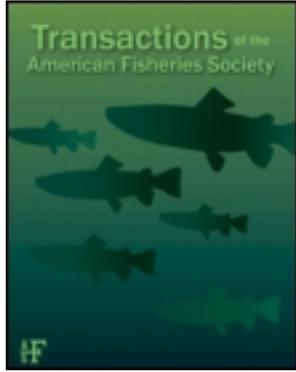


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ARTICLE

Chemical and Electrical Approaches to Sedation of Hybrid Striped Bass: Induction, Recovery, and Physiological Responses to Sedation

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Abstract

Currently, in the United States, there are few sedatives available to fisheries professionals that are safe, effective, and practical. Chemical sedatives, including tricaine methanesulfonate (MS-222), carbon dioxide (CO₂), benzocaine, and eugenol may be used to sedate fish, though none of these compounds are currently approved by the U.S. Food and Drug Administration as immediate-release fish sedatives. Another option is the use of electricity to temporarily immobilize fish. Few studies have assessed the efficacy of these options for immediate-release sedation in side-by-side comparisons. We evaluated the use of MS-222 (150 mg/L), CO₂ (~400 mg/L), benzocaine (150 mg/L), eugenol (60 mg/L), and a commercially available electrosedation unit (30 Hz pulsed DC, 60 V, 25% duty cycle, 3-s exposures) to induce hybrid striped bass (white bass *Morone chrysops* × striped bass *M. saxatilis*; 510 ± 12 g [mean ± SE]) to stage IV anesthesia or sedation. Induction times were shortest (0.2 ± 0.1 min) when electrosedation was used and longest (2.5 ± 0.1 min) when CO₂ was used; the induction times for the other chemical sedatives varied (<2 min). Recovery times were longest for eugenol (5.2 ± 0.4 min postinduction) and benzocaine (4.0 ± 0.4 min); however, the difference in recovery time between these two treatments was not significant or between recovery times for benzocaine and the remaining sedatives (~3–4 min). Physiological responses varied but were consistent with the generalized stress response. Circulating levels of cortisol, glucose, and lactate increased after sedation, and though response magnitude and duration varied somewhat among these variables, these changes were resolved within 6 h. Changes in plasma osmolality and hematocrit were less overt and varied less among the sedatives. Electrodesation may be a suitable tool for quickly sedating hybrid striped bass; however, all of the sedatives evaluated were effective at the doses and strengths used and some may be better suited to certain applications than to others.

The availability of safe and effective fish sedatives is crucial to fisheries researchers, managers, and culturists. Fisheries professionals sedate fish for a variety of purposes, ranging from simple handling to invasive surgical procedures. Compared

with terrestrial animals, the skin of most fishes is delicate and prone to damage (Ross and Ross 2008). As a result, fish cannot be restrained in the same manner as terrestrial animals without causing mechanical damage. Fish are innately difficult

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to handle, and when they actively resist restraint, epithelial damage or other physical injury is more likely (Ross and Ross 2008). If fish are sedated before handling, risk to both fish and handler is minimized.

The terms “anesthesia,” “sedation,” and “immobilization” are used somewhat interchangeably with respect to fish, but the terms have distinct definitions. 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 the acuity of sensory perception. Although the term “electroanesthesia” is used to describe the effects of DC electricity on fish, Ross and Ross (2008) have suggested that true anesthesia may not occur with this method. It could be argued that none of these terms perfectly describe the processes we investigated in the present work. However, we use the terms “electrosedation” and “sedatives” herein to best reflect our behavioral observations and the current understanding of the processes of sedation and anesthesia in fish.

In addition to mechanical damage, fish that are handled without proper sedation may also be subject to the physiological consequences of a heightened generalized stress response. Stress has been defined as a natural reaction to a negative stimulus leading to the mobilization and redirection of energetic resources to support “fight or flight” (Selye 1950), or more recently, as factors inducing “predictive” and “reactive” responses intended to maintain homeostasis, which may have positive or negative consequences depending on the relative success or failure of these responses (Romero et al. 2009). During a stress response, energy otherwise devoted to important, but immediately non-critical functions is redirected to fuel the metabolic demands of the response. These functions can include osmoregulation, exclusion and clearance of pathogens, reproduction, and feeding behavior; as a result, stressed fish may become compromised and suffer increased vulnerability to disease, reduced reproductive performance, and reduced growth (Barton 2002).

Currently, there are few sedative options available to fisheries professionals that are safe, effective, and practical to use. There is only one sedative compound that is currently approved by the U.S. Food and Drug Administration (FDA): tricaine methanesulfonate (MS-222) to temporarily immobilize fish (two products are currently approved in the United States). However, use of MS-222 is restricted to four families of fish (Ictaluridae, Salmonidae, Esocidae, and Percidae) or other laboratory or hatchery fish held at water temperatures greater than 10°C, and users must adhere to a 21-d withdrawal period (a holding period deemed necessary to allow for drug residue depletion before fish are processed for consumption or released). Tricaine methanesulfonate is believed to exert its sedative effect by preventing generation and conduction of nerve impulses, similar

to many other local anesthetics (Frazier and Narahashi 1975). Another option is the use of carbon dioxide (CO₂), which is considered by the FDA to be a drug of low regulatory priority. The sedative effect of CO₂ is based on interference with respiratory exchange and CO₂ excretion. When environmental concentrations of CO₂ are high, excretion is slowed or reversed, causing CO₂ to build within the central nervous system and other tissues (hypercapnia). Gradually, widespread central nervous system depression occurs, resulting in the loss of consciousness and voluntary motor function. There are at least two additional compounds currently being investigated for use as immediate-release fish sedatives: benzocaine and eugenol. Both compounds elicit sedative effects by interfering with conduction of nervous stimuli (Kozam 1977; Neumcke et al. 1981). All of these chemical sedatives has positive and negative attributes associated with its use, including approval status (approved drug versus low regulatory priority drug versus Investigational New Animal Drug status), allowable use patterns (immediate release versus 3-d withdrawal period versus 21-d withdrawal period), disposal considerations, cost, ease of use, and efficacy. While chemical sedatives are well suited to some fish sedation applications, they may not be appropriate for all circumstances. An alternative option is the use of electricity to temporarily immobilize fish. Electrofishing has been used for decades as a means of capturing fish in field studies, but only recently has this approach been modified specifically for sedating or anesthetizing fish and its use commercialized (Zydlewski et al. 2008). “Electroanesthesia,” or more accurately, electrosedation, can immobilize fish via electronarcosis (stunning) or electrotetany (tetanic muscle contraction) caused by electrically induced interference with neurotransmission. Electrosedation may offer several advantages over chemical sedatives in terms of withdrawal periods, chemical disposal, and potentially ease of use.

