

# Exclusion of fish and invertebrates from benthic patches of artificial aquatic environments across water conductivity levels using high-frequency (10 Hz) pulses and adjustable electrical settings

Ryan M. Utz<sup>1,5</sup>, Scott D. Cooper<sup>2,6</sup>, Keith B. Gido<sup>3,7</sup>, and Jenna R. Stewart<sup>4,8</sup>

<sup>1</sup>Falk School of Sustainability, Chatham University, 6035 Ridge Road, Gibsonsia, Pennsylvania 15044 USA

<sup>2</sup>Department of Ecology, Evolution, and Marine Biology, University of California Santa Barbara, Santa Barbara, California 93106 USA

<sup>3</sup>Division of Biology, Kansas State University, 116 Ackert Hall, Manhattan, Kansas 66506 USA

<sup>4</sup>Department of Civil, Environmental, and Architectural Engineering, University of Colorado, Boulder, Colorado 80309 USA

**Abstract:** Livestock fence chargers are often used to generate pulsed electrical fields in freshwater environments to exclude fish and invertebrates from benthic patches so that their ecological function can be investigated. Such devices appear to be effective, but the precise characteristics of the electrical fields they generate and specific settings needed to exclude organisms across water conductivity levels have not been described. We present an electrical engineering and experimental framework to predict and evaluate the effectiveness of a modified device at different combinations of electrical settings and water conductivities. We avoided toxic materials and considered safety issues. We conducted laboratory experiments to identify the electrical fields with a 10-Hz pulse frequency needed to exclude adult Fathead Minnows (*Pimephales promelas*) and crayfish (*Procambarus clarkii*) from electrical enclosures across a range of water conductivities (13–800  $\mu\text{S}/\text{cm}$ ). Fish and crayfish in waters of low conductivity ( $\leq 50$   $\mu\text{S}/\text{cm}$ ) were excluded from enclosures at  $\geq 200$  V of electric potential. In water of higher conductivity ( $\geq 250$   $\mu\text{S}/\text{cm}$ ), similar settings caused consumer immobilization. Electrical pulse durations of 150  $\mu\text{s}$  were more effective than 50- $\mu\text{s}$  pulse durations at excluding organisms. Further refinement toward standardized methods requires analogous experimentation in the field, but our findings emphasize the importance of comprehensively considering electrical fields (voltage, pulse frequency and duration), water conductivity, and electrode configuration a priori when using these devices to optimize designs.

**Key words:** electrical enclosure, conductivity, *Procambarus clarkia*, *Pimephales promelas*

Many ecological investigators have sought to quantify the effects of large-bodied aquatic organisms on the benthic zone via experimental exclusion. The goals of such inquiries can be diverse because the largest organisms in aquatic ecosystems (typically fishes or amphibians) can exert strong direct or indirect trophic cascade effects (Gido et al. 2010, Klemmer and Richardson 2013) and can influence and physically structure the benthic zone through bioturbation (Usio and Townsend 2004, Pledger et al. 2016). Experimental exclusion can be achieved with physical barriers (Flecker 1996), but the barrier itself may induce undesired effects, such as accumulation of debris because of entrainment, on response variables. To avoid this shortcoming,

investigators have long used livestock fence chargers that deliver pulses of electricity through small-scale ( $\sim <1$  m<sup>2</sup>) electrode arrays to exclude organisms from patches (Pringle and Blake 1994). However, little attention has been paid to the electrical characteristics of these devices and the precise settings that are effective at excluding organisms at different water conductivities. This lack of standardization has uncertain effects on experimental results. Many authors have not reported or have misreported factors, such as electric potential (voltage), pulse frequency or duration, or water conductivity that may significantly influence enclosure performance. For example, several investigators (Pringle and Hamazaki 1997, Effenberger et al.

E-mail addresses: <sup>5</sup>rutz@chatham.edu; <sup>6</sup>scott.cooper@lifesci.ucsb.edu; <sup>7</sup>kgido@ksu.edu; <sup>8</sup>jenna.r.stewart@colorado.edu

2011) stated that their livestock fence chargers delivered 2-ns electrical pulses, but nearly all commercial livestock fence chargers deliver pulses that are ~50 to 10,000  $\mu$ s in duration. Replicating study designs and drawing quantitative conclusions about enclosure efficacy is difficult unless authors report a basic suite of electrical settings and environmental conditions. A rigorous framework for describing and using such devices, including the experimental work to identify the electrical fields needed for effective operation, would help ensure methodological consistency among sites or studies.

The goals of our study were to: 1) use an electrical engineering perspective to describe the electric properties of enclosure devices comprehensively and 2) experimentally identify the electrical settings needed for a 10-Hz electrical field to exclude fish and invertebrates from small patches across a wide range of water conductivities. Many factors influence enclosure effectiveness in a particular setting: target organism size; electric pulse duration, frequency, and intensity; water conductivity and temperature; and the presence of clays or other suspended particles that influence electric currents. To examine how some of these factors affect enclosure effectiveness, we measured fish and crayfish responses to different combinations of voltage and pulse duration across different water conductivities. We set frequency at 10 Hz because frequencies this high can produce the exclusion effect with less current. However, pulse frequency could be manipulated as a factor that controls efficacy.

### Enclosure design

Researchers began using electrical livestock fence chargers to manipulate stream organisms experimentally >20 y ago. However, few researchers have outlined critical design features needed to quantify and replicate experimental results. Our goal was to provide an electrical engineering overview of the operation of these devices. We also created a modified device tailored for aquatic ecological investigations to conduct our experimental work. We sought to ensure that experiments were repeatable, operational features were standardized, safety hazards were minimized, and toxic or ineffective materials were not used.

