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North American Journal of Aquaculture

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/unaj20>

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Available online: 11 Apr 2012

To cite this article: Jesse T. Trushenski, James D. Bowker, Bonnie L. Mulligan & Brian R. Gause (2012): Induction, Recovery, and Hematological Responses of Largemouth Bass to Chemo- and Electroshock, North American Journal of Aquaculture, 74:2, 214-223

To link to this article: <http://dx.doi.org/10.1080/15222055.2012.675990>

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ARTICLE

Induction, Recovery, and Hematological Responses of Largemouth Bass to Chemo- and Electro sedation

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Abstract

Sedating fish before handling minimizes the risk of injury to both fish and handler and may also minimize the fish's stress response. We conducted two experiments to quantitatively compare induction and recovery times of largemouth bass *Micropterus salmoides* sedated with tricaine methanesulfonate (MS-222), eugenol, benzocaine, carbon dioxide (CO₂), or electro sedation (pulsed DC). We also assessed the fish's hematological profile following sedation with MS-222, eugenol, and electro sedation. Induction times varied significantly among the sedatives evaluated; electro sedation yielded the fastest inductions (0.2 ± 0.1 min; mean \pm SE) and CO₂ yielded the slowest (3.6 ± 0.1 min). Times to recovery of equilibrium and responsiveness to tactile and visual–auditory stimuli also varied, ranging from 1.8 ± 0.3 to 3.7 ± 0.3 min and from 2.3 ± 0.3 to 4.0 ± 0.3 min, respectively, depending on the sedative used. Plasma cortisol concentrations were elevated at 0.5 h post sedation among fish sedated with eugenol and MS-222, whereas cortisol levels of electro sedated fish were comparatively low and stable throughout the experiment. Conversely, plasma glucose and lactate levels increased markedly from 0.5 to 2 h post sedation among electro sedated fish, whereas the responses among fish treated with eugenol or MS-222 were weak or negligible. Our results indicate that electro sedation, benzocaine, eugenol, and MS-222 are all effective in quickly sedating largemouth bass. Physiological and behavioral data suggest that largemouth bass generally recover within 6 h of sedation using MS-222, eugenol, or electro sedation.

Fisheries professionals, including researchers, managers, and culturists, need access to safe and effective options to sedate or anesthetize fish for a variety of purposes ranging from simple handling to invasive surgical procedures. The terms “anesthesia” and “sedation” are used somewhat interchangeably with respect to fish, largely because the compounds used to restrain fish can act as sedatives (causing “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,” Ross and Ross 2008) or anesthetics (causing “a reversible, generalized loss of sensory perception accompanied by a sleep-like state induced by drugs or by physical means,” Ross and Ross 2008) depending on the dosage and exposure time. Fish are innately difficult to handle, cannot be restrained in the same manner as terrestrial animals without causing physical damage, and are therefore prone to epithelial damage or other physical injury if restrained without proper sedation. Sedating fish before handling minimizes the

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Received April 28, 2011; accepted November 5, 2011

risk of injury to both fish and handler and may also minimize the fish's stress response. Stress is defined as a natural reaction to a negative environmental stimulus and is often associated with the "fight or flight" response (Selye 1950). During or after a stress response, energy is diverted to support fight or flight and important, but noncritical, processes are often suppressed. As a result, stressed individuals may suffer longer-term consequences including increased vulnerability to disease, reduced reproductive performance, and reduced growth (Barton 2002). Proper use of sedatives may attenuate the primary stress response and, in turn, minimize the occurrence of negative consequences after handling.

Currently, there are two sedative options readily available to fisheries professionals: tricaine methanesulfonate and carbon dioxide (CO₂). Tricaine methanesulfonate (commonly referred to MS-222) products (99.5% tricaine methanesulfonate; Finquel, Argent Laboratories, Redmond, Washington, and Tricaine-S, Western Chemical, Ferndale, Washington) are approved by the U.S. Food and Drug Administration (FDA) to temporarily immobilize fish. However, use of these products is restricted to four families of fish (Ictaluridae, Salmonidae, Esocidae, and Percidae) or other laboratory or hatchery fishes at water temperatures greater than 10°C, and users must adhere to a 21-d withdrawal period. The sedative action of MS-222 is related to its ability to prevent generation and conduction of nervous impulses, similar to many other local anesthetics (Frazier and Narahashi 1975). Carbon dioxide is considered by FDA to be an unapproved drug of low regulatory priority and can be used as an "immediate-release" sedative (no withdrawal period) for fish, provided that certain criteria are met regarding the grade and purity of the drug and the intended use pattern (FDA 2011). The sedative mode of action for CO₂ is based on the ability of high environmental concentrations to slow or reverse excretion at the gill, causing CO₂ to build within the central nervous system and other tissues. Gradually, widespread central nervous system depression occurs, resulting in the loss of consciousness and voluntary motor function. There are several additional compounds currently being investigated for use as immediate-release fish sedatives, including benzocaine and eugenol. The sedative mode of action for both of these compounds is associated with their ability to interfere with changes in membrane permeability necessary to conduct nervous stimuli. Currently, Benzoak (20% benzocaine, Frontier Scientific Laboratories, Logan Utah) and AQUI-SE (50% eugenol) or AQUI-S 20E (10% eugenol; AQUI-S New Zealand, Lower Hutt, New Zealand) can be legally used as unapproved drugs under the U.S. Fish and Wildlife Service (USFWS) compassionate Investigational New Animal Drug (INAD) exemption. However, there is a 3-d withdrawal period associated with use of either compound under these INAD exemptions. There are pros and cons associated with use of each of these chemosedatives, including approval status (approved drug versus low regulatory priority drug versus INAD status), allowable use patterns (immediate-release versus 3-d withdrawal period versus 21-d withdrawal period),

