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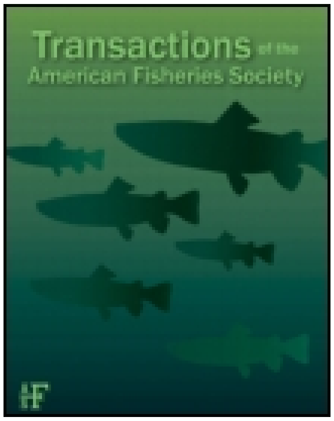

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Orientation and Position of Fish Affects Recovery Time from Electrosedation

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NOTE

Orientation and Position of Fish Affects Recovery Time from Electrosedation

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Abstract

Commercially available electrosedation apparatuses (e.g., the Smith-Root Portable Electroanesthesia System [PES]) are growing in popularity within the fisheries research community. This technology can be used to immobilize fish rapidly and does not require a withdrawal period before fish are released. A number of studies examined how various settings (e.g., duration, frequency, voltage) influence the performance of the PES for fish sedation, but comparatively less is known about the role of fish orientation and position on the efficacy of electrosedation within the PES. We compared recovery times of Bluegill *Lepomis macrochirus* upon manipulation of three variables: orientation of fish, electric field size (i.e., spacing between the anode and cathode), and fish proximity relative to the anode. Fish were individually exposed to pulsed DC with a standardized frequency (100 Hz), voltage (90 V), and shock duration (3 s). Full recovery time was significantly longer for fish oriented at horizontal angles (0° and 180°) than at acute angles (45° and 135°). Significant interactions were found between orientation and electrode spacing, as well as between orientation and fish proximity. These findings are pertinent to researchers in the field looking to optimize recovery time for a quick release after surgery, tagging, or any other time fish sedation is required.

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The availability of a safe and effective sedative for fish is critical for fisheries research and management as it enables researchers to perform surgeries, external tagging, and various other invasive and noninvasive procedures (reviewed in Trushenski et al. 2013). Currently, tricaine methanesulfonate (commonly referred to as MS-222) remains the predominant method of fish sedation in North America (Ross and Ross 2008; Trushenski et al. 2013). However, MS-222 has several limitations: induction time is lengthy, dose time is often subjective, and sedated fish must be held for 21 d prior to release to avoid accidental ingestion of MS-222 by harvesters (Carter et al. 2011). There are a variety of other sedative chemical compounds (reviewed in Ross and Ross 2008), although most cannot be used on food fish. There have been calls for a zero-withdrawal sedative (Marking and Meyer 1985; Bowker and Trushenski 2011; Trushenski et al. 2013) with some interest in CO_2 (Ross and Ross 2008) and more recently with AQUI-S 20E (Bowker and Trushenski 2013). However, the effects of CO_2 are unpredictable and occasionally ineffective in some species of fish (Trushenski et al. 2013), not to mention that hypercapnia is a recognized fish stressor (Bernier and Randall 1998). AQUI-S 20E is not universally approved and is unavailable in some jurisdictions. Moreover, CO_2 and AQUI-S 20E do not yield immediate sedation and recovery can be prolonged (Ross and Ross 2008; Trushenski et al. 2013).

Electrosedation (note that it is sometimes referred to as electroanesthesia—see Trushenski et al. 2012b for a discussion of terminology; we use "sedative" throughout this paper) represents a promising zero-withdrawal approach in that it provides a viable method of sedation while avoiding the common constraints that accompany the use of chemical sedatives such as MS-222, AQUI-S 20E, and CO_2. Electrosedation is a method of fish sedation that involves the use of electric currents (Ross and Ross 2008) to rapidly immobilize fish and does so without leaving any chemical residue (Trushenski and Bowker 2012). Electrosedation can be tailored to allow for six stages of sedation (Summerfelt and Smith 1990): the strongest stage (stage VI) euthanizes fish, while the weakest (stage I) does not alter fish behavior or activity. Stage IV is typically the stage desired by those intending to conduct procedures, such as the implantation of electronic tags (Trushenski et al. 2013).

