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ARTICLE

A Local Analgesic, Lidocaine, Did Not Affect Short-Term Welfare during Electroanesthesia of a Teleost Fish

Connor H. Reid,* Alice E. I. Abrams, and Aaron J. Zolderdo
Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental and Interdisciplinary Science, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

Jonathan D. Midwood
Fisheries and Oceans Canada, 867 Lakeshore Road, Burlington, Ontario L7R 4A6, Canada

E. Don Stevens
Biomedical Science, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada

Thomas W. Moon
Department of Biology, University of Ottawa, 75 Laurier Avenue East, Ottawa, Ontario K1N 6N5, Canada

Steven J. Cooke
Fish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental and Interdisciplinary Science, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

Abstract

In recent decades, fisheries research has benefited from the use of various forms of electroimmobilization to facilitate fish handling through rapid induction and recovery times, capacity to allow immediate release, and other advantages not shared by pharmaceuticals. However, as electroimmobilization becomes increasingly prevalent, it is expected that animal care committees may require electroimmobilized fish to also receive chemical anesthetics or analgesics. We experimentally investigated whether the administration of lidocaine (a local analgesic at ~1 mg/kg body mass) to electroanesthetized fish resulted in any welfare-relevant differences in the behavior and physiology of Largemouth Bass *Micropterus salmoides* during and after standardized intracoelomic tag implantation surgeries relative to a group that received a saline sham. We also used multiple control treatments to examine potential behavioral and physiological effects of handling, electroanesthesia, surgery, and the drug administration process. We quantified voluntary movements on the surgery table, ventilation rates after surgery, reflexes, and emergence/exploration in a behavioral arena. Primary and secondary stress biomarkers also were used to evaluate physiological stress over a 2-h period postsurgery. The administration of lidocaine at the tested dose did not facilitate fish handling during surgery and did not affect changes in the physiological stress response relative to the saline control. Swimming activity postsurgery was lower in fish treated with lidocaine; however, other differences in behavior were negligible. Electroanesthesia alone was able to sufficiently facilitate the surgical procedures by limiting voluntary escape attempts without significantly exacerbating physiological stress from handling. There does not appear to be any advantage to adding lidocaine to the protocol.

*Corresponding author: connorreid@cmail.carleton.ca
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Chemical sedatives and anesthetics are commonly used in fisheries science in order to immobilize fish during handling and to improve fish welfare (Ross and Ross 2008). Despite their prevalence, there are a number of issues associated with pharmaceuticals that make them inconvenient or inappropriate in various scenarios. For work with food fish that are to be released alive, the only approved pharmaceutical for sedation/anesthesia in North America is tricaine methanesulfonate (MS-222). In Canada, fish treated with MS-222 must be held in water above 10°C for a minimum of 5 d before being released into the wild (Health Canada 2010); this holding period is extended to 21 d in the United States (USFDA 2020). Metomidate, although costly and associated with long recovery times, also is approved in Canada for veterinary use, but only for fish not being released with the possibility of human consumption (Ackerman et al. 2005). A few other drugs, such as benzocaine, can be used with government approval in investigatory research (e.g., Investigational New Animal Drugs in the USA), but these too have prerelease holding periods of multiple days (Trushenski et al. 2013). The only exception to date is eugenol, which can be used as an immediate-release option in the USA for wild fish only, with a 72-h withdrawal period still necessary for hatchery fish (USFWS 2020).

To address the need for immediate-release sedation techniques (i.e., techniques that allow for the immediate release of treated food fish into the wild or that allow for treated fish to be harvested immediately for consumption), researchers have been exploring the use of electricity to immobilize fish for short-term handling procedures (e.g., Henyey et al. 2002; Matsche 2013; Balazik 2015; Faust et al. 2017). Many have also conducted direct comparisons of the behavioral and/or physiological impacts of drugs and electroimmobilization techniques, with a number of studies recommending the latter due to rapid induction and short recovery times (e.g., Balazik et al. 2013; Keep et al. 2015; Abrams et al. 2018), normal recovery from typical stress responses (e.g., Trushenski et al. 2012a; Johnson et al. 2016), and high survival rates (e.g., Jennings and Looney 1998; Faust et al. 2017; Kim et al. 2017). Standardized definitions of the different forms of electroimmobilization (e.g., electrosedation versus electroanesthesia) have been published recently (see Reid et al. 2019 for a prototypical list of the stages of electroimmobilization). Here, we use "electroanesthesia" to refer to immobilization induced by low-voltage, low-current electricity that induces responses similar to chemical anesthesia (i.e., steady opercular rate and relaxed muscles). Electroanesthesia is associated with rapid to near-instantaneous induction and recovery times (Balazik et al. 2013; Abrams et al. 2018) and therefore has high potential to facilitate time-sensitive handling procedures in fisheries research. Since chemical anesthesia is associated with relatively long induction/recovery times as well as the aforementioned mandatory withdrawal periods that span multiple days, electroanesthesia may be seen as a more desirable practice in field scenarios where fish should be handled and released as quickly as possible. Unlike chemical anesthetics, however, the capacity for electroanesthesia to elicit analgesia (defined by the Canadian Council on Animal Care [CCAC] as a "decrease in response to noxious stimuli"; CCAC 2005) is still equivocal.

Whether or not fish feel "pain" is controversial, hard to assess experimentally, and further complicated by the need to differentiate between the general capacity for nociception and the conscious awareness of "pain" in a sense that approximates the human experience (e.g., Rose et al. 2014; Browman et al. 2019; Sneddon 2019). As a precaution, analgesics and/or anesthetics producing analgesia are sometimes required by various guidelines and authorities (e.g., the CCAC and Institutional Animal Care and Use Committees) during experimental manipulation of fish and especially when conducting invasive procedures (Ackerman et al. 2005). By extension, animal care committees may require the application of chemical anesthetics or analgesics when performing electroimmobilization on fishes. One of the main advantages of electroimmobilization is that it permits food fish to be safely released into the wild or harvested for consumption immediately after handling procedures; this is nullified by the application of drugs, necessitating otherwise-avoidable posthandling holding times or euthanasia.

