

Notes

Effect of Voltage and Exposure Time on Fish Response to Electroседation

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Abstract

Chemical sedatives requiring withdrawal (period of time where fish are held posttreatment to allow for tissue drug residues to dissipate) may be impractical for field use. As a result, fisheries professionals are beginning to investigate alternative methods that allow fish to be released immediately after treatment. To address the safety and efficacy of electroседation as an "immediate-release" sedative approach, induction, recovery times, and blood chemistry of juvenile (211 ± 4 g, 26.1 ± 0.1 cm total length [mean \pm SE]) hybrid striped bass (female *Morone chrysops* \times male *M. saxatilis*) were evaluated after sedation by exposure to 100, 150, or 200 V of pulsed direct current (30 Hz and 25% duty cycle) for 4 or 8 s. All fish were sedated to stage IV sedation within 0.3 min, regardless of voltage strength or exposure time. Recovery times varied significantly by the electroседation treatment used, but all fish recovered within 2 min postinduction. Changes in blood chemistry were consistent with an acute stress response, but these effects were transient and no differences were observed among the electroседation treatments. Results suggest that pulsed direct current electroседation is an effective strategy for quickly and easily sedating juvenile hybrid striped bass and potentially other species of conservation or management concern.

Keywords: sedation; electroanesthesia; stress, blood chemistry; sunshine bass; hybrid striped bass; *Morone* spp.

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Introduction

To effectively collect data, manage fisheries, and facilitate fish culture, fisheries professionals routinely sedate, anesthetize, or immobilize fishes for purposes ranging from simple handling to more invasive surgical procedures. Although surgeries and other invasive procedures require the use of sedative or anesthetic agents, noninvasive procedures (e.g., measuring length and weight, fin clipping) can be made easier and safer for the fish and the handler if sedatives are used to keep fish docile during handling. There are relatively few chemical sedative options available at this time for use in the

fisheries disciplines in the United States and elsewhere. Tricaine methanesulfonate products (often referred to as MS-222) are approved by the U.S. Food and Drug Administration (FDA) for temporary immobilization of ictalurids, salmonids, esocids, percids, or other laboratory or hatchery fishes at water temperatures $>10^{\circ}\text{C}$. In addition, exposed fish must be held for a 21-d withdrawal period (period of time where fish are held posttreatment to allow for tissue drug residues to dissipate) before being slaughtered for consumption or released into the wild where they may be harvested for food (FDA 2011a). Two currently unapproved drugs, benzocaine (Benzoak[®] [20% benzocaine]; manufacturer,



ACD Pharmaceuticals AS, Leknes, Norway; U.S. distributor, Frontier Scientific, Logan, UT) and eugenol (AQUI-S E® [50% eugenol] or AQUI-S® 20E [10% eugenol]; AQUI-S New Zealand Ltd., Lower Hutt, New Zealand), may be used under the authority of an Investigational New Animal Drug exemption held by the U.S. Fish and Wildlife Service's Aquatic Animal Drug Approval Partnership Program, but users must adhere to a 3-d withdrawal period before releasing catchable-sized fish that were exposed to either sedative. Carbon dioxide (CO₂) is not an approved fish sedative, but it is considered a drug of "low regulatory priority." This classification means that FDA is unlikely to exercise its regulatory authority and take action against the use of CO₂ as an unapproved animal drug as long as certain conditions are met (FDA 2011b). Under the low regulatory priority banner, CO₂ can be used as an "immediate-release" sedative (no withdrawal period necessary). However, CO₂ can be unwieldy and unpredictable, and is not an effective sedative for all fishes (e.g., marine fishes can be difficult or impossible to sedate using CO₂ without drastically altering water pH to establish effective sedative concentrations). A range of crude products and purified compounds are widely used as fish sedatives (e.g., clove, wintergreen, or spearmint oils; quinaldine [Neiffer and Stamper 2009; Danner et al. 2011]). However, these compounds are not FDA-approved and can only be used on research fish that will be buried or incinerated after euthanasia to prevent human consumption. Aquacalm™ (Western Chemical Inc., Ferndale, Washington; 100% active metomidate hydrochloride) has recently been granted "indexed status" by the FDA for the sedation and anesthesia of ornamental finfish. Indexed drugs are not approved by FDA, but they can be legally marketed for use in ornamental aquarium fish. However, indexed drugs remain illegal for use in fish intended for human or animal consumption. Current sedative options and their withdrawal periods can constrain projects conducted in the laboratory or hatchery; for field research, there are simply no legal options that allow fish to be sedated and released. Although the regulations and prohibitions outlined here relate directly to the use of chemical sedatives in the United States, similar restrictions limit access to fish sedatives in other countries.

Given the constraints associated with chemical sedatives, fisheries professionals are currently exploring alternative methods of sedating fish, including electro-sedation. Electro-fishing has been used as a field sampling technique for decades, and there are early reports of using electricity for sedation of fishes (Kynard and Lonsdale 1975; Madden and Houston 1976; Curry and Kynard 1978). Increasingly, this technique is being modified and refined specifically for the purpose of sedating fish for handling, surgery, and other purposes (Jennings and Looney 1998; Zydlewski et al. 2008; Hudson et al. 2011; Vandergoot et al. 2011; Trushenski et al. 2012), including applications involving marine fish (J. Trushenski et al., unpublished data). Although the terms "anesthesia," "sedation," and "immobilization" are used somewhat interchangeably with respect to fishes, each has a distinct definition as do the terms used to describe the response of fish to electricity. Ross and Ross

(2008) define anesthesia as "a reversible, generalized loss of sensory perception accompanied by a sleep-like state induced by drugs or by physical means," and sedation as "a preliminary level of anesthesia, in which response to stimulation is greatly reduced and some analgesia is achieved, but sensory abilities are generally intact and loss of equilibrium does not occur." Immobilization generally refers to prevention of movement and does not imply any status regarding sensory acuity. Although the term "electroanesthesia" is sometimes used to generally describe the response of fish to electricity, the term "electroimmobilization" is perhaps more accurate, because it has been suggested that true anesthesia may not occur when using electricity in fish (Ross and Ross 2008). Although the terms electroanesthesia or "electronarcosis" are most often used to describe the effects of alternating current, the term "galvanarcosis" is typically used to describe the effects of continuous or pulsed direct current. Each of these different waveforms has a different mode of action and effect on fish (Ross and Ross 2008). In lower voltage direct current (usually used with continuous waveforms), immobilization may be the result of a general narcotic effect and forced swimming toward the anode. In higher voltage direct current (usually used with pulsed waveforms), immobilization may be achieved by stunning or electro-tetany (tetanic muscle contraction; Ross and Ross 2008; M. Holliman, formerly of Smith-Root, Inc., personal communication). Arguably, none of these terms perfectly describe the process investigated in the present work; however, attempts to redefine these terms or establish new terminology for the responses of fish to electricity is outside the scope of the present manuscript. As such, we have elected to use the terms "electrosedation" and "sedatives" throughout this article to best reflect the behavioral observations made and the current understanding of sedation and anesthesia in fish.