Early attempts to use electrical currents to sedate fish required development of a custom apparatus (e.g., Madden and Houston 1976; Gunstrom and Bethers 1985; Walker et al. 1994; Gaikowski et al. 2001) or modification of electrofishing equipment (e.g., Vandergoot et al. 2011). There was not a widespread adoption of these devices given the concerns over operator safety and liability and the lack of an off-the-shelf tool. However, there have been recent developments such that there is now a commercially available electrosedation system (i.e., the Portable Electroanesthesia System [herein called PES]; U.S. patent 8,739,736; see Holliman 2010; produced by Smith-Root, Vancouver, Washington). According to the manufacturer, the PES is a "portable device intended for the generation of electrical energy to be used for the electrical stunning of aquatic organisms... designed for use in field applications, in hatcheries and on boats" (Smith-Root 2009). The PES consists of a control unit and an insulated container (140 L, 107 cm × 48 cm × 47 cm) with anode and cathode plates (27.5 cm × 34 cm). Fish are placed in the water between the anode and cathode plates and a remote switch is used to deliver a preprogramed current from the PES control unit. In the short period since the PES has been on the market (i.e., 2009), there have been a number of studies evaluating its performance and efficacy on freshwater (Bowzer et al. 2012; Gause et al. 2012; Trushenski and Bowker 2012; Trushenski et al. 2012a, 2012c) and marine (Trushenski et al. 2012b; Duryea 2014) fish. In addition, there is also indication of the adoption of the PES by natural resource agencies, universities, and other organizations for use in routine fisheries monitoring and tagging programs (e.g., used for the tagging of Walleye *Sander vitreus* in Lakes Erie, Huron, and Ontario and in the Winnipeg River in Manitoba; S. J. Cooke, personal observation).

The majority of research to date on fish electrosedation using the PES has focused on manipulating the voltage, frequency, and duration of the electroshock while standardizing environmental conditions (e.g., water conductivity, temperature) and biotic aspects (e.g., fish size) and evaluating both the efficacy of the technique and the consequences (e.g., stress, injury) to the fish (e.g., Trushenski et al. 2012a, 2012b, 2012c). Based on preliminary work by our team, it was apparent that other aspects appeared to influence efficacy, including the orientation of the fish when the electric current was applied, as well as the position of the fish relative to the anode and cathode. Research emanating from electrosedation as a fish sampling technique (i.e., electrofishing; Kolz 1989; Snyder 2003) suggests that fish orientation is a critical variable influencing the efficacy of the electric field because it influences the voltage gradient across the length of the fish (P. Cooney, Smith-Root, personal communication). To that end, in this experiment we examined the effects of varying the orientation and proximity of the fish within the PES, as well as the distance between the electrodes (hereafter called "electrode spacing") on the efficacy of electrosedation. Understanding how those parameters affect the recovery time of fish following treatment in the PES is important to researchers who wish to sedate fish efficiently and effectively. By refining methods of electrosedation with the PES, this study provides important practical information needed for those interested in electrosedation as an alternative to chemical sedatives.

METHODS

Experimental organism.—The study focused on Bluegill *Lepomis macrochirus*, used as a representative freshwater teleost as it is a common sunfish found throughout North America. We used 330 individuals for this study: 90 individuals (mean ± SD length, 178 ± 21 mm) in the orientation experiment, 120 individuals (177 ± 22 mm) in the electrode spacing experiment, and 120 individuals (176 ± 22 mm) in the anode proximity experiment. Individuals were captured in Lake Opinicon using fyke nets between May 1, 2014, and May 7, 2014. After capture, fish were held in 1,000-L round outdoor tanks at the Queen's University Biological Station and fed to satiation with earthworms up to 24 h prior to experimentation. A submersible pump supplied tanks with a continuous flow-through of lake water. Ambient water conductivity was approximately 650 μS/cm. Experiments were performed between May 4, 2014, and May 7, 2014, during which time water temperatures ranged from 8°C to 10°C.

Experimental protocol.—Prior to the experiments, a nonconductive frame was constructed to support a fine-mesh net to hold the fish in a fixed position (Hudson et al. 2011; Figure 1a). The frame was constructed using chlorinated PVC (CPVC) 1.3-cm piping, electrical tape, and magic wrap. A net pocket (made from plastic insect window screen) was used to restrain the fish. The netting consisted of a 27.9-cm × 27.9-cm net bag with two lengths of CPVC 1.3-cm-diameter pipe, 55.9 cm long, fitted through the top and a fiberglass rod at the bottom of the bag to keep the net structured and weighted. The fish placement section of the netted bag was small enough to prevent lateral movement of the fish during trials.

