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ARTICLE

Chemical and Electrical Approaches to Sedation of Cobia: Induction, Recovery, and Physiological Responses to Sedation

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Abstract

 To support the growing interest in marine fisheries research in areas such as biotelemetry, tagging, and tracking, we assessed the ability of various sedatives to facilitate this research in juvenile cobias *Rachycentron canadum* (∼300 g), namely, tricaine methanesulfonate (MS-222; 150 mg/L), carbon dioxide (CO_2; ∼750 mg/L), eugenol (60 mg/L), benzocaine (150 mg/L), and pulsed-DC electrosedation (100 V, 30 Hz, 25% duty cycle, 5-s exposure). Induction times (CO_2 [z] > benzocaine [y] > eugenol [y] > MS-222 [y] > electrosedation [x]), recovery of equilibrium (CO_2 [z] > eugenol [z] > MS-222 [y] > benzocaine [y] > electrosedation [x]), and responsiveness to tactile stimulus (eugenol [z] > MS-222 [y] > benzocaine [y] > CO_2 [xy] > electrosedation [x]) differed significantly among the sedative treatments (treatments with the same letters are not significantly different). Total handling time from initial sedative exposure to recovery differed among the sedatives as well (CO_2 [z] > eugenol [y] > benzocaine [x] > MS-222 [x] > electrosedation [w]), with cumulative means ± SEs of 5.9 ± 0.2 min for CO_2, 4.1 ± 0.2 for eugenol, 2.7 ± 0.2 min for benzocaine and MS-222, and 1.0 ± 0.2 min for electrosedation. Physiological responses differed significantly over time, with transient increases in plasma cortisol, glucose, osmolality, and lactate that were resolved within 6 h. The overall magnitude of the physiological responses differed among sedatives, depending on the response variable; however, in each case, CO_2 elicited the greatest response. Although variations in induction and recovery times were observed, it is likely that these differences can be reasonably accommodated within the context of typical research by adjusting the sedative treatments or allowing for longer induction and recovery times as needed.

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

Although the specific constraints differ from one situation to the next, ideally a fish sedative is safe and easy to administer, is effective at low doses (minimizing the amount needed for field applications), sedates fish quickly and predictably, has a

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reasonable margin of safety with respect to oversedation, can be used over a broad range of water chemistries, is inexpensive, and allows for rapid recovery from sedation and its effects (Bowker and Trushenski 2011). Additionally, in field research, it is particularly advantageous if sedative use does not require treated fish to be held to complete a withdrawal period prior to release (i.e., they can be released immediately).

At this time, there are few legal options for sedating fish, and those that are available are not always ideal in terms of their safety, efficacy, and practicality of use. Currently, there is only one sedative compound that is approved by the U.S. Food and Drug Administration (FDA) for the temporary immobilization of fish: tricaine methanesulfonate (commonly referred to as MS-222). Two MS-222 products are currently approved in the United States, but the use of these products is restricted to ictalurid, salmonid, esocid, and percid fishes (though they are approved for other fishes in laboratory or hatchery settings only) treated at water temperatures $>10^{\circ}C$. Use of MS-222 is further restricted by the 21-d withdrawal period deemed necessary to allow for drug residue depletion prior to releasing treated fish into the wild (or otherwise making them available for human consumption). Although not approved by the FDA, carbon dioxide (CO_2) is considered a drug of "low regulatory priority" (USFDA 2011) and its use allows fish to be released immediately after sedation. However, CO_2 can be difficult to apply uniformly and is typically slow-acting; adverse effects have also been reported (Neiffer and Stamper 2009). There are at least two additional drugs currently being investigated for use as fish sedatives, specifically, benzocaine and eugenol. These drugs can currently be used under the Investigational New Animal Drug exemptions held by the U.S. Fish and Wildlife Service with an associated 3-d withdrawal period. In the meantime, there is an effort by the drug sponsors and researchers to gain FDA approval of one or both of these compounds as immediate-release fish sedatives. Another option which is not subject to the rigors of FDA animal drug oversight is the use of electricity to sedate fishes. Electrofishing has been used for decades as a field technique in fisheries, but only recently has it been modified specifically for sedating/anesthetizing fish and commercialized (Zydlewski et al. 2008; Hudson et al. 2011; Trushenski et al. 2012a, 2012b).