The purpose of this experiment was to evaluate whether an analgesic affected fish welfare during the electroanesthesia of a teleost fish. Specifically, we tested the hypothesis that the welfare of electroanesthetized fish, defined using behavioral and physiological endpoints, was improved or hampered by the additional application of a local analgesic (lidocaine) at a standardized, recommended dosage (Chatigny et al. 2017) during and after a standardized electronic tag implantation-type surgery in electroanesthetized Largemouth Bass *Micropterus salmoides*. Largemouth Bass are important in recreational fisheries and are frequently used as experimental subjects over a wide variety of research topics (e.g., Savino and Stein 1982; Oberdörster 2004; Hasler et al. 2016; Twardek et al. 2017), making this species highly relevant for our experiment. We used primary (i.e., plasma cortisol) and secondary (i.e., blood glucose, blood lactate, plasma osmolality, and hematocrit) metrics to evaluate physiological stress. Cortisol is the main glucocorticoid hormone associated with stress in teleost fish, and elevations in plasma cortisol may be followed by increases in blood glucose (to provide energy for responding to stress) and blood lactate (with higher, stress-induced activity changes; Raposo de Magalhães et al. 2020). In freshwater teleosts, cortisol may play a role in osmoregulation by augmenting

ion uptake at the gills (McCormick 2001); therefore, increases in plasma cortisol could be associated with higher plasma osmolality levels. Hematocrit can increase in response to stress either through the swelling of red blood cells (erythrocytes) after adrenaline release (e.g., Nascimento et al. 2012) or through the release of new erythrocytes from the spleen (e.g., Pearson and Stevens 1991). We also measured a number of behaviors and reflex indicators (1) during and after surgical procedures to assess how adequately the fish were immobilized across treatments and (2) after surgeries to evaluate fish recovery and behavioral impacts associated with each treatment. We predicted that physiological responses would increase (as a sign of stress) in response to handling relative to baseline levels but would not differ among handled treatments. We also predicted that during surgeries, fish would remain "calmer" (i.e., exhibit fewer lateral thrashes) in all electroanesthetized treatments relative to the handling control and that the posthandling behavior would differ from typical baseline responses but would not differ with the addition of incisions and a saline sham or lidocaine infiltration.

METHODS

Study site, experimental subjects, and overview of treatments.— Our experiment was conducted from July 14 to August 24, 2019, at Queen's University Biological Station (Elgin, Ontario). One-hundred seventy-two adult Largemouth Bass (288–405 mm TL) were angled from Lake Opinicon (44.5590°N, 76.3280°W) after surface water temperatures stabilized at about 24°C (~mid to late June). Fish were transported in coolers of fresh lake water back to Queen's University Biological Station and were held individually in holding chambers (blackened plexiglass boxes) for 24–48 h to permit acclimatization and to control for stress associated with capture and captivity (Suski et al. 2006; Newby et al. 2007; Galvez et al. 2008). The holding chambers (~78.7 × 15.2 × 15.2 cm [length × width × height]) were placed in a large circular tank (inner diameter = 3.5 m) filled with lake water to an approximate depth of 110 cm, with each chamber sitting on a rack roughly 30 cm from the water's surface. Fresh lake water was added to the tank during experimental trials, and an air stone bubbler ran constantly in each holding chamber to ensure adequate oxygen saturation. An oxygen probe (Handy Polaris; OxyGuard, Farum, Denmark) was used to monitor oxygen concentrations in the holding chambers themselves (immediately after removing the fish), showing oxygen concentrations consistently above 92% saturation. Due to physical constraints at the experimental site, only 10 fish could be held in holding chambers at a time, and angling efforts did not always yield 10 fish to test each day. As a result, we were not able to collect data for all 12 treatment × track combinations (tracks are defined below) on any given day of testing (Figure S1 available in the Supplementary File 1 in the online version of this article).

Fish were randomly divided into either a behavioral experimental protocol or a physiological protocol (henceforth, "behavior track" and "physiology track," respectively). Here, we focus on comparing the two main treatments of interest: surgery with electroanesthesia and lidocaine (SELi) and surgery with electroanesthesia and a saline sham (SESa). However, to quantify potential effects of handling, electroanesthesia, surgery, and drug administration on our results, we also included the following control groups for which protocols and results are detailed in Supplementary File 1: handling control (HC), electroanesthesia (E), surgery with electroanesthesia (SE), and baseline groups (BB for behavior-track fish and BP for physiology track-fish; Figure S1). Detailed descriptions of the SESa and SELi protocols follow in the next subsection (Surgical Setup and Protocol). This work was conducted in accordance with CCAC guidelines and approved by the Carleton University Animal Care Committee (Animal Use Protocol 110557).

Surgical setup and protocol.— Surgeries were conducted on the inverted lid of a plastic bin (~50 L) filled with lake water from the holding tank. Fish were placed on top of the lid, between two bricks covered by a soft-mesh mat in a trough-like shape for stability. A recirculation pump was placed inside the bin and connected to tubing that extended to the fish's mouth, permitting water to flush over the gills during the procedure (Figure S2). Fresh water was used for each fish, and water temperature and conductivity were recorded once per fish by using a digital water quality meter (Aquapro AP-2; HM Digital, Redondo Beach, California).

Regardless of track, SESa and SELi fish were individually removed from the holding chambers and measured in a water-filled plastic trough with a built-in ruler. Fish were then given a 0.25-mL infiltration of sterile physiological saline (Eyesaline; Honeywell, Charlotte, North Carolina) or lidocaine (lidocaine HCl injection [2%] with preservative; Teligent, Mississauga, Ontario) diluted in sterile saline, respectively, via sterile 1-mL syringes and 23-gauge needles. In an infiltration, the solution is gradually expelled as the needle is withdrawn as opposed to an injection where solution is injected at the deepest point of needle puncture. To give the lidocaine time to take effect, SELi fish were held for 5 min (e.g., Oswald 1978) in a cooler (~45 L) full of fresh lake water. The SESa fish were also held for 5 min under the same conditions to control for the drug administration process. Four diluted lidocaine stock solutions were prepared prior to experiments such that one solution would provide an approximate dose of 1 mg/kg body mass (based on recommendations by Chatigny et al. [2017]) for fish with TLs of 300–325, 326–350, 351–375, and 376–400 mm; the masses