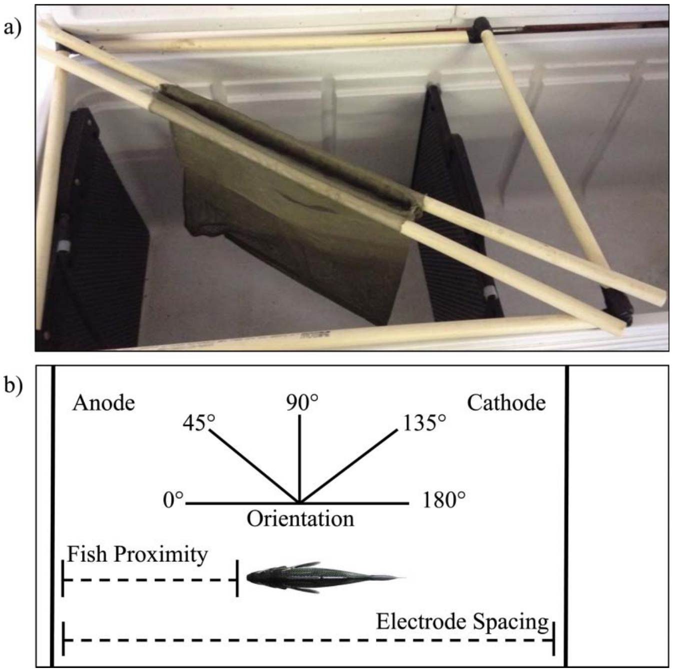

FIGURE 1. **(a)** Photograph of the netting and frame apparatus that was created to hold fish at specific orientations and distances in the Portable Electroanesthesia System (PES), and **(b)** schematic diagram of the PES describing the orientation, the electrode spacing, and the fish proximity parameters. The thick black lines represent the anode and cathode. [Figure available online in color.]

For each experiment, the PES insulated container was filled with lake water (depth = 29 cm). Individual fish were randomly netted from a holding tank (quickly captured, i.e., in < 30 s) and transferred to the PES insulated container. The fish was placed in the defined position and orientation within the frame. The control operator administered the electrosedation; dose settings were maintained constant for all experiments (pulse type = standard pulsed DC, frequency = 100 Hz, voltage = 90 V, duty cycle = 25%, and duration = 3 s). The dose used was based on extensive trials by our group on a variety of warmwater fishes (S. J. Cooke, unpublished data). After induction, fish were transferred to an individual recovery tank and monitored. The stage of sedation and time of induction were recorded. Two Reflex Action Mortality Predictor (RAMP) tests (Davis 2010), righting response and tail grab, were performed on each fish 5 min after induction to assess recovery. The RAMP is becoming increasingly common as a means of evaluating fish responses to various fisheries-induced stressors (e.g., Raby et al. 2012; Brownscombe et al. 2013) and is relevant to recovery from sedation given that reflexes require coordinated neurological and physiological function (Davis 2010). The progression of each fish through the stages of recovery was continually observed and assessed using the RAMP tests. The recovery time to each stage was recorded, as were any injuries or trauma symptoms (e.g., bruising, tail perfusion). Full recovery was defined as normal equilibrium (righting ability) and responsiveness to tactile stimuli (tail grab). Fish that did not recover within 30 min were scored a recovery time of 30 min. To reduce bias in determining the induction stage and time and the recovery time, the same observer was used for all experiments. Once recovered, all fish were measured, dorsal fin-clipped (per treatment), returned to the holding tank, and monitored for 24 h to record morbidity and mortality.

The effects of orientation, electrode spacing, and fish proximity.—First, we tested whether fish orientation affects the recovery time of fish following electrosedation. The orientation treatments were 0, 45, 90, 135, and 180° and a control treatment that was not electrosedated (Figure 1b). An orientation of 0° represents a fish that is upright and facing the anode. Angles of 0° and 180° were termed horizontal angles, angles of 45° and 135° were termed acute angles, and 90° a right angle. There were 15 fish assigned to each treatment (the first 15 fish captured from the holding tank were assigned to 0°, and so on). The electrode spacing was 55.8 cm. Fish were positioned with their nearest body part 14.8 cm from the anode for each treatment. Second, we tested whether the electrode spacing affects recovery time. The electrode spacing treatments were 29.6, 42.7, 55.8, and 68.9 cm. Fish were positioned in the approximated center of the electric field for each treatment. For each electrode spacing, 15 fish were tested in each of two orientations, 0° and 45°. Lastly, we tested whether the proximity of fish to the anode affects recovery time. The fish proximity treatments were 8.3, 14.8, 21.4, and 27.9 cm.

Proximity was measured from the anode to the snout of the fish. The electrode spacing was 68.9 cm. For each proximity treatment, 15 fish were tested in each of two orientations, 0° and 45°. Body voltage is equal to the exposed fish length (cm) divided by the voltage gradient (V/cm), where exposed fish length is dependent on the orientation of the exposed fish.

