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MANAGEMENT BRIEF

Electric Fish Handling Gloves Provide Effective Immobilization and Do Not Impede Reflex Recovery of Adult Largemouth Bass

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Abstract
Electric fish handling gloves (FHGs) have been developed to immobilize fish during handling, with the potential benefit of reducing the time needed for sedation and recovery of fish relative to chemical anaesthetics. We examined the secondary stress responses (i.e., hematocrit, blood glucose, lactate, and pH) and reflex responses of Largemouth Bass *Micropterus salmoides* that were immobilized in water using electric FHGs for multiple durations (0, 30, and 120 s) relative to fish that were handled using only bare hands in water. We also evaluated the efficacy of the immobilization by quantifying the number of volitional movements that were observed during handling. Our findings suggested that when FHGs were used, fish tended to remain still (i.e., to show full reflex impairment) during handling

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relative to controls. Fish that were held with FHGs showed negligible reflex impairment immediately after the electricity was terminated. After a 30-min posttreatment retention period, blood chemistry and ventilation rates were similar between fish held with FHGs and those held with bare hands. This study supports the notion that electric FHGs are a safe and effective tool for practitioners who need to temporarily immobilize fish for handling, enumeration, or performing various scientific procedures.

Fish handling is a routine practice in aquaculture, fisheries assessment, and research on live fish. Practitioners often need to immobilize or restrain fish for enumeration, measurement, health assessment, tissue sampling, or tagging. Chemical agents, such as MS-222 (tricaine methanesulfonate), metomidate, benzocaine, and clove oil, are often used to anaesthetize and immobilize fish (see Iwama and Ackerman 1994; Ross and Ross 2009). However, chemical agents require time to adequately anaesthetize an individual, may degrade over time, and remain in fish tissue for an extended period, thus resulting in protracted behavioral impairment (Ross and Ross 2009). Moreover, some jurisdictions regulate or restrict the use of such chemical anaesthetics if treated fish could eventually be consumed by humans (Trushenski et al. 2013). Recently, there has been a growing interest in using electricity to sedate fish, with typical approaches employing one of two distinct methods of electrical immobilization. Pulsed DC has typically been used to induce effects ranging from electrotetany to electronarcosis and has seen application in open-water settings (i.e., electrofishing) or using some form of insulated container equipped with an anode and cathode to which pulsed DC is applied (e.g., Vandergoot et al. 2011; Trushenski and Bowker 2012; Trushenski et al. 2012). However, this approach has drawbacks, such as requiring (1) electrical isolation of the operator; (2) manipulations of voltage intensity, anode spacing, and fish orientation; and (3) a variable recovery period for anaesthetized fish (Rous et al. 2015). In contrast, non-pulsed-DC techniques immobilize fish only when the fish is in contact with the electric field, and recovery is almost instantaneous once the field is removed (e.g., Kynard and Lonsdale 1975; Gunstrom and Bethers 1985; Hudson et al. 2011; Keep et al. 2015). There are now commercially available electric fish handling gloves (FHGs) that purport to immobilize fish only when both gloves are in contact with the fish and to allow immediate volitional swimming ability of fish upon release. With the growing adoption of these gloves by fisheries professionals, there is a need to understand their physical and physiological impacts on fish and to identify factors influencing their fish immobilization efficiency.

Handling a fish can lead to physical damage if the fish thrashes, is dropped, or is held too tightly (Houston et al. 1971; Pickering et al. 1982; Trushenski et al. 2012). As such, immobilizing fish during handling can reduce the amount of time that the fish is exposed to handling (and, often, air; see Cook et al. 2015) and can thereby limit the inherent risks associated with fish handling. However, it is also conceivable that the method used to immobilize the fish causes physiological disturbance (Cooke et al. 2016). Handling without using some form of immobilization leads to elevated stress in fish, as a number of previous studies have observed alterations in physiological parameters attributed to handling (reviewed by Barton 2002). Acute stress from handling has been shown to activate the hypothalamic–pituitary–adrenal (HPA) axis, resulting in an increase of cortisol, the primary glucocorticoid stress hormone in fishes (Wendelaar Bonga 1997). Secondary stress responses, such as changes in blood glucose, lactate, pH, and hematocrit, have also been proposed as indicators for approximating the stress response of fishes (Barton et al. 2002). Acute stressors (including electrical induction; reviewed by Snyder 2003) can also influence the rate of recovery (return to physiological homeostasis), resulting in behavioral and reflex impairments. Therefore, a method of mitigating handling stress is needed in applications where fish are handled routinely or where the subsequent health and condition of live-released fish are a concern. Some of the aforementioned bioindicators can be used to determine the extent to which electric FHGs cause homeostatic disturbance.

