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Cardiac activity in walleye (*Sander vitreus*) during exposure to and recovery from chemical anaesthesia, electroanaesthesia and electrostunning

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Abstract

Handling and conducting invasive procedures are necessary for aspects of fisheries science, invariably inducing a stress response and imposing energetic demands on fish. Anaesthesia or immobilisation techniques are often used in an attempt to mitigate stress and improve welfare, yet these also come with their own impacts on post-release recovery. Here, the authors investigated whether changes in cardiac activity (heart rates over time, heart rate maxima, and scopes) differed in adult walleye (*Sander vitreus*) anaesthetised with AQUI-S® 20E (eugenol), electroanaesthetised with a transcutaneous electrical nerve stimulation (TENS) unit or electrostunned with a commercially developed stunning unit. This experiment was divided into two trials. In the first trial, fish were implanted with heart rate loggers and left to recover for *c.* 4 days. In the second trial, fish were implanted with heart rate loggers, given 3 days to recover and re-exposed to their initial treatments (excluding surgery). Post-treatment cardiac activity was quantified for both trials. Although highly variable across individuals, the authors found no significant differences in heart rate changes over time or recovery times among treatments. Maximum heart rates were consistent among treatment groups, yet significant differences in heart rate scope provided further evidence of strong interindividual variation in the second trial. Based on these results, the authors did not identify any welfare-relevant differences or concerns associated with one treatment over another. Further investigations of the relationships between measures of cardiac function and other physiological stress markers would be beneficial towards identifying best practices for fish handling in fisheries science.

KEYWORDS
anaesthesia, cardiac function, electro-immobilisation, handling, heart rate, walleye

1 | INTRODUCTION

Fish are frequently subjected to handling stress associated with fisheries research, management, and aquaculture activities with stressors varying from relatively brief and minor (*e.g.*, transfer from one holding tank to another) to more extensive or invasive procedures (*e.g.*, surgery, non-lethal gamete removal). The generalised stress response, the cascade in physiological and behavioural changes as the fish attempts to regain homeostasis (Wendelaar Bonga, 1997), ensues, with the magnitude of the stress response and its effects on an organism being

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generally reflective of the severity and duration of the stressor (Barton, 2002; Gesto *et al.*, 2015). Maximising welfare is therefore a key consideration when capturing, handling, and releasing fish, thereby reducing the potential for adverse effects of acute and potentially chronic stress (Iwama, 2007; Volpato, 2009). Thus, for intensive handling or invasive procedures, various sedation or anaesthesia techniques are used to promote welfare and survival (Ross & Ross, 2009). Tricaine methanesulfonate (MS-222) is the only anaesthetic currently approved for use on wild fish that may be released with the possibility of human consumption in Canada and the U.S.A. (where fish must be held for 5 and 21 days in water above 10°C before release, respectively; Health Canada, 2010; Trushenski *et al.*, 2013), but other substances with shorter holding periods are being investigated as well. One such substance is eugenol, which is one of the active anaesthetic ingredients in clove oil, and is being considered for use (*e.g.*, in the U.S.A.) as an anaesthetic in fish with no required pre-release holding period (Bowker *et al.*, 2015; Meinertz *et al.*, 2016). Alternatively, researchers have turned to electrical methods of fish restraint (electro-immobilisation) that allow for fish to be released immediately (*e.g.*, Hayden *et al.*, 2014; Kim *et al.*, 2017), are generally associated with rapid induction and recovery times (Balazik *et al.*, 2013; Keep *et al.*, 2015) and do not currently face the same logistical challenges and legal ambiguities associated with chemical anaesthesia (Faust *et al.*, 2017; Topic Popovic *et al.*, 2012; Trushenski *et al.*, 2013; Vandergoot *et al.*, 2011). Electro-immobilisation may be administered in several ways, with the most common and effective options being electroanaesthesia (where immobilisation and recovery are quickly achieved with a weak current) and electrostunning (where rapid immobilisation and prolonged recovery follow a brief exposure to a more intense current, usually pulsed direct current; reviewed in Reid *et al.*, 2019). In any case, fish will exhibit a stress response following handling and anaesthesia/electro-immobilisation; however, the stress response may appear to be mitigated, exacerbated or unaffected by each technique depending on the species, anaesthetic or electric current type, and the chosen methods for quantifying stress (Olsen *et al.*, 1995; Zahl *et al.*, 2012). Moreover, "welfare" itself may be defined and measured in different ways, and the presence or absence of physiological stress alone does not necessarily correspond with the welfare state of an individual (Volpato, 2009). Markers of stress and welfare must therefore be selected with careful consideration of what they do and do not represent, and how useful they are for a particular research question.

Numerous metrics are available to measure aspects of physiological stress over various timescales, each with their own advantages and disadvantages (reviewed by Sopinka *et al.*, 2016). In teleosts, blood sampling is used extensively to determine cortisol, glucose, and other well-established stress marker levels in laboratory (*e.g.*, Ramsay *et al.*, 2009) and field (*e.g.*, Meka & McCormick, 2005) settings. Yet, when a sampling technique is itself a stressor (*e.g.*, phlebotomy, see Lawrence *et al.*, 2018), other methods of quantifying stress in individuals over extended time periods must be explored. Some non-invasive approaches, such as water-borne cortisol (*e.g.*, Ellis *et al.* 2004), may be useful if experimental and holding conditions allow for their use. However, these are not always viable, particularly for field experiments where fish are kept with other individuals and/or released in the wild to provide large-scale ecological (*e.g.*, movement) data. Biologgers and transmitters may offer a diverse suite of solutions to this problem (Cooke *et al.*, 2012); although some capture and invasive handling is inevitable, fish may be left to recover and return towards homeostasis while the implanted logger or transmitter continues to collect data. Electronic tags currently are only capable of measuring less direct (secondary and tertiary) metrics of stress (*e.g.*, heart rate) but are nonetheless incredibly useful (Cooke *et al.*, 2016).

Heart rate loggers can be implanted in fish to record cardiac activity *via* electrocardiograms (ECGs) (Muller *et al.*, 2020; Skeeles *et al.*, 2020). In many fishes, changes in the stroke volume of the heart can contribute more to the regulation of cardiac output than changes in stroke frequency (*i.e.*, heart rate; Farrell, 1991; Thorarensen *et al.*, 1996), but the latter is still a useful indicator of stress and metabolic rate in fish under certain circumstances (Brodeur *et al.*, 2001). Heart rate (a tertiary or whole-organism metric of stress) is directly related to metabolic rate in some fishes, and by extension is influenced by the endocrine and cellular demands that follow natural circadian rhythms, swimming activity, and feeding/digestion (Anderson *et al.*, 1998; Priede, 1983; Sopinka *et al.*, 2016). Changes in heart rate following exposure to a stressor can correspond to the changes observed in other common stress markers (*e.g.*, cortisol and other blood chemistry changes; Svendsen *et al.*, 2021). Stress associated with various handling procedures has been shown to correspond with increased heart rates in fishes such as northern pike *Esox lucius* L. (Armstrong, 1986), brown trout *Salmo trutta* L. (Laitinen & Valtonen, 1994) and largemouth bass *Micropterus salmoides* (Lacepède 1802; Cooke *et al.*, 2004). During anaesthesia, heart rate typically increases rapidly (tachycardia) during induction and decreases (bradycardia) as anaesthesia reaches the point where equilibrium and reactivity to external stimuli are lost, though this is not always the case (Cooke *et al.*, 2004; Sneddon, 2012). Heart rate loggers have logistical benefits in that they allow for continuous data collection from individuals over an extended period of time without adding additional handling stress with every collected data point, as opposed to more traditional invasive techniques such as blood sampling where each repeated sampling may confound recovery.

