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Evaluating immobilisation thresholds and suitability of conductive glove electrodes for largemouth bass electroanaesthesia

Connor H. Reid [a,*], Albana I. Berberi [a,b], Kara M. Scott [a], Sam J. Woods [a,c], Jonathan D. Midwood [d], Steven J. Cooke [a]

[a] Fish Ecology and Conservation Physiology Laboratory, Department of Biology, Carleton University, Ottawa, ON K1S 5B6, Canada
[b] Social Ecology and Conservation Collaborative, Carleton University, Ottawa, ON K1S 5B6, Canada
[c] Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada
[d] Fisheries and Oceans Canada, Great Lakes Laboratory for Fisheries and Aquatic Science, 867 Lakeshore Rd., Burlington, ON L7S 1A1, Canada

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ABSTRACT

Although electroanaesthesia (immobilising fish with weak currents and direct electrode contact for short-term handling and surgeries) is becoming more common, detailed data on electroanaesthesia immobilisation thresholds and potential morphological predictors of such thresholds remain sparse. We administered electroanaesthesia to largemouth bass (*Micropterus nigricans*) over a large range of body sizes using conductive mesh gloves connected to a power supply with fine-scale current control and real-time output display, noting current strengths at which tetany occurred. We also investigated whether a range of morphological indices were correlated with the currents required to induce tetany as a proxy for electroanaesthesia. Larger fish required stronger currents before tetany was observed, and larger fish were also more likely to fail to reach tetany before the maximum output on the apparatus (30 V) was reached, a finding that was slightly offset by increasing condition factor. Body length was a suitable predictor of the current required to induce tetany; no other morphometric indices examined were superior to length in this regard. However, there was very high variability in current strengths required to induce electroanaesthesia, likely attributable to consistency challenges associated with the use of conductive mesh gloves as electrodes (e.g., location of contact, wear-and-tear on gloves). We explore the implications of our results for applications of electroanaesthesia in the field, and make recommendations for the development and implementation of novel technologies and best practices when using electroanaesthesia on fish.

1. Introduction

In research and aquaculture, fish often need to be sedated or anaesthetised for handling and invasive procedures (e.g., tag implantation surgeries). When anaesthesia is necessary, chemical anaesthetics such as tricaine methanesulfonate (MS-222) or clove oil (eugenol/isoeugenol) are regularly used on a variety of fishes (Ross and Ross, 2008; Aydın and Barbas, 2020). Alternatively, researchers may consider using electrical methods of fish immobilisation (henceforth “electro-immobilisation”). Electro-immobilisation techniques are generally associated with rapid overall handling times, and allow for fish to be immediately released into the wild upon recovery with no concerns of contamination or drug clearance times (reviewed in Reid et al., 2019). While the terminology used to refer to various electro-immobilisation practices is not consistent (e.g., “electronarcosis”, “electrosedation”, etc.; cf. Hudson et al., 2011; Trushenski and Bowker, 2012; Kim and Mandrak, 2019), here we use the term “electroanaesthesia” to refer to the practice of immobilising fish with weak currents administered via direct contact with electrodes (e.g., conductive mesh gloves, electrode straps) while the fish is above water, and alleviated upon cessation of current (i.e., fish regain equilibrium and motor control almost instantaneously, facilitating rapid handling and release).

Just as chemical anaesthetics should be administered to fish using consistent doses grounded in empirical data from dose-response curves in the most similar species, sizes, and conditions (e.g., water temperature) as possible, the necessary “doses” for electro-immobilisation methods should also be reasonably consistent in fish of the same species and similar sizes, assuming environmental variables like water

* Corresponding author.
E-mail address: connorreid@cmail.carleton.ca (C.H. Reid).

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conductivity are consistent. Electrically underdosing fish will lead to inadequate immobilisation, while overdosing fish can lead to injuries and death (similar to well-studied phenomena in the electrofishing literature; Snyder, 2003; Dolan and Miranda, 2004). Pulsed DC currents, such as those commonly employed in electric stunning for temporary immobilisation or euthanasia, have many variable parameters (primarily voltage; pulse frequency, width, shape, and pattern; and exposure time to induce stunning; Trushenski and Bowker, 2012; Grimsbø et al., 2016). Continuous DC as used in electroanaesthesia is much simpler because voltage (which mediates current output) is often the main setting that needs adjustment, as exposure time is continuous for as long as fish need to be immobilised and there are no pulse waveforms to modify. Thus, electroanaesthesia offers a potential advantage over electrostunning and chemical anaesthesia in that real-time control over the dose is both possible and relatively straightforward. Most of what is known about how fish respond to electric currents, however, comes from electrofishing research, where behavioural responses have long been characterised and studied (even if the underlying physiological explanations offered at the time are inadequate from a modern lens; Vibert, 1963; Snyder, 2003). How fish respond to more recently developed electro-immobilisation techniques (especially electroanaesthesia), tailored towards immobilising individual fish in relatively controlled settings for handling and invasive procedures, is less well understood.

