maintain their motility for extended periods (e.g., >48 h) in the water column when contained in the spermatozeugmatum and that this structure allows fertilization of eggs in female mussels far removed from the males. Thus, the spermatozeugmatum also might play a critical role in protecting spermatozoa from waterborne chemical exposure. However, the possibility that environmental contaminants might adversely affect sperm viability within the sphere has implications for reproductive success and should be a topic of research.

Certain contaminants could interfere with the ability of a female to achieve fertilization or brood glochidia to maturity. However, the marsupial region of a female gill is thought to be physiologically isolated from respiratory activities (Kays et al. 1990, Richard et al. 1991), and this isolation might provide a measure of protection from waterborne toxicants (Ingersoll et al. 2007, Keller et al. 2007). However, limited evidence indicates that nutritional and ionic exchange might occur between a brooding female and her glochidia (Silverman et al. 1985, Schwartz and Dimock 2001), providing a possible route for accumulated or waterborne chemicals to disrupt critical biochemical and physiological pathways, such as those involved in maternal Ca transport for construction of the glochidial shell (Silverman et al. 1985). Certain metals, such as Cd and Cu, that are known to interfere with ionic regulation have the potential to decrease the viability of glochidia (Huebner and Pynnonen 1992, Jacobson et al. 1997).

Chemicals that act directly on the neuroendocrine pathways controlling reproduction are a major exception to the assertion that brooding glochidia in the marsupial gill are protected from waterborne (and potentially sediment) toxicant exposure. These chemicals can cause premature release (parturition) of viable or nonviable glochidia. For example, serotonin (5-hydroxytrypamine), an important neurotransmitter in vertebrate and invertebrate systems, has been used to induce spawning in freshwater bivalves for aquaculture (Cunha and Machado 2001) and has been investigated as a potential chemical control mecha-

nism (i.e., disruptor of reproduction) for exotic bivalves, such as the zebra mussel (*Dreissena polymorpha*; Ram et al. 1992, 1996, Fong et al. 1994). The active ingredient in many human prescription antidepressant drugs belonging to the class of selective serotonin reuptake inhibitors (SSRIs) is now found in measurable concentrations in surface waters (Kolpin et al. 2002, 2004, Johnson et al. 2005), and like serotonin (Gibbons and Castagna 1984) has the potential to affect reproduction in bivalves (Cunha and Machado 2001). Thus, environmental exposures from this class of human pharmaceuticals are an imminent threat to native mussels. A related type of chemical exposure that could influence successful attachment of glochidia to host fish comes from compounds like the SSRIs that have the potential to alter mussel behavior because of their action on serotonin and other neuroendocrine pathways. Some species of mussels have specialized mantle flaps and adaptations that mimic prey items of fish to lure host fish and increase the probability of host infestation (Haag et al. 1995). Waterborne chemicals that alter these behaviors in females could affect glochidial infestation rates on host fish, and thus, have population-level implications.

The glochidia (and juveniles) of mussels are the most tested life stages for contaminant toxicity because they are important to the life cycle, have demonstrated sensitivity to some contaminants, and can be obtained or cultured in large numbers for laboratory testing (Barnhart 2006, Ingersoll et al. 2007, Keller et al. 2007). Use of ASTM standard procedures for water-only laboratory toxicity tests with glochidia and juveniles (ASTM 2006) has addressed the procedural issues regarding acceptability of data for regulatory purposes (e.g., derivation of water-quality criteria; Ingersoll et al. 2007). Toxicity tests with glochidia generally are conducted for 24 h (ASTM 2006). This test duration is based largely on the presumed length of time glochidia are in the water between release from the female and encystment onto a host fish and their survival time in water. A 24-h test duration ensures 90% survival in control treatments at the end of the test (required by ASTM protocols; ASTM 2006, Ingersoll et al. 2007). The actual longevity of glochidia after release from the female and before attachment onto a host fish varies with species and water temperature (Zimmerman and Neves 2002), but can range from several days to several weeks (ASTM 2006, Ingersoll et al. 2007). For example, we measured survival of glochidia, based on valve-closure response with addition of saturated NaCl solution (ASTM 2006), from triplicate subsamples (50–100 glochidia/subsample) of 20 species of mussels held separately in containers of hard water (ASTM 2006) at 20°C daily for 10 d. More than 90% of

