



NOTE

Comparing Immobilization, Recovery, and Stress Indicators Associated with Electric Fish Handling Gloves and a Portable Electro sedation System

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Abstract

Fish sedation facilitates safer handling of fish during scientific research or fisheries assessment practices, thus limiting risk of injury to fish and reducing stress responses. In recent years, there has been growing interest in using electricity to sedate fish; two methods include (1) lower-voltage, non-pulsed-DC fish handling gloves (FHGs) that tend to only sedate fish while the gloves are touching the animal; and (2) a comparatively high-voltage, pulsed-DC Portable Electro sedation System (PES) that leads to galvanonarcosis. This study compared the physiological consequences of exposure to FHGs and PES in teleost fish. Bluegills *Lepomis macrochirus* and Largemouth Bass *Micropterus salmoides* were exposed to FHGs, PES, or a handling control for a 3-min simulated surgery. Blood was then sampled at 0.5 and 4.5 h postexposure and was analyzed for blood glucose, blood lactate, and plasma cortisol concentrations. Opercular rates were monitored during surgery, at 2 min postsurgery, and 0.5 h postsurgery. At 24 h postsurgery, time to exhaustion (via a standardized swimming chase protocol) was assessed. Fish exposed to FHGs tended to exhibit lower opercular rates than fish that were sedated with the PES during simulated surgery. Cortisol levels of Largemouth Bass treated with FHGs were higher than those of fish sedated with the PES. Glucose levels recorded for Bluegills at 4.5 h postsurgery were higher with FHGs than with the PES. In both species, lactate was lower for fish treated with FHGs than for those treated with the PES. At 24 h posttreatment, Bluegills sedated with FHGs exhibited a longer time to exhaustion than those subjected to the PES, whereas Largemouth Bass sedated with the PES exhibited a

longer time to exhaustion than those sedated with FHGs. Physiological responses to treatments were inconsistent between species. Further investigation to determine the optimal electro sedation method is required.

In recent decades, anesthesia has become an increasingly important tool in contemporary fisheries science. Anesthesia includes a variety of chemical (e.g., clove oil and tricaine methanesulfonate [MS-222]) and physical agents (e.g., electricity) and serves two purposes: (1) immobilization of the animal for complex surgical manipulations (Ross and Ross 2008) and (2) maintaining animal welfare through reducing the degree of stress the animal experiences (Trushenski et al. 2013). However, limitations do arise when using chemicals for fish sedation. For example, the metabolic clearance of MS-222 is often slow, resulting in an extended duration of impairment (Pirhonen and Schreck 2003; reviewed by Popovic et al. 2012). Furthermore, high MS-222 retention in sport fish released back into the wild can result in animals that are not suitable for consumption by the general public, especially when metabolic turnover rates are relatively low (Marking

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Received July 4, 2017; accepted January 10, 2018

and Meyer 1985; Trushenski et al. 2013). In Canada, restrictions associated with chemical anesthetics such as MS-222 require a holding period of 5 d after exposure, with water temperatures of at least 10°C to allow for anesthetic to be metabolized prior to release (Health Canada 2010); in the USA, a holding period of 21 d prior to release is required (Carter et al. 2011). These holding periods are often logistically challenging and can conflict directly with the objectives of certain studies (e.g., telemetry tagging) in which fish must be immediately released after surgery. Limitations accompanying chemical anesthetics reveal the need for alternative methods of sedation that alleviate requirements for metabolic clearance and allow for release immediately after sedation.

As an alternative to chemical anesthetics, electrosedation presents benefits in field settings where low contamination, quick recovery times, and immediate release options are advantageous. In contrast to chemical sedatives (e.g., MS-222), electrosedation has been found to produce the quickest induction times and recovery times (Keep et al. 2015; Johnson et al. 2016) and to reduce handling stress in comparison with chemical sedatives, and the fish may be released immediately posttreatment (Bowzer et al. 2012; Trushenski et al. 2012b). Advancements in electrosedation technology have provided fisheries scientists with various commercially available systems. Electricity may be used to sedate fish in open-water applications by means of backpack electrofishing and boat electrofishing, where the operator is isolated from the electrical field. This form of electrosedation is beneficial for fish capture; however, alternative methods of electrosedation intended for fish surgeries are required. Smith-Root has developed electric fish handling gloves (FHGs; patent pending) and the Portable Electrodesation System (PES), offering easily portable electrosedation options to fisheries scientists (Smith-Root 2015, 2017). The PES has been commercially available since 2009 and is a self-contained, portable device that quickly renders individual fish or batches of fish unconscious (Trushenski et al. 2012b) through exposure to DC or pulsed DC (PDC); application of the PES involves no withdrawal period and permits immediate release after treatment (Smith-Root 2017). The FHGs are a more recent addition to commercially available methods of electrosedation for use by fisheries scientists. The FHGs offer the ability to immobilize an individual fish in the hands of the fisheries scientist by using comparatively lower-voltage DC, with the ability to wear the portable control box on-person (Smith-Root 2017). The FHGs immobilize fish upon contact with both of the insulated conductive gloves; upon removal of one glove from the fish, the current is broken and immediate recovery occurs (Smith-Root 2017).