Here, we evaluate the efficacy of electric FHGs as a means of immobilization by examining the physiological, behavioral, and reflex status of adult Largemouth Bass *Micropterus salmoides* when handled using FHGs relative to bare hands. We contrast the supposed benefits of immobilization (i.e., effectiveness as a restraint) with the potential negative effects of electronarcosis from electric FHGs (i.e., the potential to cause physiological disturbance) against the bare-handed treatment. Posttreatment recovery was also investigated by quantifying ventilation rates during a 30-min recovery period. Our objective was to quantify the effectiveness of electric FHGs for immobilization as well as the recovery of physiological, behavioral, and reflex status. By understanding the effects of electric FHGs on fish, fisheries practitioners—including researchers, assessment biologists, hatchery managers, and aquaculturists—can make informed decisions about the potential efficacy of such devices for improving fish handling procedures and promoting animal welfare.

METHODS

Study area and specimen collection.—Largemouth Bass represented a suitable study species, as they are easily captured, ubiquitous, and well-studied with regard to the physiological effects of angling (e.g., Cooke et al. 2003; White et al. 2008; Gingerich and Suski 2012; O'Connor et al. 2013). Largemouth Bass are also cultured throughout North America, where they are frequently stocked to support recreational fisheries (Morris and Clayton 2009), rendering this study valuable to aquaculturists and hatchery managers.

Our study was conducted out of the Queen's University Biological Station on Lake Opinicon (44°35′6.4278″N, −76°17′ 47.6622″W), Ontario, Canada. Fish were collected from May 1 to May 4, 2015, at water temperatures of 12–19°C (mean = 17.0 ± 2.0°C) by using medium-action fishing rods, spinning reels, and 6.8-kg-test fishing line. Terminal tackle was standardized to size-1/ 0 octopus hooks (Cooke et al. 2003), which were baited with pink, artificial soft-plastic worms rigged in the "wacky" fashion (i.e., the hook placed through the center of the plastic worm). Fight duration was limited to 30 s or less, and angling time was recorded as the time between the moment of hooking and the moment the fish was landed in a rubberized net (following Lennox et al. 2015).

*Experimental design.—*Fish were divided into five treatment groups: control ($n = 14$), handling with bare hands for 30 s ($n = 13$), handling with bare hands for 120 s ($n = 15$), handling with electric FHGs for 30 s ($n = 13$), and handling with electric FHGs for 120 s ($n = 14$). "Control" fish received no handling treatment and were immediately placed in holding tanks and monitored via the same protocol as treatment fish. Fish in both the electric FHG treatments and the bare-handed treatments were experimentally handled immediately after landing and de-hooking while in the water. Handling protocol consisted of holding the fish around the caudal peduncle and posterior to the opercular cover with wetted hands or gloves, as per recommendations of the electric FHG manufacturer (Smith-Root, Inc., Vancouver, Washington). Gloves were set to deliver a current of 4 mA, the lowest power setting (higher settings included 6.3, 10, 16, and 25 mA). Fish were held in a padded, V-shaped sampling trough (see Cooke et al. 2005) with their heads submerged in lake water to allow gill ventilation. "Escape attempts" were counted as the number of volitional movements made by the handled fish, indicating suboptimal immobilization. After the handling treatment, fish were immediately transferred to 60-L, onboard holding tanks filled with lake water and were monitored for 30 min. Lake water was transferred to the holding container immediately before holding, and the temperature was monitored during the holding period. Over the recovery period, ventilation rates for all treatment groups were measured by observing opercular movements at seven time intervals: 0, 2, 5, 10, 15, 20, and 30 min (following White et al. 2008). Ventilation rates were monitored for 30 s at each time point and were doubled to calculate the number of ventilations per minute (White et al. 2008).