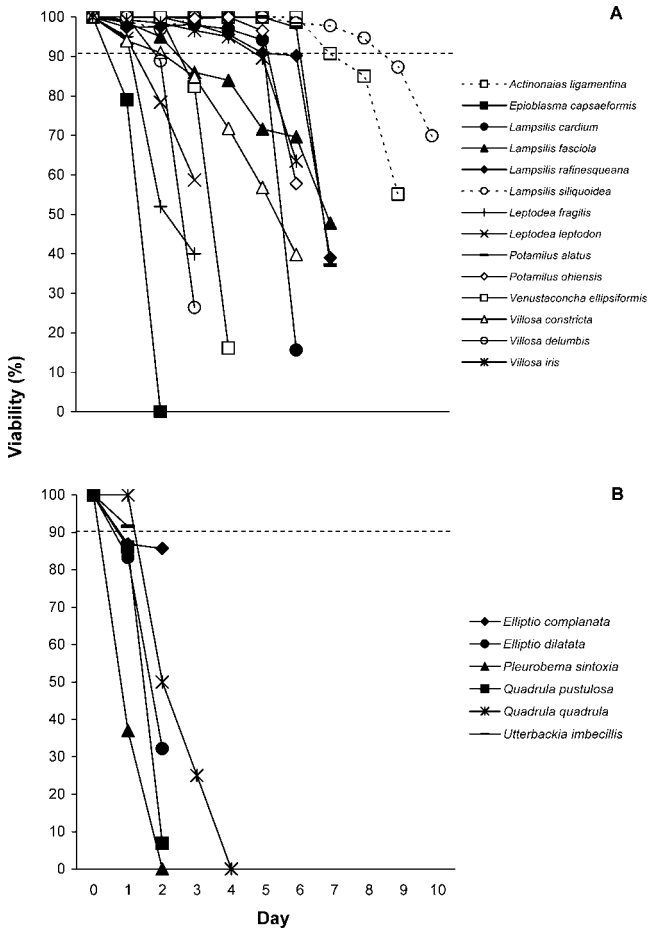

FIG. 3. Viability (survival) curves for glochidia of the unionid tribes Lampsilini (A) and Pleurobemini, Quadrulini, and Anodontini (B) in water at 20°C after release from female mussels. Dashed line at 90% viability represents the ASTM (2006) criteria for control viability in standard 24-h acute toxicity tests.

the species tested had viable glochidia for at least 24 h after release from female mussels (Fig. 3A, B). Glochidia of species belonging to the tribe Lampsilini (Campbell et al. 2005) generally lived longer than those belonging to the tribes Anodontini, Pleurobemini, and Quadrulini (Fig. 3A, B), although fewer species were represented in the latter group. For example, 13 of 14 species from the tribe Lampsilini had >90% survival at 24 h, and 6 of 14 species had >90% survival for 6 to 10 d (Fig. 3A). None of the species tested in the tribes Anodontini, Pleurobemini, and Quadrulini had viabilities >90% for >2 d (Fig. 3B). Thus, 24-h toxicity tests are ecologically relevant for glochidia of most species tested, based on longevity in water, and might underestimate toxicity to longer-lived glochidia. However, the duration of a toxicity test with glochidia may be adjusted to longer or shorter than 24 h, based on the life history of the particular species of interest (ASTM

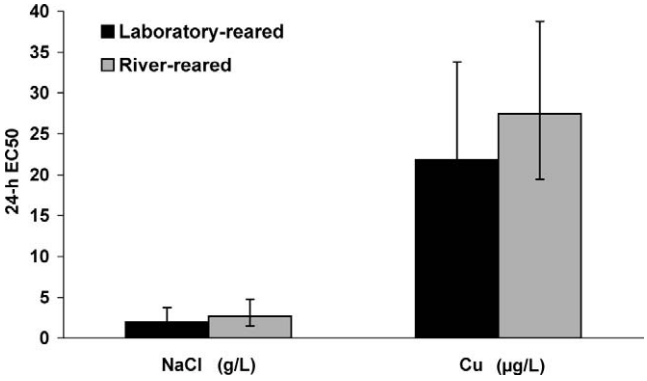

FIG. 4. Toxicity (median effective concentrations [EC50] ± 95% CI) of NaCl and Cu in water-only tests to *Lampsilis siliquoidea* glochidia taken from gravid female mussels held in the laboratory for 6 mo and from gravid female mussels newly collected from the river. The collection site of the brooding females for both groups tested was Silver Fork of Perche Creek, Boone County, Missouri.

2006). A portion of the glochidia released from some species with mantle-flap lure display or other fish-attracting behaviors might attach to host fish within seconds to hours (Ingersoll et al. 2007). However, the recommended 24-h test duration (ASTM 2006) is relevant because many more of the released glochidia from these species can remain viable and infective in the water for days (Zimmerman and Neves 2002). Moreover, many species of mussels snare or lure host fish and increase the probability of infestation by releasing glochidia contained within mucus strands or conglutinate packets resembling prey items of fish (Watters 2007). Thus, glochidia of these species might remain in the water or on the sediment surface for extended periods of time. No studies are available on the toxicity of waterborne or sediment-associated contaminants to glochidia contained in these structures.

The relative sensitivity of glochidia to contaminants varies with species and chemical but is similar to that of newly transformed juveniles (Ingersoll et al. 2007, Keller et al. 2007). For example, the 24-h median effective concentrations (EC50s) for glochidia (9 species) and 96-h EC50s for newly transformed juveniles (6 species) were similar for Cu (glochidia: 39 µg/L, juveniles: 22 µg/L), NH_3 (glochidia: 10 mg N/L, juveniles: 8.6 mg N/L), and Cl (glochidia: 83 µg/L, juveniles: >100 µg/L) (Wang et al. 2007c). These results demonstrate the predictive value of tests on glochidia for estimating the toxicity of chemicals to juveniles.

Tests on glochidia are especially valuable when juveniles of a particular species of mussel are unavailable. One benefit of testing glochidia, relative to juveniles, is that glochidia can be obtained easily in large numbers from gravid females, some of which can be held in the laboratory for extended periods of time. Testing glochidia might be particularly useful when glochidia of endangered species are extracted in the field and the female mussels are returned immediately to their habitat or when methods have not been developed to culture juvenile mussels of a particular species of interest.