disposal considerations, cost, ease of use, safety to user and fish, and efficacy. Another option for temporarily immobilizing fishes is the use of electricity. Electrofishing is a common scientific survey method that has been used for decades to sample fish populations to determine abundance, density, and species composition. When performed correctly, electrofishing results in no permanent harm to most fish species (some "finely boned" taxa may be more prone to injury than others), which return to their natural state in as little as 2 min after being stunned. More recently, this approach has been modified specifically for sedating or anesthetizing individual fish. "Electroanesthesia," or more accurately, electrosedation, immobilizes fish by interfering with neurotransmission and causing electronarcosis (stunning) or electrotetany (tetanic muscle contraction). Electrodesation may offer several advantages over chemosedation in terms of withdrawal periods, chemical disposal, and potentially, ease of use. In addition, use of electrodesation equipment to immobilize fish is not regulated by FDA as a drug, and its legal use by fisheries professionals would not be contingent upon generating myriad data sets to demonstrate safety and effectiveness to support approval by FDA.

Many sedatives frequently used by fisheries professionals (e.g., clove oil, metomidate, MS-222, CO₂) have been well researched (Gilderhus and Marking 1987; Mattson and Riple 1989; Hseu et al. 1998; Iversen et al. 2003; Small 2003; Davis and Griffin 2004; Weber et al. 2009). However, not all of these compounds are necessarily legal for such uses and few comprehensive studies have been conducted to compare chemical and electrical sedative options in terms of their efficacy or physiological effects (Trushenski et al. 2012). Therefore, we conducted two experiments to quantitatively compare induction and recovery times of fish sedated with MS-222 (Finquel), eugenol (AQUI-SE), benzocaine (Benzoak), CO₂, or electrodesation (pulsed DC). We also assessed the fish's hematological profile following sedation with MS-222, eugenol, and electrodesation. Largemouth bass *Micropterus salmoides* were selected as a model fish because they are a popular sport and food fish, a fish species commonly reared by natural resource agencies (Halverson 2008), and broadly considered a representative warmwater finfish.

METHODS

As indicated above, the various terms used to describe chemically or physically induced restraint are often used interchangeably with respect to fish. Arguably, none of these terms perfectly describe the processes we investigated in the present study, particularly with respect to electrodesation. However, we use the terms "electrodesation" and "sedatives" herein to best reflect our behavioral observations and the current understanding of the sedation and anesthesia in fish.

Experiment 1: induction and recovery times.—Individual largemouth bass (508 ± 20 g, 31.8 ± 0.4 cm total length [TL], mean \pm SE) were transferred from holding tanks in a

recirculating aquaculture system and placed into a sedation chamber filled with 70 L of culture water and either dosed with a chemosedative (114-L cooler, water depth of ~10 cm) or equipped with the electrosedation unit (142-L cooler, water depth of ~8 cm). Fish were the experimental unit (sedated once and not reused) and were individually sedated in one of the following five sedative regimens listed below:

