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[Article]

Effects of Electroshock on Cyprinid Embryos: Implications for Threatened and Endangered Fishes

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Abstract.—The purpose of this study was to evaluate the effects of electroshock on survival to hatching in embryos of Cyprinidae, which includes numerous fish species designated as threatened or endangered under the U.S. Endangered Species Act. Embryos of three cyprinids were exposed for 20 s to a homogeneous electric field (DC or 60-Hz pulsed DC [PDC], 3-ms pulse width) at voltage gradients similar to those used during electrofishing. Exposure occurred only once and was at a specific stage of development. Zebrafish *Danio rerio* were electroshocked at numerous stages of embryonic development, and the most sensitive developmental stage was determined and used to guide subsequent experiments. Embryos of two minnows native to the southeast United States, the spotfin chub *Erimonax monachus* and whitetail shiner *Cyprinella galactura*, were exposed to a range of DC (3–15 V/cm) and 60-Hz PDC (8–15 V/cm) voltage gradients, and survival to hatching was evaluated. Additionally, the potential for electrofishing to induce premature hatching in late-stage cyprinid embryos was investigated by exposing eyed spotfin chub embryos to 8-V/cm DC electroshock. Embryos were most vulnerable to electroshock-induced mortality early in development, particularly near epiboly, and DC was more harmful than 60-Hz PDC. At older developmental stages, embryos were less vulnerable to electroshock-induced mortality, although premature hatching was induced in some older developmental stages of electroshocked spotfin chub embryos. Whitetail shiner embryos were more vulnerable (lethal voltage gradient predicted to induce 50% mortality [LV50], 5 V/cm) to electroshock-induced mortality than were spotfin chub embryos (LV50, 6 V/cm). Results indicate that cyprinid embryos can be killed by DC electroshock at electric field intensities commonly generated by electrofishing equipment, and electrofishing near spawning grounds of threatened or endangered cyprinids should be avoided when embryos are present.

Electrofishing equipment generates electric fields for capture of fish from many freshwater environments. The electric field is distributed around electrodes, and the intensity (e.g., voltage gradient, V/cm) decreases with distance from the electrodes (Reynolds 1996). Both efficiency of electrofishing (Miranda and Dolan 2003) and potential for fish injury increase with increasing intensity of the electric field (e.g., Henry et al. 2004). The intensity of voltage gradients around the anodes of electrofishing boats (e.g., 20 V/cm at 5 cm; Henry et al. 2003) and backpack electrofishing units (e.g., 13 V/cm at 16 cm; J. W. Habera, Tennessee Wildlife Resource Agency, unpublished) is similar and sufficient to induce injuries in exposed fish (e.g., Henry and Grizzle 2003; Snyder 2003; Henry et al. 2004).

Negative effects of electrofishing on fish are a concern for both target and nontarget species and life history stages (Snyder 2003). While it is recognized that some fish populations must be sampled for effective management of fisheries and that sampling frequently involves electrofishing, it is also recognized

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that negative effects on fish must be reduced when possible. In most situations, electrofishing does not place unreasonable stress on fish or lead to population-level effects (McMichael et al. 1998). However, for threatened and endangered fishes that reside in small streams, a large proportion of the population could be exposed to electric fields during a single electrofishing event and population-level effects are possible. For threatened and endangered fishes, the loss of even one individual can be unacceptable and considered as "take" under the U.S. Endangered Species Act (ESA; Nielsen 1998).

Fish embryos are not targets of electrofishing but can be unintentionally exposed to electric fields during routine electrofishing, and negative effects on embryo survival can occur. Electroshock-induced mortality of fish embryos has been related to developmental stage at time of electroshock and the duration and characteristics of the electric field during exposure (Snyder 2003). Survival of electroshocked embryos can be improved by reducing electric field intensity (Godfrey 1957; Henry and Grizzle 2004) and frequency of pulsed electric currents (Muth and Rupert 1997). Only one previous study has directly evaluated differences in susceptibility to electroshock-induced mortality of embryos between species (Henry and Grizzle 2004), and comparisons among different studies are often difficult because of differences in experimental design. Embryos of numerous species can be exposed to electric fields during electrofishing, and effects on threatened and endangered species are particularly important. Cyprinidae is the largest family of fishes (Helfman et al. 1997), and this group includes numerous threatened and endangered species (e.g., spotfin chub *Erimonax monachus*, Cape Fear shiner *Notropis mekistocholas*, blue shiner *Cyprinella caerulea*, slender chub *Erimystax cahni*) that are present in small streams where electrofishing can occur, but currently there is no information on the effects of electroshock on cyprinid embryo survival.