We did not euthanize fish to examine potential internal hemorrhaging or spinal compression. However, we did assess every fish for incidences of external bruising, spinal damage (inferred from permanently impaired swimming or misalignment of body), and short-term mortality for 1 h after treatment. Longer-term (24-h) mortality was assessed by holding fish in a 1,000-L circular flow-through tank.

*Statistical analysis.—*In each experiment, the amount of time spent in stage IV of sedation, as well as full recovery time (time to stage I), was recorded. For the orientation experiment, a one-way analysis of variance (ANOVA) test was used to determine the significance of orientation on both time in stage IV and time to full recovery. For the electrode spacing experiment, a two-way ANOVA test was used to determine the significance of electrode spacing and orientation, as well as the interaction of electrode spacing and orientation on both time in stage IV and time to full recovery. For the fish proximity experiment, a two-way ANOVA test was used to determine the significance of the proximity of fish to the anode and orientation, as well as the interaction of proximity and orientation on both time in stage IV and time to full recovery. In all cases, Tukey's honestly significant difference (HSD) post hoc tests were used for pairwise comparisons of means. The assumptions of normality and homoscedasticity of residuals were tested to ensure that the parametric test was appropriate and robust for this experiment. The body voltage analysis was tested using a linear regression model. All individuals that reached stage IV of sedation in treatment groups from the orientation experiment ($N = 65$) and the electrode spacing experiment ($N = 118$) were included in the regression analysis. Differences were considered at $P < 0.05$, set a priori. All analyses were performed using R version 3.0.3 (R Development Core Team 2014).

RESULTS

The orientation of the fish had a significant effect on the time to full recovery (Table 1). The mean time to recovery was longest for the horizontal angles (0° and 180°; 1,447 ± 355 s [mean ± SD] and 1,404 ± 448 s, respectively) and was significantly different than the mean time to recovery for the acute angles (45° and 135°; 945 ± 412 s and 885 ± 316 s, respectively; Figure 2a). There were no significant differences within the paired horizontal angles or the paired acute angles. Body voltage was highest for fish that were oriented at horizontal angles (Table 2). Orientation did not have a significant effect on the time in stage IV of sedation ($F_{3,\ 55} = 2.236$, $P >$ 0.05; Figure 2b). All individuals in the 0, 135, and 180° treatments and all but one individual in the 45° treatment reached stage IV of sedation. No individuals from the 90° treatment reached stage IV. Individuals that did not reach stage IV of sedation were not included in the ANOVA test. Across all treatments, six individuals (7%) were dead or moribund 24 h after the experiment.

TABLE 1. Results of ANOVA tests assessing the effects of orientation, electrode distance, and fish proximity on the full recovery time. The electrode spacing treatments were 29.6, 42.7, 55.8, and 68.9 cm. The fish proximity position treatments were 8.3, 14.8, 21.4, and 27.9 cm. For the fish proximity experiment, the electrode spacing was 68.9 cm. Values presented in bold italics are significant at $P < 0.05$.

Treatment	df	*F*	*P*-value
Orientation experiment			
Orientation	3	8.827	***<0.001***
Electrode spacing experiment			
Orientation	1	0.082	0.770
Electrode spacing	3	10.713	***<0.001***
Orientation × electrode spacing	3	4.864	***0.003***
Fish proximity experiment			
Orientation	1	0.007	0.930
Fish proximity	3	3.324	***0.020***
Orientation × fish proximity	3	5.813	***0.001***

There was a significant interaction between electrode spacing and orientation on the time to full recovery (Table 1). The effect of electrode spacing depends on the orientation of the fish in the PES unit (Figure 3a). At electrode spacing treatments of 29.6, 42.7, and 68.9 cm, fish oriented at 0° had shorter recovery times. In contrast, fish oriented at 45° had shorter recovery times when the electrode spacing was 55.8 cm. Fish oriented at 0° had higher body voltage exposures than fish oriented at 45° in each respective electrode spacing treatment (Table 2). There were no significant differences in the time spent in stage IV with electrode spacing and orientation ($F_{3,\ 108} = 1.73$, $P > 0.05$). All individuals in the 29.6-cm treatment reached stage IV of sedation. All but one individual in each of the 42.7-cm and 55.8-cm treatments, and all but two individuals in the 68.9-cm treatment, reached stage IV. Individuals that did not reach stage IV of sedation were not included in the ANOVA test. Both individuals that did not reach stage IV in the 68.9-cm treatment failed to exit stage I (i.e., no recovery time). Across all treatments, four individuals (3%) were dead or moribund 24 h after the experiment.

