Transactions of the American Fisheries Society 136:1593–1606, 2007

DOI: 10.1577/T07-006.1

[Article]

Exposure of Unionid Mussels to Electric Current: Assessing Risks Associated with Electrofishing

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Abstract.—Electric current is routinely applied in freshwater for scientific sampling of fish populations (i.e., electrofishing). Freshwater mussels (families Margaritiferidae and Unionidae) are distributed worldwide, but their recent declines in diversity and abundance constitute an imperilment of global significance. Freshwater mussels are not targeted for capture by electrofishing, and any exposure to electric current is unintentional. The effects of electric shock are not fully understood for mussels but could disrupt vital physiological processes and represent an additional threat to their survival. In a controlled laboratory environment, we examined the consequences of exposure to two typical electrofishing currents, 60-Hz pulsed DC and 60-Hz AC, for the survival of adult and early life stages of three unionid species; we included fish as a quality control measure. The outcomes suggest that electrical exposure associated with typical electrofishing poses little direct risk to freshwater mussels. That is, adult mussel survival and righting behaviors (indicators of sublethal stress) were not adversely affected by electrical exposure. Glochidia (larvae that attach to and become parasites on fish gills or fins) showed minimal immediate reduction in viability after exposure. Metamorphosis from glochidia to free-living juvenile mussels was not impaired after electric current simulated capture-prone behaviors (stunning) in infested host fish. In addition, the short-term survival of juvenile mussels was not adversely influenced by exposure to electric current. Any minimal risk to imperiled mussels must be weighed at the population level against the benefits gained by using the gear for scientific sampling of fish in the same waters. However, scientists sampling fish by electrofishing should be aware of mussel reproductive periods and processes in order to minimize the harmful effects to host fish, especially in areas where mussel conservation is a concern.

Electrofishing (i.e., fishing with electric current in surface waters) is an effective method for scientific sampling of freshwater fish populations (Reynolds 1996); it is used globally and is increasingly being employed for collection of aquatic invertebrates (Rabeni et al. 1997). Freshwater mussels are not targeted for capture by electrofishing; however, those residing in shallow waters may be exposed to electric currents during collection efforts targeting other organisms. The physical, physiological, and behavioral effects of exposure to electric current on fish have been studied extensively (Schreck et al. 1976; Sharber and Carothers 1988; Holliman et al. 2003a). In contrast, the direct or indirect consequences of exposure to electric current on freshwater mussels have not been widely investigated.

Freshwater mussels belonging to the families Margaritiferidae and Unionidae are globally distributed but are declining in abundance and diversity (Bogan 1993; Lydeard et al. 2004). The International Union for the Conservation of Nature and Natural Resources (IUCN)–World Conservation Union recently catego-

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[1] The Unit is jointly supported by North Carolina State University, the North Carolina Wildlife Resources Commission, the U.S. Geological Survey, and the Wildlife Management Institute.

Received January 8, 2007; accepted July 23, 2007
Published online November 8, 2007

rized 126 of the 306 (41%) unionoidean mussels occurring in North America as critically endangered, endangered, or vulnerable, placing them on the 2004 IUCN Red List (Baillie et al. 2004). Imperilment of this magnitude has global significance. The peril may, however, be even greater because Williams et al. (1993) estimated that up to 70% of the unionoidean species in the United States may be endangered, threatened, or of special concern. The decline of the freshwater mussel fauna has led to national and international efforts to identify research, management, and conservation measures for maintenance and recovery (National Native Mussel Conservation Committee 1998; Lydeard et al. 2004).

The survival of mussel populations is directly related to the timely presence and abundance of host fish (Neves et al. 1997). The life cycle of unionoid mussels includes an obligate parasitic stage, where glochidia (larvae) attach to the gills or fins of a host fish. After attachment, the encysted parasitic stage metamorphoses into a free-living juvenile stage. Hosts may be a taxonomically diverse assortment of fish, or only one or two closely related species, depending on the taxa of mussels. The relationship between fish and mussels may lead to the integration of fish conservation plans with those for mussels (National Native Mussel Conservation Committee 1998). Ironically, electrofishing surveys to assess the population of fish species that host the mussel life cycle could disrupt the brood cycle of glochidia in gravid mussels, glochidia attachment to fish, and metamorphosis of the encysted stage to free-living juvenile.

The use of electric current for the collection of imperiled fish, in which deleterious effects on individual fish may influence survival of the population, is a topic of concern and ongoing research (Nielsen 1998; Holliman et al. 2003a, 2003b). Little is known about the physiological effects of electroshock on mussel health, reproduction, and juvenile recruitment. To our knowledge, the work conducted by Hastie and Boon (2001), which showed no adverse effects on adult eastern pearlshell *Margaritifera margaritifera*, a coldwater margaritiferid species, is the only published work addressing the effects of electric current on native freshwater mussels. Their investigation was a field study and subject to the suite of uncontrolled variables commonly influencing electrofishing (Zalewski and Cowx 1990).

The aim of our study was to examine the risks of exposure to electric currents commonly associated with electrofishing for freshwater mussels. Accordingly, we investigated the effects of electric current of varied strength and duration on the four life stages (glochidia, encysted, juvenile, and adult) of three warmwater unionid species in a controlled laboratory environment.

Methods

Treatments.—Mussels were exposed to electric fields (experimental treatments) defined by type of electric current, electric power per volume of water, and duration (period) of exposure. Mussels designated as controls were subjected to experimental protocols and procedures but not electrical treatment. Treatments were administered in one of two dielectric exposure chambers, the size of the organism by life stage determining the chamber used for treatment. The electric currents used to generate electric fields were pulsed direct current (PDC) or alternating current (AC). The PDC was characterized by 60 pulses/s (Hz), a period of 16.6 ms, a 6-ms pulse width, and a 36% duty cycle (ratio of pulse time to the period). The AC was a single-phase sine-wave cycling at 60 Hz. Quantities of AC were measured and reported as root mean square (RMS) values. Adult mussels, largemouth bass *Micropterus salmoides* and bluegills *Lepomis macrochirus* received treatments in a fiberglass tank (168 × 42 cm; water depth = 40 cm). Electric fields were described by the equation $y = 1.84 + 0.78x$, where y is the percent of applied voltage x cm from the anode (Holliman and Reynolds 2002). Treatments were administered to glochidia, juvenile mussels, and eastern mosquitofish *Gambusia holbrooki* in a second nonconductive exposure chamber, for which the electric fields were described by the equation $y = 1.37x$. Electrical treatments were described by the peak density of electrical energy ($W \cdot s/cm^3$), the product of peak power density (D or W/cm^3; Kolz 1989) and time (s).

Adult life stage.—Eastern elliptio *Elliptio complanata* and paper pondshell *Anodonta imbecillis* were collected from streams in the central Piedmont region of North Carolina during spring and summer 2004 and 2005. Eastern elliptios averaged 82 mm in length (SD, 11), 25 mm in width (3), and 45 mm in height (6), and wet weight averaged 68 g (23). Paper pondshells averaged 57 mm in length (SD, 8), 19 mm in width (3), and 28 mm in height (4), and wet weight averaged 14 g (6). The marsupial gills of the eastern elliptios were examined (valves were gently opened with reverse pliers) to detect the presence of brooded glochidia before removal from collection sites. Mussels were transported cool, in ice chests with dampened fabric, which prevented direct contact with ice (Cope et al. 2003), to the College of Veterinary Medicine at North Carolina State University, Raleigh.

