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Contrasting the effects of immobilisation and anaesthesia on the stress physiology and behaviour of juvenile lake sturgeon (*Acipenser fulvescens*)

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ABSTRACT

Anaesthesia and/or immobilisation are typically required to facilitate experimental procedures on fishes such as the surgical implantation of electronic tags. Yet, optimal anaesthetic or immobilisation methods have primarily been developed and tested in teleosts, with relevant information on efficacy and suitability of these methods lacking for basal ray-finned fishes such as imperilled sturgeons (Acipenseridae). Here, we investigated the behavioural and physiological responses of hatchery-origin juvenile lake sturgeon (*Acipenser fulvescens*) to four immobilisation or anaesthetic methods. We conducted an experiment wherein fish were held in a fashion designed to induce tonic immobility, exposed to electroanaesthesia with a TENS unit, anaesthetised with MS-222, or anaesthetised with clove oil, and then subjected to a simulated intracoelomic tagging surgery. Following reflex indicator scoring, fish were "released" into a circular tank arena for behaviour trials and blood sampling at 0.5, 2, and 4 h post-release. Both tonic immobility and electroanaesthesia were ineffective at immobilising fish, and surgery under these methods led to relatively little behavioural impairment but the highest plasma cortisol peaks. Fish anaesthetised with MS-222 or clove oil had greater post-release behavioural impairment but lower cortisol levels. We discuss the implications of our findings for the practical application of these methods, and the challenges with identifying the most suitable method for immobilising or anaesthetising sturgeons in laboratory and field settings.

1. Introduction

Fish often have to be sedated or anaesthetised in research and aquaculture settings during procedures that are invasive or that require fish to be immobilised (e.g., for intracoelomic implantation of electronic tags, tissue biopsies). There are several chemical substances available to anaesthetise fish, although whether these are legal or approved for use varies across jurisdictions (Topic Popovic et al., 2012; Trushenski et al., 2013). The only chemical anaesthetics or sedatives in Canada approved for use on fish that may be consumed by humans are tricaine methanesulfonate, also known as TMS or MS-222, and Aqui-S (50 % isoeugenol) which has recently been approved for salmonids. Legally, fish exposed to MS-222 must be held for at least 5 days in water that is 10 °C or warmer before being released or slaughtered (Health Canada, 2010), while fish treated with Aqui-S must be held for at least 6 degree-days (e.g., one day at 6 °C, two days at 3 °C; minimum of 15 h above 10 °C) before slaughter (Health Canada, 2023). MS-222 is also the only fully approved anaesthetic for fish in the United States, but the pre-release/slaughter period is extended to 21 days (FDA, 2023). Other options, such as clove oil or its derivatives (eugenol, isoeugenol and commercially available AQUI-S-20E), may be used experimentally in research with shorter or no pre-release holding times (Trushenski et al., 2012b; Trushenski and Bowker, 2012). However, typically less is known about the welfare impacts of newer anaesthetics on fish, especially as it pertains to their effects on stress indicators such as plasma cortisol levels or behavioural impairments.

An alternative to chemical anaesthesia is electro-immobilisation, a suite of methods in which fish are immobilised with electric currents rather than being exposed to chemical substances (reviewed in Reid et al., 2019). Support for the use of electro-immobilisation methods is

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largely grounded in the logistical benefits associated with these methods, such as the potential for immediate immobilisation, the ability to release fish immediately upon recovery, and the absence of drug residues that contaminate fish and the environment. One such method, electroanaesthesia, involves passing a weak current (typically between ~4–40 mA, depending on the species, size, and type of current) through a fish that is held out of water in direct contact with the electrodes (e.g., conductive mesh gloves; Abrams et al., 2018). Once a fish is removed from the current, behavioural recovery (voluntary movement, swimming, etc.) is virtually instantaneous and fish can be released immediately into recovery tanks or the wild (Ward et al., 2017). Moreover, the physiological stress response and behavioural effects are generally similar to those seen following chemical anaesthesia (e.g., Trushenski and Bowker, 2012; Soltani and Mirzargar, 2013; Prystay et al., 2017). Electroanaesthesia is typically induced using constant DC with devices designed for use on fish (e.g., Smith-Root Fish Handling Gloves, Smith-Root, Vancouver, WA, U.S.A.; Abrams et al., 2018), but other commercial devices have also been used (e.g., transcutaneous electrical nerve stimulation (TENS) units designed for use on humans that emit a biphasic pulsed current; Dembkowski et al., 2021). When electroanaesthesia is being employed, institutional animal care committees may sometimes recommend using local analgesics such as lidocaine in conjunction with electroanaesthesia. However, currently no local analgesics are legally approved in Canada or the United States for use in fish that may be consumed by humans. The above features make electroanaesthesia a potentially desirable alternative to chemical anaesthesia in scenarios where the latter is impractical (e.g., large-bodied fish) or otherwise unfeasible (e.g., if immediate release is necessary; Trushenski et al., 2013).

The sturgeon family (Acipenseridae) includes approximately 25 large, long-lived extant fish species that are more threatened than any other group of species on the International Union for Conservation of Nature's Red List (IUCN, 2022). Despite sturgeons having been the focus of research and conservation/management efforts for decades (e.g., Ferguson et al., 1993; Hildebrand et al., 1999; Gross et al., 2002; Vélez-Espino and Koops, 2009; Hilton et al., 2016), researchers working with sturgeon may lack suitable anaesthetic options on account of the large body size of sturgeons, lack of rigorous studies, and/or major logistical challenges impeding the use of approved chemical anaesthetics in wild populations. Such challenges have led to cases where surgeries have been conducted on juvenile and adult sturgeons in the wild without anaesthesia or immobilisation (Kessel et al., 2018; Lilly et al., 2020; McLean et al., 2020; Maskill et al., 2022) despite some evidence indicating more severe physiological stress from handling without anaesthesia (Balazik et al., 2013). While chemical anaesthetics are commonly used for larval (Crossman et al., 2014) and juvenile sturgeons associated with hatchery programs (Mohler et al., 2012), there are specific complications associated with applications of chemical anaesthetics to larger fishes in field settings. Given the large size of most species (e.g., > 1 m long), the use of chemical anaesthesia would necessitate substantially longer handling times in individuals exposed to pharmaceuticals. In addition, there can be greater risks to fish (e.g., unreliable recovery prospects, lingering behavioural impairment leading to increased susceptibility to predation) and/or significant impracticalities (e.g., legal concerns, inability to meet anaesthetic bath size requirements) with attempting to administer chemical anaesthetics, as is often the case for elasmobranchs and some larger teleosts such as tunas (Kessel and Hussey, 2015; Leroy et al., 2023). In endangered populations, there may be zero tolerance for inestimable mortalities from sampling efforts in conservation work. If fish cannot be safely anaesthetised (i.e., with minimal risk to post-release health and mortality), any elevated risk of mortality may therefore be unacceptable. In order to improve welfare for sturgeons during handling and tagging procedures, rigorous assessments of potential alternatives for immobilisation or anaesthesia are therefore required.

Attempts to explore alternatives to MS-222 anaesthesia in sturgeon species have been limited compared to efforts in other taxa. Clove oil has shown promise in some *Acipenser* spp. (e.g., Taylor and Roberts, 1999; Feng et al., 2011) but has only been tested under hatchery conditions and is not currently a fully approved substance for use in wild fish in most if not all jurisdictions where such substances are regulated. Electro-immobilisation methods have also been investigated, but typically using ad hoc/custom-built devices lacking necessary information for replication and/or without including direct experimental comparisons to chemical anaesthetics other than MS-222 (Henyey et al., 2002; Balazik et al., 2013). To improve welfare when conducting invasive procedures on sturgeons, and inform relevant animal care and use guidelines, more data are needed on the viability and welfare impacts of possible alternative chemical anaesthetics and electro-immobilisation options. Here, we first performed a pilot project to test the efficacy of electroanaesthesia using two different devices, as to date there is no comparable literature on electroanaesthesia in sturgeons. We then investigated the effects of four immobilisation and anaesthesia methods on the behaviour and physiology of juvenile lake sturgeon (*Acipenser fulvescens*) in conjunction with intracoelomic surgery intended to simulate the implantation of electronic tags. We selected MS-222 and clove oil as chemical anaesthetics. Both are used frequently in fisheries research, and clove oil is also more common and accessible than commercial clove oil derivatives (e.g., AQUI-S). Results from this experiment improve our understanding of which method(s) might improve welfare outcomes based on changes in behaviour and physiology, with potential for scaling up to use on larger bodied sturgeons in field or research settings.

2. Methods

2.1. Site and experimental subjects

Experiments were conducted at the Grand Rapids Fish Hatchery in Grand Rapids, Manitoba, between 1 April and 17 April 2024. This experiment was divided into two parts. The first part was a pilot project evaluating the efficacy of electroanaesthesia using constant DC or biphasic pulsed currents from a TENS unit. The second part comprised the main experiment evaluating behaviour and stress physiology following surgeries under four chemical anaesthesia and immobilisation treatments. All fish used in experiment 1 ($n = 48$) and experiment 2 ($n = 135$) were juvenile (~9-month-old, mean fork lengths of 219 and 233 mm, respectively) lake sturgeon kept in circular holding tanks of ~152 cm (5 ft) internal diameter maintained between ~14–15 °C on the hatchery system. The hatchery system used for the experiments consisted of a recirculating aquaculture system supplied with groundwater from two on-site wells, with a built-in water heater and chiller. The system contained a line of twelve holding tanks sharing the same water filtration and recirculation equipment (i.e., isolated from other similar systems in the hatchery), of which eight tanks were used exclusively for behavioural trials in experiment 2; one served exclusively as a pre-trial holding tank for fish to be tested the next day; and, three tanks were used for holding all other fish pre- and post-experimentation. Fish were initially held in two tanks at an approximate density of ~110 fish per tank. Average water depth in the tanks ranged from ~33–35 cm. Additional information on fish holding, feeding, and tanks can be found in Supplementary File 1.

This experiment was conducted under approval from the Carleton University animal care committee (AUPs #118872 and #119111). All pieces of equipment that came into contact with fish and/or hatchery water systems (e.g., coolers, nets, surgery tools and setup) were sanitised with Virkon® upon arrival at the site per hatchery biosecurity protocols.

2.2. Experiment 1: Electroanaesthesia pilot

To assess whether and how electroanaesthesia may be reliably induced in juvenile lake sturgeon, we tested two devices (a TENS unit emitting asymmetrical biphasic pulsed current; (TENS 7000®,

Middleburg Heights, OH, U.S.A.) and a low-voltage power supply emitting constant DC; KD3005D, KORAD, Dongguan, China) on individuals with focus responses to the current and immobilisation thresholds. Methods (including data analyses) and results for this pilot can be found in Supplementary File 2. TENS units elicited more consistent tetany thresholds and were selected for use in experiment 2.

2.3. Experiment 2: Physiology and behaviour following surgery under anaesthetic or immobilisation treatments

Fish were transferred from a general holding tank to the pre-trial holding tank in the late afternoon for testing the next day. Each day, twelve fish were taken to provide one fish per treatment/time point combination (detailed below), and two to four fish were used for baseline physiology or behaviour data. During the data collection phase, all fish transfers (e.g., pre-trial holding tank to anaesthetic bath, surgery table to behaviour arena) were done using buckets that were rinsed and refilled with fresh water for each fish.

In total, 15 fish were used for the baseline physiology group. Three fish did not yield usable samples and were excluded from analyses. Usable samples were obtained from six sampled as soon as the lights were switched on in the room (~08:00–08:05), and another six sampled between ~12:25–13:00 to account for potential shifts in baseline blood chemistry during the day. Baseline physiology fish were netted from the pre-trial holding tank, and a ~ 0.15 ml blood sample was withdrawn from the caudal vasculature within 2–3 min (Lawrence et al., 2018) using a heparinised 23G needle and a 1 ml syringe.

