Comparison of the Behavioral Consequences and Recovery Patterns of Largemouth Bass Exposed to MS-222 or Electrosedation

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Comparison of the Behavioral Consequences and Recovery Patterns of Largemouth Bass Exposed to MS-222 or Electrosedation

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Abstract

Fish are commonly sedated to render them immobile and thus easier to handle for research, veterinary, and aquaculture practices. Since sedation itself imposes a significant challenge on the targeted fish, the selection of sedation methods that minimize physiological and behavioral disturbance and recovery time is essential. Two popular sedation methods include the chemical tricaine methanesulfonate (MS-222) and electrosedation. Although many studies have already investigated the physiological consequences of these methods, there is limited research examining the latent behavioral effects on fish. Using Largemouth Bass *Micropterus salmoides* as a model species, we compared the postsedation behaviors of fish that were sedated with either MS-222 or electrosedation to those of a control group exposed to the same handling protocol. Immediately after sedation, fish exposed to either treatment demonstrated lower reflex scores than the control group. Time to resume regular ventilation did not differ between chemically sedated and electrosedated fish; however, electrosedated fish regained equilibrium faster (mean ± SE = 154 ± 20 s) than fish that were exposed to MS-222 (264 ± 30 s). Locomotor activity and swimming performance were assessed at 5-, 30-, or 60-min intervals, beginning after individuals had recovered from sedation sufficiently to regain equilibrium. For all postsedation intervals, locomotor activity was two times greater in the electrosedated group than in the control and MS-222 groups. Other behavioral measures (refuge emergence time, activity level, and flight initiation distance) and swimming performance did not differ at 5, 30, or 60 min postrecovery for any of the treatment groups. Our results indicate that while both chemical and electrical sedation methods result in impairment (i.e., sedation) immediately after treatment, these behavioral effects do not persist beyond 5 min postrecovery, and the two methods have similar impacts on Largemouth Bass. However, we caution that these results cannot be extrapolated to other fish species without further study.

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There are multiple reasons for sedating fish, including facilitating surgical procedures, minimizing handling and escapes, maintaining fish welfare, and increasing the general safety of the fish handling and/or surgical procedures for the handler or surgeon (Neiffer and Stamper 2009; Trushenski et al. 2012). Understanding the ecological endpoints of procedures like sedation techniques is critical for maintaining the welfare status of fish and ensuring that fish regain "normal" behavior rapidly after being subject to sedation. Nevertheless, it is widely acknowledged that the use of sedatives has its own consequences (reviewed by Cooke et al. 2016). The ideal sedative is one that exerts minimal negative impacts on the animal and the environment, is fast acting, can be effectively used in small quantities, and can be used across a variety of environmental conditions without altering the behavior or physiology of the subject (Ross and Ross 2008; Trushenski et al. 2012).

One of the most frequently used methods of sedating fish is with a buffered solution of tricaine methanesulfonate (MS-222), which is especially popular in North America (Popovic et al. 2012; Trushenski et al. 2013). An important factor contributing to the adoption of MS-222 as a sedative is that it is usable on food fishes provided that users follow the appropriate withdrawal procedures (Carter et al. 2011; Trushenski et al. 2013). Furthermore, MS-222 can maintain an animal under sedation for extended periods, allowing for more complex procedures. However, the use of MS-222 as a sedative requires a postapplication holding period to reduce the MS-222 concentration in the sedated fish before releasing them into nature. The length of the holding period is 5 d in Canada (Health Canada 2010) but varies with the laws of different governments (Carter et al. 2011; Popovic et al. 2012; Trushenski et al. 2013). Observed physiological consequences associated with sedation via MS-222 include hyperglycemia, hypoxia, respiratory acidosis, hypercapnia, and tachycardia (Sladky et al. 2001; Cotter and Rodnick 2006; Popovic et al. 2012). Such physiological alterations presumably have negative consequences for fish behavior (see Schreck et al. 1997), but relatively few studies have examined the behavioral consequences of different sedation methods. Among these, Anderson et al. (1997) demonstrated that neither clove oil nor MS-222 had an effect on the critical swimming behavior of Rainbow Trout *Oncorhynchus mykiss*; however, fish that were sedated with clove oil required a longer recovery time than MS-222-sedated fish. Similarly, Pirhonen and Schreck (2003) reported that steelhead (anadromous Rainbow Trout) sedated with MS-222 and clove oil showed reduced feeding behaviors compared with nonsedated fish. Furthermore, Cooke et al. (2004) reported that in Largemouth Bass *Micropterus salmoides*, the gilling rate decreased with increased sedative (clove oil) concentration; the fish spent more time on the bottom of the enclosure; and at all concentrations, sedated fish required a longer recovery period than nonsedated fish, ranging between 10 and 30 min

for recovery after attaining stage IV or stage V sedation (stage IV includes the loss of equilibrium, no body movement, and reduced ventilation; stage V involves the loss of reactivity and reflexes, irregular or no opercular movements, and a slowed heart rate; Summerfelt and Smith 1990). Such studies show that fish are behaviorally affected after sedation. Wild fish that are sedated (e.g., for tagging studies or stock assessment) are often released back into the wild, where they must be able to avoid predators and obtain food resources; thus, even short-term behavioral impairments could influence their fitness or survival.

An alternative to chemical sedation is electrosedation, whereby an electrical current is used to incapacitate the fish almost immediately (Sterritt et al. 1994). Electrosedation may be preferred over chemical sedatives because it reduces handling stress and allows fish to be released immediately (Trushenski et al. 2012). In Striped Bass *Morone saxatilis*, induction and recovery from stage IV sedation occurred sooner when the fish were exposed to electrosedation than to chemical sedatives (i.e., CO_2, eugenol, benzocaine, and MS-222; Trushenski et al. 2012). In an experiment with Largemouth Bass, Trushenski et al. (2012) observed that on average, electrosedation acted eight times faster than the sedatives MS-222 and CO_2. Additionally, those authors noted that electrosedated Largemouth Bass had (1) relatively low cortisol levels compared to fish sedated with MS-222 or CO_2 but (2) relatively high blood glucose and lactate levels in comparison with MS-222- or eugenol-treated fish. These physiological alterations may negatively affect the sedated individuals, but there is limited knowledge as to how electrosedation and its associated physiological consequences affect the postsedation behavior of fish. Past research investigating recovery after electrosedation has measured the time to resumption of opercular movement, fin movement, and equilibrium and the survival rate (Vandergoot et al. 2011; Trushenski et al. 2012) but has ignored the effects on swimming performance and character (i.e., boldness).