Whether toxicity data generated from glochidia recently harvested from river-collected females is similar to data generated from glochidia harvested from females held in the laboratory for several months has been a concern. Recent data indicate that sensitivity of glochidia obtained from both sources is similar. For example, the relative sensitivity of mature glochidia from female *L. siliquoidea* held unfed in the laboratory at 10°C for 6 mo in aerated water (changed weekly) and from females sampled in the field was compared in standardized acute toxicity tests (ASTM 2006) with 2 reference toxicants, NaCl and Cu. The brooding females in both groups were collected from Silver Fork of Perche Creek, Boone County, Missouri. Glochidia from both sources were tested within 1 wk of the time they were flushed from the gills.

The NaCl 24-h EC50 for glochidia from females held in the laboratory (2.0 g/L; 95% confidence interval [CI]: 1.0–3.8) did not differ (based on overlapping 95% CIs) from the NaCl 24-h EC50 for glochidia from freshly collected females (2.7 g/L; 95% CI: 1.5–4.8) (Fig. 4). These values fall within the range of NaCl 24-h EC50 values from previous toxicity tests on 5 species of mussel glochidia (0.6–3.3 g/L, mean = 1.9 g/L; $n = 7$; Bringolf et al. 2007b). The Cu 24-h EC50 for glochidia from females held in the laboratory (21.9 µg/L; 95% CI: 14.1–33.8) did not differ from the Cu 24-h EC50 for glochidia from freshly collected females (27.5 µg/L; 95% CI: 19.5–38.8) (Fig. 4). These values fall within the range of Cu 24-h EC50 values from previous toxicity tests with *L. siliquoidea* glochidia (33.0 µg/L; 95% CI: 32.0–35.0; Wang et al. 2007a). Thus, results of both toxicity tests suggest that sensitivity did not differ between the 2 groups of glochidia. Moreover, the 24-h EC50s are similar to values obtained in toxicity tests with other mussel glochidia (Wang et al. 2007c). Therefore, glochidia from gravid *L. siliquoidea* (and possibly other species) held in the laboratory for extended periods appear suitable for conducting standardized toxicity tests.

Based on the available information for glochidia, the primary route of exposure for contaminants is through surface water, and occurrence of the exposure can be when the larvae are free in the water or packaged in mucus and conglutinates over the course of seconds to

days (Table 1). However, other routes and mechanisms of exposure, such as in the marsupial gill, are plausible and require further investigation.

Encysted glochidia

Glochidia on the gills or fins of a host fish become encysted in the fish tissue within 2 to 36 h (Ingersoll et al. 2007). The general hypothesis has been that the cyst provides a measure of protection from waterborne toxicants (Jacobson et al. 1997, Rach et al. 2006). Therefore, exposure to waterborne chemicals that could influence successful transformation into juveniles is restricted to the period before full encystment occurs. However, fish body burdens of certain contaminants (e.g., dichlorodiphenyldichloroethylene [DDE], toxaphene, and atrazine) might adversely affect transformation success of glochidia into juveniles (Ingersoll et al. 2007). This type of effect and route of exposure is consistent with our current understanding of the physiology of encysted glochidia as true parasites on host fish. Glochidia feed on host tissue entrapped between the glochidial shells and autolyze their own adductor muscle (Watters 2007). Glochidia remain encysted on host fish for a period of a week to several months, and during this period, exposure to contaminant burdens contained in host fish tissues represents a potential route of contamination. The information available for encysted glochidia indicates that the primary potential routes of exposure for contaminants are through surface water until encystment (2–36 h) and host fish tissue burdens thereafter (weeks to months) (Table 1). Without research on this potential mechanism for contaminant exposure, no further assessment is possible.

Juvenile mussels

The transformation from glochidia encysted on a host fish to free-living juveniles generally takes place in several stages over a 2- to 6-wk period in most North American species; actual transformation time depends upon the mussel species, host fish species, and water temperature (Watters 2007). Once free of the host fish, juvenile mussels become infaunal benthic organisms that typically remain burrowed beneath the sediment surface through the first 2 to 4 y of life (Strayer et al. 2004, Schwalb and Pusch 2007). Adult mussels exhibit vertical movement patterns in the substrate with periods at, above, or below the sediment surface, but juveniles (<3 y old) tend to remain burrowed within the sediment (Balfour and Smock 1995). This infaunal existence might greatly influence exposure routes and subsequent uptake of contaminants during the first several years of life. For example, residence beneath the sediment surface necessitates deposit feeding, and juveniles ingest algae and other interstitial organic matter for nutrition and rely on pore water for O_2 (Watters 2007). Pedal feeding, a form of deposit-feeding that uses ciliary currents on the foot, is the primary method of feeding in early juvenile mussels because gills are rudimentary and incapable of filtering particles (Yeager et al. 1994). The exact composition of the juvenile diet is unknown, but a mixture of fine particulate organic matter such as detritus, bacteria, and algae is likely, and contaminants that are in or on these food items and ingested by the juveniles represent a potentially substantial source of exposure. The relative importance of exposure of juvenile mussels to contaminants in overlying surface water, pore water, whole sediment, or food has not been adequately assessed. However, during the first 0–4 y of life, exposure to contaminants from each of these routes might be important and probably varies with certain periods and environmental conditions (Table 1).