Commercial availability of the PES has allowed researchers to study the physiological and behavioral

consequences associated with this electrosedation device. Recent availability of the FHGs, however, reveals little knowledge as to how exposure to this method of electrose- dation affects the fish physiologically. There is a require- ment for comparative studies to assist fisheries researchers in making better-informed decisions for selecting a fish sedation method that does not negatively affect the wel- fare of the animal. Therefore, in this study, we evaluate the effect of exposure to FHGs and the PES through the analysis of immobilization, recovery, and physiological stress indicators displayed by Bluegills *Lepomis macro- chirus* and Largemouth Bass *Micropterus salmoides*. Our objective was to determine whether physiological stress indicators differed for Bluegills and Largemouth Bass that were exposed to two different electrosedation methods. To examine this, we quantified blood physiological paramet- ers, opercular rates, and 24-h recovery (exhaustive chase protocol). Further understanding of the effects of exposure to FHGs in comparison to the PES may assist best prac- tice protocols for sedation methods used by fisheries prac- titioners, thus limiting the risk of injury to fish and reducing the degree of stress associated with sedation.

METHODS

Study area and species.—All fish were collected from shallow, vegetated bays of Lake Opinicon (Chaffey's Lock, Ontario, Canada; 44°33'32.3994"N, 76°19'40.8"W) between June and July 2016. Bluegills (mean \pm SD = 159.9 \pm 12.7 mm TL) and Largemouth Bass (306.6 \pm 34.1 mm TL) were captured using rod and reel with a variety of plastic and live baits. These species were selected for the present study due to an abundance of litera- ture that concentrates on centrarchids' physiological response to stress (Mommensen et al. 1999; Trushenski et al. 2012b; Lawrence et al., in press). All experimental prac- tices were approved by the Carleton University Animal Care Committee under guidance from the Canadian Council on Animal Care (Number 1082340).

Experimental protocol.—Individual fish were placed into blacked-out holding cells (Bluegills: 4.2 L; Large- mouth Bass: 18.3 L) that were maintained on a flow- through of natural lake water and independent aeration (McConnachie et al. 2012). Animals were allowed to accli- mate for 24 h prior to any experimental proceedings. Treatment groups were randomly assigned to each fish and included either a bare-hands control (i.e., latex gloves; Bluegills: $n = 12$; Largemouth Bass: $n = 12$), anesthesia by way of FHGs (Bluegills: $n = 13$; Largemouth Bass: $n = 12$), or anesthesia with the PES (Bluegills: $n = 11$; Largemouth Bass: $n = 13$). All fish treated with the bare- hands control and the PES unit were handled with latex gloves during simulated surgery. Control fish received no anesthesia and were immediately placed dorsal-side down

in a surgery trough, with the gills submerged under continuously flowing water, to begin the trial. Fish exposed to FHGs were placed dorsal-side down in a surgery trough, with the gills submerged underwater (continuous flow). The FHGs were positioned on the fish's head and caudal peduncle (suggested glove position; Smith-Root 2016) and were turned on at the lowest current setting (4 mA). The current setting was increased (6.3, 10, 16, and 25 mA) until full-body flinches stopped (as per instructions; Smith-Root 2016), eliciting stage IV sedation with complete immobilization and continuous opercular respiration (Summerfelt and Smith 1990). Fish that were exposed to the PES treatment were placed in the exposure tank that was filled with lake water. The fish was positioned perpendicularly to the unit's electrodes (Rous et al. 2015) before administering treatment. At this time, the PES unit was activated, and the fish was exposed to an electrical current (pulse type = standard PDC; frequency = 40 Hz; voltage = 200 V; duty cycle = 25%; duration = 3 s). After sedation with the PES, the fish was transferred to a surgery trough, with the gills submerged underwater (continuous flow).

Simulation of surgery was administered anterior to the pelvic girdle by using stroking motions with the blunt end of a scalpel handle. Strokes were administered every 15 s for 3 min (Kim et al. 2017), during which time opercular beats were quantified. Fish were then transferred to a cooler filled with lake water and independent aeration. After 1 min in the cooler, the lid was removed and the number of opercular beats displayed in 1 min was counted before the lid was replaced. At 29 min posttreatment, the cooler lid was again removed, and opercular beats were quantified for 1 min. The fish were then transferred to the surgery trough and placed dorsal-side down, submerged in lake water with a continuous flow. Prior to use, syringes (1 mL) and needles (Bluegills: 23 gauge; Largemouth Bass: 21 gauge) were rinsed with heparin and placed on ice. The first blood sample was drawn (0.5 h posttreatment) from the caudal vein, with the needle ventrally entering the midline posterior to the anal fin. This first blood sample was extracted closer to the caudal fin to allow room for a second blood sample to be extracted closer to the anal fin. A 0.3-mL blood sample was drawn from Bluegills, and a 0.5-mL sample was drawn from Largemouth Bass; each sample was obtained in less than 3 min to avoid the presentation of handling stress in blood parameters (Lawrence et al., in press). The TL of the fish was recorded, and then the fish was returned to its original, uniquely labeled blacked-out holding cell. Blood was immediately measured for glucose and lactate concentrations (discussed below). The remainder was placed on ice for later isolation of the plasma. Consistent with the protocol above, the second blood sample was drawn at 4.5 h posttreatment (Wood 1991), and each fish was once again

returned to its labeled holding cell for 24 h. Whole blood was kept in ice (<1 h) before centrifugation (3 min at 6,000 rotations/min); subsequently, the plasma was decanted for flash-freezing and storage at -80°C .