used in the dose calculations were estimated from those size ranges based on the length–weight relationships provided by Schneider et al. (2000). Two SELi fish (one behavior-track fish and one physiology-track fish) had TLs slightly under 300 mm and received the stock solution for 300–325-mm fish. The use of stock solutions and estimated masses reflects the reality of many field scenarios, where it may not be feasible to record the mass of every captured fish and prepare a dose tailored to each individual (Chatigny et al. 2018). There are no clear or established safe withdrawal times for lidocaine in Canada (or, to our knowledge, any other jurisdictions), and because SELi fish would have been released with the potential for human consumption, these fish were euthanized via cerebral percussion and disposed of at the end of experimental protocols. When not in use, all surgical tools (scalpel, hemostat, and sutures) were kept in diluted 10% povidone-iodine (Betadine; Purdue Pharma, Pickering, Ontario) for disinfection. After drug administration, SESa and SELi fish were placed on the surgery setup and held upside down for approximately 240 s by an assistant wearing Smith-Root Electric Fish Handling Gloves (EFHGs) with a standard output of about 32 V and five current settings (4.0, 6.3, 10.0, 16.0, and 25.0 mA; Smith-Root 2016). Per Abrams et al. (2018), the current setting was selected by beginning at the lowest setting, increasing the current strength until full-body spasms (tetany) were observed, and then returning one setting lower to achieve electroanesthesia (muscle relaxation, normal opercular movement, complete loss of equilibrium and reactivity; Reid et al. 2019). A ventral incision (~2 cm) was made with a scalpel and closed with three interrupted 3-0 sutures (PDS II; Ethicon, Somerville, New Jersey).

A video camera mounted on a tripod was used to record the surgeries for all fish regardless of track, allowing for many more samples of behavior during surgery. Surgery videos were used to score the number of voluntary thrashes (rapid lateral flexes) during the procedures, and mean ventilation rates (VRs; calculated from the number of opercular movements during the surgery) over a 15-s period postincision (VR_1) were also quantified from the videos. This 15-s period began as soon as possible after the incision had been made, based on visibility in the videos. After the handling procedures were completed, each fish was subjected to either a behavioral evaluation or a physiological evaluation of posthandling stress. Fish masses were recorded on an electronic balance (OHAUS, Parsippany, New Jersey) after data collection was complete. The average estimates of fish mass were equal to about $100.7 \pm 8.3\%$ (mean $\pm$ SD) of the actual recorded masses for all fish (i.e., an estimate equal to 100% of the actual mass of a fish would be fully accurate).

Experimental procedure: behavior track.— In total, 96 Largemouth Bass (mean TL $\pm$ SD = 330 ± 29.3 mm) were subjected to the behavior-track protocol. After the treatment handling procedures, each individual was transferred to a white cooler (~45 L) filled with lake water from the holding tank. As soon as the fish was settled (~3–5 s), the number of opercular movements over a 30-s period was recorded. This was followed by a reflex action mortality predictor (RAMP) test consisting of five quick binary scores evaluating whether (1) the fish initiated a flight response when the tail was lightly pinched; (2) the fish's eyes followed the handler when held at the water's surface and rotated laterally; (3) the fish attempted regular opercular movement during 5 s of air exposure; (4) the fish attempted to free itself when held sideways in the air and gripped in the middle of its body; and (5) the fish regained equilibrium within 3 s when flipped upside down in the water. The RAMP score (e.g., Raby et al. 2012; Prystay et al. 2017), calculated as the sum of the five binary reflex scores, can act as a predictor of short-term mortality. The fish was then allowed to rest in the cooler for 1 min, after which another 30-s opercular movement count and subsequent RAMP test were performed (hereafter, the first and second RAMP tests are denoted RAMP 1 and RAMP 2). When the opercula were fully open during normal breathing, they were sometimes observed to rapidly twitch half-closed, open fully again, and then shut normally in unison; the frequencies of these twitches were also recorded during both opercular movement counts and were then pooled. Both of the opercular movement counts were converted to mean VRs (VR_2 and VR_3) by dividing the number of counts observed by 30 s, giving VRs in ventilations per second. The difference between the two cooler VRs (ΔVR) was also calculated. After the RAMP 2 test, the fish was placed in a refuge within a behavioral arena for acclimatization and refuge emergence and line cross trials. The refuge ($30 \times 60 \times 30$ cm [length $\times$ width $\times$ height]) was constructed with black spray-painted plastic sheeting and had a hinged door and removable floor operated by pulley systems to allow for fish to be inserted with minimal escape risk and to permit refuge opening and lifting.

Behavioral trials were conducted in one of two large circular tanks with an inner diameter of 3.5 m and filled with lake water to an approximate depth of 110 cm, as with the holding tank. The arenas were intersected with lateral and longitudinal lines at every 0.5 m with black spray-painted rebar (the crossing of which was used to provide an index of exploratory behavior; e.g., Cooke et al. 2017). The refuge was placed along the edge of the tank facing toward the center. A 9-m^2 canvas gazebo was erected above each behavior tank in an attempt to standardize lighting and prevent environmental debris (leaves, twigs, caterpillars, etc.) from falling into the tanks and contaminating the water. At the apex of each gazebo, a camera was raised via a pulley system to record the behavioral trials and mitigate observer effects.

Once a fish was placed inside the refuge, the door was closed and the refuge was lowered into the arena. Using a long strand of fishing line tied to one end of the refuge floor,

the floor of the refuge was slid out from beneath the refuge so that the refuge could be lifted later without hitting the fish. After this, fish were given 10 min to acclimatize in the dark refuge. The door to the refuge was then opened via a pulley, and fish were given up to 10 min for voluntary refuge emergence. If a fish emerged during the 10 min, the refuge remained in the tank; if not, then the refuge was slowly lifted straight upwards and out of the tank after the 10-min mark. After emergence or refuge removal, the number of gridlines crossed over a 10-min period was recorded. All behavioral metrics (binary refuge emergence score, time taken to emerge [if applicable], whether the raised refuge was considered to be a threat, number of lines crossed, and number of refuge re-entries [if applicable] and total time spent back in the refuge) were scored through video analysis. A positive refuge emergence score was assigned when the fish's full body had left the refuge. Whether the lifting of the refuge was considered to be a threat was scored positive if the fish initiated a flight response (fast swimming) away from the refuge as it was being lifted; if this was observed, line crosses were not counted until the flight response ended (i.e., the fish came to rest). During the line cross trial, a cross was counted only when the fish's whole body had crossed over the line. After the behavioral trials were completed, fish were netted out of the arena, weighed, and returned to their holding chambers.