Electrosedation may offer several advantages over chemical sedatives in terms of withdrawal periods, chemical disposal, and potentially, ease of use. In addition, electro-sedation of fish is currently not subject to FDA enforcement as a drug and can be used legally without having to go through the arduous, multiple-year, multiple million-dollar, drug-approval process. However, it is important to ensure safe, effective electro-sedation treatments are developed to minimize the risk of negative postsedation outcomes, including mortality. It is also important to quantify the response of fishes to electro-sedation and assess postsedation effects such that institutional animal care and use committees or other oversight bodies may gauge the appropriateness of electro-sedation under different research scenarios. The present study was conducted to evaluate the effect of different voltage strengths and exposure durations on induction and recovery times and postsedation blood chemistry of hybrid striped bass (female *Morone chrysops* × male *M. saxatilis*).

Methods

Electrosedation procedures

Eighteen groups comprising five fish each (211 ± 4 g, 26.1 ± 0.1 cm total length [mean \pm SE]) were randomly



collected from a reference population of juvenile hybrid striped bass and transferred to individual holding tanks (130 L) in a recirculating aquaculture system 1 d before the experiment (Figure 1). Groups were established to facilitate easy capture and transfer into an electrosedation chamber. The chamber consisted of a 142-L cooler that contained 70 L of aerated culture water (water depth, ~8 cm) and was equipped with an electrosedation unit (Portable Electroanesthesia System[®]; Smith-Root, Inc., Vancouver, WA). Triplicate groups of fish were exposed to 100, 150, or 200 V of pulsed direct current (30 Hz, 25% duty cycle, electrodes positioned ~77 cm apart) for 4 or 8 s in a 3 × 2 factorial design (100 V–4 s; 100 V–8 s; 150 V–4 s; 150 V–8 s; 200 V–4 s; and 200 V–8 s; Figure 1). Although the groups were randomly established, they were assigned to electrosedation treatments (three groups per treatment) in systematic manner, that is, groups 1–3 were assigned to 100 V–4 s, groups 4–6 were assigned to 100 V–8 s; groups 7–9 were assigned to 150 V–4 s; groups 10–12 were assigned to 150 V–8 s, and so on. To apply the electrosedation treatments (Figure 1), each group was quickly captured (<1 min) and transferred into the electrosedation chamber. The electrosedation treatment was applied, and fish were monitored to determine induction to stage IV as defined by Summerfelt and Smith (1990). Stage IV is associated with the total loss of equilibrium, muscle tone, and responsiveness to visual and tactile stimuli, but maintenance of a slow, steady opercular rate. After the loss of equilibrium, fish were stimulated with slight manual dorsoventral compression as a tactile stimulus. Fish were considered induced to stage IV when they no longer responded to this stimulus, but the opercular rate remained steady, albeit reduced. A tremor was observed immediately after electrosedation and was generally characterized by rapid but mild, involuntary muscle twitches along the length of the body. Although fish were not responsive to tactile or other stimuli during this tremor (and were perhaps momentarily in stage V or VI of sedation), opercular movement did not resume until after the tremor had ceased. Thus, induction was considered complete after the tremor was over and fish had achieved or regained stage IV. After induction, a blood sample from one fish was collected (see below), whereas the other fish were returned to their original holding tank to assess recovery from sedation and for subsequent blood collection at 0.5, 1, 2, and 6 h postsedation (one fish per group at each time, where each individual was sampled once; Figure 1). Fish were monitored during recovery to determine when recovery of normal equilibrium and responsiveness to various stimuli occurred. Responsiveness to visual and auditory stimuli was assessed by tapping the airstone gently against the side of the tank near the fish's heads. The group was considered fully recovered when all four fish exhibited avoidance behavior to this stimulus and were responsive to tactile stimulus (i.e., recovery time = time for last fish to recover). Observer bias was minimized by having the same observers apply all stimuli and assess when fish were sedated (one observer) or had recovered from sedation (one observer). The observers practiced

and standardized their approaches before assessing fish as part of this experiment.

Blood samples were collected from individual fish according to typical methods. Before blood collection, fish were immersed in a bath of metomidate hydrochloride (Aquacalm[™], ~3–5 mg/L for ~30 s; Western Chemical, Ferndale, WA) to facilitate handling. After exposure to the metomidate hydrochloride bath, fish were weighed (to the nearest gram) and measured (total length to the nearest 0.5 cm), and a blood sample was collected from the caudal vasculature by using heparinized, evacuated blood collection assemblies (Vacutainer[®]; BD Biosciences, Franklin Lakes, NJ). Although metomidate hydrochloride may prevent corticosteroid increase during sampling (Olsen et al. 1995; Davis and Griffin 2004), all blood samples were collected within 5 min of capture to minimize the possibility of other confounding responses of handling and puncturing the caudal vasculature. Fish were then returned to adjacent tanks in the same recirculation system, held according to replicate group, and monitored 48 h for survival. All fish were transferred to a separate recirculation system and group-housed for several weeks after completion of the 48-h monitoring period. In addition to fish sampled at set time points after sedation, two fish from the reference population also were sampled 0, 3, and 6 h after the start of the experiment (Figure 1). Although these fish did not represent a true control group, they were used to establish resting blood chemistry profiles for the purposes of qualitative comparison. Blood samples were kept on wet ice (<6 h) until subsequent analysis.

Blood samples were prepared and analyzed for a variety of parameters associated with the stress response in fishes. Hematocrit (Statspin[®] centrifuge; Thermo Fisher Scientific, Waltham, MA) and glucose (Freestyle Freedom Lite[®] glucose meter; Abbott Laboratories, Abbott Park, IL) were determined, and then remaining whole blood was centrifuged (3000 × g, 4°C, 45 min). Resultant plasma was collected and stored at –80°C until further analysis. Plasma samples were analyzed to determine lactate (Accutrend[®] lactate meter; Roche, Mannheim, Germany), osmolality (Vapro 5520 osmometer; Wescor, Inc., Logan, UT), and cortisol (EIA 1887 kit; DRG International, Mountainside, NJ). Although portable lactate and glucose meters have been shown to slightly underestimate metabolite levels in fish blood relative to laboratory methods, they are considered precise and reliable for generating comparative data (Wells and Pankhurst 1999; Venn Beecham et al. 2006). The cortisol kit used has a range of 0–800 ng/mL, with a sensitivity of 2.5 ng/mL for human samples, and it has been validated and used successfully to measure cortisol in samples from a variety of fish species (Delaney et al. 2005; Woods et al. 2008; Owen et al. 2009; Sepici-Dinçel et al. 2009; Trushenski et al. 2010). All procedures were conducted under the guidance and approval of the Southern Illinois University Carbondale Institutional Animal Care and Use Committee under animal care and use protocol 10-028.