Electroanaesthesia differs from electrofishing in several notable ways. In boat electrofishing, much stronger currents are applied such that the net power (voltage [V] × current [A]) delivery to fresh water of low conductivity is ideally ~2750–3250 W at low conductivity (~100–150 μS; Miranda, 2009), versus (as an example) 0.12–0.3 W (4–10 mA at 30 V) delivered in electroanaesthesia. Modern electrofishing usually employs pulsed DC and at a general setting that cannot be tailored to the immobilisation thresholds of individual fish, as opposed to electroanaesthesia which typically administers continuous DC to individual fish with real-time "dose" control. Fish are also not meant to come into contact with electrofishing electrodes, while this is ideal for safe electroanaesthesia (n.b., some setups such as that reported by Hudson et al., (2011) do not require electrode contact but, as the authors mention, handling fish in water between live electrodes leads to current passing through researchers' hands, which invokes a number of health and safety concerns). Lastly, fish captured through electrofishing are often stunned and require time to recover equilibrium and motor control (Dolan and Miranda, 2004), whereas recovery following removal of the current in electroanaesthesia happens virtually instantaneously (Abrams et al., 2018).

Electroanaesthesia is typically administered using bespoke commercially-developed devices emitting continuous direct current (DC) for the purposes of facilitating fish handling during surgeries and other extensive procedures where immobilisation of fish is required (e.g., Vandergoot et al., 2011; Ward et al., 2017; Abrams et al., 2018). Some other ad hoc options have been explored for electroanaesthesia, such as TENS (transcutaneous electrical nerve stimulation) units designed for physiotherapy applications in humans that output an asymmetrical pulsed alternating current (AC) (e.g., Dembkowski et al., 2021), but the success of such methods in adequately immobilising fish is inconsistent and appears to vary across species and other factors that are not yet understood (CHR, unpublished data). Moreover, both commercial and ad hoc devices can vary considerably in current output control capabilities; most TENS units offer very coarse control with ~0–80 mA outputs mediated by a small dial (Izzo et al., 2023), while commercial devices may offer only several discrete settings (Ward et al., 2017). We are unaware of any available devices designed or easily adaptable for field conditions that can display relevant current outputs and parameters in real-time.

A standard protocol for administering electroanaesthesia with continuous DC is to hold fish with conductive mesh gloves or straps such that the anode (positive) is over the hindbrain area and the cathode (negative) is on the caudal peduncle region, increase the voltage slowly until tetany (full-body muscle contraction) is observed, and then decrease the voltage slightly until the body is relaxed (Reid et al., 2021). This should allow for proper, complete immobilisation for as long as the fish is in contact with the electrodes. The purpose of this experiment was to evaluate the efficacy and consistency of electroanaesthesia using a conductive glove electrode approach. We assessed the quality and consistency of relationships between current strength and the induction of tetany (as a proxy for electroanaesthesia) in relation to fish body size and morphometry in largemouth bass (*Micropterus nigricans*, formerly *M. salmoides*; Kim et al., 2022).

2. Methods

This experiment was conducted in accordance with animal use protocol #110723, approved by the Carleton University Animal Care Committee under Canadian Council on Animal Care guidelines.

2.1. Field site and experimental protocol

Forty largemouth bass (total length: 184–403 mm; mass: 77.5–920.0 g) were angled from Lake Opinicon, Ontario, Canada on 30 August 2022. Fish were divided into two large circular holding tanks on the docks of Queen's University Biological station (Elgin, Ontario) and tested on 31 August 2022. Two fish at a time were netted from one of the tanks and transferred individually to 45 L holding coolers prior to testing. Testing was performed on a set-up consisting of a 45 L plastic bin with an inverted perforated lid, on top of which sat a foam mat spread over two tape-covered bricks to form a makeshift surgical trough. A submersible pump was placed inside the bin and connected to a tube that extended through the bin's side to the foam trough and into the mouths of the fish, allowing irrigation of the gills before the water returned into the bin through holes in the lid. Water was refreshed between every second or third fish, and the mean water temperature during trials was 24.2 °C (range: 23.3–24.7 °C).