The purpose of this experiment was to quantify the relative effects of anaesthesia and electro-immobilisation on heart rate and cardiac function in adult walleye (*Sander vitreus* Mitchill 1818), with the ultimate goal of assessing how suitable anaesthesia by eugenol, electroanaesthesia, and electrostunning are as immediate-release options for use on fish with respect to recovery and welfare. To do this, the authors surgically implanted *S. vitreus* with heart rate loggers and exposed them to chemical anaesthesia with eugenol, electroanaesthesia with a transcutaneous electrical nerve stimulation (TENS) unit, or electrostunning with a commercially developed stunning unit. *S. vitreus* is an ecologically, culturally, and economically significant fish in North America, and is often surgically implanted with transmitters for research purposes, necessitating some form of

anaesthesia/immobilisation (Hayden *et al.*, 2014; Li *et al.*, 1996; Peat *et al.*, 2015; Pothoven *et al.*, 2017; Raby *et al.*, 2018). The authors began with a proof-of-concept trial to examine recovery from each handling technique after implanting the heart rate loggers. The authors then conducted a second trial where a separate cohort of fish was implanted with heart rate loggers under chemical anaesthesia, electroanaesthesia, or electrostunning, and then re-exposed to their individual treatments *c.* 3 days after heart rate loggers were implanted, allowing the authors to quantify heart rate changes over the course of each anaesthesia/immobilisation protocol. Depending on the dose and degree of prior handling, chemical anaesthesia is capable of eliciting sharp decreases in heart rate over several minutes (*e.g.*, Dziaman *et al.*, 2005; Hill & Forster, 2004). The authors therefore predicted that the tachycardia–bradycardia trend generally associated with chemical anaesthesia would be apparent in chemically anaesthetised fish, reaching lower heart rates sooner than fish exposed to electroanaesthesia or electrosty. The authors also expected TENS fish to exhibit elevated heart rates and subsequent recovery patterns typical of fish handled without anaesthesia, and PES fish to show rapidly reduced heart rates during their "unconscious" phase post-stunning before a return to normal levels; however, these predictions are speculative and based on visible symptoms of immobilisation and recovery, as the authors are unaware of any other literature investigating electro-immobilisation and cardiac function.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

This work was conducted in adherence to the American Fisheries Society's *Guidelines for the Use of Fishes in Research* (Jenkins *et al.*, 2014).

2.2 | Field site and experimental subjects

This experiment was performed in 2018 over two separate trials between 19 and 23 March (trial 1) and 3 and 9 April (trial 2). Adult *S. vitreus* were collected from the Sandusky River near Fremont, Ohio, U.S.A. by boat electrofishing surveys and transported in a Castalia State Fish Hatchery vehicle with a *c.* 1136 l transport tank filled with fresh water (mean temperature *c.* 3–4°C before trial 1, and *c.* 5–6°C before trial 2) and an aeration system to the Ohio Department of Natural Resources (ODNR) Sandusky Fisheries Research Station in Sandusky, Ohio, U.S.A. A total of 48 fish were collected for the first trial and 37 fish were collected for trial 2. Mean ± S.D. masses were 934 ± 336 g (AQUI-S® 20E, henceforth "AQUI-S 20E"; please see below for treatment descriptions), 1156 ± 423 g (PES) and 1199 ± 548 g (TENS) in trial 1; and 1029 ± 155 g (AQUI-S 20E), 1061 ± 274 g (PES) and 1299 ± 215 g (TENS) in trial 2. Only one female was captured during this experiment, allocated to the AQUI-S 20E treatment in trial 1.

2.3 | Treatments and handling

In both trials, fish were implanted with passive integrated transponder (PIT) tags into the isthmus (see Vandergoot *et al.*, 2012 for specific protocol) and alternately allocated to one of four treatment groups: AQUI-S 20E, TENS, PES, and a reference group. Fish in the reference group were placed in a floating net pen (4.6 × 6.1 × 3.7 m; #18 nylon net dipped with ultraviolet coating treatment, 19 mm bar measure) in a sheltered boat slip in Sandusky Bay immediately after PIT tagging to account for ambient survival and were not used for any heart rate analyses. Fish in the AQUI-S 20E, TENS and PES treatments were individually subjected to heart rate logger implantation surgeries (Star-Oddi, DST centi-HRT, 19 g in air, 46 mm long × 15 mm diameter), and likewise placed together in the net pen upon recovery. An inherent limitation with using ECG loggers is that they have limited battery life (estimated at *c.* 19 months by the manufacturer, depending on sampling programming) and on-board storage (Muller *et al.*, 2020). For this reason, the loggers were programmed to record ECG traces, heart rate (BPM; recorded to the nearest whole number) and temperature at 80 Hz over a 7.5 s period once every 5 min, with raw ECG traces saved once every 2 h. Loggers also record quality indices (QIs) associated with each heart rate, ranging from 0 (highest quality/least noisy) to 3 (lowest quality/most noisy; Bjarnason *et al.*, 2019).

AQUI-S 20E fish were anaesthetised in a 30 mg l^{-1} bath of AQUI-S® 20E (AQUI-S 20E; distributed by AquaTactics Fish Health, Kirkland, WA, U.S.A.), a general anaesthetic with 10% eugenol (a clove oil extract) as its active ingredient. The selected dose was based on U.S. Fish and Wildlife Service recommendations and previous pilot work on walleye in this system and environmental conditions (C.S. Vandergoot, unpubl. data). Once stage IV anaesthesia had been achieved (*i.e.*, loss of equilibrium and unable to respond to external stimulation; Summerfelt & Smith, 1990), the fish were then placed on a surgical trough for heart rate logger surgeries with an irrigation pump recirculating continuously aerated water over the gills. After surgery, fish were placed in a well-aerated, *c.* 114 l recovery tank. Once the AQUI-S 20E fish had regained equilibrium, they were transferred to the same floating net pen in the adjacent boat slip. Fish in the TENS group were removed from the holding tank and immediately placed on the same surgical set-up. The TENS fish were immobilised by a TENS 9 V DC unit with adjustable current outputs of 0–80 mA connected to TENS electrode pads (330 × 38 mm; placed anterior to the pectoral fins and around the caudal peduncle) and were transferred directly to the net pen immediately after surgery as individual fish instantaneously regained equilibrium after the electric current produced by the TENS unit ceased. Electric current was adjusted manually for each fish until immobilisation was achieved, and so the current outputs used varied across individuals. Fish in the PES group were removed from the holding tank and transferred to a commercially available electrostunning system (PES; Smith-Root, Vancouver, WA, U.S.A.). The PES fish were stunned by a 3 s exposure to 30 V pulsed DC at 100 Hz and implanted with heart rate loggers on the aforementioned surgical set-up; fish were transferred to the net pen