Knowledge of postsedation behavior and the application of appropriate recovery measures can aid in reducing postrelease mortality due to a compromised swimming ability, which can result in predation or downstream drift (Cury and Kynard 1978). Ideally, prior to release, fish should be allowed to recover until their original physiological conditions and behaviors have returned. However, even short-term holding (i.e., a few hours) can be stressful for the fish and can result in deleterious consequences (reviewed by Portz et al. 2006), thus limiting the feasibility of an extended holding period. Primary and secondary stress responses induced by suboptimal stocking density, water quality, temperature, and other holding characteristics can ultimately lead to consequences such as stunted growth, reduced fitness, decreased survivability, and increased vulnerability to illness. On the other hand, an insufficient holding period can result in releasing the fish while it is still affected by the sedative, either due to residual sedative

that is still being metabolized by the body or due to physiological disturbance, leaving the fish vulnerable to lethal consequences of behavioral impairment, such as reduced predator avoidance ability (Portz et al. 2006; Trushenski et al. 2013).

Although many studies have compared the efficacy of various fish sedatives with a focus on physiological endpoints (Iwama et al. 1989; Thomas and Robertson 1991; Popovic et al. 2012; Trushenski et al. 2012), little is known about the behavioral consequences of chemical sedation and electrosedation—specifically, the effects on activity and swimming performance. Using Largemouth Bass as a model species, we sought to determine (1) how MS-222 and electrosedation influence fish behavior (reflex impairment and swimming performance) at 5-, 30-, and 60-min intervals after sedation and (2) how long a fish should be held to permit behavioral recovery prior to release, with "recovery" defined as the demonstration of behaviors that do not differ significantly from those of nonsedated fish. Because Largemouth Bass are among the most popular recreationally angled fish species in North America (Quinn and Paukert 2009), they are often studied due to their economic and ecological importance and are thus commonly exposed to both MS-222 and electrosedation (Marking and Meyer 1985; Leitner and Isely 1994; Demers et al. 1996; Matsche 2013).

METHODS

Fish Collection

Largemouth Bass were collected from Lake Opinicon (44.5590°N, 76.3280°W), Ontario, Canada, during June 2015. Fish were collected via angling by using a variety of lure types, and landing time was limited to less than 20 s to reduce stress associated with capture and anaerobic exercise (Cooke et al. 2003). Test fish (all between 300 and 400 mm TL) were held for 24 h in offshore floating net-pens (1.2 × 1.2 × 1.2 m) to allow for acclimation to captivity (Wilson et al. 2015). Surface water temperature within the net-pens was measured three times daily with an alcohol thermometer and ranged from 15°C to 23°C.

Experimental Protocol

Sedation.—Sample sizes and size ranges of the Largemouth Bass in each treatment group are summarized in Table 1. In all treatments, we recorded the latency between the attainment of stage IV sedation (or the end of the 5-min control treatment) and the resumption of regular ventilation activity (at least once every 5 s) and equilibrium, which we refer to as the "postsedation recovery time." Fish in the MS-222 treatment were placed individually into coolers (66 × 34 × 31 cm) filled with lake water and dissolved MS-222 at 100 mg/L and were held in the coolers until they reached stage IV sedation. The fish were then transferred to a recovery cooler that received a constant flow of freshwater. Fish in the electrosedation treatment were placed into a Smith-Root Portable Electrosedation System (PES) unit (Smith-Root, Inc., Vancouver, Washington) that was installed in an insulated container (107 × 48 × 47 cm), with the electrodes placed 69 cm apart. The PES unit was operated with a constant setting (3-s operation with standard pulsed DC at 100 Hz, 90 V, and a 25% duty cycle; after Rous et al. 2015). Fish were placed perpendicular to the electrodes during sedation (Rous et al. 2015). Control fish were divided into two equal groups and placed in a cooler filled with lake water to the same depths as the sedative treatments for 5-min periods. Initial examination revealed no behavioral differences attributable to cooler dimensions, so the two control groups were pooled into a single group.

Reflex impairment index.—After the fish regained equilibrium, we applied a five-stage reflex impairment test (reflex action mortality predictors [RAMP]; Raby et al. 2012). Reflexes require the coordination of neurological and physiological functions (Davis 2010), making them a relevant metric for this study. We assigned RAMP scores based on five independent reflex assessments that were scored on a binary scale (0 = impaired; 1 = unimpaired), resulting in an overall range of 0–5. The five reflexes consisted of (1) regaining orientation within 3 s after being flipped upside down; (2) avoidance behavior, evidenced as an attempt to burst swim away during pinching of the caudal fin; (3) ocular control, demonstrated by a fish's ability to roll its eye to maintain level pitch when turned on its side; (4) body flex, shown by a fish's attempt to struggle free while being held out of water by two hands positioned on the middle of its body; and (5) a positive head complex response, evidenced by a regular ventilation pattern of opening and closing the lower jaw within 5 s while the fish was held out of water.

Behavioral trials at three recovery intervals.—Immediately after the reflex assessment, fish were transferred to a flow-

TABLE 1. Numbers and TLs (mean ± SE) of electrosedated, MS-222-sedated, and control Largemouth Bass.

Postsedation recovery interval (min)	Electrosedation		MS-222		Control	
	n	TL (mm)	*n*	TL (mm)	*n*	TL (mm)
5	10	354 ± 7	11	350 ± 11	12	353 ± 7
30	10	351 ± 7	10	352 ± 9	13	346 ± 10
60	10	353 ± 11	10	348 ± 10	13	348 ± 10

through tank supplied with lake water, where they were held for a 5-, 30-, or 60-min recovery interval. At the end of the recovery interval, each fish was placed in an opaque-gray plastic isolation box inside a rectangular test arena (2.6 × 6.0 × 0.65 m [width × length × depth]) located in Lake Opinicon and was allowed to acclimate for 5 min. During all transfer periods, fish were held in water to eliminate air exposure and stress associated with handling. The arena was surrounded with black plastic sheets outfitted with 30- × 30-cm windows to allow for observation and data collection without disturbing the fish. The surface of the test arena was divided into six equal sections of 1-m intervals by using cords strung above the water.

We quantified the behavior of Largemouth Bass by using a common three-component assay consisting of refuge emergence, activity in an open-field test, and flight initiation distance (FID) to an approaching novel object (Wilson and Godin 2009; Jones and Godin 2010). After the fish's 5-min acclimation period in the isolation box, we opened the exit by manually removing the enclosure, and we recorded the time taken by the fish to emerge from the box. If the fish did not emerge after 10 min, we recorded the emergence time as 600 s; whether or not the fish emerged, the refuge box was removed from the arena. Next, we recorded the number of grid lines the fish crossed over a period of 10 min. At the end of this open-field test, we introduced a novel object—consisting of an orange and yellow plastic ball attached to a 2-m-long dowelling rod—into the arena approximately 2 m from the test fish in a location that was likely to be in the fish's field of vision. We approached the novel object directly toward the head of the fish, and we estimated the distance (cm) at which the fish moved to avoid contact. These methods and the trial arena have previously been used to describe the behaviors of Largemouth Bass from Lake Opinicon (Wilson et al. 2015).