An exhaustive chase protocol was conducted at 24 h posttreatment. An individual fish was placed in a circular tub (50-cm interior diameter) that had been partitioned into eight pie-shaped sections using contrasting colored tape. The tub was filled with lake water, and a round container was secured in the center to hinder fish from changing directions. A GoPro HERO4 was used to document each test, and a timer was used for quantifying an individual's time to exhaustion. Fish were manually chased with a pole until exhaustion was observed (three tail-grabs with no presence of burst swimming). The tail-grab assessment is a reflex action mortality predictor (i.e., RAMP) test and is considered a common form of evaluating exhaustion in fish (Davis 2010). Exhaustion time and the number of lines crossed were recorded; the fish was then released into Lake Opinicon. Video footage was utilized to validate the total number of lines crossed.

Blood and plasma analysis.—Blood glucose (mmol/L) and blood lactate (mmol/L) concentrations were obtained in the field using a commercially available portable glucose meter (Accu-Chek Compact Plus; Hoffman-La Roche Ltd., Mississauga, Ontario) and lactate meter (Lactate Plus; Nova Biomedical Corp., Mississauga, Ontario), which have been considered acceptable for use with teleost fishes (reviewed by Stoot et al. 2014). Cortisol was analyzed in the lab using a radioimmunoassay (RIA) kit to measure plasma cortisol concentration (ng/mL; Immuno-Chem Cortisol Coated Tube RIA Kit; MP Biomedicals, Solon, Ohio).

Statistical analyses.—All parameters for Bluegills and Largemouth Bass were statistically analyzed using IBM SPSS Statistics version 24. Blood glucose, blood lactate, plasma cortisol, and opercular beats were analyzed using a repeated-measures factorial ANOVA (generalized linear model) to determine the significance of between-subject factors (i.e., treatment: control, FHGs, or PES) as well as within-subject factors (time periods). Assumptions were tested by assessing Levene's test for equality of variances, Box's test for equality of covariance matrices, and Wilks' lambda test statistic for multivariate testing (within-subject effect). Bluegill blood lactate concentration was log transformed to normalize skewed data (4.5 h posttreatment). If Wilks' lambda displayed a statistically significant main effect, then Tukey's post hoc test was used to analyze between-group differences. A test of between-subject effects was analyzed for statistical significance, and a pairwise comparison was used to determine statistical significance across time periods. All statistical assessments were conducted at an α level of 0.05. The time-to-exhaustion metric was analyzed using a Kaplan–Meier survival curve,

and the following assumptions were met: (1) event status with two mutually exclusive and collectively exhaustive states, (2) the “survival time” was clearly defined, (3) left-censoring was avoided, (4) independence, and (5) no secular trends. The log-rank (Mantel–Cox) test was analyzed for statistical significance ($\alpha = 0.05$).

RESULTS

Plasma Cortisol

During the first blood sample (0.5 h posttreatment), the mean plasma cortisol concentration (\pm SD) for Bluegills ranged from 165.42 ± 203.12 ng/mL (PES) to 179.88 ± 114.71 ng/mL (control; Table 1; Figure 1). There was no significant main effect of time ($F = 2.34$, $P = 0.14$) and no significant difference between treatments ($F = 0.75$, $P = 0.48$) for Bluegills. Furthermore, the treatment \times time interaction was not significant ($F = 1.15$, $P = 0.33$). The mean plasma cortisol concentration (\pm SD) for Largemouth Bass sampled at 0.5 h posttreatment ranged from 51.96 ± 44.74 ng/mL (control) to 117.20 ± 77.66 ng/mL (FHGs). The control and FHG treatments displayed an increase in plasma cortisol concentration at the second blood sample (4.5 h posttreatment), while the PES treatment exhibited a decrease (Table 1). There was no significant effect of time ($F = 0.002$, $P = 0.97$), and the treatment \times time interaction effect was not significant ($F = 0.11$, $P = 0.90$). The FHGs elicited a significantly higher plasma cortisol concentration than the control ($P < 0.05$) and the PES ($P < 0.05$) for both blood samples (0.5 and 4.5 h posttreatment; Figure 1).