An exclusion device creates an electrical field that deters aquatic organisms by delivering pulsating direct current (DC) to an anode and a cathode (electrodes) separated by some distance in the water. Water in such systems acts as a resistor that is several orders of magnitude less conductive than the electrodes, which typically are made of metal. The electrical resistance of water varies directly with dissolved ion concentrations, so the conductivity of water will directly affect the strength of an electrical field delivered by an enclosure. If water conductivity is low, a livestock fence charger will have to deliver a high voltage to the device to create an electrical field sufficient to exclude

organisms. Livestock fence chargers are capable of delivering high current pulses because they can store hundreds of volts of electric potential in a capacitor during the period in which current is not delivered. The intensity of electrical current traveling through water between 2 electrodes also is influenced by the frequency and duration of electrical pulses and by the orientation and size of the electrodes. Thus, many physical and chemical variables can directly affect the intensity of the electric current between electrodes and its effectiveness for excluding organisms. Consideration and reporting of each of these variables is necessary to ensure that the devices are as effective as intended and that experiments are methodologically consistent and, hence, reproducible across sites.

Livestock fence chargers and electrofishing devices affect organisms by delivering electrical pulses of similar magnitude through the water, but one key electrical property differs between these devices. Maximum electrofishing effectiveness is achieved at a 10 to 50% duty cycle (i.e., delivery of electricity for 10–50% of a given period of time; Miranda and Dolan 2004), whereas livestock fence chargers deliver high current pulses that are 50 to 10,000  $\mu$ s in duration. A livestock fence charger delivering 100- $\mu$ s pulses at a 1-Hz frequency operates at a 0.01% duty cycle. However, electric potential and current ranges overlap between electrofishing devices and fence chargers. Commonly used electrofishers may be set to 100 to 1000 V of potential (with a 40 A maximum current; Smith-Root™ 2009). Kurlle and Cardinale (2011) reported that 500 V of potential delivered from a livestock fence charger was effective at excluding fish from electrical enclosures, but they did not report the pulse duration, pulse frequency, or water conductivity. Livestock fence chargers are effective at excluding aquatic organisms from enclosures despite their relatively short duty cycles because: 1) the distances between electrodes in enclosures are typically less than for backpack electrofishers, so the electrical current is spatially concentrated; 2) the deterrence or exclusion of organisms requires less current intensity than that required for immobilization (Lamarque 1990); 3) transitioning between the presence and lack of electrical current (pulses) enhances the physiological stress induced by electricity (Miranda and Dolan 2004), so the rapid pulses produced by livestock fence chargers effectively provoke a response from organisms. Therefore, the fundamental physics of electrofishing devices have received considerably more attention in the literature (Bohlin et al. 1989, Kolz 2006, Martinez and Kolz 2009) than the physics of enclosures, but conclusions drawn from such studies have only limited utility for enclosure devices.

The short pulse duration and duty cycle of fields produced by livestock fence chargers give these fields a key advantage over fields generated by electrofishing devices by limiting the extent of electrolysis. When an electric current passes through an ionic solution, the current causes electrons to be absorbed or released from the surfaces of the

electrodes. Oxidation occurs at the anode, whereas reduction occurs at the cathode. As a consequence, anodes corrode and lose mass over time. Corrosion and mass loss usually are not problems for electrofishing devices because units are turned on for, at most, hours in an operating day, and electrode components can easily be abraded to remove corrosive buildup or replaced when needed. Experimental enclosures typically are deployed continuously for weeks or months, so the short duty cycle limits electrolysis and allows long periods of deployment without noticeable electrode dissolution.

Nevertheless, an unknown amount of electrolysis does occur during brief pulses and, therefore, electrode materials should be chosen to minimize unintended ecological effects. Most researchers have made electrodes of Cu wire (Pringle and Blake 1994, Schofield et al. 2004, Kurlle and Cardinale 2011), but the differences in resistivity among

metals are insignificant compared to resistivity differences between metals and water (Serway 1998). Thus, most commercially available metals can be used to construct enclosures. Dissolved Cu is acutely toxic to many aquatic organisms (Brix et al. 2001), so we selected 0.3175-diameter 316L stainless steel (a particularly corrosion-resistant alloy) for the enclosures described below. Steel is also less valuable and, therefore, less susceptible to theft than Cu.

The aquatic components of the enclosure include a 10-cm-diameter stainless steel circular ring that serves as the anode centered within a 25-cm stainless steel ring (Fig. 1) that serves as the cathode. Each pair of rings is held in its concentric configuration with rigid plastic spacers. Charges are delivered to the rings by 12-gauge Cu wire soldered to a small Cu tab, which is welded to each ring and coated with plastic resin to prevent electrolysis. We chose 2 electrical pulse durations (50 and 150  $\mu$ s) to span the values de-