Finally, we assessed swimming performance in the test fish at the end of the behavioral observations. The fish were transferred into a flow-through, circular holding tank (1.3 × 0.3 m [diameter × depth]) filled with lake water. The tank was divided into eight wedge-shaped sections, and a GoPro digital camera was suspended above the tank (Fobert et al. 2009). We chased each fish with a wooden rod in circles in the tub and recorded the time until the fish reached exhaustion, defined as follows: fish refused to swim and would not actively avoid being touched or pushed with the chasing rod three consecutive times; or the fish could be rolled onto its side for 3 s (Kieffer 2000). Using the video footage, relative swimming speed (determined by the number of lines crossed during the first 20 s of the chase) and the distance swam (measured by the total number of laps swam) were recorded. Individual physiological and morphological conditions were not recorded because the objective of this assessment was simply to determine the relative differences in swimming performance between treatments (Portz 2007).

Largemouth Bass treated with electrosedation in the PES unit and fish belonging to the control group were allowed 1 h to recover before being released directly into the lake. Fish that were treated with MS-222 were held for an additional 7 d prior to release to allow the sedative to leave their bodies, as required by Health Canada (2010).

Statistical Analyses

All of the response variables (time to resume ventilation, time to regain equilibrium, RAMP score, refuge emergence time, activity in the arena, FID, and time to fatigue during simulated chasing) deviated from a normal distribution (Shapiro–Wilk test: all $P < 0.05$) and were therefore individually rank-transformed to allow for the use of parametric tests (after Scheirer et al. 1976). The ranked response variables were analyzed in three groups based on their order of recording and multicollinearity between them. First, the time to resume ventilation, the time to regain equilibrium, and the RAMP scores after regaining equilibrium were combined into a multivariate response in a factorial multivariate ANCOVA (MANCOVA) against the sedation method (control: $n = 38$; MS-222 treatment: $n = 31$; PES treatment: $n = 30$) and the time of day (early or late morning; early or late afternoon) as categorical factors and with fish size (TL, mm) and water temperature (°C) as linear covariates. Second, the behavioral measures (refuge emergence time [s], activity level [number of lines crossed in an open-field test], and FID [cm]) were examined in a factorial MANCOVA against the sedation method, the time between sedation and behavioral testing (5, 30, or 60 min), and the time of day as fixed factors and with fish size and water temperature as linear covariates. Third, time to fatigue was examined as a univariate response against the sedation method and the time of day as fixed factors and with fish size, water temperature, and the total elapsed time since sedation (i.e., time between sedation and behavioral testing, plus refuge emergence time and the 10-min activity period) as linear covariates.

Stepwise model simplification based on probability (α = 0.05) was used to remove nonsignificant interaction terms while retaining all main effect factors and covariates to identify the most parsimonious, ecologically relevant models. The two multivariate models were then decomposed into individual ANCOVAs and were examined with Tukey's honestly significant difference post hoc test to identify differences between treatments. We also performed post hoc power analyses (after Cohen 1988) on effect sizes in each of the treatment groups. Any significant linear relationships were examined by using Spearman's rank correlations. All analyses were performed in R version 3.2.4 (R Core Team 2016) and the "pwr" package (Champely 2016), and figures were created using SigmaPlot version 11.0 (SYSTAT Software, San Jose, California).

RESULTS

Recovery Immediately after Sedation

Sedation treatment had a significant overall effect on the three short-term recovery measures (time to resume ventilation, time to regain equilibrium, and RAMP score; Pillai's trace = 0.937, $P < 0.0001$; Table 2). Largemouth Bass that were sedated with MS-222 or the PES unit demonstrated significant differences in recovery time compared with the control group (Table 3). On average, the time to resume ventilation was significantly greater for sedated fish (MS-222: mean ± SE = 47 ± 7 s; PES: 49 ± 11 s) than for the control group (0 s), and there was no significant difference between the means for the MS-222 and PES treatments (Figure 1a). Sedated fish also took longer to regain equilibrium (MS-222: mean ± SE = 264 ± 30 s; PES: 154 ± 20 s) than control fish (0 s), and recovery was significantly faster for PES-treated fish than for MS-222-treated fish ($P < 0.001$; Figure 1b). Based on the RAMP assessment conducted immediately after recovery, both sedation methods resulted in lower RAMP scores (MS-222: mean ± SE = 3.2 ± 2; PES: 2.6 ± 0.2) than the control (4.3 ± 0.1), and the PES treatment group's RAMP score was significantly lower than that of MS-222-treated fish ($P = 0.015$; Figure 1c).

Behavioral Assessment at Postsedation Recovery Intervals

At the 5-, 30-, and 60-min postsedation intervals, there was no overall effect of sedation method on the three behavioral measures evaluated (refuge emergence time, activity level, and FID), although temperature covaried significantly with the multiple response (Pillai's trace = 0.13, $P = 0.0071$; Table 2). Refuge emergence time did not differ among treatments (Figure 2a), among recovery intervals, or among times of day and did not covary with fish size (TL) or temperature

TABLE 2. Summary results from two MANCOVAs examining six behavioral measures in adult Largemouth Bass that were exposed to chemical sedation (MS-222) or electrosedation versus an unsedated control group. Significant factors are indicated by P-values in bold italics.

Factor	Pillai's trace	F	df	P
Recovery immediately after sedation				
Treatment	0.937	25.56	6, 174	***<0.0001***
Time of day	0.078	0.78	9, 264	0.639
Size (TL)	0.018	0.52	3, 86	0.669
Temperature	0.024	0.71	3, 86	0.546
Later recovery after sedation (5, 30, or 60 min)				
Treatment	0.13	1.91	6, 170	0.082
Recovery interval	0.05	0.77	6, 170	0.59
Time of day	0.08	0.78	9, 258	0.64
Size (TL)	0.08	2.39	3, 84	0.074
Temperature	0.13	4.3	3, 84	***0.0071***

TABLE 3. Summary results from the MANCOVAs and post hoc power analyses examining the time to recovery immediately after sedation for adult Largemouth Bass that were exposed to MS-222 or electrosedation versus an unsedated control group (ventilation = time [s] to resumption of ventilation; equilibrium = time [s] to regain equilibrium; RAMP = reflex action mortality predictors [see Methods]). Significant factors are indicated by P-values in bold italics.

Response	F	P	Power
Treatment (df = 2, 88)			
Ventilation	84.19	***<0.0001***	1
Equilibrium	115.14	***<0.0001***	1
RAMP score	32.56	***<0.0001***	1
Time of day (df = 3, 88)			
Ventilation	0.14	0.93	
Equilibrium	1.46	0.23	
RAMP score	0.33	0.81	
TL (mm) (df = 1, 88)			
Ventilation	0.37	0.54	
Equilibrium	1.52	0.22	
RAMP score	0.004	0.95	
Temperature (°C) (df = 1, 88)			
Ventilation	0.05	0.82	
Equilibrium	0.002	0.97	
RAMP score	1.32	0.25	

(all $P > 0.05$; Table 4). Activity level (number of lines crossed) differed significantly among treatments ($P = 0.016$), with the PES treatment group crossing more lines on average (mean ± SE = 24.8 ± 3.9) than the MS-222 treatment group (15.8 ± 3.2 lines) or the control group (12.7 ± 2.4 lines; Figure 2b). The FID was positively correlated with fish size ($P = 0.04$; Figure 3a) and negatively correlated with temperature ($P < 0.001$; Figure 3b) but did not differ among treatments (Figure 2c). Time to fatigue during a forced chase was not influenced by treatment, time of day, fish size, or total time elapsed since sedation (all $P > 0.05$; Figure 4a). Time to fatigue did, however, decrease significantly as water temperature increased ($F_{1,\ 87} = 10.48$, $P = 0.002$; Figure 4b).