This understanding of life history and ecology of juvenile mussels points to the immediate need to develop a standardized sediment-based toxicity test method for juvenile mussels, so that toxicity of contaminants in whole sediment and in pore water within whole sediment can be assessed in an ecologically relevant manner. The ASTM standard guide for conducting laboratory toxicity tests with juveniles (and glochidia) deals exclusively with water exposures (ASTM 2006), and the toxicity of the relevant sediment porewater compartment and of the surface water to juveniles can best be assessed with the current ASTM protocol. However, a standardized sediment toxicity test for early life stages of mussels probably would generate data that provide greater protection, if it were based on probable exposure routes in the habitat occupied by juveniles.

A limited number of acute or chronic whole-sediment toxicity tests have been done with freshwater mussels (reviewed by Ingersoll et al. 2007). However, a study by Newton and Bartsch (2007) that compared the toxicity of NH_3 to juvenile mussels between water-only and sediment exposure routes might be useful for advancing the concept of a sediment-based test. Newton and Bartsch (2007) found that the mean 96-h LC50 for juvenile *Lampsilis cardium* (Rafinesque, 1820) was ~2 to 3× lower in sediment exposures (124 μg NH_3-N/L) than in water-only exposures (215–372 μg NH_3-N/L), depending on pH. Their results indicate that a sediment-based exposure might provide a more realistic evaluation of toxicity for some toxicants, such as NH_3. However, the pH and NH_4^+ concentration of the sediments influence the proportion of ammonia in

the more toxic NH_3 form that is available to juveniles, and uncertainty exists regarding the best way to measure pH and NH_4^+ in sediment microhabitats occupied by juvenile mussels. The results obtained by Newton and Bartsch (2007) also emphasize a point made by Watters (2007); i.e., efforts to protect adult mussels might not be adequate to protect juveniles because of their unique developmental biology and feeding ecology. An added benefit of establishing a sediment toxicity test for juvenile mussels would be that growth, an important measure of sublethal toxicity and potential indicator of long-term health and survival, could be evaluated more appropriately in sediment tests than in water-only tests. For example, Newton and Bartsch (2007) showed that growth rates of juveniles were greater in sediment exposures than in water-only exposures, presumably because of the nutritive components within the sediments and juvenile feeding strategies.

The relative sensitivity of newly transformed juveniles to contaminants varies with mussel species and chemical, but sensitivity to some toxicants also might vary with the age of the juvenile. In toxicity tests conducted with 2- to 4-mo-old juveniles reared in the laboratory, older juveniles had similar sensitivity to NH_3 but were less sensitive to Cu than were newly transformed juveniles (Wang et al. 2007c). Wang et al. (2007c) completed 96-h Cu toxicity tests with newly transformed or 2-mo-old juvenile mussels, transferred the juveniles to clean water, and held them for an additional 24 h. The 96-h EC50s for newly transformed mussels did not change, whereas EC50s for 2-mo-old mussels increased $\geq 3\times$. This result indicates that the older juveniles avoided exposure to Cu in the acute 96-h tests with relatively high concentrations by temporarily closing their valves (see discussion of the toxicant avoidance behavior in *Adult mussels* above). However, similar NH_3 EC50s between newly transformed and 2-mo-old mussels suggest that older juveniles might be unable to avoid exposure to NH_3. Additional studies are needed to determine why mussels can avoid exposure to some chemicals and not others.

Another consideration is potential differences in sensitivity between laboratory-reared juveniles and juveniles of a similar age taken from nature because their relative health and condition might differ as a result of differences in diet. Recent toxicity tests were done to determine potential effects of laboratory holding on juvenile survival and to assess the potential influence of relative size (at a given age) on toxicity. Glochidia were collected from 4 gravid females of *L. siliquoidea*, pooled, and inoculated onto largemouth bass (*Micropterus salmoides* [Lacepede, 1820], average length = 14 cm) at the Genoa National Fish Hatchery (US Fish and Wildlife Service, Genoa, Wisconsin). A subsample of 100 infested fish was shipped promptly to the Mussel Culture Facility at Missouri State University for propagation of the juvenile mussels. When the newly transformed juveniles were harvested, 2 subsamples were obtained: 1 for culture in a laboratory recirculating system (Barnhart 2006) and 1 for deployment in cages called mussel silos in the James River, Missouri (MCB, unpublished data). Infested fish retained at the Genoa National Fish Hatchery were transported to the Upper Mississippi River at Dubuque, Iowa, and placed in culture cages suspended in a floating rack. The cages were fitted with solid bottoms that were covered with ~3 cm of dredged river sand to catch and hold the transformed juveniles. Host fish were placed in the cages at a density of 30 fish/cage, and 3 cages were held suspended ~1.3 m below the water surface. Mussels were harvested from the cage bottoms for toxicity testing after 111 d. A total of 1600 juvenile mussels were collected from the 3 cages with a mean (±1 standard deviation [SD]) length of 25.7 ± 3.7 mm (n = 100). The juvenile mussels reared in the James River in mussel silos, which had no direct contact with river sediment for burrowing, and the juveniles grown in the laboratory culture system at Missouri State University were harvested for toxicity testing at the same time as the juveniles grown in cages in the Upper Mississippi River. The mean length of the laboratory-reared mussels was 1.07 ± 0.2 mm (n = 42), and the mean length of the James River–reared mussels was 1.29 ± 0.3 mm (n = 42). Acute 96-h toxicity tests with NaCl were conducted on all 3 batches of juveniles simultaneously according to standard methods (ASTM 2006).