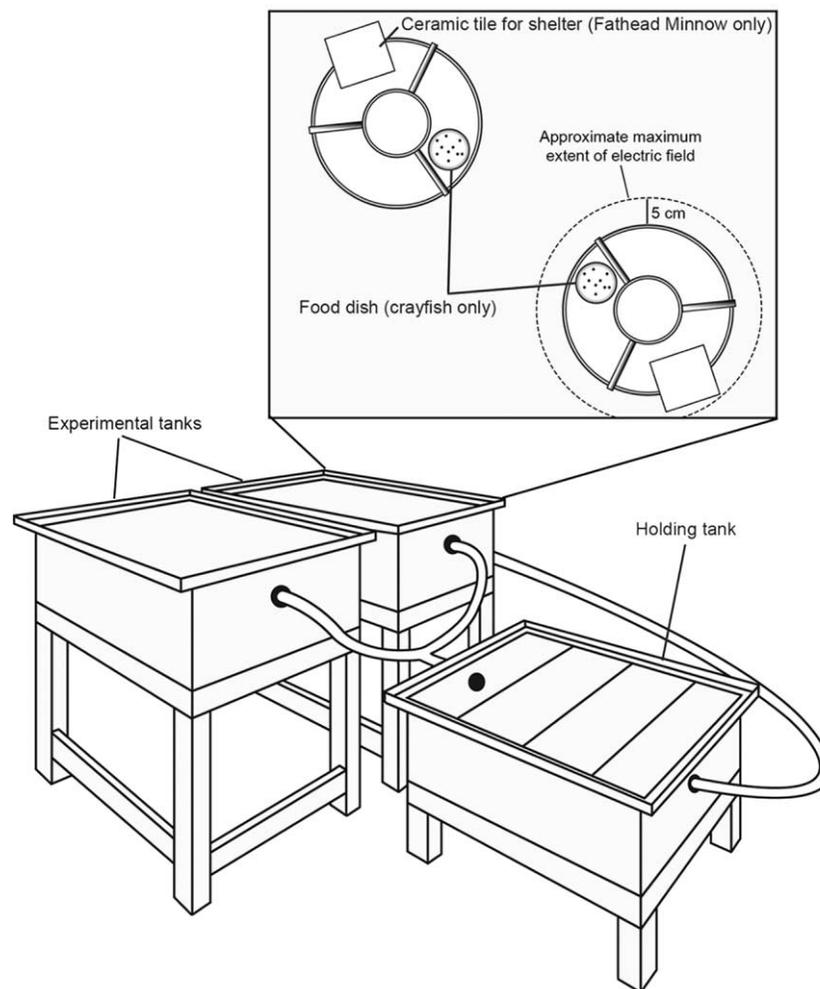


Figure 1. Layout of experimental apparatus. A pump delivered water continuously from the holding tank to the 2 experimental tanks with return flows back into the holding tank. Two enclosures were placed in opposite corners of the experimental tanks: one was electrified and the other (control) was not. One 8-cm-diameter ceramic dish holding food was positioned between the 2 rings of each enclosure in crayfish trials. One 10.7- × 10.7-cm ceramic tile raised above the bottom by 4 dowel rods provided refuge habitat in each enclosure in Fathead Minnow trials.

livered by the Unigizer™ 1000 electric fence charger (Speedrite, Mineral Wells, Texas), a model used in past electrical exposure experiments (C. Pringle, University of Georgia, personal communication). We chose the ring sizes to ensure the device could be deployed easily in small, 1<sup>st</sup>-order streams. However, we stress that the specific dimensions of the electrodes that we used do not necessarily convey an advantage. Larger areal dimensions or different shapes could be and have been used in past studies.

We used the 1000 Unigizer as our starting model for exposure development, but we opted to apply less energy intensity than delivered by this fence charger per pulse for several reasons. The 1000 Unigizer, like most similar models, is designed to repel large livestock from several kilometers of fencing. We surmised that the charge intensity delivered per pulse of electricity would be beyond what is necessary to repel aquatic organisms in patches of aquatic habitat. Preliminary analyses with an oscilloscope revealed that the device delivers as much as 1 J of energy to an organism and 9800 V of electrical potential per pulse. Such intense energy was deemed an unacceptable safety hazard to humans who might enter the electric field intentionally or accidentally. Additional preliminary analyses on organisms suggested that  $\leq 0.1$  J is needed to deter organisms. Therefore, we created a circuit that limited energy pulses to this value. Electrical potential (V) is far more easily measured than energy per pulse and remains consistent in different water conductivities, so we tracked and adjusted potential rather than energy per pulse. The voltage settings (100, 200, and 400 V) we used reflected the potential delivered by the capacitor.

We maintained a pulse frequency of 10 Hz in all trials. Although devices used in previous field experiments typically delivered pulses at frequencies of 0.5 to 1 Hz, we used a 10-Hz frequency in this experiment because: 1) we expected that a high-frequency pulse would be needed to exclude test animals in water with very low ( $< 50$   $\mu\text{S}/\text{cm}$ ) conductivity and 2) we posited that reduced current delivered with lower voltage settings would deliver the desired effect if the pulse frequency was high. Therefore, we designed the voltage and current applied by our exposure to deliver an amount of current per unit time comparable to that achieved with higher current by increasing frequency an order of magnitude while reducing the overall current per pulse by an order of magnitude.

## METHODS

We examined the effects of electrical field voltage and pulse duration on the feeding rates, survivorship, or behavioral reactions of red swamp crayfish (*Procambarus clarkii*) and Fathead Minnows (*Pimephales promelas*) across a range of water conductivities. We selected these relatively small-bodied species because they represent a size range likely to

be targeted for exclusion by electrical fields in experimental field studies. We tested both vertebrates and invertebrates because both are targets for exclusion by electrical devices, but may respond differently to electrical treatments because of their different physiological attributes. Animal responses to electrical fields consistently increase with body size (Dolan and Miranda 2003, Peterson et al. 2004), so pulse and voltage settings affecting small individuals will have even greater effects on larger organisms. We used crayfish with carapace length =  $37.4 \pm 8.9$  mm (mean  $\pm$  SD) and Fathead Minnows with total length =  $59.1 \pm 7.4$  mm.

For both study species, we used a complete 3-way factorial experimental design with 2 levels of electrical pulse duration (50 and 150  $\mu\text{s}$ ) crossed with 3 levels of electrical pulse voltage (100, 200, 400 V). We crossed these 6 treatment combinations with 4 levels of conductivity (13, 50, 250, and 800  $\mu\text{S}/\text{cm}$ ). The pulse frequency was 10 Hz for all trials.

## Experimental apparatus and treatments

We conducted all experiments in an indoor laboratory. Water was continuously circulated among three 129-L, 70-  $\times$  60-  $\times$  30-cm polyethylene tanks, with 2 acting as arenas for experimental trials and 1 functioning as a holding tank for acclimating organisms (Fig. 1). A magnetic drive pump forced water through plastic tubing from the holding tank to each experimental tank at a rate of  $\sim 282.5$  L/h. We excluded experimental animals from tank inlets and outlets by fitting intakes and drains with 1.5-mm-slot strainers. We positioned experimental tanks 35 cm above the holding tank and routed water through tubing back to the holding tank via gravity flow. Each tank bottom was covered with a 3-cm layer of  $\sim 5$ -mm-diameter pebbles.

We filled tanks with deionized water, and salt residue on the gravel raised the conductivity to  $\sim 800$   $\mu\text{S}/\text{cm}$ . We adjusted conductivity within the system to target treatment levels by adding deionized water to depress levels or Instant Ocean™ aquarium salt to elevate levels. Conductivity levels were set at  $\sim 13$ , 50, 250, and 800  $\mu\text{S}/\text{cm}$ , reflecting a reasonable range of conductivities found throughout streams of the continental USA (Griffith 2014). Temperatures were maintained at  $\sim 20^\circ\text{C}$  ( $20.7 \pm 0.5^\circ\text{C}$  during crayfish trials and  $19.5 \pm 0.8^\circ\text{C}$  during Fathead Minnow trials).