Experimental procedure: physiology track.— Overall, 76 Largemouth Bass (mean TL ± SD = 334 ± 27 mm) were subjected to the physiology-track protocol. After the relevant handling procedures, all fish were placed back into their chambers in the holding tank. Blood samples (~200 μL) were taken from the caudal vasculature of each fish at 30 and 120 min after return to the chambers to quantify physiological stress (cortisol, glucose, lactate, osmolality, and hematocrit) at peak values and during recovery. Previous work on Largemouth Bass has shown that cortisol tends to peak near the 30-min mark (Trushenski et al. 2012b) and that by 2 h postexposure, fish should be well into the recovery phase (Suski et al. 2006). Our experimental setup was not conducive to holding fish for longer periods of time (e.g., 6 h; Trushenski et al. 2012b) to measure complete recovery. All blood samples were taken using heparinized (sodium heparin, 10,000 U.S. Pharmacopeia units/mL; Sandoz, Boucherville, Quebec) 21-gauge needles and 1-mL syringes.

Blood glucose and lactate concentrations were measured using Accu-Chek Compact Plus (Hoffman-La Roche Ltd., Mississauga, Ontario) and Lactate Plus (Nova Biomedical Corporation Canada Ltd., Mississauga, Ontario) handheld meters. These medical-grade devices have been validated for use on whole-blood samples from teleost fish (Stoot et al. 2014). Both glucose and lactate meters display "LO" when the measured glucose or lactate concentration falls below the 0.3-mM detection limit. Therefore, any "LO" readings were set at 0.3 mM in the analyses so as to allow for a conservative comparison of these data across treatments. No "LO" glucose readings occurred. Several "LO" lactate readings occurred in the baseline group, two occurred in the SESa group (one at 30 min and one at 120 min), one occurred in the E group (at 120 min), and one occurred in the SELi group (at 120 min). To measure hematocrit, blood was collected in 40-mm, heparinized (ammonium heparin) microhematocrit capillary tubes (Iris Diagnostics, Chatsworth, California) and centrifuged at 13,700 × *g* for 2 min (StatSpin CritSpin; Iris Sample Processing, Westwood, Massachusetts). The remaining blood was held on ice for less than 3 h and centrifuged at 2,000 × *g* for 5 min (Benchmark MyFuge Mini; Mandel Scientific, Guelph, Ontario). The plasma was decanted, flash frozen, and stored in a −80°C freezer until plasma cortisol and osmolality assays could be carried out. Osmolality assays were conducted using a vapor pressure osmometer (VAPRO 5600; ELITech Group, Puteaux, France). Commercial radioimmunoassay kits (ImmuChem Cortisol Coated Tube RIA Kit; MP Biomedicals, Solon, Ohio) were used to estimate plasma cortisol concentrations, with an intra-assay variability of 8.26%.

Statistical analyses.— Analyses were carried out for each treatment pair (BB/BP versus HC, HC versus E, E versus SE, SE versus SESa, and SESa versus SELi). Although we maintain focus on the results and discussion of SESa versus SELi, the analyses were consistent for each comparison except for BP versus HC (detailed in Supplementary File 1). Summary statistics (e.g., normality tests) were conducted in PAST version 3.25 (Hammer et al. 2001). All other statistical analyses were performed in RStudio version 1.1.463 (RStudio Team 2016) with R version 3.6.0 (R Core Team 2019). Figures were generated using the ggplot2 package (Wickham 2016). General linear models were fitted for (1) all VR data ($VR_{1–3}$; ΔVR), with fish mass and water temperature initially included as linear covariates, and (2) continuous measures of behavior in the arena (refuge emergence time, time in the re-entered refuge), with fish length and water temperature initially included as linear covariates. Generalized linear models (GLMs) with Poisson error distributions were fitted for (1) the number of lateral thrashes during surgery, with fish mass and water temperature initially included as linear covariates, and (2) behavioral responses measured as counts, with water temperature and either mass (for RAMP scores and opercular twitches) or length (for line crosses and refuge re-entries) initially included as linear covariates. Generalized linear models with binomial error distributions were fitted for binary responses (refuge emergence and threat perception from refuge lifting), with fish length and water temperature initially included as linear covariates. For fish that did not emerge from the refuge, line cross count analyses also included a binary predictor of whether the lifting of the refuge elicited a visible flight

response. Blood chemistry data were analyzed using linear mixed-effects models (LMMs) fitted with the "lmer" function from the lme4 package (Bates et al. 2015), including treatment, time (30 or 120 min), and the two-way interaction of treatment and time as categorical predictors; fish mass as a linear covariate; and individual fish identity as a random effect. All models underwent model selection based on Akaike's information criterion corrected for small sample size (AIC_c) to test whether covariates (e.g., fish mass and water temperature) contributed significantly to model variance; if they did not, they were removed from the model. Each global model and the nested models within were sorted by AIC_c using the "dredge" function from the MuMIn package (Barton 2019). The model with the lowest AIC_c value that still contained treatment as a predictor was selected for subsequent analysis. General linear models, GLMs, and LMMs were analyzed using the "Anova" function in the car package (Fox and Weisberg 2019), with *F*-tests calculated for general linear models and LMMs and likelihood-ratio χ^2 tests performed for the GLMs. Random effects were analyzed using the "ranova" function from the lmerTest package (Kuznetsova et al. 2017) and by computing intraclass correlation coefficients (ICCs) from variance outputs from the base R "summary" function. Post hoc analyses of pairwise comparisons between categorical predictor levels were conducted using the "lsmeans" function from the emmeans package (Lenth 2019) to generate asymptotic 95% CIs.

Wasserstein et al. (2019) summarized an increasingly popular shift in researchers' attitudes toward *P*-values and the limitations of arbitrary notions of "statistical significance." We follow several of the recommendations therein toward a more comprehensive means of interpreting results. To that end, we make use of complementing *P*-values with the inclusion of local effect sizes as well as comparisons to findings from similar research where available. For categorical predictors (e.g., treatment), Cohen's f^2 is given; as an approximate guideline, Cohen (1988) recommended considering $f^2 \geq 0.02$ as a small effect size, $f^2 \geq 0.15$ as a medium effect size, and $f^2 \geq 0.35$ as a large effect size. Predictors with low effect sizes explain little of the variation in the observed data, while those with medium and large effect sizes explain moderate and high amounts of variation in the observed data, respectively. Full statistical analysis and model selection outputs are available upon request from the corresponding author.

RESULTS

Does Lidocaine Increase or Decrease Stress and Influence Behavior?