Effects of electroshock on embryos vary with developmental stage at time of exposure (Snyder 2003). For all species considered, mortality was highest for embryos electroshocked early in development. The developmental period near epiboly, the period when the blastoderm overgrows the yolk (Warga and Kimmel 1990), has been identified as a particularly sensitive stage in embryonic development (Godfrey 1957; Dwyer et al. 1993; Muth and Rupert 1997; Henry and Grizzle 2004). While survival of embryos improves as development progresses, electroshock-induced premature hatching has been reported for late-stage embryos in a centrarchid (bluegill *Lepomis macrochirus*; Henry and Grizzle 2004) and in two salmonids (vendace *Coregonus albula* and powan *Coregonus lavaretus*; Luczynski and Kolman 1987). The consequences of electroshock-induced premature hatching for survival of fish are unknown.

The objectives of this research were to (1) evaluate effects of electroshock on survival of embryos in three species of cyprinids, (2) identify the relative effect of DC and pulsed DC (PDC) waveforms on embryo survival, (3) determine the developmental stages that are most vulnerable to electroshock, and (4) evaluate premature hatching following electroshock. The zebrafish *Danio rerio* was included in our study because it is an excellent model organism that is well-adapted to laboratory conditions, and considerable information is available regarding its embryonic development for comparison with other cyprinids. Spotfin chub (listed as threatened under the ESA) and whitetail shiners *Cyprinella galactura* were used in our study because they are cyprinids native to the southeastern United States. These species spawn readily in captivity and can be used as surrogates for closely related threatened and endangered cyprinids (e.g., blue shiner, which is listed as threatened under the ESA).

Methods

Experimental fish.—Zebrafish embryos were obtained by spawning zebrafish broodstock maintained in the Zebrafish Research Facility at the University of Tennessee in Knoxville. Water for holding embryos and conducting experiments (designated as "fish water") was prepared with purified MilliQ water (Millipore Corp., Bedford, Massachusetts) with the following ions added: $NaHCO_3$ at 19 mg/L, sea salt at 1 mg/L (Instant Ocean Synthetic Sea Salt, Mentor, Ohio), $CaSO_4$ at 10 mg/L, $MgSO_4$ at 10 mg/L, and KCl at 2 mg/L. Fish water had the following characteristics: pH of 7.3–7.9; dissolved oxygen greater than 6 mg/L; total alkalinity of 30–40 mg/L as $CaCO_3$; and total hardness of 15–20 mg/L as $CaCO_3$. Spawning procedures (see Bohl 2008 for specific details) were such that spawning occurred for no more than 15 min to ensure that all embryos collected were at similar developmental stages. When spawning was complete, embryos were rinsed with clean fish water and all dead embryos were discarded.

Spotfin chub and whitetail shiner embryos were obtained by spawning adult fish held at Conservation Fisheries, Inc. in Knoxville. Adult fish used for spawning were obtained from the Little Tennessee River system in Tennessee (whitetail shiner) or from the Buffalo River system in Tennessee (spotfin chub). All available whitetail shiner adults (two male and two female fish) were spawned for experiments with whitetail shiner embryos, while 21 of the available

TABLE 1.—Descriptions of developmental stages for preserved (10% neutral buffered formalin) spotfin chub and whitetail shiner embryos collected during electroshock experiments.

Stage name	Approximate time post-fertilization (h)	Description of developmental period
Cleavage	0–24	Early cleavage of the blastoderm.
Blastula	0–24	Blastoderm appears as a ball of cells on top of the yolk sac, and cleavage is nearly completed through the onset of epiboly (<30% epiboly).
Epiboly	0–24	30% epiboly through 90% epiboly.
Tail free	24–48	Period from embryo formation (tail is distinguishable from head and is free from yolk sac) through eyed stage.
Eyed	48–168	Period from eye pigmentation through hatching.

spotfin chub adults that appeared to be healthiest were spawned for experiments with spotfin chub embryos (ratio of males to females was unknown, but at least seven males were present). Spawning tanks were checked for embryos every 24 h, and all embryos were collected (for details of the spawning method, see Bohl 2008). A soft brush was used to gently detach embryos that adhered to the spawning tiles. After collection, embryos were observed at 10× magnification and all unfertilized eggs and dead or damaged embryos were discarded.