We placed 2 electrical exposures, 1 charged and 1 uncharged, in each tank to create identical areas that did and did not contain electrical fields. The exposures were placed in opposite corners of each experimental tank with  $\geq 2.5$  cm of space between outer rings and tank walls (Fig. 1). The maximum extent of the electrical field, which occurred with the strongest electrical settings, was  $\sim 5$  cm beyond the cathode. This placement allowed movement between and around the control exposures without inducing the effects from the treatment exposure. In trials with crayfish, we placed an 8-cm-diameter ceramic dish containing food

between the 2 enclosure electrodes, and in trials with Fathead Minnows, we placed a 10.7- × 10.7-cm shelter between the 2 electrodes (Fig. 1). A shelter consisted of a ceramic tile with two 8.0-cm dowel rods affixed to 2 adjacent corners and two 5.0-cm dowel rods affixed to the opposite corners. The uneven dowel lengths supporting the tiles facilitated observations of habitat use. Tiles served as overhanging habitat because Fathead Minnows reside under such structures in laboratory settings (Pollock and Chivers 2004) (Fig. 1). We tested a total of 24 treatment combinations (4 levels of water conductivity × 3 voltage settings × 2 pulse duration settings), with 6 replicate trials of each treatment combination.

### Trial procedure

**Crayfish** We recorded food depletion (quantitative) and behavioral observations (qualitative) to assess enclosure effectiveness. We added a preweighed mix of protein- and algae-based sinking wafer food (Aqueon™ Sinking Algae Wafers and Bottom Feeder Tablets; ½ of a tablet of each; Aqueon, Franklin, Wisconsin) to the control- and treatment-enclosure ceramic dishes prior to the start of each trial. Crayfish were starved for 24–28 h in the conditioning tank and immediately transported to the experimental tanks in a nylon net at the start of each trial. We added 2 individuals (to promote interactions spurring movement) to each experimental tank in the control-enclosure corner of the tank. We used a 0.8-mm-opening wire-mesh lid placed immediately above the tanks to minimize visual disturbances caused by investigators tending to experiments. We acclimated individuals in the experimental tanks to experimental conditions without observation for 1 h with the treatment enclosure on.

After this period, we observed individuals visually at ~30-min intervals for 120 s on 5 separate occasions. We lifted the lid slowly to minimize disturbance and noted crayfish positions relative to the treatment enclosures and behavioral responses to the electric field. Behavioral responses were measured based on a 0 to 10 scale (Table 1). Behavioral scores for the control enclosures were consistently 0. If we never observed crayfish approaching a treatment ring dur-

ing a trial, then behavioral data for that trial were not recorded. We moved immobilized individuals in treatment enclosures to a position outside the treatment area to allow recovery. We recorded the total number of immobilized or dead individuals in each trial, and we did not reuse individuals in subsequent trials to ensure that learned behavior did not affect the results.

We removed crayfish from experimental tanks at the end of each 4-h trial period and measured carapace lengths to the nearest mm. We dried the food remaining in the ceramic dish at room temperature (~20°C) for 48 h on a preweighed plastic tray and subsequently weighed it to quantify the dry mass of food consumed in each trial. To control for food depletion not related to consumption, we measured the amount of food lost in 5 trials when crayfish were not present and found that <10.2% of food dry mass apparently dissolved over 4 h.

**Fathead Minnows** Enclosure effectiveness was assessed with quantitative observations of habitat use. At the start of each trial, we transferred 5 randomly selected individuals from the holding tank to the control-enclosure corner of each experimental tank and left them to acclimate for 1 h with the treatment enclosure on. After the acclimation period, we recorded the number of individuals under each tile at ~30-min intervals on 5 separate occasions by slowly lifting the lid to minimize disturbance. We moved immobilized individuals to a position outside of the treatment enclosure area to allow recovery. We recorded the total number of immobilized and dead individuals, and we did not reuse individuals in subsequent trials. We removed Fathead Minnows at the end of the trial, euthanized them by immersion in 65% ethanol, and measured their total lengths to the nearest mm.

We attempted to include a quantitative measure of food depletion similar to that used in the crayfish trials, but we repeatedly observed Fathead Minnows displacing food from the ceramic dishes when swimming above them, so we omitted feeding rates from Fathead Minnow analyses. We ran 10 trials with the treatment enclosures turned off to estimate mean tile use when no electrical field was present in the tanks. We observed a mean of 1.3 individuals

Table 1. Qualitative scoring system for crayfish behavioral responses to treatment (charged) enclosures. Scores for both crayfish individuals in each trial were rated on a 0 to 10 scale, with scores intermediate to those listed here assigned when appropriate.

Score	Description
0	No apparent reaction from crayfish; travel through or rest within enclosure
3	Some aversion to field noted; crayfish twitched while traveling within enclosures but did not quickly exit space within treatment enclosure
7	Crayfish responded strongly if they entered the space within rings but traveled within 5 cm of enclosure outer ring with little difficulty; no immobilization within enclosure observed
10	Complete exclusion within 2.5 cm of enclosure outer ring; consistent immobilization of individuals within enclosure

under tiles during these control trials (treatment tiles: 1.1 individual, control tiles: 1.4 individuals).

### Statistical analyses

We used 3 dependent variables to assess exposure effectiveness. We quantified the response effect ( $r_e$ ) of exposures (crayfish trials: food consumption, Fathead Minnow trials: habitat use) by taking the natural log of the ratio of control response to the treatment response:

$$r_e = \ln \frac{r_c}{r_t}, \quad (\text{Eq. 1})$$

where  $r_c$  represents the total mass (g) of food consumed or proportion of fish observed in the control and  $r_t$  the total mass or proportion in the treatment exposure. The above equation offered a nearly normally distributed, simple metric of response where values near 0 reflected no response to exposures and positive values indicated treatment-exposure avoidance. Preliminary analyses revealed that ~12.7% of food mass depleted could be accounted for by dissolution. Therefore, we scaled all pretrial dry mass values accordingly. We also used qualitative crayfish behavior scores (Table 1), which depicted reactions to the treatment exposures, as a complementary means of assessing exposure effectiveness.