Mussels were transferred to an indoor 1.1-kL water recirculating system upon arrival at the test facility. Gravid and nongravid eastern elliptios were segregated in water flow-through enclosures to detect individuals potentially releasing glochidia. Mussels used in these trials were maintained in this system over the course of experiments. Mussels were fed a mixed phytoplankton culture that was delivered to the water recirculating system by peristaltic pump at a rate of 300 mL/h. The physiochemical variables of the water in the system were measured with standard methods every 2–3 d over the course of the trials; means were 23.0°C (SD = 1.6) for temperature, 7.5 mg/L (0.6) for dissolved oxygen, 0.06 mg/L (0.10) for ammonia, 0.01 mg/L (0.008) for total ammonia nitrogen , 2.0 mg/L (1.0) for nitrate, and 51 mg/L (5) for alkalinity. The substrate in the system was coarse sand. Lighting conditions were regulated to 14 h light and 10 h dark. Gravid eastern elliptios were monitored for 48 h after transport to identify individuals releasing glochidia, which were excluded from subsequent trials.

We conducted four trials, two with adult eastern elliptios and two with paper pondshells. In each trial we used 48 individuals or 8 in each of six treatments, and each treatment group was equally divided into two replications of 4 mussels each. Mussel assignment to treatment and sequences of treatment application were randomized. In one trial, mussels of each species were exposed to 3 s of 60-Hz PDC at 0.4, 0.7, 1.5, 2.8, or 5.2 V/cm or electrical energies of 112, 343, 1,370, 5,480, or 18,901 peak $\mu W \cdot s/cm^3$, respectively; controls were not exposed. The randomization scheme of this trial on eastern elliptios included the constraint that each experimental group include two gravid mussels. In another trial, mussels of each species were exposed to 60-Hz PDC at 6,300 $\mu W/cm^3$ for 6 s (peak $\mu W \cdot s/cm^3$ = 37,802), 12 s (75,604), 24 s (151,208), 48 s (302,415), or 96 s (604,831); controls were not exposed. Water in the exposure tank was 23–24°C and conductivity was 229–242 μS/cm (mean, 233 μS/cm).

To suspend the mussels within the electric field during treatments, they were centered between both the electrodes and the tank bottom and water surface. The holding tank was nonconductive and made of nylon and plastic. Each mussel received treatment while positioned on the left valve, posterior end toward the anode, without contact with other mussels. Mussels were monitored during exposures and any responses noted. After treatment, mussels were returned to the water recirculating system and segregated by treatment and replication into randomly assigned, flow-through enclosures ($30 \times 20 \times 12$ cm) containing a coarse sand substrate. All mussels were positioned in enclosures on the surface of the substrate on the left valve to aid detection of righting behaviors (or burrowing) after treatment. We monitored release of glochidia by gravid eastern elliptios at 1, 24, and 48 h after treatment. During this period, mucous threads or pseudo-feces released by gravid mussels were sampled by pipette and examined under magnification. Glochidia, when present, were exposed to a saturated salt (NaCl) solution to evaluate viability (ASTM 2005).

Mussel behavior and survival was monitored every 2–3 d for 30 d after treatment. Righting behaviors were defined as the assumption of an upright position or burrowing under the surface of the substrate. The criterion described by Dietz et al. (1997) was used to assess mussel survival (i.e., those gaping widely, not responding to tactile stimulation of valves or soft tissue, and showing no ability to maintain valve closure when partially closed were considered dead and removed from the system).

Immobilization (tetany), a response by fish to electric current, enables scientists to use electrofishing to sample fish by facilitating capture with dip nets. The response may be recognized by an absence of swimming motions accompanied by a loss of equilibrium, without efforts to upright, during and briefly after exposure. To ensure adequate current levels reflective of those used for sampling fish populations were being reached in our tests, 24 juvenile largemouth bass (57–110 mm total length, mean = 84 mm, SD = 15) were exposed to the same quantities of electrical energy administered to adult mussels in the trials testing for effects of electric field amplitudes (i.e., 60-Hz PDC at 0, 0.4, 0.7, 1.5, 2.8, or 5.2 V/cm or 0, 112, 343, 1,370, 5,480, or 18,901 $\mu W \cdot s/cm^3$, respectively). Four fish were assigned to each of the six treatment groups, and the sequence of treatment delivery was random. In addition to monitoring fish during treatment, observations were taken 5 min, 3 h, and 24 h after treatment.

Glochidia life stage.—Gravid paper pondshells and eastern elliptios were segregated by taxon into separate aquaria. Water temperature was maintained at approximately 15°C to minimize release of larvae in response to collection and transport. Aquaria were swept by siphon daily, and collected water and materials were sieved through a 250-μm mesh. Afterward, the mesh was washed into tissue culture dishes and the contents were examined under magnification. When siphoning and sieving yielded glochidia in adequate numbers for experimentation, glochidia were subsampled via a 1-mL pipette and their maturity and viability were evaluated by exposure to a saturated NaCl solution.

We conducted four independent trials with glochidia, three with paper pondshells and one with eastern elliptios. Water conductivity in the exposure chamber ranged from 244 to 292 μS/cm; the mean, 271 μS/cm, was used in calculating electrical energy. Glochidia were transferred to the exposure chamber via a 1-mL pipette. An average of 145 glochidia of eastern elliptios (SD, 80) and 123 of paper pondshells (SD, 53) were exposed, in 10 replicates, to a treatment of 60-Hz PDC at 0.4, 0.8, or 1.6 V/mm for 3 s (13, 52, or 208 mW•s/cm^3, respectively). Three adult mosquitofish were exposed to 60-Hz PDC at 13 mW•s/cm^3 (0.4 V/mm for 3 s) for reference of glochidia exposures relative to induced fish response. In the third trial, 86 (SD, 33) glochidia of paper pondshells were exposed to 60-Hz PDC at 1.6 V/mm in 10 replicates for either 6 or 12 s (416 or 833 mW•s/cm^3). In the fourth trial, 56 (SD, 24) paper pondshell glochidia were exposed to 60-Hz AC for 3 s in four replicates at 0.2, 0.3, 0.5, or 0.6 V/mm (3, 7, 20, or 29 mW•s/cm^3). Each trial included similar groups of glochidia designated as experimental controls. Glochidia in these groups were subjected to the same protocols and procedures as the other experimental groups, except for application of electric current.

Live glochidia are usually in an open position (valves are gaped) and clamp shut when exposed to a saline solution, a phenomenon used to estimate viability (Zimmerman and Neves 2002). In each of the trials, the experimental groups of glochidia were gently washed from the exposure chamber into plastic petri dishes after treatment. Viability, or the proportion of responsive glochidia, was determined by enumerating glochidia responses before and after the addition of a saturated NaCl solution (ASTM 2005). Preliminary testing showed that exceeding a 1:3 ratio of the saline solution to water (in the petri dishes) resulted in closure of all glochidia having the ability to close; thus, we exceeded that ratio in all tests.