All fish were sampled for blood by caudal puncture; a blood sample of 1–2 mL was taken using a 3-mL, lithium-heparinized Vacutainer (Becton, Dickinson, and Company, Franklin Lakes, New Jersey) and an 18-gauge needle. Blood samples were held on ice and returned to the laboratory for later analysis. Blood samples from control and treatment fish were collected after the 30-min recovery period. This timing for measurement of secondary stress indicators (i.e., 30 min after the stressor) coincides with the peak of the standard response curve of circulating cortisol in Largemouth Bass (Cook et al. 2011). A further group of "baseline" fish was angled, and their blood was sampled within 3 min of hooking to discern levels of secondary stress metrics prior to the angling-induced HPA axis cascade (Wendelaar Bonga 1997). Fish were measured for TL and girth (mm) at the broadest point of the body, were marked by a caudal fin clip to avoid using recaptured fish in the study, and were released.

Blood physiology metrics were measured using the following previously validated (reviewed by Stoot et al. 2014) point-of-care devices: blood plasma lactate (mmol/L) was measured using a Lactate Pro LT-1710 (ARKRAY, Inc., Kyoto, Japan), blood glucose (mmol/L) was measured with an Accu-Chek Compact Plus (F. Hoffmann-La Roche AG, Basel, Switzerland), hematocrit was measured using a CritSpin microhematocrit centrifuge (Beckman Coulter, Inc., Brea, California), and pH was measured with an HI99161 pH meter (Hanna Instruments, Woonsocket, Rhode Island). Blood glucose levels can serve as a secondary indicator of stress, as blood glucose levels may rise in concert with circulating stress hormone levels (Wendelaar Bonga 1997). During acute stress and exhaustive behavior (e.g., burst swimming or exhaustive exercise), there may be a buildup of anaerobic metabolites (e.g., lactate) in fishes, and consequently blood pH levels may decrease, indicating disturbance of homeostasis (Wood 1991). Hematocrit (or packed cell volume) is the ratio of red blood cells to all other blood constituents and is implicated in increasing the oxygen carrying capacity of red blood cells in centrarchids and thus may also be elevated in chronically or acutely stressed fish.

*Statistical analysis.—*Largemouth Bass physiological metrics and escape attempts were statistically analyzed using JMP version 9.0.1 (SAS Institute, Cary, North Carolina) and R version 3.2.4 (R Core Team 2016). Response variables (blood glucose, lactate, pH, and hematocrit) were tested for normality, homoscedasticity, and independence using Levene's test, the unequal-variances test, and tests for collinearity among predictor variables, respectively. Metrics that were found to meet these assumptions (blood pH and hematocrit) were compared between treatments using one-way ANOVA models. Blood glucose and lactate were analyzed using standard least-squares linear regression models with treatment and body size as predictors. Number of escape attempts was evaluated using a generalized linear model with a Poisson distribution and a log-link function, wherein treatment type (factor: bare hands or electric FHGs) and duration (factor: 30 or 120 s) were used as fixed effects.

Largemouth Bass ventilation rates were log-transformed and fitted with a linear mixed-effects model using the "nlme" package (Pinheiro et al. 2016) in R, with treatment, time period, and the treatment × time period interaction as predictors. Backward stepwise model selection using log-ratio tests was conducted to determine the final model. Pairwise differences were assessed using a post hoc Tukey's honestly significant difference (HSD) test with the "multcomp" package (Hothorn et al. 2008). The model was validated following the procedure outlined by Zuur et al. (2009).

RESULTS

Physiological Stress Indicators

Fish TL was normally distributed within each treatment group and was weakly correlated with blood glucose ($r^2 = 0.07$, $P = 0.03$) and lactate ($r^2 = 0.12$, $P > 0.01$; Figure 1). Multiple regression resulted in a relationship between blood lactate and TL ($F_{1,\ 1}$ = 6.10, $P = 0.02$), with no effect of treatment ($F_{4,\ 4} = 1.03$, $P = 0.40$). Multiple regression analysis of blood glucose showed that neither treatment ($F_{1,\ 1} = 4.29$, $P = 0.04$) nor TL ($F_{4,\ 4} = 0.81$, $P = 0.52$) was a significant predictor. Similarly, blood pH and hematocrit did not significantly differ among treatment groups ($F_{4,\ 60} = 0.74$, $P = 0.57$; and $F_{4,\ 59} = 0.74$, $P = 0.57$, respectively; Figure 2).