Each of the aforementioned sedatives has positive and negative attributes associated with its use, including approval status (approved, low regulatory priority, or Investigational New Animal Drug status), allowable use patterns (immediate-release versus 3- and 21-d withdrawal periods), disposal considerations, cost, ease of use, and efficacy. Additionally, each of these sedatives has proven effective in numerous freshwater fish (Trushenski et al. 2012a, 2012b; J. D. Bowker, U.S. Fish and Wildlife Service, unpublished data). However, it is unclear whether these approaches can be effectively applied to marine species with the same degree of safety and efficacy. Furthermore, it is unclear whether the differences in physiological responses to sedation observed in freshwater

taxa extend to marine fishes. Traditionally, marine species have received less attention in terms of sedatives research: a recent review of MS-222, CO_2, eugenol and related compounds, and benzocaine research reported studies of 10 freshwater taxa and only 5 marine taxa (Trushenski et al. 2012a). This is particularly true in the case of strategies for electrical immobilization, which is generally less effective in brackish and saltwater than in freshwater. This is a particularly critical information gap, given the growing interest in biotelemetry and other tagging and tracking approaches to marine fisheries research and management efforts (Silbert and Nielsen 2001) and the concomitant demand for effective sedatives to facilitate this type of research. Accordingly, we evaluated the effectiveness of Finquel (MS-222; 100% tricaine methanesulfonate; Argent Laboratories, Redmond, Washington), AQUI-S E (50% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand), Benzoak (20% benzocaine; Frontier Scientific, Inc., Logan, Utah), CO_2, and pulsed-DC electrosedation in sedating juvenile cobias *Rachycentron canadum*. The metrics measured included induction and recovery times and physiological responses to sedation. Cobia was selected as a model species for this assessment because it is found in warm coastal waters throughout the world except for the eastern Pacific Ocean (Shaffer and Nakamura 1989) and is commonly targeted in assessments of commercial and recreational marine fisheries (Lucy and Bain 2000; Williams 2001; Smith et al. 2003; Mahon and McConney 2004).

The terms "sedation," "anesthesia," and "immobilization" are used somewhat interchangeably with respect to fish, but they have distinct meanings: 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. However, the definitions of Ross and Ross differ somewhat from the medical profession's understanding of sedatives and anesthetics; according to the *Medline Plus Medical Dictionary*, a sedative is an agent or drug "tending to calm, moderate, or tranquilize nervousness or excitement," whereas an anesthetic is a substance that causes the "loss of sensation and usually of consciousness without loss of vital functions," specifically substances that "block the passage of pain impulses along nerve pathways to the brain" (NLM 2012). Both sources appear to agree that sedation and anesthesia represent progressions in the loss of the ability to perceive and respond to stimuli, but they disagree regarding the issue of pain. Given the controversy as to whether fish are even capable of perceiving pain (Rose 2002; Braithwaite and Huntingford 2004), the use of definitions which rely on the relative ability to do so seem inappropriate. Although one could argue that none of these terms or definitions perfectly describe the processes we evaluated in the present work, "sedative" (at least as defined by

TABLE 1. Water quality in experiments 1 and 2. The values are the means of composite samples 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.

Parameter	Sedative					
	Holding system	Eugenol	Benzocaine	CO_2	MS-222	Electrosedation
Temperature (°C)	27	27	27	27	27	27
Dissolved oxygen (mg/L)	6.2	6.2	6.2	6.2	6.2	6.2
Alkalinity (mg/L)	88	97	103	104	98	45
Hardness (mg/L)	3,650	3,390	3,245	3,520	3,540	280
Salinity (‰)	20	20	20	20	20	0
Conductivity (mS)	>1,999 (over range)	>1,999 (over range)	>1,999 (over range)	>1,999 (over range)	>1,999 (over range)	700
pH	9.5	9.2	9.0	8.2	8.9	8.9

the medical community) seems the best choice. Thus, for consistency, we have elected to use the term "sedation" throughout this article.