TABLE 1 Induction and recovery times for fish anaesthetised with AQUI-S 20E, and recovery times for fish stunned with the PES, in trial 2

Trial 2	AQUI-S 20E induction time [min–max; mean ± s.d. (s)]	AQUI-S 20E recovery time [min–max; mean ± s.d. (s)]	PES recovery time [min–max; mean ± s.d. [s])
First exposure	220–543; 342 ± 114; $n = 8$	379–1880; 1084 ± 433; $n = 9$	207–657; 443 ± 157; $n = 9$
Second exposure	345–604; 493 ± 86; $n = 8$	762–1611; 1053 ± 310; $n = 10$	139–423; 222 ± 115; $n = 9$

Note: Because of missing/incomplete data, two fish were excluded from AQUI-S 20E induction times and one fish from AQUI-S 20E recovery times in the first treatment exposure of trial 2, and two fish were excluded from AQUI-S 20E induction times in the second treatment exposure of trial 2.

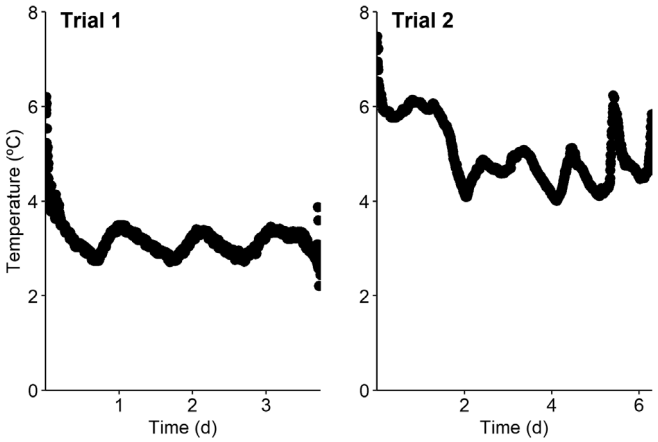

FIGURE 1 Mean temperatures recorded by the heart rate loggers over the course of trial 1 (19 March 2018 to 23 March 2018) and trial 2 (3 April 2018 to 9 April 2018). Elevated initial temperatures are present as, immediately after handling and surgery, the loggers were recording accurate heart rates even though both the loggers and the fish had not finished cooling down to ambient water temperatures

after a short (< *c.* 1 h) monitoring period in the recovery tank containing fresh lake water. Induction and recovery times for AQUI-S 20E fish and recovery times for PES fish in trial 2 are presented in Table 1. Because of missing/incomplete data, induction and recovery times for these groups in trial 1 could not be included.

Heart rate logger surgeries began with a *c.* 2–2.5 cm incision on the ventral body wall, positioned on the fish's left side between the pelvic and pectoral fins such that the anterior end of the incision was *c.* 2 cm posterior to the pericardial membrane. Each logger was inserted into the cavity and nestled against the pericardium, with the two electrodes on the loggers' surfaces oriented to be as close as possible to the pericardium as in other perciform fishes (*e.g.*, Muller *et al.*, 2020; Prystay *et al.*, 2019). The logger was anchored to the ventral body wall by a suture attached to one end of the tag as per Prystay *et al.* (2019). The incision was closed using two interrupted sutures. In trial 1, fish were undisturbed once placed in the net pens (19 March), and heart rate and temperature data were collected by the loggers until 23 March when fish were collected, euthanised *via* cerebral percussion, and the heart rate loggers were removed for download, sterilisation, and re-use. The authors assume that, as poikilotherms, the fish's internal body temperatures as recorded by the loggers were adequate proxies of water temperature. Average temperatures during both trials are shown in Figure 1. In trial 2, fish were initially placed in the net pen to recover from surgery from 3 April until 6 April, at which point they were removed from the net pen, subjected to the same handling treatment that they had received 3 days before during surgery (*i.e.*, AQUI-S 20E fish were anaesthetised with AQUI-S 20E, and so forth), and returned to the net pen until 9 April. The fish were then removed from the net pen and euthanised as described above. Heart rate loggers were retrieved from each fish, and it was noted whether the position of the heart rate logger had been compromised during the trial (*i.e.*, shifted in such a way that may have adversely impacted data collection and quality).

2.4 | Data and statistical analyses

Survival was not analysed as no mortalities (*i.e.*, among treatment and reference fish) were observed throughout either trial during this experiment. The data from each heart rate logger were downloaded and processed using Mercury software (AnimaLab, Poznań, Poland). Statistical analyses were performed in RStudio v. 1.4.1717 (RStudio Team, 2021) with R v. 4.1.0 (R Core Team, 2021). All plots were generated using the "ggplot2" (Wickham, 2016), "tidymv" (Coretta, 2021) and "ggpubr" (Kassambara, 2020) packages. Heart rate data were filtered in several steps for both trial 1 and trial 2. First, all heart rate data associated with QIs of 2 or 3 (indicative of poorer quality data; Bjarnason *et al.*, 2019; Muller *et al.*, 2020) were removed, as well as any heart rate values greater than 50 BPM (based on visual inspection of the data, where such occurrences were sporadic and unrealistic) and/or deviating by more than 10 BPM from both the previous and subsequent values (Doherty *et al.*, 2022), which seemed biologically implausible under the current experimental conditions. Heart rate plots were examined for each fish, and individual fish were excluded from the analyses if there were major inconsistencies in the data over the course of their respective trials (*e.g.*, dramatic, implausible fluctuations in heart rate that were not caught in the filtering process). Using this criterion, one fish from the AQUI-S 20E treatment in trial 1 and one fish from the TENS treatment in trial 2 were removed. Data filtering was performed manually and through the "filter()" function from

the "dplyr" package (Wickham *et al.*, 2021). This filtering process yielded 8 AQUI-S 20E, 12 TENS and 7 PES fish in trial 1, and 10 AQUI-S 20E, 8 TENS and 9 PES fish in trial 2 for subsequent analyses. In addition, 12 reference fish (*i.e.*, those that were not exposed to one of the three treatments described earlier) were placed into the submerged net pen for trial 1 and 9 fish for trial 2. Collectively, this meant that 75% (trial 1) and 96% (trial 2) of fish yielded useful data sets from their heart rate loggers, a marked improvement from some other studies using these or other heart rate loggers (*e.g.*, Doherty *et al.*, 2022; Raby *et al.*, 2015), suggesting the logger placement technique used was effective and/or that *S. vitreus* are a tractable species for this technology.