There was a significant interaction between fish proximity and orientation on the time to full recovery (Table 1). The effect of proximity to the anode depends on the orientation of the fish in the PES unit (Figure 3b). At fish proximity treatments of 14.8, 21.4, and 27.9 cm, fish oriented at 0° had shorter recovery times. In contrast, fish oriented at 45° had shorter recovery times when they were closest to the anode (8.3-cm treatment). Fish oriented at 0° had greater body

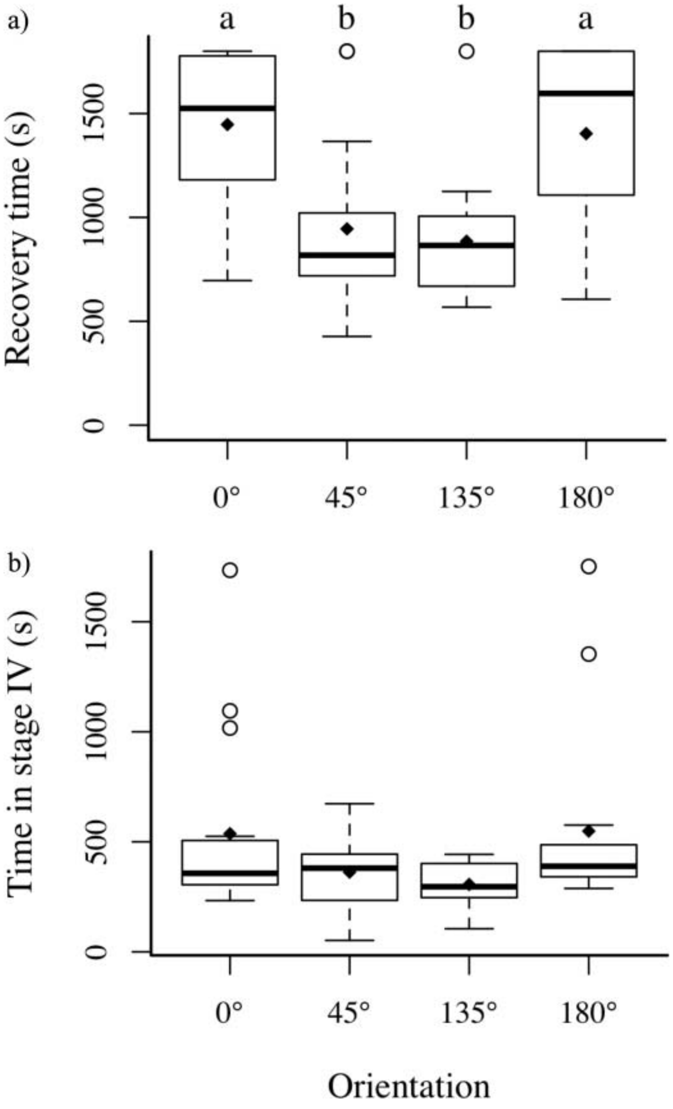

FIGURE 2. **(a)** Full recovery time and **(b)** time spent in stage IV of sedation after treatment for fish orientation within the PES. Sedation level was determined by two Reflex Action Mortality Predictor tests: righting ability and tail grab response. Full recovery was defined as normal equilibrium (righting ability) and responsiveness to tactile stimuli (tail grab). The black diamonds represent the means, the black lines represent the medians, the box dimensions represent the 1st and 3rd quartiles, the whiskers represent the upper and lower ends of the nominal data range (1.5× the interquartile range), and the open circles denote values that lie outside the nominal range. Different letters above the boxes denote significant differences between groups (considered at $P < 0.05$). Each orientation treatment had 15 replicate individuals (total $N = 60$).

voltage exposures than fish oriented at 45° in all anode proximity treatments (Table 2). There were no significant differences in the time spent in stage IV with fish proximity and orientation ($F_{3,\ 102} = 1.13$, $P > 0.05$). Four individuals in the 8.3-cm treatment, and two individuals in each of the other three treatments, failed to reach stage IV of sedation and, therefore, were not included in the ANOVA test. All of these individuals failed to exit stage I (i.e., no recovery time). Across all treatments, one individual (0.8%) was found dead or moribund 24 h after the experiment. Across all experiments, there was no evidence of bruising or impaired swimming and no fish died during the 1-h posttreatment observation period.

For body voltage up to 26 V, there was a significant effect of body voltage on the mean time to full recovery ($y = 23.92x + 482.02$, $R^2 = 0.709$, $P < 0.05$; Figure 4). Beyond 26 V, there was no relationship between body voltage and the mean time to full recovery (Figure 4).