In total, 16 fish were used for baseline behaviour (no blood sampling). These fish were netted from the pre-trial holding tank and transferred to an arena to undergo the same behavioural assays as treatment fish, described below. Once blood sampling or behaviour trials were complete, fish length, mass, and PIT tag number were recorded before transfer to a recovery tank.

Treatment fish (handling, TENS, MS-222, and clove oil) were netted from the pre-trial holding tank and exposed to their appropriate handling, anaesthesia, or immobilisation protocols in a randomised order. Fish in the "handling" group were transferred to the surgery table and held supine without anaesthesia or current administered, mimicking the handling procedures and reliance on tonic immobility alone that is often used on larger sturgeons in the field (e.g., Kessel et al., 2018; McLean et al., 2020). Fish in the TENS treatment were moved to the surgery table and held supine, and current was gradually increased until tetany (full-body contractions) was observed, after which current strength was decreased until fish became relaxed. Fish in the MS-222 and clove oil treatments were transferred to a cooler containing the appropriate anaesthetic solution. MS-222 baths (100 mg/l) were prepared by dissolving 2 g MS-222 and 4 g sodium bicarbonate (Topic Popovic et al., 2012) in 20 l of water from the hatchery system. Clove oil baths (60 mg/l) were prepared by dissolving 1.2 ml of clove oil in 10.8 ml of ethanol (a 1:9 ratio; Anderson et al., 1997; Sladky et al., 2001; Woody et al., 2002), which was added to 20 l of water. MS-222 and clove oil fish were immersed in the bath until stage IV anaesthesia was achieved (total loss of equilibrium and responsiveness to external stimuli; Summerfelt and Smith, 1990) and then transferred to the surgery setup.

The small size of the sturgeon made irrigation of the gills (e.g., Kolarevic et al., 2016; Durhack et al., 2020) impractical. Fish were therefore placed supine on an angled foam mat such that the head and gills were submerged while the remainder of the body was above the waterline for surgery. Fish were held on the surgery table by one researcher, with one hand each supporting the head and caudal peduncle, while a second researcher conducted all surgeries. Surgeries were timed from the moment fish were supine and at rest.

A 1 cm incision was made on the ventral body wall with a sterile scalpel, to one side of the ventral midline and between the ventral scutes slightly anterior of the halfway point between the pectoral and pelvic girdles. The incision was further anterior than is typical for tagging surgeries in juvenile sturgeons (e.g., Miller et al., 2014; Hegna et al., 2019). We elected to avoid midline incisions because such wounds may be more prone to dehiscence and generally poorer healing (Hegna et al., 2019). In many of the study fish, the bases of the ventral scutes nearer to the pelvic girdle were very close to the midline, leaving insufficient space for consistent offline incisions. Because we were not implanting tags (i.e., no concerns about tag retention or tags damaging the viscera) or collecting other samples (e.g., gonad biopsies; Webb et al., 2019) and merely wanted a standardised and relevant surgical procedure, we used the more anterior position to allow for consistent incision placement. There were no indications that damage to the viscera occurred while making incisions. Surgical tools and sutures were kept in a dilute povidone-iodine (Betadine) solution and rinsed with clean water before each use. A new scalpel blade was used after every second or third fish. The incision was closed with a single 3–0 PDS-II (polydioxanone) suture tied with a surgeon's knot. Surgery times were stopped when the fish was transferred to a recovery cooler. Water in the recovery cooler was changed after every fish that had received an anaesthetic, or otherwise after every 2–3 fish.

Fish reflexes were assessed as a measure of the degree of impairment following surgery (Davis, 2010; Raby et al., 2012). The reflexes scored (1 = reflex present, 0 = absent) were tail grab response (does the fish actively attempt to swim when the tail is pinched), body flex response (does a fish flex laterally when held on the flanks, rotated on its side, and gently squeezed), equilibrium resumption (does the fish regain equilibrium within 3 s when inverted), and vestibular-ocular response (does the fish's eye maintain tracking of the handler as the fish is slowly rotated on the anteroposterior axis). Fish that received four out of four positive scores were considered to have recovered and were ready for "release" (behavioural trials). Fish that failed at least one of the four scores were kept in the cooler until all reflexes had been regained (at least 20–30 s were given between reflex assessments, depending on the status of individual fish at the time of testing). Reflex recovery times were recorded, beginning from the end of surgery and ending when all four reflex tests were passed; reflex recovery times of 0 s were assigned for fish that passed all tests on the first assessment.

Following reflex recovery, fish were transferred to one of eight tanks serving as arenas for 5 min behaviour trials (Fig. 1). Arenas were divided into eight slices (octants) by electrical tape markings. Octants were selected to provide sufficiently fine resolution for behaviours (e.g., directional movements) while being large enough to encompass the length of the fish in most of their area. Fish were gently placed in the arena at the surface opposite the water inflow hose, facing the central standpipe (i.e., on the border between octants 4 and 5, facing perpendicular to the current). A stopwatch was started, and one observer (CHR) recorded whether the fish initially exhibited positive or negative rheotaxis, and then the position and orientation of the fish every 5 s for the duration of the trial. Position and orientation data consisted of the octant number the fish occupied; whether the head of the fish was within the edge zone (~5 cm from the arena edge), the outer zone (between the edge zone and half of the arena radius), or the inner zone (between half of the arena radius and the central standpipe); whether the fish was exhibiting positive, negative, or no rheotaxis; and whether the fish was at or close to the water's surface (as opposed to on or just above the substrate). At the same time, a second observer (RD) recorded the total time the fish spent actively swimming, the number of 180° turns, and the number of independent swimming events. Positive rheotaxis is a typical behaviour in sturgeons manifesting early in their development (Richmond and Kynard, 1995) and was expected to be observed in the fish in our experiment. Sturgeon will preferably engage in benthic station-holding behaviour (i.e., attempting to remain in place), but may display pelagic (free-swimming) behaviours depending on the strength of current flow (Boysen and Hoover, 2009). Benthic station-holding (substrate coupling) involves positioning the body to "rest" on the substrate, while pelagic station-holding behaviour in benthic fishes may be a consequence of currents that are too strong for fish to maintain

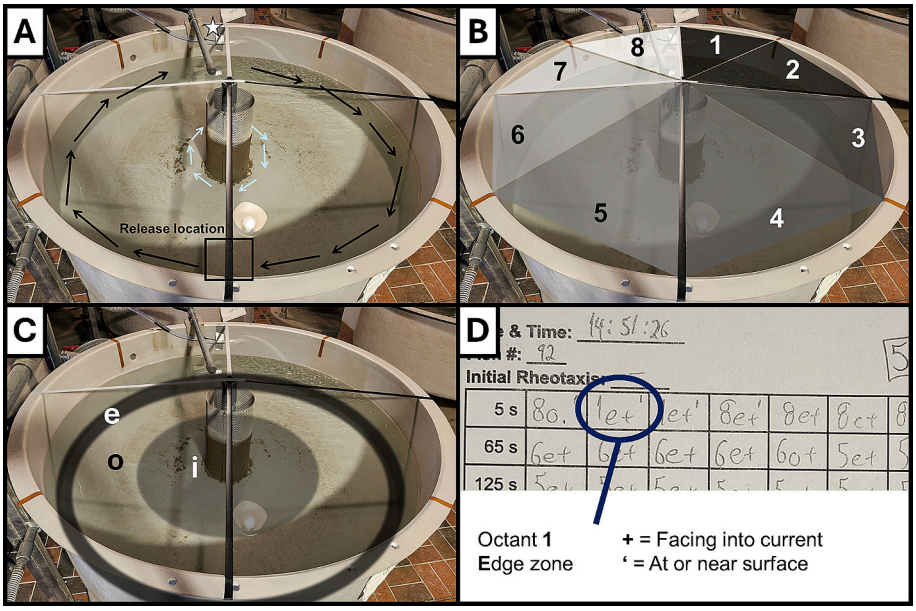

Fig. 1. Example of one of the trial arenas used for behavioural assays and fish holding prior to blood sampling in experiment 2. (A) View of an arena tank with electrical tape forming gridlines. The water inflow location is denoted by a star; flow direction and relative strength are shown by arrows. The release location was always opposite the inflow location. (B) Octants as laid out by gridlines, with the inflow location always considered octant 1 and the release location always between octants 4 and 5 (all arenas had clockwise octant number assignments). (C) Approximate illustration of the edge (e), outer (o), and inner (i) zones of the arena. (D) Example of behavioural data collection notation during a 300 s (5 min) trial, showing scores collected every 5 s.

position through substrate coupling (Coombs et al. 2020). With current flow rates at acceptable levels, deviations in the typical benthic station-holding behaviours could be interpreted as escape or refuge-seeking behaviours following the stress of capture and handling.

After each trial, fish were left in the arenas undisturbed for one of 30 min, 2 h, or 4 h (including the 5-min trial time) prior to blood sampling. The fish were then netted out of the arena and a blood sample was drawn from the caudal vasculature within 2–3 min; most samples took 30–60 s. Fish were then measured for fork lengths to the nearest mm, mass to the nearest 0.1 g, and PIT tag number before transfer to a recovery tank. Based on the approximate water inflow rates and volumes, the mean tank turnover time was ~40–45 min. To minimise the risk of metabolites or cues released from tested fish (e.g., water cortisol; reviewed in Sadoul and Geffroy, 2019) confounding subsequent behavioural assays, arenas used for more than one fish on a given day were either drained to the halfway mark and given at least ~30 min to refill plus one turnover time, or were given two to three turnover times before being used for the next fish.

Whole-blood glucose and lactate were measured in the field immediately after sampling using portable handheld meters (Contour® Next Gen, Ascensia Diabetes Care, Mississauga, ON, Canada; and Lactate Plus™, Nova Biomedical Canada, Mississauga, ON, Canada). Such meters have been validated for use in many fishes (Stoot et al., 2014), though whole blood titres for these responses tend to be lower than measurements of plasma (Holtkamp et al., 1975; Kim, 2016). In sturgeon, such meters may provide less accurate absolute values of blood glucose or lactate than certain laboratory methods but are still effective for evaluating relative differences between treatments or groups (Hagen et al. 2022). The remaining blood was then centrifuged (Mandel Mini Microcentrifuge; Mandel Scientific, Guelph, Ontario, Canada) for 5 min at 2000 *g*. The plasma was decanted into a clean tube that was flash frozen in liquid nitrogen; samples remained in lN_2 for transport to a − 80 °C freezer at Carleton University, Ottawa, Canada. Frozen plasma samples were transferred to the University of Ottawa, Ottawa, Canada, and stored in a − 80 °C freezer until assayed for cortisol. Cortisol assays were performed using the ELISA (enzyme-linked immunosorbent assay) method (Neogen®, Lexington, KY, U.S.A.). Samples were assayed in duplicate. Mean intra-assay variability was 3.8 % (range: 0.1–13.9 %), and inter-assay variability was 14.4 %. For assay validation, serial dilutions of a pooled plasma sample yielded a curve that was parallel to the standard curve (Welch's $T = 0.91$, $P = 0.387$).

All fish were monitored several times daily for approximately one month post-experimentation (i.e., until they were stocked into the wild), and sutures were removed once wounds had sufficiently healed.

2.4. Statistical analyses

All statistical analyses and figure generation were performed in RStudio v. 2024.04.2 (764; Posit Team, 2024) with R v. 4.4.1 Patched (R Core Team, 2024). Unless otherwise specified, all fitted models (generalised linear models (GLMs), multinomial regression models, etc.) were analysed using the "Anova" function from the "car" package (Fox and Weisberg, 2019) and, if necessary, post-hoc comparisons (with least-squares means and asymptotic 95 % confidence intervals) were performed using the "emmeans" function with Tukey's HSD correction from the "emmeans" package (Lenth, 2024). GLM assumptions were examined and appropriate families were selected following recommendations in Zuur et al. (2010). One fish in the MS-222 treatment group was excluded because full anaesthesia was not achieved based on evidence of some behavioural recovery as the suturing was being performed. Also, eight fish tested on day 1 were excluded from blood chemistry analyses only, due to inadequate heparinization that rendered glucose and lactate values suspect and prevented plasma collection for cortisol assays.