Despite the need for proper sedatives in fisheries and the varying suitability of available options for different applications, relatively few studies have directly compared the efficacy of these sedatives in fish or their responses to sedation. Several studies have been conducted to compare the induction and recovery times associated with using MS-222, CO₂, and other chemical sedatives (e.g., Gilderhus and Marking 1987; Sladky et al. 2001; Wagner et al. 2002; Pirhonen and Schreck 2003; Altun et al. 2006; Cotter and Rodnick 2006). Although the physiological effects of electrosedation have been investigated (Barton and Dwyer 1997), the efficacy of electrosedation has not been quantitatively compared with traditional chemical sedatives. Although several studies have assessed the stress response of fish sedated with chemical anesthetics, both individually and in side-by-side comparisons, the corresponding effects of electrosedation have not been described in comparison with chemical sedatives. Accordingly, we conducted two experiments to quantitatively compare fish sedated with Fiquel (MS-222; 100% tricaine methanesulfonate; Argent Laboratories, Redmond, Washington), AQUI-SE (50% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand), Benzoak (20% benzocaine;

Frontier Scientific, Logan, Utah), CO₂, or electrosedation in terms of (1) induction and recovery times, and (2) hematological profile following sedation. For these experiments, freshwater-reared hybrid striped bass (white bass *Morone chrysops* × striped bass *M. saxatilis*) were selected as a model fish. Hybrid striped bass are popular as both a sport and food fish, are commonly used in both laboratory and field-based fisheries research, and are considered by fisheries biologists to be a representative coolwater–warmwater, euryhaline finfish.

METHODS

Experiment 1: induction and recovery times.—Hybrid striped bass were obtained as fingerlings from a commercial vendor (Keo Fish Farm, Keo, Arkansas), and cultured according to typical in-house production methods at the Fisheries and Illinois Aquaculture Center at Southern Illinois University Carbondale until they reached a size of 510 ± 12 g (mean \pm SE) and 33.7 ± 0.2 cm total length (TL). Feed was withheld for 24 h before starting the experiment. Individual fish were transferred from holding tanks in a freshwater recirculating aquaculture system and placed into a sedation chamber (142-L cooler for electrosedation, 114-L cooler for all others). The chambers were filled with 70 L of culture water (water depth of ~ 8 cm for electrosedation, ~ 10 cm for all others) and contained either a sedative solution or was equipped with the electrosedation unit. Chemical sedation baths were prepared with aerated culture water from the holding system as follows:

1. CO₂: approximately 400-mg/L solutions prepared according to the sodium bicarbonate–sulfuric acid method described by Post (1979)
2. benzocaine: 750-mg/L solution of Benzoak (150 mg/L benzocaine)
3. eugenol: 120-mg/L solution of AQUI-SE (60 mg/L eugenol)
4. MS-222: 150-mg/L solution of Finquel (150 mg/L tricaine methanesulfonate)
5. electrosedation: pulsed DC (60 V, 30 Hz, 25% duty cycle, 3-s exposure) delivered via Portable Electroanesthesia System (Smith-Root, Vancouver, Washington)

Although the culture water used to prepare these baths was aerated before use, baths were not aerated after addition of the chemical sedative or during use. Sufficient volumes of culture water were treated with benzocaine, eugenol, and MS-222 to allow sedative baths to be exchanged from a single stock source after five fish had been treated in the sedative chamber; culture water was similarly exchanged after five fish had been treated in the electrosedation chamber. In the case of CO₂, sedative baths were also exchanged after treating five fish; however, each bath was individually prepared as needed because of the potential loss of volatile CO₂ from the sedative baths over time. Composite water samples were collected by combining aliquots collected from the sedative baths before and after use, and analyzed in duplicate along with water samples collected from the

holding recirculation system at the beginning and end of the study period. Dissolved oxygen (YSI 550 dissolved oxygen–temperature meter, Yellow Springs Instruments, Yellow Springs, Ohio), conductivity, pH, salinity (Multi-Parameter PCSTestr 35, Eutech Instruments, Oakton, Vernon Hills, Illinois), hardness, alkalinity (digital titrator and reagents; Hach, Loveland, Colorado), total ammonia nitrogen, nitrite-nitrogen, and nitrate-nitrogen (spectrophotometer and reagents; Hach, Loveland, Colorado) were maintained within ranges appropriate for hybrid striped bass culture (Kohler 2000) throughout the experiment (Table 1).

During sedation, each fish was monitored to determine the time (from the time of sedative exposure) to achieve stage IV anesthesia (as described 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 continuously challenged with tactile stimuli (slight manual dorsoventral compression). Fish were considered induced to stage IV when they no longer responded to this stimulus, but the opercular rate was maintained at approximately 30–45 beats/min. In the case of the electrosedative treatment, a tremor was observed following electrical exposure; although fish were not responsive during this tremor (and were perhaps momentarily in stage V or VI of anesthesia), induction was considered complete after the tremor had ceased. After induction, fish were weighed (to the nearest 0.1 g) and measured to determine TL (to the nearest 0.5 cm) and then transferred to an aerated recovery tank that was plumbed into the recirculation aquaculture system in which the fish had been housed. In the recovery tank, fish were monitored by using the same techniques mentioned above to determine time to recovery of normal equilibrium and tactile responses. To assess responsiveness to visual and auditory stimuli, the airstone was gently tapped against the side of the tank near the fish's head. When fish exhibited avoidance behavior to this stimulus, they were considered fully recovered. Recovered fish were returned to a holding system and monitored for survival for 48 h. Since assessment of induction and recovery can be somewhat subjective, bias was minimized by having the same observer make all assessments.

Experiment 2: hematological responses to sedation.—In this experiment, sedative baths were prepared as previously described. However, based on the lack of water chemistry changes during the course of experiment 1, a single bath was prepared and used throughout the experiment for sedating all fish groups in benzocaine, eugenol, and MS-222, or by electrosedation. Fresh sedative baths were prepared for each group of fish sedated with CO₂ because the volatile loss of CO₂ probably would be exacerbated by fish movement during group sedation. Composite water samples were collected and analyzed as described for experiment 1. Water chemistry did not vary considerably between experiments (Table 1).

Feed was withheld for 24 h before the start of experiment. Groups of five fish (509 ± 9 g, 33.8 ± 0.5 cm TL) were

TABLE 1. Water quality in experiments 1 and 2. The values are the means of composite samples obtained by combining the aliquots collected from the sedative baths before and after use and analyzed in duplicate along with water samples collected from the holding recirculation system at the beginning and end of the study period.

Variable	Experiment	Holding system	Sedative				
			Eugenol	Benzocaine	CO ₂	MS-222	Electrosedation
Temperature (°C)	1	18.6	18.2	17.8	18.3	18.2	18.1
	2	21.0	20.8	20.8	21.0	20.9	20.7
Dissolved oxygen (mg/L)	1	8.2	7.8	7.8	9.6	8.6	7.8
	2	9.9	9.9	9.9	9.9	10.0	10.0
Total ammonia nitrogen (mg/L)	1	0.17	0.47 ^a	0.06	0.28	0.30	0.35
	2	0.27	0.90 ^a	0.39	0.33	0.28	0.41
Nitrite-nitrogen (mg/L)	1	0.05	0.03	0.02	0.07	0.07	0.08
	2	0.02	0.12	0.08	0.02	0.02	0.03
Nitrate-nitrogen (mg/L)	1	2.55	2.50	2.65	2.75	2.70	3.40
	2	3.80	4.90	4.60	4.80	4.90	5.65
Alkalinity (mg/L)	1	173	247	251	197	176	182
	2	232	254	230	260	220	240
Hardness (mg/L)	1	62	61	62	63	63	67
	2	72	79	70	67	68	74
Salinity (‰)	1	1.5	1.9	1.8	2.2	1.8	1.8
	2	5.5	5.5	5.5	5.9	5.6	5.4
Conductivity (mS/cm)	1	3.0	3.7	3.4	4.2	3.4	3.4
	2	9.9	9.9	9.9	10.6	10.0	9.7
pH	1	8.3	8.4	8.5	6.5	7.5	8.4
	2	8.4	8.4	8.3	6.0	7.3	8.4