Behavioral Responses to Handling

Most Largemouth Bass (81%) did not attempt escape during handling, and the handling duration did not differ between treatments ($Z = 1.66$, $P = 0.10$). However, handling Largemouth Bass with electric FHGs resulted in statistically fewer escape attempts ($Z = -2.20$, $P = 0.03$) than handling the fish with bare hands alone (Figure 3).

Largemouth Bass ventilation rates were not significantly affected by treatment ($\chi^2 = 2.5$, $P = 0.64$) or the treatment × time period interaction ($\chi^2 = 29.1$, $P = 0.21$). However, time period did influence ventilation rates ($\chi^2 = 227.5$, $P < 0.001$). Ventilation rates were significantly lower 60 and 120 s after the treatment period than at all other time periods; ventilation rates were also lower at 60 s than at 120 s (Tukey's HSD test: $z > 3.4$, $P < 0.01$). Ventilation rates increased with time for the first 300 s and did not vary significantly among treatment groups or among fish sizes at any time period observed.

DISCUSSION

To our knowledge, this study is the first to evaluate the physiological and behavioral consequences of handling fish with commercially available, purpose-built electric FHGs. Blood glucose, lactate, pH, and hematocrit, which serve as secondary indicators of the stress response in Largemouth Bass, did not significantly vary as a function of treatment type or handling duration (Figure 2), and we did not detect an effect on posthandling ventilation rate across treatment groups. Our results indicate that little or no physiological or behavioral impairment occurred in Largemouth Bass after they were handled with electric FHGs. In addition, the electric FHGs effectively restrained fish during handling, reducing the number of escape attempts relative to bare-handed treatments (Figure 3).