For experiment 2, a chi-square test with Monte Carlo-simulated *p*-values was conducted to evaluate the distribution of fish from each treatment allocated to each of the trial arenas. Generalised linear models were fitted for responses quantified during anaesthesia and surgery for all fish unless specified otherwise, namely: induction times for MS-222 and clove oil only, the number of voluntary movements during surgery (excluding MS-222 fish), surgery duration (including time to adjust the TENS dial in the TENS treatment), summed reflex impairment scores immediately following surgery, and the total recovery time for MS-222 and clove oil fish (calculated as the surgery time plus the time required

to pass all four reflex tests). The Gaussian family was used for time responses, while the Poisson family was used for movement data and reflex score sums. Treatment was the sole predictor variable for each of the above GLMs except that for anaesthetic induction times, which also included fish mass.

A Gaussian GLM was fitted for the total time spent swimming in the arena during each 5-min trial, while quasi-Poisson GLMs were fitted for the number of times a fish started swimming and the number of 180° directional changes in each trial, with treatment and Fulton's condition factor (previously used for juvenile lake sturgeon; Beamish et al., 1996; Barth and Anderson, 2015; calculated by dividing fish mass by fork length cubed and multiplying the result by 100; Froese, 2006) as predictor variables. A second chi-square test with Monte Carlo-simulated p-values was performed on the initial rheotaxis score (positive, negative, or neutral) that a fish exhibited upon being released into the arena. The proportion of time fish spent in the edge zone, the outer zone, or the inner zone of the arena, the proportion of time spent in positive, negative, or neutral rheotaxis, and the proportion of time spent at or near the water surface were calculated from the trial data scored every 5 s and fitted with quasi-binomial GLMs with treatment as the sole predictor variable.

To identify different behaviour patterns in the arena, the number of octants by which fish moved upstream and downstream and the total time spent swimming for each individual were normalised for 3D cluster analysis. K-means cluster number selection was performed using both "wss" and "silhouette" methods in the "fviz_nbclust" function from the "factoextra" package (Kassambara and Mundt, 2020). The optimal number of identifiable clusters was three, generally comprising individuals that were active swimmers with proportionally more upstream movements ("active upstream swimmers"), somewhat active swimmers with proportionally more downstream movements ("downstream swimmers"), and relatively inert/inactive fish with proportionally fewer upstream or downstream movements ("inert fish"). Clusters were visualised using the "plotly" package (Sievert, 2020). A multinomial regression was performed on the clusters to which each fish was assigned as a function of treatment using the "multinom" function from the "nnet" package (Venables and Ripley, 2002). Post-hoc tests were performed to compare the estimated marginal probabilities of fish being assigned to a given cluster both within and between treatments.

Blood glucose concentrations were analysed using a Gaussian GLM with treatment, sampling time, and the two-way interaction effect of treatment and sampling time as predictor variables. Blood lactate concentrations could not be analysed as the vast majority of the values (89/108) fell below the lactate meter's detection limit (0.3 mmol/l) and were therefore unquantifiable. All plasma cortisol analyses were conducted using Gaussian GLMs with $\log_{10}$-transformed cortisol values and treatment, sampling time, and the two-way interaction of treatment and sampling time as predictor variables. Because baseline blood samples were taken from a unique subset of fish at a single time point (0 h) not represented in the matrix of main treatments (handling, TENS, MS-222, and clove oil) and sampling times (0.5, 2, and 4 h), analyses of treatment and time including potential interaction effects could not directly include the baseline data. Baseline glucose and cortisol were therefore excluded from these models and separate GLMs were used to compare log-transformed plasma cortisol values of baseline fish and all treatments at each time point separately (i.e., one model comparing treatment cortisol values with baseline values each at 0.5, 2, or 4 h, with treatment included as the sole predictor variable).

3. Results

In experiment 1, mean ± SD fork lengths and fish masses were 219 ± 15 mm and 58.0 ± 12.7 g, respectively. In experiment 2, mean ± SD fork lengths and fish masses were 233 ± 15 mm and 67.6 ± 13.8 g, respectively. Mean fish lengths and masses were similar between treatments in both experiment 1 (length $F_{2,45} = 0.60$, $P = 0.555$; mass $F_{2,45} = 0.86$, $P = 0.431$) and experiment 2 (length $F_{5,128} = 0.33$, $P = 0.896$; mass $F_{5,127} = 0.35$, $P = 0.883$). There was no statistical association between the individual behaviour trial arenas and the number of fish from each treatment allocated to each arena ($\chi^2 = 23.87$; Monte Carlo simulated $P = 0.717$). No mortalities were observed during the experiment or in the monitoring/pre-stocking period that followed.

3.1. Experiment 2: Physiology and behaviour following surgery under anaesthetic or immobilisation treatments

Average induction times to stage IV anaesthesia were longer for fish anaesthetised with clove oil (~393 s) than MS-222 (~352 s; Table 1; $F_{1,48} = 5.13$; $P = 0.028$), and were not influenced by fish mass ($F_{1,48} = 0.68$; $P = 0.415$). The number of voluntary movement events during surgery was higher for handling and TENS fish than MS-222 or clove oil fish (LR $\chi^2 = 108.6$; DF = 2; $P < 0.0001$). Handling and TENS fish averaged 3.1 and 2.7 movement events, respectively ($P = 0.809$), while no MS-222 fish exhibited any movement. Only one clove oil fish had a movement that was scored as potentially voluntary during the surgery but may have been a larger version of small, localised twitches that were occasionally observed in clove oil fish, because this fish failed all reflex tests immediately after surgery. Reflex scores following surgery were dependent on treatment (LR $\chi^2 = 214.2$; DF = 3; $P < 0.0001$). All handling and TENS fish exhibited maximum scores (no reflex impairment), while average scores were close to zero for clove oil and MS-222 fish (0.1/4 vs. 0.4/4, respectively; $P = 0.144$). Recovery times were therefore only assigned to clove oil and MS-222 fish, and were longer in the clove oil treatment (mean: 415 s) than the MS-222 treatment (mean: 265 s; $F_{1,35} = 40.1$; $P < 0.0001$).

The initial rheotaxis score upon placement in the arena, perpendicular to the direction of current, did not differ as a function of treatment ($\chi^2 = 10.52$; Monte Carlo simulated $P = 0.179$). The least-squares mean total amounts of time spent swimming in each 5-min behaviour trial (Fig. 2a) differed among treatments ($F_{4,104} = 22.36$; $P < 0.0001$) but was not a function of condition factor ($F_{1,104} = 0.02$; $P = 0.877$). Baseline behaviour fish spent the most time swimming on average (267 s) compared to the treatments that underwent surgery (all $P < 0.0001$). Swimming times were variable overall in handling, TENS, MS-222, and clove oil fish. Handling and TENS fish had similar mean amounts of time spent swimming (142 and 149 s, respectively; $P = 0.997$) and both were higher than the mean time for clove oil fish (61 s; $P \leq 0.001$). The mean

Table 1
Least-squares means and asymptotic 95 % confidence intervals (in parentheses) for induction and recovery times, voluntary movement events during surgery, surgery duration, reflex scores immediately following surgery, and the total recovery times (surgery time plus reflex recovery time) for fish held without anaesthesia or electric currents ("handling"), fish held with electric current from a TENS unit, and fish anaesthetised with either MS-222 or clove oil.

Treatment	Induction time (s)	Movement events	Surgery time (s)	Reflex score (/4)	Total recovery time (s)
Handling	NA	3.1 (2.4, 3.9)	152 (147, 158)	4 (NA)	NA
TENS	NA	2.7 (2.1, 3.5)	164 (159, 170)	4 (NA)	NA
MS-222	352 (326, 378)	0 (NA)	151 (145, 158)	0.4 (0.2, 0.7)	265 (229, 301)
Clove oil	393 (367, 418)	0.04 (0.01, 0.3)	155 (149, 160)	0.1 (0.02, 0.3)	415 (383, 447)

Handling and TENS fish had no real "induction time" (apart from ~10–20 s to adjust the TENS dial included in the TENS surgery time) and all passed reflex tests following surgery, therefore some data are not presented for these treatments.

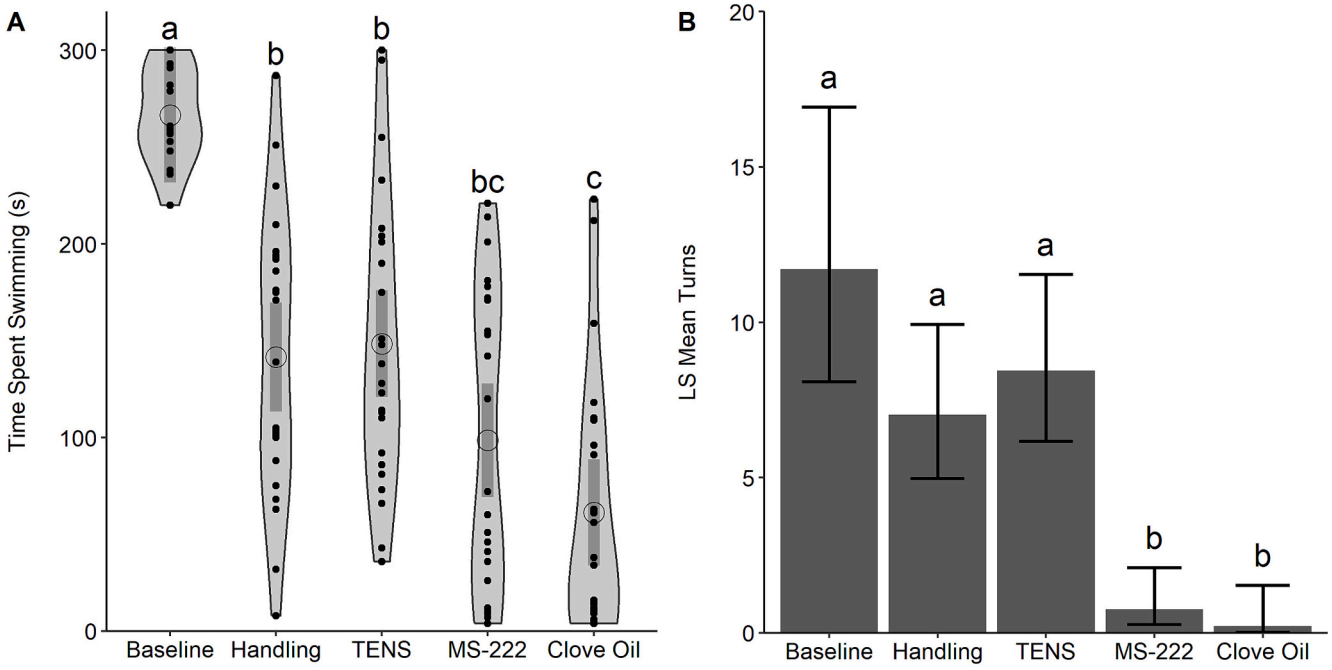

Fig. 2. The total time spent swimming (A) and the least-squares mean numbers of turns (B) during 300 s behaviour trials following surgery. Individual data points in (A) denote the total swimming times for each fish, while open circles and shaded rectangles denote the least-squares (LS) means and asymptotic 95 % confidence intervals for each treatment. In (B), bars and whiskers denote the LS means and asymptotic 95 % confidence intervals, respectively. In both panels, different lowercase letters identify treatments for which pairwise comparisons of LS means yielded $P < 0.05$. Sample sizes were $n = 16$ for baseline fish, $n = 24$ for TENS and clove oil fish, and $n = 23$ for handling and MS-222 fish in (A), and n = 16 for baseline fish and n = 24 for all treatments in (B).

swimming time for MS-222 fish (99 s) fell between the means of handling and TENS fish and that of clove oil fish (all $P \geq 0.109$).