Electric fields.—Homogeneous electric fields were generated in the laboratory with an electrofishing pulse box (TEG-10 Proto 1; Coffelt, Flagstaff, Arizona) modified to be powered by 110-V AC and to produce DC or square-pulse PDC. The electric fields tested included DC and 60-Hz PDC (3-ms pulse width) over a range of voltage gradients (0–16 V/cm). Voltage gradient and waveform were confirmed using a voltage gradient probe connected to a digital oscilloscope (THS 720A; Tectronix, Beaverton, Oregon). Embryos were exposed to electric fields in plastic troughs (38.1 × 7.7 cm; 5.1 cm deep) that contained 1 L of water. Electric fields were generated in exposure troughs by energizing aluminum plate electrodes that conformed to the trough cross-section and were separated by 30 cm. All electric equipment had been used in previously published electrofishing injury studies on early life stages of fish, including embryos (Henry and Grizzle 2003, 2004, 2006).

Experimental design.—To avoid natural variation in percent hatch between different spawning events within a species, each experiment was conducted with embryos that were collected from the same 24–48-h spawning period unless otherwise stated. All experiments included three separate, randomly selected troughs as unshocked controls. Unenergized electrodes were placed in each control trough for 20 s at each exposure period, ensuring that differences in survival between treatment and control groups were due to electroshock and not linked to physical disturbance associated with placing electrodes into the troughs. Treatment troughs were exposed to electric fields at the same time after fertilization (±15 min) except in (1) the zebrafish experiment, which had time postfertilization as the independent variable, and (2) one spotfin chub experiment, which evaluated electroshock-induced premature hatching at two separate times. Embryos were monitored daily after stocking and any obviously dead (opaque) embryos were removed (as in Henry and Grizzle 2004). Survival of embryos was calculated by counting the number of embryos that survived through hatching and dividing by the number initially stocked into the trough. Before each exposure period, a random sample of embryos was preserved in 10% neutral buffered formalin for later examination to establish the developmental stage at which embryos were electroshocked. All embryos were observed under a stereomicroscope, and developmental stage was described following Kimmel et al. (1995) for zebrafish embryos. A staging table appropriate for these experiments was developed for spotfin chub and whitetail shiner embryos (Table 1). Experimental design for all experiments is summarized in Table 2.

The effect of developmental stage on survival of electroshocked cyprinid embryos was investigated using zebrafish embryos in two separate experiments. Embryos were collected and stocked into exposure troughs by 0.3 h postfertilization. Static water in troughs with zebrafish embryos had temperature and ambient conductivity of 26–28°C and 40–50 μS/cm, respectively. Each trough was randomly assigned a time of electroshock (h postfertilization) or designated as an unshocked control. At the beginning of the experiment, each treatment trough contained 35 embryos and each control trough contained 25 embryos. Embryos were exposed once to 20 s of DC electroshock (16 V/cm) from 0.3 to 24 h postfertilization. An exposure duration of 20 s was selected to ensure that data from the present study would be comparable with results obtained by Henry and Grizzle (2004).

The effect of voltage gradient on survival to hatching was investigated for early and late-stage spotfin chub

TABLE 2.—Summary of experiments conducted to determine the effects of electroshock on cyprinid embryos. The independent variables considered included electric current type (DC or 60-Hz pulsed DC [PDC]), voltage gradient (V/cm), and the stage of embryonic development when electroshock occurred. The dependent variables considered were either survival through hatching or rate of premature hatching (experiment 9 only).

Species	Experiment number	Independent variable[a]	N_E[b]	N_P[c]	Current type	V/cm	Developmental stage
Zebrafish	1	Stage	25–35	10	DC	16	Pre-cleavage through eyed
	2	Stage	25–35	10	DC	16	Pre-cleavage through eyed
Whitetail shiner	3	Voltage	25	36	DC	3–15	Blastula (100%)
	4	Voltage	20	7	DC	5–14	Blastula (85%), epiboly (15%)
	5	Voltage	25	22	DC or PDC	10	Blastula (100%)
Spotfin chub	6	Voltage	9–10	9	DC	3–9	Cleavage (56%), blastula (44%)
	7	Voltage	15	9	DC	7–15	Tail free (33%), eyed (33%), blastula (33%)
	8	Voltage	9–20	12	DC or PDC	8–15	Blastula (40%), tail free (30%), eyed (30%)
	9	Voltage	8	9	DC	8	Tail free, eyed

[a] Independent variable includes either stage of embryonic development (stage) or voltage gradient (voltage).
[b] N_E is the number of embryos exposed in each experimental treatment.
[c] N_P is the number of embryos preserved for evaluation of developmental stage.

and whitetail shiner embryos. After stocking into troughs (9–15 spotfin chub embryos per trough, 20–25 whitetail shiner embryos per trough), embryos were exposed to 20 s of DC electroshock ranging in intensity from 3 to 15 V/cm. Exposure troughs were provided with flow-through water, and water temperature and conductivity ranged from 24°C to 26°C and from 92 to 108 μS/cm for these and all subsequent experiments.