We hypothesized that each of the sedatives assessed would be effective but that cobias would respond differently to each in terms of their induction and recovery times and physiological responses to exposure.

METHODS

All of the procedures described below were conducted under the guidance and approval of the Southern Illinois University–Carbondale Institutional Animal Care and Use Committee (IACUC; protocol 10-028).

Experiment 1: induction and recovery times.—Juvenile cobias were obtained as eggs from a commercial vendor (Troutlodge Marine Farms LLC, Vero Beach, Florida) and cultured at the Virginia Seafood Agricultural Research and Extension Center until they reached an advanced fingerling stage. Feed was withheld for 24 h prior to the experiment. Individual fish (297 ± 9 g, 38.0 ± 0.5 cm total length [mean ± SE]) were transferred from holding tanks in a brackish-water (20‰ salinity) recirculating aquaculture system (Table 1) and placed into a sedation chamber (142-L cooler for electrosedation, 30-L cooler for all others) filled to a depth of approximately 8 cm. Although the fish had been held in several separate tanks within the recirculation system, they were from the same population of fish that had been arbitrarily stocked among the holding tanks approximately 24 h prior to starting the experiment. The electrosedation chamber was filled with freshwater, whereas the chemical sedation baths were prepared using aerated culture water from the holding system (see the description of water quality testing below; Table 1). Sedation treatments were prepared as described in Table 2. To avoid the potential variability associated with different sources for the chemical sedatives, a single lot was used for each product. The chemical sedative concentrations and electrosedation settings were chosen based on our previous experience to achieve a level of sedation appropriate for basic handling

(see the description of sedation procedures below) in less than 5 min. We chose concentrations of MS-222, eugenol, and benzocaine and an electrosedation protocol that have achieved the desired effect in freshwater taxa, though we employed a higher concentration of CO_2 (~750 mg/L, compared with ~400 mg/L) in this case to compensate for the reported difficulties in achieving sedation with CO_2 in saltwater. The chemical sedatives were not tested with cobias beforehand, but we tested the electrosedation protocol prior to experimentation to ensure that the settings would yield appropriate levels of sedation. Although the culture water used to prepare these baths was aerated prior to use, the baths were not aerated following the addition of the chemical sedative or during use. Fresh chemical sedative baths were prepared after treating 5 individual fish; however, the water in the electrosedation unit was not exchanged during the treatment of individual fish. After extended use, sedative baths

TABLE 2. Sedative treatments in experiments 1 and 2.

Sedative	Preparation details
Eugenol	120-mg/L solution of AQUI-S E (60 mg/L eugenol)
Benzocaine	750-mg/L solution of Benzoak (150 mg/L benzocaine)
CO_2	~750-mg/L solutions prepared according to the sodium bicarbonate–sulfuric acid method described by Post (1979) (analytically verified as 736 ± 21 mg/L [mean ± SE of replicate baths])
MS-222	150-mg/L solution of Finquel (150 mg/L tricaine methanesulfonate)
Electrosedation	Pulsed direct current (100 V, 30 Hz, 25% duty cycle, 5-s exposure) delivered via Portable Electroanesthesia System (Smith-Root, Inc., Vancouver, Washington)