Blood Glucose

At 0.5 h posttreatment, the mean blood glucose concentration (\pm SD) for Bluegills ranged from 3.91 ± 0.94 mmol/L (control) to 4.93 ± 2.83 mmol/L (FHGs), and the glucose

concentration increased for all three treatments at 4.5 h posttreatment (Table 1). The main effect of time was significant ($F = 15.62$, $P < 0.05$), and there was a significant difference between the Bluegill FHG and PES treatment groups ($P = 0.05$) at 4.5 h posttreatment. The effect of the treatment \times time interaction was also significant ($F = 4.09$, $P < 0.05$). The mean blood glucose concentration (\pm SD) for Largemouth Bass sampled at 0.5 h posttreatment ranged from 6.26 ± 1.64 mmol/L (control) to 7.11 ± 1.52 mmol/L (FHGs; Table 1). The second blood sample (4.5 h posttreatment) displayed negligible differences in mean glucose concentration for Largemouth Bass (Table 1). Analysis did not reveal a significant effect of time ($F = 0.09$, $P = 0.76$) or a significant treatment \times time interaction effect ($F = 0.05$, $P = 0.95$) for Largemouth Bass.

Blood Lactate

For Bluegills, the mean blood lactate concentration (\pm SD) ranged from 1.18 ± 0.77 mmol/L (control) to 2.58 ± 2.02 mmol/L (PES) at the first blood sample (0.5 h posttreatment) and subsequently decreased for all three treatments at 4.5 h posttreatment (Table 1). The effect of the treatment \times time interaction was not significant ($F = 0.04$, $P = 0.96$); however, a significant main effect of time ($F = 11.01$, $P < 0.05$) was revealed for the three treatments, with the first blood analysis revealing a higher blood lactate concentration than the second analysis (4.5 h posttreatment; Figure 1). At 0.5 h posttreatment, the mean blood lactate concentration (\pm SD) for Largemouth Bass ranged from 3.48 ± 1.21 mmol/L (control) to 6.84 ± 0.54 mmol/L (PES; Table 1). A significant treatment \times time interaction was observed ($F = 7.66$, $P < 0.01$). The first blood sample (0.5 h posttreatment) indicated that the blood lactate concentration in Largemouth Bass was significantly higher for the FHG treatment than for the control ($P < 0.05$); likewise, the PES treatment exhibited a significantly higher lactate concentration than the

TABLE 1. Blood parameters (mean \pm SD; plasma cortisol, blood glucose, and blood lactate concentrations) sampled at two time periods (0.5 and 4.5 h posttreatment [PT]) from Bluegills and Largemouth Bass that were exposed one of three treatments (control, fish handling gloves [FHG], and Portable Electrodesation System [PES]). Sample size is shown in parentheses.

Treatment	Plasma cortisol (ng/mL)		Blood glucose (mmol/L)		Blood lactate (mmol/L)	
	0.5 h PT	4.5 h PT	0.5 h PT	4.5 h PT	0.5 h PT	4.5 h PT
Bluegill						
Control	179.88 ± 114.71 (8)	188.23 ± 236.98 (8)	3.91 ± 0.94 (12)	7.37 ± 3.26 (12)	1.18 ± 0.77 (12)	0.29 ± 0.38 (12)
FHG	174.58 ± 130.10 (11)	134.93 ± 101.64 (11)	4.93 ± 2.83 (13)	10.22 ± 7.06 (13)	1.63 ± 0.94 (13)	0.81 ± 1.46 (13)
PES	165.42 ± 203.12 (8)	52.10 ± 34.59 (8)	4.51 ± 2.51 (11)	4.61 ± 2.51 (11)	2.58 ± 2.02 (11)	1.86 ± 3.91 (11)
Largemouth Bass						
Control	51.97 ± 44.74 (10)	58.08 ± 44.57 (10)	6.26 ± 1.64 (12)	5.96 ± 1.95 (12)	3.48 ± 1.21 (12)	0.74 ± 0.42 (12)
FHG	117.20 ± 77.66 (10)	119.23 ± 78.02 (10)	7.11 ± 1.52 (12)	7.17 ± 2.79 (12)	5.47 ± 2.04 (12)	1.05 ± 0.60 (12)
PES	67.06 ± 65.63 (12)	56.92 ± 46.95 (12)	6.70 ± 2.22 (13)	6.52 ± 2.41 (13)	6.84 ± 0.54 (13)	1.47 ± 1.62 (13)