Water samples were collected from the holding system 0, 3, and 6 h after the start of the experiment and

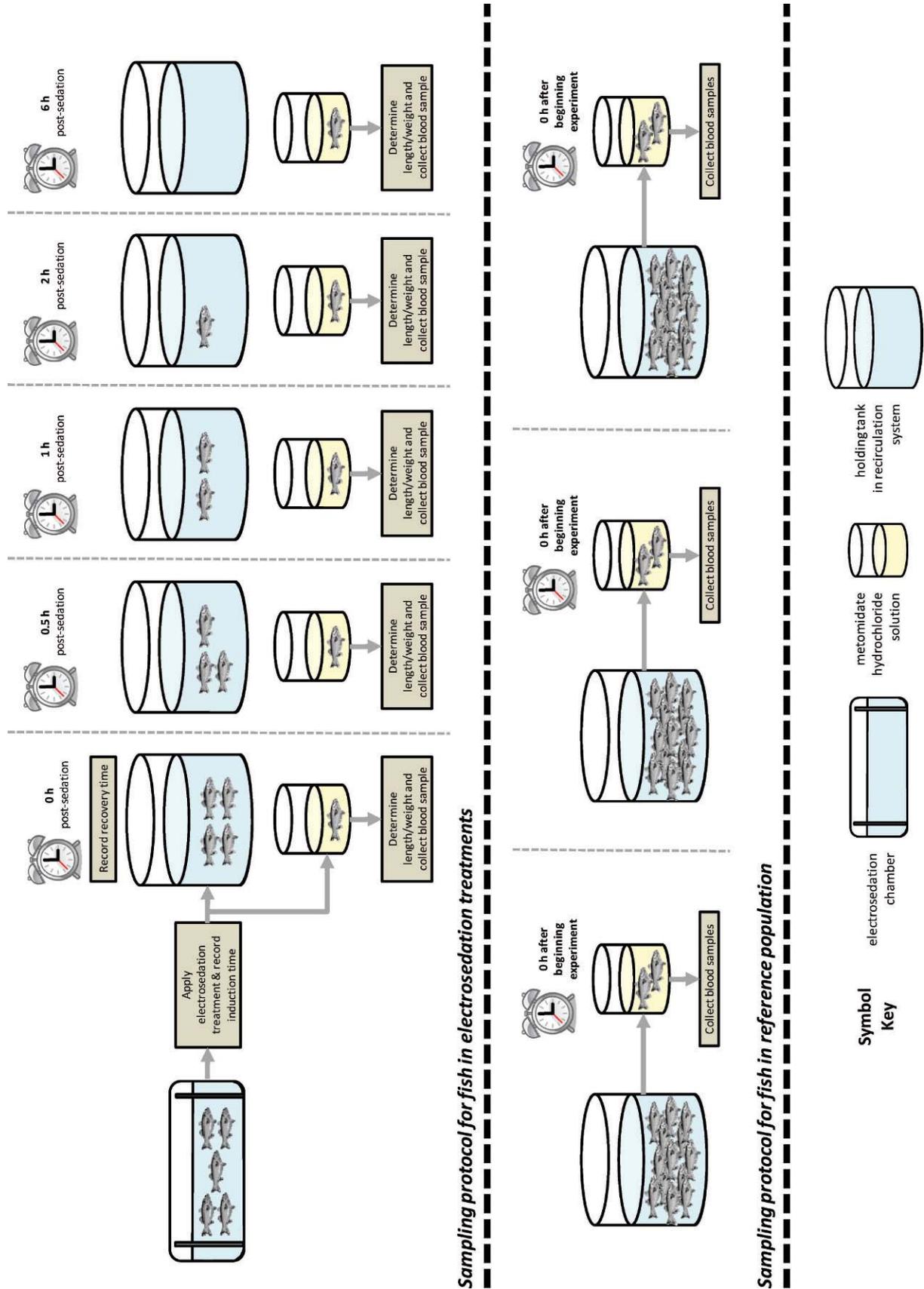


Figure 1. Schematic illustrating sampling protocols for juvenile hybrid striped bass *Morone chrysops* × *M. saxatilis* electrosedated to stage IV sedation by using pulsed direct current of various voltage strengths and exposure durations or sampled directly from the reference population. For simplicity, only one replicate group (comprising five fish) is shown for the electrosedation sampling protocol; three replicate groups in total were sampled for each of the six electrosedation treatments assessed.

Table 1. Water quality measured during an experiment to determine the effects of pulsed direct current electrosedation on juvenile hybrid striped bass *Morone chrysops* × *M. saxatilis*. Values represent means ± SE of water quality parameters measured in water samples collected at time = 0, 3, and 6 h from the holding recirculation system. Due to equipment sensitivity, values <0.1 are reported as 0.0. Observed variability was due to replacing water lost when recirculation system filters were backflushed during the course of the experiment.

Parameter	Value
Temperature (°C) ^a	22.3 ± 0.1
Total ammonia nitrogen (mg/L) ^b	0.3 ± 0.1
Nitrite nitrogen (mg/L) ^b	0.0 ± 0.0
Nitrate nitrogen (mg/L) ^b	4.0 ± 0.3
Alkalinity (mg/L) ^c	217 ± 34
Hardness (mg/L) ^c	149 ± 4
Salinity (g/L) ^d	2.4 ± 0.1
Conductivity (mS) ^d	4.6 ± 0.1
pH ^d	8.5 ± 0.0

^a YSI 550 meter (Yellow Springs Instruments, Yellow Springs, OH).

^b DR 2800 spectrophotometer and reagents (Hach, Loveland, CO).

^c Digital titrator and reagents (Hach).

^d Oakton® Multi-Parameter PCSTestr™ 35 (Eutech Instruments, Vernon Hills, IL).

analyzed in duplicate to ensure water quality was within ranges appropriate for hybrid striped bass (Kohler 2000; Table 1; Table S1, *Supplemental Material*, “Water Quality Data” tab). Dissolved oxygen concentrations are not available because the meter malfunctioned during the experiment; however, dissolved oxygen readings in recirculation systems used typically exceed 5 mg/L when operated normally under similar biological oxygen demand (J. Trushenski, unpublished data). Culture water in the sedation chamber was aerated between fish groups but was not exchanged over the course of the experiment. The chamber housed fish for <18 min over the period of time (~3 h) needed to treat all 18 groups; thus, we assumed water quality would not degrade to the point where water exchanges were necessary. Feed was withheld for 24 h before, during, and 24 h after the experiment to minimize nitrogenous waste excretion during the experiment and monitoring period.