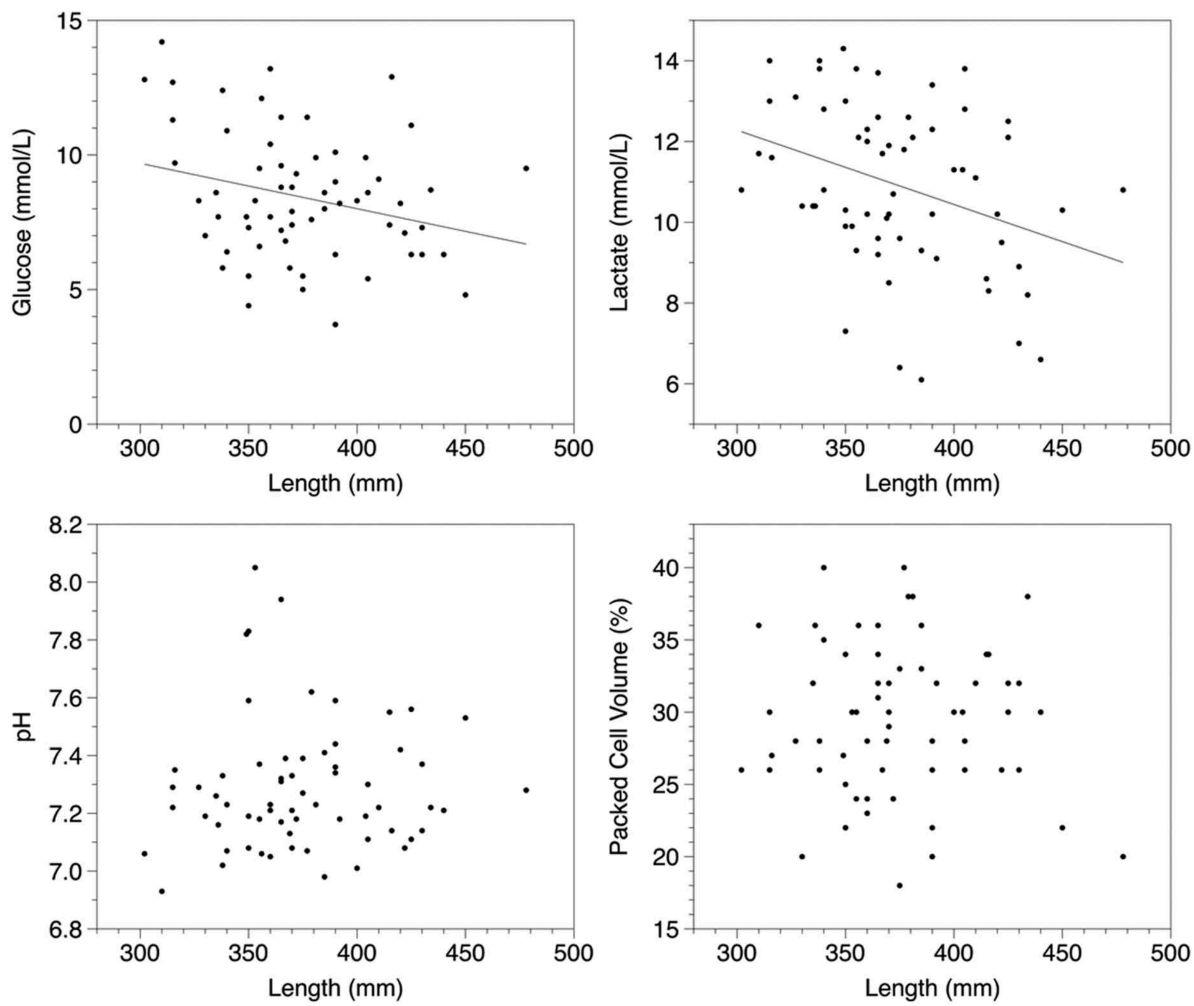

FIGURE 1. Blood physiology metrics of Largemouth Bass in relation to fish total length for all treatments pooled: glucose ($R^2 = 0.07$, $P = 0.03$), lactate ($R^2 = 0.11$, $P > 0.01$), pH ($R^2 > 0.01$, $P = 0.67$), and packed cell volume (i.e., hematocrit; $R^2 > 0.01$, $P = 0.79$).