1. CO₂: approximately 400 mg/L solutions prepared according to the sodium bicarbonate–sulfuric acid method described by Post (1979) (concentration analytically verified as 384 mg/L; digital titrator and reagents, Hach, Loveland, Colorado)
2. Benzocaine: 150 mg/L benzocaine (750 mg/L Benzoak)
3. Eugenol: 60 mg/L eugenol (120 mg/L solution of AQUI-SE)
4. MS-222: 150 mg/L tricaine methanesulfonate (150 mg/L Fiquel)
5. Electrodesation: pulsed DC (100 V, 30 Hz, 25% duty cycle, 3-s exposure) delivered via a 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 the addition of the chemosedatives or during use. Sufficient volumes of culture water were treated with benzocaine, eugenol, and MS-222 to allow for sedative baths to be exchanged from a single stock source after five fish had been exposed to each sedative in the sedative chamber; culture water was similarly exchanged after five fish had been treated in the electrodesation chamber. In the case of CO₂, sedative baths were also exchanged after treating five fish; however, each bath was individually prepared

immediately before use to minimize the loss of volatile CO₂. Water samples were collected from the sedative baths before and after use, and the composite samples were analyzed in duplicate along with water samples collected from the holding system. Conductivity, pH, salinity (Multi-Parameter PCSTestr 35, Eutech Instruments, Oakton, Vernon Hills, Illinois), hardness, alkalinity (digital titrator and reagents, Hach), total ammonia nitrogen, nitrite-nitrogen, and nitrate-nitrogen (Hach DR 2800 spectrophotometer and reagents) were measured and maintained within ranges appropriate for largemouth bass (Tidwell et al. 2000) throughout the experiment (Table 1). Dissolved oxygen readings were taken periodically during the experiments; however, an equipment malfunction resulting in inaccurate measurements was subsequently discovered. Although dissolved oxygen values are not available, the recirculation systems used were operating normally during the course of experiments 1 and 2, and dissolved oxygen readings typically exceed 5 mg/L for these systems when under similar biological oxygen demand (J. Trushenski, unpublished data).

Each fish was monitored during sedation to determine induction to Stage IV of sedation (Summerfelt and Smith 1990; “anesthesia” is the term used by these authors). 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 challenged with tactile stimuli (slight manual dorso-ventral compression, tactile stimulation of ocular–opercular area). Fish were considered induced to Stage IV when they no longer responded to this stimulus, but the opercular rate remained steady

TABLE 1. Water quality variables measured in experiments 1 and 2. Values represent the means of composite samples analyzed in duplicate.

Variable	Experiment	Holding system	Sedative treatment				
			Eugenol	Benzocaine	CO ₂	MS-222	Electrodesation
Temperature (°C)	1	21.2	21.3	21.2	21.4	21.1	21.5
	2	19.9	20.5			19.5	19.4
Total ammonia nitrogen (mg/L)	1	0.1	1.2	0.5	0.2	0.2	0.2
	2	0.5	0.9			0.4	0.4
Nitrite-nitrogen (mg/L)	1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1
	2	<0.1	<0.1			<0.1	0.1
Nitrate-nitrogen (mg/L)	1	7.0	8.4	8.0	8.4	8.4	7.9
	2	4.2	4.0			4.0	4.6
Alkalinity (mg/L)	1	494	480	498	494	460	512
	2	198	226			180	196
Hardness (mg/L)	1	58	62	59	56	61	59
	2	80	62			60	60
Salinity (‰)	1	3.6	3.6	3.6	4.1	3.6	3.5
	2	4.2	4.2			3.7	3.7
Conductivity (mS/cm)	1	6.6	6.7	6.6	7.4	6.6	6.5
	2	7.7	7.7			6.9	6.9
pH	1	8.9	8.9	8.9	6.0	8.6	8.9
	2	8.5	8.5			7.7	8.6

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 sedation), induction was considered complete after the tremor had ceased. After induction, fish were weighed (to the nearest 0.1 g), measured to determine TL (to the nearest 0.5 cm), and then transferred to an aerated recovery tank linked to the holding system. In the recovery tank, fish were monitored to determine recovery of normal equilibrium. To assess responsiveness to visual–auditory stimuli, a hand was placed gently over the fish's head to block vision and apply gentle tactile stimulation of the eye and opercular areas (noninvasive, but irritating and elicits avoidance behavior in nonsedated largemouth bass). When fish exhibited avoidance behavior to this stimulus, they were considered fully recovered. Recovered fish were returned to a holding system and survival was monitored for 24 h. Since assessment of induction and recovery can be somewhat subjective, bias was minimized by having the same observer apply all stimuli and make assessments under the supervision of two additional observers.