Each fish was individually hand-netted from the cooler and placed on the handling bin. All fish were held ventral side-up by the same handler (CHR) wearing insulated rubber lineworker gloves under conductive mesh gloves designed for use with TENS units. All fish were held such that the palm and fingers of the anode (+) glove were under the fish facing upwards, supporting the head/opercular region, while the thumb was in contact with the lower jaw. The cathode (−) glove gently gripped the caudal peduncle region in the same hand position as one would use to grip a hammer, for example. The conductive gloves were connected to a low-voltage benchtop power supply unit (PSU; KD3005D, KORAD, Dongguan, China) capable of providing maximum voltage and current outputs of 30 V and 5 A, respectively. The power supply unit allowed for constant voltage (current output adjusts to meet a desired voltage) or constant current (voltage adjusts to ensure a constant current) modes, with an adjustable current resolution of 1 mA. The PSU was always off when a fish was positioned in the hands of the handler wearing the conductive gloves. The soft plastic irrigation tube was placed in the fish's mouth through the fingers of the anode glove, and the fish was repositioned until regular opercular movements were observed (a process that was not quantified but generally took approximately 5–10 s).

Next, the power supply was switched on and current strength was gradually increased by individual increments on the current dial. The PSU was used under constant current mode (i.e., the current is manually adjustable and the voltage adjusts automatically to ensure that the desired current setting remains constant) and displayed the actual output voltage and current in real-time. Theoretically, each increment should have increased current by 1 mA, however in practice the current increases were less than 1 mA per turn (e.g., setting the PSU to 5 mA could result in 3 mA running through the fish, which was known based on the output display that showed the actual current values in real time). Current strength was increased until fish exhibited tetany in the form of full-body muscle contractions, as in practice this constitutes the endpoint for current increases in electroanaesthesia and a subsequent, slight decrease in current to achieve muscle relaxation. Output voltage was validated independently with a multimeter where the electrodes were placed against the metal snaps on the conductive gloves. The current strength was then dialed back down to 0 mA on the PSU, and fish were transferred to a measuring trough to collect total length and then weighed using a digital scale (OHAUS, Parsippany, NJ, U.S.A.). Given that decreasing current very slightly upon achieving tetany is a standard practice for inducing electroanaesthesia (Reid et al., 2021), as described above, the currents required to induce tetany were used as a proxy for currents required to induce electroanaesthesia since these should differ by only a small magnitude and be proportionally consistent across fish. Current and voltage readings from the device for fish that reached tetany are shown in Table 1.

Bin water temperatures were recorded after each trial with a digital thermometer (TM-KIT; axGear, Blaine, WA, U.S.A.), and the water was changed after every second or third fish, or sooner if the water began to appear foamy. From length and mass data, we calculated Fulton's condition factor (K = 100 × mass/(lengthˆ3)), where mass is measured in g and length in cm (per Froese, 2006).

For morphometry images, fish were placed laterally on a white corrugated plastic board illuminated with two headlamps for photographs. A ruler was placed near the fish and adjusted in height by adding or removing thin plastic sheets until it sat at a height in line with the sagittal plane of the fish (i.e., the ruler was the same distance from the camera lens as the maximum visible surface area of the fish). Fish were positioned as straight as possible on the median plane (i.e., minimal curvature up or down from head to tail) and a photo was taken with a phone camera (iPhone 8 Plus, wide camera; 28 mm *f*1.8; 3 MP). Fish were then tagged externally with an anchor tag as part of a separate project and then released off the docks ensuring that fish would not be re-used in the experiment. Total handling and processing time for each individual ranged from 4 to 19 min (mean = 8 min), largely depending on how quickly or slowly fish reached the tetany stage and including the time taken by attempts to quantify voluntary movement in fish as described below.