For each trial, a generalised additive mixed model (GAMM) was fitted using the "gam()" function from the "mgcv" package (Wood, 2011), with heart rate (BPM) as the response variable and the following predictor variables: a smoothing parameter for time, grouped by treatment; treatment as a parametric term; individual smoothing parameters for water temperature and fish mass; and individual fish ID incorporated as a random effect, using a smoothing function with "re" as the basis for smooths. Time was modelled as seconds since the start of each trial. GAMM fits were evaluated visually and through "gam.check()" from "mgcv" (Wood, 2011), with the basis dimensions (k) increased for time and temperature smoothing parameters as necessary. Both GAMMs used restricted maximum likelihood (REML) methods because of the presence of random effects and had Poisson distribution families on account of heart rate being recorded to the nearest whole (positive) number. GAMM fits were assessed and compared using the base "summary()" and "anova()" functions as well as "wald_gam()" from the "itsadug" package (van Rij *et al.*, 2020). To examine the rates at which heartbeats increased or decreased over time, the first-order derivatives of each trial's GAMM were computed along with 95% C.I. using the "derivatives()" function from the "gratia" package (Simpson, 2021).

Because actual baseline heart rates for these fish under the described environmental conditions are unknown, the authors defined recovery points (*i.e.*, when heart rates were low enough for a fish to be considered "recovered") as the time required for a moving average of seven consecutive heart rates to reach or fall below the 10th percentile of heart rate values for each fish (Prystay *et al.*, 2017). Heart rate recovery time, defined as the time required for elevated heart rate to return to approximately baseline values, was quantified for each fish. In trial 1, recovery times were quantified over the course of the trial following initial handling and surgeries. In trial 2, recovery times were quantified after both initial handling and the treatment re-exposure period. Recovery times were fitted with a general linear model (LM) for trial 1 (with treatment and fish mass included as predictor variables), and a general linear mixed model (LMM) for trial 2, with treatment, exposure (first *vs.* second handling treatment) and the interaction of treatment and exposure included as fixed effects. In trial 2, individual fish ID was included as a random effect to account for repeated sampling, while mass was not included because of collinearity with fish ID. Fish sex was excluded from the analyses as only one female was captured during this experiment. Fish mass was included in trial 1 (where there was no repeated sampling and therefore no fish ID in the model) as theoretically, vertebrate heart rates should decrease with increasing body mass (West *et al.*, 1997), though in fishes this is not necessarily true (Clark & Farrell, 2011). LM(M)s were analysed using "Anova()" from the "car" package (Fox & Weisberg, 2019), and *post hoc* tests performed using "emmeans()" from the "emmeans" package (Lenth, 2021).

Heart rate scopes associated with each recovery window were also calculated by taking the difference between mean heart rate during the first 3 h of data collection in trial 1, the first 3 h in trial 2, or the treatment re-exposure period in trial 2, and the 10th percentile values. In trial 1, heart rate scopes were analysed *via* a Kruskal–Wallis test because of non-normal residuals. In trial 2, maximum heart rates during peaks associated with initial handling treatments and treatment re-exposures were quantified, and both scopes and maximum heart rates for trial 2 fish were analysed using the LMM method described earlier for trial 2 recovery times.

3 | RESULTS

3.1 | Trial 1

In all treatments, heart rates changed significantly over time [AQUI-S 20E $\chi^2 = 1075.4$; estimated degrees of freedom (EDF) = 17.0; $P < 0.001$; TENS $\chi^2 = 1222.1$; EDF = 17.4; $P < 0.001$; PES $\chi^2 = 1544.0$; EDF = 12.9; $P < 0.001$; Figure 2a–c]. All fish exhibited elevated heart rates at the beginning of the trial, recorded immediately after the surgical implantation of the heart rate loggers (Figure 2). The smoothing trends in heart rate over the subsequent holding period were not significantly different between treatments ($\chi^2 = 0.469$; DF = 2; $P = 0.791$) and on average, heart rates at a given point in time were largely consistent between fish anaesthetised with AQUI-S 20E, immobilised with TENS or stunned with PES throughout trial 1. The mean rates of heart rate change (first-order derivatives from the GAM) differed significantly over time ($F = 327.4$; DF = 1; $P < 0.001$) but did not differ significantly between treatments ($F = 2.72$; DF = 2; $P = 0.067$; Figure 2d–f). Heart rates also changed significantly with temperature, generally increasing with temperature but also increasing below 2°C when fish would have been handled out of the water and therefore stressed ($\chi^2 = 22.4$; EDF = 4.5; $P < 0.001$). Interindividual variation in heart rate was likewise significant and accounted for *c.* 20% of the deviance explained (*c.* 27% of the total deviance explained by the model; $\chi^2 = 2424.2$; EDF = 23.6; $P < 0.001$; Table 2).

Mean heart rate recovery times did not differ between treatments ($F = 0.20$; DF = 2; $P = 0.822$), nor was fish mass a significant predictor of recovery time ($F = 0.80$; DF = 1; $P = 0.381$). On average, recovery times were *c.* 33.5, 29.4 and 30.7 h for AQUI-S 20E, PES and TENS fish, respectively. However, all treatments were associated with highly variable heart rate recovery times, ranging from *c.* 8 to 13 h for the fastest-recovering fish to *c.* 53 to 64 h for fish that recovered the slowest (Figure 3). Maximum heart rates were consistent

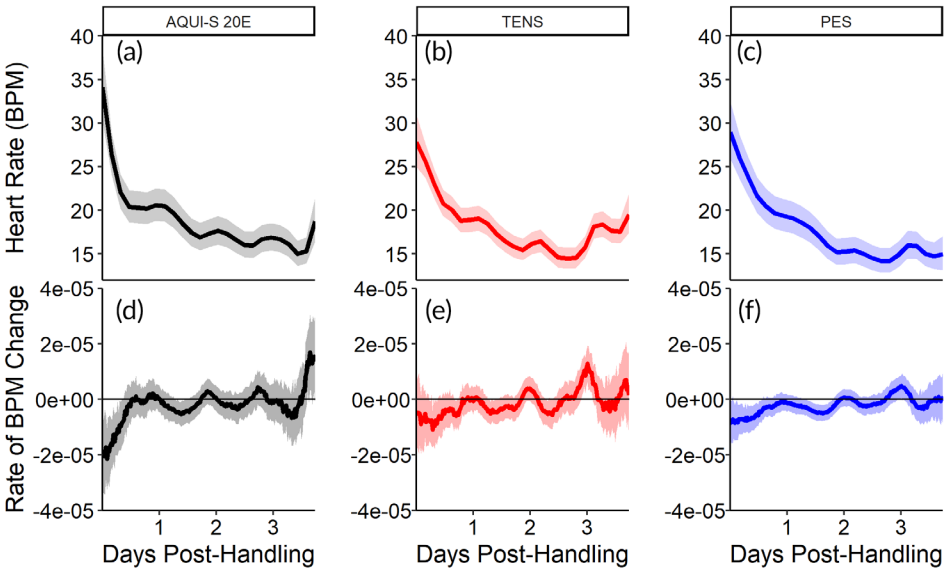

FIGURE 2 Heart rate and rates of change for AQUI-S 20E, TENS and PES fish in trial 1. (a–c) Mean heart rate (BPM; plus 95% confidence intervals) over the <4 days holding period, beginning from the first recorded heart rate (post-filtering) in fish once the loggers had been implanted. (d–f) Mean rates of change for heart rates, calculated as first derivatives of the curves in (a–c), with 95% C.I. A derivative of zero represents no change in heart rate, whereas positive values indicate increasing heart rate and negative values indicate decreasing heart rate. No significant differences were found between each treatment's heart rates over time