^aIt was assumed that the presence of eugenol in the water interfered with the Nessler reagent ammonia assay, which is affected by alcohols and other substances that create turbidity or yellowish-green colors in water (as eugenol does).

transferred from the same holding tanks in a freshwater recirculating aquaculture system and placed into the sedation chamber and sedated en masse as previously described for experiment 1. Immediately after induction to stage IV, one fish per group was transferred to a bath of metomidate hydrochloride (Aquacalm, Western Chemical, Ferndale, Washington; ~3–5 mg/L for ~30 s). Although fish sampled at time = 0 did not require further sedation in order to collect blood samples, sedation was required to facilitate blood sampling at later time points in compliance with our Institutional Animal Care and Use Committee (IACUC)-approved animal care and use protocol. Metomidate hydrochloride blocks corticosteroid synthesis (Mattson and Rippe 1989; Olsen et al. 1995; Davis and Griffin 2004), and is therefore a particularly useful sedative to use in this context because it minimizes the effects of handling and sample collection on circulating cortisol levels. For consistency, all fish sampled, regardless of sampling time (including those sampled immediately after sedation), were transferred to a solution of metomidate hydrochloride. After exposure to the metomidate hydrochloride bath, TL and weight were measured, and a blood sample was collected from the caudal vasculature with heparinized, evacuated blood collection assemblies (Vacutainer; Becton Dickinson, Franklin Lakes, New Jersey). All blood samples were collected within 5 min of capture to minimize the

possibility of confounding responses of handling and sampling via the caudal vasculature as acute stressors. The remaining four fish in each group were returned to a holding tank in the source recirculation aquaculture system. One fish was then sampled from each group at 0.5, 1, 2, and 6 h postsedation. After blood collection, fish were placed into an adjacent recirculation system (similar water temperature and quality) and monitored for survival for 48 h. During the sampling period, fish were sampled periodically from the reference population. Tubes containing blood samples were kept on wet ice (<6 h) until analysis. Subsamples of whole blood were used to determine hematocrit (Statspin centrifuge, Fisher Scientific, Pittsburgh, Pennsylvania) and glucose levels (Freestyle Freedom Lite glucose meter, Abbott Laboratories, Abbott Park, Illinois). Whole blood samples were then centrifuged (3,000 × g, 45 min, 4°C) and the resultant plasma was stored at –80°C until further analysis. Plasma samples were analyzed to determine lactate (Accutrend lactate meter, Roche, Mannheim, Germany), osmolality (Vapro 5520, Wescor, Logan, Utah), and cortisol levels (EIA 1887, DRG International, Mountainside, New Jersey). Although portable lactate and glucose meters, such as those used in this study, can slightly underestimate metabolite levels in fish blood relative to laboratory methods, they are considered precise and reliable for use in generating comparative data (Wells and Pankhurst 1999;

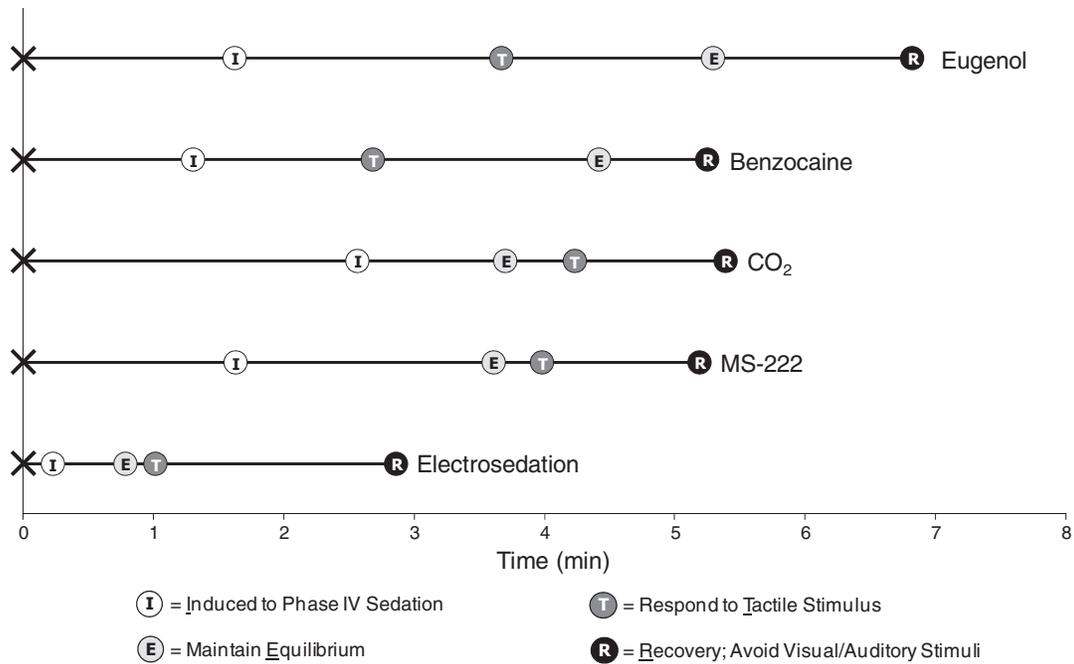


FIGURE 1. Schematic illustrating induction and various stages of recovery of hybrid striped bass sedated to stage IV of anesthesia using various chemical sedatives or electrosedation.

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 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; Sepici-Dinçel et al. 2009; Owen et al. 2010).

Statistical analyses.—For experiment 1 data analysis, individual fish were considered experimental units ($n = 9$). Induction and recovery times in fish from experiment 1 were analyzed by one-way analysis of variance (ANOVA) (PROC GLM) with the Statistical Analysis System, version 9.1 (SAS Institute, Cary, North Carolina) to detect significant differences among the sedatives relative to induction and recovery times. For variables exhibiting significant treatment effects, post hoc Tukey's honestly significant difference (HSD) tests were used for pairwise comparisons of means. Fish weight and TL were assessed as potential covariates (PROC CORR), but no significant correlations between body size and induction or recovery times were observed. For experiment 2 data analysis, replicate groups were considered experimental units ($n = 3$). Thus, fish sampled at each time point represented repeated observations made on the same experimental unit (i.e., sedation group or tank). Accordingly, hematological data from experiment 2 were analyzed by one-way, repeated measures ANOVA (PROC MIXED) with the Statistical Analysis System. For variables exhibiting significant treatment effects, treatment means were compared at individual time points with posthoc Tukey's HSD tests for pairwise comparisons. In all cases, differences were considered significant at $P < 0.05$ and no data were transformed before analysis.