The differences in mean numbers of times that fish initiated swimming from rest or drifting were not likely attributable to treatment overall (LR $\chi^2 = 8.41$; DF = 4; $P = 0.078$); pairwise comparisons between treatment means yielded $P \geq 0.300$ except for the comparison of clove oil and handling fish ($P = 0.046$), representing the lowest (4.0) and highest (7.8) mean times fish initiated swimming. The mean number of times fish turned around (180°) differed as a function of treatment (LR $\chi^2 = 89.7$; DF = 4; $P < 0.0001$; Fig. 2b). Mean turn counts were highest in the baseline group (11.7) followed by the TENS (8.4) and handling (7.0) treatments (all pairwise $P \geq 0.267$). Baseline, TENS, and handling means were higher than those of fish treated with MS-222 (0.8; all $P \leq 0.0005$) and clove oil (0.2; all $P \leq 0.006$); MS-222 and clove oil means were similar to one another ($P = 0.788$). Condition factor did not appear to influence swim initiation counts (LR $\chi^2 = 0.07$; DF = 1; $P = 0.798$) or the number of turns (LR $\chi^2 = 1.58$; DF = 1; $P = 0.209$).

Mean proportions of time spent in the edge zone of the arena differed

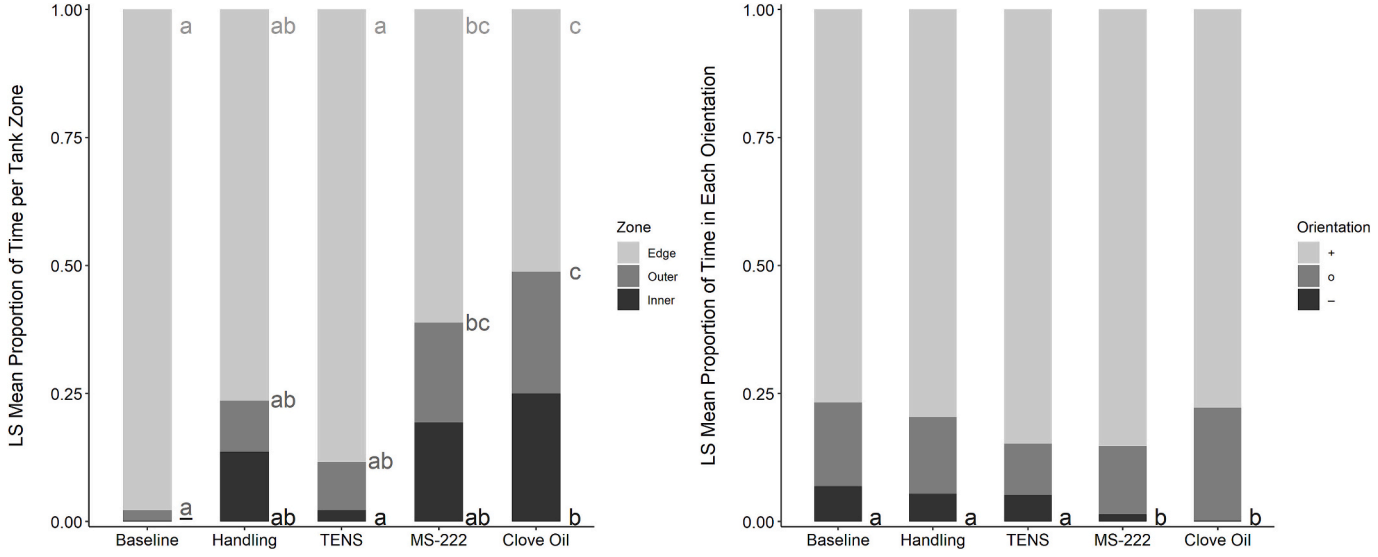

Fig. 3. Mean proportions of time fish spent (A) in each zone of the arena and (B) exhibiting positive, negative, or no rheotaxis during 5 min behaviour trials (edge = outer ~5 cm of the arena; outer = between the edge zone and half of the arena radius; inner = between half of the arena radius and the central standpipe; see Fig. 1 for a visualisation). Letters denote differences in proportions among treatments within each zone (A) or orientation (B; negative rheotaxis only) where $P < 0.05$; a "–" is listed for baseline fish in the inner zone in (A) as only one baseline fish had one recorded score in the inner zone, precluding meaningful pairwise comparisons of means with other treatments. Sample sizes were n = 16 for baseline fish, $n = 26$ for handling and TENS fish, and $n = 25$ for MS-222 and clove oil fish.

as a function of treatment (LR $\chi^2 = 50.44$; DF = 4; $P < 0.0001$; Fig. 3a), with chemically anaesthetised fish generally spending less time in the edge zone than other groups. Mean proportions of time spent in the edge zone were similar among the baseline group and handling and TENS treatments (all $P \geq 0.138$), and between MS-222 and clove oil fish ($P = 1.00$). The differences most likely attributable to treatment effects were those between baseline and TENS fish versus MS-222 and clove oil fish (all $P \leq 0.015$). Mean proportions of time spent in the outer zone also differed among treatments (LR $\chi^2 = 33.52$; DF = 4; $P < 0.0001$), with the differences between clove oil fish and baseline, handling, and TENS fish (all $P \leq 0.019$) and between MS-222 and baseline fish ($P = 0.013$) being most likely to be attributable to treatment; other pairwise comparisons yielded $P \geq 0.116$. Treatment likewise influenced the mean proportions of time spent in the inner zone (LR $\chi^2 = 30.78$; DF = 4; $P < 0.0001$). There was only one occurrence of an inner zone score in the baseline group, precluding pairwise comparisons with other treatments. Otherwise, the greatest difference between treatments was between TENS and clove oil fish ($P = 0.016$); other comparisons yielded $P \geq 0.054$.

Treatment did not appear to affect the mean proportion of time spent exhibiting positive rheotaxis (LR $\chi^2 = 4.12$; DF = 4; $P = 0.390$), which ranged from 77 % in the baseline group to 85 % in the MS-222 treatment, or neutral/no rheotaxis (LR $\chi^2 = 7.25$; DF = 4; $P = 0.123$), which ranged from 10 % in the TENS treatment to 22 % in the clove oil treatment (Fig. 3b). The mean proportion of time exhibiting negative rheotaxis was smaller for all treatments groups but also differed among them (LR $\chi^2 = 50.67$; DF = 4; $P < 0.0001$). Baseline, handling, and TENS fish had higher mean proportions of negative rheotaxis time (6.9 %, 5.4 %, and 5.1 %, respectively) than MS-222 and clove oil fish (1.4 % and 0.2 %, respectively; all $P \leq 0.023$), while differences were negligible between baseline, handling, and TENS fish (all $P = 1.00$) and between MS-222 and clove oil fish ($P = 0.739$). The mean proportion of time fish spent at or near the water surface varied across treatments (LR $\chi^2 = 43.0$, DF = 4; $P < 0.0001$). Specifically, the mean surface proportions were highest in the baseline group (32.2 %) and lowest in MS-222 (6.7 %; pairwise $P = 0.0002$ vs. baseline) and clove oil (3.6 %; $P < 0.0001$) fish. Mean surface proportions aligned with baseline values in the handling (19.8 %; $P = 0.503$) and TENS (19.4 %; $P = 0.419$) groups.

As described above, three groups were identified through cluster analysis of swimming activity patterns in the arena: active upstream swimmers, downstream swimmers, or inert fish (Fig. 4). Treatment influenced the identities of the cluster to which each fish was assigned (LR $\chi^2 = 65.99$; DF = 8; $P < 0.0001$). On average, baseline fish were roughly evenly distributed between the active upstream swimmer (56 %) and downstream swimmer (44 %) clusters ($P = 0.871$), whereas handling fish were roughly evenly distributed into the downstream swimmer (57 %) and inert (43 %) clusters ($P = 0.807$; Fig. 5). TENS fish fell primarily into the downstream swimmer cluster (50 %), followed by the inert (37 %) and active upstream swimmer (13 %) clusters (all $P \geq 0.059$). MS-222 and clove oil fish were distributed unevenly into the inert (78 % and 88 %, respectively) and downstream swimmer (22 % and 12 %, respectively; $P = 0.020$ and 0.006) clusters. Within clusters, all treatments were represented in the downstream swimmers cluster, with the greatest difference in means between handling and clove oil fish ($P = 0.03$; all other pairwise comparisons had $P \geq 0.071$). No baseline fish fell into the inert cluster, but similar proportions were observed between handling and TENS fish ($P = 0.993$). MS-222 and clove oil fish means were similar as the most represented treatments in the inert cluster ($P = 0.910$). Differences in the mean proportions of MS-222 fish and handling and TENS fish had $P = 0.147$ and 0.066, respectively, while differences between clove oil fish and handling and TENS fish yielded $P = 0.033$ and 0.013, respectively. The active upstream swimmers cluster primarily consisted of baseline fish and a smaller proportion of TENS fish ($P = 0.068$), but no handling, MS-222, or clove oil fish were assigned to this cluster.

Plasma cortisol levels differed as a function of both treatment ($F_{3,84} = 8.46$; $P < 0.0001$) and sampling time ($F_{2,84} = 62.15$; $P < 0.0001$) but not the interaction of these factors ($F_{6,84} = 0.60$; $P = 0.728$; Fig. 6). Fish in all treatments exhibited the highest mean plasma cortisol at the 0.5 h mark, and values decreased substantially by 2 h post-handling (from 0.5 to 2 h, all $P \leq 0.007$ within treatments). Mean log plasma cortisol at 0.5 h was higher in the TENS group (55.0 ng/ml) than in clove oil (11.7 ng/ml; $P = 0.031$) and MS-222 (10.0 ng/ml; $P = 0.013$) fish. Handling fish had middling mean log cortisol levels relative to the other treatments at 0.5 h (40.5 ng/ml; $P = 0.117$ versus clove oil fish, $P = 0.059$ vs. MS-222 fish, and $P = 0.944$ vs. TENS fish). At 2 h, differences in mean cortisol values among treatments and overall variation in cortisol had largely

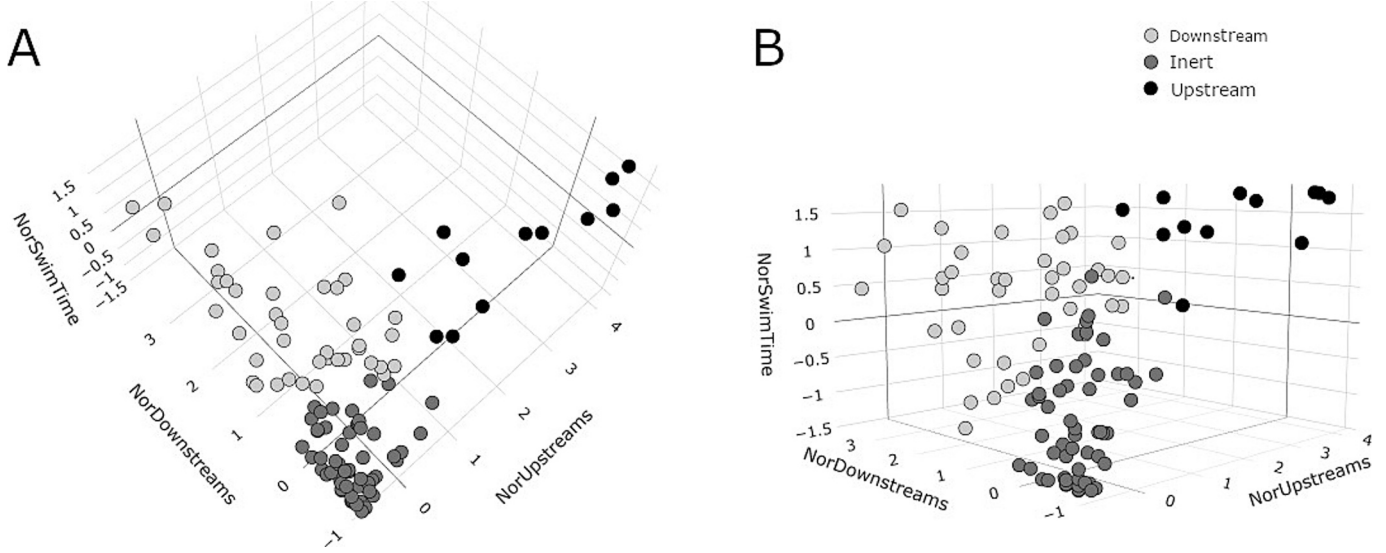

Fig. 4. Two views of the same cluster analysis plot, showing three identified clusters by colour where each point represents one fish in experiment 2. Axes represent normalised scores for time spent swimming, number of "downstream" octant movements (in the direction of current flow), and number of "upstream" octant movements (against the current flow). Light grey points denote fish that, on average, spent more time swimming downstream and had moderate-to-high swimming activity scores ("downstream swimmers"). Medium grey points denote fish that, on average had moderate-to-low swimming activity scores and tended to move little in either upstream or downstream directions ("inert" fish). Black points denote fish that, on average, had high swimming activity scores and tended to swim primarily upstream ("active upstream swimmers").