TABLE 2 Log likelihood and deviance explained by generalised additive models for heart rate (BPM), with and without random effects ("Fish. ID", italicised) for both trials

Trial	Model	Log likelihood	Deviance explained (%)	Random effects *P*
1	BPM ~ s(Timestamp, by = Treatment, k = 50) + Treatment + s(Temp, k = 15) + s(Fish.ID, bs = "re")	−28,409.0	72.1	<0.001
	BPM ~ s(Timestamp, by = Treatment, k = 50) + Treatment + s(Temp, k = 15)	−29,662.6	52.4	NA
2	BPM ~ s(Timestamp, by = Treatment, k = 100) + Treatment + s(Temp, k = 200) + s(Fish.ID, bs = "re")	−85,605.8	77.1	<0.001
	BPM ~ s(Timestamp, by = Treatment, k = 100) + Treatment + s(Temp, k = 200)	−91,264.9	45.5	NA

Note: "Timestamp" refers to time since each trial's beginning, in s; "Temp" refers to water temperature. *P* values for significance of random effects are listed where appropriate. For all models, the Poisson family and REML methods were selected.

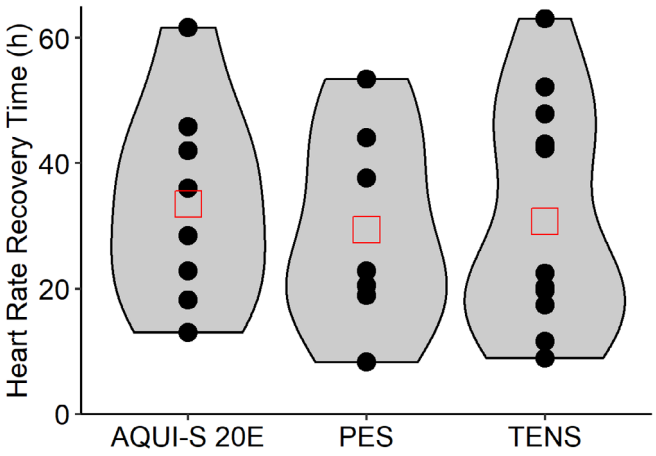

FIGURE 3 Heart rate recovery times associated with each treatment in trial 1. Individual recovery times are shown by black circles, whereas empty squares denote the mean recovery times for each treatment. Note that TENS fish in particular seem to be divided into either relatively rapid or slow recovery times

between treatments (27.0, 26.5 and 26.8 BPM for AQUI-S 20E, PES and TENS fish, respectively; $F = 0.05$; DF = 2; $P = 0.951$), but a significant negative relationship was observed with fish mass. On average, maximum heart rate decreased by *c.* 0.33 BPM for every 100 g increase in mass for the fish used in this experiment ($F = 4.66$; DF = 1; $P = 0.041$). Rank sums of heart rate scope during trial 1 were similar among treatments (Kruskal–Wallis $\chi^2 = 0.59$; DF = 2; $P = 0.745$), with mean differences between average peak heart rate and 10th percentiles on the order of *c.* 9.7–10.6 BPM for all treatments.

3.2 | Trial 2

In all treatments, heart rates changed significantly over time (AQUI-S 20E $\chi^2 = 1368.5$; EDF = 44.8; $P < 0.0001$; TENS $\chi^2 = 1315.5$; EDF = 27.6; $P < 0.0001$; PES $\chi^2 = 2784.2$; EDF = 43.4; $P < 0.0001$; Figure 4a–c) and increased with temperature ($\chi^2 = 811.7$; EDF = 82.7; $P < 0.0001$). All fish exhibited elevated heart rates at the

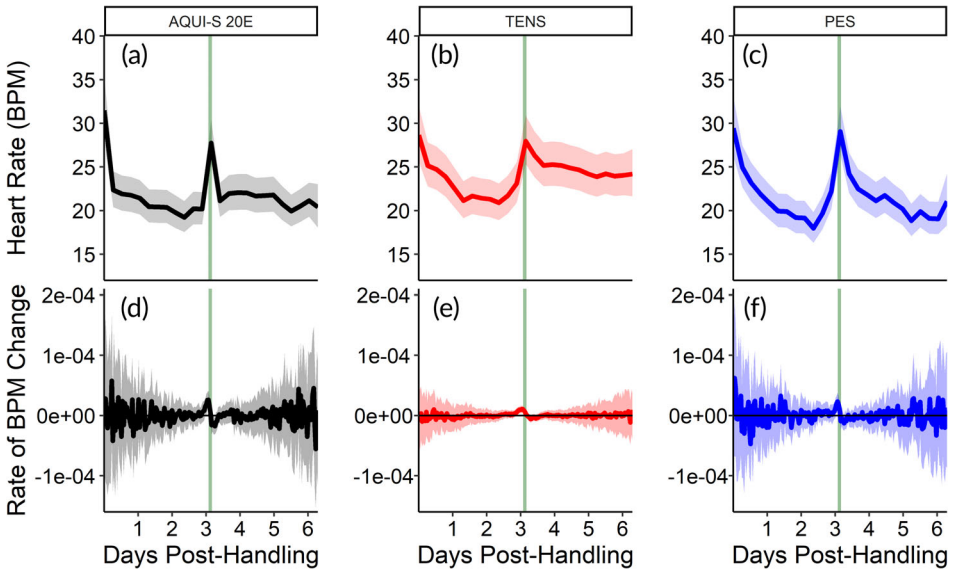

FIGURE 4 Heart rate and rates of change for AQUI-S 20E, TENS and PES fish in trial 2. (a–c) Mean heart rate (BPM; plus 95% C.I.) over the <7 days holding period, beginning from the first recorded heart rate (post-filtering) in fish once the loggers had been implanted. (d–f) Mean rates of change for heart rates, calculated as first derivatives of the curves in (a–c), with 95% C.I.. A derivative of zero represents no change in heart rate, whereas positive values indicate increasing heart rate and negative values indicate decreasing heart rate. The narrow vertical window in each plot denotes the period of time in which fish were re-exposed to their handling treatments (AQUI-S 20E, TENS or PES); note the earlier increases in heart rate corresponding with the required time for collective capture and removal of fish from the net pens before the re-sampling period