RESULTS

Induction times varied significantly among the sedatives evaluated (electroanesthesia [z] < CO₂ [z] < MS-222 [z] < benzocaine [yz] < eugenol [y]), where different letters indicate significant differences in the mean values; Figure 1). Briefly, the induction time for electroanesthesia was 0.2 ± 0.1 min (mean \pm SE), that for eugenol, MS-222, and benzocaine ranged from 1.3 to 1.6 ± 0.1 min, and that for CO₂ was 2.5 ± 0.1 min. Time to recovery of equilibrium (electroanesthesia [z] < CO₂ [y] < MS-222 [x] < benzocaine [w] < eugenol [w]) and responsiveness to tactile (electroanesthesia [z] < benzocaine [yz] < CO₂ [xy] < eugenol [wx] < MS-222 [w]) and visual–auditory stimuli (electroanesthesia [z] < CO₂ [yz] < MS-222 [y] < benzocaine [y] < eugenol [x]) also varied significantly among the sedative treatments. All benchmarks of recovery were achieved most rapidly in the electroanesthesia treatment: mean time to regain equilibrium, tactile responsiveness, and avoidance of visual–auditory stimuli were 0.6 ± 0.1 , 0.8 ± 0.2 , and 2.6 ± 0.4 min postinduction, respectively. Equilibrium was regained among fish treated with MS-222 and CO₂ in 1.1 – 2.0 ± 0.1 min postinduction, followed by tactile responsiveness at 1.7 – 2.4 ± 0.2 min, and visual–auditory responsiveness at 2.8 – 3.6 ± 0.4 min. Fish treated with benzocaine and eugenol exhibited a different recovery pattern, regaining tactile responsiveness first at 1.4 – 2.0 ± 0.2 min postinduction, followed by equilibrium at 3.1 – 3.7 ± 0.1 min, and visual–auditory responsiveness at 4.0 – 5.2 ± 0.4 min. Total handling time from the beginning of sedative exposure to full recovery was 2.9 ± 0.4 min for

electrosedation, $5.2\text{--}5.4 \pm 0.4$ min for CO₂, MS-222, and benzocaine, and 6.8 ± 0.4 min for eugenol.

Hematology varied significantly among the sedatives evaluated and over time (trends are summarized in Figure 2; see Table 2 for mean \pm SE values and results of pairwise comparisons). Plasma cortisol concentrations increased, though not significantly in all cases, within 0.5 h after sedation, but returned to levels consistent with the reference populations within 2 h for all sedatives except eugenol. A similar response pattern was observed for lactate, though lactate levels remained elevated through the 2-h time point. Plasma glucose levels also increased 0.5–1 h postsedation, but remained elevated throughout the 6-h sampling period. Although a significant treatment effect was observed for hematocrit readings, differences between the sedatives were not readily apparent, and the response patterns appeared to more greatly reflect a generalized decline from 0 to 6 h postsedation. Plasma osmolality in fish did not vary among the sedatives, but also appeared to decline over the course of the sampling period.

Several anecdotal observations were made during the course of the experiments with respect to behavioral responses to the sedatives. Fish exhibited opercular flaring, fin extension, and body rigidity during electrosedation, but appearance returned to normal after resolution of the postsedation tremor. During exposure to eugenol and CO₂, fish were hyperactive and observed to “pipe” at the water surface; although piping was more pronounced among fish exposed to eugenol, hyperactivity was not as apparent. Although some hyperactivity was observed during sedation using MS-222 and benzocaine, it was less pronounced than in the other treatments. During the course of the two experiments involving sedation and handling of 120 individuals, only three mortalities were observed: in experiment 2, two fish failed to recover from electrosedation and one fish died within a few hours of recovering from sedation with CO₂. No mortalities were observed after experiment 1.

DISCUSSION

Our results indicate that electrosedation, benzocaine, CO₂, eugenol, and MS-222 are all effective in sedating hybrid striped bass to stage IV in less than 3 min at the doses or strengths evaluated. However, electrosedation yielded faster induction and recovery times than any of the chemical sedatives evaluated. It is likely that faster induction times would have been observed with the chemical sedatives if greater concentrations had been used. However, sedating fish to the desired endpoint with higher concentrations of a sedative often results in a longer recovery period. It is somewhat difficult to compare induction times across experiments, given the variability in sedation times associated with taxon, size, water temperature, and other variables. For example, Lemm (1993) sedated striped bass (300–1,500 g) to stage IV (described as stage II–plane 2 in Lemm 1993) with 150 mg/L MS-222 and reported mean induction and recovery times at 18°C and 23°C that ranged from 2.1 to 2.5 min and

from 4.21 to 5.95 min, respectively. We achieved similar levels of sedation in shorter periods of time, which is somewhat surprising if one assumes that induction and recovery times should be similar among similarly sized *Morone* spp. However, induction and recovery times are known to vary with water conditions, particularly water temperatures. Differences in water conditions aside, the induction and recovery times we observed are largely consistent with the observations of others (Table 3). Our anecdotal behavioral observations (e.g., hyperactivity before induction with chemical sedatives, piping at the water surface, body rigidity and flexion during electrosedation) are also consistent with previous reports of fish sedation (Ross and Ross 2008); the occurrence of these normal, in some cases reflexive, responses before induction is reassuring in that it suggests the fish were not stressed or compromised at the onset of the experiment (Davis 2010).

Although the specific hematological patterns varied somewhat according to the sedative used, each elicited changes consistent with the generalized stress response. Although relatively few fish were sampled at each time point, (i.e., three fish per treatment per time point) and the resultant power of the design is somewhat limited, the patterns we observed are interesting and broadly consistent with the reported observations of others, but warrant further investigation. Sedatives are commonly used to reduce stressor severity (Sandodden et al. 2001; Finstad et al. 2003; Iversen et al. 2003; Wagner et al. 2003; Cooke et al. 2004; Small 2004; Palić et al. 2006); however, sedation itself can elicit a mild to moderate stress response and induce departures from normal physiological states (Table 3), particularly if these are accompanied by changes in water chemistry associated with sedative treatment (i.e., pH shifts associated with CO₂ and MS-222). Depending on the sedative concentrations used, a transient cortisol response has been observed in fish following sedation with MS-222, CO₂, and various clove derivatives (Davidson et al. 2000; Wagner et al. 2002; Davis and Griffin 2004; King et al. 2005; Bolasina 2006; Zahl et al. 2010). Although higher sedative concentrations may be expected to elicit greater cortisol responses, even concentrations several times lower than we used can induce responses of a comparable magnitude (Davis and Griffin 2004). Similarly, increases in plasma glucose and lactate are also commonly associated with exposure to sedatives (Bourne 1984; Bernier and Randall 1998; Sladky et al. 2001; Cho and Heath 2000; Wagner et al. 2002), as are various other hematological perturbations including changes in hematocrit readings (Sladky et al. 2001; Cho and Heath 2000), plasma ion levels (Bourne 1984), and partial pressures of respiratory gases (Sladky et al. 2001). In terms of hematological responses, most of the various sedatives we evaluated were relatively similar. Eugenol was an exception, however, and was associated with a greater cortisol response. Chiba et al. (2006) investigated various forms of electrosedation and sedation with MS-222 and 2-phenoxyethanol and found that cortisol responses were generally lower when sedation was achieved in a shorter period of time. Similar results were observed by