Experiment 2: hematological responses to sedation.—Sedative baths were prepared as previously described, but because of limited numbers of fish available for experiment 2, only electrosedation, MS-222, and eugenol were evaluated in experiment 2. Based on the lack of water chemistry changes observed during the course of experiment 1, a single bath was prepared and used throughout the experiment for all sedatives. Composite water samples were collected and analyzed as described for experiment 1. Although a different recirculation system was used for holding and recovering fish for experiment 2, most aspects of water chemistry did not vary considerably between the experiments (Table 1). The largest differences in water chemistry noted were associated with different levels of nitrate-nitrogen and alkalinity (both lower in experiment 2 owing to a greater water exchange rate in this system, though all measured values were within ranges suitable for largemouth bass culture and, in the case of alkalinity, sufficient to yield adequate buffering capacity in the system). Although water temperature and chemistry are known to influence the response to sedation in fishes (Cherkin and Catchpool 1964; Belaud et al. 1977; Sylvester and Holland 1982; Woolsey et al. 2004), the differences observed between experiments 1 and 2 were relatively minor, and it was assumed that the responses observed from one experiment to the next would be reasonably similar. This assumption was borne out, in that the mean group induction times (time for the last fish in the group to become induced to Stage IV) recorded for MS-222, eugenol, and electrosedation in experiment 2 ranged from 96% to 135% of the mean times recorded for these sedatives in experiment 1 (data not shown).

An experimental unit consisted of a group of five fish (492 ± 20 g, 31.9 ± 0.4 cm TL) that were transferred from holding tanks in a recirculating aquaculture system, placed into the sedation chamber, and sedated en masse using one of the sedative

regimens as previously described for experiment 1. Each sedative regimen was tested in triplicate. Immediately after all fish had reached Stage IV sedation, one fish was sampled (see below) while the others were returned to a holding tank in the holding system for subsequent sampling at 0.5, 1, 2, and 6 h postsedation (one fish per group per timepoint, individuals were only sampled once). For qualitative comparison purposes, one fish from the reference population was sampled every hour during the course of experiment 2. Before sampling, all fish (including those sampled immediately following sedation) were immersed in a bath of metomidate hydrochloride (Aquacalm, Western Chemical, Ferndale, Washington; $\sim 3\text{--}5$ mg/L for ~ 30 s) to facilitate handling. Metomidate hydrochloride blocks corticosteroid synthesis (Olsen et al. 1995; Davis and Griffin 2004) and is therefore particularly useful in minimizing the effects of handling and sample collection on circulating cortisol levels. After exposure to the metomidate hydrochloride bath, fish length and weight were measured, and a blood sample was collected from the caudal vasculature by using heparinized, evacuated blood collection assemblies (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey). Although metomidate hydrochloride was used to prevent a corticosteroid increase during sampling, all blood samples were collected within 5 min of capture to minimize the possibility of other confounding responses of handling and venipuncture. After blood collection, fish were placed into an adjacent recirculation system (similar water temperature and quality) and monitored for 48 h for survival.

Blood samples were kept on wet ice (<6 h) until analysis. Subsamples of whole blood were used for the determination of hematocrit (Statspin centrifuge, Fisher Scientific, Pittsburgh, Pennsylvania) and glucose (Freestyle Freedom Lite glucose meter, Abbott Laboratories, Abbott Park, Illinois). Whole blood samples were then centrifuged 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 (EIA kit 1887, DRG International, Mountainside, New Jersey). Although portable lactate and glucose meters 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; Venn Beecham et al. 2006) and are commonly used in fisheries research because of their ease of use. 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; Owen et al. 2009; Sepici-Dinçel et al. 2009).

Statistical analyses.—Induction and recovery times 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