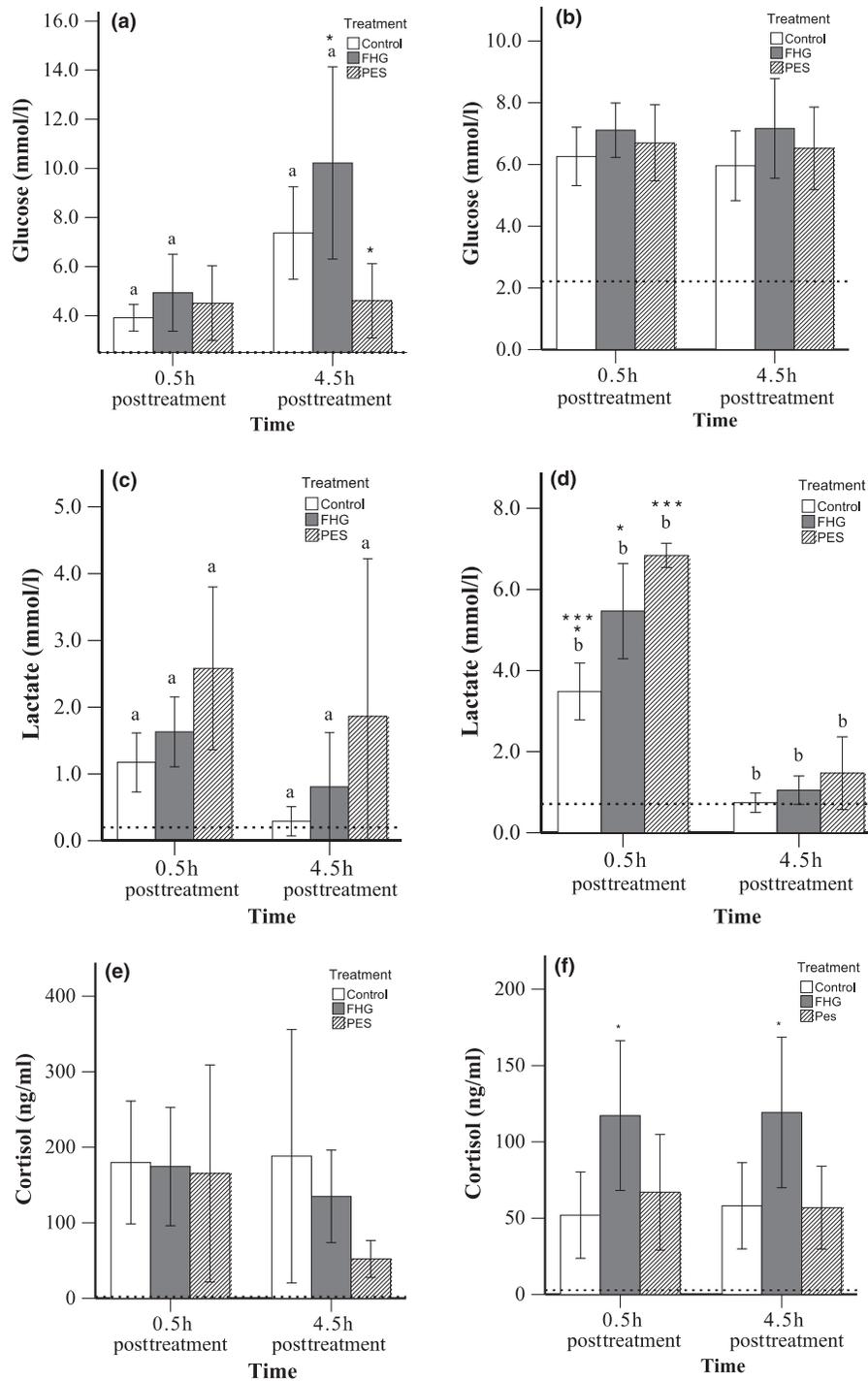


FIGURE 1. Blood parameters of Bluegills (left) and Largemouth Bass (right) subjected to one of three treatments (control, fish handling gloves [FHG], and Portable Electrosedation System [PES]) measured during two time periods (0.5 and 4.5 h posttreatment): (A), (B) mean blood glucose concentration (mmol/L); (C), (D) mean blood lactate concentration (mmol/L); and (E), (F) mean plasma cortisol concentration (ng/mL). Dotted line denotes the absolute baseline level (Lawrence et al., in press); asterisks denote significant differences between groups ($*P < 0.05$; $***P < 0.001$); and letters ($^aP < 0.05$; $^bP < 0.001$) denote significant differences within a treatment group.

control ($P < 0.001$). Blood lactate at 4.5 h posttreatment significantly decreased for all three treatments ($F = 224.96$, $P < 0.001$).

Opercular Rates

Bluegills that were exposed to FHGs exhibited the greatest range in opercular beats during minute 3 of surgery

TABLE 2. Opercular beats (mean \pm SD) of Bluegills and Largemouth Bass exposed to one of three treatments (control, fish handling gloves [FHG], and Portable Electro sedation System [PES]) and observed for 1-min time intervals over five time periods (surgery minutes 1–3; 2 min postsurgery; and 0.5 h postsurgery). Sample size is shown in parentheses.