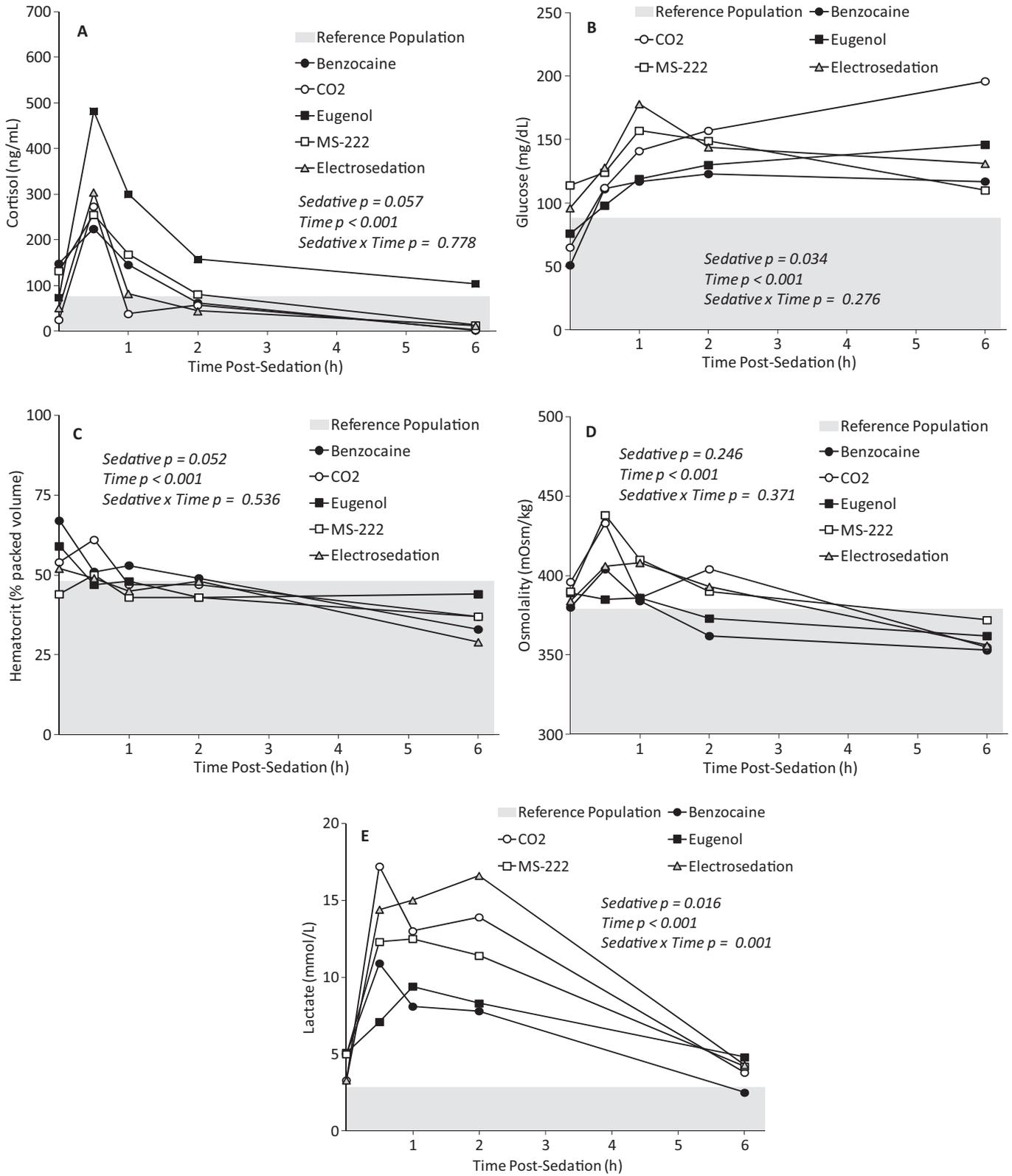


FIGURE 2. Time course of hematological responses (A = cortisol, B = glucose, C = hematocrit, D = osmolality, and E = lactate) of hybrid striped bass after sedation to stage IV of anesthesia using various chemical sedatives or electrosedation. Points represent means reported in Table 2; gray reference bars represent means of values observed for fish sampled from the reference population throughout the course of the experiment.

TABLE 2. Hematological responses of hybrid striped bass after sedation to stage IV anesthesia using various chemical sedatives or electrosedation. The values are the means \pm SEs of triplicate samples at each time point–treatment combination. Means within a time point with different letters are significantly different ($P < 0.05$); the absence of letters indicates that the pairwise comparisons within the time point were not statistically significant. P -values generated by repeated-measures ANOVA are provided for each hematological variable; values for glucose (mmol/L) are provided in brackets.

Hematological variable	Time (h)	Sedative					
		Eugenol	Benzocaine	CO ₂	MS-222	Electrosedation	
Cortisol (ng/mL)	0	74 \pm 37	148 \pm 69	25 \pm 15	132 \pm 27	51 \pm 36	
	0.5	482 \pm 187	224 \pm 19	273 \pm 45	255 \pm 92	304 \pm 93	
	Sedative $P = 0.057$	1	300 \pm 98	145 \pm 21	38 \pm 9	168 \pm 21	82 \pm 30
	Time $P < 0.001$	2	158 \pm 80	62 \pm 36	57 \pm 26	81 \pm 28	44 \pm 35
	Sedative \times time $P = 0.778$	6	104 \pm 48	2 \pm 2	2.8 ^a	13 \pm 5	12 \pm 5
Glucose (mg/dL) [mmol/L]	0	76 \pm 12 [4.2]	51 \pm 5 [2.8]	65 \pm 1 [3.6]	114 \pm 12 [6.3]	96 \pm 9 [5.3]	
	0.5	98 \pm 7 [5.4]	111 \pm 12 [6.2]	112 \pm 8 [6.2]	124 \pm 12 [6.9]	128 \pm 15 [7.1]	
	Sedative $P = 0.034$	1	119 \pm 6 [6.6]	117 \pm 2 [6.5]	141 \pm 17 [7.8]	157 \pm 7 [8.7]	178 \pm 12 [9.9]
	Time $P < 0.001$	2	130 \pm 8 [7.2]	123 \pm 7 [6.8]	157 \pm 41 [8.7]	149 \pm 14 [8.3]	143 \pm 32 [7.9]
	Sedative \times time $P = 0.276$	6	146 \pm 23 [8.1]	117 \pm 34 [6.5]	198 [11.0] ^a	110 \pm 17 [6.1]	131 \pm 12 [7.3]
Hematocrit (%)	0	59 \pm 1	67 \pm 3	54 \pm 3	44 \pm 17	52 \pm 5	
	0.5	47 \pm 5	51 \pm 1	61 \pm 1	50 \pm 5	49 \pm 3	
	Sedative $P = 0.052$	1	48 \pm 1	53 \pm 2	47 \pm 4	43 \pm 3	45 \pm 1
	Time $P < 0.001$	2	43 \pm 2	49 \pm 7	47 \pm 4	43 \pm 3	49 \pm 11
	Sedative \times time $P = 0.536$	6	44 \pm 3	33 \pm 2	36 ^a	37 \pm 2	30 \pm 4
Osmolality (mOsm/kg)	0	389 \pm 3	380 \pm 3	396 \pm 5	390 \pm 24	384 \pm 8	
	0.5	385 \pm 5	404 \pm 13	433 \pm 3	438 \pm 34	406 \pm 5	
	Sedative $P = 0.246$	1	386 \pm 20	384 \pm 5	386 \pm 4	410 \pm 9	408 \pm 3
	Time $P < 0.001$	2	373 \pm 6	362 \pm 2	404 \pm 14	390 \pm 14	395 \pm 22
	Sedative \times time $P = 0.371$	6	362 \pm 3	353 \pm 4	349 ^a	372 \pm 9	358 \pm 18
Lactate (mmol/L)	0	5.1 \pm 0.2	5.0 \pm 1.4	3.3 \pm 1.2	5.0 \pm 0.9	3.3 \pm 0.3	
	0.5	7.1 \pm 2.1 z	10.9 \pm 1.3 yz	17.2 \pm 0.2 y	12.3 \pm 1.6 yz	14.4 \pm 0.9 yz	
	Sedative $P = 0.016$	1	9.4 \pm 1.9	8.1 \pm 0.8	13.0 \pm 1.6	12.5 \pm 1.5	15.0 \pm 1.2
	Time $P < 0.001$	2	8.3 \pm 0.3 yz	7.8 \pm 0.7 z	13.9 \pm 2.7 yz	11.4 \pm 1.4 yz	16.5 \pm 1.4 y
	Sedative \times time $P = 0.001$	6	4.8 \pm 1.2	2.5 \pm 0.8	3 ^a	4.2 \pm 0.8	4.2 \pm 2.5

^aBecause of two mortalities occurring in this treatment group, values for time 6 are based on a single individual fish.