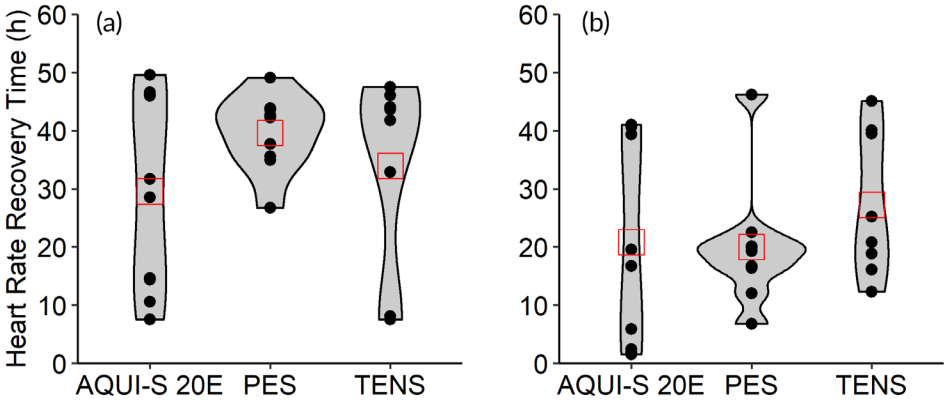

FIGURE 5 Heart rate recovery times for each treatment after initial handling (a) and handling re-exposure (b) in trial 2. Individual recovery times are denoted by black circles, whereas means for each treatment are shown as empty squares. In (b), the single PES fish with a very long recovery time was stunned twice

beginning of the trial (as with the first trial), as well as during the handling period where they were re-exposed to their respective treatments (*i.e.*, AQUI-S 20E, TENS and PES). None of the smoothing trends of heart rates for anaesthesia/immobilisation treatments differed significantly from one another in trial 2 ($\chi^2 = 3.43$; DF = 2; $P = 0.180$). Intra-individual differences accounted for *c.* 32% of the deviance in heart rate throughout the trial (41% of the total deviance explained by the model; $\chi^2 = 12{,}459$; estimated DF = 23.9; $P < 0.0001$; Table 2). The rates at which heart rate changed over the first recovery period, treatment re-exposure and second recovery period were not significantly different between treatments ($F = 0.34$; DF = 2; $P = 0.715$), and even differences in overall rates of change over time were not statistically significant ($F = 0.02$; DF = 1; $P = 0.899$; Figure 4d–f).

Recovery times after the initial exposure to treatments were correlated (Pearson $r = 0.62$; DF = 25; $P < 0.001$), and *c.* 68% of the variance in the mixed model for recovery times was explained by interindividual differences. As in trial 1, time to heart rate recovery varied considerably within all treatments (Figure 5). The fastest recovery times ranged from *c.* 7.5 to 26.6 h and *c.* 1.5 to 12.3 h, whereas the slowest times ranged from *c.* 47.5 to 49.6 h and *c.* 41.0 to 46.2 h, after the first and second exposures to treatments, respectively. Recovery times did not differ across treatments overall in trial 2 ($F = 0.47$; DF = 2; $P = 0.633$); however, the initial recovery times were significantly longer than those that followed treatment re-exposures for AQUI-S 20E fish (29.6 *vs.* 20.8 h; DF = 32.9; $P = 0.023$) and PES fish (39.6 *vs.* 20.0 h; DF = 32.9; $P < 0.001$), but not TENS fish (33.9 *vs.* 27.2 h; DF = 32.9; $P = 0.109$; Figure 5).

First and second maximum heart rates were strongly correlated (Pearson $r = 0.84$; DF = 25; $P < 0.001$), and interindividual variation accounted for *c.* 79% of the mixed model's total variance. Maximum heart rates during the second trial were fairly consistent across

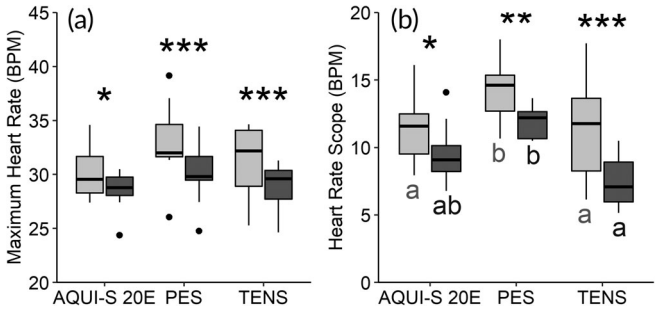

FIGURE 6 Maximum heart rates (a; calculated as the average heart rate during peaks associated with first and second exposures to handling treatments) and heart rate scopes (b; calculated as the difference between maximum and 10th percentile heart rates for each fish) during trial 2. Asterisks denote statistically significant differences between first and second handing exposures for a given treatment ("*": $P < 0.05$; "**": $P < 0.01$; "***": $P < 0.001$). For heart rate scope, lowercase letters denote statistically significant differences between treatments for a given exposure period; no such differences were observed for maximum heart rate. Exposure: First, Second

treatments ($F = 1.57$, DF = 2; $P = 0.228$) and were significantly lower during handling the re-exposure compared to the initial handling and surgery period ($F = 41.8$; DF = 1; $P < 0.001$; Figure 6a). During initial handling, mean maximum heart rates were 30.1, 32.9 and 31.2 BPM for AQUI-S 20E, PES and TENS fish, and during re-exposure to treatments these decreased to 28.5, 30.1 and 28.8 BPM, respectively (Figure 6a). The interaction between treatment and first *vs.* second handling exposure was not significant ($F = 1.15$; DF = 2; $P = 0.332$); that is, the decrease in maximum heart rate was roughly consistent for all treatments.

Heart rate scopes during the first and second halves of trial 2 were strongly correlated with one another (Pearson $r = 0.61$; DF = 25; $P < 0.001$), with interindividual variation accounting for *c.* 47% of the mixed model's variance. Heart rate scopes differed significantly between treatments ($F = 7.13$; DF = 2; $P = 0.004$) and between first and second halves of trial 2 ($F = 28.1$; DF = 1; $P < 0.001$; Figure 6b). PES fish had higher mean scopes during the first half of trial 2 compared to AQUI-S 20E (14.3 *vs.* 11.2 BPM) and TENS (11.4 BPM) fish, and in the second half PES fish also had higher mean scopes than TENS fish (11.9 *vs.* 7.5 BPM) (though neither of these differed significantly from the mean scopes of AQUI-S 20E fish, at 9.5 BPM). The interaction term of handling exposure and treatment was not statistically significant ($F = 1.66$; DF = 2; $P = 0.211$); all treatments exhibited similar trends of heart rate scopes decreasing by *c.* 1.7–3.9 BPM in the second half of trial 2 relative to the first half (Figure 6b).

