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Electrosedation of freshwater fishes for the surgical implantation of transmitters

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Running head: Electrosedation of freshwater fishes

Abstract

Although electrosedation is becoming a frequently used technique to anaesthetize fish for a variety of commercial, management, and research applications, limited information is available on the effectiveness of different settings (e.g. voltage, frequency, shocking duration) and target species. We examined recovery times and short-term survival while using a commercially available electroanaesthesia unit to prepare fishes for transmitter implantation surgeries and ran experimental tests without surgery on different electrosedation settings. Our results indicate that electrosedation is a quick and efficient form of anaesthesia, with full anaesthesia achieved, surgeries completed, and recovery in under 10 min for Common Carp *Cyprinus carpio* L., 1758, Goldfish *Carassius auratus* L. 1758, and others. We had zero mortality over at least 24 h associated with electrosedation. We found no significant differences in recovery times related to species or weight, nor did we find any significant changes to recovery time when changing voltage applied, frequency, shocking duration, or when changing the type of pulse applied (e.g. burst pulses vs. standard pulse). Although further researches are needed to address the sub-lethal and long-term effects of eletrosedation, these findings are important to researchers for selecting suitable target species and optimizing recovery time and survival for a quick release after surgery, tagging, or any time fish sedation is required.

Keywords: electroanaesthesia, induction, sedation, recovery, Common Carp *Cyprinus carpio*

43 <A> Introduction

44 Anaesthetics are the standard method used in a variety of fishery management practices
45 to immobilize fish for handling and to reduce stress, particularly during procedures that would
46 otherwise cause substantial stress in non-anaesthetised fishes (Muench 1958; McFarland 1959).
47 Historically, chemicals such as tricainemethanesulfonate (commonly referred to as MS-222),
48 quinaldine, eugenol (clove oil), and gases or other physical processes, such as CO₂, temperature,
49 and tonic immobility, have been used as anaesthetics for fishes (Hisaka et al. 1986; Anderson et
50 al. 1997; Ackerman et al. 2005; Gosset and Rives 2005; Vandergoot et al. 2011; Kessel and
51 Hussey 2015). The benefits and drawbacks of these various sedation techniques have been
52 debated over the years; however, more recently, the use of electrosedation has gained interest in
53 the fisheries and scientific community as a potential alternative (Jennings and Looney 1998;
54 Ackerman et al. 2005; Renault et al. 2011; Rous et al. 2015).

55 Compared to chemical anaesthetics, electricity offers quicker recovery times with fewer
56 post-anaesthetic impacts on fish physiology and biochemistry (Gosset and Rives 2005; Renault
57 et al. 2011). Levels of cortisol and the transcription of genes related to oxidative-stress proteins
58 has been shown to be higher when using clove oil compared to electrosedation (Gosset and Rives
59 2005; Trushenski et al. 2012). With chemicals, there are recommended and mandatory
60 withdrawal periods where fishes must be maintained in captivity prior to releasing them back
61 into the wild or processing for human consumption, whereas fishes that undergo electrosedation
62 may be released immediately (i.e., no withdrawal period). While there are new immediate-
63 release candidate substances such as benzocaine, eugenol, and isoeugenol, these are not
64 universally approved and are unavailable in some jurisdictions (Trushenski et al. 2013; Rous et
65 al. 2015). Electrosedation is being increasingly used in the food processing industry as an

alternative to CO₂ and other chemicals for harvest as a means to prepare fish for euthanization (Zydlewski et al. 2008).

Drawbacks of electrosedation include risk of injury to both the handler and to the fishes. For example, prolonged muscle tetany may lead to spinal injuries in fishes (Dalbey et al. 1996; Gaikowski et al. 2001; Zydlewski et al. 2008; Vandergoot et al. 2011). Other drawbacks include increased glucose levels, although the effects are considered fairly minimal (Gosset and Rives 2005; Trushenski et al. 2012). For instance, Largemouth Bass *Micropterus salmoides* (Lacepède, 1802) had elevated plasma glucose and lactate levels from 0.5 to 2 h following electrosedation (Trushenski et al. 2012).