Statistical analyses

Although electrosedation treatments were applied to triplicate groups of multiple individuals, it was determined that groups, not individuals, would serve as experimental units. By definition, experimental units represent independent observations. Given that the presence, position, or both of other fish within the electrosedation chamber can alter the waveform and the way in which it affects each fish, we determined that individuals sedated within the same group should not be considered independent of one another. Thus, to maintain a reasonably conservative statistical approach, for each statistical procedure, fish group was considered the experimental unit and used as the level of replication ($N = 3$). Induction and recovery data were analyzed

using a 2-way ANOVA in the Statistical Analysis System, version 9.1 (PROC MIXED; SAS Institute, Cary, NC) to determine the significance of voltage and exposure time as main effects, and to test for a significant interaction effect. Because blood sampling was associated with multiple observations made on the same experimental unit (i.e., multiple samples taken through time from each fish group), a repeated measures 2-way ANOVA procedure was used to analyze blood chemistry data. Tukey’s honestly significant difference post hoc test was used for all pairwise comparisons of means when main or interactive effects were determined. In all cases, differences were considered significant at $P < 0.05$ (α set at 0.05 a priori). Blood chemistry data associated with fish sampled from the reference population were not included in the statistical analysis.

Results

Regardless of voltage strength and exposure time, all fish were successfully electrosedated to stage IV within 0.3 min, with minimal variability among treatment groups (Table 2; Figure 2; Table S1, *Supplemental Material*, “Induction-Recovery Data” tab). Slightly longer induction times were associated with higher voltages and longer exposure durations. However, differences were not statistically significant or practically relevant (range = 0.13–0.29 min). Fish were rendered unresponsive almost immediately; thus, variation in induction times was associated with differences in the time of initiation and duration (~5–15 s) of the observed postexposure tremors. Although recovery times varied significantly by the voltage and exposure duration treatment combinations used for sedation, all fish recovered in <2 min (range = 0.37–1.90 min). Significant voltage and exposure duration effects were observed for recovery of equilibrium and responsiveness to tactile stimulus. However, the interaction effects were not significant for either recovery parameter. Voltage strength and exposure duration were positively related to time to recovery of equilibrium and responsiveness to tactile and visual and auditory stimuli, although the longest equilibrium and recovery times observed were among fish in the intermediate 150 V–8 s treatment group. Time elapsed between recovery of equilibrium and full recovery (range = 0.30–0.41 min) did not vary by treatment combination (voltage, $P = 0.67$; exposure duration, $P = 0.38$; voltage × exposure, $P = 0.52$; data not shown), suggesting that time to recovery of equilibrium is the primary determinant of total recovery time. Regardless of the electrosedation treatment, all fish were sedated and recovered within 2 min (range = 0.54–2.03 min).

Blood chemistry varied significantly over time after sedation (Figures 3A–3E; Table S1, *Supplemental Material*, “Blood Chemistry Data” tab); however, significant effects of voltage strength or exposure duration were not detected. No trends were observed suggesting a relationship between voltage strength or exposure duration and the magnitude of the physiological responses. Cortisol, lactate, glucose, and osmolality levels

Table 2. Induction and recovery times (minutes) for groups of juvenile hybrid striped bass *Morone chrysops* × *M. saxatilis* (N = 3) electrosedated using pulsed direct current (30 Hz, 25% duty cycle) of different voltage strengths and exposure durations. Means ± SE are shown for each treatment factor combination in normal text; means across voltage strength and exposure duration treatment factors are shown in italics. Treatment factor means with different letter labels (i.e., y, z) are significantly different (P > 0.05); the absence of letter labels indicates the absence of a significant treatment factor effect. P values for each response parameter and their interaction also are provided.

	Exposure time (s)	Voltage			Mean	Voltage	P values from 2-way ANOVA	
		100 V	150 V	200 V			Exposure time	Voltage × exposure
Induction ^a	4	0.24 ± 0.03	0.23 ± 0.03	0.24 ± 0.03	0.24	0.04 ^e	0.06	0.05
	8	0.29 ± 0.03	0.13 ± 0.03	0.13 ± 0.03	0.18			
	Mean	0.27	0.18	0.18				
Equilibrium ^b	4	0.11 ± 0.11	0.44 ± 0.11	0.62 ± 0.11	0.39 _y	<0.01	<0.01	0.10
	8	0.69 ± 0.11	1.53 ± 0.11	1.18 ± 0.11	1.13 _z			
	Mean	0.40 _y	0.98 _z	0.90 _z				
Recovery ^c	4	0.37 ± 0.15	0.74 ± 0.12	0.95 ± 0.12	0.69 _y	0.01	<0.01	0.15
	8	1.00 ± 0.12	1.90 ± 0.12	1.59 ± 0.12	1.50 _z			
	Mean	0.68 _y	1.32 _z	1.27 _z				
Total time ^d	4	0.54 ± 0.15	0.97 ± 0.12	1.19 ± 0.12	0.90 _y	0.01	<0.01	0.20
	8	1.29 ± 0.12	2.03 ± 0.12	1.72 ± 0.12	1.68 _z			
	Mean	0.92 _y	1.50 _z	1.46 _z				

^a Time from beginning of exposure to stage IV sedation.

^b Time from induction to recovery of ability to maintain equilibrium.

^c Time from induction to recovery of responsiveness to tactile and visual and auditory stimuli (full recovery).

^d Time from beginning of exposure to full recovery.

^e Although the 2-way ANOVA indicated a significant voltage effect, more conservative Tukey's honestly significant difference pairwise comparisons indicated no significant difference among the voltage means.