Results for SESa versus SELi analyses are presented in Table 1. The administration of lidocaine to the surgery site in SELi fish neither improved nor hindered fish handling on the surgery table relative to SESa fish. Changes in physiological stress markers after surgery were consistent between the two treatments. Postsurgery behavioral responses were largely consistent as well except for arena activity, which was lower in SELi fish than in SESa fish.

Mean thrash counts during surgery did not differ between SESa and SELi fish ($P = 0.25$, $df = 1$, $f^2 = 0.03$; Figure 1). Mean thrash counts increased by approximately 46% for every 1°C increase in water temperature ($P = 0.002$). We do not report results for VR data analyses. During surgeries, there was a number of instances in which fish were being prevented from exhibiting normal opercular movements due to random differences in handling technique between fish and between handlers. Ventilation rates recorded in the cooler were likewise imprecise since the time between the stressor and the visible response was highly variable and subject to influence from different handling times for each treatment and observer effects (Barreto and Volpato 2004).

In SESa and SELi fish, treatment did not affect (or explain much variation in) mean differences in plasma cortisol, blood glucose, blood lactate, plasma osmolality, or hematocrit (Figure 2). For both SESa and SELi fish, mean plasma cortisol increased slightly from 30 to 120 min postsurgery ($P = 0.024$, $df = 1$, $f^2 = 0.05$; Figure 2A). Decreases in mean values from 30 to 120 min were observed for blood lactate ($P = 0.003$, $df = 1$, $f^2 = 0.02$; Figure 2C), plasma osmolality ($P = 0.044$, $df = 1$, $f^2 = 0.05$; Figure 2D), and hematocrit ($P < 0.0001$, $df = 1$, $f^2 = 0.23$; Figure 2E). Time was excluded from glucose analyses between these two treatments. Intraindividual variation outweighed the variation explained by all fixed effects for plasma cortisol (ICC = 0.554), blood glucose (ICC = 0.824), blood lactate (ICC = 0.790), plasma osmolality (ICC = 0.624), and hematocrit (ICC = 0.628).

The SELi and SESa fish did not differ in any responses measured in the cooler. Treatment had no effect on and explained little variation in mean opercular twitch counts ($P = 0.49$, $df = 1$, $f^2 = 0.02$) or either RAMP score (RAMP 1: $P = 0.68$, $df = 1$, $f^2 = 0.005$; RAMP 2: $P = 0.81$, $df = 1$, $f^2 = 0.002$) between SESa and SELi fish.

In the arena, no SESa fish emerged voluntarily from the refuge; therefore, we could not quantify differences in refuge emergence, refuge emergence time, refuge re-entry counts, and time in the re-entered refuge. Treatment explained little variation in whether the refuge lifting elicited a flight response, and there was no observable difference between SESa and SELi fish ($P = 0.85$, $df = 1$, $f^2 = 0.001$). Mean line cross counts were 12% lower for SELi fish than for SESa fish, with treatment explaining moderate variation in the observed counts ($P = 0.032$, $df = 1$, $f^2 = 0.20$; Figure 3). Fish that perceived the lifting refuge as a threat had higher mean line cross counts than those that did not, with threat perception explaining a

TABLE 1. Effects of treatment—and, for blood chemistry data, time and treatment × time interactions—on responses of Largemouth Bass that received lidocaine (SELi) or a saline sham (SESa) prior to undergoing surgery under electroanesthesia (RAMP 1, RAMP 2 = reflex action mortality predictor scores). Both *P*-values and effect sizes (Cohen's f^2) are reported when applicable; "NA" denotes that the predictor was not included in the analysis either because it was excluded during model selection (time or the treatment × time interaction for blood chemistry data) or because no refuge emergences occurred in the SESa group. None of the metrics showed a large effect ($f^2 > 0.35$), and only one showed a moderate effect (line cross count; $f^2 > 0.15$).

Stage	Response	Treatment *P*	Treatment f^2	Time *P*	Time f^2	Interaction *P*
Surgery	Thrash count	0.25	0.03			
Physiology track	Plasma cortisol	0.103	0.11	0.024	0.05	0.872
	Blood glucose	0.149	0.09	NA	NA	NA
	Blood lactate	0.415	0.05	0.003	0.02	NA
	Plasma osmolality	0.405	0.04	0.044	0.05	0.301
	Hematocrit	0.55	0.007	<0.0001	0.23	NA
Behavior track–cooler	Opercular twitches	0.49	0.02			
	RAMP 1	0.68	0.005			
	RAMP 2	0.81	0.002			
Behavior track–arena	Refuge emergence	NA	NA			
	Emergence time	NA	NA			
	Threat perception	0.85	0.001			
	Line cross count	0.032	0.20			
	Refuge re-entry count	NA	NA			
	Time in refuge	NA	NA			

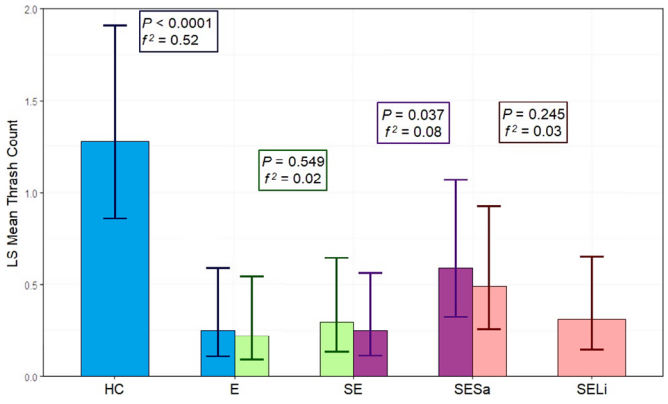

FIGURE 1. Least-squares (LS) mean thrash counts (with asymptotic 95% CIs) observed in Largemouth Bass during surgeries for each treatment. Treatments are represented by letter codes (HC = handling control [$n = 27$]; E = electroanesthesia [$n = 27$]; SE = surgery and electroanesthesia [$n = 28$]; SESa = surgery, electroanesthesia, and saline infiltration [$n = 28$]; SELi = surgery, electroanesthesia, and lidocaine infiltration [$n = 28$]). Column color or shading denotes each treatment comparison, with corresponding *P*-values and effect sizes (Cohen's f^2) in matching boxes; LS means were generated during each analysis, so two columns are present for all treatments but HC and SELi. [Color figure can viewed at afsjournals.org.]

very high proportion of the observed variation in line cross counts ($P < 0.001$, df = 1, $f^2 = 8.22$). Mean line cross counts tended to decrease by about 1% per 1-mm increase in fish length ($P = 0.01$). In summary, only 1 of the 15 metrics showed a large effect size; 14 metrics did not.