Encysted (parasitic) life stage.—We conducted two trials to determine whether exposure to 60-Hz PDC would prevent metamorphosis from the parasitic to free-living juvenile stages. Each trial consisted of infestation of glochidia on host fish, incubation, electric treatment, and subsequent observation. Fish were acquired from a commercial vendor and quarantined for at least 30 d before use in the trials. Infestation was accomplished by placing host fish in a container filled with 5 L of highly aerated water and paper pondshell glochidia for a period of 10 min. We exposed 28 bluegills to glochidia in the first trial and 32 bluegills and 6 largemouth bass in the second trial. After infestation, fish were held in glass aquaria at 24°C.

The incubation period for metamorphosis from attachment of glochidia to excystation of juveniles was uncertain. Thus, aquaria housing infested fish were searched for juveniles every 1–2 d after infestation by sweeping the bottoms of the tanks with a siphon. Collected water and material were filtered through 250-μm mesh, which was then rinsed into a petri dish for examination by stereomicroscopy. In the first trial, the first excysted juvenile mussels were found 5 d after infestation, so fish were exposed to experimental treatments on day 6. In the second trial, counts of sloughed glochidia, which were included in data collections, began 2 d after infestation, so fish were exposed to the experimental treatments at day 3. Trials were concluded when a marked decrease in production of juveniles was observed.

Fish were exposed to 60-Hz PDC at 0.8 V/cm (532 μW•s/cm^3) or were untreated controls. Immobilization was accomplished in each fish exposed to electric current. Water temperature in the exposure chamber was 24°C and had a mean conductivity of 277 μS/cm. Control fish were subjected to the same protocols as those assigned to electrical treatment, but were not exposed to electric current. Assignment to treatment was random, as were the sequences of experimental treatments. In the first trial, 18 bluegills were exposed to 60-Hz PDC, and 10 were designated as controls. In the second trial, 18 bluegills and 3 largemouth bass were exposed to 60-Hz PDC and 14 bluegills and 3 largemouth bass were designated as controls. Sample size was greater for the electrical treatment than control groups to buffer electroshock-induced fish mortality, if it occurred. Fish were transferred to the exposure chamber, administered the assigned treatment, and then transferred to incubation aquaria. Two fish exposed to the same experimental treatment were randomly assigned to a given incubation tank.

Juvenile life stage.—Juvenile (approximately 120 d after excystation) Ozark broken-ray *Lampsilis reeviana* cultured from Beaver Creek (Taney County, Missouri) stock at Missouri State University, Springfield, were exposed, in three repetitions, to 60-Hz PDC for 3 s at 0.4, 0.8, or 1.6 V/mm (259, 1,036, or 4,147 μW•s/cm^3) or for 6 or 12 s at 1.6 V/mm (8,294 or 16,588 μW•s/cm^3). Others were exposed, in two repetitions, to alternating current at 0.2, 0.3, 0.5, or 0.6 V/mm for 3 s (64, 146, 405, or 583 μW•s/cm^3). Each replication in each experimental group consisted of seven treated and seven untreated (experimental control) mussels. Mean water conductivity in the exposure chamber was 540 μS/cm and temperature was 22°C.

Exposure protocols were similar to those previously described for glochidia. The mussels were held in a common 500-mL beaker filled with water. Seven mussels were selected from the beaker using a 1-mL pipette and transferred to the exposure chamber. A randomly assigned treatment was administered, and mussel response was monitored by stereomicroscopy. After treatment, the mussels were flushed from the exposure chamber into 250-mL tissue culture dishes filled with 100 mL of water. The mussels, in dishes, were transferred to a laboratory incubator held at 22°C for the remainder of the trial. Survival was evaluated by stereomicroscopy once every 2–3 d for the first 14 d, then on days 19 and 25. The water was changed (75% replacement) in the dishes after each survival evaluation. A commercial preparation of *Nannochloropsis*, *Isochrysis*, *Pavlova*, and *Tetraselmis* spp. and *Thalassiosira weissflogii* (Reed Mariculture, Campbell, California) that is commonly used in shellfish culture was added to each dish at a rate of 10 mL every 2–3 d.

Data analysis.—In the trials with adult mussels, the endpoints of interest were righting and burrowing behaviors and survival. In trials with juvenile mussels (60-Hz-PDC and 60-Hz-AC exposures), the endpoint of interest was survival. Data analysis, in each case, was based on time-to-event methodology, where treatments were applied at time 0 and time to event was treated as a discrete variable. Data were type I, singly right-censored (i.e., the monitoring period was fixed by trial, the monitoring period was the same for all observations within a trial, and events of interest occurring after termination of monitoring were missing; Allison 1995). Species and trials were evaluated individually, with experimental treatment, time to event, and a censoring indicator variable composing each data set.

Product-limit methods were used to estimate distribution functions and 95% confidence intervals for the endpoints of interest (Kaplan and Meier 1958). In these initial analyses, independent variables were treated as univariates and log-rank tests used to evaluate statistical significance. The Cox regression model for discrete-time data was used for confirmation of log-rank tests (Cox 1972). When event data were sparse (i.e., numerous zero cells), data were pooled within treatments over the observation period and evaluated with Fisher's exact test.

In trials with glochidia, the proportion of viable glochidia in the experimental groups was the endpoint of interest. Within each trial and replicate, viability in control groups was compared to viability in groups exposed to electric current; control groups were used as a baseline. The relative risk (RR), the ratio of the proportions being compared, was used to estimate the risk (probability) for an exposure to electric current to influence viability. An RR exceeding 1.0 indicates a reduction in the viability of glochidia exposed to electric current, that is, an increased risk for the harmful effect. An RR less than 1.0 indicates greater viability in groups exposed to electric current than in controls. If RR = 1.0 or if 1.0 is within the bounds of the confidence interval, there is no indication of a difference in risk between groups.

In the trials on the encysted life stage, the proportion of transformation was compared between fish immobilized by electric current and those designated as controls (second trial only). The proportion of transformation was calculated from counts of glochidia and excysted mussels recovered from the incubation tanks, that is, number of transformed juveniles/(number of juveniles recovered + number of sloughed glochidia).

The statistical hypothesis of no association between experimental treatment and proportion of transformation was tested in a 2×2 table via Pearson's chi-square test. Data analysis for this study was conducted with version 9.1 of the SAS System for PC (SAS, Cary, North Carolina).

Results

Adult Life Stage

Despite a closed appearance, a slight shift in position accompanied by a release of air bubbles from the dorsal-posterior region of the mussels, proximate to the incurrent siphon, was often observed in adult mussels upon application of electric current (Table 1). About 70 s after the exposure of eastern elliptios to 60-Hz PDC at the cumulative energy of 604,831 $\mu W \cdot s/cm^3$ (96-s exposure), approximately 38% of the mussels released streams of very small air bubbles from their anterior region for several seconds.

Exposure to 60-Hz PDC at the equivalent of 343 $\mu W \cdot s/cm^3$ or more induced tetany in each fish within the experimental groups of largemouth bass (Table 1). The percentage of fish within an experimental group righting within 5 min after cessation of treatment was inversely related to the quantity of electrical energy applied. Survival was 100% in fish exposed to 343 $\mu W \cdot s/cm^3$ or less, decreasing to 75% in groups exposed to greater quantities of electrical energy. Those fish expiring in the study died 3 h after treatment.