Spotfin chub and whitetail shiner embryos were used to compare effects of DC and 60-Hz PDC electroshock on survival to hatching of cyprinid embryos. Embryos were stocked into exposure troughs and exposed to 20 s of DC or PDC electroshock. For spotfin chub embryos, each control and DC treatment trough contained 20 embryos and each 60-Hz PDC treatment trough contained nine embryos. Each treatment trough was exposed to a different voltage gradient of DC or 60-Hz PDC electroshock (8–15 V/cm). For whitetail shiner embryos, each exposure trough was exposed to 10-V/cm DC or 60-Hz PDC electroshock. Three replicate troughs were used for both treatment groups, and each trough contained 25 embryos.

Susceptibility of spotfin chub embryos to electroshock-induced premature hatching was evaluated. Eight embryos were stocked into each trough, and treatment embryos were exposed for 20 s to 8-V/cm DC electroshock at one of two time periods (1–2 d postfertilization or 5–6 d postfertilization, three troughs each). Embryos were checked every 24 h, and the number hatched was divided by the number initially stocked to obtain percent hatch.

Statistics.—Statistical analyses were conducted using the Statistical Analysis System (SAS Institute, Cary, North Carolina). For the experiment evaluating differences between the effects of DC and PDC on survival of whitetail shiner embryos, survival data were arcsine-transformed (Zar 1984) and homogeneity of variance was assessed by Levene's test before performing analysis of variance (ANOVA). Tukey's honestly significant difference (HSD) test was used post hoc to evaluate survival differences between DC and 60-Hz PDC groups. For all other experiments, changes in survival as a function of independent variables developmental stage or voltage gradient were modeled by logistic regression. The model was

$$\text{logit}(p) = a + bx,$$

where logit(p) is the probability of a fish surviving, a is the intercept value, b is a parameter estimate, and x is either time of electroshock (h postfertilization) or electric field intensity (V/cm). A model was generated by iterative maximumization of the likelihood function and was considered significant at P-values of 0.05 or less based on the Wald chi-square statistic. Model selection was based on the likelihood ratio test (using the chi-square statistic of Hosmer and Lemeshow 2000), and effects were selected for inclusion in the model if they significantly ($P \leq 0.05$) improved the predictive ability of the model.

The estimate of logit(p) was used to obtain the predicted probability of embryo survival (p) as follows:

$$p = e^{\text{logit}(p)}/[1 + e^{\text{logit}(p)}],$$

and voltage gradients that reduced embryo survival to 50% (LV50 values) were calculated based on p.

Results

Control survival among all species and experiments ranged from 74% to 93%. Exposure to electroshock within 48 h postfertilization did not affect the time when hatching occurred, and no deformities of hatched

TABLE 3.—Logistic regression models for each experiment conducted to investigate the effects of electroshock (DC or pulsed DC [PDC]) on cyprinid embryos. Information on the design of each experiment is summarized in Table 2. Experiments 5 and 9 were designed for analysis of variance and are not included in the table.

Experiment number	Model	x[a]	Figure
1	$-1.39 + 0.2x$	Stage	1
2	$-1.9 + 0.2x$	Stage	1
3	$4.03 + 0.78x$	Voltage	2b
4	$2.98 - 0.27x$	Voltage	2b
6	$2.75 + 0.44x$	Voltage	2a
7	$2.86 - 0.14x$	Voltage	2a
8 (DC)	$1.37 - 0.12x$	Voltage	3a
8 (PDC)	$3.4 - 0.12x$	Voltage	3a

[a] Independent variable is stage (h postfertilization) or voltage gradient (voltage, V/cm).