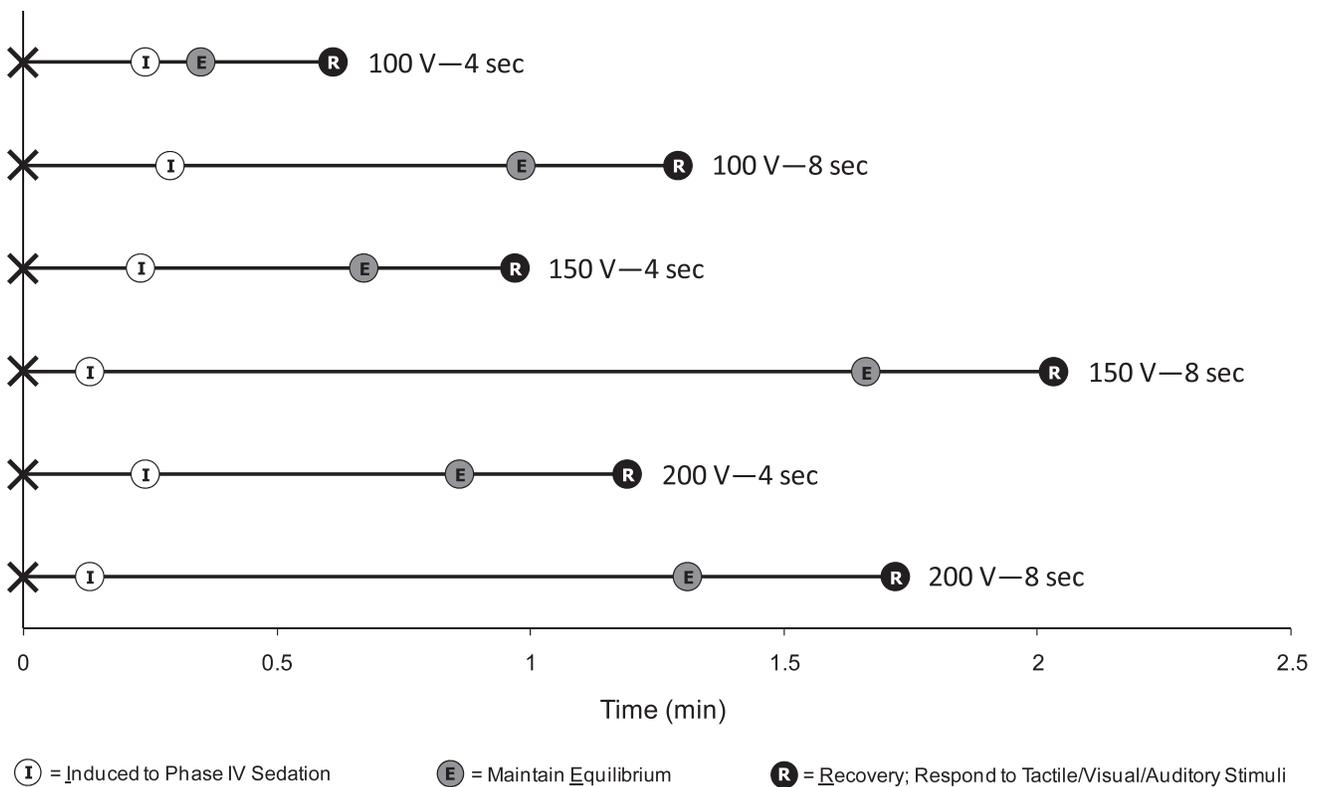


Figure 2. Schematic illustrating induction and various stages of recovery of juvenile hybrid striped bass *Morone chrysops* × *M. saxatilis* electrosedated to stage IV sedation by using pulsed direct current of various voltage strengths and exposure durations.

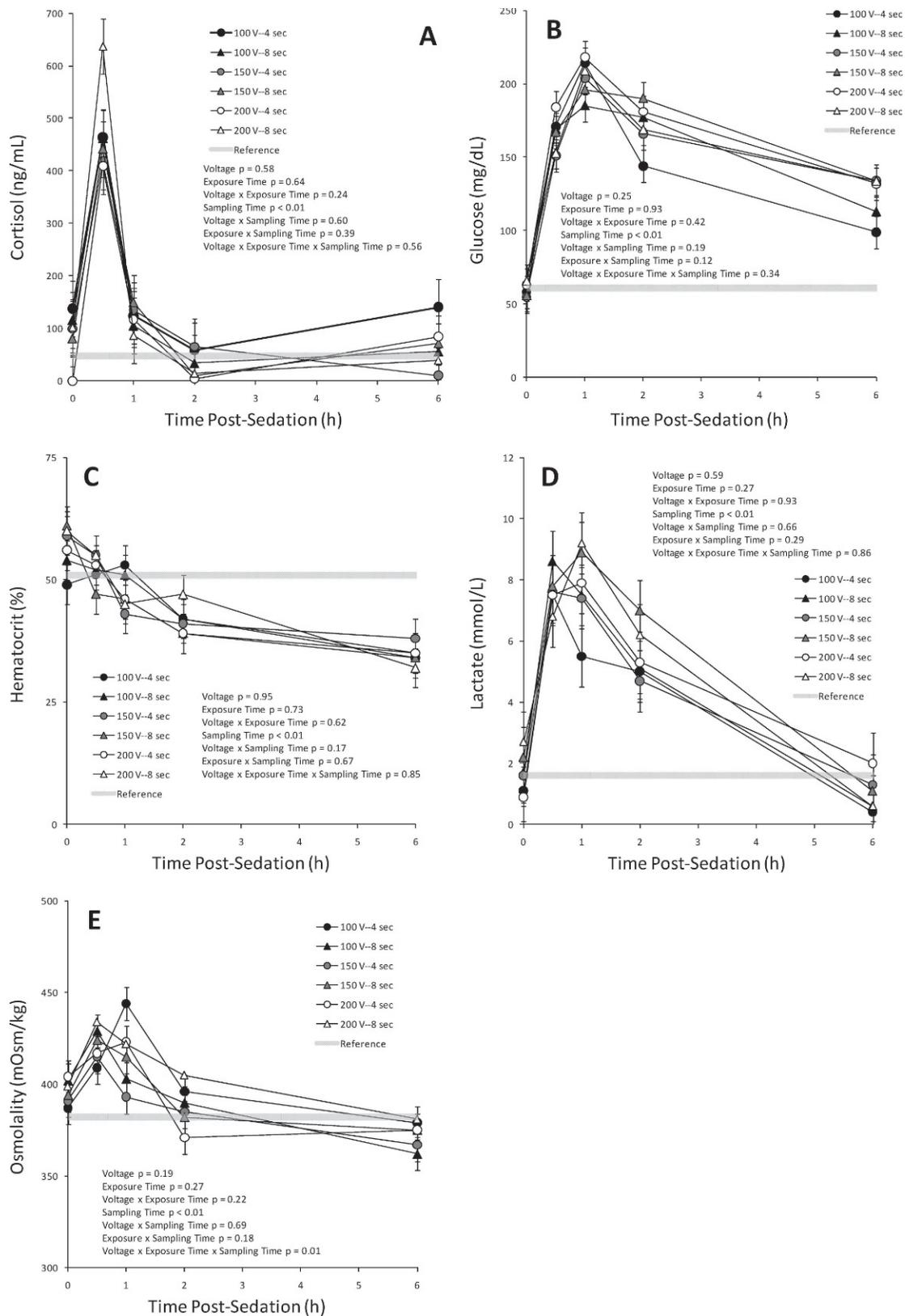


Figure 3. Time course of blood chemistry changes observed in (A = cortisol, B = glucose, C = hematocrit, D = lactate, and E = osmolality) juvenile hybrid striped bass *Morone chrysops* × *M. saxatilis* ($N = 3$) after electroshock using pulsed direct current of various voltage strengths and exposure durations. Points represent means \pm SE; gray reference bars represent means of values observed for fish sampled from the reference population throughout the course of the experiment (time = 0, 3, and 6 h). P values for main effects of voltage strength and exposure duration, sampling time effect (repeated effect), and their interactions are provided.

increased shortly after sedation and then decreased over time. The cortisol response was rapid and transient, peaking (400–630 ng/mL) at 0.5 h postsedation and returning to basal levels (0–60 ng/mL) between 1 and 2 h postsedation. Peak levels of glucose (160–225 mg/dL), lactate (6–9 mmol/L), and osmolality (380–445 mOsm/kg) were slower to develop, reaching maximum levels between 0.5 and 1 h postsedation. Glucose and lactate remained elevated beyond the duration of the cortisol pulse. Lactate returned to resting levels within 6 h of sedation, but glucose remained elevated and did not return to basal levels by the end of the sampling period. Voltage strength and exposure duration had no significant effect on hematocrit; however, values did decrease over the course of the sampling period. Significant voltage \times exposure duration effects were not observed for any of the blood chemistry parameters, although a significant voltage \times exposure duration \times sampling time effect was noted for osmolality.