The main effects of water conductivity, pulse duration, and voltage, and possible interactive effects among these variables on dependent variables were evaluated with 3-way factorial type-II analysis of variance models. Each model consisted of the following factorial terms: water conductiv-

ity, pulse duration, voltage, conductivity  $\times$  duration, and conductivity  $\times$  voltage. We included the latter 2 interaction terms to assess whether exposure settings varied significantly in terms of efficacy among water conductivity levels. We identified significant differences in trial outcomes across electrical settings within each water conductivity level with post hoc Duncan's multiple range test. We assessed variance homogeneity in response variable values among treatment groups with Bartlett's test (Bartlett 1937) and checked normality by inspecting quantile probability plots. All statistical analyses were performed using the *base* and *car* (Fox and Weisberg 2015) packages in program R (version 3.2.3; R Project for Statistical Computing, Vienna, Austria).

## RESULTS

### Crayfish trials

Crayfish readily consumed the food offered, and in some cases, they fed within seconds of release into the experimental tanks. We corrected all estimates of food consumption for nonconsumptive loss. Food was consumed in 93% of the experimental trials. In  $>1/2$  of the trials in which food was consumed,  $<15\%$  of initial food mass remained. Behavioral reactions to treatment exposures usually were observed, and we assigned qualitative behavioral scores in 91% of the trials.

Voltage and pulse duration significantly affected crayfish feeding (Table 2). In 250 and 800  $\mu\text{S}/\text{cm}$  water, all electrical settings reduced crayfish feeding in treatment exposures relative to control areas, although occasional

Table 2. Results of 3-way analysis of variance examining the effects of electrical setting (100, 200, 400 V; 50-, 150- $\mu\text{s}$  pulse duration) and water conductivity (13, 50, 250, 800  $\mu\text{S}/\text{cm}$ ) on response variables.

Organism and metric	Model term (degrees of freedom)	Statistical parameter	
		<i>F</i>	<i>p</i>
Crayfish: feeding rate	Water conductivity (3)	1.1	$\geq 0.05$
	Voltage (2)	10.1	$< 0.001$
	Pulse duration (1)	8.6	$< 0.001$
	Conductivity $\times$ pulse duration (3)	3.1	$< 0.05$
	Conductivity $\times$ voltage (6)	2.5	$< 0.05$
Crayfish: qualitative score	Water conductivity (3)	70.4	$< 0.0001$
	Voltage (2)	188.4	$< 0.0001$
	Pulse duration (1)	70.1	$< 0.0001$
	Conductivity $\times$ pulse duration (3)	3.7	$< 0.05$
	Conductivity $\times$ voltage (6)	9.2	$< 0.0001$
Fathead Minnow: habitat use	Water conductivity (3)	38.6	$< 0.0001$
	Voltage (2)	23.6	$< 0.0001$
	Pulse duration (1)	23.8	$< 0.0001$
	Conductivity $\times$ pulse duration (3)	3.2	$< 0.05$
	Conductivity $\times$ voltage (6)	8.9	$< 0.0001$

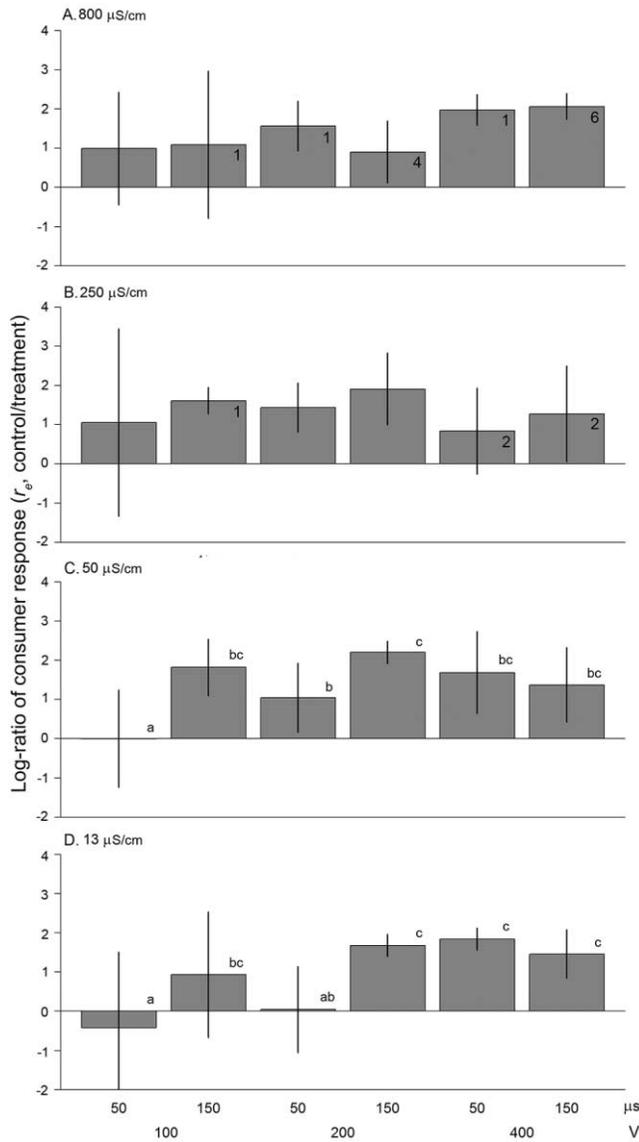


Figure 2. Crayfish feeding rates when exposed to electrical fields generated by enclosures in water with conductivities of 800 (A), 250 (B), 50 (C), and 13 μS/cm (D). Values correspond to the log response effect ( $r_e$ ) of daily feeding rates in the control and the treatment enclosures ( $\pm$ SE). Numbers in bars denote the total number of instances (maximum per treatment group = 6) where individuals were discovered immobilized in treatment enclosures. Bars with the same letters are not significantly different among electrical settings within water conductivity categories ( $p > 0.05$ ). Letters are not shown for individual bars within 800 and 250 μS/cm because the treatment effect did not differ among electrical settings.

feeding was observed in treatment enclosures at 100 V, especially at 50 μs (Fig. 2A, B). In 50 μS/cm water, crayfish regularly fed in treatment enclosures at 100 V and 50 μs, but fed less in treatment enclosures relative to control areas at higher pulse durations and voltages (Fig. 2C). In 13 μS/cm water, crayfish consistently fed in treatment enclosures at

100 and 200 V when the pulse duration was 50 μs, occasionally fed in treatment enclosures at 100 V and 150 μs, and did not feed in treatment enclosures at 200 V and 150 μs or at 400 V and 50 or 150 μs (Fig. 2D).