DISCUSSION

Based on the monitoring of Largemouth Bass behavior at 5, 30, and 60 min after sedation, the present study confirms that electrosedation and MS-222 are both effective sedatives for this species. The results demonstrated that although both the MS-222 and PES methods impair reflexes immediately after sedation, neither sedation method causes the fish to exhibit short-term behavioral alterations beyond 5 min postsedation. The implications of the behavioral changes observed immediately after sedation and recommendations for fish sedation methods are discussed below.
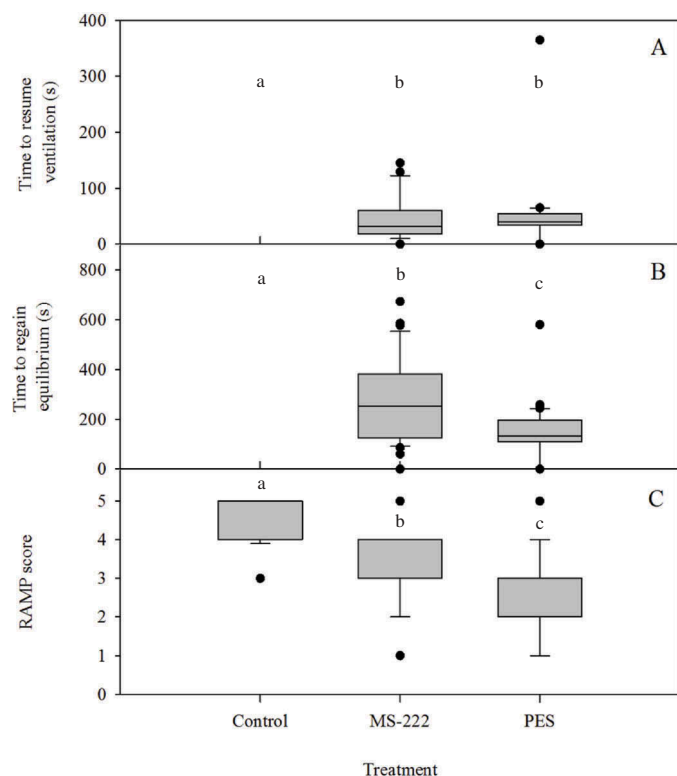
FIGURE 1. Variation in Largemouth Bass behaviors assessed immediately after chemical sedation with MS-222 ($N = 31$) or electrosedation with a Portable Electrosedation System (PES) unit ($N = 30$) versus an unsedated control ($N = 38$), according to ANCOVA analyses. The responses are (A) time (s) to resume ventilation ($P < 0.0001$), (B) time (s) to regain equilibrium ($P < 0.0001$; MS-222 versus PES: $P < 0.001$), and (C) reflex action mortality predictor (RAMP) score ($P < 0.0001$; MS-222 versus PES: $P = 0.015$). Line within box = median; ends of box = 25th and 75th percentiles; ends of whiskers = relative minimum and relative maximum; black circles = outliers. Lowercase letters summarize significant differences between treatments according to the Tukey's honestly significant post hoc analysis.

FIGURE 2. Variation in Largemouth Bass behaviors evaluated after chemical sedation with MS-222 ($N = 31$) or electrosedation with a Portable Electrosedation System (PES) unit ($N = 30$) versus an unsedated control ($N = 38$), according to ANCOVA analyses. The responses are (A) refuge emergence time (s), (B) activity level (number of lines crossed; $P = 0.016$), and (C) flight initiation distance (cm). Line within box = median; ends of box = 25th and 75th percentile; ends of whiskers = relative minimum and relative maximum; black circles = outliers. Lowercase letters summarize significant differences between treatments according to the Tukey's honestly significant post hoc analysis.

Sedatives act directly on the nervous system of the fish. Tricaine methanesulfonate temporarily reduces the function of the central nervous system and the peripheral nervous system by suppressing Na^+ transport into the nerve, thus limiting nerve excitability (Summerfelt and Smith 1990; Burka et al. 1997) and the ability to display voluntary movement. Electrosedation causes fish to experience sustained muscular convulsions (tetany) due to sudden changes in the voltage differential across nerves (Snyder 2003), thereby decreasing the fish's ability to exhibit movement. Given these disruptions to the nervous system, it is not surprising that the sedated fish in the present study exhibited immediate reflex impairment, including delayed resumption of ventilation and a longer time to regain equilibrium. However, similar to the findings of Trushenski et al. (2012) in comparing Largemouth Bass recovery after electrosedation and MS-222 sedation, the duration of these alterations was relatively short (not exceeding 5 min) for both treatments. Such recovery times suggest that once a fish is removed from the sedative, its nervous
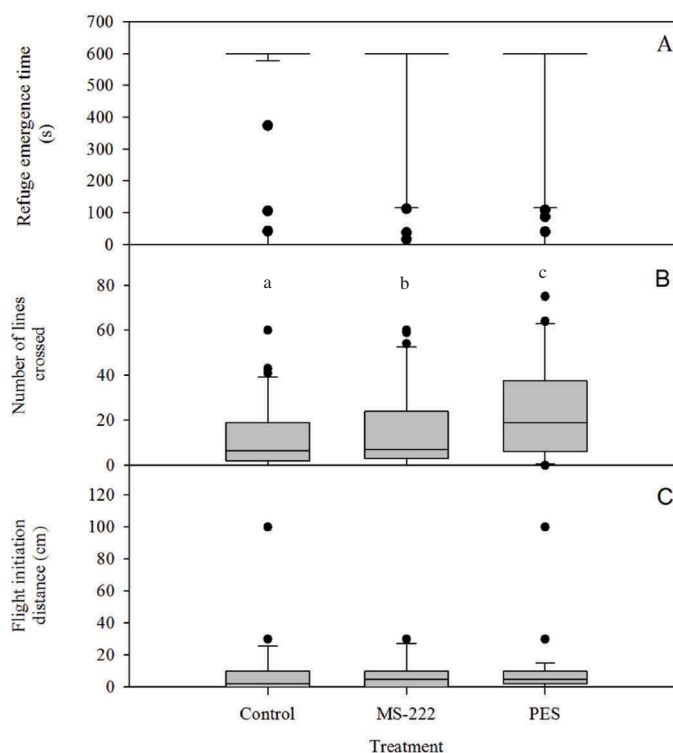
system is able to resume normal functions within minutes of being disabled. The delayed time to regain equilibrium in MS-222-sedated fish compared with electrosedated fish is likely attributable to residual sedatives being metabolized in the body even after the fish were removed from the sedative bath (Burka et al. 1997; Carter et al. 2011).