During electrosedation, fish exhibited opercular flaring, fin extension, and body rigidity, but they regained normal posture after resolution of the postexposure tremor. A single mortality was observed shortly after sedation in the 200 V–4 sec treatment. During the time after the 48-h observation period, no further mortalities or overt pathologies were observed and general behavior (e.g., swimming, feeding) of the population seemed normal.

Discussion

Electrosedation using pulsed direct current at voltage strengths of 100–200 V and exposure durations of 4 or 8 s induced stage IV sedation in juvenile hybrid striped bass and influenced recovery times. Although slight numeric differences were noted for induction times, they were not statistically significant and would probably not be considered practically meaningful to fisheries professionals. The same could be said of the equilibrium and full recovery times: although some significant differences were detected, all fish recovered within 2 min, which would probably be considered adequate for fisheries professionals sedating fish. Regardless of how long it took for fish to regain equilibrium, the elapsed time between equilibrium and full recovery was relatively short and consistent among all treatments. The observed changes in blood chemistry were consistent with an acute stress response (Barton 2002), but they were transient and either resolved or observed to diminish within 6 h of sedation. Although postsedation mortality was observed, this was associated with a single, healthy-appearing fish treated at the highest voltage but for the shortest duration that died shortly after blood sampling. This mortality represented 1% of the fish handled during the experiment and was considered incidental. Exposure to direct current electricity can cause internal injuries, depending on the fish and exposure conditions (Dolan and Miranda 2004), but we did not evaluate fish in this study for such injuries. However, the absence of delayed mortality or abnormal behavior in the weeks after the experiment suggests that if internal injuries occurred, they were minor. Based on these data, it would seem

that juvenile hybrid striped bass are resilient to electrosedation at the range of voltage strengths and exposure durations tested in this experiment and that the electrosedation treatments tested are reasonably safe with respect to postsedation survival and physiological status of these fish.

Blood chemistry results from the present study were similar to those reported by Trushenski et al. (2012) in which adult hybrid striped bass (510 ± 12 g, 33.7 ± 0.2 cm total length [mean \pm SE]) were sedated with the same Portable Electroanesthesia System at 60 V for 3 s. Trushenski et al. (2012) reported peak cortisol and glucose concentrations (regardless of time period) that were lower than observed in the present study (~ 50 and $\sim 25\%$ lower, respectively), lactate levels that were higher ($\sim 140\%$ higher), and osmolality and hematocrit levels that were nearly identical. These differences are considered to be fish size related and may have been the result of greater oxygen demand coupled with greater capacity for anaerobic metabolism among the larger fish assessed in the previous experiment. Although resting metabolic rate is known to be inversely related to body size (Kleiber 1947; Zeuthen 1953), absolute oxygen demand (not expressed as a function of body mass) is greater among larger fish (Clarke and Johnston 1999), as is the capacity for anaerobic metabolism and lactate production within the muscle tissue (Somero and Childress 1980). Thus, the adult fish previously investigated may have exhibited a greater lactate response to electrosedation simply because of their larger size.

Previous research with chemical sedatives and various methods of electrosedation (e.g., alternating current, continuous direct current, pulsed direct current) has demonstrated that fish undergo the generalized stress response (Selye 1950) after sedation (Bourne 1984; Bernier and Randall 1998; Cho and Health 2000; Davidson et al. 2000; Sladky et al. 2001; Wagner et al. 2002; Davis and Griffin 2004; Woods et al. 2008; Feng et al. 2009; Sattari et al. 2009; Trushenski et al. 2012). Circulating levels of cortisol are observed to increase quickly after sedation and typically return to resting levels within a comparatively short time frame. However, exposure to chemical sedatives has been reported to elicit cortisol pulses lasting as long as 24 h (Davis and Griffin 2004). Although the magnitude of the response is probably increased by pre- and postsedation handling, physiological responses also occur in the absence of handling, indicating that the sedatives themselves act as stressors (Zahl et al. 2010). The stress response in fish is typically characterized by the involvement of two hormonal axes or cascades: the adrenergic cascade yielding catecholamines and the corticosteroid cascade yielding primarily cortisol. Rapidly after stressor perception, stored catecholamines are released from the chromaffin tissue as a result of direct nervous stimulation. Cortisol is synthesized after stimulation by adrenocorticotropin release after stressor perception as part of the hypothalmo-pituitary-interrenal axis (Iwama 1998). The involvement of releasing hormones and the need to synthesize cortisol before release result in the corticosteroid cascade being the slower of the two stress hormone

axes (Barton and Iwama 1991). Consequently, very short-lived acute stressors may be insufficient to elicit or maintain a robust cortisol response. Given the delay between stressor perception and the synthesis and release of corticosteroids, if the stressor is present for only a very short period, the corticosteroid response may be similarly short-lived or only the adrenergic cascade may be triggered. In the present case, the electrical "stressor" was present for no more than 8 s, although the fish were handled for as long as 2 min (e.g., netting, transfer to the electrosedation chamber). Nonetheless, exposure to the primary stressor (pulsed direct current electricity) was extremely short lived, and exposure to the secondary stressors (the sedation chamber, handling) was also brief. A true control treatment in which fish were exposed to the electrosedation chamber but not exposed to pulsed direct current electricity would be necessary to differentiate between the effects of these primary and secondary stressors. Regardless, the cumulative stress of handling and electrosedation was sufficient to engage the hypothalmo-pituitary-interrenal axis and induce a sizable cortisol response, but it was expectedly transient and resolved within 1–2 h.