4 | DISCUSSION

4.1 | Changes in heart rate over time

The changes in heart rates over time did not differ significantly between treatments in trial 1 or after either handling exposure in trial 2. In all cases, heart rates elevated sharply in response to handling and decreased over an average timeframe of several days. As evidenced by the re-exposure period in trial 2 (Figure 4a–c), these increases in heart rate began before treatment-specific protocols were administered, suggesting that pre-treatment capture and handling provided the greatest contribution to heart rate changes and masked any potential effects that might have arisen because of treatment. The heart is innervated by the sympathetic branch of the autonomic nervous system (Sandblom & Axelsson, 2011), which is also implicated in catecholamine release at the beginning of the stress response (Reid *et al.*, 1998), and heart rate can increase rapidly in response to handling and other disturbances (Sopinka *et al.*, 2016). In trial 1, natural circadian rhythms began to appear beneath the recovery trends between the 1- and 2-day marks (Figure 2), corresponding approximately with the mean recovery times which (based on our criteria) ranged from *c.* 29.4 to 33.5 h. The masking of circadian rhythms normally visible in ECGs can occur following elevated heart rate (see Brijs *et al.*, 2018), though this is not always the case (Føre *et al.*, 2021). Similar fluctuations appeared near the end of each half of trial 2 however these were less consistent than those seen in trial 1. Diurnal changes in water temperature were more irregular throughout trial 2, and ambient temperature plays a crucial role in regulating heart rate, cardiac function and metabolic processes in general (Priede, 1983; Vornanen, 2016). This experiment was also conducted at low temperatures (trial 1: *c.* 1.5–6.2°C; trial 2: *c.* 3.7–8.2°C), where recovery times and other changes in the generalised stress response following handling tend to be prolonged relative to higher temperatures (Davis, 2004; Gingerich *et al.*, 2007; Hoskonen & Pirhonen, 2004). Therefore, although our assigned recovery values of heart rate were conservative, the authors cannot say how close or far they may be to the true resting heart rates of fish if recovery required more time under the experimental temperatures.

One plausible concern about the analytic approach may be that over such a relatively large timescale, along with high among-individual variation (Figure 7), there is a possibility of missing biologically relevant differences or trends that may only be present at smaller, subtler scales. Indeed, this might have been the case for trial 2; the changes in first derivatives of the GAM in trial 2 over time itself were not statistically "significant," yet for all treatments there appear to be similar clear trends of high variation and fluctuations, followed by strong and consistent changes at the treatment re-exposure period, and then returning to higher variation (Figure 4d–f). However, average heart rate recovery times (subjected to an entirely different method of analysis) were still not found to be significantly different between treatments, corroborating the interpretations of both trials' GAMs. Although recovery is often quantified and analysed in different manners, other experiments investigating heart rates following handling stress (focusing on salmonids) have reported recovery times and visible circadian rhythms over similar timescales, *e.g.*, *c.* 4 days for Atlantic salmon *Salmo salar* L. at *c.* 4–5°C (Føre *et al.*, 2021), and 3+ days for rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) at *c.* 14–16°C (Brijs *et al.*, 2018). It is worth noting that the observed recovery times show substantial variation within treatments. Although

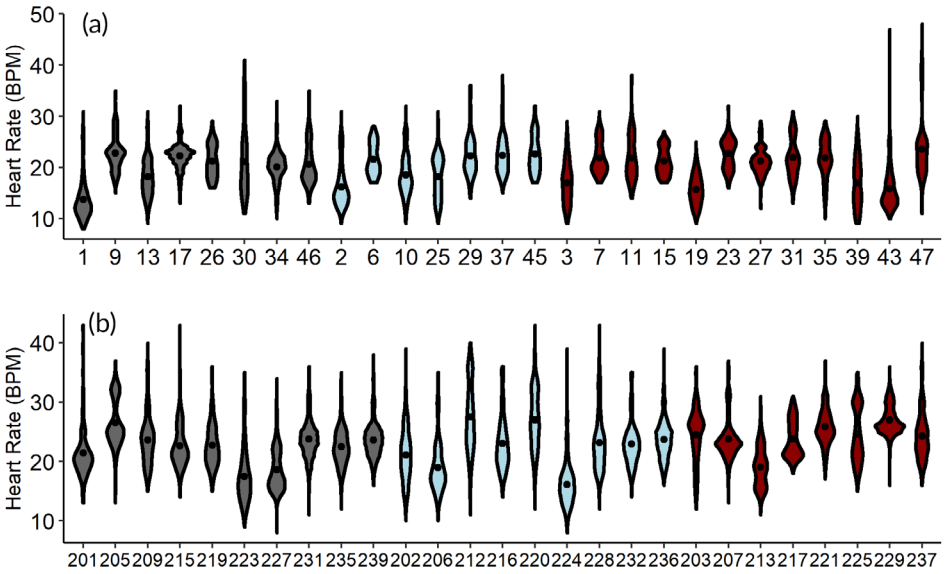

FIGURE 7 Ranges and relative frequencies of heart rates recorded within individual fish in trial 1 (a) and trial 2 (b), with mean heart rates for each fish shown as black circles. Numbers on the *x*-axes are numeric identifiers for each fish. Treatment: AQUI-S 20E, PES, TENS.

the authors did not identify any differences attributable to treatment, it appears that TENS fish leaned towards a bimodal distribution of short and long recovery times following the first exposure to electroanaesthesia (Figures 3 and 5). The bimodal distribution of TENS fish could be the result of high natural interindividual variation in that group, or interindividual variation imposed by electric currents of variable strength. PES and AQUI-S 20E fish received the same electric currents/doses within the treatment, whereas TENS fish had electric currents with amperages tailored to each fish until immobilisation was induced. The underlying physiology of electro-immobilisation is poorly understood, and the possibility of certain thresholds of stressor intensity being crossed in some of the TENS fish depending on the applied current remains open. In the case of the individual with the longest recovery in the PES group in trial 2 (Figure 5b), electrostunning was administered twice during treatment re-exposure as the initial shocking did not stun the fish (a phenomenon that occasionally occurs); otherwise, PES fish in trial 2 exhibited the greatest consistency in recovery times within each half of trial 2. The shorter recovery times following the second handling exposure relative to those seen after the first handling exposure in trial 2 appear to have been driven largely by the PES treatment, as AQUI-S 20E and TENS fish remained more variable throughout this trial (Figure 5). It could be that the second recovery time was also shorter as it followed a less stressful handling protocol (*i.e.*, there was no surgery), however this does not account for the differences in variability across treatments (which could be a function of natural interindividual variation).