Madden and Houston (1976), who reported that electrosedation of rainbow trout *Oncorhynchus mykiss* was more rapid than chemical sedation and elicited fewer long-lasting physiological perturbations. Therefore, it was not unexpected that we saw a greater hematological response with eugenol, which was associated with some of the slowest induction times. However, such results were not consistently observed, as correspondingly exaggerated hematological changes were not observed among fish sedated with CO₂, which had the longest induction times of all sedatives evaluated. Further, electrosedation was not always associated with the mildest hematological responses, despite having markedly lower induction times compared with the other sedatives. Although differences in concentrations and induction times may explain variability in the responses of fish to a particular sedative, it does not appear to fully explain the differences between sedatives observed in the present work. Regardless, it would likely be beneficial to minimize total sedation, handling,

and recovery time with the intent of limiting any resulting stress response.

The hormonal response (corticosteroid and catecholamine release) to sedation or any other stressor is generally rapid and relatively short-lived, whereas the other alterations are slower to develop, but longer lasting (Mazeaud et al. 1977; Barton 2002). In some cases this is because the alterations are induced or upregulated by the surge in circulating corticosteroids or catecholamines and therefore occur after the hormonal response; other changes may not be directly related to stress hormone release but are nonetheless slower to develop for other reasons (Barton and Iwama 1991). Regardless of the underlying mechanisms, this pattern of rapid, transient endocrine response and slower, more persistent metabolic responses is consistent with the results we observed: peak cortisol levels were observed at 0.5 h postsedation, whereas maximal responses in glucose and, in some cases, lactate were not observed until 1–2 h after

TABLE 3. Summary of induction and recovery times and physiological responses to carbon dioxide, MS-222, eugenol and related compounds, and benzocaine used to sedate fish to stage II anesthesia, except where noted.

Taxon	Sedative and exposure	Response criteria			
		Induction time	Recovery time	Physiological alterations and responses	Reference
Carbon dioxide					
Rainbow trout <i>Oncorhynchus mykiss</i>	36.5–124.8 mm HgCO ₂ 442–642 mg/L NaHCO ₃ , 6.5–7.5 pH	~5–15 min 1.2–4.8 min	5–10 min	↑ Lactate, ↑ catecholamines, acid–base disturbance, fish struggled violently when exposed to sedative.	Bernier and Randall (1998) Booke et al. (1978)
Brook trout <i>Salvelinus fontinalis</i>	442–642 mg/L NaHCO ₃ , 6.5–7.5 pH	1.5–5.0 min	10 min		Booke et al. (1978)
Common carp <i>Cyprinus carpio</i>	442–2142 mg/L NaHCO ₃ , 5.0–7.5 pH	4.0–12.0 min	15–30 min		Booke et al. (1978)
Benzocaine					
Rainbow trout	35 mg/L	2.2–2.8 min	7.2–8.5 min		Gilderhus and Marking (1987)
	108 mg/L	1 min	10.5 min	↑ heart rate variability relative to clove oil and MS-222 following extended exposure.	Cotter and Rodnick (2006)
Clove oil					
Steelhead (anadromous rainbow trout)	40 mg/L	~3 min	3.5 min	↓ feed intake following exposure.	Pirhonen and Schreck (2003)
Rainbow trout	25 mg/L	0.8 min	10 min		Cotter and Rodnick (2006)
	100 mg/L	2.4 min (stage 3) Anesthesia recovery based on Ross and Ross (2008), “surgical anesthesia”	9.1 min	↑ Glucose 6 h after exposure.	Sattari et al. (2009)
Hybrid striped bass <i>Morone chrysops</i> × <i>M. saxatilis</i>	8 mg/L			↑ cortisol following 15–30 min of exposure, elevated glucose at 24 h postexposure, ↓ chloride at 2 h postexposure.	Davis and Griffin (2004)
Isoeugenol					
Rainbow trout	40–>80 mg/L	<2.2–2.2 min (stage 4)	6.2 min	↑ Cortisol at 24 h postexposure and handling, ↑ glucose up to 24 h postexposure and handling, ↓ chloride at 7 h postexposure and handling.	Wagner et al. (2002)

(Continued on next page)

TABLE 3. Continued.

Taxon	Sedative and exposure	Response criteria			
		Induction time	Recovery time	Physiological alterations and responses	Reference
	17 mg/L	5–10 min	20 min	↑ cortisol from 0–4 and 16–24 h postexposure, ↓ potassium up to 16 h postexposure, ↑ protein at 16 h postexposure, ↑ hematocrit up to 48 h postexposure.	Davidson et al. (2000)
Hybrid striped bass	3.6 mg/L			↑ Cortisol following 15–30 min of exposure, ↓ chloride at 2 h postexposure.	Davis and Griffin (2004)
Striped bass <i>Morone saxatilis</i>	25–45 mg/L	6–14.4 min (stage IV)	5.6–15.2 min (stage IV)	↑ Cortisol, particularly at lower isoeugenol concentrations.	Woods et al. (2008)
Chinook salmon <i>Oncorhynchus tshawytscha</i>	20 mg/L	<2 min (stage V)		↑ Glucose relative to MS-222 1 h postexposure, ↑ white blood cell count relative to MS-222 up to 6 h postexposure, ↑ lysozyme relative to control up to 48 h postexposure.	Cho and Heath (2000)
European eel <i>Anguilla anguilla</i>	25–75 mg/L	5 min (only stage I achieved)	1 min		Altun et al. (2006)
	50–75 mg/L	3–4 min (stage V)	7–30 min		Altun et al. (2006)
European eel		Eugenol			
	2,250 mg/L	5 min (only stage I achieved)	2–30 min		Altun et al. (2006)
	3,375–4,500 mg/L	2–3 min (stage V)	3–30 min		Altun et al. (2006)
Red pacu <i>Piaractus brachipomus</i>		MS-222			
	50 mg/L, buffered 1:1 with NaHCO ₃	Not induced within 10 min to stage IV		↑ Blood glucose, hematocrit, and hemoglobin; ↓ pH; ↑ pCO ₂ , ↓ pO ₂ ; 8 of 15 fish reacted to venipuncture.	Sladky et al. (2001)

TABLE 3. Continued.