Appropriately anaesthetizing fishes prior to surgically implanting acoustic transmitters is necessary for telemetry studies, so improving our understanding of the effectiveness of different anaesthetic options and appropriateness for target species is important and warranted (Vandergoot et al. 2011). For example, 1 to 21 minutes may be needed to anaesthetize Common Carp *Cyprinus carpio* L., 1758 with clove oil (range 10 – 200 mg/L), depending on fish size and water temperatures (Velisek et al. 2005; Hajek et al. 2006; Bowker et al. 2015). Electrodesation may offer a faster method for sedating fishes; however, data are limited (but see Ackerman et al. 2005; Trushenski et al. 2012). Given the limited information available for freshwater fishes on electrodesation regarding effectiveness and settings, we examined: 1) if electrodesation would be a suitable anaesthetic option by assessing the recovery time and short-term mortality; and, 2) compared the various electrodesation settings.

Methods

Electrosedation trials with surgery

As part of an observational movement study, acoustic transmitters were surgically implanted into 179 fishes between July 17 and November 10 2014. Twenty fishes were tagged weekly from July 17 to August 2 and October 3 to October 31, with a further four fish tagged November 7 and three fish tagged November 10. Of these 179 fishes, 136 were Common Carp, 22 Goldfish *Carassius auratus* L., 1758, eight Largemouth Bass, eight Brown Bullhead *Ameiurus nebulosus* (Lesueur, 1819), four Bowfin *Amia calva* L., 1766, one White Sucker *Catostomus commersonii* (Lacepède, 1803), and one Golden Redhorse *Moxostoma erythrurum* (Rafinesque, 1818). All fishes were captured by boat electrofishing in Hamilton Harbour, at the western end of Lake Ontario and transported in live wells to 12 large aerated recirculating tanks (~700 to 1600 L) at Aquatic Life Research Facility of Canadian Centre for Inland Waters (CCIW), Burlington, Ontario, Canada. All fishes were housed in recirculating tanks with dechlorinated water (12 h: 12 h light: dark photoperiod; water temperature 12-15 °C) and were fed ad libitum (1-1.5% of fish weight) daily of commercial fish food (Profishent Trout Chow, Martin Mills, Inc) and beef liver or heart for Largemouth Bass (Davis 1967; Crans et al. 2015) for at least 2 weeks prior to the study.

We used a Portable Electroanaesthesia System (PES; Smith-Root, Inc., Vancouver, WA) to conduct the electrosedation trials. The system includes a large portable cooler (100 cm X 46 cm X 38 cm) with anode/cathode plates at either end (distance between anode and cathode = 80 cm), both connected to a control system capable of regulating wave form supplied, duty cycle, and time. Electrosedation settings (Smith-Root, Inc. 2009) were as follows: 1) “burst of 3”, duty cycle 62%, burst frequency 500Hz, cycle frequency 30Hz, a voltage of 100V; or, 2) “standard pulse”, duty cycle 25%, burst frequency 100 Hz, cycle frequency 30 Hz, a voltage of 100V