Behavioral reactions to treatment enclosures corroborated feeding results. At all water conductivities, avoidance behavior and immobilization were typical at 200 V and 150 μs and at 400 V and 50 or 150 μs (Fig. 3A–D). All immobilized individuals regained normal activity when

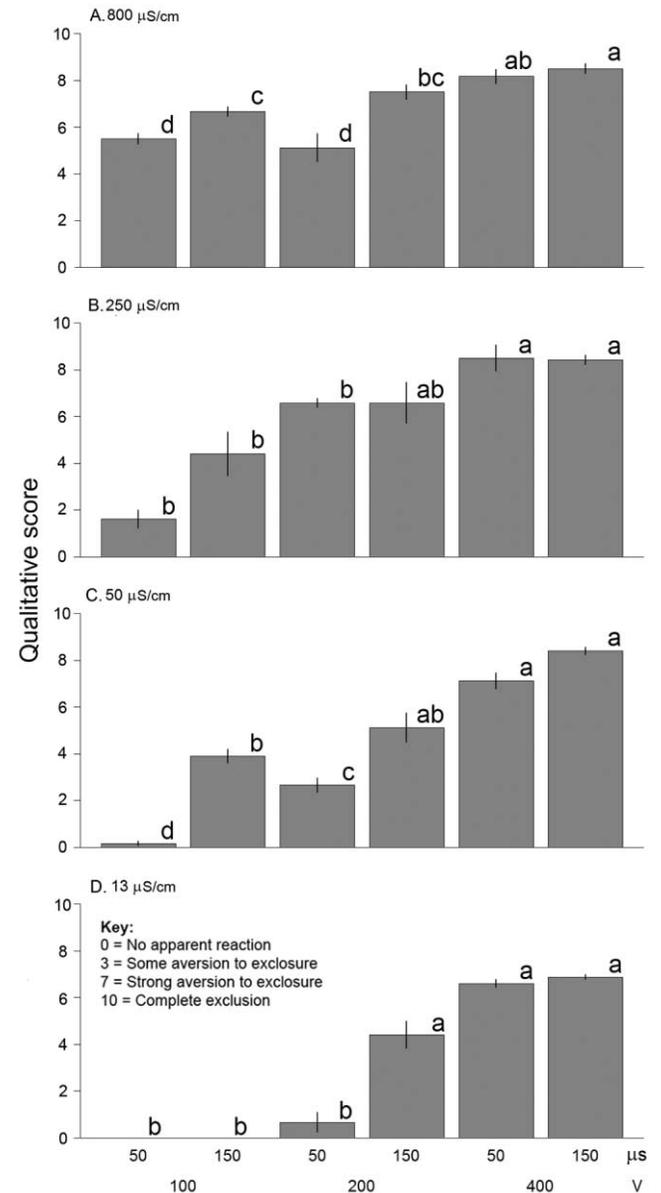


Figure 3. Qualitative scores ( $\pm$ SE) of crayfish behavioral reactions when exposed to electrical fields generated by enclosures in water with conductivities of 800 (A), 250 (B), 50 (C), and 13 μS/cm (D). Bars with the same letters are not significantly different among electrical settings within water conductivity categories ( $p > 0.05$ ).

removed from the treatment area. In 50  $\mu\text{S}/\text{cm}$  water, crayfish were observed consistently resting and moving in treatment exclosures at 100 V and 50  $\mu\text{s}$ , but treatment exclosures became more effective as voltage and pulse duration increased (Fig. 3C). In 13  $\mu\text{S}/\text{cm}$  water, crayfish were observed resting in treatment exclosures at 100 V and 50 or 150  $\mu\text{s}$  and at 200 V and 50  $\mu\text{s}$  (Fig. 3D).

### Fathead Minnow trials

Fathead Minnows often held positions under elevated ceramic tiles in control areas. At least 1 of the 5 Fathead Minnows in each trial held a position under a tile during 86.3% of the observations. Fathead Minnows did not use control or treatment tiles in 1 of 144 trials. The total mean number of individuals observed under control tiles across all observations and trials was 2.0, so the maximum value for the difference between control and treatment habitat use when treatment exclosures totally excluded minnows would be  $\sim 2$ .

The effects of electrified exclosures on Fathead Minnows tile use varied with water conductivity and electrical settings. In water of all conductivities, Fathead Minnows were excluded from treatment tiles at 400 V, and in  $>50$   $\mu\text{S}/\text{cm}$  water, Fathead Minnows were excluded from treatment tiles at 200 V (Fig. 4A, B). In 250 and 800  $\mu\text{S}/\text{cm}$  water, Fathead Minnows were excluded from treatment tiles at all electrical settings. Occasional mortality was observed at 400 V in 250  $\mu\text{S}/\text{cm}$  water and at multiple electrical settings in 800  $\mu\text{S}/\text{cm}$  water. In 50  $\mu\text{S}/\text{cm}$  water, Fathead Minnows used treatment and control tiles equally at 100 V and 50 or 150  $\mu\text{s}$  (Fig. 4C). In 13  $\mu\text{S}/\text{cm}$  water, Fathead Minnows used treatment and control tiles equally at 100 and 200 V and 50  $\mu\text{s}$  (Fig. 4D).

### DISCUSSION

Multiple electrical parameters and water-chemistry variables must be considered for the successful application of electrical exclosures. Our results suggest that electric potential and pulse duration may affect exclosure effectiveness independently and interactively across water conductivity levels. Furthermore, electrical settings (high voltages) necessary for successful consumer exclusion at low water conductivity (i.e.,  $\geq 200$  V in  $\leq 50$ - $\mu\text{S}/\text{cm}$  water) might be problematic in water with higher conductivities ( $\geq 250$   $\mu\text{S}/\text{cm}$ ) because some animals encountering exclosures could be immobilized or killed by the treatment. However, such effects may be reduced in a natural system where the electrical field would be easier to avoid. Therefore, researchers deploying patch- or reach-scale electrical exclosures should report all parameters describing the electric field and water conductivity.