Control Treatment Comparisons

Since the ultimate goal of our experiment was to test whether the welfare of electroanesthetized fish was improved or hampered by the application of a local analgesic at a standard recommended dosage, and given the volume of results generated, we present detailed results from control experiments (comparisons between BB/BP and HC, HC and E, E and SE, and SE and SESa) in Supplementary File 1. In brief, handling of fish increased stress but generally did not affect behavior relative to baseline groups. Electroanesthesia reduced voluntary movement on the surgery table (Figure 1) and swimming activity (Figure 3) without exacerbating physiological stress; surgery further depressed swimming activity. The drug administration process generally did not affect stress physiology but slightly increased activity on the surgery table.

Fish size across treatments did not differ for either the behavior track (though unequal variances were observed; Welch's $F = 1.589$, df = 41.2, $P = 0.19$) or the physiology track (ANOVA: $F = 0.335$, df = 5, $P = 0.89$). Across treatments, no differences were found between surgery temperatures for either the behavior-track fish ($F = 0.863$, df = 66, $P = 0.49$) or the physiology-track fish ($F = 1.848$, df = 61, $P = 0.13$); likewise, no differences were found between water conductivities for the behavior-track fish ($F = 0.613$, df = 66, $P = 0.65$) or the physiology-track fish ($F = 0.352$, df = 61, $P = 0.84$). Mean water conductivity ± SD throughout our experiment was 193.2 ± 8.2 μS/cm. Mean water temperatures ± SD were

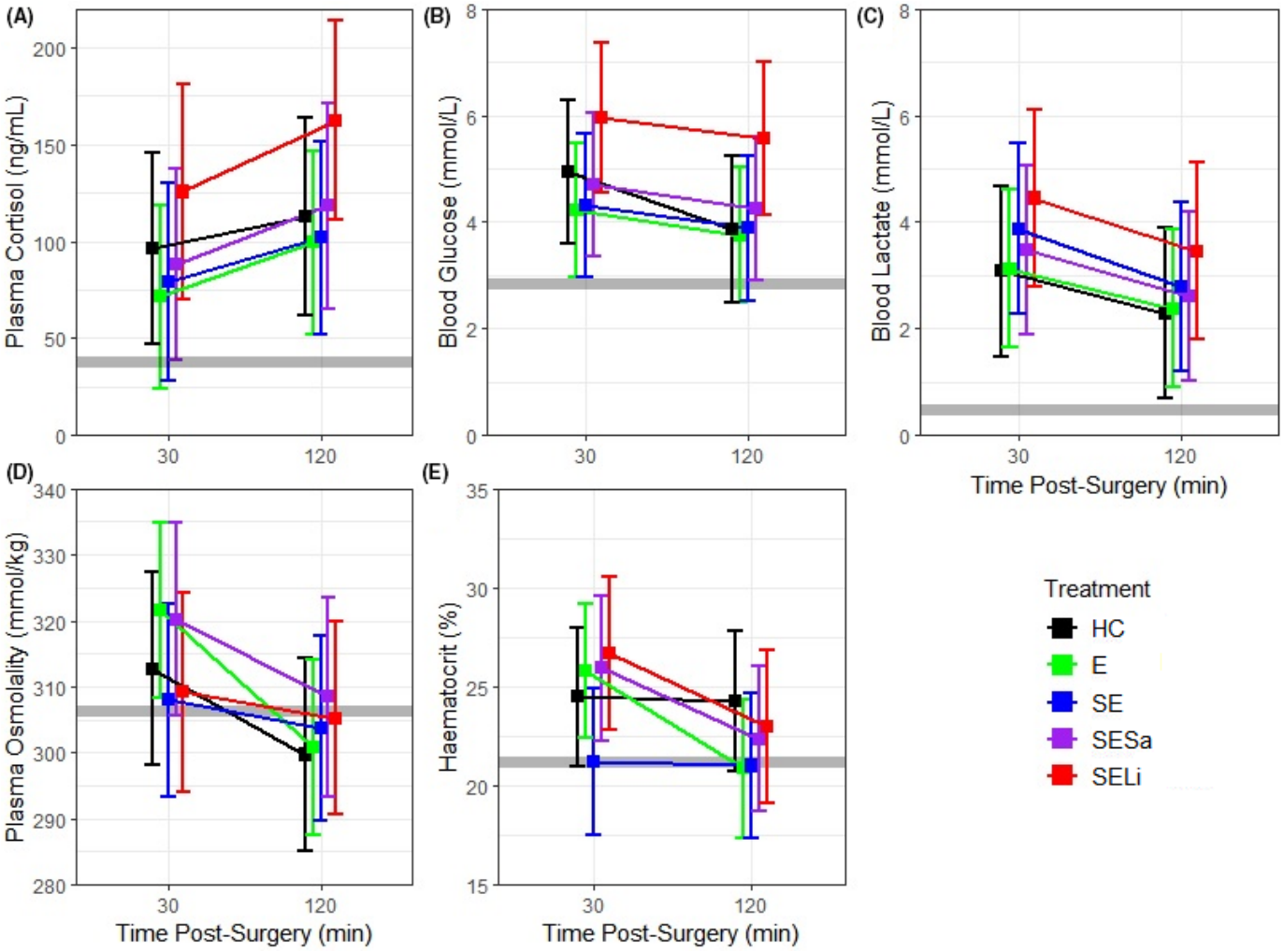

FIGURE 2. Least-squares means (with asymptotic 95% CIs) of **(A)** plasma cortisol concentrations, **(B)** whole-blood glucose concentrations, **(C)** whole-blood lactate concentrations, **(D)** plasma osmolality concentrations, and **(E)** hematocrit for Largemouth Bass in each treatment at 30 and 120 min postsurgery. Data points are jittered for clarity of viewing. The horizontal gray line represents the mean value from the baseline group for each response. Treatments are represented by letter codes (HC = handling control; E = electroanesthesia; SE = surgery and electroanesthesia; SESa = surgery, electroanesthesia, and saline infiltration; SELi = surgery, electroanesthesia, and lidocaine infiltration). Sample sizes per treatment (HC, E, SE, SESa, and SELi, respectively) were as follows: 12, 13, 11, 12, and 10 for cortisol at 30 min; 11, 13, 12, 10, and 12 for cortisol at 120 min; 12, 14, 12, 12, and 12 for glucose at 30 min; 11, 13, 11, 12, and 11 for glucose at 120 min; 12, 14, 12, 12, and 12 for lactate at 30 min and at 120 min; 11, 13, 11, 11, and 11 for osmolality at 30 min; 11, 13, 12, 10, and 12 for osmolality at 120 min; 12, 13, 11, 11, and 11 for hematocrit at 30 min; and 12, 12, 11, 11, and 11 for hematocrit at 120 min. [Color figure can viewed at afsjournals.org.]