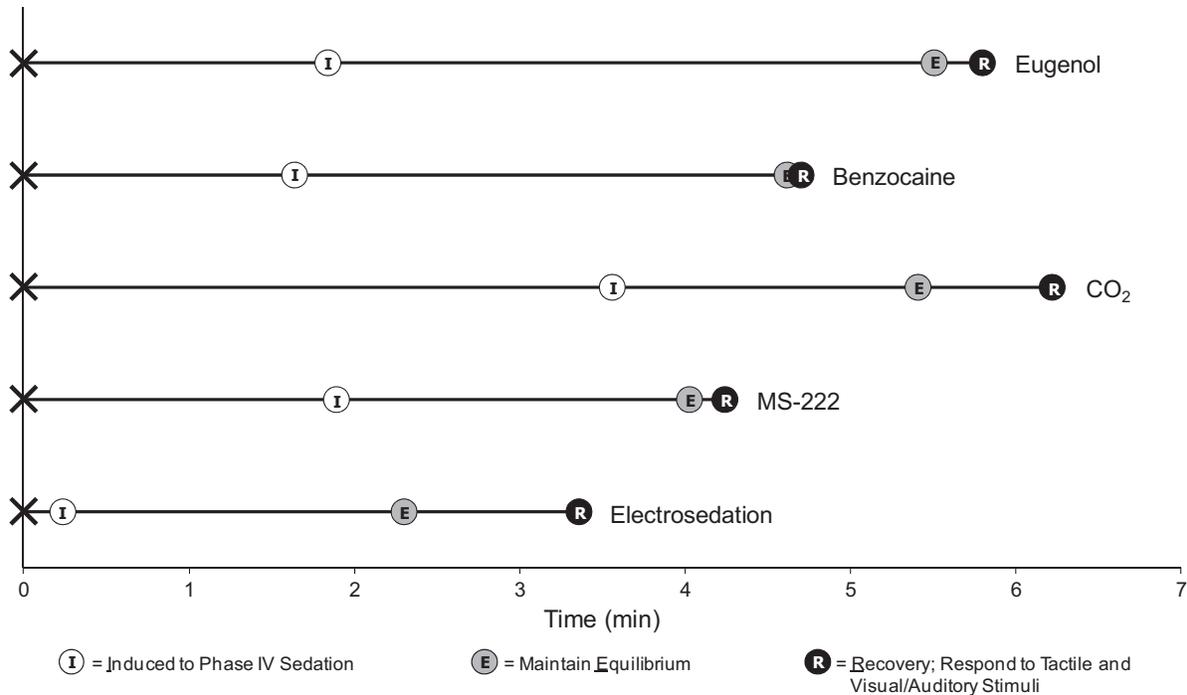


FIGURE 1. Schematic illustrating induction and various stages of recovery of largemouth bass sedated to Stage IV of sedation with various chemical sedatives or electrodesation. Significant differences in the timing of induction and recovery events were noted as follows, with significant differences among means indicated by different letters in parentheses. Induction: electrodesation (z) < benzocaine (y) < eugenol (y) < MS-222 (y) < CO₂ (x). Recovery of equilibrium: CO₂ (z) < electrodesation (yz) < MS-222 (yz) < benzocaine (xy) < eugenol (x). Recovery of responsiveness to tactile and visual–auditory stimuli: MS-222 (z) < CO₂ (z) < benzocaine (yz) < electrodesation (yz) < eugenol (y).

effects, post hoc Tukey's honestly significant difference (HSD) tests were used for pairwise comparisons of means. For experiment 1 data analysis, individual fish were considered experimental units ($n = 10$). Hematological data from experiment 2 were analyzed by one-way, repeated measures ANOVA (PROC MIXED) with the Statistical Analysis System. For experiment 2 data analysis, replicate groups were considered experimental units ($n = 3$). Although each sedative was applied to triplicate groups, each composed of five fish, we determined that groups, not individuals, should serve as experimental units. By definition, experimental units represent independent observations. Given that the presence or position of other fish within the sedation chamber could alter the waveform applied to electrodesated fish or, in general, affect the behavior of fish during sedation, we determined that individuals sedated within the same group could not be considered independent of one another. Thus, to maintain a reasonably conservative statistical approach, for each statistical procedure, sedation group was used as the level of replication ($n = 3$). Thus, fish sampled at each time point represented repeated observations made on the same experimental unit (i.e., sedation group or tank). Accordingly, physiological data from experiment 2 were analyzed by using the repeated measures approach described. Physiological data collected from fish sampled from the reference population lacked true replication and were not included in the statistical analysis; mean values are

provided for the purposes of general comparison only. For variables exhibiting significant treatment effects, treatment means were compared at individual time points with post hoc Tukey's HSD tests for pairwise comparisons. In all cases, differences were considered significant at $P < 0.05$.

RESULTS

Induction times (Table 1; Figure 1) varied significantly among the sedatives evaluated. Briefly, mean induction time using electrodesation was 0.2 ± 0.1 min (mean \pm SE), mean induction time for eugenol, MS-222, and benzocaine ranged from 1.6 to 1.9 ± 0.1 min, and mean induction time for CO₂ was 3.6 ± 0.1 min. Recovery (Table 1; Figure 1) of equilibrium and responsiveness to tactile and visual–auditory stimuli also varied significantly among the sedative treatments. Mean times to regain equilibrium and tactile–visual–auditory responsiveness, respectively, after induction were 2.1 ± 0.3 and 2.3 ± 0.3 min postinduction for MS-222, 1.8 ± 0.3 and 2.7 ± 0.3 min for CO₂, 3.0 ± 0.3 and 3.1 ± 0.3 min for benzocaine, 2.1 ± 0.3 and 3.1 ± 0.3 min for electrodesation, and 3.7 ± 0.3 and 4.0 ± 0.3 min for eugenol (Figure 1). Total handling time from the beginning of sedative exposure to full recovery was 3.4 ± 0.3 min for electrodesation, 4.2 ± 0.3 min for MS-222,

4.7 ± 0.3 min for benzocaine, 5.8 ± 0.3 for eugenol, and 6.2 ± 0.3 min for CO₂ (Figure 1).