Sampling period	Treatment	Bluegill	Largemouth Bass
Surgery minute 1	Control	79 \pm 16 (12)	67 \pm 18 (12)
	FHG	64 \pm 17 (13)	30 \pm 14 (12)
	PES	74 \pm 20 (11)	29 \pm 12 (13)
Surgery minute 2	Control	82 \pm 16 (12)	74 \pm 12 (12)
	FHG	61 \pm 16 (13)	28 \pm 14 (12)
	PES	73 \pm 26 (11)	49 \pm 10 (13)
Surgery minute 3	Control	82 \pm 13 (12)	71 \pm 10 (12)
	FHG	56 \pm 28 (13)	23 \pm 15 (12)
	PES	72 \pm 26 (11)	52 \pm 8 (13)
2 min postsurgery	Control	94 \pm 15 (12)	81 \pm 19 (12)
	FHG	80 \pm 19 (13)	76 \pm 16 (12)
	PES	81 \pm 30 (11)	57 \pm 8 (13)
0.5 h postsurgery	Control	79 \pm 16 (12)	85 \pm 11 (12)
	FHG	79 \pm 26 (13)	82 \pm 18 (12)
	PES	83 \pm 25 (11)	66 \pm 16 (13)

(mean \pm SD = 56 \pm 28) in comparison to observations made at 2 min postsurgery (80 \pm 19; Table 2; Figure 2). However, Bluegill opercular rates exhibited no significant treatment \times time interaction ($F = 0.80$, $P = 0.52$) and no significant difference among treatments ($F = 2.66$, $P = 0.09$). Largemouth Bass that were exposed to FHGs exhibited the greatest range in opercular beats between 23 \pm 15 (minute 3 during surgery) and 82 \pm 18 (at 0.5 h postsurgery; Table 2). There was a significant main effect of time ($F = 32.65$, $P < 0.001$), where the FHG and PES treatments displayed significantly lower ($P < 0.001$) opercular rates than the control during simulated surgery.

Exhaustive Chase Protocol

Bluegills exhibited no significant difference ($P = 0.86$) among the three treatments (control, FHGs, and PES) in time to exhaustion (Figure 3). Additionally, Largemouth Bass also displayed no significant difference ($P = 0.18$) in time to exhaustion for the three treatments.

DISCUSSION

Overview

To our knowledge, this is the first study to compare physiological and behavioral consequences for wild freshwater teleosts from exposure to lower voltage, non-PDC FHGs and the comparatively high-voltage PES, which uses PDC. We quantified primary and secondary

indicators of stress (plasma cortisol, blood glucose, and blood lactate concentrations), opercular rates, and chase to exhaustion to assess the potential consequences of electro sedation, the effectiveness of immobilization, and the posttreatment recovery of fish over a 24-h period. Consistent with previous findings for the PES (Trushenski et al. 2012b), our results suggest that FHGs and the PES are effective electro sedation techniques that impair reflexes immediately upon administration of appropriate current settings, as is needed to enable procedures such as implantation of electronic tags. The characteristic increase and stabilization of fish opercular rates after treatment were observed, and opercular rates at 24 h posttreatment did not significantly differ between electro sedation methods. The implications of exposure to FHGs and the PES throughout treatment and the consequences for recovery are discussed below.

Plasma Cortisol Responses

In response to a stress event, the principal corticosteroid hormone released in teleosts is cortisol, and concentrations of plasma cortisol increase considerably as a result of stress (Mømmesen et al. 1999). Cortisol levels of fish exposed to electro sedation have also been found to rapidly increase after sedation, with the maximum transient levels evident at 30 min postsedation (Trushenski and Bowker 2012). All treatments elicited an increase in circulating cortisol concentration (Figure 1E, F) above the absolute baseline cortisol level (Lawrence et al., in press); however, the Largemouth Bass that were exposed to FHGs yielded a significantly higher plasma cortisol concentration than those in the PES treatment (Figure 1F). Physiological consequences associated with exposure to electro sedation are unlikely to be the sole cause of the elevated cortisol concentration associated with FHG treatment. All fish that were exposed to FHG treatment initially received the lowest current setting (4 mA), and the magnitude of current was increased (maximum = 25 mA) at approximately 3-s intervals (which elicited full-body flinches) until the fish became unresponsive. This extended period of handling time necessary to induce complete sedation (still <30 s) with FHGs in comparison to the PES (~3 s) may have influenced the elevation of plasma cortisol concentration (Figure 1F). Teleosts subjected to handling are known to exhibit increased cortisol levels as a physiological stress response (Van Der Boon et al. 1991). Our own standardized method for FHG treatment may have negated the benefits of electro sedation and its associated reduction in handling stress (Trushenski et al. 2012a). Future investigation could focus on determining the suitable magnitude of current to initiate immediate immobilization using FHGs based on fish size, since the response time to electric current is related to the size of the fish (Snyder 2003; Siepker et al. 2010).