(Table 1). These pre-programmed settings (Smith-Root, Inc. 2009) were selected with input from Smith-Root personnel and experienced biologists at Fisheries and Oceans Canada. The burst-of-3 setting indicates that dual-frequency pulses are sent out in groups of three, with a small delay between those three pulses (burst frequency) followed by another delay between each burst, known as the cycle frequency, whereas, standard-pulse settings send out rectangular pulses (Reynolds 1996; Smith-Root, Inc. 2009). Fishes were removed from holding tanks using dipnets and placed into the PES unit, which was filled with dechlorinated water. The dechlorinated tap water quality was measured on four separate days using multiparameter sondes (YSI Inc.) and had the following ranges in water chemistry: water temperature 12.7-12.8 °C; conductivity of 273.9-275.3 $\mu\text{S}/\text{cm}$; total dissolved solids 41-56 mg/L; salinity 0.15 psu; dissolved oxygen 10.39-10.45 mg/L; pH 7.60-7.62; and, turbidity of 0.19-0.23 FNU. During electrosedation, shocking was applied to each fish until stage IV of sedation was achieved (Summerfelt and Smith 1990). In general, fish were oriented at horizontal angles towards cathode or anode plates (0° or 180°, Rous et al. 2015). Stage IV sedation is associated with the total loss of equilibrium, muscle tone, and responsiveness to visual and tactile stimuli but maintenance of a steady, although reduced, opercular ventilation rate. In general, tremor was observed following shocking (and fish were perhaps temporarily in stage V of sedation). Stage V sedation is associated with total loss of reactivity, slow and irregular opercular movements, very slow heart rate, and loss of all reflexes. The fishes then underwent a quick surgical procedure of about 2 min by several trained surgeons to implant acoustic tags (length = 17.5 – 25.0 mm, weight = 0.65 – 4.6 g; HTI 795 LG) and were promptly placed in an aerated recovery tank. For each specimen, weight, fork-length, and a photograph were taken. A 1-2 cm incision was made on the ventral side of each fish, the tag inserted, and then the incision closed using a surgeon's knot with 3-4 μm suture thread. Recovery

time was measured from the moment the fish reached stage IV anaesthesia, until the fish was able to maintain proper balance in the recovery tank including the surgery time. Once observed upright, they were turned back over to determine whether or not their balance and motor skills had returned enough for them to return to the upright position immediately (Davis 2010; Rous et al. 2015). Since the assessment of induction and recovery can be somewhat subjective, bias was minimized by having 1-3 observers making all the assessments. Shortly after motor skills were recovered, fishes were returned to their holding tanks, where they were monitored immediately and for at least 24 h (range 24 – 72 h) until they were released into an outdoor mesocosm experiment.

Electrosedation trials without surgery

Four settings were compared to evaluate whether they had any effect on the electrosedation of Common Carp (Table 1). Treatments 1 and 3 used PES settings with the goal of assessing the impact of cutting the burst and cycle frequencies in half for the burst-of-3 setting while maintaining the same duty cycle, voltage, and shocking duration. Treatments 2 and 4 used settings with the goal of assessing any major differences between burst of 3 and standard pulse, and to determine the effect of doubling the shocking duration. These particular settings were selected based on preliminary results of electrosedation trials with surgery, and to minimize the shock delivered to fishes in general.

To isolate the effect of using different electrosedation settings with the PES unit, fishes did not undergo tagging surgeries. They were shocked under the assigned settings, placed in a recovery cooler with aerated dechlorinated water, and monitored until they achieved balance, similar to

electrosedation trials with surgery. For each trial, 10 Common Carp, randomly selected (total of 40 individuals), were collected and housed under similar conditions as the electrosedation trials with surgery.

 Data analyses

For the electrosedation trials with surgery, we used a one-way ANOVA to assess recovery time differences between species for burst of 3 setting only. Bowfin, White Sucker, and Golden Redhorse were excluded in the analyses as each species had a sample size of less than five. Fishes for standard pulse setting were also excluded in the analyses as all species had a sample size of less than five except Common Carp. A multiple regression analysis was used to examine the effect of fish weight and shocking time on recovery time for Common Carp.

For electrosedation trials without surgery, we used a one-way ANCOVA analysis to assess the effects of four electrosedation settings on recovery time for Common Carp using weight as a covariate. We also examined the relationship between weight and recovery time within the four treatments via linear regression analyses to determine if the size of a fish affected its recovery time. All analyses were performed using R version 3.0.2. (R Development Core Team 2013).