can "wear out" as the sedative agent is absorbed by the fish or otherwise dissipated. Also, debris (e.g., mucus, scales, feces) and dissolved wastes (i.e., ammonia) can accumulate in the sedative bath and affect fish during sedation. Although it is unlikely that the loss of sedative efficacy or substantial waste accumulation would have occurred after treating the relatively small numbers of fish used in our study (J. T. Trushenski and J. D. Bowker, unpublished data), we exchanged the bath treatments to avoid that possibility altogether. Dissolved oxygen (measured with the YSI-85 dissolved oxygen–temperature meter; Yellow Springs Instruments, Yellow Springs, Ohio), conductivity, pH, salinity (Multi-Parameter PCSTestr 35; Oakton Instruments, Vernon Hills, Illinois), hardness, and alkalinity (digital titrator and reagents; Hach, Inc., Loveland, Colorado) were maintained within the ranges appropriate for cobia culture throughout the experiment (Table 1). Although the freshwater conditions in the electrosedation chamber would not be considered appropriate for culturing cobias, the fish were only exposed to freshwater for the short period of time associated with electrosedation (~30–45 s elapsed from stocking to completion of induction); the fish then recovered in a brackish water bath identical to that used during the recovery of fish sedated with the chemical sedatives.

During sedation, each fish was monitored to determine the time (from the time of sedative exposure) at which stage IV of sedation (Summerfelt and Smith 1990) was achieved. Stage IV is associated with the total loss of equilibrium, muscle tone, and responsiveness to visual and tactile stimuli but maintenance of a steady, though reduced, opercular ventilation rate. After the loss of equilibrium, fish were continually challenged with tactile stimuli (manual stimulation of the buccal cavity). Fish were considered induced to stage IV when they no longer responded to this stimulus but the opercular rate remained slow but steady. In the case of the electrosedation treatment, a tremor was observed following electrical exposure; although fish were not responsive during this tremor (and were perhaps temporarily in stage V or VI of sedation), induction was considered complete after the tremor ceased. After induction, fish were weighed (to the nearest 0.1 g) and measured to determine total length (to the nearest 0.5 cm) and then transferred to a static recovery tank filled with aerated culture water (exchanged at the same time as in the sedative baths). In the recovery tank, fish were monitored using the techniques mentioned above to determine the time to recovery of normal equilibrium and tactile responses. When fish exhibited normal equilibrium and began responding to the tactile stimulus (by apparent attempts to dislodge the researcher's finger from the buccal cavity), they were considered fully recovered. Recovered fish were returned to a holding system and monitored for survival for 24 h. Since the assessment of induction and recovery can be somewhat subjective, bias was minimized by having the same observers make all assessments.

Experiment 2: physiological responses to sedation.—In this experiment, sedative baths were prepared as previously described. Single working baths of benzocaine, eugenol, and MS-222 were used to sedate all groups of fish in experiment 2. How-

ever, fresh baths of CO_2 were prepared to sedate each group of fish in this treatment because of the volatile loss of CO_2 likely to be exacerbated by fish movement during group sedation. As with the chemical sedatives, the freshwater used in the electrosedation chamber was not exchanged during experiment 2. Water samples were prepared by collecting aliquots from the sedative baths before and after each use and combining these (50:50) to create a single composite water sample for each sedative treatment. Each of the composite water samples was analyzed in duplicate as described for experiment 1, along with water samples collected from the holding recirculation system at the beginning and end of the study period. With the exception of the salinity of the freshwater electrosedation bath (to which fish were only exposed temporarily), all measured values were within the ranges acceptable for cobia culture (Rodrigues et al. 2007; Atwood et al. 2008; Benetti et al. 2008; Chen et al. 2009; Table 1). Additional fish from the population used in experiment 1 (i.e., from the same cohort) were used in experiment 2 (fish were not reused in either experiment). Groups of five fish (286 ± 7 g, 37.0 ± 0.5 cm total length) were transferred from the holding tanks in the brackish-water recirculating aquaculture system previously described for experiment 1 and placed into the sedation chamber and sedated *en masse*. 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 the fish sampled at the start of the experiment 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 IACUC-approved animal care and use protocol. Using a secondary sedative in addition to the other sedatives tested did present a potential confounding effect, i.e., our observations would essentially represent the responses of fish treated with two sedatives (metomidate hydrochloride plus the sedative of interest). We considered several alternative approaches, including blood sampling without sedation and repeat use of the test sedative. However, these approaches were deemed unsuitable because they would likely have a confounding influence on the responses (i.e., fish sampled under sedation at time 0 but not at subsequent data points or fish exposed to protocols that were inconsistent among treatments and through time). Using a distinct, secondary sedative for blood sampling was the preferred approach and would facilitate the most direct comparison among treatments. Metomidate hydrochloride is known to block corticosteroid synthesis in some fish species (Mattson and Riple 1989; Olsen et al. 1995; Davis and Griffin 2004). Consequently, it can be a useful sedative for stress physiology experiments because it may minimize the effects of handling and sample collection on circulating cortisol levels. For consistency, all fish sampled, including those sampled immediately after sedation, were transferred to a solution of metomidate hydrochloride. After exposure to the metomidate hydrochloride bath for approximately 30 s, fish length and weight were measured and a blood sample was collected from the caudal vasculature