2.2. Morphometry data

Images of fish were individually loaded into ImageJ (1.53t; National Institutes of Health, Bethesda, MD, USA). Calibration of measurement length using the straight line tool was performed each time using the ruler visible in each photo. A summary of measurement locations is available in Fig. 1. Using the straight line tool, measurements were taken for head depth (vertical distance from dorsal to ventral surfaces of the head with the line falling at the posterior edge of the eye), body depth (three different measures from dorsal to ventral surfaces: one at the base

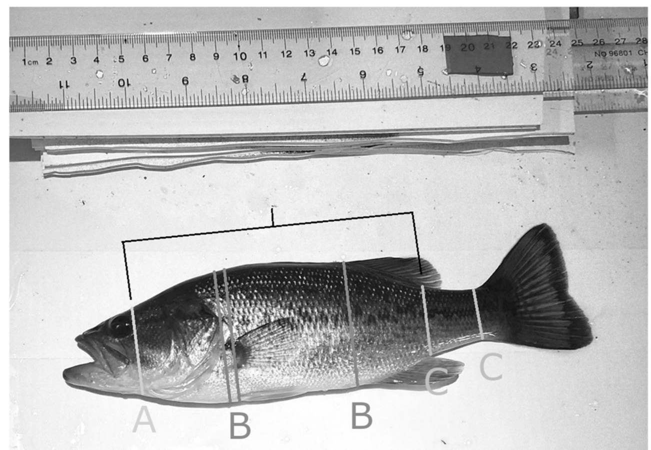

Fig. 1. Example overview of the locations of morphometry measurements taken from each fish. From anterior to posterior, straight-line dorsal-ventral depths included head depth (A), three measures of body depth (B), and two measures of caudal peduncle depth (C). The straight line distance and surface area between the head (A) and first caudal peduncle line (leftmost C) were also recorded. Note the ruler at the top of the image on sheets of plastic used to adjust the height of the ruler so that it lay at the same distance from the camera as the fish (assessed prior to photography by the handler positioning the fish).

Table 1
Current and voltage readings from the benchtop power supply at the tetany thresholds for fish that did exhibit tetany (those that failed to reach tetany are excluded here). Rows are ordered by the current strength at which tetany was observed. Note the highly variable voltage readings that do not show a clear positive relationship with current. *The 0.5 mA current value is an estimate of the actual output where the display output showed 0 mA, despite an increase in the current dial and visible response from the fish.

Fish	Length (mm)	Mass (g)	K	Current (mA)	Voltage (V)	Resistance (Ω)	Power (mW)
10	206	107.0	1.22	0.5 *	23.69	47380	11.85
4	191	89.5	1.28	1	18.90	18900	18.90
16	215	139.0	1.40	3	8.38	2793	25.14
18	184	77.5	1.24	3	14.55	4850	43.65
35	233	175.5	1.39	3	15.68	5227	47.04
32	241	200.0	1.43	4	17.66	4415	70.64
5	237	184.0	1.38	5	30.00	6000	150.00
8	321	455.0	1.38	5	29.30	5860	146.50
19	248	196.0	1.28	5	16.13	3226	80.65
14	268	247.5	1.29	6	12.64	2107	75.83
20	268	251.5	1.31	7	15.77	2253	110.38
30	219	158.0	1.50	7	17.42	2489	121.92
2	307	421.0	1.46	8	24.58	3073	196.61
7	386	828.0	1.44	8	24.62	3078	196.93
15	318	443.0	1.38	8	11.82	1478	94.53
17	259	233.5	1.34	8	11.23	1404	89.82
34	246	184.0	1.24	8	18.37	2296	146.98
6	377	805.0	1.50	10	24.94	2494	249.40
3	367	681.5	1.38	11	30.00	2727	330.03
40	233	177.5	1.40	11	20.03	1821	220.32
1	311	382.0	1.27	12	28.07	2339	336.86
39	291	322.0	1.31	12	15.32	1277	183.79
36	255	220.0	1.33	13	28.08	2160	365.04
26	281	295.0	1.33	15	17.27	1151	259.13
28	188	85.0	1.28	15	15.09	1006	226.35
29	360	682.5	1.46	22	26.8	1218	589.69

of the pectoral fin, one at the base of the pelvic fin, and one at the base of the soft dorsal fin), and caudal peduncle depth (two different vertical measures, one from the posterior end of the anal fin and one at the thinnest point of the caudal peduncle). The lines for head depth and first caudal peduncle depth were imprinted on the image, and the straight line distance between the head depth and first caudal peduncle depths was measured. Lastly, the surface area of the body was measured using the area measuring tool, covering the body between the head and first caudal peduncle lines (i.e., the approximate area that fell between where the handling gloves were located on the fish).