All paper pondshells exhibited righting–burrowing behaviors during the first observation interval after experimental treatment. In contrast, 17% of eastern elliptios failed to right or burrow (no movement detected) during the first trial (Figure 1). There was no evidence of significant treatment effects on times to first righting and burrowing by eastern elliptios yielded by log-rank tests ($\chi^2 = 2.45$, df = 5, $P = 0.78$) or Cox regression (H_0: $\beta = 0$; $\chi^2 = 0.031$, df = 1, $P = 0.95$). In the second trial, where electric power was constant and the period of exposure varied, the statistical tests yielded conflicting evidence for significant treatment effects on times to first righting and burrowing by eastern elliptios (Figure 2). The log-rank test yielded evidence of significant variation among the experi-

TABLE 1.—Occurrences of observed responses (%) in adults of two unionid mussels, paper pondshell and eastern elliptio, and juvenile largemouth bass exposed to 60-Hz pulsed DC. Experimental groups included four of each species in two separate experiments: (1) exposure to electric fields of various amplitudes (power densities) for 3 s and (2) exposure to electric fields of 6,300 μW/cm^3 for 0, 6, 12, 24, 48, or 96 s, resulting in the aggregate electrical energy exposures shown.

Electrical energy (μW•s/cm^3)	Paper pondshell		Eastern elliptio		Largemouth bass	
	No response	Expelled air	No response	Expelled air	Tetany	Righting ≤5 min
Exposed for 3 s						
0	100.0	0.0	100.0	0.0	0.0	100.0
112	87.5	12.5	100.0	0.0	0.0	100.0
343	100.0	0.0	100.0	0.0	100.0	100.0
1,370	75.0	25.0	100.0	0.0	100.0	75.0
5,480	100.0	0.0	100.0	0.0	100.0	50.0
18,901	37.5	62.5	100.0	0.0	100.0	25.0
Total	83.3	16.6	100.0	0.0	66.6	75.0
Exposed to 6,300 μW/cm^3						
0	100.0	0.0	100.0	0.0		
37,802	100.0	0.0	50.0	50.0		
75,604	100.0	0.0	62.5	37.5		
151,208	100.0	0.0	25.0	75.0		
302,415	75.0	25.0	50.0	50.0		
604,831	100.0	0.0	37.5	62.5		
Total	96.0	4.0	54.2	45.8		

mental groups ($\chi^2 = 12.86$, df = 5, $P = 0.0247$), but Cox regression failed to reject the null hypothesis that $\beta = 0$ ($\chi^2 = 0.0395$, df = 1, $P = 0.8425$).

Survival was 100% for eastern elliptio in each of the trials. The survival of paper pondshells was 96% in the first trial and 98% in the second, two dieing in the first trial (one exposed to 18,901 μW•s/cm^3 in the third observation interval, one exposed to 5,480 μW s/cm^3 in the ninth observation interval) and one in the second trial (one designated control). Fisher's exact test failed to yield evidence for significant variation in survival among the experimental groups in either trial ($P > 0.99$).

One-half (24 of 48) of the gravid eastern elliptios collected for the trial on adults released conglutinate (strands of mucous containing glochidia) during the 2-d period before initiation of the trial. The glochidia were a mixture of immature and mature individuals. Overall, 9 of the 24 gravid eastern elliptios used in the trial released glochidia within 48-h after treatment (Table 2); 6 of the 9 released viable glochidia. One of the control mussels released nonviable glochidia. In all cases, glochidia were attached to mucous strands.

Glochidia Life Stage

Exposure to 60-Hz PDC at 13 mW•s/cm^3 immobilized each of the eastern mosquitofish treated to determine relative fish response. The glochidia of paper pondshells and eastern elliptios responded to the electric current by repeatedly closing and reopening (i.e., snapping). Many glochidia closed and remained closed after a few seconds of exposure, reopening upon cessation of the treatment. Furthermore, a slight torsion of the valves was observed in some glochidia exposed to AC treatments.

In most cases, exposure of glochidia to electric current failed to elevate or reduce viability relative to the baseline (Figure 3). There was no evidence that 60-Hz PDC and cumulative electrical energies of 13, 52, or 208 mW•s/cm^3 in 3-s exposures influenced the viability of eastern elliptio glochidia (baseline = 0.57, 95% confidence interval [CI] = 0.46–0.68). Similarly, there was no evidence that 60-Hz PDC and cumulative electrical energies of 13 or 52 mW•s/cm^3 in 3-s exposures influenced the viability of paper pondshell glochidia (baseline = 0.76, 95% CI = 0.71–0.82), but viability increased 3–13% (RR = 1.08, 95% CI = 1.03–1.13) in the 208 mW•s/cm^3 exposure. There was no evidence that 60-Hz PDC and cumulative electrical energies of 416 and 832 mW•s/cm^3 in 6-s and 12-s exposures influenced the viability of paper pondshell glochidia (baseline = 0.66, 95% CI = 0.62–0.70). Finally, there was no evidence that 3-s exposures to 60-Hz AC and cumulative electrical energies of 7 and 29 mW•s/cm^3 influenced viability of paper pondshell glochidia (baseline = 0.70, 95% CI = 0.59–0.80), but viability was reduced by 6–29% in those receiving a cumulative exposure to 3 mW•s/cm^3 and 1–24% in those receiving a cumulative exposure of 20 mW•s/cm^3.

Encysted Life Stage

In the first trial, excysted juvenile mussels ($N = 130$) were found in the incubation tanks 5 d after infestation. A total of 1,166 juvenile mussels were collected after exposure of fish to the experimental