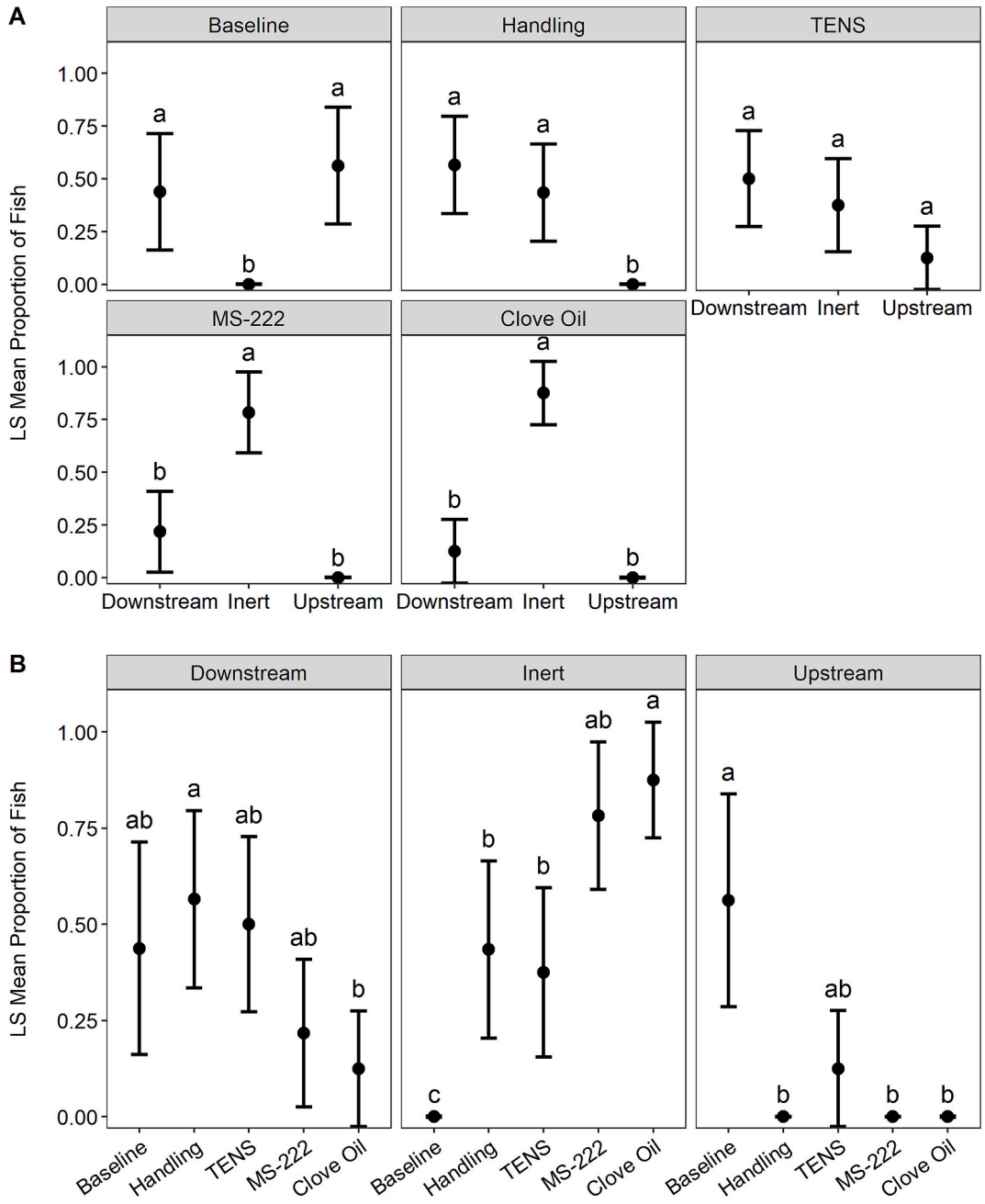

Fig. 5. Mean proportions of fish assigned to each of the three clusters (see Fig. 4), showing pairwise comparisons within (A) and among (B) treatments. Least-squares mean proportions are accompanied by asymptotic 95 % confidence intervals. Letters denote comparisons with P < 0.05 between clusters (A) or treatments (B) in each panel separately. Sample sizes were n = 16 for baseline fish, n = 24 for TENS and clove oil fish, and n = 23 for handling and MS-222 fish.

decreased and the greatest difference was reduced to handling (6.2 ng/ml) versus clove oil fish (1.6 ng/ml; $P = 0.071$; all other pairwise comparisons yielded $P \geq 0.123$). Similarly, mean plasma cortisol values at 4 h fell between 0.6 and 2.1 ng/ml and all pairwise comparisons had $P \geq 0.106$. When modelled with treatment groups at each time point separately relative to the baseline group (least-squares mean: 0.9 ng/ml), evidence of treatment effects was strongest at 0.5 h ($F_{4,39} = 28.71$; $P < 0.0001$), less strong at 2 h ($F_{4,39} = 3.84$; $P = 0.010$), and negligible by 4 h ($F_{4,39} = 0.95$; $P = 0.44$). All treatments exhibited elevated cortisol at 0.5 h with respect to baseline values (all pairwise $P < 0.0001$). At 2 h, differences in mean cortisol values compared to the baseline group were smallest for clove oil (1.6 ng/ml; $P = 0.837$) and MS-222 fish (1.8 ng/ml $P = 0.708$) while still somewhat greater in handling fish (6.2 ng/ml; $P = 0.011$) and TENS fish (4.2 ng/ml $P = 0.057$). At 4 h, all pairwise comparisons between treatment and baseline means yielded $P \geq 0.370$.

Blood glucose values (Table 2) were variable in all treatment groups and at all sampling times, including the baseline group, but changes in blood glucose were not attributable to treatment ($F_{3,82} = 1.19$; $P = 0.319$) or sampling time ($F_{2,82} = 0.11$; $P = 0.897$), nor was there a clear interaction effect between treatment and sampling time ($F_{6,82} = 1.68$; $P = 0.137$). Additionally, there was no clear correlation between ranked blood glucose and plasma cortisol levels (Kendall $\tau = 0.04$; $P = 0.564$).

4. Discussion

4.1. Efficacy of immobilisation and anaesthetic methods

Based on the incidence of voluntary movements, tonic immobility

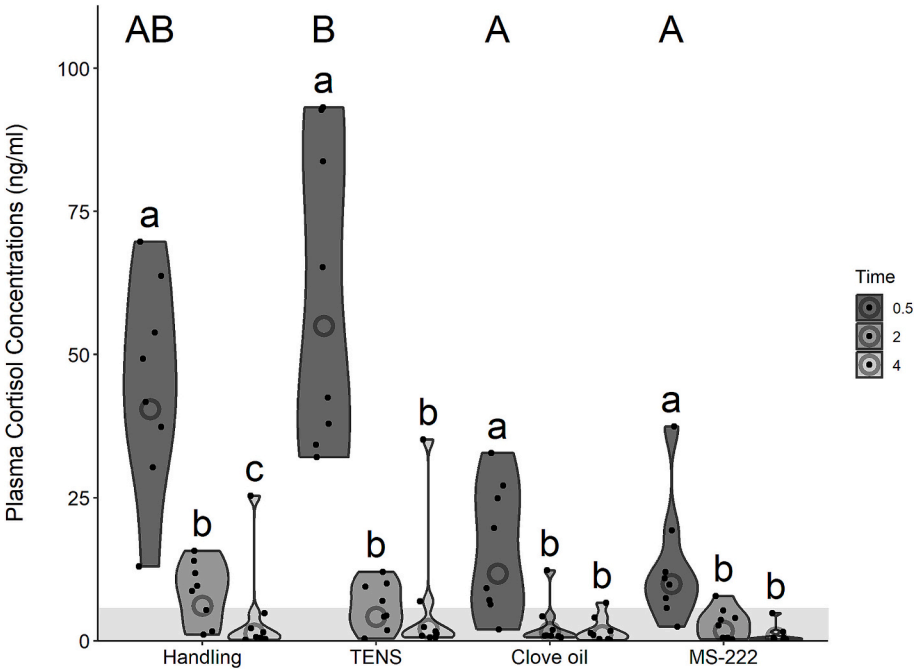

Fig. 6. Plasma cortisol values at 0.5, 2, and 4 h post-handling and surgery. The mean ± SD baseline value range is shaded in light grey (lower end truncated at 0). Black dots represent data points from individual fish, while larger hollow circles denote the least-squares means for each treatment and sampling time. Analyses (including post-hoc pairwise comparisons) were conducted on log10-transformed plasma cortisol values. Uppercase letters denote pairwise comparisons among treatments, with differing letters for comparisons that had $P < 0.05$ at 0.5 h only (not applicable for 2 or 4 h sampling times). Lowercase letters denote pairwise comparisons among sampling times within a given treatment with $P < 0.05$.

Table 2
Least-squares mean blood glucose levels (with asymptotic 95 % confidence intervals for all groups except the baseline group, which was not included in the model), as well as the numbers and proportions of blood lactate samples that were above the lower detection limit of the device (0.3 mmol/l), for fish of each treatment group and sampling time.

Treatment	Sampling time (h)	Blood Glucose (mmol/l)		Blood Lactate
		LS Mean (95 % CIs)	Range	Detectable samples (≥ 0.3 mmol/l)
Baseline	0	3.3	2.3, 4.9	1 / 12
Handling	0.5	3.9 (3.3, 4.5)	2.2, 5.8	3 / 8
	2	3.2 (2.7, 3.8)	2.2, 4.3	0 / 8
	4	3.2 (2.6, 3.8)	2.2, 4.2	0 / 8
TENS	0.5	3.3 (2.7, 3.9)	2.4, 4.0	6 / 8
	2	4.0 (3.4, 4.6)	3.2, 5.1	2 / 8
	4	4.2 (3.6, 4.8)	3.1, 5.8	0 / 8
MS-222	0.5	3.6 (3.0, 4.2)	2.6, 4.9	1 / 8
	2	3.5 (2.9, 4.0)	2.3, 4.5	2 / 8
	4	3.3 (2.7, 3.9)	2.1, 4.2	0 / 8
Clove oil	0.5	3.8 (3.2, 4.5)	3.0, 4.6	1 / 8
	2	3.5 (2.9, 4.1)	2.4, 4.7	2 / 8
	4	3.7 (3.1, 4.3)	2.7, 5.4	1 / 8

was not observed in the juvenile lake sturgeon in this experiment and cannot be recommended for individuals in the tested size range even though it appears to be effective in larger sturgeons (Lilly et al., 2020; McLean et al., 2020). Existing methods using anaesthetics in smaller juvenile sturgeons (e.g., larvae to age-0) are therefore more appropriate (e.g., Crossman et al., 2014). We found no evidence that the constant DC (cDC from a benchtop power supply) or asymmetric pulsed current (TENS) electroanaesthesia methods were effective or appropriate for use in juvenile lake sturgeon. The cDC method has previously been found capable of inducing electroanaesthesia in largemouth bass (*Micropterus nigricans*) despite some deficiencies inherent to using conductive mesh gloves as electrodes (Reid et al., 2024). Yet, the DC current thresholds required to induce electroanaesthesia in juvenile sturgeon were essentially unpredictable, and some fish could not be immobilised by the current even when briefly held mostly or entirely out of water to eliminate the possibility of current loss through the water. This difference in efficacy between previous work with DC electroanaesthesia in teleosts and our observations is difficult to explain, especially given a lack of well-established underlying physiology of electro-immobilisation (cf. Vibert, 1963; Sharber and Sharber Black, 1999). There may be effects from anatomical differences that could be relevant to electroanaesthesia. Sturgeons possess greater proportions of skeletal cartilage than teleosts (Doroshov and Cech Jr., 2011; Leprévost and Sire, 2014), and in other taxa cartilage can be more conductive than bone and muscle tissues (Binette et al., 2004; Lee et al., 2022). Our observations would be consistent with the hypothesis that currents may have largely travelled through inert cartilage rather than tissues that must be affected to induce immobilisation. However, this alone does not explain why TENS units had more consistent tetany thresholds, which may be the result of different interactions between current types and various tissues.