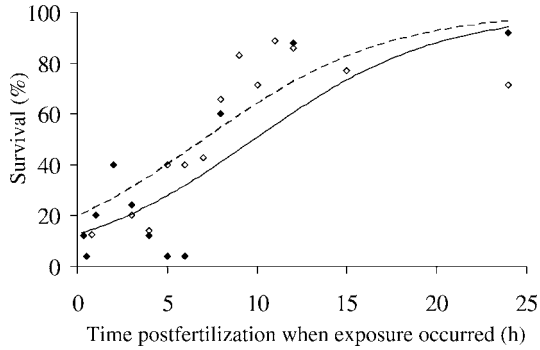

FIGURE 1.—Survival to hatching of zebrafish embryos relative to the stage of embryonic development when embryos were exposed to electroshock. Embryos were exposed for 20 s to 16-V/cm DC electroshock. Open data points and the dashed line represent observed survival and the logistic model, respectively, for the first experiment (80% control survival); filled data points and the solid line represent observed survival and the logistic model for the second experiment (93% control survival).

larvae were observed in electroshock treatments or controls. Embryos from late developmental stages moved rapidly inside the chorion during the first 5–10 s of electroshock with DC or 60-Hz PDC, and movements returned to preshock levels within 5 min following exposure.

Survival of cyprinid embryos was lowest when electroshock occurred at early developmental stages and increased when embryos were exposed at later developmental stages. In logistic regression models of embryo survival, the independent variables (stage of development and voltage gradient) were significant predictors of embryo survival. All logistic regression models of embryo survival were significant ($P < 0.0001$), and the index of rank correlation, which indicates the percent effectiveness of a model to predict a binary response (SAS Institute 1995), was 70–94% (Table 3). For zebrafish exposed before 5 h postfertilization, survival was lowest and varied from 4% to 40%; after 6 h postfertilization, survival increased and reached levels similar to controls by 9–12 h postfertilization (Figure 1). Hatching of zebrafish embryos occurred between 60 and 72 h postfertilization.

Survival to hatching of whitetail shiner and spotfin chub embryos was lower when embryos were electroshocked at earlier developmental stages (blastula period) than when embryos were exposed at older developmental stages (i.e., late epiboly, tail-free, or eyed stages; Figure 2). Results from two separate experiments with early stage (0–24 h postfertilization) spotfin chub embryos did not differ significantly ($df = 1$, $\chi^2 = 1.56$, $P = 0.21$); thus, the data were combined (logistic model: Wald $\chi^2 = 15.5$, $P \leq 0.0001$; Figure 2a). Spotfin chub embryos exposed early in development (0–24 h postfertilization) had an LV50 value of 6 V/cm. Survival to hatching of electroshocked spotfin chub embryos from a range of older developmental stages (one-third blastula, one-third tail free, one-third eyed) was related to voltage gradient (Wald $\chi^2 = 5.04$, $P = 0.02$), and was significantly greater than for early stage embryos exposed during the blastula developmental period ($df = 1$, $\chi^2 = 35.0$, $P < 0.0001$; Figure 2). For whitetail shiner embryos exposed early in development (0–24 h postfertilization), the relation between voltage gradient and survival was significant (Wald $\chi^2 = 98.6$, $P < 0.0001$), the LV50 value was 5 V/cm, and survival was significantly lower ($df = 1$, $\chi^2 = 5.17$, $P = 0.02$) than for spotfin chub embryos (LV50 = 6 V/cm) exposed at the same developmental stage. For electroshocked whitetail shiner embryos from two developmental periods (85% blastula, 15% epiboly), survival to hatching was related to voltage gradient (Wald $\chi^2 = 25.35$, $P < 0.0001$) and survival was significantly greater than for early stage whitetail shiner embryos exposed during the blastula developmental period ($df = 1$, $\chi^2 = 78.75$, $P < 0.0001$; Figure 2).

Exposure to DC resulted in lower survival than exposure to 60-Hz PDC for both spotfin chub and whitetail shiner embryos (Figure 3). Survival of spotfin chub was related to voltage gradient (Wald $\chi^2 = 23.01$, $P < 0.0001$) and was significantly higher for the embryos exposed to 60-Hz PDC than for those exposed to DC over the same range of voltage gradients (8–15 V/cm; likelihood ratio test: $df = 1$, $\chi^2 = 21.6$, $P < 0.0001$). The ANOVA model for whitetail shiner embryos exposed early in development to DC (10 V/cm) or 60-Hz PDC (10 V/cm) was significant ($df = 2$, $F = 69.8$, $P < 0.0001$), and Tukey's HSD test indicated a