2.3. Additional attempted methods

The slow, incremental approach to increasing current was used as we had attempted to quantify symptoms of electroanaesthesia (impairment of opercular movements, incidence and severity of muscle contractions) as current increased. Unfortunately, the symptom data were highly sporadic and subject to confounds inherent to the use of conductive gloves as electrodes. The exact locations and positions of hands on fish were inconsistent over large size ranges, leading to differences in physical pressures (which could conceivably affect opercular movements). Because electric currents tend to travel down the "path of least resistance", i.e., shorter distances and through extracellular fluids and tissues with lower electrical impedance/higher conductivity (Cornish, 2006), it is conceivable that even subtle inconsistencies in electrode glove placement and positioning could have led to unquantifiable changes in the paths that currents took through the fish, potentially affecting the incidence and severity of symptoms that could be sensitive to such changes.

To assess whether fish were truly immobilised by the current, we attempted to induce voluntary movements at multiple points throughout the handling process by poking and rubbing the ventral body wall between the pelvic fins and cloaca with the blunt end of a plastic scalpel handle. However, despite several minutes of attempts each on multiple fish, the fish in this experiment did not respond to any form of physical stimulation even in the total absence of electric current. This could be a result of tonic immobility, wherein fish are immobilised simply by being placed upside down (an effect most obvious in elasmobranchs; Watsky and Gruber, 1990), however the degree to which tonic immobility may manifest has not been explicitly quantified in centrarchids. Our animal care and use protocol did not allow for more invasive stimuli to be used, and so we were unable to quantify the degree or quality of immobilisation throughout this experiment and pinpoint thresholds of immobilisation.

2.4. Statistical analyses

All statistical analyses were conducted in RStudio v. 2023.03.1–446 (RStudio Team, 2023) with R v. 4.1.0 (R Core Team, 2022). Figures were generated using "ggplot2" (Wickham, 2016) and supporting packages "ggfortify" (Tang et al., 2016), "ggiraphExtra" (Moon, 2020), "ggpubr" (Kassambara, 2023), and "factoextra" (Kassambara and Mundt, 2020).

Because of the large number of correlated morphometric parameters recorded, a principal component analysis (PCA) with a correlation matrix was performed to attempt to reduce the dimensionality of the dataset using the "prcomp" function and visualised with "autoplot()". Variables included in the PCA were fish mass, total length, condition factor, head depth, mean body depth, anterior caudal depth, eye-caudal length, and eye-caudal surface area. All variables were scaled and centred. The first principal component (PC1) accounted for 86.3% of the variation in the data, and highly similar loading values were obtained for all variables except for condition factor, while the second principal component (PC2) accounted for 12.1% of the variation and was primarily influenced by condition factor (Table S1, Fig. S1). Principal components 3 through 8 each explained less that 1% of the total variances. Based on the high degree of correlation between morphometric variables and the PC loadings, fish length and condition factor were selected for use as predictor variables in subsequent analyses to minimise unnecessary autocorrelation between other potential predictors. Temperature was not included in analyses as this varied by only 1.4 °C throughout the experiment and had no relationship with fish mass ($r = -0.045$; DF = 37; $P = 0.787$).

The current required to induce tetany as a proxy for electroanaesthesia thresholds, excluding fish for which the power supply maxed out, was ln-transformed (natural logarithm) and modelled with a general linear model with fish length and condition factor as predictor variables. Whether electroanaesthesia could successfully be achieved, or if the power supply output was maxed out before electroanaesthesia could be achieved, was modelled with a generalised linear model with a binomial error distribution and fish length and condition factor as the sole predictors. General and generalised linear models were analysed with the "Anova()" function from the "car" package (Fox and Weisberg, 2019).

For one fish (#12), the final symptom/stage line appeared to be missing from the field notes, making it unclear whether the fish reached tetany or if the power supply had been maxed out. Since this information was necessary for assigning an electroanaesthesia endpoint, this fish was excluded from all analyses. For another fish (#10), tetany was observed at the lowest setting such that the power supply still displayed "0" as the current output. The current value was therefore initially recorded as 0.5 mA (between 0 mA and the lowest displayable value, 1 mA) to provide a conservative estimate of the actual amount of current travelling through the fish. When resistances were calculated based on voltage and current data for all fish in Table 1, however, fish #10 and fish #4 were found to have implausibly high resistances. These fish were therefore excluded from the analyses on the grounds that the current and/or voltage readings may have been inaccurate or confounded by some unknown factor (i.e., final $n = 37$).