Even once ventilation and equilibrium were regained, sedated Largemouth Bass continued to exhibit reflex impairment, as indicated by the RAMP assays. This implies that Largemouth Bass experienced behavioral effects at 5 min postsedation (since some fish required up to 5 min to regain equilibrium). Past research has shown that fish have elevated glucose and lactate levels after electrosedation (Popovic et al. 2012; Trushenski et al. 2012), providing a potential physiological explanation for the greater reflex impairment we noted immediately after sedation. However, the more likely justification for this trend is that reflex impairment was assessed immediately after the fish regained equilibrium, which took about 100 s longer on average in MS-222-treated fish than in the PES-treated group; this temporal confound may account for the lower reflex impairment scores

TABLE 4. Summary results from the MANCOVAs and post hoc power analyses examining the behavioral responses at postsedation recovery intervals of 5, 30, or 60 min for adult Largemouth Bass that were exposed to MS-222 or electrosedation versus an unsedated control group (FID = flight initiation distance). Significant factors are indicated by P-values in bold italics.

Response	F	P	Power
Treatment (df = 2, 80)			
Refuge emergence time (s)	0.89	0.41	1.00
Activity level (line crosses)	4.35	***0.016***	1.00
FID (cm)	1.87	0.16	0.99
Postsedation recovery interval (df = 2, 80)			
Refuge emergence	2.3	0.11	
Activity level	0.22	0.80	
FID	0.07	0.93	
Time of day (df = 3, 80)			
Refuge emergence	0.65	0.58	
Activity level	0.68	0.57	
FID	1.35	0.26	
TL (mm) (df = 1, 80)			
Refuge emergence	3.16	0.08	
Activity level	1.04	0.31	
FID	4.53	***0.04***	
Temperature (°C) (df = 1, 80)			
Refuge emergence	0.05	0.83	
Activity level	0.46	0.50	
FID	13.5	***<0.001***	

in the PES group despite their faster recovery periods for resuming ventilation and regaining equilibrium.

These immediate behavioral effects appeared to diminish after a short period of recovery. When held for 5, 30, or 60 min postsedation, Largemouth Bass from both sedation treatments showed little difference in behavior compared to non-sedated fish, implying that the sedated fish had recovered from the treatments. The only behavioral difference was that electrosedated fish were two times more active than the control fish and MS-222-sedated fish regardless of the recovery holding interval. This elevated activity level could be due to stress caused by the PES method. Trushenski et al. (2012) reported that during electrosedation, anaerobic metabolism (resulting from tetanic muscular contractions and the cessation of ventilation) caused an increase in blood lactate and glucose concentrations, which persisted for 2 h postsedation. Those responses were not observed in MS-222-sedated fish (Trushenski et al. 2012). Furthermore, it is also possible that the stress of electrosedation induces a "fight or flight" response (reviewed by Reid et al. 1998). The electrosedation process, coupled with the associated prolonged physiological alterations that require time to regulate (Heath and Pritchard

1965; Perry and Gilmour 2006), likely caused the responses to persist over a 2-h period in the Trushenski et al. (2012) study. Largemouth Bass in the present study were given a maximum recovery time of 1 h; therefore, it is likely that they were still exhibiting this stress response, potentially explaining the hyperactivity. Future studies should investigate how long hyperactivity is maintained after release. Finally, fish swimming performance evaluated after the activity assessment did not suggest any differences between the sedation treatments. We anticipated that the swimming performance assessment would only yield meaningful differences if the impairments were prolonged, which apparently was not the case here.

In addition to the sedative, other biotic and abiotic factors influence fish behavioral recovery, such as the individual's size (Suski and Phillip 2004), the time of day (Shoup et al. 2004), and the temperature (Hanson et al. 2007). Previous research investigating postsedation recovery trends in fish reported that the response to chemical sedatives, including MS-222, is more influenced by the sedative dose and water temperature than by fish size or dissolved oxygen concentration (Bowker et al. 2014). Although there was no difference between treatments, the present study showed that temperature and fish size exerted effects on FID and time to fatigue. The FID and time to fatigue were negatively correlated with temperature. Indeed, decreased water temperatures result in reduced swimming performance and behavioral changes in Largemouth Bass (Lemons and Crawshaw 1985; Randall and Brauner 1991; Hasler et al. 2009). Decreased activity at colder water temperatures is likely attributable to changes in physiological processes, reduced oxygen carrying efficiency of blood cells, and decreased muscle contractility (Randall and Brauner 1991; Hasler et al. 2009). The reduced oxygen carrying capacity of blood at colder water temperatures is also likely compounded by the use of anaesthetics, which affect the rate of gas and ion exchange between the fish and its environment (Zahl et al. 2012). Therefore, the greater FID in cooler water temperatures may serve as a predator avoidance behavior in compensation for decreased swimming performance.

In contrast, we found that FID was positively correlated with fish size. Body weight, growth rate, and sexual maturity are size-dependent characteristics that affect individual fish physiology (Zahl et al. 2012). Therefore, the effect of fish size on FID could be related to individual variation in physiology, which determines the fish's respective sensitivity to the anaesthetic (Zahl et al. 2012). In addition, a study on Rainbow Trout demonstrated that fish size can influence the efficiency of various anaesthetics, including MS-222 (Gilderhus and Marking 1987). As noted earlier, MS-222 sedation and electrosedation of Largemouth Bass have been shown to generate stress responses, including elevated levels of blood glucose, lactate, and cortisol (Trushenski et al. 2012). Smaller Largemouth Bass tend to recover faster than larger individuals from similar physiological changes caused by an exercise-induced stress response (Gingerich and Suski 2012).
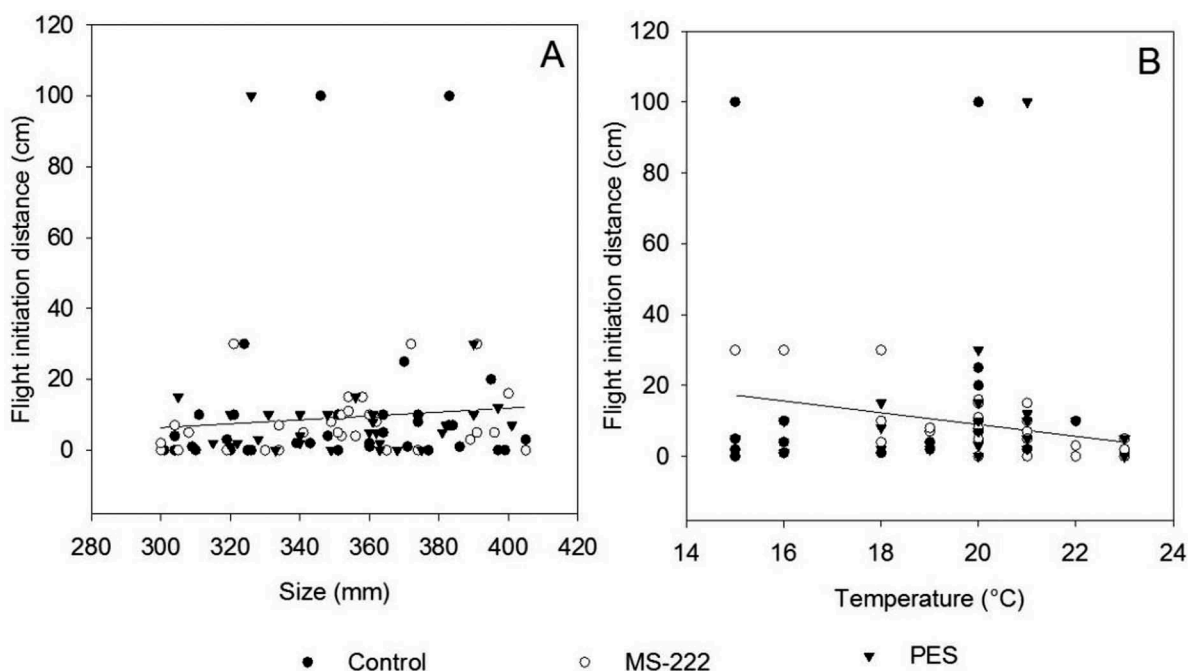
FIGURE 3. Flight initiation distance (cm) for Largemouth Bass that were chemically sedated with MS-222 ($N = 31$), electrosedated with a Portable Electrosedation System (PES) unit ($N = 30$), or unsedated (control; $N = 38$), presented as a function of **(A)** fish TL (mm; Spearman's $r = 0.22$, $P = 0.03$) and **(B)** water temperature (°C; Spearman's $r = 0.31$, $P < 0.01$).