MS-222 and clove oil both induced anaesthesia in juvenile sturgeon, though induction and recovery times were faster for MS-222 in our experiment compared to clove oil. Clove oil was found to induce anaesthesia faster than MS-222 at equal doses in rainbow trout (*Oncorhynchus mykiss*), but also yielded slower recovery times (Anderson et al., 1997; Keene et al., 1998; Wagner et al., 2003). Longer recovery times for eugenol (the main anaesthetic ingredient in clove oil) versus MS-222 were also observed when clove oil was administered at lower

doses in largemouth bass and cobia *Rachycentron canadum* (Trushenski et al., 2012a; 2012b). It is likely that the mean induction times for clove oil in our experiment were slower simply because of the lower dose used (60 mg/l) compared to that for MS-222 anaesthesia (100 mg/l). The relatively longer recovery times we observed for clove oil, despite the lower dose, are in line with existing trends in the literature and potentially attributable to clove oil having a relatively greater suppressive effect on circulation and respiration rates (Priborsky and Velisek, 2018), which could decrease the rate at which fish clear the substance during recovery.

Several of the fish treated with clove oil were observed having some muscle spasms ranging from fin twitches to rapid, whole-body jerking motions during induction, as well as sometimes during surgery while incisions or suturing were performed. Such movements were not observed in MS-222 fish. Twitching or spasms during anaesthesia have been reported in some species for several substances including metomidate, 2-phenoxyethanol, and ketamine (Matsche, 2017; Neiffer, 2021), but few works detail similar observations for clove oil or one of its derivatives. Sladky et al. (2001) anaesthetised red pacu (*Piaractus brachypomus*) with MS-222 or eugenol and observed higher rates of unspecified "behavioural reactions" to a needle puncture at higher doses of eugenol (higher doses of MS-222 led to decreased rates of reactions to the same stimulus). In addition, Barbas et al. (2021) observed epileptiform neural activity in the midbrains of juvenile tambaqui (*Colossoma macropomum*) following exposure to eugenol at a relevant anaesthetic dose. Those fish exhibited some but not all of the behavioural changes associated with exposure to a known seizurogenic drug, however "jerky swimming" and twitches of the caudal fin appeared to be absent (Barbas et al. 2021). Except for the one large movement that occurred, the twitches and spasms we observed in clove oil-treated fish did not affect the ease or length of handling and surgery. However, our results corroborate previous suggestions that clove oil as an anaesthetic may be less effective than MS-222 at depressing or eliminating nervous responses to physical stimuli.

4.2. Anaesthetic and immobilisation effects on post-release behaviour & stress physiology

Reflex impairment scoring provides a straightforward and reliable way of assessing impairment in fishes following exposure to a stressor (Davis, 2010; Raby et al., 2012) and, in field contexts with captured wild fish, a "zero impairment" score often signifies that the fish is ready to be released. All handling and TENS fish had full reflexes present (i.e., 4/4 score, "zero impairment") following surgery and would have been considered ready for release in a field setting. Fish treated with clove oil or MS-222 consistently had very low scores, with most fish having all reflexes impaired. Impaired reflexes in chemically anaesthetised fish immediately following surgery are expected, and even desirable as evidence that fish did remain anaesthetised for the duration of the surgery, though they also highlight the inherent need for prolonged recovery periods before fish can be considered suitable for release.

The behavioural assays were conducted on hatchery-reared fish in a non-ecologically relevant setup, and so the "post-release" responses observed here may not correspond to specific behaviours or actions in a natural setting. However, we identified three patterns of behavioural responses in the arenas: high swimming activity and tendencies to swim "upstream" against the current; variable but typically moderate swimming activity moving both against and with the current (i.e., downstream); and little swimming activity or directional movement. The relatively wide range in the numbers of downstream octant movements and swimming times in the downstream swimmers group may reflect the fact that it was easier for fish to accrue higher downstream movement scores than upstream ones, both because actively swimming with the current yielded greater travel distances per unit time/effort, and because many individuals would at least sometimes drift downstream even while remaining still and being oriented towards the oncoming current. Fish in all treatments spent the majority of their time exhibiting positive rheotaxis, which is typical of juveniles in many sturgeon species when placed in arenas or chambers with directional flow (Richmond and Kynard, 1995; Kynard and Horgan, 2002; Boysen and Hoover, 2009; Hoover et al., 2011).

Baseline fish were most active in the arenas, displaying higher swimming times and higher placement in the water column suggestive of an escape response. Behavioural responses in handling and TENS fish were variable, with individual swimming times spanning most of the range of the 300 s trials but few (TENS) or none (handling) falling into the cluster of fish actively swimming against the current. We found evidence of greater behavioural impairment in MS-222 and clove oil fish, even though all fish were allowed to recover to the point of having positive reflex scores prior to being transferred to the behavioural arena. Fish in both chemical anaesthesia groups were less active in terms of overall swimming times and numbers of turns, and were more likely classified as relatively inert fish that had fewer octant movements overall. Chemically anaesthetised fish also spent more time away from the edge zone (where current flow would be strongest) than baseline behaviour fish or TENS and handling fish. Fish that are released into the wild with impaired reflexes or swimming capabilities may be at greater risk of predation or other threats (Campbell et al., 2010; Brownscombe et al., 2014). In our experiment, the vigorous escape-like behavioural patterns exhibited in the baseline group may be indicative of flight behaviour and also possibly refuge-seeking (McLean et al., 2019) in an environment where no refuge may be found, while the trends towards reduced swimming activity, fewer turning movements, and generally more lethargic behaviour may be indicative of behavioural impairment caused by the treatments (particularly MS-222 and clove oil). The typical behavioural responses observed in chemically anaesthetised fish did not exactly correspond with the station-holding behaviour seen in most fish in our pre-trial holding and recovery tanks. The latter fish preferred to spend time in/near the edge zone and remain in place, while the anaesthetised fish were less likely to be in the edge zone and were often observed becoming more active towards the end of the trials. As mean cortisol levels at 0.5 h were lowest and least variable in the chemically anaesthetised treatments, the observed behavioural impairment in these fish was likely not caused by the stress of handling and surgery under anaesthesia but may have instead reflected residual effects of the anaesthetics themselves that persisted beyond the point of reflex recovery (Zahl et al., 2012). In practice, this may mean that even if MS-222 or clove oil were to some day be classified as immediate-release anaesthetics, there could still be a need to hold chemically anaesthetised sturgeon for longer periods of time before release than what is required for full recovery of reflex indicators to reduce potential risks of predation (for smaller juveniles), being swept away by currents and potentially injured (if released into high-velocity flow areas), and/or being unable to perform essential behaviours (e.g., feeding).

We failed to detect blood lactate levels in most fish (89/108), but of the 19 fish for which lactate had risen to detectable levels, almost a third (6) were in the TENS group at 0.5 h while the handling group at 0.5 h had the second-most detections (3). This may have be due to insufficient analytical sensitivity of the measuring device; too little air exposure and forced muscle activity to induce changes in blood lactate levels, particularly as sturgeons generally have relatively mild physiological stress responses (Cataldi et al., 1998; Barton et al., 2000; Allen et al., 2009; Penny et al., 2023); and/or, sampling times that did not capturing peak changes in this response (Lankford et al., 2003).

There was a trend for plasma cortisol to be higher and more variable in handling and TENS fish relative to the chemically anaesthetised fish at 0.5 h. The capacity for an anaesthetic to reduce or mitigate physiological stress responses depends in part on the dose and timing of administration with respect to other stressors, and evidence concerning whether MS-222 or clove oil is more effective at preventing or mitigating physiological stress responses to subsequent stressors appears to be equivocal and context-dependent (e.g., Cho and Heath, 2000; Wagner et al., 2003;

Sink et al., 2007; Weber et al., 2011). Here, both anaesthetics appeared to be somewhat but not entirely effective at mitigating plasma cortisol increases in juvenile lake sturgeon undergoing a standardised surgery. Cortisol had recovered to near-baseline levels in all treatment groups by 2 h, suggesting that more severe stress at the 0.5 h mark in some fish (i.e., higher cortisol levels) was not necessarily associated with longer cortisol recovery times. Chemical anaesthetic and dose selection may present trade-offs wherein higher doses can provide faster induction times and potentially more effective anaesthesia and stress response mitigation (Sladky et al., 2001; Matsche 2011), but at the expense of significantly longer recovery times (King et al. 2005) and elevated risk of overdose. It is possible that higher doses of MS-222 and/or clove oil might have resulted in lower plasma cortisol peaks at 0.5 h, but very likely yielding prolonged recovery times and potentially more severe behavioural impairment.

We failed to detect noteworthy trends or pattens in blood glucose as a function of treatment. Previous experiments on stress physiology in sturgeons have sometimes reported meaningful changes in blood glucose following exposure to stressors (Cataldi et al., 1998; Allen et al., 2009; Nelson and Small, 2014; Falahatkar and Poursaeid, 2013; Falahatkar et al., 2022), but the lack of consistency in this response suggests that glucose may not be as sensitive an indicator of stress in sturgeons as it is in other fishes. Glucose levels can also vary as a function of other factors such as diet and season, which may influence baseline values (King, 2004).

In summary, none of the handling, immobilisation, or anaesthetic methods tested here was clearly superior to the others in every respect. Attempts at inducing effective tonic immobility and electroanaesthesia for surgery failed, while chemical anaesthetics led to the most significant behavioural impairment. The latter issue could be overcome with longer recovery times and the development of more sensitive metrics for determining releasability, but legal restrictions on MS-222 would preclude its use in most practical settings. Clove oil may therefore be the most appropriate of the methods for field scenarios where anaesthesia is required for juvenile lake sturgeon. For larger sturgeons, particularly in the field, we recommend further exploration of opportunities to refine fish welfare that can be derived from and integrated with current best handling practices. As examples, the effects of different capture methods, sampling procedures (e.g., morphometry measurements, blood and tissue sampling), necessary air exposure, and overall handling times on stress and health can be studied in both laboratory and field settings. Some welfare-relevant responses can be collected through methods that are already used in existing monitoring protocols (e.g., acoustic telemetry data; Melnychuk et al., 2017), while other techniques may be easily incorporated (e.g., the use of retrievable biologgers capable of measuring responses that have historically been difficult to study in situ, such as heart rate or swimming activity; LaRochelle et al., 2021; Morgenroth et al., 2024). Future work on anaesthesia or sedation techniques that may reduce stress and improve welfare of larger sturgeons would be welcome. Evidence syntheses pertaining to anaesthesia and immobilisation techniques in the context of telemetry or other tagging studies may also provide valuable insights. However, such work must include focus on solutions to the various obstacles that would prevent their use in most applied settings (e.g., approval and permissions with respect to existing legal constraints, establishing safe and effective dosages and routes of administration, and handling and disposal in field scenarios).

CRediT authorship contribution statement

Connor H. Reid: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Raegan Davis:** Writing – review & editing, Investigation. **Kathleen M. Gilmour:** Writing – review & editing, Resources, Methodology. **Cheryl N. Klassen:** Writing – review & editing, Supervision, Resources, Project administration. **James A. Crossman:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Steven J. Cooke:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no competing or conflicting interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2025.111823.