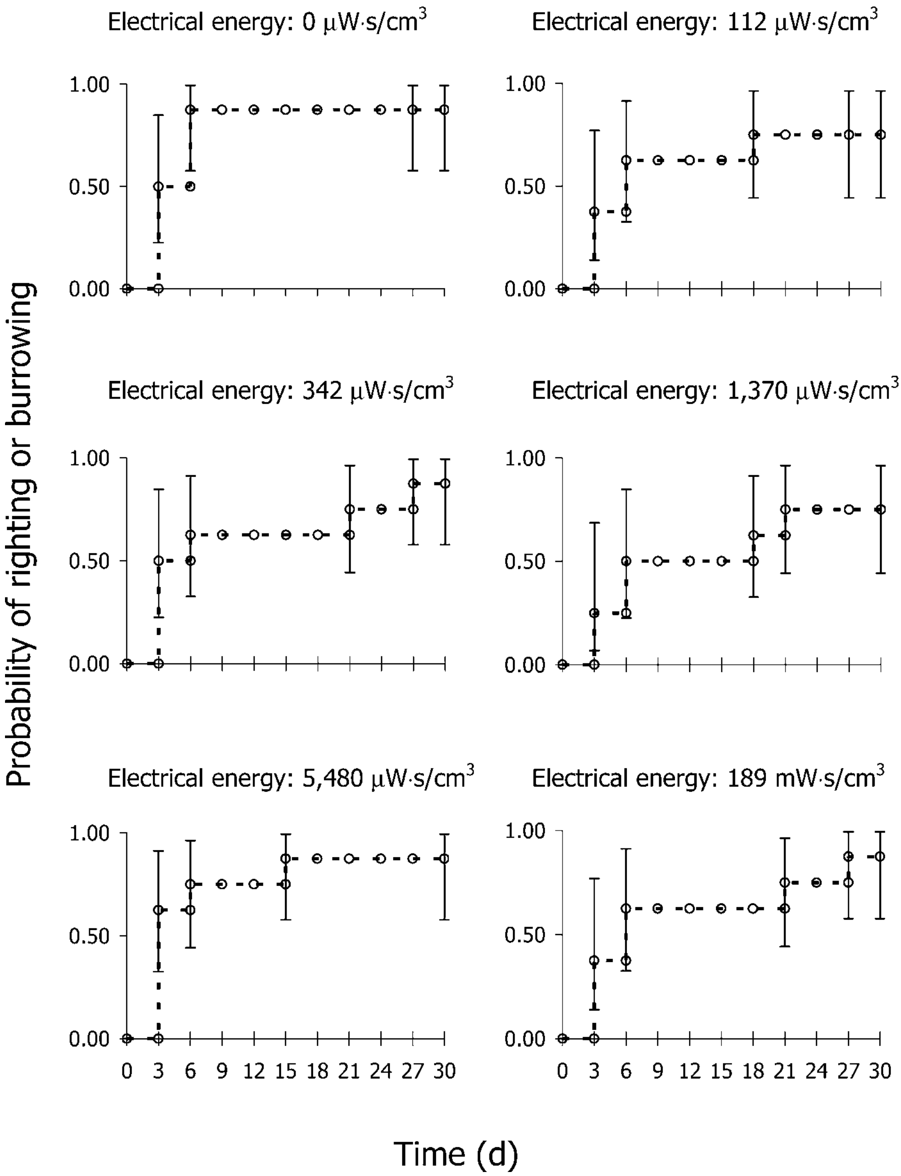

FIGURE 1.—Product-limit distribution functions of righting and burrowing behaviors and 95% confidence intervals (CI) for adult eastern elliptios exposed to 60-Hz pulsed DC at various energy levels for 3 s under laboratory conditions. Upward steps in the plots indicate behavioral events; 95% CI are reported for the observation intervals containing an event (including censor).

treatments; 79% were produced from fish exposed to electric current and 21% from fish designated as controls. The majority (65%) of the juveniles were found 7 d after infestation (1 d after experimental exposures) with numbers decreasing to 21%, 7%, and 7% on days 8, 9, and 11. The majority of the juveniles were alive and active when collected, regardless of treatment (73–75%).

In the second trial, searches of the incubation tanks 2–10 d after infestation yielded 2,887 sloughed glochidia and 966 excysted juvenile mussels (68% from bluegills and 32% from largemouth bass). Of the sloughed glochidia, 474 were recovered from the incubation tanks before infested fish received the experimental treatments. There was no statistical evidence that the proportion of transformation differed significantly between fish exposed to electric current (528 of 1,352) and those designated as

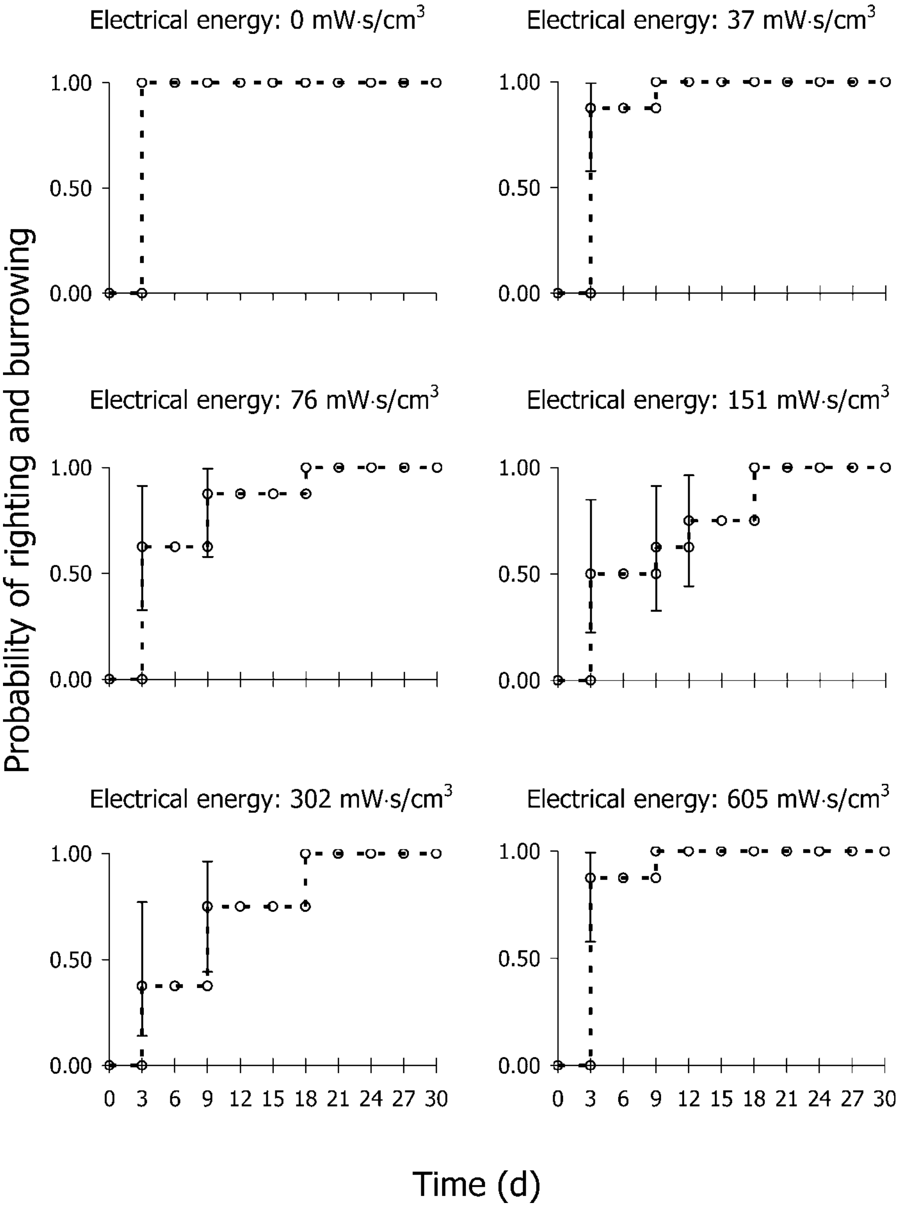

FIGURE 2.—Product-limit distribution functions of righting and burrowing behaviors and 95% confidence intervals (CI) for adult eastern elliptios exposed to 6,300 μW/cm^3 of 60-Hz pulsed DC for 0 (control), 6, 12, 24, 48, or 96 s, resulting in the aggregate exposures shown. Upward steps in the plots indicate behavioral events; 95% CI are reported for the observation intervals containing an event.

controls (438 of 1,061; $\chi^2 = 1.2298$, df $= 1$, $P = 0.2674$). Of the 346 excysted mussels alive when discovered, 68% were from bluegills exposed to electric current and 32% from control fish. There were 275 live and 35 dead juveniles excysted from largemouth bass. Of these, 43% were from fish exposed to electric current and 57% were from fish designated as controls.

Juvenile Life Stage

Juvenile Ozark broken-rays responded physically to electrical stimulation; the foot was withdrawn into the valves, soft tissues were contracted to the region of the retractor muscle, and heart contraction rates increased. In the trial exposing juveniles to 60-Hz PDC, mean time of death was 9 d (median and mode, 6 d), and

TABLE 2.—Percent of gravid eastern elliptios releasing glochidia (viable or nonviable) within 48 h of exposure to various levels of 60-Hz pulsed DC. There were four mussels per experimental group.