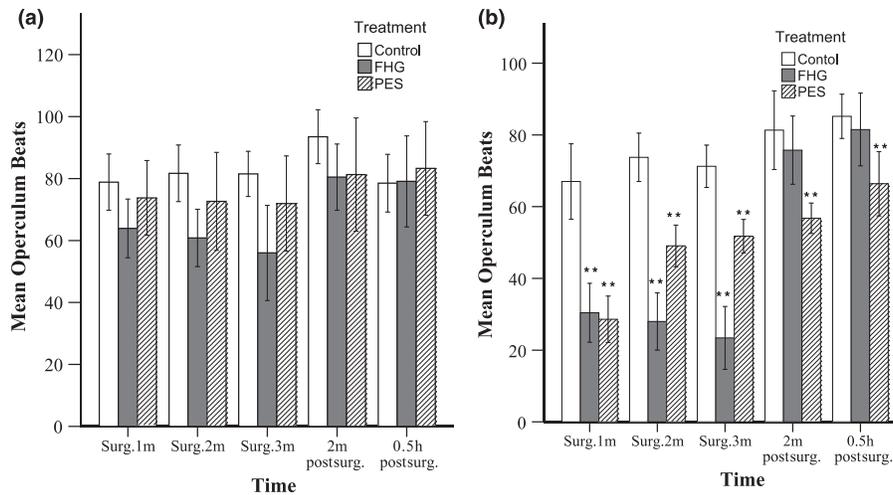


FIGURE 2. Mean number of operculum beats of (A) Bluegills and (B) Largemouth Bass subjected to one of three treatments (control, fish handling gloves [FHG], and Portable Electrosedation System [PES]). Operculum beats were counted for 1 min during five time periods (minutes 1, 2, and 3 of surgery [Surg]; 2 min postsurgery; and 0.5 h postsurgery). Asterisks denote a significant difference (** $P < 0.001$) between the treatment and the control.

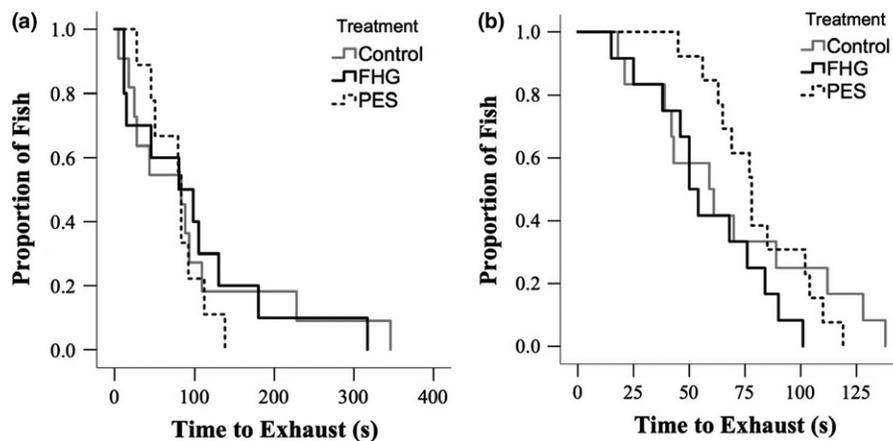


FIGURE 3. Kaplan–Meier survival curve displaying time to exhaustion (s) for (A) Bluegills and (B) Largemouth Bass exposed to one of three treatments (control, fish handling gloves [FHG], and Portable Electrosedation System [PES]).

Blood Glucose Responses

Stress in fish provokes increased requirements for energy to power cellular processes (Barton et al. 2002), and the mobilization of glucose fuels such important metabolic functioning (Mommsen et al. 1999; Barton 2002). In this study, Bluegills that were exposed to the FHG treatment exhibited significantly higher glucose levels than PES-treated fish at the 4.5-h sampling point (Figure 1A); however, all treatments did elicit elevated levels above the absolute baseline (Lawrence et al., in press) of blood glucose for Bluegills and Largemouth Bass (Figure 1A, B). In teleosts, elevated glucose concentration in circulation is often used as an indicator of the stress status of the animal (reviewed by Barton and

Iwama 1991; Barton 2002). The apparent increases in blood glucose concentration observed here across all treatments likely stem from the actions of cortisol on gluconeogenic processes. Indeed, in previous work, electrical sedation in a number of teleost species has been shown to greatly elevate blood glucose over a number of hours postexposure, with a time course comparable to that presented here (Figure 1A, B). In most instances, these changes were associated with a rise in plasma cortisol concentration, suggesting a role of the hypothalamic–pituitary–interrenal axis in regulating this response (Madden and Houston 1976; Bowzer et al. 2012; Trushenski et al. 2012a). Therefore, handling time may be the source of the observed variation in the secondary stress

response. As stated above, our standardized method for FHG treatment required longer handling times in comparison to the PES treatment. Handling of many teleosts is known to cause increased blood glucose levels, and the expression of this increase may not be apparent until 1 h after handling (Oikari and Soivio 1975). Increased blood glucose levels associated with FHGs appear to be influenced by treatment technique and not by the consequences of treatment alone. Complete sedation was achieved immediately after the PES treatment, and additional handling time was not required. The absence of PES handling stress is evident in glucose levels, as they did not significantly differ between the two sample times and were significantly lower than levels observed at 4.5 h posttreatment for FHG-treated fish (Figure 1A). This is consistent with our understanding of handling time relating to glucose concentrations (Oikari and Soivio 1975). Because handling stressors associated with these treatments were acute occurrences, the ability of the fish to recover from stressors was enabled by adaptive responses (Davis 2010).