Taxon	Sedative and exposure	Induction time	Response criteria		
			Recovery time	Physiological alterations and responses	Reference
	100 mg/L, buffered 1:1 with NaHCO ₃	~9.5 min to stage IV Anesthesia and recovery stages based on Stoskopf (1993); numerical estimates based on graphical data reporting	~5.3 min	↑ blood glucose, hematocrit, and hemoglobin; ↓ pH; ↑ pCO ₂ , ↓ pO ₂ ; 3 of 15 fish reacted to venipuncture.	Sladky et al. (2001)
	200 mg/L, buffered 1:1 with NaHCO ₃	~6.0 min to stage IV Anesthesia and recovery stages based on Stoskopf (1993); numerical estimates based on graphical data reporting	~7.6 min	↑ blood glucose, hematocrit, and hemoglobin; ↓ pH; ↑ pCO ₂ , ↓ pO ₂ ; 1 of 15 fish reacted to venipuncture.	Sladky et al. (2001)
Red drum <i>Sciaenops ocellatus</i>	40–70 mg/L	23–100% to stage 4 within 3 min Anesthesia and recovery stages based on Mattson and Ripley (1989)	77–100% recovery within 10 min		Massee et al. (1995)
Goldfish <i>Carassius auratus</i>	50–90 mg/L	63–100% to stage 4 within 3 min	97–100% recovery within 10 min		Massee et al. (1995)
Hybrid striped bass	25 mg/L			↑ Cortisol following 15–30 min of exposure, ↓ chloride up to 2 h postexposure.	Davis and Griffin (2004)
Electrosedation					
Rainbow trout	AC waveform, 91-s exposure	1.4 min (stage 3) Anesthesia and recovery based on Ross and Ross (2008), “surgical anesthesia”	0.9 min (stage 3)	↑ Glucose 6 h following exposure.	Sattari et al. (2009)
Siberian sturgeon <i>Acipenser baeri</i>	DC waveform, variable voltage and exposure time	1.1–1.6 min	0 min	Alterations in plasma K ⁺ and Mg ²⁺ , Ca ²⁺ , Na ⁺ , and Cl ⁻ , ↓ pH.	Feng et al. (2009)

sedation or later. Zahl et al. (2010) observed a similar corticosteroid response in Atlantic salmon *Salmo salar*, Atlantic cod *Gadus morhua*, and Atlantic halibut *Hippoglossus hippoglossus* exposed to MS-222, benzocaine, metomidate, and isoeugenol: sedation with these compounds, without handling or other stres-

or exposure, resulted in cortisol release into circulation peaking approximately 0.5 h postexposure and returning to basal levels within 6 h. Though plasma glucose and lactate levels continued to rise throughout sedation in American eel *Anguilla rostrata* sedated with MS-222 (Cornish and Moon 1986), these

fish were exposed to the sedatives throughout the monitoring period in an approach mimicking chronic rather than acute exposure. Regardless of these temporal differences in hormonal versus metabolic indicators of the stress response, each of the response features we evaluated returned to resting levels within 6 h of sedation, with the exception of plasma glucose, which remained elevated. Given that acute stressors, including short-term exposure to chemical sedatives, can elicit responses lasting well beyond 6 h (Soivio et al. 1977; Davis and Griffin 2004), it would seem that the sedatives we evaluated were relatively mild stressors at the concentrations or strengths used.

Three mortalities were observed throughout the course of our study, including two that were associated with electro-sedation. These two mortalities represented approximately 8% of the fish that were electro-sedated, and this level of mortality would probably be considered unacceptable to most fisheries professionals. However, our study is the first to evaluate electro-sedation in hybrid striped bass and selection of the waveform we used was somewhat arbitrary. Additional evaluations are needed to identify optimal waveforms for hybrid striped bass, as well as for other fish species. None of the sedative options evaluated induced a stress response severe enough to cause hyperosmoregulatory failure, changes in hematocrit, or other more pronounced effects of stressor exposure. Although slight differences in hematological responses were observed among the sedatives, there was little evidence to suggest that one sedative option was clearly better than the others in terms of minimizing overall hematological disturbance following sedation. Thus, despite different modes of action, the primary distinctions between the sedatives were related to induction and recovery times and ease of use. In this sense, electro-sedation may be a suitable tool for quickly inducing sedation in hybrid striped bass. However, all of the sedative options evaluated were effective in sedating fish within reasonable time frames at the doses or strengths used. However, induction and maintenance of sedation for a longer period of time (e.g., to facilitate surgical procedures) would require a different approach from those we evaluated in the present work. Longer procedures could be facilitated by modified approaches to chemical (e.g., flushing sedative-treated water across the gill) or electro-sedation (e.g., continuous rather than pulsed DC); further research to validate and optimize these approaches is warranted. The most appropriate sedative to use will depend on the fish to be sedated, the setting, as well as general usage patterns. Although the electro-sedation unit evaluated in the present work represents a significant one-time investment, there are essentially no expendable commodity costs and presumably limited maintenance costs associated with the unit. For fisheries professionals routinely sedating large numbers of fish, electro-sedation may be a cost-effective option, particularly in field settings where immediate-release and sedative bath disposal may be concerns. Conversely, chemical sedatives may be more appropriate for individuals sedating small numbers of fish, particularly in laboratory or hatchery settings where fish can be maintained for appropriate withdrawal times before release and

chemical disposal is more easily accomplished. Although all of the sedatives we evaluated were effective, their attributes may make some better suited to certain applications than others.

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REFERENCES

- Altun, T., A. Ö. Hunt, and F. Usta. 2006. Effects of clove oil and eugenol on anaesthesia and some hematological parameters of European eel *Anguilla anguilla*, L. 1758. *Journal of Applied Animal Research* 30:171–176.
- Barton, B. A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology* 42:571–525.
- Barton, B. A., and W. P. Dwyer. 1997. Physiological effects of continuous- and pulsed-DC electroshock on juvenile bull trout. *Journal of Fish Biology* 51:998–1008.
- Barton, B. A., and G. K. Iwama. 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, N. J., and D. J. Randall. 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.
- Bolasina, S. N. 2006. Cortisol and hematological response in Brazilian codling, *Urophycis brasiliensis* (Pisces, Phycidae) subjected to anesthetic treatment. *Aquaculture International* 14:569–575.
- Booke, H. E., B. Hollender, and G. Lutterbie. 1978. Sodium bicarbonate, an inexpensive fish anesthetic for field use. *The Progressive Fish-Culturist* 40: 11–13.
- Bourne, P. K. 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.
- Chiba, H., T. Hattori, H. Yamada, and M. Iwata. 2006. Comparison of the effects of chemical anesthesia and electroanesthesia on plasma cortisol levels in the Japanese eel *Anguilla japonica*. *Fisheries Science* 72:693–695.
- Cho, G. K., and D. D. Heath. 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.
- 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.
- Cornish, I. M. E., and T. W. Moon. 1986. The glucose and lactate kinetics of American eels, *Anguilla rostrata* (LeSueur), under MS 222 anaesthesia. *Journal of Fish Biology* 28:1–8.
- 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* 145A:158–165.
- Davidson, G. W., P. S. Davie, G. Young, and R. T. Fowler. 2000. Physiological responses of rainbow trout *Oncorhynchus mykiss* to crowding and anesthesia with AQUI-S™. *Journal of the World Aquaculture Society* 31: 105–114.
- Davis, K. B., and B. R. Griffin. 2004. Physiological responses of hybrid striped bass under sedation by several anesthetics. *Aquaculture* 233:531–548.