Largemouth Bass are relatively robust and resilient (Thompson et al. 2008), and fish behavioral responses to sedatives and other biotic and abiotic factors will vary depending on the species (reviewed by Neiffer and Stamper 2009).

Thus, more research is required to determine whether the observed trends are applicable across species and environments. Nonetheless, the results here are promising because irrespective of the sedation method, behavioral recovery
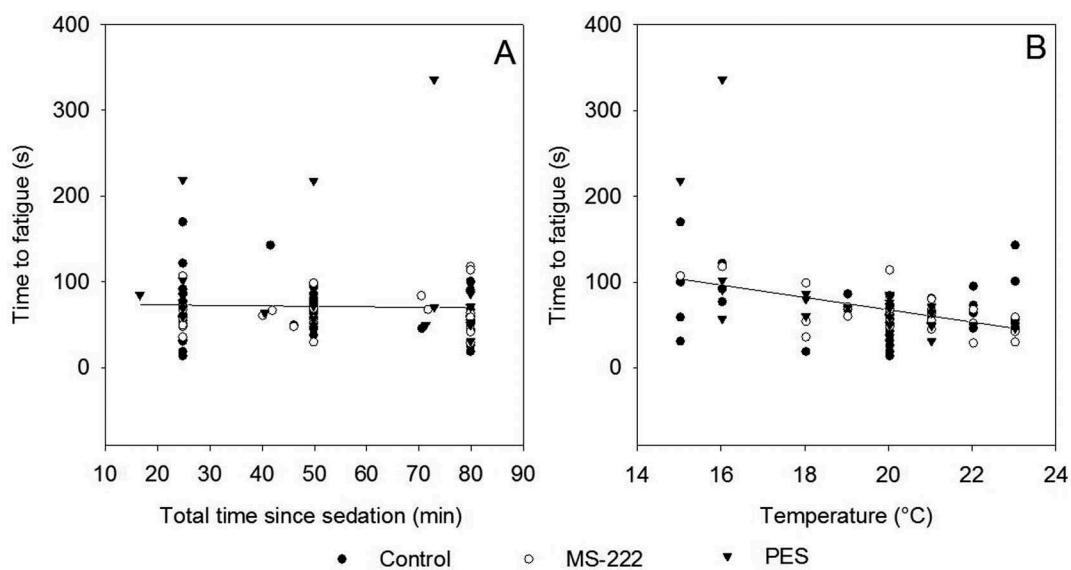

FIGURE 4. Time (s) to fatigue during a forced chase event for Largemouth Bass that were chemically sedated with MS-222 ($N = 31$), electrosedated with a Portable Electrosedation System (PES) unit ($N = 30$), or unsedated (control; $N = 38$), presented as a function of **(A)** the total time (min) since sedation occurred (Spearman's $r = 0.09$, $P > 0.05$) and **(B)** water temperature (°C; Spearman's $r = 0.38$, $P < 0.001$).

appears to be rather rapid, which is relevant given the large number of studies that involve sedation of fish prior to implanting or affixing electronic tags (e.g., Hussey et al. 2015; Cooke et al. 2016). Authors have mused about whether tracking data collected immediately after tagging (hours to days) should be discarded given the potential for behavioral alterations, but our findings suggest that such effects are relatively minor or nonexistent, as the fish regained normal behaviors within 5 min after handling and sedation.

Overall, the present results indicate that fish require a recovery period after sedation, regardless of the sedation method used. However, the results also show that a holding period as short as 5 min postsedation, during which the fish are allowed to fully regain equilibrium, may be sufficient to enable the behavioral recovery of sedated Largemouth Bass. Previous studies on recovery in Chinook Salmon *O. tshawytscha* indicated that nearly all cardiovascular variables (including heart rate and cardiac output) returned to baseline levels within 5 min of recovery (Hill and Forster 2004), which is consistent with the lack of behavioral differences observed in Largemouth Bass. Similar trends have been reported for postsedation recovery of Atlantic Sturgeon *Acipenser oxyrinchus oxyrinchus* (Balazik 2015) and Coho Salmon *O. kisutch* (Keep et al. 2015). Although electrosedated fish appeared to be behaviorally affected beyond 5 min postsedation, it is unclear whether increasing the holding time would be the correct solution given that (1) increased activity was the only behavioral difference detected and (2) handling and holding periods can also be stressful. In fact, Hill and Forster (2004) demonstrated that handling and manipulation of fish had a greater impact on cardiac physiology than the actual sedative. Applying a sufficient postsedation holding period to ensure behavioral recovery is important to optimize fish survival after release (e.g., escaping from predation and downstream drift) while avoiding unnecessary holding, which itself can be damaging to the fish (Portz et al. 2006).

Given that longer recovery periods generally resulted in no significant differences in behavior relative to shorter recovery periods, we suggest that the fish be released soon after they regain reflexes and behavioral capability so as to minimize the amount of stress incurred from holding and handling over extended time periods (Portz et al. 2006). Additionally, chemical sedatives, such as MS-222, render the fish unfit for human consumption until the chemical is fully metabolized (Marking and Meyer 1985; USOFR 1990; Health Canada 2010). The inability to release the fish until days after the sedation event makes chemical sedation methods inappropriate for immediate-release studies, such as biotelemetry research. An understanding of these limitations is crucial when choosing the appropriate sedative.

In conclusion, our data indicate that both MS-222 and electrosedation have negligible effects on fish behaviors after a 5-min recovery period, suggesting that either sedation method is appropriate for use with Largemouth Bass.