Hematology also varied significantly among the sedatives evaluated and over time (Figure 2). Plasma cortisol concentrations were elevated at 0.5 h postsedation and again at 6 h postsedation among fish sedated with eugenol and MS-222 compared with the reference population, whereas cortisol levels of electrosedated fish were comparatively low and stable throughout the sampling period. Mean cortisol concentration for the reference population was relatively high (86 ng/mL) as a result of two individual fish with cortisol levels above 248 ng/mL; all other values for the reference population were 0–89 ng/mL. Excluding these two apparent outliers, mean cortisol concentration was more consistent with expectations for unstressed fish and the rest of the data set; thus, this is the value reported for the reference population (Figure 2A). The opposite pattern was observed for plasma glucose in that values increased at 0.5 h postsedation among electrosedated fish and remained elevated throughout the sampling period compared with the reference population, whereas fish sedated with eugenol or MS-222 exhibited a relatively weak glucose response (Figure 2B). Response patterns in plasma lactate were somewhat similar to glucose; whereas lactate levels increased markedly from 0.5 to 2 h postsedation among electrosedated fish, the corresponding response among fish treated with eugenol or MS-222 was weak or negligible (Figure 2E). Hematocrit decreased during the course of the sampling period; however, no differences were observed among the sedatives (Figure 2C). Plasma osmolality did not vary significantly by sedative treatment or over time (Figure 2D).

During the course of the two experiments involving sedation and handling of 95 individuals (excluding untreated fish sampled from the reference population), no mortalities were observed.

DISCUSSION

Results from this study indicate that electrosedation, benzocaine, eugenol, and MS-222 are all effective in sedating largemouth bass to Stage IV sedation in less than 2 min at the doses-strengths evaluated, and in less than 4 min at the CO₂ concentration tested. Once sedated, all fish recover within 4 min. Total handling time, from initial sedative exposure through induction and recovery, ranged from 3.4 to 6.2 min for all treatments. Electrodesation yielded a faster induction time than any of the chemical sedatives evaluated, but yielded one of the slower recovery times. It is likely that faster induction times would have been observed with the chemical sedatives had greater concentrations been used. However, sedating fish to the desired endpoint with higher concentrations of a chemical sedative can result in a longer recovery period (Muench 1958; Gibson 1967; Waterstrat 1999; Small 2003; Cunha and Rosa 2006). Induction and recovery times from the present study are generally comparable with those reported for a similar evaluation of chemo- and electrodesation of similarly sized hybrid striped bass (white bass *Morone chrysops* × striped bass *M. saxatilis*), a representative

coolwater–warmwater finfish (Trushenski et al. 2012). However, some interspecific differences were observed. Induction times for hybrid striped bass and largemouth bass were virtually identical for electrodesation, but induction times were 0.3 min slower for largemouth bass sedated with MS-222, eugenol, and benzocaine and 1.1 min slower for CO₂. Recovery from CO₂ sedation was roughly the same for both taxa; however, largemouth bass recovered from sedation with MS-222, eugenol, and benzocaine nearly 1 min faster than hybrid striped bass, but approximately 0.5 min slower after electrodesation. Other investigators have noted significant variation among different taxa in the effects of chemosedatives (Gibson 1967; Peake 1998; Cunha and Rosa 2006). Given the extent of variability reported by others, the differences in induction and recovery times we observed between largemouth bass and hybrid striped bass exposed to similar sedation protocols were relatively small. Regardless of the differences noted, the induction and recovery times we observed for both hybrid striped bass and largemouth bass would probably be considered acceptable by most fisheries professionals.

Specific hematological patterns varied somewhat according to the sedative used, but each elicited changes that were broadly consistent with the generalized stress response. Although sedatives are commonly used to reduce stressor severity (i.e., need for physical restraint or handling time) and minimize the stress response (Limsuwan et al. 1983; Thomas and Robertson 1991; Sandodden et al. 2001; Finstad et al. 2003; Wagner et al. 2003; Cooke et al. 2004; Small 2004; Palić et al. 2006), sedation itself can elicit a mild to moderate stress response and induce departures from resting physiological states (reviewed by Trushenski et al. 2012). Depending on the sedative and concentrations used, changes in circulating levels of cortisol (Wedemeyer 1970; Strange and Schreck 1978; Iwama et al. 1989; Thomas and Robertson 1991; Davidson et al. 2000; Wagner et al. 2002; Davis and Griffin 2004; King et al. 2005; Zahl et al. 2010; Weber et al. 2011), plasma glucose and lactate (Wedemeyer 1970; Bourne 1984; Thomas and Robertson 1991; Bernier and Randall 1998; Sladky et al. 2001; Cho and Heath 2000; Wagner et al. 2002; Weber et al. 2011), hematocrit (Iwama et al. 1989; Sladky et al. 2001; Cho and Heath 2000), plasma ion levels (Bourne 1984), circulating and tissue levels of various nutrients (Wedemeyer 1970), oxidative stress (Velisek et al. 2011), and partial pressures of respiratory gases (Iwama et al. 1989; Sladky et al. 2001) have been reported following sedation. Often, these effects are exacerbated if coupled with changes in water chemistry, such as pH shifts, which are commonly associated with the use of CO₂ and MS-222 (Trushenski et al. 2012). Our results are generally consistent with these reports, though the magnitude of hematological responses exhibited by largemouth bass was somewhat smaller than what has been reported for other species. The responses associated with exposure to MS-222 were not notably exaggerated despite the reduction in water pH (7.7 versus 8.5–8.6) observed in experiment 2 for this treatment. However, the magnitude of the shift was relatively small because of the high alkalinity (≥ 180 mg/L) and associated buffering capacity of the