22.6 ± 1.6°C during surgeries and 22.9 ± 1.7°C in the behavioral arena.

DISCUSSION

Does Lidocaine Affect the Welfare of Electroanesthetized Fish?

Overall, we found insufficient evidence to suggest that the welfare of electroanesthetized Largemouth Bass undergoing surgeries was improved or diminished by the infiltration of lidocaine at approximately 1 mg/kg at the surgery site, given the general consistencies between physiological and behavioral responses in SESa and SELi fish. It is unclear, however, whether this dose of lidocaine (~1 mg/kg) was sufficient to induce an analgesic effect in our experimental fish. There is insufficient information on appropriate doses for locally administered lidocaine in fish, with recommendations such as the dose used in our experiment (~1 mg/kg fish) based on work in other taxa (i.e., mammals; Chatigny et al. 2017). A higher lidocaine dose of 10 mg/kg was infiltrated in the dorsal musculature of Rainbow Trout *Oncorhynchus mykiss* and resulted in more damage to muscular tissues over a 15-d period

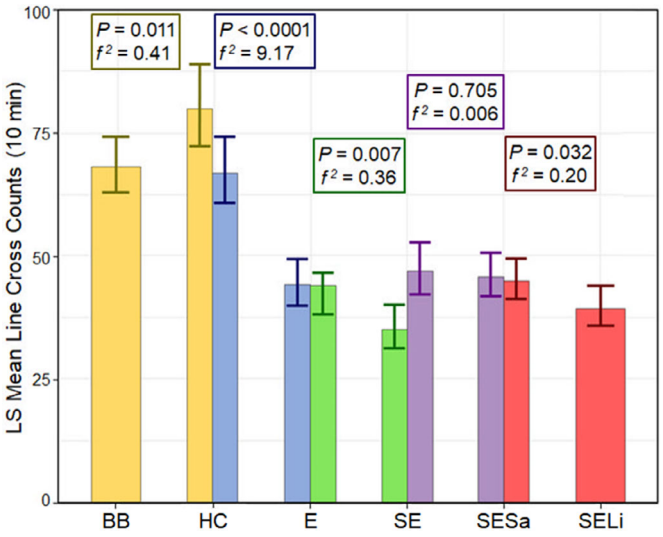

FIGURE 3. Least-squares (LS) mean line cross counts (with asymptotic 95% CIs) for Largemouth Bass in each treatment. Treatments are represented by letter codes (BB = baseline behavior [$n = 14$]; HC = handling control [$n = 14$]; E = electroanesthesia [$n = 12$]; SE = surgery and electroanesthesia [$n = 14$]; SESa = surgery, electroanesthesia, and saline infiltration [$n = 13$]; SELi = surgery, electroanesthesia, and lidocaine infiltration [$n = 16$]). Column color or shading denotes each treatment comparison, with corresponding *P*-values and effect sizes in matching boxes; LS means were generated during each analysis, so two columns are present for all treatments but BB and SELi. [Color figure can viewed at afsjournals.org.]

relative to a saline control group (Chatigny et al. 2018), but Chatigny et al. (2017) also reported that the use of 20-mg/kg infiltrated lidocaine in Rainbow Trout had no apparent adverse impacts on survival. Lamglait and Lair (2021) evaluated postsurgical changes in blood chemistry (plasma cortisol, lactate, and creatine kinase concentrations) in adult Brook Trout *Salvelinus fontinalis* that were held with the same EFHGs used in our experiment with or without 6 mg/kg lidocaine infiltrated at the surgery site or Brook Trout that were held under general anesthesia (MS-222 or eugenol) followed by lidocaine infiltration. Despite several differences between our methodologies (e.g., lidocaine dose, species, and selection of EFHG current strength), Lamglait and Lair (2021) also found no difference in postsurgery cortisol or lactate levels between electroanesthetized fish with or without a lidocaine infiltration and highlighted the need for more information about dose requirements for similar procedures. The plasma creatine kinase concentrations were elevated in both electroanesthesia treatments relative to the chemically anesthetized groups (Lamglait and Lair 2021), but the authors attributed this to altered energy processes rather than structural damage in the muscles. Researchers attempting to replicate such experiments or to carry out similar work in another species would benefit greatly from an evidence-based understanding of appropriate doses for lidocaine (and other local analgesics), such as species-specific dose–response curves for various taxa. In addition, the legally mandated euthanasia of SELi fish did little to promote individual fish welfare and was not practical given the context of the surgical procedure we simulated (which aims to put tracking tags in live fish).

Surgery Behavior

The application of lidocaine did not add to or improve upon the ease of handling attributable to electroanesthesia alone at the tested dose. Increases in mean thrash counts with increasing water temperature could be an indication of the general increase in exploration activity with increasing temperature (e.g., Hasler et al. 2009). We are unaware of any studies to date on the specific effects of temperature on lidocaine metabolism in fish, although drug metabolism (and therefore induction/recovery times) are generally faster with increasing temperature (Neiffer and Stamper 2009). It is plausible that the effects of the drug were not consistent as temperatures changed in our experiment, which might have then reduced the observed difference between SESa and SELi fish thrash counts. The positive relationship between mean thrash counts and fish mass might be explained by aggression/antipredator behavior, which, by some metrics, increases with size (e.g., Cooke et al. 2017). In the only other known experiment to also quantify rapid lateral thrashes using the same electroimmobilization equipment, Ward et al. (2017) did not find a relationship between the number of escape attempts and fish size, although substantial differences exist between that experiment and the one presented here (e.g., water temperature, selected current strengths, range of fish sizes, presence/absence of invasive treatments, and handling times).