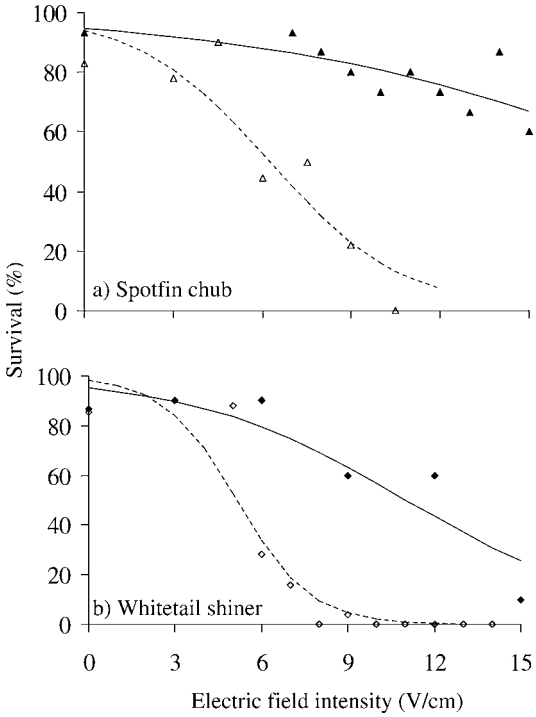

FIGURE 2.—Survival of **(a)** spotfin chub embryos and **(b)** whitetail shiner embryos exposed to DC electroshock. Points at 0 V/cm represent survival of unshocked control embryos. The open data points and gray line in (a) represent observed survival and the logistic model of survival, respectively, for electroshocked early stage (56% cleavage, 44% blastula) spotfin chub embryos collected from two separate spawning periods. Filled data points and black line in (a) represent observed survival and the logistic model, respectively, for electroshocked later-stage (33% blastula, 33% tail free, 33% eyed) embryos. The open data points and the gray line in (b) represent observed survival and the logistic model, respectively, for electroshocked early stage (100% blastula) whitetail shiner embryos. Filled data points and black line in (b) represent observed survival and the logistic model, respectively, for electroshocked later-stage (85% blastula, 15% early epiboly) embryos.

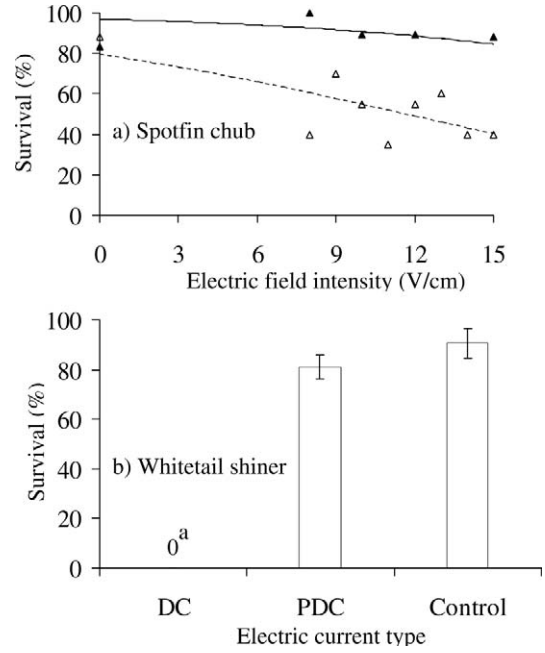

FIGURE 3.—Survival to hatching of **(a)** spotfin chub embryos and **(b)** whitetail shiner embryos after DC and pulsed DC (PDC) electroshock. Spotfin chub embryos from a single spawn and a range of developmental stages (30% eyed, 30% tail free, 40% blastula) were exposed to voltage gradients from 8 to 15 V/cm. The filled data points and black line in (a) represent the observed survival and corresponding logistic model, respectively, for embryos exposed to PDC. The open data points and gray line represent the observed survival and corresponding logistic model, respectively, for embryos exposed to DC. Whitetail shiner embryos in (b) were from the blastula developmental stage and were exposed to 10-V/cm DC or 10-V/cm 60-Hz PDC. The survival (mean ± SE) denoted by a letter was significantly different from the mean for unshocked controls.

significant ($P \leq 0.05$) difference in survival between the DC and 60-Hz PDC groups, but not between embryos exposed to 60-Hz PDC (10 V/cm) and unshocked control embryos.