Despite the substantial differences in length among the juveniles from the different sites, sensitivities to NaCl at 96 h (WGC, unpublished data; Fig. 5) did not differ among rearing treatments (based on overlapping 95% CIs). The largest juveniles (from the cages in the Upper Mississippi River) were less sensitive than juveniles from the other sites after 48 h of exposure to NaCl, presumably because they were able to avoid exposure by closing their valves (see discussion in *Adult mussels* above). However, by 96 h (the standard length of a toxicity test with juvenile mussels; ASTM 2006), sensitivity of the large mussels (4.8 g/L; 95% CI: 3.4–6.7) was similar to sensitivities of the smaller mussels cultured in the laboratory (3.2 g/L; 95% CI: 2.2–4.5) and in the James River (2.9 g/L; 95% CI: 2.0–4.3) (Fig. 5). These results are consistent with other tests (e.g., mean 96-h EC50 = 4.5 g/L; 95% CI: 3.8–5.4) with 3 species of juvenile mussels exposed to NaCl

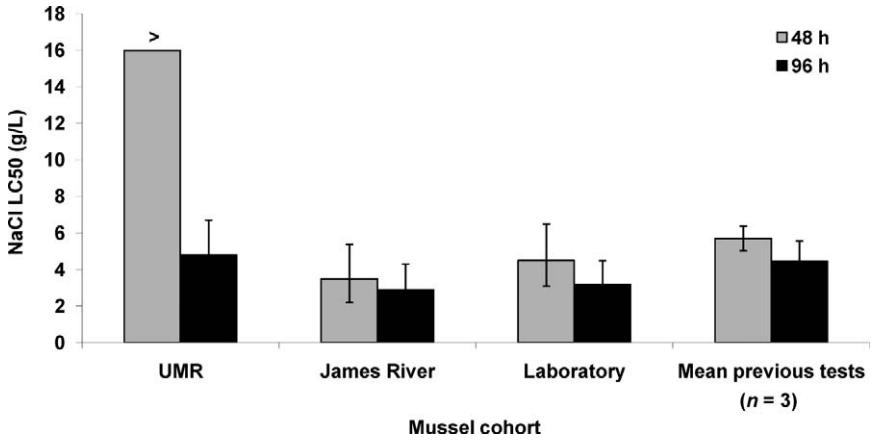

FIG. 5. Toxicity (median effective concentrations [EC50] ± 95% CI) of NaCl in water-only tests to 3 subsamples of juvenile *Lampsilis siliquoidea*, each produced from the same host fish infestation and reared separately in cages in the Upper Mississippi River (UMR) and James River, Missouri, and in a laboratory recirculating system (Barnhart 2006). All mussels were 12 wk old when tested. Mean (±1 SD) EC50s of previous toxicity tests conducted with 3 mussel species are shown for comparison (data from Bringolf et al. 2007b). > symbol indicates that the EC50 was greater than the highest test concentration of NaCl.

(Bringolf et al. 2007b; Fig. 5). These results suggest that sensitivity of juvenile mussels cultured in the laboratory is similar to that of mussels grown in a more natural setting, despite their smaller size for a given age, and that laboratory-reared juveniles are sufficiently representative of wild-caught mussels for purposes of toxicity testing.

Research Needs

Advances have been made in laboratory toxicity-testing methods and in developing toxicant-specific information that can be used to derive water-quality criteria protective of mussels (Augspurger et al. 2003, March et al. 2007), but much remains to be done. The important results of single-chemical laboratory tests, such as for NH_3, and in situ caging studies with juveniles, might help to explain the declines observed in populations in certain locations. In situ exposures also might help address the effects on mussels of multiple chemical stressors, an area of research that has received little attention (e.g., Milam et al. 2005). Existing acute, water-only laboratory test methods should provide a solid framework for assessing the toxicity of chemical mixtures in the laboratory or of complex effluent samples taken from specific locations. Chronic (21- or 28-d) studies with juveniles have been conducted in laboratory settings (Bringolf et al. 2007a, b, c, Wang et al. 2007b), but longer-term studies with juveniles and adults would be especially useful because most mussels have life spans of decades. Developing standardized test methods for adult mussels and sediment-based testing protocols for juveniles and adults should be research priorities, given recent evidence of the importance of habitat, diet, and feeding strategies to routes of contaminant exposure. For example, current US Environmental Protection Agency water-quality criteria for aquatic life for most metals that are not bioaccumulative assume that metals dissolved in the water are the primary cause of toxicity in exposed aquatic organisms. This assumption might require additional study for freshwater mussels because a significant portion of their exposure to metals might occur through their diet. Any future tests should strive to advance endpoints from lethality to meaningful sublethal measures (e.g., Newton and Cope 2007) of fitness, such as physiological health and reproductive success, that might be linked to population-level consequences. Determining the role of host-fish contaminant burdens on transformation success from encysted glochidia to free-living juveniles is imperative. Much new knowledge of the biology, ecology, and toxicology of native mussels has been gained over the past 5 to 10 y, and state and federal resource management agencies should continue to fund the necessary research on this imperiled group of organisms so that science-based information can guide improved management, regulatory, and policy decisions to curtail further losses of this valuable fauna in North America and throughout the world.