Data availability

The data and analysis script are accessible in a GitHub repository, the link to which has been provided in the acknowledgements section of the paper.

References

Abrams, A.E.I., Rous, A.M., Brooks, J.L., Lawrence, M.J., Midwood, J.D., Doka, S.E., Cooke, S.J., 2018. Comparing immobilization, recovery, and stress indicators associated with electric fish handling gloves and a portable electrosedation system. Trans. Am. Fish. Soc. 147 (2), 390–399.

Allen, P.J., Barth, C.C., Peake, S.J., Abrahams, M.V., Anderson, W.G., 2009. Cohesive social behaviour shortens the stress response: the effects of conspecifics on the stress response in lake sturgeon *Acipenser fulvescens*. J. Fish Biol. 74 (1), 90–104.

Anderson, W.G., McKinley, R.S., Colavecchia, M., 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. N. Am. J. Fish Manag. 17 (2), 301–307.

Balazik, M.T., Langford, B.C., Garman, G.C., Fine, M.L., Stewart, J.K., Latour, R.J., McInich, S.P., 2013. Comparison of MS-222 and electronarcosis as anesthetics on cortisol levels in juvenile Atlantic sturgeon. Trans. Am. Fish. Soc. 142 (6), 1640–1643.

Barbas, L.A.L., Torres, M.F., da Costa, B.M.P., Feitosa, M.J.M., Maltez, L.C., Amado, L.L., Toda, Y.P.S., dos Santos Batista, P., Cabral, D.A.C., Hamoy, M., 2021. Eugenol induces body immobilization yet evoking an increased neuronal excitability in fish during short-term baths. Aquat. Toxicol. 231, 105734.

Barth, C.C., Anderson, W.G., 2015. Factors influencing spatial distribution and growth of juvenile lake sturgeon (*Acipenser fulvescens*). Can. J. Zool. 93 (11), 823–831.

Barton, B.A., Bollig, H., Hauskins, B.L., Jansen, C.R., 2000. Juvenile pallid (*Scaphirhynchus albus*) and hybrid pallid × shovelnose (*S. albus* × *platorynchus*) sturgeons exhibit low physiological responses to acute handling and severe confinement. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 126 (1), 125–134.

Beamish, F.W.H., Jebbink, J.-A., Rossiter, A., Noakes, D.L.G., 1996. Growth strategy of juvenile lake sturgeon (*Acipenser fulvescens*) in a northern river. Can. J. Fish. Aquat. Sci. 53 (3), 481–489.

Binette, J.S., Garon, M., Savard, P., McKee, M.D., Buschmann, M.D., 2004. Tetrapolar measurement of electrical conductivity and thickness of articular cartilage. J. Biomech. Eng. 126 (4), 475–484.

Boysen, K.A., Hoover, J.J., 2009. Swimming performance of juvenile white sturgeon (*Acipenser transmontanus*): training and the probability of entrainment due to dredging. J. Appl. Ichthyol. 25, 54–59.
Brownscombe, J.W., Nowell, L., Samson, E., Danylchuk, A.J., Cooke, S.J., 2014. Fishing-related stressors inhibit refuge-seeking behavior in released subadult great barracuda. Trans. Am. Fish. Soc. 143 (3), 613–617.
Campbell, M.D., Patino, R., Tolan, J., Strauss, R., Diamond, S.L., 2010. Sublethal effects of catch-and-release fishing: measuring capture stress, fish impairment, and predation risk using a condition index. ICES J. Mar. Sci. 67 (3), 513–521.
Cataldi, E., Di Marco, P., Mandich, A., Cataudella, S., 1998. Serum parameters of Adriatic sturgeon *Acipenser naccarii* (Pisces: Acipenseriformes): effects of temperature and stress. Comp. Physiol. Physiol. Part A 121 (4), 351–354.
Cho, G.K., Heath, D.D., 2000. Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile Chinook salmon *Oncorhynchus tshawytscha* (Walbaum). Aquac. Res. 31 (6), 537–546.
Coombs, S., Bak-Coleman, J., Montgomery, J., 2020. Rheotaxis revisited: a multi-behavioral and multisensory perspective on how fish orient to flow. J. Exp. Biol. 223 (23), jeb223008.
Crossman, J.A., Scribner, K.T., Davis, C.A., Forsythe, P.S., Baker, E.A., 2014. Survival and growth of lake sturgeon during early life stages as a function of rearing environment. Trans. Am. Fish. Soc. 143 (1), 104–116.
Davis, M.W., 2010. Fish stress and mortality can be predicted using reflex impairment. Fish Fish. 11 (1), 1–11.
Dembkowski, D.J., Isermann, D.A., Vandergoot, C., Hansen, S.P., Binder, T.R., 2021. Short-term survival of lake whitefish following surgical implantation of acoustic transmitters using chemical anesthesia and electroimmobilization. Adv. Limnol. 66, 173–187.
Doroshov, S.I., Cech Jr., J.J., 2011. Sturgeon | physiology of sturgeon. In: Farrell, A.P. (Ed.), Encyclopedia of Fish Physiology, vol. 3. Academic Press, London, U.K.
Durhack, T.C., Jeffrey, J.D., Enders, E.C., 2020. In search of an anaesthesia alternative for field-based research. Aquaculture 525, 735285.
Falahatkar, B., Poursaeid, S., 2013. Stress responses of great sturgeon *Huso huso* subjected to husbandry stressors. Aquac. Int. 21, 947–959.
Falahatkar, B., Abdollahpour, H., Jafari, N., 2022. Physiological responses to air exposure stress in Siberian sturgeon (*Acipenser baerii* Brandt 1869) injected with dexamethasone. J. Appl. Ichthyol. 38 (3), 311–319.
FDA [United States Food and Drug Administration], 2023. Approved Aquaculture Drugs. Available from. https://www.fda.gov/animal-veterinary/aquaculture/approved-aquaculture-drugs.
Feng, G., Zhuang, P., Zhang, L., Kynard, B., Shi, X., Duan, M., Liu, J., Huang, X., 2011. Effect of anaesthetics MS-222 and clove oil on blood biochemical parameters of juvenile Siberian sturgeon (*Acipenser baerii*). J. Appl. Ichthyol. 27 (2), 595–599.
Ferguson, M.M., Bernatchez, L., Gatt, M., Konkle, B.R., Lee, S., Malott, M.L., McKinley, R. S., 1993. Distribution of mitochondrial DNA variation in lake sturgeon (*Acipenser fulvescens*) from the Moose River basin, Ontario, Canada. J. Fish Biol. 43, 91–101.
Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, Third edition (Sage, Thousand Oaks, CA, U.S.A).
Froese, R., 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. 22 (4), 241–253.
Gross, M.R., Repka, J., Robertson, C.T., Secor, D.H., Van Winkle, W., 2002. Sturgeon conservation: insights from elasticity analysis. In: American Fisheries Society Symposium, 28, pp. 13–30.
Hagen, K.B., Marie Lima, K., Ang, J., Montealegre-Golcher, F., Alonso, F.H., Soto, E., 2022. Glucose and lactate reference intervals for white sturgeon and evaluation of two point-of-care devices in sturgeon infected with *Veronaea botryosa*. J. Aquat. Anim. Health 34 (1), 28–37.
Health Canada, 2010. List of Veterinary Drugs that are Authorized for Sale by Health Canada for Use in Food-Producing Aquatic Animals - Health Canada. Available from. https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/legislation-guidelines/policies/list-veterinary-drugs-that-authorized-sale-health-canada-use-food-producing-aquatic-animals.html (Accessed 2024-10-21).
Health Canada, 2023. Notice of Compliance Information for Aqui-S. Available from. https://health-products.canada.ca/noc-ac/nocInfo?no=31324 (Accessed 2024-10-21).
Hegna, J., Scribner, K., Baker, E., 2019. Evaluation of optimal surgical techniques for intracoelomic transmitter implantation in age-0 lake sturgeon. Fish. Res. 218, 198–208.
Henyey, E., Kynard, B., Zhuang, P., 2002. Use of electronarcosis to immobilize juvenile lake and shortnose sturgeons for handling and the effects on their behavior. J. Appl. Ichthyol. 18, 502–504.
Hildebrand, L., McLeod, C., McKenzie, S., 1999. Status and management of white sturgeon in the Columbia River in British Columbia, Canada: an overview. J. Appl. Ichthyol. 15 (4–5), 164–172.
Hilton, E.J., Kynard, B., Balazik, M.T., Horodysky, A.Z., Dillman, C.B., 2016. Review of the biology, fisheries, and conservation status of the Atlantic sturgeon, (*Acipenser oxyrinchus oxyrinchus* Mitchill, 1815). J. Appl. Ichthyol. 32, 30–66.
Holtkamp, H.C., Verhoef, N.J., Leijnse, B., 1975. The difference between the glucose concentrations in plasma and whole blood. Clin. Chim. Acta 59 (1), 41–49.
Hoover, J.J., Boysen, K.A., Beard, J.A., Smith, H., 2011. Assessing the risk of entrainment by cutterhead dredges to juvenile lake sturgeon (*Acipenser fulvescens*) and juvenile pallid sturgeon (*Scaphirhynchus albus*). J. Appl. Ichthyol. 27 (2), 369–375.
IUCN, 2022. The IUCN Red List of Threatened Species. Version 2022-1. Available from: www.iucnredlist.org. (Accessed 21 October 2024).
Kassambara, A., Mundt, F., 2020. Factoextra: extract and visualize the results of multivariate data analyses. In: R Package Version 1.0.7.
Keene, J.L., Noakes, D.L.G., Moccia, R.D., Soto, C.G., 1998. The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res. 29 (2), 89–101.
Kessel, S.T., Hussey, N.E., 2015. Tonic immobility as an anaesthetic for elasmobranchs during surgical implantation procedures. Can. J. Fish. Aquat. Sci. 72 (9), 1287–1291.
Kessel, S.T., Hondorp, D.W., Holbrook, C.M., Boase, J.C., Chiotti, J.A., Thomas, M.V., Wills, T.C., Roseman, E.F., Drouin, R., Krueger, C.C., 2018. Divergent migration within lake sturgeon (*Acipenser fulvescens*) populations: multiple distinct patterns exist across an unrestricted migration corridor. J. Anim. Ecol. 87 (1), 259–273.
Kim, H.S., 2016. Blood glucose measurement: is serum equal to plasma? Diabetes Metab. J. 40 (5), 365–366.
King, K., 2004. Growth, survival, and body composition of juvenile Atlantic sturgeon fed five commercial diets under hatchery conditions. N. Am. J. Aquac. 66 (1), 53–60.
King, W., Hooper, B., Hillsgrove, S., Benton, C., Berlinsky, D.L., 2005. The use of clove oil, metomidate, tricaine methanesulphonate and 2-phenoxyethanol for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.). Aquac. Res. 36 (14), 1442–1449.
Kolarevic, J., Aas-Hansen, Ø., Espmark, Å., Baeverfjord, G., Terjesen, B.F., Damsgård, B., 2016. The use of acoustic acceleration transmitter tags for monitoring of Atlantic salmon swimming activity in recirculating aquaculture systems (RAS). Aquatic Engineer. 72, 30–39.
Kynard, B., Horgan, M., 2002. Ontogenetic behavior and migration of Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*, and shortnose sturgeon, *A. brevirostrum*, with notes on social behaviour. Environ. Biol. Fish 63 (2), 137–150.
Lankford, S.E., Adams, T.E., Cech Jr., J.J., 2003. Time of day and water temperature modify the physiological stress response in green sturgeon, *Acipenser medirostris*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 135 (2), 291–302.
LaRochelle, L., Chhor, A.D., Brownscombe, J.W., Zolderdo, A.J., Danylchuk, A.J., Cooke, S.J., 2021. Ice-fishing handling practices and their effects on the short-term post-release behaviour of largemouth bass. Fish. Res. 243, 106084.
Lawrence, M.J., Jain-Schlaepfer, S., Zolderdo, A.J., Algera, D.A., Gilmour, K.M., Gallagher, A.J., Cooke, S.J., 2018. Are 3 minutes good enough for obtaining baseline physiological samples from teleost fish? Can. J. Zool. 96 (7), 774–786.
Lee, J.H., Yoon, Y.C., Kim, H.S., Lee, J., Kim, E., Findeklee, C., Katscher, U., 2022. In vivo electrical conductivity measurement of muscle, cartilage, and peripheral nerve around knee joint using MR-electrical properties tomography. Sci. Rep. 12 (1), 73.
Lenth, R., 2024. Emmeans: estimated marginal means, aka least-squares means. In: R Package Version 1.10.3.
Leprévost, A., Sire, J.-Y., 2014. Architecture, mineralization and development of the axial skeleton in Acipenseriformes, and occurrences of axial anomalies in rearing conditions; can current knowledge in teleost fish help? J. Appl. Ichthyol. 30 (4), 767–776.
Leroy, B., Scutt Phillips, J., Potts, J., Brill, R.W., Evans, K., Forget, F., Holland, K., Itano, D., Muir, J., Pilling, G., Nicol, S., 2023. Recommendations towards the establishment of best practice standards for handling and intracoelomic implantation of data-storage and telemetry tags in tropical tunas. Anim. Biotel. 11 (1), 4.
Lilly, J., McLean, M.F., Dadswell, M.J., Wirgin, I., Comolli, P., Stokesbury, M.J., 2020. Use of social network analysis to examine preferential co-occurrences in Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* Mitchill, 1815. Anim. Biotel. 8, 14.
Maskill, P.A., Crossman, J.A., Webb, M.A., Marrello, M.M., Guy, C.S., 2022. Accuracy of histology, endoscopy, ultrasonography, and plasma sex steroids in describing the population reproductive structure of hatchery-origin and wild white sturgeon. J. Appl. Ichthyol. 38 (1), 3–16.
Matsche, M.A., 2011. Evaluation of tricaine methanesulfonate (MS-222) as a surgical anesthetic for Atlantic Sturgeon *Acipenser oxyrinchus oxyrinchus*. J. Appl. Ichthyol. 27 (2), 600–610.
Matsche, M.A., 2017. Efficacy and physiological response to chemical anesthesia in wild hickory shad during spawning season. Marine Coastal Fish. 9 (1), 296–304.
McLean, M.F., Litvak, M.K., Cooke, S.J., Hanson, K.C., Patterson, D.A., Hinch, S.G., Crossin, G.T., 2019. Immediate physiological and behavioural response from catch-and-release of wild white sturgeon (*Acipenser transmontanus* Richardson, 1836). Fish. Res. 214, 65–75.
McLean, M.F., Litvak, M.K., Stoddard, E.M., Cooke, S.J., Patterson, D.A., Hinch, S.G., Welch, D.W., Crossin, G.T., 2020. Linking environmental factors with reflex action mortality predictors, physiological stress, and post-release movement behaviour to evaluate the response of white sturgeon (*Aciepenser transmontanus* Richardson, 1836) to cahtc-and-release angling. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 240, 110618.
Melnychuk, M.C., Dunton, K.J., Jordaan, A., McKown, K.A., Frisk, M.G., 2017. Informing conservation strategies for the endangered Atlantic sturgeon using acoustic telemetry and multi-state mark–recapture models. J. Appl. Ecol. 54 (3), 914–925.
Miller, E.A., Froehlich, H.E., Cocherell, D.E., Thomas, M.J., Cech, J.J., Klimley, A.P., Fangue, N.A., 2014. Effects of acoustic tagging on juvenile green sturgeon incision healing, swimming performance, and growth. Environ. Biol. Fish 97, 647–658.
Mohler, J.W., Sweka, J.A., Kahnle, A., Hattala, K., Higgs, A., DuFour, M., Breece, M.W., Fox, D.A., 2012. Growth and survival of hatchery-produced Atlantic sturgeon released as young-of-year into the Hudson River, New York. J. Fish Wildl. Manag. 3 (1), 23–32.
Morgenroth, D., Kvaestad, B., Økland, F., Finstad, B., Olsen, R.E., Svendsen, E., Rosten, C., Axelsson, M., Bloecher, N., Føre, M., Gräns, A., 2024. Under the sea: how can we use heart rate and accelerometers to remotely assess fish welfare in salmon aquaculture? Aquaculture 579, 740144.
Neiffer, D.L., 2021. Anesthesia and analgesia. In: In Hadfield, C.A., Clayton, L.A. (Eds.), Clinical Guide to Fish Medicine. John Wiley & Sons, Hoboken, NJ, U.S.A, pp. 198–212.