4.2 | Heart rate maxima and scopes

Maximum heart rates did not differ between treatments in this experiment. Interestingly, both maximum heart rates and scope decreased significantly within each treatment over the course of trial 2 (Figure 6). The *S. vitreus* used in this experiment were captured during their spring spawning migration, an energetically demanding time following reduced food intake during the wintertime (Quist *et al.*, 2002), and were not fed (barring any small forage fishes that might have swum into the net pen). Cardiac output (the product of heart rate and stroke volume) in fish increases in response to elevations in metabolic demands, including digestion, and the decreases in maximum observed heart rates over time may be related to reduced oxygen consumption as fish are fasting (Armstrong, 1986; Ivarsen *et al.*, 2010). As above, the lack of surgery during treatment re-exposures may have also lessened the degree of stress that fish experienced during trial 2. Scopes were consistently higher in PES fish than in AQUI-S 20E fish after initial handling and in TENS fish throughout trial 2. As PES fish had higher scopes while maximum heart rates were similar between treatments, it is likely that the PES fish naturally achieved lower mean resting heart rates than TENS and AQUI-S 20E fish. This may also partially explain why interindividual variation was prominent throughout all of the analyses, or, perhaps, the timescales used to assess recovery may have been too short in this experiment and the TENS and AQUI-S 20E fish might have actually required longer recovery times than the PES fish. In that case, the heart rate scopes (calculated between mean maxima and 10th percentiles) might not have been different between treatments if TENS and AQUI-S 20E fish had been able to reach their true resting heart rates.

4.3 | Other considerations and limitations

The authors are unaware of any other published experiments detailing cardiac responses to any method of electro-immobilisation in fish. In addition, the literature on cardiac responses to chemical anaesthesia in fish generally focuses on different taxa and/or warmer water temperatures (*e.g.*, Anderson *et al.*, 1998; Cooke *et al.*, 2004), making direct comparisons difficult. The authors were not able to quantify potential sex-specific differences in cardiac responses throughout this

experiment, although they are unaware of any evidence suggesting that fish sexes respond differentially to electro-immobilisation. The results corroborate previous findings concerning the need for adequate timescales when assessing post-anaesthesia recovery using heart rate (Altimiras & Larsen, 2000), and the authors extend this to other capture and handling stressors, especially when water temperatures and/or metabolic rates are expected to be low as they were in this experiment. The authors were not able to collect enough data to reliably quantify potential differences in ECG waveforms during electric current exposure, and therefore cannot eliminate the possibility that some small treatment differences may manifest on very short timescales such as impacts on the speeds or timings of cardiac muscle contractions. In an experiment simulating electrofishing on *O. mykiss* (Schreer *et al.*, 2004), elevations in stroke volume rather than heart rate were observed following shocking, and periods of cardiac arrest (roughly proportional to the duration of shocking) were followed by cardiac arrhythmias lasting seconds to minutes as heart rate recovered. The currents applied during electrofishing are often more intense and typically meant for indiscriminate capture of fish rather than being tailored to particular species or individuals. Schreer *et al*. (2004) used higher voltages than the electrostunned (PES) fish in this experiment (100–400 V *vs*. 30 V), and also reported more variable behavioural recovery times (including many fish regaining equilibrium almost immediately following shocking, which would not be seen in electrostunning). The possibility of electrostunning eliciting cardiac arrest and other injuries inducible by electrofishing currents certainly deserves consideration, but it is inappropriate to directly compare observations without very cautious appraisals of the various methods used in electrofishing *vs*. electro-immobilisation experiments. In this experiment it did not appear that either the TENS or PES treatments elicited tangible, lasting effects on heart rate, and the lack of mortalities in all treatments suggests that no handling treatment investigated herein is more welfare-adverse than another in terms of survival and heart function.

The authors acknowledge that heart rate is not a direct indicator of stress or oxygen consumption. Relationships with oxygen consumption may not be linear and may change in response to the severity of stress (Thorarensen *et al.*, 1996), nor does heart rate fully account for cardiac output (which may also be heavily modulated by changes in stroke volume; Farrell, 1991). Killen *et al.* (2006) reported significant elevations in heart rate but not stroke volume in *S. vitreus* following simulated angling stress, however, water temperatures and differences in handling protocols were very different to those in this experiment. Even under the assumption that heart rate was the primary contributor to cardiac output in this experiment, it may only account for a fraction of the actual (unknown) oxygen consumption rates in these fish. For example, in bowfin *Amia calva* Linnaeus 1766 and *M. salmoides* held at 19°C, heart rate accounted for less than one-third of the observed variation in oxygen consumption rates following a brief handling stressor (Doherty *et al.*, 2022). Future research linking passive heart rate monitoring with long-term sampling of other stress markers would be worthwhile to allow broader conclusions about the welfare states of fish following intensive handling. Water cortisol, for example, is released slowly and can be used to non-invasively measure stress in captive fish when held in isolation (Dallas *et al.*, 2010; Ellis *et al*., 2004). The experimental set-up was not conducive to such a procedure, yet it could be arranged for a variety of other taxa and systems. Heart rate may not scale consistently with body mass in fishes as it does in other vertebrates (*e.g*., Clark & Farrell, 2011), although the authors did observe a slight negative relationship between fish mass and maximum heart rates in trial 1. As the authors could not simultaneously account for fish mass and repeated sampling during the analyses of trial 2, mass may have contributed to some degree to the observed interindividual variation in the results if a relationship between mass and resting/maximum heart rates manifested in the fish at experimental temperatures. Suboptimal sample sizes were likewise a possible contributor to the high observed interindividual variation.

5 | CONCLUSIONS

Currently, AQUI-S 20E has not been approved as an anaesthetic appropriate for immediate release of consumable wild fishes in most jurisdictions, whereas electro-immobilisation techniques are often received with caution in the absence of more sufficient data on welfare. Overall, the authors did not find evidence of differential effects of chemical anaesthesia with AQUI-S 20E, electroanaesthesia with a TENS unit, or electrostunning with a commercially developed stunning unit on post-handling heart rates in adult *S. vitreus*. Importantly, all three methods of immobilisation showed 100% short-term survival with fish apparently recovering to "resting" heart rates within 30 h, consistent with expected recovery times at low temperatures for any handling/capture stressor. Although trials 1 and 2 were conducted at somewhat different ambient temperatures reflective of warming that occurs during spring, and the latter included an element of repeated sampling, the trials were largely consistent in that considerable variability existed in recovery times for all treatments. This likely outweighed any potential differences in treatment effects and was driven by interindividual variation in heart rate and cardiac function. While more common metrics of fish welfare (*e.g*., corticosteroids and blood chemistry changes) were not assessed in this experiment, the authors illustrate changes in heart rate as a proxy for metabolic rate (a secondary response to stress) before, during and after three handling procedures relevant to fisheries science and field research.

AUTHOR CONTRIBUTIONS

C.H.R.: manuscript preparation and statistical analyses. G.D.R.: project conceptualisation, data collection, statistical analyses and manuscript editing. M.D.F.: data collection, provision of resources, statistical analyses and manuscript editing. S.J.C.: project conceptualisation, provision of resources and manuscript editing. C.S.V.: project conceptualisation, data collection, statistical analyses and manuscript editing.

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SIGNIFICANCE STATEMENT

Using surgically implanted heart rate loggers, the authors examined changes in cardiac function and recovery in response to chemical anaesthesia (eugenol) or two forms of electro-immobilisation (electroanaesthesia and electrostunning) in adult walleye (*Sander vitreus*). Overall, the authors found no significant differences between treatments in terms of survival and heart rate recovery, and inter-individual variation in cardiac function was high throughout this experiment.

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