Literature Cited

AMYOT, J.-P., AND J. A. DOWNING. 1997. Seasonal variation in vertical and horizontal movement of the freshwater bivalve *Elliptio complanata* (Mollusca: Unionidae). Freshwater Biology 37:345–354.

ASTM (AMERICAN SOCIETY FOR TESTING AND MATERIALS). 2006.

Standard guide for conducting laboratory toxicity tests with freshwater mussels (E2455-06). ASTM annual book of standards. Volume 11.05. American Society for Testing and Materials, West Conshohocken, Pennsylvania.

AUGSPURGER, T., F. J. DWYER, C. G. INGERSOLL, AND C. M. KANE. 2007. Advances and opportunities in assessing contaminant sensitivity of freshwater mussel (Unionidae) early life stages. Environmental Toxicology and Chemistry 26: 2025–2028.

AUGSPURGER, T., A. E. KELLER, M. C. BLACK, W. G. COPE, AND F. J. DWYER. 2003. Water quality guidance for the protection of freshwater mussels (Unionidae) from ammonia exposure. Environmental Toxicology and Chemistry 22: 2569–2575.

BALFOUR, D. L., AND L. A. SMOCK. 1995. Distribution, age structure, and movements of the freshwater mussel *Elliptio complanata* (Mollusca: Unionidae) in a headwater stream. Journal of Freshwater Ecology 10:255–268.

BARNHART, M. C. 2006. Buckets of muckets: a compact system for rearing juvenile freshwater mussels. Aquaculture 254: 227–233.

BOGAN, A. E. 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): a search for causes. American Zoologist 33: 599–609.

BRINGOLF, R. B., W. G. COPE, M. C. BARNHART, S. MOSHER, P. R. LAZARO, AND D. SHEA. 2007a. Acute and chronic toxicity of pesticide formulations (atrazine, chlorpyrifos and permethrin) to glochidia and juveniles of *Lampsilis siliquoidea* (Unionidae). Environmental Toxicology and Chemistry 26:2101–2107.

BRINGOLF, R. B., W. G. COPE, C. B. EADS, P. R. LAZARO, M. C. BARNHART, AND D. SHEA. 2007b. Acute and chronic toxicity of technical-grade pesticides to glochidia and juveniles of freshwater mussels (Unionidae). Environmental Toxicology and Chemistry 26:2086–2093.

BRINGOLF, R. B., W. G. COPE, S. MOSHER, M. C. BARNHART, AND D. SHEA. 2007c. Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of *Lampsilis siliquoidea* (Unionidae). Environmental Toxicology and Chemistry 26:2094–2100.

CAMPBELL, D. C., J. M. SERB, J. E. BUHAY, K. J. ROE, R. L. MINTON, AND C. LYDEARD. 2005. Phylogeny of North American amblemines (Bivalvia, Unionoida): prodigious polyphyly proves pervasive across genera. Invertebrate Biology 124:131–164.

CHRISTIAN, A. D., B. N. SMITH, D. J. BERG, J. C. SMOOT, AND R. H. FINDLAY. 2004. Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca: Unionidae) in 2 small Ohio streams. Journal of the North American Benthological Society 23:101–113.

CUNHA, E. M., AND J. MACHADO. 2001. Parturition in *Anodonta cygnea* induced by selective serotonin reuptake inhibitors (SSRIs). Canadian Journal of Zoology 79:95–100.

FONG, P. P., J. DUNCAN, AND J. L. RAM. 1994. Inhibition and sex specific induction of spawning by sertonergic ligands in the zebra mussel *Dreissena polymorpha* (Pallas). Experientia 50:506–509.

GIBBONS, M. C., AND M. CASTAGNA. 1984. Serotonin as an inducer of spawning in six bivalve species. Aquaculture 40:189–191.

GUSTAFSON, L. L. 2002. Nonlethal health assessment of the freshwater mussel *Elliptio complanata*. PhD Thesis, North Carolina State University, Raleigh, North Carolina.

HAAG, W. R., R. S. BUTLER, AND P. D. HARTFIELD. 1995. An extraordinary reproductive strategy in freshwater bivalves: prey mimicry to facilitate larval dispersal. Freshwater Biology 34:471–476.

HORNE, F. R., AND S. MCINTOSH. 1979. Factors influencing distribution of mussels in the Blanco River of central Texas. Nautilus 94:119–133.

HUEBNER, J. D., AND K. S. PYNNONEN. 1992. Viability of glochidia of two species of *Anodonta* exposed to low pH and selected metals. Canadian Journal of Zoology 70: 2348–2355.

INGERSOLL, C. G., N. J. KERNAGHAN, T. S. GROSS, C. D. BISHOP, N. WANG, AND A. ROBERTS. 2007. Laboratory toxicity testing with freshwater mussels. Pages 95–134 *in* J. L. Farris and J. H. Van Hassel (editors). Freshwater bivalve ecotoxicology. CRC Press, Boca Raton, Florida, and SETAC Press, Pensacola, Florida.

ISHIBASHI, R., A. KOMARU, AND T. KONDO. 2000. Sperm sphere in unionid mussels (Bivalvia: Unionidae). Zoological Science 17:947–950.

JACOBSON, P. J., R. J. NEVES, D. S. CHERRY, AND J. L. FARRIS. 1997. Sensitivity of glochidial stages of freshwater mussels (Bivalvia: Unionidae) to copper. Environmental Toxicology and Chemistry 16:2384–2392.