However, electrosedation may be the preferable method given that (1) we observed faster immediate recovery periods relative to MS-222 sedation; and (2) there are no withdrawal period requirements for holding or consuming electrosedated fish. Nevertheless, we strongly recommend that future study designs reflect both species- and system-specific requirements in the selection of an appropriate sedation method. Regardless of the method used, sedated fish should be held for a minimum of 5 min after they regain equilibrium and resume regular ventilation to ensure behavioral recovery to a level that will not jeopardize the fish's postrelease survival.

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REFERENCES

Anderson, W. G., R. S. McKinley, and M. Colavecchia. 1997. The use of clove oil as an anesthetic for Rainbow Trout and its effects on swimming performance. North American Journal of Fisheries Management 17:301–307.

Balazik, M. T. 2015. Capture and brief invasive procedures using electronarcosis does not appear to affect postrelease habits in male Atlantic Sturgeon during the spawning season. North American Journal of Fisheries Management 35:398–402.

Bowker, J. D., J. T. Trushenski, D. C. Glover, D. G. Carty, and N. Wandelear. 2014. Sedative options for fish research: a brief review with new data on sedation of warm-, cool-, and coldwater fishes and recommendations for the drug approval process. Reviews in Fish Biology and Fisheries 25:147–163.

Burka, J. F., K. L. Hammell, T. E. Horsberg, G. R. Johnsons, J. J. Rainnie, and D. J. Speares. 1997. Drugs in salmonid aquaculture—a review. Journal of Veterinary Pharmacology and Therapeutics 20:333–349.

Carter, K. M., C. M. Woodley, and R. S. Brown. 2011. A review of tricaine methanesulfonate for anesthesia of fish. Reviews in Fish Biology and Fisheries 21:51–59.

Champely, S. 2016. PWR: basic functions for power analysis. R package version 1.2-0. Available: //CRAN.R-project.org/package=pwr. (February 2017).

Cohen, J. 1988. Statistical power analysis for the behavioral sciences, 2nd edition. Lawrence Erlbaum Associates, Mahwah, New Jersey.

Cooke, S. J., K. G. Ostrand, C. M. Bunt, J. F. Schreer, D. H. Wahl, and D. P. Philipp. 2003. Cardiovascular responses of Largemouth Bass to exhaustive exercise and brief air exposure over a range of water temperatures. Transactions of the American Fisheries Society 132:1154–1165.

Cooke, S. J., C. D. Suski, K. G. Ostrand, B. L. Tufts, and D. H. Wahl. 2004. Behavioral and physiological assessment of low concentrations of clove oil anaesthetic for handling and transporting Largemouth Bass (*Micropterus salmoides*). Aquaculture 239:509–529.

Cooke, S. J., A. D. Wilson, C. K. Elvidge, R. J. Lennox, N. Jepsen, A. H. Colotelo, and R. S. Brown. 2016. Ten practical realities for institutional animal care and use committees when evaluating protocols dealing with fish in the field. Reviews in Fish Biology and Fisheries 26:123–133.

Cotter, P. A., and K. J. Rodnick. 2006. Differential effects of anesthetics on electrical properties of the Rainbow Trout (*Oncorhynchus mykiss*) heart. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 145:158–165.

Cury, D. K., and B. Kynard. 1978. Effect of extended galvanonarcosis on behavior of Rainbow Trout, *Salmo gairdneri*, and Channel Catfish, *Ictalurus punctatus*. Journal of the Fisheries Research Board of Canada 35:1297–1302.

Davis, M. W. 2010. Fish stress and mortality can be predicted using reflex impairment. Fish and Fisheries 11:1–11.

Demers, E., R. S. McKinley, A. H. Weatherley, and D. J. McQueen. 1996. Activity patterns of Largemouth and Smallmouth bass determined with electromyogram biotelemetry. Transactions of the American Fisheries Society 125:434–439.

Fobert, E., P. Meining, A. Colotelo, C. O'Connor, and S. J. Cooke. 2009. Cut the line or remove the hook? An evaluation of sublethal and lethal endpoints for deeply hooked freshwater recreational fish. Fisheries Research 99:38–46.

Gilderhus, P. A., and L. L. Marking. 1987. Comparative efficacy of 16 anesthetic chemicals on Rainbow Trout. North American Journal of Fisheries Management 7:288–292.

Gingerich, A. J., and C. D. Suski. 2012. The effect of body size on post-exercise physiology in Largemouth Bass. Fish Physiology and Biochemistry 38:329–340.

Hanson, K. C., S. J. Cooke, C. D. Suski, G. Niezgoda, F. J. S. Phelan, R. Tinline, and D. P. Philipp. 2007. Assessment of Largemouth Bass (*Micropterus salmoides*) behaviour and activity at multiple spatial and temporal scales utilizing a whole-lake telemetry array. Hydrobiologia 582:243–256.

Hasler, C. T., C. D. Suski, K. C. Hanson, S. J. Cooke, D. P. Philipp, and B. L. Tufts. 2009. Effect of water temperature on laboratory swimming performance and natural activity levels of adult Largemouth Bass. Canadian Journal of Zoology 87:589–596.

Health Canada. 2010. List of veterinary drugs that are authorized for sale by Health Canada for use in food-producing aquatic animals. Available: www.hc-sc.gc.ca/dhp-mps/vet/legislation/pol/aquaculture_anim-eng.php. (July 2016).

Heath, A. G., and A. W. Pritchard. 1965. Effects of severe hypoxia on carbohydrate energy stores and metabolism in two species of fresh-water fish. Physiological Zoology 38:325–334.

Hill, J. V., and M. E. Forster. 2004. The effects of settlement and anaesthesia on oxygen consumption in the New Zealand Spotty (*Notolabrus celidotus*) measured in an automated flow-through respirometry system. New Zealand Natural Sciences 29:39–48.

Hussey, N. E., S. T. Kessel, K. Aarestrup, S. J. Cooke, P. D. Cowley, A. T. Fisk, R. G. Harcourt, K. N. Holland, S. J. Iverson, J. F. Kocik, and J. E. M. Flemming. 2015. Aquatic animal telemetry: a panoramic window into the underwater world. Science 348:1221–1231.

Iwama, G. K., J. C. McGeer, and P. Pawluk. 1989. The effects of five fish anaesthetics on acid–base balance, hematocrit, blood gases, cortisol, and adrenaline in Rainbow Trout. Canadian Journal of Zoology 67:2065–2073.

Jones, K. A., and J.-G. J. Godin. 2010. Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. Proceedings of the Royal Society of London B: Biological Sciences 277:625–632.

Keep, S. G., M. B. Allen, and J. S. Zendt. 2015. Comparison of electronarcosis and carbon dioxide sedation effects on travel time in adult Chinook and Coho salmon. North American Journal of Fisheries Management 35:906–912.

Kieffer, J. D. 2000. Limits to exhaustive exercise in fish. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 126:161–179.

Leitner, J. K., and J. J. Isely. 1994. A liver and muscle biopsy technique for electrophoretic evaluation of Largemouth Bass. Progressive Fish-Culturist 56:288–290.