<A> Results

Electrosedation trials with surgery

All 179 fishes recovered within 160 to 2660 s of electrosedation and survived for at least 24 h using both settings (Table 2). Shocking durations were in the ranges of 4-24 s and 5-12 s for burst of 3 and standard pulse settings, respectively (Table 2). Recovery time was faster on

average when using the burst of 3 (mean $\pm$ standard deviation (SD) = 405 ± 294 ; $n = 155$; Fig. 1A) compared to standard pulse (mean $\pm$ SD = 508 ± 235 ; $n = 15$; Fig. 1B). Using the burst of 3, Brown Bullhead had the longest mean recovery time followed by Largemouth Bass, Common Carp, and Goldfish (Fig. 1A). The recovery time did not differ significantly between species while using the burst-of-3 settings (one-way ANOVA: $F_{3,151} = 0.78$, $P = 0.51$; Fig. 1A). Although sample sizes were low ($n < 5$), we observed that White Sucker, Golden Redhorse, and Bowfin had similar recovery times (Table 2). In addition, there were no significant relationships for Common Carp between either weight ($P = 0.98$) or shocking duration ($P = 0.18$) and recovery time (multiple regression: $F_{2,118} = 0.906$, $R^2 = 0.015$).

Electrosedation trials without surgery

Pooling individuals from all four treatments, average recovery time for Common Carp was 222 s (Fig. 2). There were no significant differences in recovery times of Common Carp between the four electrosedation settings evaluated with using weight as a covariate (one-way ANCOVA: $F_{3,35} = 2.13$, $P = 0.11$; Fig. 2). Linear regressions determined that, regardless of the electrosedation settings, weight did not demonstrate a significant relationship with recovery time (Treatment 1: $R^2 = 0.34$, $P = 0.075$, Treatment 2: $R^2 = 0.12$, $P = 0.33$, Treatment 3: $R^2 = 0.04$, $P = 0.57$, Treatment 4: $R^2 = 0.08$, $P = 0.44$; Fig. 3).

<A> Discussion

Our results indicate that electrosedation for Common Carp, Goldfish, and others is an effective and efficient option for immobilizing fish prior to surgical procedures associated with

transmitter implantation. The mean recovery times, including time spent during surgery, for Common Carp (using burst of 3), Goldfish (burst of 3), and Common Carp (standard pulse) were 399 s, 373 s, and 508 s, respectively (Table 2). All fishes reached stage IV of sedation (Summerfelt and Smith 1990) with an average ~8 shocking seconds and maximum of 24 shocking seconds. The recovery times in this study were quicker than the 15 – 30 min in Common Carp anaesthetized by clove oil (Velisek et al. 2005), but not subject to surgical procedure. However, other studies (Hajek et al. 2006; Bowker et al. 2015) have shown comparable recovery times of 4 to 19 mins without surgical procedures. Of the chemicals available for anaesthesia, clove oil has both favourable induction and recovery times relative to MS-222 and Quinaldine (Anderson et al. 1997; Woody et al. 2002; Ackerman et al. 2005; Bowker et al. 2015). Yet, to reach the level of anaesthesia required for invasive procedures such as surgeries, induction times regularly take several minutes, and recovery times reported were either similar or longer than those seen for electrosedation in our study (Prince and Powell 2000; Cooke et al. 2004; Bowker et al. 2015). In addition, electrosedation (Ackerman et al. 2005; Vandergoot et al. 2011; Trushenski et al. 2012) had both the fastest induction and recovery times relative to CO₂, MS-222, benzocaine, and clove oil (Bowker et al. 2015). Our study demonstrates the effectiveness of electrosedation in adult large-bodied freshwater fishes, while other studies have demonstrated its effectiveness with both juveniles (Trushenski and Bowker 2012) and marine species (Trushenski et al. 2012).

There was no short-term mortality in our study following electrosedation among the 158 fishes that underwent surgical tagging procedures. In addition, all 40 Common Carp subjected to different electrosedation settings, but no surgery, survived for at least 24 h. Other studies have indicated mortality associated with electrosedation, including rates of 6% over 84 days for Lake