Blood Lactate Responses

Lactate, which is associated with the secondary stress response, is produced in an effort to satisfy increased demands for energy and oxygen (Barton et al. 2002); maximum levels are detected 30 min after application of an acute stressor (Samaras et al. 2016). Stressed fish enduring extensive anaerobic metabolism accumulate lactate in their blood plasma (Raby et al. 2013) as a byproduct of producing energy in the absence of oxygen (Trushenski and Bowker 2012). At 0.5 h posttreatment, concentrations of lactate in Largemouth Bass that were exposed to the PES or FHGs were significantly higher than the lactate concentrations in the control group (Figure 1D). This is consistent with the findings of Trushenski et al. (2012b), who determined that exposure to electrosedation influences a transition to anaerobic metabolism caused by tetanic muscle contractions. Moreover, the FHG treatment may have reduced glycogen reserves and subsequently accumulated lactate due to the anaerobic glycolysis that is necessary for burst movements (Wood 1991). Burst movements were present during the FHG treatment (due to our standardized techniques), which presumably contributed to the accumulation of lactate. Increasing respiration would be necessary to correct cellular hypoxia associated with FHGs and the PES, yet the tetanic muscular contractions associated with electrosedation are known to decrease gas exchange through a reduction in ventilation (Figure 2A, B; Trushenski and Bowker 2012).

The cost of restoring homeostasis after stress-induced anaerobic exercise is termed “EPOC” (excess postexercise oxygen consumption), and the inadequacies of available oxygen require tissues to implement more anaerobic glycolysis to satisfy demands for ATP (Wood 1991; Suski

et al. 2004). Elevated lactate levels above the absolute baseline (Lawrence et al., in press) at 4.5 h posttreatment (Figure 1C, D) are likely the result of postexhaustion EPOC and may have been restored to resting levels by 6 h posttreatment, as was observed by Scarabello et al. (1992) and Trushenski et al. (2012b). Additionally, the clearance rate of lactate was slower in Bluegills than in Largemouth Bass (Figure 1C, D), which parallels previous findings (Heath and Pritchard 1962; Suski et al. 2006) and does not necessarily relate to one species being more stressed than the other.

Opercular Rates and Exhaustive Chase Responses

Opercular rates of Bluegills did not differ significantly among electrosedation treatments and the control (Figure 2A), which is consistent with the findings of Ward et al. (2017). However, Largemouth Bass that were exposed to FHGs and the PES had significantly lower opercular rates than those in the control treatment during simulated surgery (Figure 2B); after treatment, their opercular rates increased toward the rate observed in the control group. Similar to the findings of Trushenski et al. (2012b) and Prystay et al. (2017), suppressed opercular rates associated with electrosedation were relatively short-lived once the fish were removed from sedation and their nervous system was able to recover.

At 24 h posttreatment, fish were chased to exhaustion, with the endpoint clearly observed as the incapability of burst swimming (Wood 1991). This type of exercise relies on anaerobic metabolism and is sustained for only a brief amount of time (Kieffer 2000). No significant interaction between treatment and time to exhaustion was revealed (Figure 3A, B), suggesting no short-term (24-h) effect of treatment on swimming performance. This implies that both electrosedation methods have limited negative consequences for Bluegills and Largemouth Bass.

Summary and Recommendations

Our results indicate negligible differences between electrosedation methods for Bluegills (based on plasma cortisol and blood lactate concentrations) and Largemouth Bass (based on blood glucose and blood lactate concentrations). Characteristic suppression of opercular rates associated with electrosedation was detected more so in Largemouth Bass during treatment; however, after 24 h of recovery, neither species exhibited a significant difference between treatment types. Both FHGs and the PES enabled immediate immobilization upon administration of appropriate current settings, and prompt recovery was observed. Our results suggest that the use of FHGs or the PES by scientific researchers and fisheries assessment practitioners would permit safe handling, limit risk of injury to fish, and allow for quick recovery with high survival after treatment.

Inconsistencies between treatment type and associated stress responses may have been influenced by our own standardized method for FHG treatment, which involved increased handling time to achieve complete immobilization. Therefore, we suggest future research be focused on determining appropriate FHG current settings based on fish size to ensure immediate sedation. Additionally, it would be beneficial for future research of FHGs to be focused on other fishes with different-sized vertebrae (e.g., salmonids) subjected to the same study. We attempted to include a coolwater species, the Northern Pike *Esox lucius*, in the present study; however, holding facilities were unsuitable for Northern Pike, thus preventing their inclusion in the study. In general, however, both methods seem to maintain fish welfare and constitute practical additions to the fisheries science “toolbox.”

ACKNOWLEDGMENTS

We thank the Queen's University Biological Station for providing holding facilities; Aaron Zolderdo, Dana Dusevic, and Ian Jason Byerley for their assistance in fish collection; and two anonymous reviewers for constructive comments. All work complied with Protocol Number 1082340 approved by the Animal Care Committee at Carleton University. There is no conflict of interest declared in this article.

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