- Davis, M. W. 2010. Fish stress and mortality can be predicted using reflex impairment. *Fish and Fisheries* 11:1–11.
- Delaney, M. A., P. H. Klesius, and R. A. Shelby. 2005. Cortisol response of Nile tilapia, *Oreochromis niloticus* (L.), to temperature changes. *Journal of Applied Aquaculture* 16:95–104.
- Feng, G. P., P. Zhuang, L. Z. Zhang, N. N. Chen, Z. F. Yao, and Y. Y. Men. 2009. Effects of electroanesthesia on behavior and serum iron concentration of juvenile *Acipenser baeri*. *Marine Fisheries* [online serial] 1. DOI: CNKI:SUN:HTYY.0.2009-01-005.
- Finstad, B., M. Iversen, and R. Sandodden. 2003. Stress-reducing methods for releases of Atlantic salmon (*Salmo salar*) smolts in Norway. *Aquaculture* 222:203–214.
- Frazier, D. T., and T. Narahashi. 1975. Tricaine (MS-222): effects on ionic conductances of squid axon membranes. *European Journal of Pharmacology* 33:313–317.
- 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.
- Iversen, M., B. Finstad, R. S. McKinley, and R. A. Eliassen. 2003. The efficacy of metomidate, clove oil, AQUI-STM and Benzoak[®] as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. *Aquaculture* 221:549–566.
- King, W., B. Hooper, S. Hills Grove, C. Benton, and D. L. Berlinsky. 2005. The use of clove oil, metomidate, tricaine methanesulphonate and 2-phenoxyethanol for inducing anaesthesia and their effects on the cortisol stress response in black sea bass (*Centropristis striata* L.). *Aquaculture Research* 36:1442–1449.
- Kohler, C. C. 2000. Striped bass and hybrid striped bass culture. Pages 898–907 in R. R. Stickney, editor. *Encyclopedia of aquaculture*. Wiley, New York.
- Kozam, G. 1977. The effect of eugenol on nerve transmission. *Oral Surgery, Oral Medicine, Oral Pathology* 44:799–805.
- Lemm, C. A. 1993. Evaluation of five anesthetics on striped bass. U.S. Fish and Wildlife Service Resource Publication 196.
- Madden, J. A., and A. H. Houston. 1976. Use of electroanaesthesia with freshwater teleosts: some physiological consequences in the rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology* 9:457–462.
- Massee, K. C., M. B. Rust, R. W. Hardy, and R. R. Stickney. 1995. The effectiveness of tricaine, quinaldine sulfate and metomidate as anesthetics for larval fish. *Aquaculture* 134:351–359.
- Mattson, N. S., and T. H. Riple. 1989. Metomidate, a better anesthetic for cod (*Gadus morhua*) in comparison with benzocaine, MS-222, chlorobutanol, and phenoxyethanol. *Aquaculture* 83:89–94.
- Mazeaud, M. M., F. Mazeaud, and E. M. Donaldson. 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.
- Neumcke, B., W. Schwarz, and R. Stämpfli. 1981. Block of Na channels in the membrane of myelinated nerve by benzocaine. *European Journal of Physiology* 390:230–236.
- Olsen, Y. A., I. Einarsdottir, and K. J. Nilssen. 1995. Metomidate anaesthesia in Atlantic salmon, *Salmo salar*, prevents plasma cortisol increase during stress. *Aquaculture* 134:155–168.
- Owen, M. A. G., S. J. Davies, and K. A. Sloman. 2010. Light colour influences the behaviour and stress physiology of captive tench (*Tinca tinca*). *Reviews in Fish Biology and Fisheries* 20:375–380.
- Palić, D., D. M. Herolt, C. B. Andreasen, B. W. Menzel, and J. A. Roth. 2006. Anesthetic efficacy of tricaine methanesulfonate, metomidate and eugenol: effects on plasma cortisol concentration and neutrophil function in fathead minnows (*Pimephales promelas* Rafinesque, 1820). *Aquaculture* 254:675–685.
- Pirhonen, J., and C. B. Schreck. 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.
- Post, G. 1979. Carbonic acid anesthesia for aquatic organisms. *Progressive Fish-Culturist* 41:142–144.
- Romero, L. M., M. J. Dickens, and N. E. Cyr. 2009. The reactive scope model—a new model integrating homeostasis, allostasis, and stress. *Hormones and Behavior* 55:375–389.
- Ross, L. G., and B. Ross. 2008. *Anaesthetic and sedative techniques for aquatic animals*, 3rd edition. Blackwell Scientific Publications, Oxford, UK.
- Sandodden, R., B. Finstad, and M. Iversen. 2001. Transport stress in Atlantic salmon (*Salmo salar* L.): anaesthesia and recovery. *Aquaculture Research* 32:87–90.
- Sattari, A., S. Mirzargar, A. Abrishamifar, R. Lourakzadegan, A. Bahonar, H. E. Mousavi, and A. Niasari. 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* 1950(1):1383–1392.
- Sepici-Dinçel, A., A. Çağlan Karasu Benli, M. Selvi, R. Sarikaya, D. Şahin, I. Ayhan Özkul, and F. Erkoç. 2009. Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio* L.) fingerlings: biochemical, hematological, histopathological alterations. *Ecotoxicology and Environmental Safety* 72:1433–1439.
- Sladky, K. K., C. R. Swanson, M. K. Stoskopf, M. R. Loomis, and G. A. Lewbart. 2001. Comparative efficacy of tricaine methanesulphonate and clove oil for use as anesthetics in red pacu (*Piaractus brachyomus*). *American Journal of Veterinary Research* 62:337–342.
- Small, B. C. 2004. Effect of isoleugenol sedation on plasma cortisol, glucose, and lactate dynamics in channel catfish *Ictalurus punctatus* exposed to three stressors. *Aquaculture* 238:469–481.
- Smit, G. L., J. Hattingh, and A. P. Burger. 1979. Haematological assessment of the effects of the anaesthetic MS-222 in natural and neutralized form in three freshwater fish species: interspecies differences. *Journal of Fish Biology* 15:633–643.
- Soivio, A., K. Nyholm, and M. Huhti. 1977. Effects of anaesthesia with MS 222, neutralized MS 222 and benzocaine on the blood constituents of rainbow trout, *Salmo gairdneri*. *Journal of Fish Biology* 10:91–101.
- Stoskopf, M. K. 1993. Clinical pathology. Pages 113–131 in M. Stoskopf, editor. *Fish medicine*. Saunders, Philadelphia.
- 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.
- Venn Beecham, R., B. C. Small, and C. D. Minchew. 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., R. Arndt, and B. Hilton. 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.
- Wagner, G. N., T. D. Singer, and R. S. McKinley. 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquaculture Research* 34:1139–1146.
- Wells, R. M. G., and N. W. Pankhurst. 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, L. C., I. D. D. Theisen, and S. He. 2008. Efficacy of AQUI-S as an anesthetic for market-sized striped bass. *North American Journal of Aquaculture* 70:219–222.
- Zahl, I. H., A. Kiessling, O. B. Samuelsen, and R. E. Olsen. 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.
- Zydlewski, G. B., W. Gale, J. Holmes, J. Johnson, T. Brigham, and W. Thorson. 2008. Use of electroshock for euthanizing and immobilizing adult spring Chinook salmon in a hatchery. *North American Journal of Aquaculture* 70: 415–424.