DISCUSSION

To our knowledge this is the first study to systematically evaluate the influence of fish orientation and position relative to the efficacy of electrosedation. Most previous studies that examined the efficacy of using a portable electrosedation device have either standardized such factors, ignored them as factors, or failed to report sufficient details to know what was done with respect to fish orientation and position (Walker et al. 1994; Gaikowski et al. 2001; Hudson et al. 2011; Trushenski et al. 2012a, 2012c). Our work revealed that fish orientation played a significant role in determining the extent to which fish were immobilized (i.e., using stage of sedation criteria; Summerfelt and Smith 1990), as well as influencing the time for fish to recover from sedation. Using the two most promising orientations, (i.e., 0° and 45°) we further explored the role of fish position. The electrode spacing influenced recovery time and that effect was mediated by orientation. Finally, the proximity of the fish within the PES relative to the anode influenced recovery time, again with the effect mediated by fish orientation. Clearly fish orientation and position are important factors to consider when using the PES to immobilize fish, as well as when evaluating its effectiveness in focused PES research studies.

In order to understand why the orientation and position of fish in the PES affects its efficacy, it is critical to first understand how electricity interacts within the study organism. The state of electronarcosis, achieved through use of the PES, is a result of the inhibition of the electrical activity of the cerebellum and diencephalon (Gualtierotti et al. 1949; Martini et al. 1950). This disrupts cerebral messaging to the motor pathways causing a loss of equilibrium and muscle movement in fish (Hudson et al. 2011). According to the present study, there is a significant relationship between fish orientation within the PES and the length of time it takes for the fish to recover. This is contrary to the findings of Lines and Kestin (2004), who investigated stunning for humane slaughter, which may be due to species-specific differences in that they studied salmonids. Furthermore, in their study a change in orientation (i.e., 90°) represented a fish rotated onto its side but still parallel to the anode, but in our study orientation represents a change in the angle between the fish and the anode. Lamarque (1967) acknowledged the significance of orientation with respect to the amount of electric current required to sedate the fish,

TABLE 2. Voltage gradient, exposed fish length, and body voltage for each experiment. Body voltage is equal to the exposed fish length (cm) divided by the voltage gradient (V/cm), where exposed fish length is dependent on the orientation of the exposed fish. Voltage was 90 V for all treatments. The mean ± SD fish length was 178 ± 21, 177 ± 22, and 176 ± 22 mm for the orientation, electrode spacing, and fish proximity experiments, respectively. Recovery time is given as mean ± SD.

Fish orientation	Electrode spacing (cm)	Fish proximity (cm)	Number of fish	Voltage gradient (V/cm in water)	Mean exposed fish length (mm)	Body voltage (V)	Recovery time (s)
Orientation experiment							
0°	55.8	14.8	15	1.6	178	28.7	1,447 ± 355
45°	55.8	14.8	15	1.6	126	20.3	945 ± 412
90°	55.8	14.8	5	1.6	3[a]	4.8	543 ± 710
135°	55.8	14.8	15	1.6	126	20.3	885 ± 316
180°	55.8	14.8	15	1.6	178	28.7	1,404 ± 448
Electrode spacing experiment							
0°	29.6	6.3	15	3.0	177	53.8	1,496 ± 443
0°	42.7	12.9	15	2.1	177	37.3	1,349 ± 407
0°	55.8	14.8	15	1.6	177	28.5	1,447 ± 355
0°	68.9	14.8	14	1.3	177	23.1	1,014 ± 451
45°	29.6	6.3	15	3.0	125	38.1	1,705 ± 253
45°	42.7	12.9	15	2.1	125	26.4	1,501 ± 487
45°	55.8	14.8	15	1.6	125	20.2	945 ± 411
45°	68.9	14.8	14	1.3	125	16.4	1,074 ± 399
Fish proximity experiment							
0°	68.9	8.3	15	2.1	176	23.0	1,219 ± 345
0°	68.9	14.8	14	2.1	176	23.0	1,014 ± 451
0°	68.9	21.4	15	2.1	176	23.0	733 ± 238
0°	68.9	27.9	15	2.1	176	23.0	730 ± 187
45°	68.9	8.3	11	2.1	125	16.3	716 ± 200
45°	68.9	14.8	14	2.1	125	16.3	1,074 ± 399
45°	68.9	21.4	13	2.1	125	16.3	899 ± 484
45°	68.9	27.9	13	2.1	125	16.3	935 ± 352

[a] Estimated body width.