To date, no authors of studies in which electric fence chargers were used to exclude organisms have reported all of the electric field parameters we manipulated, but

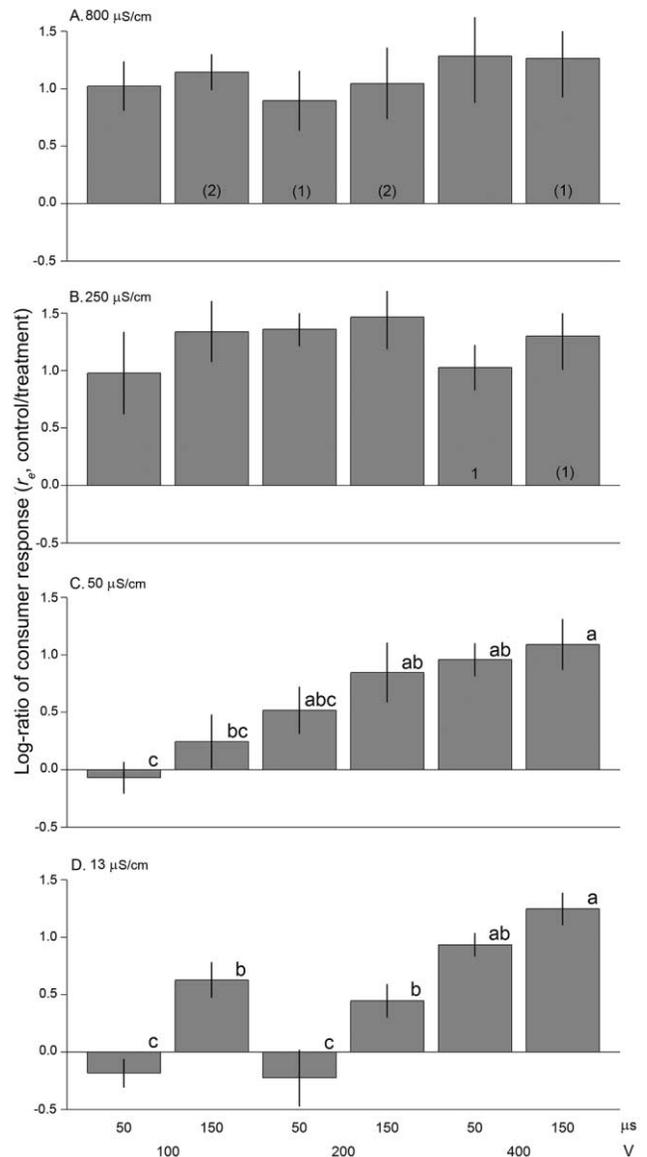


Figure 4. Tile shelter use by Fathead Minnows when exposed to electrical fields generated by exclosures in water with conductivities of 800 (A), 250 (B), 50 (C), and 13  $\mu\text{S}/\text{cm}$  (D). Values correspond to the difference in tile use by Fathead Minnows between paired control and electrified exclosure treatments as quantified by the log response effect ( $r_e$ ) ( $\pm$ SE). Values in parentheses (maximum per treatment = 30, 5 minnows per trial  $\times$  6 trials per treatment combination) represent the number of dead minnows for each treatment combination, whereas the number not in parentheses denotes an instance where an immobilized individual became active after being removed from the treatment exclosure area. Bars with the same letters are not significantly different among electrical settings within water conductivity categories ( $p > 0.05$ ). Letters are not shown for individual bars for 800 and 250  $\mu\text{S}/\text{cm}$  because the treatment effect did not differ among electrical settings.

electric field descriptors could be obtained easily with the use of an oscilloscope connected to the anode and cathode wires. We think our results demonstrate that different electrical settings and water conductivities can affect enclosure performance in ways that could influence the results of studies in which these devices are used. Therefore, an empirically based framework that conveys how different electrical settings, organism body sizes, and water conductivities affect enclosure efficacy would help researchers use enclosures with replicability and assurance that the intended effect is delivered.

The limited documentation and operational adjustments provided with most livestock fence chargers restrict investigator knowledge of and adjustments to electrical fields, primarily because these devices were designed to enclose livestock rather than exclude fish or aquatic invertebrates. The electrical field generated by a particular device, especially if designed to deliver a constant voltage rather than a constant current, may be applicable in one setting but not another, depending on water conductivity. Water conductivity values in a given study can span orders of magnitude, so a device that includes adjustable electrical controls probably should be used for aquatic ecological studies. Until such a device is commercially available, researchers probably will use commercial livestock fence chargers for their experimental work. They should note a basic suite of electrical parameters that describe the electrical field delivered by their devices and the mean water conductivity at their study sites. Such information is necessary to convey the probable effectiveness of these devices and will be needed if subsequent researchers attempt to replicate designs. Further refinement of related devices will lead to a commercially available, adjustable device that is specifically designed for aquatic science research.

Our results carry implications for the use of electrical enclosures, but our findings must be considered in the context of our experimental design. Our work was confined to the laboratory, so many of the conditions encountered by our subjects would not be experienced in a natural setting. In a field setting, organisms would be freer to avoid the electrical field, which extended ~5 to 8 cm outside the outer electrode of our device (for a total spatial effect of 30–33 cm). The confined space in our design may have led to the immobilizations and deaths we observed. In the field, the operative distance of effect relative to the electrodes may be much broader or narrower because of the large volume of water. Organisms like crayfish may be capable of burrowing under the apparatus and inducing an effect on variables within the enclosure. Continued development of electrical enclosures as a standardized device with reproducible effects will require experimental work in multiple field settings. Meanwhile, application of off-the-shelf fence chargers should involve preliminary field trials to ensure the intended effect is delivered. The intent of our study was to offer a maximally controlled investiga-

tion of parameters that merit consideration in subsequent developmental efforts.