3. Results and discussion

3.1. To what extent can the current strengths required to induce tetany be expected to scale with fish morphology?

Fish body length was weakly correlated with the ln-transformed current required to induce tetany ($\beta = 0.005$; $F = 5.92$; DF = 1; $P = 0.024$), used here as a proxy for the current required to induce electroanaesthesia (Fig. 2). No relationship was found between condition factor and ln-transformed tetany current strength ($F = 0.63$; DF = 1;

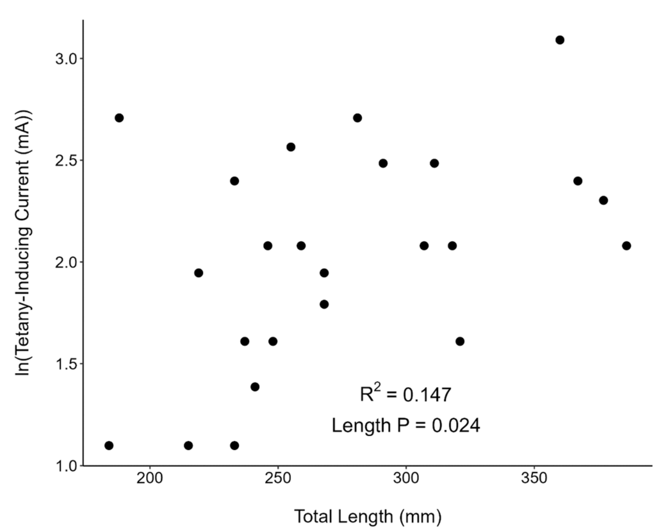

Fig. 2. Natural logarithm-transformed current strength at which fish exhibited tetany (full-body muscle contractions) as a function of fish total length.

$P = 0.436$), and overall, the model explained little variation in this response (adjusted $R^2 = 0.147$). There were, however, relationships between both fish length and condition factor with respect to whether the power supply reached maximum output before tetany could be achieved (Fig. 3). For each 1 mm increase in length, the odds ratio (expected multiplicative change) of whether fish would reach the tetany stage was 0.955 (likelihood ratio [LR] $\chi^2 = 21.75$; DF = 1; $P < 0.0001$); i.e., the expected probability of reaching tetany decreases by ~4.5% per 1 mm increase in length at a given condition factor. For every 0.1 increase in condition factor, the odds ratio of whether fish would achieve tetany was 1.36 (LR $\chi^2 = 4.07$; DF = 1; $P = 0.044$); i.e., the expected probability of reaching tetany increases by 36% per 0.1 increase in condition factor at a given length.

Based on the principal component analysis, length was the only direct measurement of fish size/shape selected for use as a predictor in subsequent analyses, as all correlated size/shape measures (excluding condition factor) yielded similar loadings on PC1. Length also contributed more than any other direct morphometry measurements to PC2, which was primarily influenced by condition factor (Table S1). Revisiting analyses with another variable in place of fish length yielded only very minor changes in statistical outputs with either no impacts on the interpretation of our results, or leading to models with poorer fit and less satisfactory in meeting testing assumptions. For example, in the model for ln-transformed current strength at which tetany was induced, replacing length with mean body depth changed P values for length/mean body depth and condition factor from 0.024 to 0.011 and 0.436 to 0.226, respectively, and changed adjusted R^2 of the model from 0.147 to 0.201. Replacing length with mass reduced adjusted R^2 of the model from 0.147 to 0.126.

3.2. Insights for electroanaesthesia applications in the field and other research settings

We did not find evidence that any particular measure of fish shape/size is superior for predicting the thresholds of major symptoms under weak continuous DC currents in largemouth bass. Researchers employing electroanaesthesia may therefore be able to use more basic measurements such as total length for the purposes of predicting required current outputs rather than more complex morphometric measurements. It is unclear whether this principle holds true for fishes with vastly different body shapes (e.g., fish that are elongate, deep-bodied or

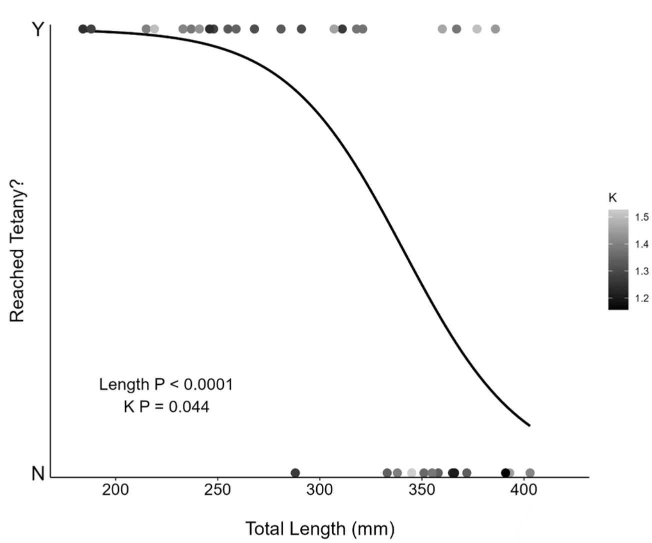

Fig. 3. Predicted probability of whether fish reached full-body tetany as a proxy for electroanaesthesia-inducing current strength or if the power supply would achieve maximum output before this stage was reached, as a function of total body length. Points are shaded based on condition factor.