JOHNSON, D. J., H. SANDERSON, R. A. BRAIN, C. J. WILSON, K. T. BESTARI, AND K. R. SOLOMON. 2005. Exposure assessment and microcosm fate of selected selective serotonin reuptake inhibitors. Regulatory Toxicology and Pharmacology 42:313–323.

KAYS, W. T., H. SILVERMAN, AND T. H. DIETZ. 1990. Water channels and water canals in the gill of the freshwater mussel, *Ligumia subrostrata*: ultrastructure and histochemistry. Journal of Experimental Zoology 254:256–269.

KELLER, A., M. LYDY, AND D. S. RUESSLER. 2007. Unionid mussel sensitivity to environmental contaminants. Pages 151–167 *in* J. L. Farris and J. H. Van Hassel (editors). Freshwater bivalve ecotoxicology. CRC Press, Boca Raton, Florida, and SETAC Press, Pensacola, Florida.

KELLER, A. E. 1993. Acute toxicity of several pesticides, organic compounds, and a wastewater effluent to the freshwater mussel, *Anodonta imbecillis, Ceriodaphnia dubia*, and *Pimephales promelas*. Bulletin of Environmental Contamination and Toxicology 51:696–702.

KELLER, A. E., AND D. S. RUESSLER. 1997. The toxicity of malathion to unionid mussels: relationship to expected environmental concentrations. Environmental Toxicology and Chemistry 16:1028–1033.

KOLPIN, D. W., E. T. FURLONG, M. T. MEYER, E. M. THURMAN, S. D. ZAUGG, L. B. BARBER, AND H. T. BUXTON. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environmental Science and Technology 36:1202–1211.

KOLPIN, D. W., M. SKOPEC, M. T. MEYER, E. T. FURLONG, AND S.

D. ZAUGG. 2004. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. Science of the Total Environment 328:119–130.

LYDEARD, C., R. H. COWIE, W. F. PONDER, A. E. BOGAN, P. BOUCHET, S. A. CLARK, K. S. CUMMINGS, T. J. FREST, O. GARGOMINY, D. G. HERBERT, R. HERSHLER, K. E. PEREZ, B. ROTH, M. SEDDON, E. E. STRONG, AND F. G. THOMPSON. 2004. The global decline of nonmarine mollusks. BioScience 54: 321–330.

MARCH, F. A., F. J. DWYER, T. AUGSPURGER, C. G. INGERSOLL, N. WANG, AND C. A. MEBANE. 2007. An evaluation of freshwater mussel toxicity data in the derivation of water quality guidance and standards for copper. Environmental Toxicology and Chemistry 26:2066–2074.

MCMAHON, R. F., AND A. E. BOGAN. 2001. Mollusca: Bivalvia. Pages 331–429 *in* J. H. Thorp and A. P. Covich (editors). Ecology and classification of North American freshwater invertebrates. 2nd edition. Academic Press, San Diego, California.

MILAM, C. D., J. L. FARRIS, F. J. DWYER, AND D. K. HARDESTY. 2005. Acute toxicity of six freshwater mussel species (glochidia) to six chemicals: implications for daphnids and *Utterbackia imbecillis* as surrogates for protection of freshwater mussels (Unionidae). Archives of Environmental Contamination and Toxicology 48:166–173.

NAIMO, T. J. 1995. A review of the effects of heavy metals on freshwater mussels. Ecotoxicology 4:341–362.

NEWTON, T. J., AND M. R. BARTSCH. 2007. Lethal and sublethal effects of ammonia to juvenile *Lampsilis* mussels (Unionidae) in sediment and water-only exposures. Environmental Toxicology and Chemistry 26:2057–2065.

NEWTON, T. J., AND W. G. COPE. 2007. Biomarker responses of unionid mussels to environmental contaminants. Pages 95–134 *in* J. L. Farris and J. H. Van Hassel (editors). Freshwater bivalve ecotoxicology. CRC Press, Boca Raton, Florida, and SETAC Press, Pensacola, Florida.

NICHOLS, S. J., AND D. GARLING. 2000. Food-web dynamics and trophic-level interactions in a multi-species community of freshwater unionids. Canadian Journal of Zoology 78: 871–882.

ORTMANN, A. E. 1909. The destruction of the fresh-water fauna in western Pennsylvania. Proceedings of the American Philosophical Society 48:90–110.

RACH, J. J., T. BRADY, T. M. SCHREIER, AND D. ALOISI. 2006. Safety of fish therapeutants to glochidia of the plain pocketbook mussel during encystment on largemouth bass. North American Journal of Aquaculture 68:348–354.

RAIKOW, D. F., AND S. K. HAMILTON. 2001. Bivalve diets in a Midwestern U.S. stream: a stable isotope enrichment study. Limnology and Oceanography 46:514–522.

RAM, J. L., P. FONG, R. P. CROLL, S. J. NICHOLS, AND D. WALL. 1992. The zebra mussel (*Dreissena polymorpha*), a new pest in North America: reproductive mechanisms as possible targets of control strategies. Invertebrate Reproduction and Development 22:77–86.

RAM, J. L., P. P. FONG, AND D. W. GARTON. 1996. Physiological aspects of zebra mussel reproduction: maturation, spawning, and fertilization. American Zoologist 36: 326–338.