using heparinized, evacuated blood collection assemblies (Vacutainer; Becton Dickinson and Co., Franklin Lakes, New Jersey). Although metomidate hydrochloride was used, in part, as a potential corticosteroid blocker, all blood samples were collected within 5 min of capture (<5 min elapsed from netting the fish to placing the blood sample on ice) to minimize the possibility of other 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 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 euthanized by immersion in an ice water bath until all voluntary and involuntary movement ceased and disposed of in the local landfill. Every 2 h during the sampling period, three fish were sampled from the reference population to represent untreated, resting conditions. These fish were also treated with metomidate hydrochloride to facilitate blood sampling. These fish did not represent true controls (which would not have been treated with any sedative whatsoever) but were intended to provide a reference by which the effects of the test sedatives could be qualitatively assessed.

Necessary hematological testing equipment was not available at the Virginia Seafood Agricultural Research and Extension Center, so the tubes containing blood samples were kept on wet ice during transport from Hampton, Virginia, to Carbondale, Illinois (total time between collection and analysis, <36 h). Subsamples of whole blood were used for the determination of hematocrit (Statspin centrifuge; Fisher Scientific, Pittsburgh, Pennsylvania). Whole blood samples were then centrifuged (3,000 × g for 45 min at 4°C), and the resultant plasma was stored at –80°C until further analysis. Plasma samples were analyzed to determine glucose (glucose test reagent; Pointe Scientific, Inc., Canton, Michigan; test adapted for 96-well plates using external standards), lactate (Accutrend lactate meter; Roche, Mannheim, Germany), osmolality (Vapro 5520; Wescor, Inc.; Logan, Utah), and cortisol (EIA 1887; DRG International, Mountainside, New Jersey). Although the portable meters, such as the one we used to measure lactate, have been shown to 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). 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.—In experiment 1, individual fish were considered the experimental units ($n = 10$). Induction and recovery times were analyzed by one-way analysis of variance (ANOVA; PROC MIXED) using SAS, version 9.1 (SAS Institute, Cary, North Carolina) to detect significant differences among the sedatives relative to induction and recovery times. For parameters exhibiting significant treatment effects, post

hoc Tukey's honestly significantly different (HSD) tests were used for pairwise comparisons of the least-squares means. Fish weight and length were assessed as potential covariates (PROC CORR), but no significant correlations between body size and induction and recovery times were observed. In experiment 2, replicate groups of fish were considered the experimental units. Although each sedative was applied to triplicate groups (each comprising five fish), it was determined that groups, not individuals, should serve as the experimental units. By definition, experimental units represent independent observations. We determined that individuals sedated in the same group could not be considered fully independent observations because the presence and/or position of other fish within the sedation chamber could affect the general behavior of the group or, in the case of electrosedated fish, alter the way in which the waveform was applied to individuals. Thus, to maintain a reasonably conservative statistical approach, sedation group was used as the level of replication or experimental unit for each statistical procedure ($n = 3$). 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 were analyzed by one-way, repeated-measures ANOVA (PROC MIXED; SAS 9.1). For parameters exhibiting significant treatment effects, treatment least-squares means were compared at individual time points using post hoc Tukey's HSD tests for pairwise comparisons. In all cases, differences were considered significant at $P < 0.05$ and no data were transformed prior to analysis.