concluding that more current is required to sedate fish when the angle between the neuron and the flow of current is increased. This supports why Bluegills that were placed at 0° and 180° with respect to the anode experienced the longest recovery periods (i.e., were most effectively sedated). At these orientations, the current and the neurons of the fish are directly aligned, resulting in the greatest sedative effect of all treatments. Furthermore, as the fish were oriented away from the current, the recovery time of the fish decreased. Fish oriented 90° to the anode experienced the shortest recovery times and were often not fully sedated. The 90° treatment represents the greatest angle between neurons and current; though 135° and 180° treatments are at a greater angle with respect to the anode, they are at the same angle to the current as 45° and 0° treatments. Therefore, we can conclude that orientation does affect efficacy of electrosedation and as such should be considered in field settings.

The electrode spacing influenced recovery time and that effect was mediated by orientation. The electric field intensity can be described by the voltage gradient, a quantity that can be measured directly or estimated based on the applied voltage and the distance between the electrodes (Snyder 2003). Given that voltage is positively correlated with current, increasing the current within the PES will increase the voltage to which fish are subjected:

$$E = I \cdot R,$$

where E is the voltage, I is the current, and R is the resistance. The efficacy of electrosedation is voltage dependent; therefore, fish that are subjected to higher voltages will reach deeper stages of sedation and, as a result, experience longer recovery periods (e.g., Trushenski et al. 2012a, 2012b, 2012c). Both fish orientation and electrode spacing influence the body voltage exposure on fish. The significant positive relationship between body voltage and recovery time from electrosedation observed in our experiment has also been observed for electronarcosis duration and applied voltage in Northern Pike *Esox lucius* (Walker et al. 1994) and Lake Trout *Salvelinus namaycush* (Gaikowski et al. 2001).

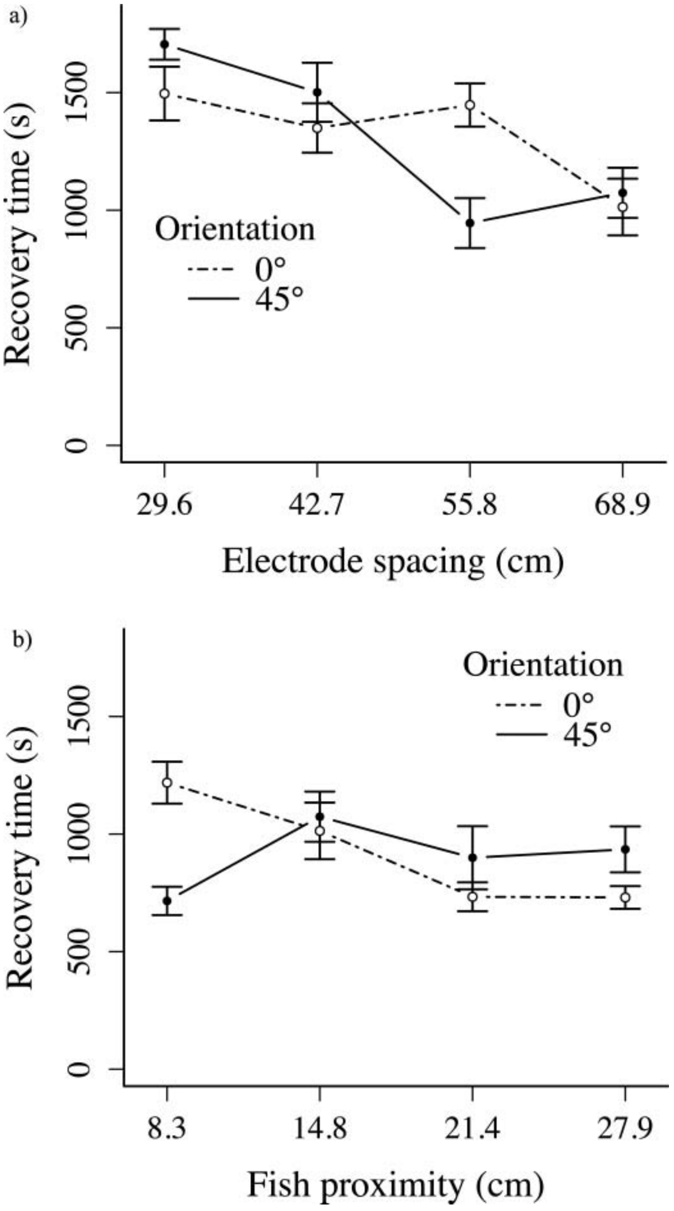

FIGURE 3. The interaction of **(a)** electrode spacing and orientation within the PES and **(b)** fish proximity and orientation within the PES on the full recovery time of fish. Full recovery was defined as normal equilibrium (righting ability) and responsiveness to tactile stimuli (tail grab). The electrode spacing treatments were 29.6, 42.7, 55.8, and 68.9 cm. The fish proximity position treatments were 8.3, 14.8, 21.4, and 27.9 cm. For the fish proximity experiment, the electrode spacing was 68.9 cm. The dots represent means, and the error bars represent ± 1 SE (total $N = 116$ individuals for the electrode spacing experiment, and total $N = 110$ individuals for the fish proximity experiment).