	Electrical energy (μW·s/cm³)					
Glochidia status	0	112	343	1,370	5,480	18,901
Viable	0	50	50	0	25	25
Nonviable	25	0	25	0	25	0

39% of the mussels survived to 25 d (Figure 4). There was no evidence of significant differences in mussel survival among the replicates ($\chi^2 = 0.16$, df = 2, P = 0.92) or treatments ($\chi^2 = 7.36$, df = 5, $P = 0.20$) according to the log-rank tests ($\chi^2 = 1.77$, df = 2, P = 0.41) or the Cox regression model (H_0: $\beta = 0$; χ^2 = 1.77, df = 2, $P = 0.41$).

In the trial exposing juveniles to AC, the mean time of death was 9 d (median and mode, 6 d), and 27% of the mussels survived to day 25 (Figure 5). Log-rank tests failed to detect statistical differences in survival between replicates ($\chi^2 = 0.766$, df = 1, $P = 0.7820$) or among experimental treatments ($\chi^2 = 1.0941$, df = 4, P = 0.8952). The Cox regression model yielded similar results (H_0: $\beta = 0$; $\chi^2 = 0.772$, df = 2, $P = 0.9621$).

Discussion

Our study demonstrates that exposure to 60-Hz PDC at quantities greatly exceeding those immobilizing 55–110-mm largemouth bass does not adversely affect the survival or righting and burrowing behaviors of adult eastern elliptios and paper pondshells. The species used in our study are common representatives that differ in subfamily (Ambleminae versus Unioninae), habitat, distribution, morphology, and fish hosts, thereby bracketing the extremes of the family Unionidae. The eastern elliptio is thick-shelled and common in the coarse substrates of streams and rivers. The paper pondshell is thin-shelled and associated with fine sediments in backwaters, ponds, and impoundments. Our findings on adult unionid mussels are in accord with those of the field exposures by Hastie and Boon (2001), who noted no effect, extending the null effects of electric current exposure from adult freshwater pearl mussels to additional species, with more conclusive results from controlled exposures.

Voltage gradients ranging from 0.1 to 1.0 V/cm are often used to define the "effective" zone of electric fields used for fishing (Reynolds 1996). We induced tetany in largemouth bass exposed to 60-Hz PDC at 343 μW·s/cm³. Although we exposed adult mussels to electric fields of up to seven times the amplitude that

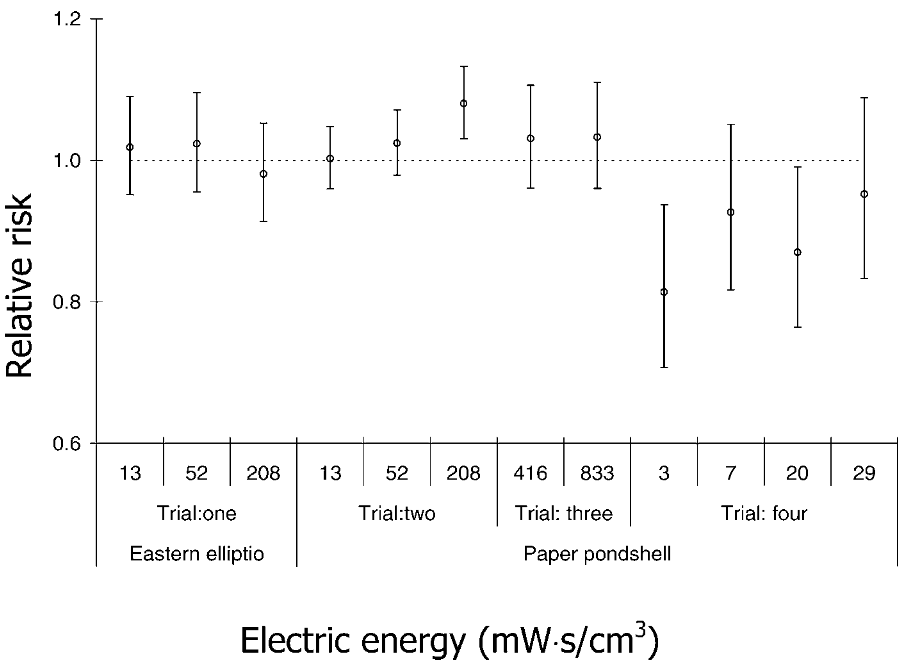

FIGURE 3.—Risk to glochidia viability in eastern elliptio and paper pondshell groups exposed to electric current. The relative risk reflects the probability that a given exposure will alter viability relative to that of unexposed controls. Values exceeding 1.0 indicate less viability in the exposed group (i.e., an increased risk of harmful effects), values less than 1.0 greater viability, and values of exactly 1.0 (or within the bounds of the confidence interval) no difference in risk between the groups.