RESULTS

Induction times differed significantly among the sedatives evaluated (CO_2 [z] > benzocaine [y] > eugenol [y] > MS-222 [y] > electrosedation [x]; treatments with the same letters are not significantly different; Figure 1). Briefly, the induction time using CO_2 was 2.7 ± 0.1 min, those for benzocaine, eugenol, and MS-222 ranged from 1.2 to 1.4 ± 0.1 min, and that for electrosedation was 0.2 ± 0.1 min (least-squares means ± SEs). Recovery of equilibrium (CO_2 [z] > eugenol [z] > MS-222 [y] > benzocaine [y] > electrosedation [x]) and responsiveness to tactile stimulus (eugenol [z] > MS-222 [y] > benzocaine [y] > CO_2 [xy] > electrosedation [x]) also differed significantly among the sedative treatments. With the exception of fish treated with CO_2, which exhibited a more protracted recovery and regained tactile responsiveness before equilibrium, the general recovery pattern was to regain equilibrium, then tactile responsiveness in rapid succession. All benchmarks of recovery were achieved most rapidly in the electrosedation treatment: the mean times to regain equilibrium and tactile responsiveness were 0.6 ± 0.1 min and 0.8 ± 0.1 min postinduction, respectively. Equilibrium was regained among fish treated with benzocaine in 1.2 ± 0.1 min; the values for the other treatments were as follows: MS-222, 1.3 ± 0.1 min; eugenol, 2.7 ± 0.1; and CO_2, 3.2 ± 0.1 min. Tactile responsiveness was regained among fish treated with CO_2 in 1.0 ± 0.1 min; the values for the
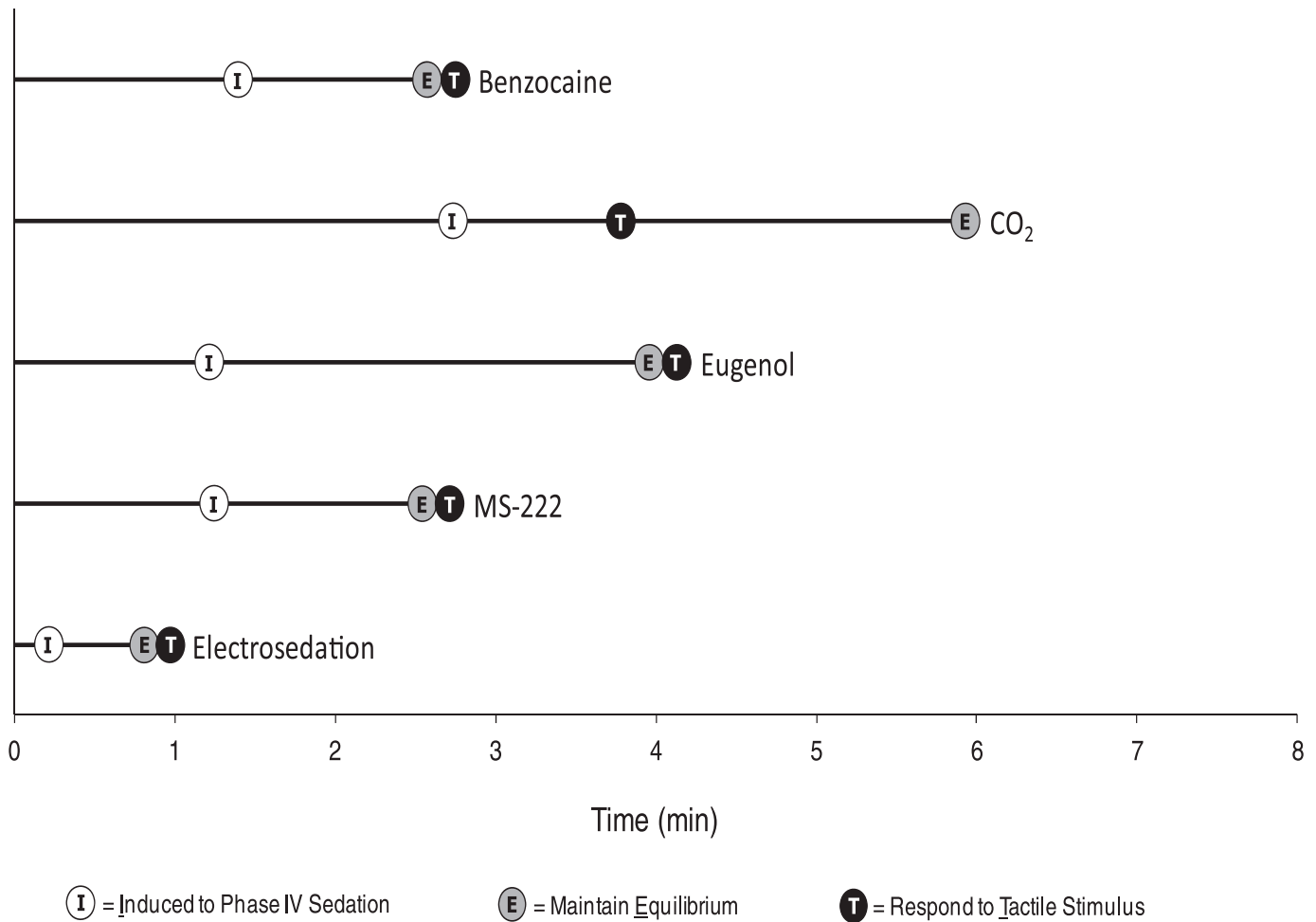
FIGURE 1. Schematic illustrating mean times to induction and various stages of recovery of cobias sedated to stage IV of anesthesia using various chemical sedatives or electrosedation ($n = 10$). Note that events are shown in terms of total elapsed time, i.e., event times reported in the text are displayed in an additive fashion in the figure.