Behavior Track

Opercular twitches have only been documented in an Antarctic fish as a response to reaching critical thermal maxima (Bilyk et al. 2012), but the fish in our experiment were exposed to lake water at temperatures to which they were accustomed, and they were not incapacitated as would be expected in a severe heat stress event. Since these twitches were observed across all treatments (including BB), and with no discernable mechanism to explain the occurrence of opercular twitches in otherwise normally breathing fish, we can only recommend that this phenomenon be investigated in future work. In the event that it is a proxy for stress, opercular twitches would corroborate the increase in stress from more intense handling (HC) compared with minimal handling (BB) as well as the overall lack of differences in physiological stress responses induced across treatments in our experiment. The lack of differences in RAMP scores across all

treatments suggests that under the environmental conditions of our experiment, none of the procedures used would be expected to increase short-term mortality in Largemouth Bass.

Although the statistical analyses yielded further decreasing line cross counts from SESa to SELi fish, the biological relevance of these differences should be brought into question. As is clearly visible in Figure 3, the least-squares means that were used to estimate population means and generate CIs for each treatment were calculated separately during each analysis and were therefore not always consistent (e.g., the least-squares means for SE fish differed starkly from one another). As a result, the differences in line cross counts between SESa and SELi fish could very well be a result of the statistical methods that had to be employed for these data, and in practice the differences are small enough to not be considered biologically relevant. For fish that did not emerge from the refuge, the frequency with which the raising of the refuge elicited a flight response did not differ between SESa and SELi fish. Despite controlling for this in the analysis of line crosses and allowing for fish that did flee to finish their flight response before beginning line cross counts, higher line cross counts were still observed for fish that fled compared with those that did not. The brief flight response observed may have been coupled with other, relatively long-lasting physiological processes that are linked to relevant behavioral responses (e.g., heart rate or cardiac output; Johnsson et al. 2001; Cooke et al. 2003).

Physiological Stress Responses

The magnitude and direction of changes in plasma cortisol (Figure 2A), blood glucose (Figure 2B), and blood lactate (Figure 2C) between 30 and 120 min were similar for SESa and SELi fish as well as for control treatments. The increase in plasma cortisol from 30 to 120 min was surprising, as we expected that plasma cortisol would peak roughly 30 min after exposure to a stressor (Barton 2002; e.g., Trushenski et al. 2012a, 2012b). Increased cortisol levels at 120 min postsurgery may be the result of the cumulative stress of repeated sampling, which was not performed in experiments that predicted a 30-min peak. The possibility of stress due to conspecific water cortisol release (e.g., Ellis et al. 2004; Fanouraki et al. 2008) also cannot be ruled out.

In general, high intraindividual variation was a much more important factor than treatment in influencing the responses of all hematological stress metrics, as individual fish often accounted for more than half of the observed variation in each response. Intraindividual variation in physiological changes to various stimuli has been previously reported in other fish (e.g., Cook et al. 2012), with these changes possibly being grounded in genetic differences among individuals (Prunet et al. 2008), which were not assessed here. There is a paucity of research on the extent of intraindividual variation in physiological stress responses under circumstances comparable to our experiment, thus hampering our ability to interpret the relative contributions of intraindividual variation in a broader context.

A post hoc power analysis was conducted on blood chemistry responses to determine the power of each analysis (i.e., the probability of detecting a treatment effect that is truly present) given our relatively low experimental sample sizes. Statistical power for SESa versus SELi comparisons of blood chemistry parameters ranged from 65% to 69%, which is not as high as would be ideal (>80%) but is still well above the averages of many biological field studies (Jennions and Møller 2003; Lemoine et al. 2016). We advise readers to consider these statistics as an integral part of interpreting results in this and other experiments.

Improving the Electroanesthesia Apparatus

The electroanesthesia apparatus used in our experiment is effective but could be improved in several ways. First, the EFHGs have limited flexibility in that there are only five discrete current output settings (4.0, 6.3, 10.0, 16.0, and 25.0 mA) that do not provide as much sensitivity in eliciting electroanesthesia as a continuous dial would; therefore, they may not always be able to provide the ideal current to achieve electroanesthesia. The voltage output (~32 V) also cannot be controlled and may be higher than necessary (a transcutaneous electrical nerve stimulation unit, although emitting different current types, is also effective on many species while operating with a 9-V battery). Lastly, the fabric/metal mesh gloves that serve as electrodes in contact with the fish are heavily subject to wear and tear, causing diminished efficacy as the metal wires snap and lose connectivity from stretching over various hands, getting caught on fish teeth, etc. Muscle spasms were occasionally observed during electroanesthesia in our experiment, and Lamglait and Lair (2021) reported similar observations, which may be mitigated by expanding the degree of operator control and customization of EFHG settings for individual fish. Overcoming these obstacles could improve the efficacy of electroanesthesia as administered in our experiment and might have allowed for greater precision in the symptom-based administration of electroanesthesia. Suggested improvements include the integration of a continuous spectrum of current outputs (rather than discrete values) and more structurally sound gloves that can conform to diverse hand sizes (possibly via electrode plates on the palms and/or fingertips rather than thin mesh). Researchers building their own electroanesthesia setups or parts may wish to refer to the methods of others like Hudson et al. (2011) or Vandergoot et al. (2011). The development of an alternative system to the EFHGs as they currently exist could improve researchers' ability to administer electroanesthesia in a more consistent and controlled fashion.

Conclusion

We found no evidence that infiltrated lidocaine at a dose of about 1 mg/kg affected the welfare of electroanesthetized adult Largemouth Bass undergoing a brief surgical procedure. Although our experiment does not show that local analgesics are definitively ineffective when used alongside electroanesthesia, there are currently no experiments that provide evidence to support this practice. Our findings corroborate those of Lamglait and Lair (2021) in that changes in the stress physiology of electroanesthetized fish do not appear to be affected by the infiltration of lidocaine at the surgery site; however, we also acknowledge that very little work has been conducted so far in this area. For hatchery fish or for wild fish released during active fishing seasons, it is also important to consider the food safety issues imparted by lidocaine (and other pharmaceuticals) in the event that the fish are consumed. The electroimmobilization literature is still riddled with knowledge gaps, and electroimmobilization has yet to be truly incorporated into animal care guidelines. Further research like the experiment described herein would be of substantial benefit toward the formulation of evidence-based guidelines for the use of this technology.

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ORCID


Connor H. Reid https://orcid.org/0000-0001-9431-9044

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.