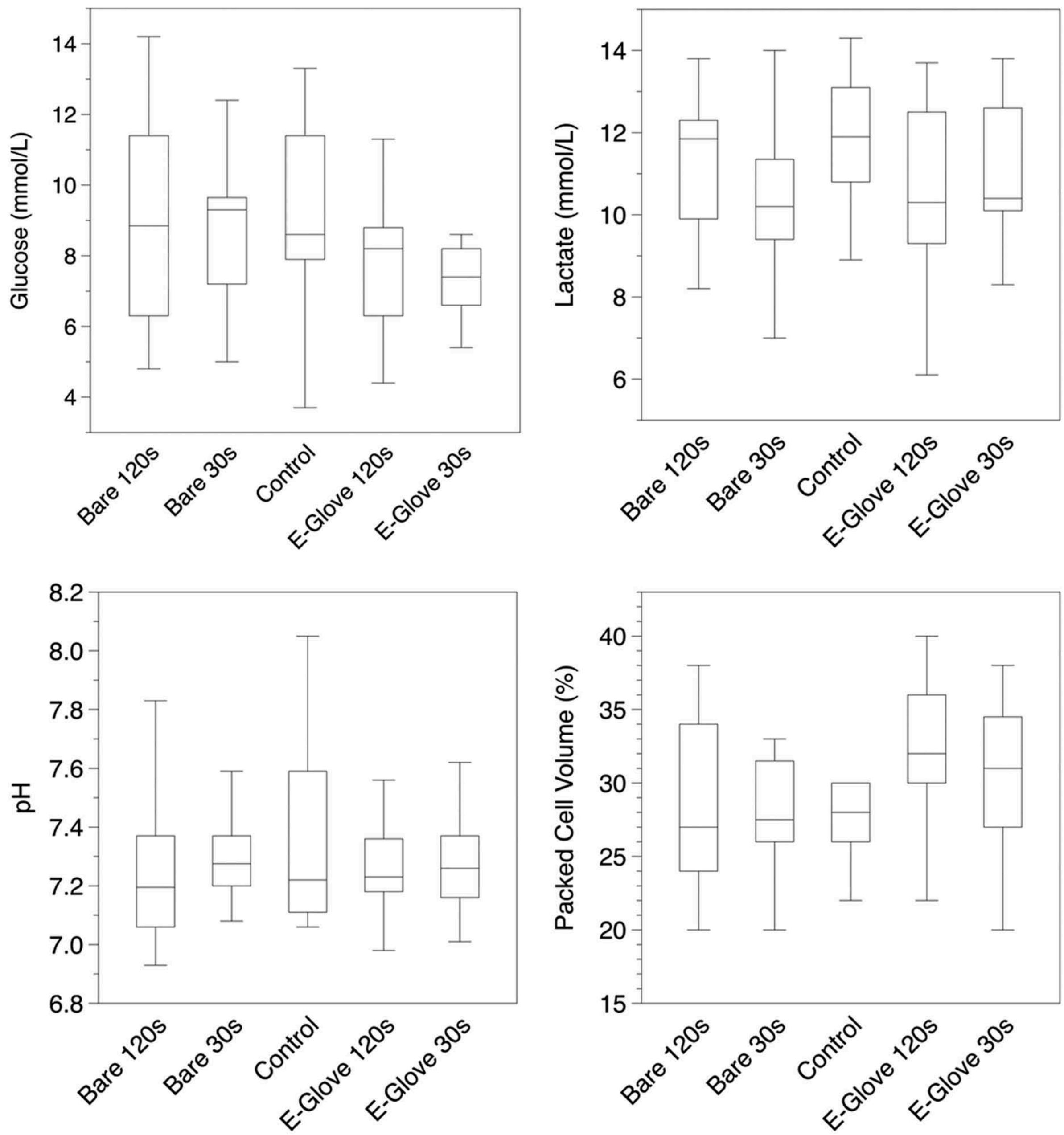

FIGURE 2. Blood physiology metrics of Largemouth Bass after being handled with electric fish handling gloves (E-gloves) or with bare hands for 30 s or 120 s (packed cell volume = hematocrit). Box-and-whisker plots denote interquartile ranges. There was no effect of treatment or time period on any of these metrics (see Results for a statistical summary).

Exposing fish to an electrical current results in a suite of physiological responses that can range from benign to fatal depending on the method and intensity of application (Vibert 1967; Snyder 2004). Improper electrofishing practices (using pulsed DC) have been found to cause hemorrhages, spinal injury, and death in a variety of fish species (Dolan and Miranda 2004). However, when properly employed, both pulsed DC and nonpulsed DC represent safe and highly effective methods of fish capture, sedation, and immobilization (Snyder 2003). In this study, low-power nonpulsed DC was used to induce immobilization. Although the lowest available current setting (4 mA) was used, some fish were still able to make occasional volitional movements at this amperage. Higher current settings may produce more reliable immobilization, as per the manufacturer's recommendations; however, this might also induce a greater stress response (Snyder 2004). Examining the relative effects of electro-immobilization using varied current settings across a variety of fish species is a logical next step in the evaluation of this technique.

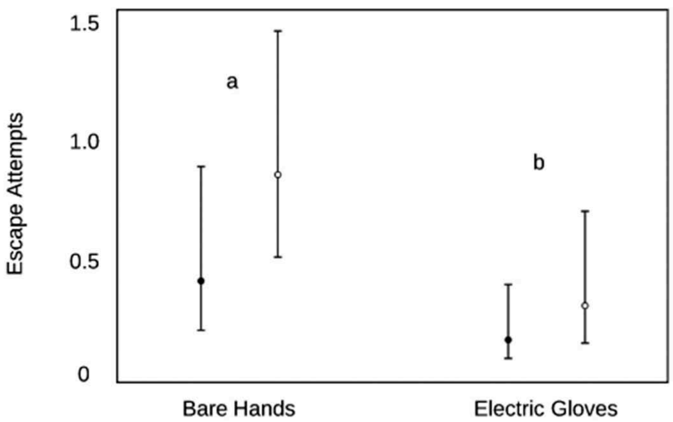

FIGURE 3. Estimated number of escape attempts (±95% confidence interval [CI]) for Largemouth Bass that were handled for 30 s (black circles) or 120 s (open circles) by using bare hands or electric fish handling gloves. Letters denote significant differences between handling treatments.

Nevertheless, at the low amperage used in this study, electric FHG treatments yielded fewer escape attempts than the bare-handed trials, indicating effective immobilization even at low amperage.