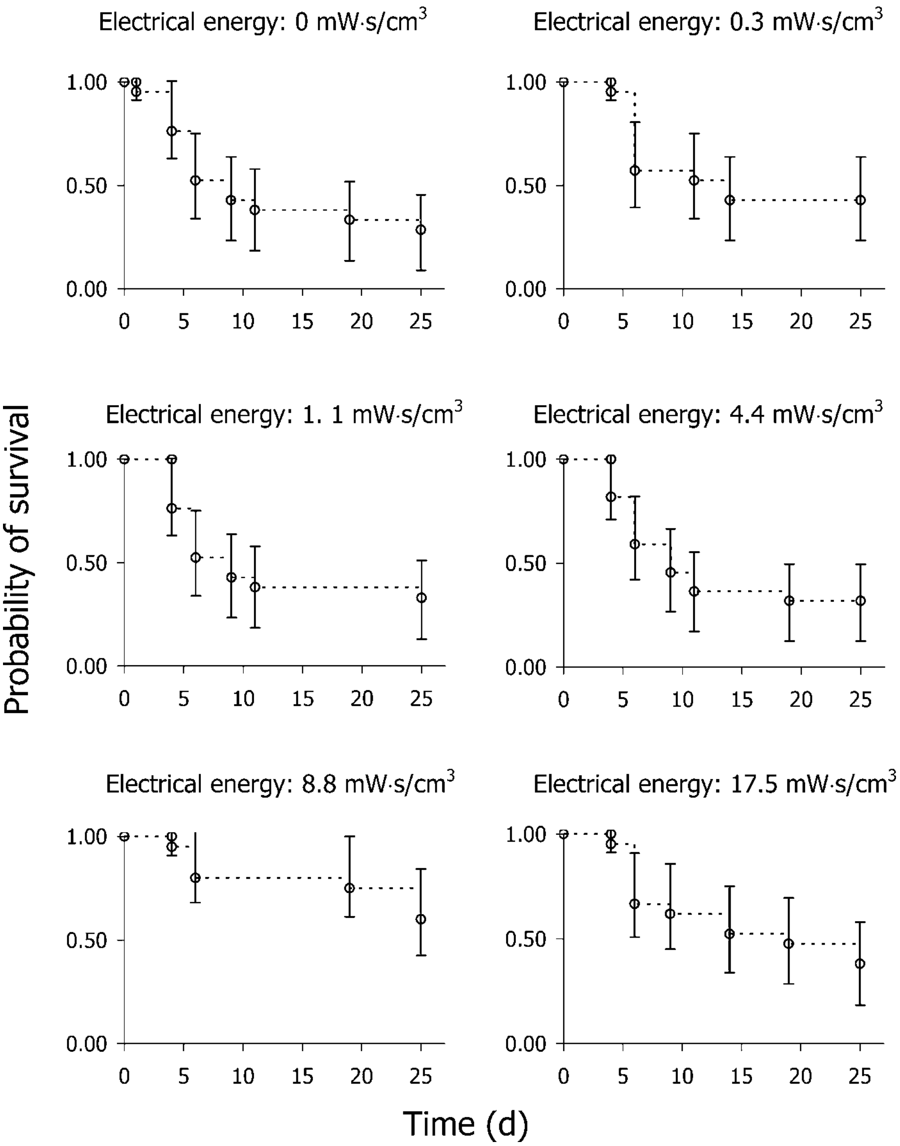

FIGURE 4.—Product-limit survival distribution functions for juvenile unionid mussels exposed to 60-Hz pulsed DC at various energy levels for up to 12 s.

immobilized the largemouth bass and to cumulative electrical energies up to 1,700 times the immobilization level, we found no significant effects on mussel survival or behavior through 30 d postexposure. Some adult mussels did, however, exhibit physical response to electric stimulation: movement of the valves and release of air bubbles upon initiation of current flow.

The bubbles expelled by the mussels were probably environmental air (rather than a physiological product) that had been trapped within the shell by tissue folds or in the siphons during emersion and transport to the exposure chamber. Rapid closure of the valves (the physical shift observed) is the most likely mechanism for the expulsion of the air bubbles. Whether this reaction to electric current flow was initiated by electrical stimulation of a sensory system (e.g., photoreceptor cells, tactile receptors on the siphons, sensory cells on the mantle) or resulted from direct action of electric current on nerve or muscle is not known. Regardless of the biological mechanism, the

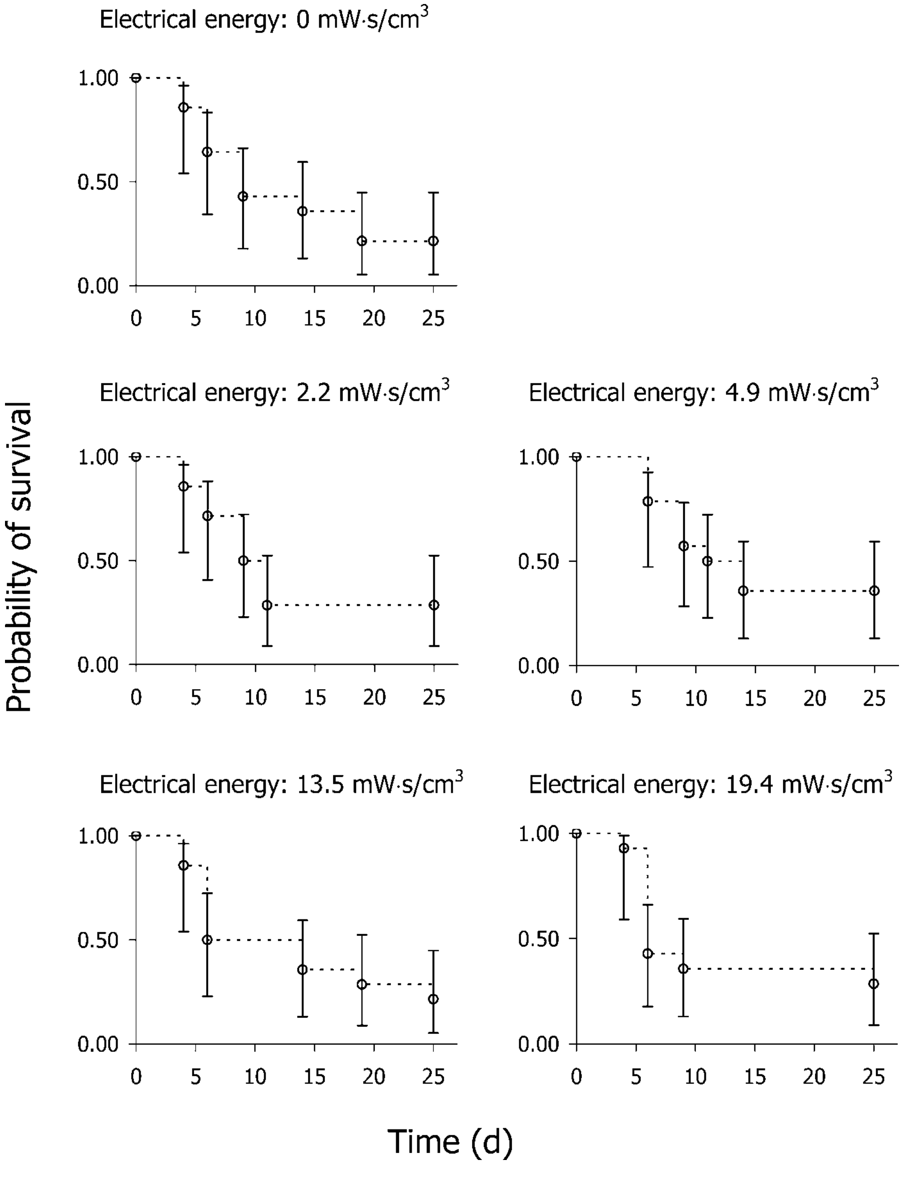

FIGURE 5.—Product-limit survival distribution functions for juvenile unionid mussels exposed to 60-Hz AC at various energy levels for 3 s.

responses demonstrated by the mussels suggest potential for gravid females to release glochidia upon electrical stimulation.

Freshwater mussels retain developing embryos in marsupial pouches of the gills (brood chambers). Certain lampsiline species (e.g., plain pocketbook *Lampsilis cardium*) are known to extend or conspicuously display gravid marsupial gills outside of the shell margin to lure potential host fish (Haag and Warren 1999). Other mussels (e.g., the paper pondshell) maintain gravid marsupial pouches within the valves, using a rapid closure of the valves to eject glochidia into the external environment during spawning (Tankersley and Dimock 1992). Rapid valve closure resulting from exposure to electric current stimulation could therefore compress swollen marsupial pouches in a gravid mussel within the valves or displayed beyond the shell margins, resulting in premature expulsion of glochidia.

Tests on the retention of glochidia by gravid eastern elliptios were inconclusive in this study. About half of the gravid mussels collected for the trial released glochidia within 48 h of collection and transport (likely due to handling stress) and could not be effectively included in the experiment. Of the gravid mussels used in the trial, one individual treated as control released glochidia within 48 h of treatment. Thus, the methods we used in this study were inadequate for direct testing of the effects of electrical exposure on the retention of glochidia, even though we took special care to reduce stress to experimental mussels. Our results demonstrate the difficulty of working with gravid eastern elliptios with regard to handling and transport induced release of glochidia. Alternative experimental approaches or tests with other species like lampsilines that generally retain glochidia until directly stimulated by a fish host will be required to elucidate the effects of electrical exposure on gravid mussels and associated glochidia.