otherwise have a body form unlike a typical fusiform fish) where the electric current densities and dispersal throughout the body might vary substantially. Indeed, most knowledge about the passage of current through fishes comes primarily from research on electrofishing (e.g., Dolan and Miranda, 2003; Snyder, 2003) and aquaculture stunning practices (e.g., Robb et al., 2002) where current types (e.g., pulsed DC), settings (e.g., voltages), fish position and orientation with respect to the electrodes, a lack of contact with the electrodes (i.e., currents also pass through water), and other factors differ markedly from standard electroanaesthesia practices. In electrofishing and electric stunning, currents must spread and pass through waters of varying conductivity. Stronger currents may be required to induce longer immobilisation in fish of a given size (Walker et al., 1994) and stronger currents should also be needed to immobilise larger fishes (Dolan and Miranda, 2003), but relationships between fish size and stunning duration are not always observed (Kim et al., 2017).

Our results corroborate the existence of a relationship between fish body size and the current strength required to induce electroanaesthesia, but with considerable variability in our data. Fish body length was positively associated with higher currents required to induce tetany (as our most direct proxy for electroanaesthesia thresholds; Fig. 2), and the power supply was more likely to reach maximum output before tetany could be induced for larger fish (Fig. 3). Interestingly, while no associations with condition factor were evident for the thresholds of current strength required to induce tetany, fish with higher condition factors (i.e., more muscular/fatty) were actually more likely to reach tetany before maximum current output was reached than more slender fish. This apparent contradiction may be the result of a genuine but weak effect size for condition factor. For the analyses on specific current strengths required to induce tetany, fish size in general was a much more important determining factor, yet the data were also very scattered due to imprecision and inconsistency in the administration of electroanaesthesia with mesh gloves as electrodes. On the other hand, analysing whether tetany was achieved as a binary outcome provided a different response, with a different distribution, that may have been more sensitive to the effects of fish size and morphometry (including length). Still, it remains difficult to explain the mechanics of this observation in the absence of more knowledge on the specific current pathways and densities throughout fish tissues. Higher levels of fatty tissues contribute significantly to increasing condition factor in many but not all fishes (McComish et al., 1974; Costopoulos and Fonds, 1989; Herbinger and Friars, 1991; Salam and Davies, 1994; Mozsár et al., 2014), and fats are poor conductors of electricity in fish as in other animals (Hartman et al., 2015). Although speculative, it could be the case that fish with higher condition factors in this experiment were slightly more insulated in certain regions (most adipose tissues tend to be distributed subcutaneously and in/around the coelom; Weil et al., 2013; Ren et al., 2018), and that current paths could therefore have been slightly more concentrated into tissues with lower impedances, such as those in the central nervous system that are thought to play a role in the induction of tetany (Sharber and Sharber Black, 1999). This effect, if true, would have to have been great enough to slightly counteract the negative relationship between fish size and likelihood of tetany induction (i.e., larger fish were more likely to fail to reach the tetany stage, but more insulated large fish were less likely to fail than poorly insulated fish), but not so great as to actually affect the thresholds at which tetany was induced. Internal tissue composition such as lipid distributions will vary between males and females (Brown and Murphy, 1995; O'Connor et al., 2013), and since fish were released alive, we have no data on fish sex in this experiment.

While the conductivity of whole fish bodies can vary across sizes and species (Miranda and Dolan, 2003; Reynolds, 2021), this has only been studied in an electrofishing context where the amount of current passing through fish relative to surrounding waters is not known; since the power supply we used was set to deliver (and display) a particular current value in real time, it is unlikely that whole-fish body conductivity affected our results in any meaningful way.