Nelson, L.R., Small, B.C., 2014. Stress responses in pallid sturgeon following three simulated hatchery stressors. N. Am. J. Aquac. 76 (2), 170–177.
Penny, F.M., Bugg, W.S., Kieffer, J.D., Jeffries, K.M., Pavey, S.A., 2023. Atlantic sturgeon and shortnose sturgeon exhibit highly divergent transcriptomic responses to acute heat stress. Comp. Biochem. Physiol. Part D 45, 101058.
Posit Team, 2024. RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA, U.S.A.
Priborsky, J., Velisek, J., 2018. A review of three commonly used fish anesthetics. Rev. Fish. Sci. Aquacult. 26 (4), 417–442.
Prystay, T.S., Elvidge, C.K., Twardek, W.M., Logan, J.M., Reid, C.H., Clarke, S.H., Foster, J.G., Cooke, E.L.L., Cooke, S.J., 2017. Comparison of the behavioral consequences and recovery patterns of largemouth bass exposed to MS-222 or electrosedation. Trans. Am. Fish. Soc. 146 (3), 556–566.
R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Raby, G.D., Donaldson, M.R., Hinch, S.G., Patterson, D.A., Lotto, A.G., Robichaud, D., English, K.K., Willmore, W.G., Farrell, A.P., Davis, M.W., Cooke, S.J., 2012. Validation of reflex indicators for measuring vitality and predicting the delayed mortality of wild coho salmon bycatch released from fishing gears. J. Appl. Ecol. 49 (1), 90–98.
Reid, C.H., Vandergoot, C.S., Midwood, J.D., Stevens, E.D., Bowker, J., Cooke, S.J., 2019. On the electroimmobilizaiton of fishes for research and practice: opportunities, challenges, and research needs. Fisheries 44 (12), 576–585.
Reid, C.H., Berberi, A.I., Scott, K.M., Woods, S.J., Midwood, J.D., Cooke, S.J., 2024. Evaluating immobilisation thresholds and suitability of conductive glove electrodes for largemouth bass electroanaesthesia. Fish. Res. 272, 106931.
Richmond, A.M., Kynard, B., 1995. Ontogenetic behavior of shortnose sturgeon, *Acipenser brevirostrum*. Copeia 1995, 172–182.
Sadoul, B., Geffroy, B., 2019. Measuring cortisol, the major stress hormone in fishes. J. Fish Biol. 94 (4), 540–555.
Sharber, N.G., Sharber Black, J., 1999. Epilepsy as a unifying principle in electrofishing theory: a proposal. Trans. Am. Fish. Soc. 128 (4), 666–671.
Sievert, C., 2020. Interactive Web-Based Data Visualization with R, Plotly, and Shiny. Chapman and Hall/CRC, Boca Raton, FL, U.S.A.
Sink, T.D., Strange, R.J., Sawyers, R.E., 2007. Clove oil used at lower concentrations is less effective than MS-222 at reducing cortisol stress responses in anesthetized rainbow trout. N. Am. J. Fish Manag. 27 (1), 156–161.
Sladky, K.K., Swanson, C.R., Stoskopf, M.K., Loomis, M.R., Lewbart, G.A., 2001. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). Am. J. Vet. Res. 62 (3), 337–342.
Soltani, M., Mirzargar, S.S., 2013. Effect of tricainemethanesulfonate (MS222), clove oil and electro-anaesthesia on respiratory burst activity in whole blood and serum alternative complement response in rainbow trout (*Oncorhynchus mykiss*), during the narcosis stage. Fish Shellfish Immunol. 34 (2), 692–696.
Stoot, L.J., Cairns, N.A., Cull, F., Taylor, J.J., Jeffrey, J.D., Morin, F., Mandelman, J.W., Clark, T.D., Cooke, S.J., 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. Conserv. Physiol. 2 (1), cou011.
Summerfelt, R.C., Smith, L.S., 1990. Anaesthesia, surgery and related techniques. In: Schreck, C.B., Moyle, P.B. (Eds.), Methods for Fish Biology. American Fisheries Society, Bethesda, MD, U.S.A, pp. 213–272.
Taylor, P.W., Roberts, S.D., 1999. Clove oil: an alternative anaesthetic for aquaculture. N. Am. J. Aquac. 61 (2), 150–155.
Topic Popovic, N., Strunjak-Perovic, I., Coz-Rakovac, R., Barisic, J., Jadan, M., Persin Berakovic, A., Sauerborn Klobucar, R., 2012. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. J. Appl. Ichthyol. 28 (4), 553–564.
Trushenski, J.T., Bowker, J.D., 2012. Effect of voltage and exposure time on fish response to electrosedation. J. Fish Wildl. Manag. 3 (2), 276–287.
Trushenski, J.T., Bowker, J.D., Mulligan, B.L., Gause, B.R., 2012a. Induction, recovery, and hematological responses of largemouth bass to chemo- and electrosedation. N. Am. J. Aquac. 74 (2), 214–223.
Trushenski, J.T., Bowzer, J.C., Bowker, J.D., Schwarz, M.H., 2012b. Chemical and electrical approaches to sedation of cobia: induction, recovery, and physiological responses to sedation. Marine Coastal Fish. 4 (1), 639–650.
Trushenski, J.T., Bowker, J.D., Cooke, S.J., Erdahl, D., Bell, T., MacMillan, J.R., Yanong, R.P., Hill, J.E., Fabrizio, M.C., Garvey, J.E., Sharon, S., 2013. Issues regarding the use of sedatives in fisheries and the need for immediate-release options. Trans. Am. Fish. Soc. 142 (1), 156–170.
Vélez-Espino, L.A., Koops, M.A., 2009. Recovery potential assessment for lake sturgeon in Canadian designatable units. N. Am. J. Fish Manag. 29 (4), 1065–1090.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth edition. Springer, New York, NY, U.S.A.
Vibert, R., 1963. Neurophysiology of electric fishing. Trans. Am. Fish. Soc. 92 (3), 265–275.
Wagner, G.N., Singer, T.D., McKinley, S., 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquac. Res. 34 (13), 1139–1146.
Ward, T.D., Brownscombe, J.W., Gutowsky, L.F., Ballagh, R., Sakich, N., McLean, D., Quesnel, G., Gambhir, S., O'Connor, C.M., Cooke, S.J., 2017. Electric fish handling gloves provide effective immobilization and do not impede reflex recovery of adult largemouth bass. N. Am. J. Fish Manag. 37 (3), 652–659.
Webb, M.A.H., Van Eenennaam, J.P., Crossman, J.A., Chapman, F.A., 2019. A practical guide for assigning sex and stage of maturity in sturgeons and paddlefish. J. Appl. Ichthyol. 35 (1), 169–186.
Weber, R.A., Pérez-Maceira, J.J., Peleteiro, J.B., García-Martín, L., Aldegunde, M., 2011. Effects of acute exposure to 2-phenoxyethanol, clove oil, MS-222, and metomidate on primary and secondary stress responses in Senegalese sole (*Solea senegalensis* Kaup 1858). Aquaculture 321 (1–2), 108–112.
Woody, C.A., Nelson, J., Ramstad, K., 2002. Clove oil as an anaesthetic for adult sockeye salmon: field trials. J. Fish Biol. 60 (2), 340–347.
Zahl, I.H., Samuelsen, O., Kiessling, A., 2012. Anaesthesia of farmed fish: implications for welfare. Fish Physiol. Biochem. 38, 201–218.
Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol. Evol. 1 (1), 3–14.