To date, most of the research on the effects of electric current exposure on freshwater mussels has focused on the prevention of settlement by veligers of the zebra mussel *Dreissena polymorpha* and on eradicating adult zebra mussels in hydropower facilities. Difficulties encountered in that work led investigators to postulate that the shell may provide zebra mussels a measure of protection from electric current (Fears et al. 1994; Ostlie et al. 1994). Our results and those of Hastie and Boon (2001) support their postulation. The shells of freshwater mussels are composites of calcite, aragonite, or calcium carbonate crystals in a proteinaceous matrix with an outer layer of concholin (i.e., tanned protein; McMahon 1991, Checa and Rodriguez-Navarro 2001). Cortical bone, also called compact bone, has several characteristics in common with mollusk shells (e.g., layered, dense, low porosity). Consisting mainly of collagen and hydroxipatite, bone has high electrical resistivity (Lee 1991) and is a poor conductor; the skull, in particular, has been a topic of research. Weaver et al. (1976) reported the skull to account for approximately 85% of the loss of applied electrical energy during electroconvulsive therapy (ECT).

We did not directly measure the insulating characteristics of the unionid shell, but our results suggest that the shell provides protection from electric current by preventing or reducing electric current transfer from water to soft tissues, functioning similar to the skull during ECT. Many freshwater mussels have the capacity to close their valves for extended periods (e.g., eastern elliptio); this behavioral and physiological adaptation should enable the valves to protect the mussels from the ambient aquatic environment should electrical exposure interrupt homeostasis and the capacity for osmoregulation. Freshwater mussels routinely stop siphoning and close their valves when disturbed. Stream disruption during electrofishing may trigger valve closure and ensure protection from administered currents. Some unionid taxa, however, such as Lampsiline species, have mantle flaps and foot soft tissue extending beyond the shell margin, preventing complete closure of the valves (McMahon 1991). Mussels with such morphology may be more vulnerable to electric shock and physiological upset than those able to close completely.

The glochidia of eastern elliptios and paper pondshells respond to electric current by repeatedly closing and reopening. These actions persisted for several seconds during treatment, but glochidia eventually closed and remained closed during extended exposures, most reopening after flow of current was terminated. The response of glochidia to electric current may provide a nonlethal alternative to testing viability of glochidia, in contrast to lethal concentrations of salt solution.

Significant differences in the risk to glochidia viability (acute measures) were evident in some of our trials. Although significant differences occurred between some treatment and control groups, the results were not consistent and did not follow the trends expected for electric shock (i.e., that the effects would increase with the amplitude and duration of exposure). Glochidia were exposed to 60-Hz PDC at voltage gradients as great as 16 V/cm and for periods of up to 12 s. Voltage gradients of this magnitude occur only in a small volume of water near the anode in typical electrofishing operations and 12-s exposures to glochidia within this volume are unlikely.

Young and Williams (1984) estimated the probability of metamorphosis of freshwater pearl mussel glochidia to be as low as 0.000001%. If a similar rate of metamorphosis is true for unionids, reductions in viability of the magnitudes demonstrated in our study are probably inconsequential at the population level. However, our tests evaluated acute, short-term effects on viability, rather than delayed effects. The physical reactions of the glochidia to electric current leads us to postulate that the impacts on viability may amplify with time. Because glochidia have a finite source of energy to sustain life until attachment to a fish host, we hypothesize that exposure to electric current depletes their energy reserves, thereby decreasing the periods over which they are viable. Furthermore, the repeated snapping during exposure probably constitutes severe activity for glochidia, potentially upsetting oxygen requirements and abilities to osmoregulate. We suggest that exposure to electric current may pose a latent risk to glochidia viability and is a direction for future research.

Our findings also show that electric shock does not prevent metamorphosis from encysted parasite to excysted juvenile mussel or influence the rate at which successful metamorphosis is accomplished. Bluegills and largemouth bass hosts, after being infested with glochidia, were immobilized (tetany) by exposure to 60-Hz PDC. Fish response to electric fields is governed by in vivo electric current, the amount of current transferred from the external electric field to the internal tissues (Kolz 1989). The efficiency of the energy transfer is determined by the ratio of water and fish conductivity, but the in vivo electrical energy thresholds for responses are regarded as consistent (Kolz and Reynolds 1989). Our study shows that when in vivo electric current exceeds the threshold for immobilization of a typical host fish, metamorphosis by encysted glochidia is not prevented or impeded.

Our results on the electrical exposure of juvenile Ozark broken-rays did not reveal a significant effect on survival in that species, but these results are equivocal because of the reduced survival in both treatment and control groups in both trials (AC and 60-Hz PDC). No statistically significant differences in survival were demonstrated among the treatment groups in either trial. The similarity in survival and the shapes of the survival curves at the conclusion of the experiment support the outcomes of the statistical tests. Overall, survival of the juvenile mussels was lower than expected, but the underlying causes were unknown. Extensive culture and holding techniques (e.g., flow-through water systems) are generally necessary for long-term survival and growth of juvenile mussels in the laboratory (e.g., Barnhart 2006). The reduction in survival in our study was probably due to the husbandry techniques required in our test, which may have resulted in inadequate nutrition and less than optimal culture conditions. Regardless of cause of death, survival was similar among experimental treatments and between the trials.

The survival of freshwater mussels is intimately linked with fish by an obligate parasitic larval phase. Often, the populations of both the host and the parasite, fish and mussel, are imperiled. Freshwater fish and mussels are similar in having tissue and blood (or hemolymph in the case of mussels) osmotic concentrations greater than the ambient freshwater medium; survival is dependent on the ability to osmoregulate (i.e., prevent excessive ion loss and water gain). Electric shock can induce stresses in fish similar to those of hypoxia or severe muscular activity (Schreck et al. 1976), fatally increasing gill permeability to water by altering osmoregulatory capacity through actions on gill function (Mazeaud et al. 1977). Hence, electrofishing-induced fish mortality is a concern, especially for small fish; death of infested fish will prevent successful metamorphosis of encysted glochidia, so death of potential hosts may negatively influence the survival of mussel populations. Because voltage gradient and the period of exposure influence the probability of death in small fish exposed to electric current (Holliman et al. 2003a), we recommend minimizing exposure time and electrical output when electrofishing the host fish of freshwater mussels.

Our cumulative results suggest that exposure to pulsed direct current at energy levels typical of electrofishing does not pose a significant risk to the survival of unionid mussel adults, juveniles, or encysted or free-living glochidia. The effects that electric current exposure may have on the survival of aquatic animals have not been widely studied apart from fish. Our research represents an important advance in that knowledge for freshwater mussels.

Acknowledgments

We thank Chris Eads, Erin Shubert, and Leroy Humphries for their assistance in the field. Nolan Banish, Robert Curry, Shannon Deaton, Steve Fraley, Ryan Heise, Kent Nelson, Rob Nichols, Angie Rodgers, and Scott Van Horn of the North Carolina Wildlife Resources Commission provided input into study design, assisted with collection of mussels, and aided with funding administration. John Barr and Will Wilkinson provided assistance in the laboratory. We thank Chris Barnhart of Missouri State University for providing juvenile mussels for testing. Smith-Root Inc., Vancouver, Washington, supported this research by providing the electrofishing power supply used in the study. Mark Hove and two anonymous reviewers provided constructive reviews of an earlier draft of this manuscript. This research was funded by the North Carolina Wildlife Resources Commission through State Wildlife Grant funding (Project T03-01).

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