other treatments were as follows: benzocaine, 1.4 ± 0.1 min; MS-222, 1.5 ± 0.1 min; and eugenol, 2.9 ± 0.1 min. Total handling time, from initial sedative exposure to recovery, differed among the sedatives as well: CO_2 [z] > eugenol [y] > benzocaine [x] > MS-222 [x] > electrosedation [w]), with a total of 5.9 ± 0.2 min for CO_2, 4.1 ± 0.2 min for eugenol, 2.7 ± 0.2 min for benzocaine and MS-222, and 1.0 ± 0.2 min for electrosedation.

Physiological responses differed significantly among the sedatives evaluated and over time (Figure 2; Table A.1 in the appendix). Plasma cortisol concentrations increased within 0.5 h after sedation but began returning to resting levels within 1 h postsedation for all sedatives except CO_2, which remained elevated through 2 h postsedation. Similar response patterns were observed for osmolality and lactate, though lactate levels remained somewhat elevated 2 h after sedation with pulsed-DC electricity and CO_2. Plasma glucose levels increased following sedation, in most cases peaking between 0.5 and 1 h postsedation, though a second, higher peak was observed among the

electrosedated fish at 2 h postsedation. Nonetheless, glucose gradually decreased following the peak response in each treatment, returning to near-resting levels within 6 h of sedation. The overall magnitude of the physiological responses differed to a greater (cortisol, lactate, glucose) or lesser (glucose) degree among the sedatives tested; however, in each case, CO_2 elicited the greatest response. Although a significant time effect was observed for hematocrit, reflecting a generalized decline from 0–0.5 h to the end of the sampling period, differences were not observed between the sedatives.