The response time of Largemouth Bass to electric currents has previously been shown to be dependent on the size of the fish (Siepker et al. 2010) and has also been noted in relation to electric FHGs in a recent technical report (K. W. Johnson, personal communication). Thus, fish size may be expected to influence the effectiveness of electric FHGs. In this study, body size (TL) was normally distributed across treatment groups and was not a predictor of volitional movements during handling with electric FHGs. Although body size was found to influence lactate levels, the handling method was not, indicating that electric FHGs do not significantly influence lactate levels. This relationship was also observed to a lesser extent for blood glucose, suggesting that for these metrics, body size may have been a stronger predictor of physiological stress than treatment. The nutritional status of angled fish can also play a moderating role in blood glucose concentrations and the timing and intensity of HPA axis responses, which can alter the interpretation of blood glucose data (Vijayan and Moon 1992). Note, however, that given the lack of serial replication within individuals through time, blood physiology parameters do not reflect the magnitude of stress response per se: variation related to fish body size may reflect size-specific underlying natural variance in baseline metabolite levels, although this relationship is weak for both blood glucose and lactate (Figure 1). The utility of hematocrit as an indicator of acute stress in teleost fishes is tenuous due to various factors that can confound interpretation of elevated values and given that splenic contractions can occur within seconds of a stressor such that it is nearly impossible to assess the baseline state (Barton et al. 2002). Nevertheless, hematocrit values observed in this study did not vary significantly across treatment groups ($F_{4,\ 59}$ = 0.74, P = 0.57). To more appropriately identify the primary stress response associated with handling fish by use of electric FHGs, we recommend that future investigators evaluate circulating corticosteroid levels. Future research should examine the long-term physiological effects of short-term electro-immobilization in fishes. In addition, another area that requires further study is the effect of electro-immobilization on the ionic composition of the blood as well as longer-term effects associated with electric FHGs.

The practice of angling can result in periods of exercise and air exposure, factors that are known to have a significant effect on Largemouth Bass physiology (White et al. 2008). Largemouth Bass have also demonstrated stress-induced blood chemistry alterations as a consequence of hooking (Gustaveson and Wydoski 1991); they showed more pronounced physiological responses to hooking in warmer water (28–30°C) than in cool (11–13°C) or intermediate-temperature (16–20°C) waters. To ameliorate the contribution of angling stress, future investigations could focus on aquaculture and hatchery settings, where the fish experience controlled conditions and where handling is routine. In this study, angling protocol was standardized across treatment groups, and angling time and water temperature did not vary during the study. To evaluate stress induced by handling techniques in isolation, we also controlled for the confounding effect of air exposure stressors by submerging fish in water during treatment, thus limiting their air exposure. Notably, fish were observed to continue ventilating while they were immobilized by the gloves. In studies of Rainbow Trout *Oncorhynchus mykiss*, pulsed DC was found to interfere with cardiac function, particularly over longer exposure durations (Schreer et al. 2004). It is not clear what effect low-voltage continuous DC has on Largemouth Bass; although fish in this study appeared to ventilate during handling, it cannot be confirmed from this study that immobilization through electric FHGs does not interfere with Largemouth Bass respiration. However, blood lactate and pH values did not indicate substantial anaerobic metabolism typical of impaired cardiac function. Furthermore, we found no significant difference in ventilation rates among treatment groups at any time point of measurement, and all groups (including controls) exhibited a characteristic increase and stabilization of ventilation rates after 300 s posttreatment.

The stress associated with angling may have masked any potential effects induced by handling technique. Nonetheless, what is apparent from our findings is that handling, whether with bare hands or electric FHGs and for either short (30-s) or long (2-min) durations, does not appear to significantly impair fish for the 30-min period after treatment. Moreover, for Largemouth Bass, electric gloves appear to be a more effective method of immobilization than bare hands alone. For example, attempts to implant a telemetry tag, take photos for morphometric assessment, or obtain a blood sample from fish held by bare hands would allow the fish to be too active, thus leading to welfare impairments. However, use of the electric FHGs immobilized the fish in such a way that would permit data collection and procedures to be conducted by fisheries practitioners. The results of this study can be used to inform best handling practices that maximize fish welfare (Fraser 2003) in settings where fish are handled for various reasons.

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