3.3. Insights for electroanaesthesia apparatus designs and equipment

It is most likely that much of the variation in our data is a consequence of employing conductive mesh gloves as electrodes for electroanaesthesia. Conductive gloves are theoretically very useful as they allow for considerable hand mobility and control when handling fish. However, standardising placement of the hands and fingers can be difficult. The exact positioning of the conductive gloves, serving as the electrodes in contact with the fish, may be highly variable with respect to the anatomy of fish, particularly over a wide range of fish sizes where hand positions must be shifted and altered to ensure the fish is secure and that gills are properly irrigated during handling or surgeries.

Based on the assumption that the ability to engage in osmoregulation should correspond to fairly constant conductivity within whole fish bodies (Kolz, 2006), fish of similar sizes and dimensions should have similar immobilisation thresholds and similar resistances when held consistently (i.e., both current and voltage output from a power supply should be reasonably similar). We observed a clear lack of a clean relationship between current output and voltage output on the PSU when tetany was achieved (Table 1), suggesting significant fluctuations in resistance of the whole circuit. The most likely, non-mutually exclusive causes of fluctuating resistance are stretching and breaking of the metal mesh within the conductive gloves (wear and tear), and artefacts of different hand positions while securing fish over a large size range. Build-up of relatively non-conductive materials on the gloves is also conceivable, however the water used in this experiment was clean and the only substantial build-up that could be observed in places was fish mucus, which tends to have relatively high conductivity (Guardiola et al., 2015). Although we changed gloves whenever possible to minimise the effects of conductivity loss through metal mesh breakage, even "large" sized conductive gloves must be stretched to fit over the insulated rubber gloves and each trial entailed more movement and stretching of the mesh gloves. Some degree of conductive mesh breakage was highly likely to occur within the span of only a few trials, however the fine scale of the steel mesh embedded within the fabric meant quantifying breakage was unfeasible. Unfortunately, these are unavoidable realities for field applications of electroanaesthesia with currently available technologies. Alternative electrodes include fixed options like electrode pads or straps (e.g., Reid et al., 2022), yet these are also subject to wear and tear and may not be suitable for very small or very large fishes.

Future bespoke electroanaesthesia apparatus designs might take into consideration a way of standardising electrode positioning and contact on fish (e.g., solid, non-abrasive electrode plates rather than conductive mesh pads or gloves) to improve handling consistency. If the tools and methods of electroanaesthesia can be further refined and standardised to minimise potential confounding effects such as those attributable to physical handling variation, scoring symptomatic endpoints with respect to fish size and other parameters of interest may be considerably easier and more reliable. For example, tetany is generally the key symptomatic endpoint denoting that current should no longer increase and instead needs to decrease slightly until muscle relaxation returns (e.g., Reid et al., 2021; Izzo et al., 2023; C.S. Vandergoot, personal communication), but ventilatory movements might yet prove to be another valuable symptomatic marker of central nervous system impairment. Ventilatory movements are a complex product of efferent (motor) outputs and afferent (sensory) feedback through multiple cranial nerves innervating the face and gill regions (Shelton, 1970; Taylor et al., 1999), and electroanaesthesia is likely no different from electrofishing (and other circumstances of exogenous electric currents being applied to fish) where the underlying mechanisms are, in essence, very likely facets of epilepsy operating on the central nervous system (Sharber and Sharber Black, 1999).

Another recommendation we suggest for future electroanaesthesia devices is finer control over current output and real-time displays, which would provide data that could be paired with fish size and other parameters and incorporated into routine methodological reporting in the literature. Even bespoke commercially developed devices may not provide adequate immobilisation for surgeries in all contexts (Lamglait and Lair, 2021), and a lack of fine-scale control over current strength and/or inability to provide sufficiently strong currents for larger fish could at least partially explain this. Like other low-voltage benchtop power supplies, the one used in this experiment provided a decent resolution of current control and displayed both current and voltage output in real time. For fieldwork, such power supplies are often not viable and/or genuinely hazardous as they are not designed to be used in or around water, nor are they intended to be connected to living organisms. Future research is highly recommended to include detailed reporting of electric current settings, immobilisation thresholds, and relevant environmental variables. We encourage researchers and industry professionals to collaborate in efforts to develop and share "dose-response" data and work together to design equipment that is safe (for fish and users), effective, and versatile while ensuring that best practices are maintained during field applications of electroanaesthesia.

CRediT authorship contribution statement

Berberi Albana I.: Data curation, Investigation, Writing – review & editing. **Reid Connor H.:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Woods Sam J.:** Investigation, Writing – review & editing. **Scott Kara M.:** Investigation, Writing – review & editing. **Cooke Steven J.:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Midwood Jonathan D.:** Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fishres.2023.106931.

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