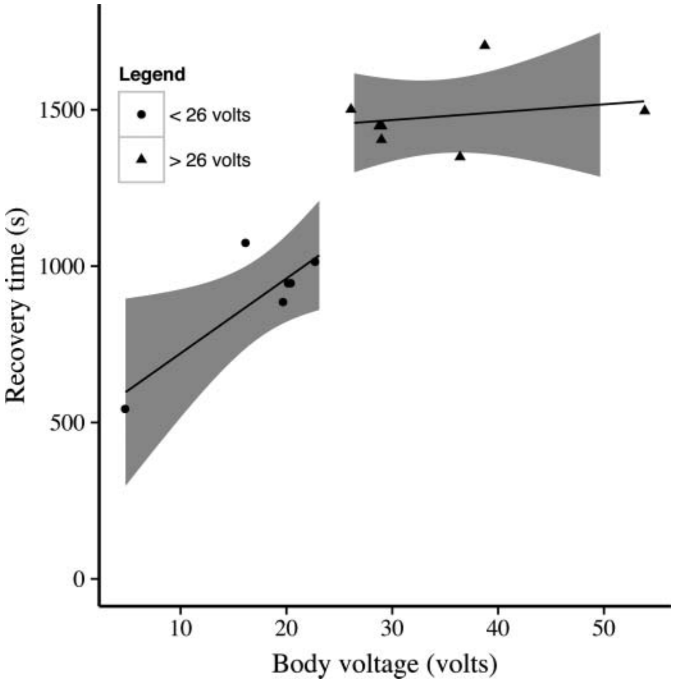

FIGURE 4. Plot of the effect of body voltage on the mean full recovery time of fish. All treatment groups from the orientation experiment and the electrode spacing experiment were included in the analysis. Body voltage is equal to the exposed fish length (cm) divided by the voltage gradient (V/cm), where exposed fish length is dependent on the orientation of the exposed fish. Full recovery was defined as normal equilibrium (righting ability) and responsiveness to tactile stimuli (tail grab). The shaded area represents the 95% confidence region. Each body voltage treatment had 15 replicate individuals (total $N = 183$ individuals).

The proximity of the fish within the PES relative to the anode influenced recovery time, again with the effect mediated by fish orientation. In theory, electric fields generated from parallel plates (like those generated by the anode and cathode paddles of the PES) extend in straight lines, perpendicular to the plates, creating a uniform electric field. In practice, however, field lines originating from the center of the paddles remain straight, while the surrounding field lines can curve slightly outwards, resulting in a nonuniform electric field (Snyder 2003). In a uniform electric field, the voltage gradient would be constant, regardless of the location between the electrodes. Based on our experimental findings that full recovery time (and the extent to which fish were sedated) varied depending on the fish proximity relative to the anode, we must conclude that the electric field within the PES unit was nonuniform. Because the neural systems of fish in the 0° and 45° orientations are oriented differently within the field, they are likely affected differently by the electric field. Given our findings on the importance of the orientation of fish with respect to the electric field, we conclude that fish may then be sedated to varying degrees due to the way the field lines are oriented with respect to their neural systems at each position (Lamarque 1967). With the development of the PES and the presumed interest in this tool for fish sedation, additional research on the neurophysiological aspects of the electrosedation seems warranted to improve our understanding of the mechanisms of action and recovery processes.

Depending on the reason for sedating fish, it may be possible to exploit this knowledge to achieve a desired level of sedation and duration of sedation (i.e., recovery period). Our work also emphasizes the importance of standardizing orientation and position within a study to reduce variation in PES performance that would be driven by those factors. The use of netting material to hold fish in position prior to shocking, such as what we used here, seems prudent but must use materials that minimize the alteration of the electric field. We also encourage researchers that use portable electroanesthesia systems or those that explicitly study their performance to report the orientation and position of the fish within the system, given the role of orientation and position in fish recovery times.

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