Spotfin chub embryos hatched prematurely when exposed to DC electroshock at approximately 5–6 d postfertilization. These embryos were electroshocked with 8 V/cm DC and 100% hatched within 30 min of the exposure, whereas 100% of embryos electroshocked 1–2 d postfertilization hatched on the same day as control embryos (7–8 d postfertilization, 48 h later than those electroshocked at 5–6 d postfertilization). All embryos used in this experiment survived through hatching.

Discussion

Survival to hatching of all three cyprinid species considered was related to developmental stage at time of electroshock and was lowest when embryos were electroshocked early in development. Survival improved as development progressed beyond epiboly, which is consistent with results of investigations on embryos of rainbow trout *Oncorhynchus mykiss* (Godfrey 1957; Dwyer et al. 1993), razorback suckers *Xyrauchen texanus* (Muth and Rupert 1997), and largemouth bass *Micropterus salmoides*, bluegills, and channel catfish *Ictalurus punctatus* (Henry and Grizzle 2004). The developmental period most susceptible to electroshock included early epiboly in zebrafish

and is consistent with other studies (Muth and Rupert 1997; Henry and Grizzle 2004). However, some previous investigations have reported high survival (similar to control survival) of embryos electroshocked at some developmental stages before epiboly (Dwyer et al. 1993; Cho et al. 2002). In zebrafish, there was considerable variability in survival when exposure occurred before epiboly, and only 4–40% of embryos survived when electroshock occurred during these earlier stages. Survival of zebrafish embryos did not approach control levels until epiboly was nearly completed. In contrast to other studies, Cho et al. (2002) found that highest sensitivity of Chinook salmon *O. tshawytscha* embryos occurred during the early eyed stage (145 accumulated thermal units [ATU], 8.1°C), and low sensitivity was noted during both precleavage (0 ATU) and pre-eyed (57 ATU) developmental stages. The differences between our results and those of Dwyer et al. (1993) and Cho et al. (2002) could be related to species differences or to differences between the electric fields to which embryos were exposed, as both of those studies tested effects of PDC electroshock on salmonid embryos, whereas the present study tested DC electroshock on cyprinid embryos.

Our experiments with zebrafish to determine the embryonic stage most susceptible to electroshock-induced mortality were focused on the first 12 h postfertilization (fertilization through epiboly) to more precisely define the stage of highest sensitivity. No other study has electroshocked embryos at as many early developmental stages as the present study; however, the variance in sensitivity during the first 12 h of development prevented precise identification of the most sensitive developmental stage. Zebrafish embryos develop rapidly (hatching occurred between 60 and 72 h postfertilization at 28°C), and the variability observed early in development could partly be due to differences in developmental stages among individual embryos that were not detected in the staging process. Despite the variability of embryo sensitivity, it was clear that zebrafish embryos were most sensitive to DC electroshock early in development and that early epiboly was a particularly sensitive developmental stage.

The effect of developmental stage on survival of electroshocked embryos was difficult to determine precisely for spotfin chub and whitetail shiners because both species spawn fractionally and because obtaining embryos that were all at the same stage of development was not possible. In experiments with these species, the mixture of developmental stages of embryos was determined by examining a sample of the embryos preserved at the time electroshock occurred. While this approach allowed the stages of exposed embryos to be determined, it did not enable determination of which of the stages represented in the sample had higher mortality. However, survival of spotfin chub and whitetail shiner embryos exposed to a range of DC voltage gradients was related to age at the time of exposure, as indicated by differences in survival between experiments containing embryos from different developmental stages. For experiments in which embryos of spotfin chub and whitetail shiners were exposed to DC voltage gradients, survival was higher when embryos from later developmental stages (late epiboly, tail free, or eyed) were present in the exposure troughs. Because of the fractional spawning strategy of the native cyprinids tested in this study (Etnier and Starnes 1993), a range of developmental stages is probably present in the nests of these species, which increases the likelihood that both highly sensitive and less-sensitive stages will be exposed to electric fields during electrofishing.

Voltage gradient of DC was inversely related to survival of spotfin chub and whitetail shiner embryos. The highest voltage gradients tested in these studies were within the range commonly produced by electrofishing equipment (Henry et al. 2003; J. W. Habera, unpublished.), and 0% of spotfin chub and whitetail shiner embryos survived 10-V/cm DC electroshock when exposed early in development (cleavage or blastula period). Also, DC voltage gradients are known to reduce survival of exposed fish embryos (Dwyer and Erdahl 1995; Henry and Grizzle 2004). Henry and Grizzle (2004) exposed embryos of three warmwater fishes to DC voltage gradients similar to those tested in this study and found that survival decreased with electric field intensity and that level of response differed among species.