We did not manipulate pulse frequency in our study, but higher frequencies should elicit stronger responses from organisms, thereby influencing enclosure effectiveness. Some authors did not report pulse frequency (Greathouse et al. 2006, Bobeldyk and Lamberti 2010, Ludlam and Magoulick 2010), but default frequency settings on livestock fence chargers typically range from 0.5 to 1.0 Hz (Rosemond et al. 1998, Bassar et al. 2012, Marshall et al. 2012). We chose to use a high frequency to assess whether exclusion could be achieved by reducing current per pulse (thereby reducing safety hazards) while applying an elevated frequency. Livestock fence chargers can exclude organisms successfully, but they are not designed for such use, and we conjectured that they deliver an electric load greater than what is needed. However, the high frequency we used in conjunction with the confining nature of the artificial habitat may have contributed to the small number of immobilizations and deaths in our trials. Investigators seeking to further refine electrical enclosures should test pulse frequency as a potential adjustable setting.

Our design consisted of components configured on a breadboard to deliver the desired adjustability for experiments. For researchers seeking to replicate our model without having to build a device from basic electrical components, we offer the following information. Solar-powered chargers, such as the Parmak models STD-12 and DF-SP-SS (Parker McCrory Manufacturing, Kansas City, Missouri), emit reduced charge intensities relative to the Speedrite model and have been used successfully to exclude larger-bodied aquatic organisms (Pringle and Blake 1994, Schofield et al. 2004). One commercially available livestock fence charger, the Speedrite model AN90 Energizer is not adjustable but does emit a degree of energy per pulse (~0.12 J, averaged over time) comparable to the 250 V, 150  $\mu$ s setting of our unit.

Other factors can affect enclosure performance. Enclosure effectiveness varies with the body size of organisms among and within species (Dolan and Miranda 2003). Settings capable of excluding small fish and crayfish in our study probably will exclude larger-bodied organisms. Clays and other suspended material and biofouling can conduct current and affect enclosure charges and effectiveness (Revil and Glover 1998, Malvankar et al. 2012). A final critical attribute of enclosures is the distance between electrodes. The strength of an electric field is strongest between electrodes and diminishes exponentially with distance outside of this space. Even the space within the inner electrode of concentrically designed devices may have lower electrical potential than the space between electrodes. Reporting the distance between electrodes would improve the reproducibility of studies.

Further experimentation, including in natural settings, will be needed to develop a comprehensive methodological

framework for patch-scale enclosures. Several researchers have conducted a priori tests to confirm enclosure effectiveness before attempting long-term electrical exclusion (Greathouse et al. 2006, Kurle and Cardinale 2011). Quantitative reporting of the results of such tests, accompanied by information on all the electrical, mechanical, biological, and water-quality attributes listed above, would enhance guidelines for the application of patch-scale electrical enclosures. For example, our enclosure settings probably would not be effective for organisms smaller than those included in our study, but the weakest settings we applied may be quite effective for excluding organisms larger than those we tested. Studies that address the effects of voltage and pulse frequency across a range of organism sizes would be of particular importance.

An additional consideration that requires research attention is the potential effect of electrical enclosures on nontarget organisms. Greathouse et al. (2006) reported that organic matter, algal, and macroinvertebrate levels in patches where large-bodied fish and shrimp were excluded by electrical enclosures matched values in areas in the same stream where the same organisms were excluded by a dam. Less attention has been directed at the effects of electrical enclosures on the community composition of periphyton and smaller invertebrates, such as insect larvae. Electrical devices similar to those we used have been applied to manipulate or collect small-bodied invertebrates, such as aquatic insect larvae (Taylor et al. 2002, Lento and Morin 2014), and electrofishing devices can elevate drift rates (Elliot and Bagenal 1972). Thus, enclosure devices may be capable of excluding nontarget invertebrates at strong electrical settings and could introduce undesired effects in studies in which the goal is to manipulate only larger organisms. The ability of electrical enclosures to affect small invertebrates suggests that such devices might be useful in studies of the ecological effects of smaller organisms when adjusted to high-current settings. Last, electric currents may stimulate microbial metabolism (Thrash and Coates 2008), and some aquatic fungi generate weak electric currents to achieve hyphal growth (Gow 1984). To our knowledge, no researchers have investigated the effects of electrical enclosures on the abundance or biomass of microbial or fungal assemblages or on leaf-litter breakdown rates. Most electrical enclosure experiments are designed to investigate the effects of large-bodied organisms on other components of the community, so the direct effects of electrical enclosures on all groups of aquatic organisms that are present should be known. Without such data, quantitative investigations of the role of aquatic organisms in benthic ecosystem processes may prove incomplete.

In summary, electrical enclosures have been used effectively to manipulate stream organisms, but more research is needed to establish the sizes and identities of organisms excluded by enclosures and to identify the effects of enclosures on nontarget organisms at different electrical set-

tings and water conductivities. Such work has been done to standardize methods for electrofishing (Bohlin et al. 1989, Martinez and Kolz 2009). Rigorous standardization of methods for electrical enclosures may help establish them as a sensible alternative to mesh cages, which can concentrate organic debris and indirectly affect target organisms (Compson et al. 2013), to exclude aquatic consumers. Our results suggest that electric pulses at a 10-Hz frequency at 200 V and 150  $\mu$ s and at 400 V at 50 and 150  $\mu$ s effectively exclude small-bodied fish and adult crayfish across all water conductivities, but may have the undesirable effect of killing or immobilizing organisms. Enclosures function with compromised efficiency at lower voltages (100 V) or pulse durations (50  $\mu$ s at 200 V), particularly at low conductivities ( $\leq 50 \mu$ S/cm). The effectiveness of enclosure devices may vary across systems, depending on electrical settings and water conductivity. We think that with additional work that identifies the sizes and identities of organisms excluded by electrical enclosures across levels of water conductivity and observations of enclosure effects on nontarget organisms, the method can be developed into a more rigorous, standardized, and repeatable tool.

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Author contributions: RMU wrote most of the manuscript, conducted some of the laboratory trials, and performed the data analysis. SDC and KBG helped craft the experimental design and analytical decisions. JRS performed some of the laboratory trials and created manuscript figures. All coauthors offered written content and constructive suggestions on various drafts of the manuscript.

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