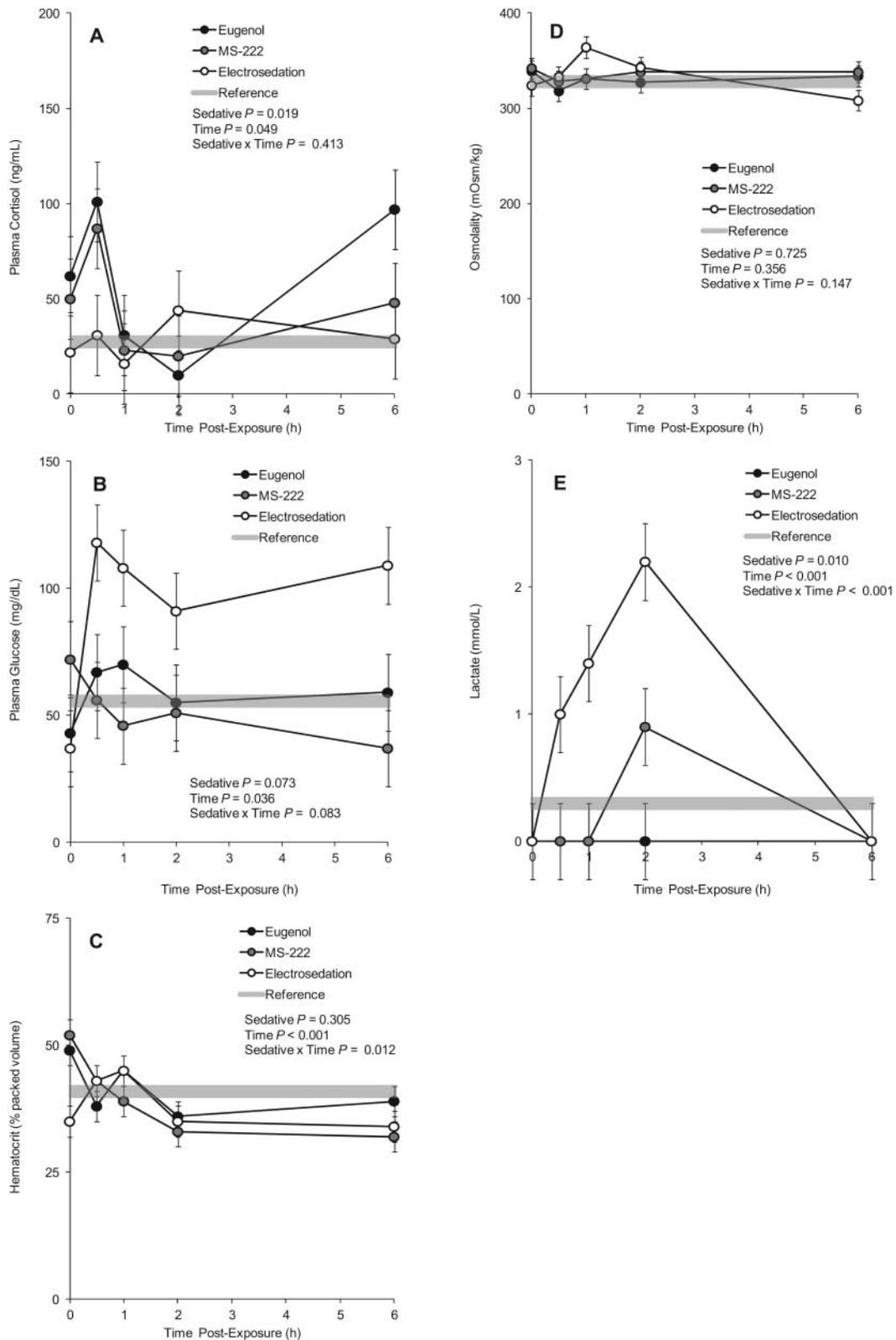


FIGURE 2. Time course of (A) cortisol, (B) glucose, (C) hematocrit, (D) osmolality, and (E) lactate in largemouth bass after sedation to Stage IV of sedation with eugenol, tricaine methanesulfonate (MS-222), or electrosedation. Points represent means reported in Table 2; gray reference bars represent means of values observed for fish sampled from the reference population throughout the course of the experiment.