Trout *Salvelinus namaycush* (Gaikowski et al. 2001) and immediate mortality rates of 8.3% for Walleye *Sander vitreus* (Vandergroot et al. 2011). While difficult to detect (Vandergroot et al. 2011), it is possible that fishes in our study may have been susceptible to sub-lethal effects of electrosedation such as vertebral injuries or tissue damage (Gaikowski et al. 2001; Snyder 2003; but see Culver and Chick 2015) and/or post-surgical stress (Moore et al. 1990; Jepsen et al. 2001), which we did not evaluate in this study. However, there were only four confirmed mortalities (2%) of unknown causes from the telemetry studies during the study, suggesting that most tagged fishes survived at least 1 week and up to 8 weeks, prior to being released back into the wild (J. Kim, Fisheries and Oceans Canada, unpublished data). While further research on sub-lethal effects, including physiology and behaviour of fishes and the long-term effects and survival, are necessary, our study provides support that electrosedation is a safe method for anaesthetizing fishes for tagging studies, while minimizing acute mortality.

Electrosedation represents a promising zero-withdrawal approach where it provides viable sedation while avoiding the common constraints of chemical sedatives (Kessel and Hussey 2015; Rous et al. 2015). Most of the 179 fishes in our study recovered quickly (under 10 min) and were ready to be released immediately with no chemical residue (Ackerman et al. 2005; Trushenski and Bowker 2012) following electrosedation and surgical implantation of acoustic transmitters. However, there were some fishes that took longer than 10 minutes to recover (Fig. 1), which warrants further research for refinement of electrosedation techniques, and minimizing of any potential sub-lethal and/or long-term effects of electrosedation (CCAC 2005; AVMA 2008; Rous et al. 2015). In contrast, with MS-222, the predominant method of fish sedation in North America (Ross and Ross 2008; Trushenski et al. 2013), 21 days of a withdrawal period in food fish is required to avoid accidental ingestion by harvesters (Summerfelt and Smith 1990;

FDA 2002; Carter et al. 2011). There are a variety of other chemical sedatives (reviewed in Ross and Ross 2008; Trushenski et al. 2013; Bowker et al. 2015) including new and growing use of sedatives such as Aqui-S 20E (10 % eugenol) and Benzoak (20% benzocaine), although most cannot be used on food fish. While the FDA is currently evaluating AQUIS 20E as an immediate release sedative (approved under INAD authority), both Aqui-S 20E and Benzoak are neither universally approved nor available in some jurisdictions (Trushenski et al. 2013; Bowker et al. 2015; Rous et al. 2015). Hence, electrosedation provides a safe and efficient method of sedating and releasing fishes for various invasive and noninvasive procedures such as surgeries and external tagging (Trushenski et al. 2013).

Our results indicate that electrosedation is similarly effective across a range of body sizes of Common Carp and Goldfish. We did not observe any significant differences in recovery times or short-term mortalities between the four treatments used to test the electrosedation settings, making it difficult to recommend which settings to use in the future. Doubling shocking duration from 5 to 10 s, halving the burst and cycle frequencies, and switching from burst-of-3 to standard-pulse settings all produced similar results. Further research with potentially larger sample sizes and a broader range of fish sizes and species would provide additional insights on effects of specific electrosedation settings (Trushenski et al. 2012; Bowker et al. 2015; Rous et al. 2015).

Electrosedation is a viable method for surgical anaesthetization, and the results of our study indicate that fishes can undergo sedation, an invasive tagging surgery and recover in under 10 min with no immediate mortality. With no withdrawal period required before release, as is the case with some forms of chemical sedation (Anderson et al. 1997; Bowker et al. 2016), electrosedation offers a quick method that can be used in the field, where fishes may be released

immediately upon recovery. While further research expanding upon current sample sizes and species studied is required to determine optimal shocking settings and evaluate post-release behaviour or physiology, animal welfare, and long-term survival (CCAC 2005; AVMA 2008), our research indicates that the use of pulsed direct electric current was sufficient to immobilize large-bodied freshwater fishes of varying sizes long enough to allow surgical implantation of telemetry transmitters.