Metabolic responses to stressors, such as elevated plasma glucose and lactate, are generally slower to develop than cortisol, but they can persist for a longer period after sedation (Mazeaud et al. 1977; Barton 2002). Whereas cortisol often peaks and begins to decrease within 1–2 h of exposure to an acute stressor, glucose and lactate may not peak until after the cortisol response is beginning to resolve (Davis and Small 2006; Trushenski et al. 2010). The present results are therefore consistent with previous work sedating adult fish by using both chemical sedative techniques and electrosedation (Trushenski et al. 2012) and the majority of published works on the subject. It is somewhat surprising that the lactate and glucose responses were so robust and persistent, given that the cortisol response was fully resolved within 1–2 h postsedation. This may be explained, in part, by the different timelines observed for the primary and secondary physiological effects of stressor exposure. Although metabolic indicators of stress often covary with hormonal responses, this does not necessarily reflect a direct or causal relationship between the parameters (Barton and Iwama 1991). Although lactate has been observed to increase as a result of direct, cortisol-stimulated lactate production (Mommsen et al. 1999), increasing lactate also may reflect a transition to anaerobic metabolism in sedated fish. This phenomenon results from reduced ventilation and impaired gas exchange during sedation and is not strictly a metabolic response to cortisol release. It is likely that tetanic muscle contraction occurring during electrosedation exacerbates the effect of reduced or absent ventilation, leading to even greater lactate accumulation within the tissues. In electrosedation, the lactate pulse may be disconnected from the cortisol pulse. Presumably, lactate may increase shortly after sedation as a result of elevated cortisol, but the elevated levels may be sustained after cortisol returns to resting levels. The elevated lactate levels may persist because of

the physical consequences of electrosedation, that is, reduced ventilation, tetanic muscle contraction, and increased anaerobic metabolism. Clearance of metabolic acidosis, respiratory acidosis, or both is known to take time and significant investment of energetic resources in fishes (Heath and Pritchard 1965; Perry and Gilmour 2006). Resolution of acidosis may create sufficient energetic demand to maintain up-regulated glucose trafficking long after resolution of the primary stress response. Alternatively, circulating levels of glucose may remain elevated for a longer period than lactate because of the preferential tissue uptake and use of lactate for resynthesis of glycogen after physical stress such as exertion (Milligan 1996). Thus, although electrosedation offers significant advantages in terms of rapid induction and recovery and a brief, transient stress response, it also may lead to lactate accumulation and acidosis. Regardless, this effect is not restricted to electrosedation (Trushenski et al. 2012), and lactate accumulation we observed in our study was resolved within 6 h.

In conclusion, pulsed direct current electrosedation is an effective strategy for sedating juvenile hybrid striped bass quickly and easily. Although slight differences in induction and recovery times were associated with different voltage strengths and exposure durations, all of the treatments yielded sedation patterns that would be considered acceptable by fisheries professionals for routine handling procedures. Given the rapid induction and recovery times associated with electrosedation, this approach would be useful for collection of morphometric data, fin clipping, placement of external tags, or other noninvasive procedures, particularly in circumstances where high throughput and immediate release of the fish are priorities. Electrosedation elicited changes in blood chemistry consistent with the generalized stress response and metabolic acidosis. However, none of the treatments elicited responses that were considered extreme compared with the others or the reported effects of chemical sedatives. We recommend using the lowest voltage strength and exposure duration yielding effective electrosedation to minimize the incidence of unforeseen injuries or physiological alterations. Although researchers should practice caution in applying electrosedation to untested taxa, similar results observed in several other cool- and warmwater fishes (J. Trushenski, J. Bowker, A. Johnson, and M. Schwarz, unpublished data) suggest that electrosedation may be developed as a safe and effective approach to sedation of other fish species of conservation or management concern.

Supplemental Material

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Table S1. All data are organized into three tabs, "Induction-Recovery Data," "Blood Chemistry Data," and "Water Quality Data." The Blood Chemistry Data tab



includes data for fish exposed to the electrosedation treatments, as well as fish sampled from the Reference population (reference fish blood chemistry is provided to the left of the main dataset). In several circumstances, additional information about individual data points is provided as remarks. These remarks are denoted by red earmarks in the upper right-hand corner of the cell and may be viewed by holding the cursor over the earmark. All tabs are formatted with data in plain text and headings/labels in bold, and all-caps font for clarity; all headings and terms used in this data file are defined in the first tab, "Read Me – Data File Explanation."

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Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

- Barton BA. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology* 42:571–525.
- Barton BA, Iwama GK. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases* 1:3–26.
- Bernier NJ, Randall DJ. 1998. Carbon dioxide anaesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anesthetic treatment. *Journal of Fish Biology* 52:621–637.
- Bourne PK. 1984. The use of MS-222 (tricaine methanesulphonate) as an anaesthetic for routine blood sampling in three species of marine teleosts. *Aquaculture* 36:313–321.
- Cho GK, Heath DD. 2000. Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile Chinook salmon *Oncorhynchus tshawytscha* (Walbaum). *Aquaculture Research* 31:537–546.
- Clarke A, Johnston NM. 1999. Scaling of metabolic rate wit body mass and temperature in teleost fish. *Journal of Animal Ecology* 68:893–905.
- Curry KD, Kynard B. 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* 35:1291–1302.
- Danner GR, Muto KW, Zieba AM, Stillman CM, Seggio JA, Ahmad ST. 2011. Spearmint (*l*-carvone) oil and wintergreen (methyl salicylate) oil emulsion is an effective immersion anesthetic of fishes. *Journal of Fish and Wildlife Management* 2:146–155.
- Davidson GW, Davie PS, Young G, Fowler RT. 2000. Physiological responses of rainbow trout *Oncorhynchus mykiss* to crowding and anesthesia with AQU-I-S™. *Journal of the World Aquaculture Society* 31:105–114.
- Davis KB, Griffin BR. 2004. Physiological responses of hybrid striped bass under sedation by several anesthetics. *Aquaculture* 233:531–548.
- Davis KB, Small BC. 2006. Rates of cortisol increase and decrease in channel catfish and sunshine bass exposed to an acute confinement stressor. *Comparative Biochemistry and Physiology* 143C:134–139.
- Delaney MA, Klesius PH, Shelby RA. 2005. Cortisol response of Nile tilapia, *Oreochromis niloticus* (L.), to temperature changes. *Journal of Applied Aquaculture* 16:95–104.
- Dolan CR, Miranda LE. 2004. Injury and mortality of warmwater fishes immobilized by electrofishing. *North American Journal of Fisheries Management* 24: 118–127.
- [FDA] Food and Drug Administration. 2011a. Approved drugs (aquaculture). Food and Drug Administration. Available: <http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/Aquaculture/ucm132954.htm> (March 2012).
- [FDA] Food and Drug Administration. 2011b. Enforcement priorities for drug use in aquaculture. Center for Veterinary Medicine Program Policy and Procedures Manual. Food and Drug Administration. Available: <http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/PoliciesProceduresManual/UCM046931.pdf> (March 2012).
- Feng GP, Zhuang P, Zhang LZ, Chen NN, Yao ZF, Men YY. 2009. Effects of electroanesthesia on behavior and serum ion concentration of juvenile *Acipenser baeri*. *Marine Fisheries* 1, English language abstract. Available: http://en.cnki.com.cn/Article_en/CJFDTotat-HTYY200901005.htm (March 2012).
- Heath AG, Pritchard AW. 1965. Effects of severe hypoxia on carbohydrate energy stores and metabolism in two species of fresh-water fish. *Physiological Zoology* 38: 325–334.
- Hudson JM, Johnson JR, Kynard B. 2011. A portable electronarcosis system for anesthetizing salmonids and other fish. *North American Journal of Fisheries Management* 31:335–339.
- Iwama GK. 1998. Stress in fish. *Annals of the New York Academy of Sciences* 851:304–310.
- Jennings CA, Looney GL. 1998. Evaluation of two types of anesthesia for performing surgery on striped bass.