system water; in experiment 1, the alkalinity was substantially higher (≥ 460 mg/L) and no pH shift was observed. Regardless, it seems that the pH shift observed in experiment 2 was insufficient to elicit a more pronounced response to reduced pH as a secondary stressor. Barton (2002) cautioned against interspecies comparisons in hematological responses to stressors and argued against the conclusion that fish exhibiting lesser responses are therefore "less stressed." Although direct numeric comparisons are perhaps unwise, we can conclude that largemouth bass exhibited hematological responses to MS-222, eugenol, and electrosedation that are broadly consistent with those observed for other taxa following sedation.

In comparing physiological responses to the sedatives we evaluated, the chemosedatives were relatively similar, but electrosedation was associated with a reduced postsedation cortisol pulse and increased lactate and glucose responses. This may be related to differences among the sedatives in terms of exposure times and modes of action. Lactate is commonly used as an indicator of stress in fish, and circulating lactate levels can increase in fish as a result of endogenous corticosteroid release or treatment with exogenous cortisol (reviewed by Mommsen et al. 1999). In this context, cortisol and lactate are probably linked indirectly via the stimulatory effect of cortisol on muscle glycogenolysis (Mommsen et al. 1999). Cortisol and glucose are similarly linked via the catabolic actions of cortisol as well as catecholamines released during the generalized stress response (Barton and Iwama 1991). However, lactate can also be produced in the absence of cortisol, and in the case of sedated fish, increasing lactate may be a consequence of the physical effects of sedation (e.g., hyperactivity during induction, reduced ventilation and respiratory exchange, tetanic muscle contraction [in the case of electrosedation]) as well as the generalized stress response. Our hematological data seem to support this hypothesis: whereas electrosedated fish exhibit virtually no cortisol response, their glucose and lactate responses were substantially greater than those of fish sedated with MS-222 and eugenol. Conversely, measurable cortisol responses among fish sedated with MS-222 and eugenol were not associated with marked changes in metabolic response indicators (i.e., lactate and glucose).

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. Because of the involvement of releasing hormones and the need to synthesize cortisol before release, the corticosteroid cascade is the slower of the two stress hormone axes involved in the stress response in fish (Barton and Iwama 1991). As a result, 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 of time, the corticosteroid response may be similarly short-lived or only the adrenergic cascade may be triggered. In the present case, the electrosedation stressor was

present for, at most, a few seconds, though the fish were handled for a couple of minutes postsedation to assess recovery; total exposure times for experiment 2 were approximately 3–4 min. Groups of fish sedated with chemosedatives, however, were exposed to the sedative and handling for a longer period of time (approximately 7–8 min for experiment 2). It is possible that the longer duration of stressor exposure among the MS-222 and eugenol treatment groups is the reason a stronger cortisol response was recorded for these fish. Although exposure to pulsed DC and handling may be sufficient to engage the hypothalamic–pituitary–interrenal axis and induce a cortisol response in fish (Trushenski et al. 2012), in the present work with largemouth bass it may have been too short-lived (i.e., resolved within 30 min postsedation) to have been recorded using our sampling timeline. The greater responses in metabolic indicators of stress among electrosedated fish may be related to the greater physical effects of electrosedation, particularly muscle tetany in the absence of normal respiratory exchange. Regardless of the specific causal relationships, the hematological profiles were generally observed to return to normal. Exceptions to this observation included elevated glucose levels among electrosedated fish, and to a lesser extent, cortisol levels among fish sedated with eugenol. These aberrations aside, the hematological data coupled with behavioral observations of the fish, suggest that largemouth bass will recover within 6 h of sedation when MS-222, eugenol, or electrosedation are used. Further research to elucidate the complex physiological responses of fish to sedation is recommended, as are studies to assess the varying responses of different species to different types of sedatives.

ACKNOWLEDGMENTS

We thank Smith-Root, Inc., for providing access to a portable electroanesthesia system (PES), and Jack Wingate and Mike Holliman for providing training and technical support in using the PES unit. We also thank Kenson Kanczuzewski and Curtis Crouse for their assistance with data collection. We also thank Bruce Barton and Steven Chipps for feedback they provided during preparation of this manuscript, and Jack Wingate for providing a technical review of our draft manuscript.

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