Lemons, D. E., and L. I. Crawshaw. 1985. Behavioral and metabolic adjustments to low temperatures in the Largemouth Bass (*Micropterus salmoides*). Physiological Zoology 58:175–180.

Marking, L. L., and F. P. Meyer. 1985. Are better anesthetics needed in fisheries? Fisheries 10(6):2–5.

Matsche, M. A. 2013. A portable electro-immobilization and laparoscopy system for sex determination and gonadal biopsy in Largemouth Bass *Micropterus salmoides*. Journal of Fish Biology 83:1391–1400.

Neiffer, D. L., and M. A. Stamper. 2009. Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. ILAR (Institute for Laboratory Animal Research) Journal 50:343–360.

Perry, S. F., and K. M. Gilmour. 2006. Acid–base balance and CO_2 excretion in fish: unanswered questions and emerging models. Respiratory Physiology and Neurobiology 154:199–215.

Pirhonen, J., and C. B. Schreck. 2003. Effects of anaesthesia with MS-222, clove oil and CO_2 on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*). Aquaculture 220:507–514.

Popovic, B. N. T., J. Barisic, M. Jadan, and A. P. Berakovic. 2012. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. Journal of Applied Ichthyology 28:553–564.

Portz, D. E. 2007. Fish-holding-associated stress in Sacramento River Chinook Salmon (*Oncorhynchus tshawytscha*) at south delta fish salvage operations: effects on plasma constituents, swimming performance, and predator avoidance. Doctoral dissertation. University of California, Davis.

Portz, D. E., C. M. Woodley, and J. J. Cech Jr. 2006. Stress-associated impacts of short-term holding on fishes. Reviews in Fish Biology and Fisheries 16:125–170.

Quinn, S., and C. Paukert. 2009. Centrarchid fisheries. Pages 312–359 *in* S. J. Cooke and D. P. Phillip, editors. Centrarchid fishes: diversity, biology, and conservation. Blackwell Publishing, Oxford, UK.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Raby, G. D., M. R. Donaldson, S. G. Hinch, D. A. Patterson, A. G. Lotto, D. Robichaud, K. K. English, W. G. Willmore, A. P. Farrell, M. W. Davis, and S. J. Cooke. 2012. Validation of reflex indicators for measuring vitality and predicting the delayed mortality of wild Coho Salmon bycatch released from fishing gears. Journal of Applied Ecology 49:90–98.

Randall, D., and C. Brauner. 1991. Effects of environmental factors on exercise in fish. Journal of Experimental Biology 160:113–126.

Reid, S. G., N. J. Bernier, and S. F. Perry. 1998. The adrenergic stress response in fish: control of catecholamine storage and release. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology, and Endocrinology 120:1–27.

Ross, L. G., and B. Ross. 2008. The features of anesthetic agents. Pages 53–56 *in* L. G. Ross and B. Ross, editors. Anaesthetic and sedative techniques for aquatic animals, 3rd edition. Blackwell Scientific Publications, Ames, Iowa.

Rous, A. M., A. Forrest, E. H. McKittrick, G. Letterio, J. Roszell, T. Wright, and S. J. Cooke. 2015. Orientation and position of fish affects recovery time from electrosedation. Transactions of the American Fisheries Society 144:820–828.

Scheirer, C. J., W. S. Ray, and N. Hare. 1976. The analysis of ranked data derived from completely randomized factorial designs. Biometrics 32:429–434.

Schreck, C. B., B. L. Olla, and M. W. Davis. 1997. Behavioral responses to stress. Fisheries Stress and Health in Aquaculture 62:145–170.

Shoup, D. E., R. E. Carlson, and R. T. Heath. 2004. Diel activity levels of centrarchid fishes in a small Ohio lake. Transactions of the American Fisheries Society 133:1264–1269.

Sladky, K. K., C. R. Swanson, M. K. Stoskopf, M. R. Loomis, and G. A. Lewbart. 2001. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in Red Pacu (*Piaractus brachypomus*). American Journal of Veterinary Research 62:337–342.

Snyder, D. E. 2003. Electrofishing and its harmful effects on fish. U.S. Government Printing Office, sInformation and Technology Report USGS/BRD/ITR-2003-0002, Denver.

Sterritt, D. A., S. T. Elliott, and A. E. Schmidt. 1994. Electrical anesthesia for immobilizing adult Coho Salmon in freshwater. North American Journal of Fisheries Management 14:453–456.

Summerfelt, R. C., and L. S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213–272 in C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Suski, C. D., and D. P. Phillip. 2004. Factors affecting the vulnerability to angling of nesting male Largemouth and Smallmouth bass. Transactions of the American Fisheries Society 133:1100–1106.

Thomas, P., and L. Robertson. 1991. Plasma cortisol and glucose stress responses of Red Drum (Sciaenops ocellatus) to handling and shallow water stressors and anesthesia with MS-222, quinaldine sulfate and metomidate. Aquaculture 96:69–86.

Thompson, L. A., S. J. Cooke, M. R. Donaldson, K. C. Hanson, A. Gingerich, T. Klefoth, and R. Arlinghaus. 2008. Physiology, behavior, and survival of angled and air-exposed Largemouth Bass. North American Journal of Fisheries Management 28:1059–1068.

Trushenski, J. T., J. D. Bowker, S. J. Cooke, D. Erdahl, T. Bell, J. R. Macmillan, R. P. Yanong, J. E. Hill, M. C. Fabrizio, J. E. Garvey, and S. Sharon. 2013. Issues regarding the use of sedatives in fisheries and the need for immediate-release options. Transactions of the American Fisheries Society 142:156–170.

Trushenski, J. T., J. D. Bowker, B. L. Mulligan, and B. R. Gause. 2012. Induction, recovery, and hematological responses of Largemouth Bass to chemo- and electrosedation. North American Journal of Aquaculture 74:214–223.

USOFR (U.S. Office of the Federal Register). 1990. Food and drugs. Code of Federal Regulations, Title 21, Part 529. U.S. Government Printing Office, Washington, D.C.

Vandergoot, C. S., K. J. Murchie, S. J. Cooke, J. M. Dettmers, R. A. Bergstedt, and D. G. Fielder. 2011. Evaluation of two forms of electro-anesthesia and carbon dioxide for short-term anesthesia in Walleye. North American Journal of Fisheries Management 31:914–922.

Wilson, A. D. M., J. W. Brownscombe, B. Sullivan, S. Jain-Schlaepfer, and S. J. Cooke. 2015. Does angling technique selectively target fishes based on their behavioural type? PLOS (Public Library of Science) ONE [online serial] 10:e0135848.

Wilson, A. D. M., and J.-G. J. Godin. 2009. Boldness and behavioral syndromes in the Bluegill sunfish, Lepomis macrochirus. Behavioral Ecology 20:231–237.

Zahl, I. H., O. Samuelsen, and A. Kiessling. 2012. Anaesthesia of farmed fish: implications for welfare. Fish Physiology and Biochemistry 38:201–218.