- North American Journal of Fisheries Management 18: 187–190.
- Kleiber M. 1947. Body size and metabolic rate. *Physiological Reviews* 27:511–541.
- Kohler CC. 2000. Striped bass and hybrid striped bass culture. Pages 898–907 in Stickney RR, editor. *Encyclopedia of aquaculture*. New York: Wiley.
- Kynard B, Lonsdale E. 1975. Experimental study of galvanonarcosis for rainbow trout (*Salmo gairdneri*) immobilization. *Journal of the Fisheries Research Board of Canada* 32:300–302.
- Madden JA, Houston AH. 1976. Use of electroanaesthesia with freshwater teleosts: some physiological consequences in rainbow trout, *Salmo gairdneri*, Richardson. *Journal of Fish Biology* 9:457–462.
- Mazeaud MM, Mazeaud F, Donaldson EM. 1977. Primary and secondary effects of stress in fish, some new data with a general review. *Transactions of the American Fisheries Society* 106:201–212.
- Milligan CL. 1996. Metabolic recovery from exhaustive exercise in rainbow trout. *Comparative Biochemistry and Physiology* 113A:51–60.
- Mommsen TP, Vijayan MM, Moon TW. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries* 9:211–268.
- Neiffer DL, Stamper MA. 2009. Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. *Institute of Laboratory Animal Research Journal* 50:343–360.
- Olsen YA, Einarsdottir I, Nilssen KJ. 1995. Metomidate anaesthesia in Atlantic salmon, *Salmo salar*, prevents plasma cortisol increase during stress. *Aquaculture* 134:155–168.
- Owen MAG, Davies SJ, Sloman KA. 2009. Light colour influences the behaviour and stress physiology of captive tench (*Tinca tinca*). *Reviews in Fish Biology and Fisheries* 20:375–380. doi: 10.1007/s11160-009-9150-1
- Perry SF, Gilmour KM. 2006. Acid-base balance and CO₂ excretion in fish: unanswered questions and emerging models. *Respiratory Physiology and Neurobiology* 154:199–215.
- Pirhonen J, Schreck CB. 2003. Effects of anaesthesia with MS-222, clove oil and CO₂ on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*). *Aquaculture* 220:507–514.
- Ross LG, Ross B. 2008. *Anaesthetic and sedative techniques for aquatic animals*. 3rd edition. Oxford, UK: Blackwell Publishing.
- Sattari A, Mirzargar S, Abrishamifar A, Lourakzadegan R, Bahonar A, Mousavi HE, Niasari A. 2009. Comparison of electroanesthesia with chemical anesthesia (MS222 and clove oil) in rainbow trout (*Oncorhynchus mykiss*) using plasma cortisol and glucose responses as physiological stress indicators. *Asian Journal of Animal and Veterinary Advances* 4:306–313.
- Selye H. 1950. Stress and the general adaptation syndrome. *British Medical Journal* 1(4667):1383–1392.
- Sepici-Dinçel A, Çağlan Karasun Benli A, Selvi M, Sarikaya R, Şahin D, Ayhan Özkul I, Erkoç F. 2009. Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio* L.) fingerlings: biochemical, hematological, histopathological alterations. *Ecotoxicology and Environmental Safety* 72:1433–1439.
- Sladky KK, Swanson CR, Stoskopf MK, Loomis MR, Lewbart GA. 2001. Comparative efficacy of tricaine methanesulphonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). *American Journal of Veterinary Research* 62:337–342.
- Small BC. 2004. Effect of isoeugenol sedation on plasma cortisol, glucose, and lactate dynamics in channel catfish *Ictalurus punctatus* exposed to three stressors. *Aquaculture* 238:469–481.
- Somero GN, Childress JJ. 1980. A violation of the metabolism-size scaling paradigm: activities of glycolytic enzymes in muscle increase in larger-size fish. *Physiological Zoology* 53:322–337.
- Summerfelt RC, Smith LS. 1990. Anesthesia, surgery, and related techniques. Pages 213–272 in Schreck CB, Moyle PB, editors. *Methods for fish biology*. Bethesda, Maryland: American Fisheries Society.
- Trushenski JT, Schwarz M, Takeuchi R, Delbos B, Sampaio LA. 2010. Physiological responses of cobia *Rachycentron canadum* following exposure to low water and air exposure stress challenges. *Aquaculture* 307:173–177.
- Trushenski JT, Bowker JD, Gause BR, Mulligan BL. 2012. Chemical and electrical approaches to sedation of hybrid striped bass: induction, recovery, and hematological responses to sedation. *Transactions of the American Fisheries Society* 141:455–467.
- Vandergoot CS, Murchie KJ, Cooke SJ, Dettmers JM, Bergstedt RA, Fielder DG. 2011. Evaluation of two forms of electroanesthesia and carbon dioxide for short-term anesthesia in walleye. *North American Journal of Fisheries Management* 31:914–922.
- Venn Beecham R, Small BC, Minchew CD. 2006. Using portable lactate and glucose meters for catfish research: acceptable alternatives to established laboratory methods? *North American Journal of Aquaculture* 68:291–295.
- Wagner E, Arndt R, Hilton B. 2002. Physiological stress responses, egg survival and sperm motility for rainbow trout broodstock anesthetized with clove oil, tricaine methanesulfonate or carbon dioxide. *Aquaculture* 211:353–366.
- Wells RMG, Pankhurst NW. 1999. Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. *Journal of the World Aquaculture Society* 30:276–284.
- Woods LC, Theisen DD, He S. 2008. Efficacy of Aquic-S as an anesthetic for market-sized striped bass. *North American Journal of Aquaculture* 70:219–222.
- Zahl IH, Kiessling A, Samuelsen OB, Olsen RE. 2010. Anesthesia induces stress in Atlantic salmon (*Salmo*

- salar*, Atlantic cod (*Gadus morhua*), and Atlantic halibut (*Hippoglossus hippoglossus*). *Fish Physiology and Biochemistry* 36:719–730.
- Zeuthen E. 1953. Oxygen uptake as related to body size in organisms. *Quarterly Review of Biology* 28:1–12.
- Zydlowski GB, Gale W, Holmes J, Johnson J, Brigham T, Thorson W. 2008. Use of electroshock for euthanizing and immobilizing adult spring Chinook salmon in a hatchery. *North American Journal of Aquaculture* 70: 415–424.