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410 <A> Figure captions

411 Figure 1. Boxplot of recovery times using A) burst of 3, and B) standard pulse settings in the
412 electrosedation trials with surgery. A) Species include Common Carp *Cyprinus carpio* (CC; $n =$
413 121), Goldfish *Carassius auratus* (GF; $n = 20$), Largemouth Bass *Micropterus salmoides* (LMB;
414 $n = 8$), and Brown Bullhead *Ameiurus nebulosus* (BB; $n = 6$). B) Common Carp (CC; $n = 15$).
415 Box limits indicate lower and upper quartiles (25% and 75%), whiskers indicate the range of the
416 distribution excluding outliers, and the solid line indicates the median.

417 Figure 2. Boxplot of recovery times for Common Carp (CC; $n = 10$ for each treatment) using
418 four different electrosedation settings in the electrosedation trials without surgery outlined in
419 Table 1. Box limits indicate lower and upper quartiles (25% and 75%), whiskers indicate the
420 range of the distribution excluding outliers, and the solid line indicates the median.

421 Figure 3. Relationship between recovery time (s) and weight (kg) for Common Carp using four
422 electrosedation settings in the electrosedation trials without surgery outlined in Table 1. The
423 treatments are labeled as blue squares (Treatment 1), orange triangles (Treatment 2), grey
424 diamonds (Treatment 3), and yellow open circles (Treatment 4).

Figure 1. Boxplot of recovery times using A) burst of 3, and B) standard pulse settings in the electrosedation trials with surgery. A) Species include Common Carp *Cyprinus carpio* (CC; $n = 121$), Goldfish *Carassius auratus* (GF; $n = 20$), Largemouth Bass *Micropterus salmoides* (LMB; $n = 8$), and Brown Bullhead *Ameiurus nebulosus* (BB; $n = 6$). B) Common Carp (CC; $n = 15$). Box limits indicate lower and upper quartiles (25% and 75%), whiskers indicate the range of the distribution excluding outliers, and the solid line indicates the median.

198x97mm (288 x 288 DPI)

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162x157mm (288 x 288 DPI)

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164x157mm (288 x 288 DPI)

Table 1: Settings used with Portable Electroanesthesia System (PES).

Treatments		Pulse type	Duty cycle	Burst frequency (Hz)	Cycle frequency (Hz)	Voltage (V)	Shocking duration (s)
Electrosedation trials with surgery	1	burst of 3	62%	500	30	100	4-24
	2	standard pulse	25%	100	30	100	5-12
Electrosedation trials without surgery	1	burst of 3	62%	500	30	300	5
	2	standard pulse	25%	250	30	100	5
	3	burst of 3	62%	250	15	300	5
	4	standard pulse	25%	100	30	100	10

Table 2. Descriptive statistics for the 179 fishes that underwent electrosedation trials with surgery. Values are presented with their means $\pm$ standard deviation. Note that species with a sample size of one do not have a standard error.

1) burst of 3					2) standard pulse			
Species	<i>n</i>	Weight (kg)	Shocking duration (s)	Recovery time (s)	<i>n</i>	Weight (kg)	Shocking duration (s)	Recovery time (s)
Bowfin <i>Amia calva</i>	3	2.94 ± 0.84	5.0 ± 1.0	413 ± 89	1	2.21	5	180
Brown Bullhead <i>Ameiurus nebulosus</i>	6	0.42 ± 0.08	4.0 ± 1.8	564 ± 251	1	0.43	6	377
Common Carp <i>Cyprinus carpio</i>	121	3.10 ± 1.27	8.6 ± 3.84	399 ± 303	15	3.76 ± 1.48	8.87 ± 2.17	508 ± 235
Golden Redhorse <i>Moxostoma erythrurum</i>	1	1.51	10	415				

Goldfish <i>Carassius auratus</i>	20	0.77 ± 0.17	7.2 ± 2.8	373 ± 249	2	1.01 ± 0.22	8.5 ± 2.1	675 ± 573
Largemouth Bass <i>Micropterus salmoides</i>	8	0.98 ± 0.30	4.0 ± 1.20	464 ± 106				
White Sucker <i>Catostomus</i> <i>commersonii</i>	1	1.36	7	525				