RICHARD, P. E., T. H. DIETZ, AND H. SILVERMAN. 1991. Structure of the gill during reproduction in the unionids *Anodonta grandis*, *Ligumia subrostrata*, and *Carunclina parva texasensis*. Canadian Journal of Zoology 60:1722–1727.

RICHTER, B. D., D. P. BRAUN, M. A. MENDELSON, AND L. L. MASTER. 1997. Threats to imperiled freshwater fauna. Conservation Biology 11:1081–1093.

SCHWALB, A. N., AND M. T. PUSCH. 2007. Horizontal and vertical movements of unionid mussels in a lowland river. Journal of the North American Benthological Society 26:261–272.

SCHWARTZ, M. L., AND R. V. DIMOCK. 2001. Ultrastructural evidence for nutritional exchange between brooding unionid mussels and their glochidia larvae. Invertebrate Biology 120:227–236.

SILVERMAN, H., W. L. STEFFENS, AND T. H. DIETZ. 1985. Calcium from extracellular concretions in the gills of freshwater unionid mussels is mobilized during reproduction. Journal of Experimental Zoology 236:137–147.

STARRETT, W. C. 1971. A survey of the mussels (Unionacea) of the Illinois River, a polluted stream. Illinois Natural History Survey Bulletin 30:265–403.

STRAYER, D. L., J. A. DOWNING, W. R. HAAG, T. L. KING, J. B. LAYZER, T. J. NEWTON, AND S. J. NICHOLS. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. BioScience 54:429–439.

USEPA (US ENVIRONMENTAL PROTECTION AGENCY). 2007. Current national recommended water quality criteria. Office of Water, US Environmental Protection Agency, Washington, DC. (Available from: http://www.epa.gov/waterscience/criteria/wqcriteria.html)

VAN HASSEL, J. H., AND J. L. FARRIS. 2007. A review of the use of unionid mussels as biological indicators of ecosystem health. Pages 19–49 *in* J. L. Farris and J. H. Van Hassel (editors). Freshwater bivalve ecotoxicology. CRC Press, Boca Raton, Florida, and SETAC Press, Pensacola, Florida.

VAUGHN, C. C., AND C. C. HAKENKAMP. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46:1431–1446.

WALLER, D. L., AND B. A. LASEE. 1997. External morphology of spermatozoa and spermatozeugmata of the freshwater mussel *Truncilla truncata* (Mollusca: Bivalvia: Unionidae). American Midland Naturalist 138:220–223.

WANG, N., T. AUGSPURGER, M. C. BARNHART, J. R. BIDWELL, W. G. COPE, F. J. DWYER, S. GEIS, I. E. GREER, C. G. INGERSOLL, C. M. KANE, T. W. MAY, R. J. NEVES, T. J. NEWTON, A. D. ROBERTS, AND D. W. WHITES. 2007a. Intra- and interlaboratory variability in acute toxicity tests with glochidia and juveniles of freshwater mussels (Unionidae). Environmental Toxicology and Chemistry 26:2029–2035.

WANG, N., C. G. INGERSOLL, I. E. GREER, D. K. HARDESTY, C. D. IVEY, J. L. KUNZ, W. G. BRUMBAUGH, F. J. DWYER, A. D. ROBERTS, T. AUGSPURGER, C. M. KANE, R. J. NEVES, AND M. C. BARNHART. 2007b. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). Environmental Toxicology and Chemistry 26:2048–2056.

WANG, N., C. G. INGERSOLL, D. K. HARDESTY, C. D. IVEY, J. L. KUNZ, T. W. MAY, F. J. DWYER, A. D. ROBERTS, T. AUGSPURGER, C. M. KANE, R. J. NEVES, AND M. C. BARNHART. 2007c. Acute toxicity of copper, ammonia, and chlorine to glochidia and juveniles of freshwater mussels (Unionidae). Environmental Toxicology and Chemistry 26: 2036–2047.

WATTERS, G. T. 2007. A brief look at freshwater mussel (Unionacea) biology. Pages 51–64 *in* J. L. Farris and J. H. Van Hassel (editors). Freshwater bivalve ecotoxicology. CRC Press, Boca Raton, Florida, and SETAC Press, Pensacola, Florida.

WATTERS, G. T., S. H. O'DEE, AND S. CHORDAS. 2001. Patterns of vertical migration in freshwater mussels (Bivalvia: Unionoida). Journal of Freshwater Ecology 16:541–549.

WILLIAMS, J. D., AND R. J. NEVES. 1995. Freshwater mussels: a neglected and declining aquatic resource. Pages 177–197 *in* E. T. LaRoe, G. S. Farris, C. E. Puckett, P. D. Doran, and M. J. Mac (editors). Our living resources: a report to the nation on the distribution, abundance, and health of U.S. plants, animals, and ecosystems. National Biological Service, US Department of the Interior, Washington, DC.

WILLIAMS, J. D., M. L. WARREN, K. S. CUMMINGS, J. L. HARRIS, AND R. J. NEVES. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18(9):6–22.

YEAGER, M. 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.

ZIMMERMAN, L. L., AND R. J. NEVES. 2002. Effects of temperature on duration of viability for glochidia of freshwater mussels (Bivalvia: Unionidae). American Malacological Bulletin 17:31–35.

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