Exposure to 60-Hz PDC did not reduce survival of spotfin chub or whitetail shiner embryos. Other studies have also indicated that PDC has less effect on survival of embryos than DC (Dwyer and Erdahl 1995; Henry and Grizzle 2004). Although survival of embryos in the present study was not affected by PDC voltage gradients as high as 15 V/cm, Muth and Rupert (1997) found that survival of razorback sucker embryos was reduced after exposure to 1.2-V/cm PDC (conductivity, 650 μS/cm) of various pulse frequencies including 60 Hz, indicating that PDC can reduce survival of embryos in some species.

Whitetail shiner and spotfin chub embryos responded similarly to DC electroshock, with LV50 values of 5 and 6 V/cm, respectively. Although the susceptibility to electroshock-induced mortality of these species was significantly different, the difference could be due to subtle differences in developmental stage at the time of

electroshock. Differences in developmental stage between these species could not be determined precisely due to the small number of spotfin chub embryos ($n = 9$) that were preserved for staging. However, it was evident that two different developmental periods were present in the preserved sample of spotfin chub embryos (five of the nine embryos were from the cleavage period and four of the nine were from the blastula period), while all 36 preserved whitetail shiner embryos were from the blastula period, indicating that there were some differences in developmental stage between these experiments.

Spotfin chub and whitetail shiners deposit their eggs into natural crevices in bedrock and boulder for protection from predators (Etnier and Starnes 1993), and developing embryos might receive protection from the full intensity of electrofishing fields because of this spawning adaptation. Godfrey (1957) found that buried (8 cm of gravel) Atlantic salmon *Salmo salar* embryos exposed during precleavage had significantly higher survival than embryos left uncovered when exposed to similar DC electric fields, but voltage gradient was not reported in that study. Dwyer et al. (1993) reported that significant mortality occurred when cutthroat trout *O. clarkii* eggs in artificial redds (buried under 15 cm of gravel) were exposed to 340 V of 250-Hz PDC and that electric field intensity was similar between artificial redds and exposure chambers (1 V/cm). Spotfin chub and whitetail shiners can deposit their eggs more than 7 cm into crevices between ceramic tiles during hatchery spawning, but most naturally spawning fish deposit eggs into crevices ranging from 1 to 3 cm deep (P. L. Rakes, personal observation). There is insufficient evidence that crevice spawning will provide any protection from electrofishing for cyprinid embryos, and perhaps embryos in crevices are more vulnerable if the boundary effect increases the electric field intensity.

Sublethal effects of electrofishing can occur in posthatching life history stages of fish but have rarely been reported for fish embryos. Embryos in the present study that survived through hatching did not have gross external deformities, and this observation is consistent with other studies that have reared electroshocked embryos through hatching (Muth and Rupert 1997; Henry and Grizzle 2004). In the present study, premature hatching was induced in spotfin chub embryos exposed to electroshock at 5–6 d postfertilization, and this result is consistent with previous observations of electroshock-induced premature hatching (Luczynski and Kolman 1987; Henry and Grizzle 2004). The observations of Henry and Grizzle (2004) in electroshock experiments with bluegill embryos were consistent with the release of enzymes from hatching glands within 30 min of electroshock. Premature hatching did not directly affect survival in their laboratory experiment, but they suggested that increased predation risk could result from premature hatching. The effect on survival after premature hatching should be investigated before reaching the conclusion that later stages of embryonic development are less vulnerable to consequences of electroshock than early stages.

Results from this study indicate that electrofishing in the spawning habitat of threatened and endangered cyprinids may significantly reduce embryo survival if sensitive stages are present and if DC electric fields are used. Direct current has been suggested as a means of minimizing the negative effects of electrofishing (Reynolds 1996); however, based on the results of the present study and others (Dwyer et al. 1993; Henry and Grizzle 2004), use of DC should be avoided when fish embryos may be present. In light of the results from this research, it is recommended that electrofishing not be conducted when embryos of threatened or endangered cyprinids are known to be present in a sampling location. As suggested by Nielsen (1998), some endangered species that lay eggs in nests may be particularly vulnerable to the negative effects of electrofishing. Because cyprinids employ a range of spawning strategies (e.g., nesting and aggregating to spawn), threatened or endangered cyprinid populations could be negatively affected if a significant proportion of embryos are exposed to harmful electric fields during electrofishing.

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