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 posture returned to normal after resolution of the postexposure tremor. Blanching of the skin was observed among some fish sedated with CO_2, particularly among electrosedated fish. During exposure to CO_2, fish were hyperactive and observed to pipe at the water surface. Although some hyperactivity was observed during sedation with
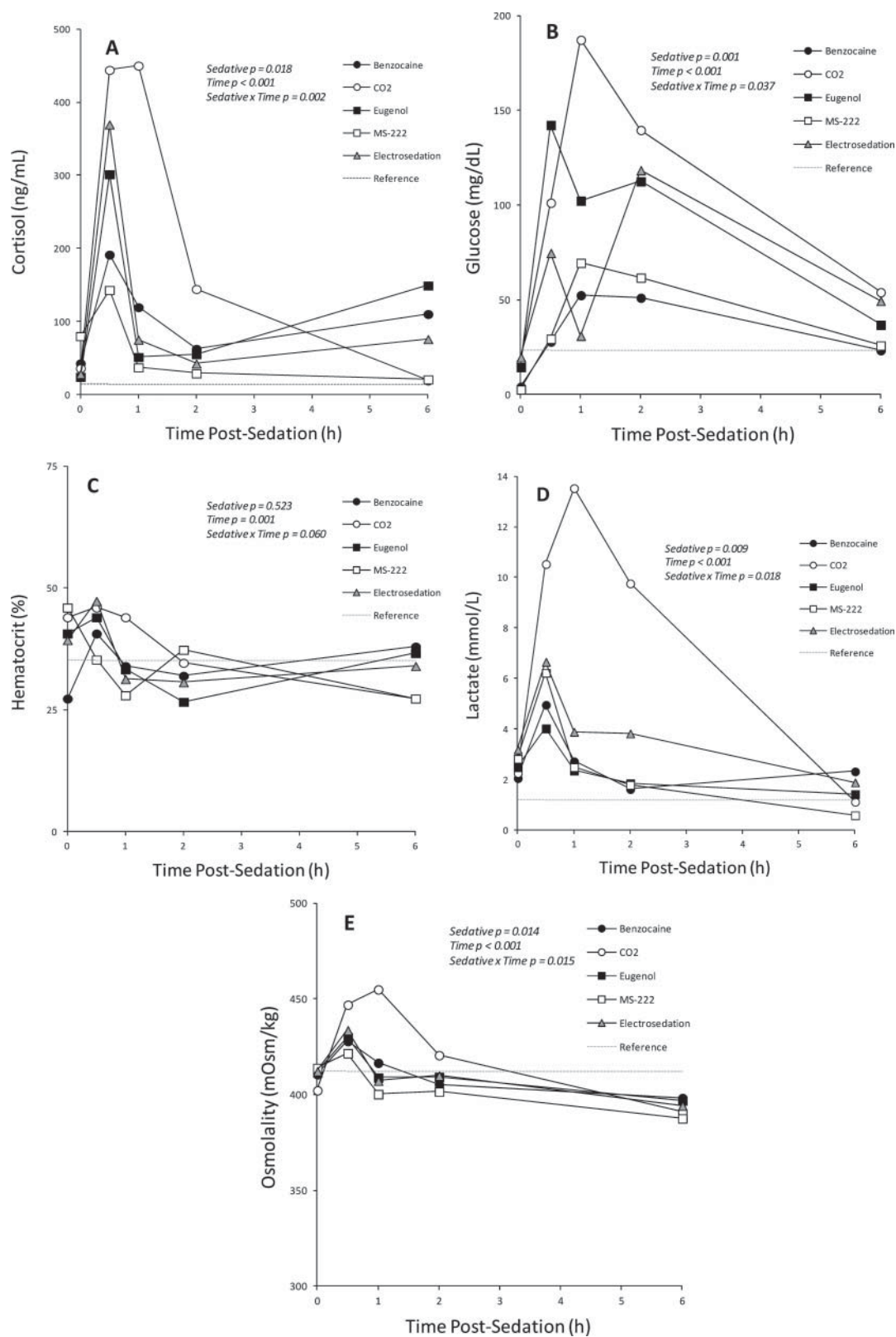
FIGURE 2. Time courses of physiological responses ([**A**] cortisol, [**B**] glucose, [**C**] hematocrit, [**D**] lactate, and [**E**] osmolality) of cobias following sedation to stage IV of anesthesia using various chemical sedatives or electrosedation. The points represent the least-squares means reported in Table A.1 ($n = 3$); the gray reference bars represent the least-squares means of the values for fish sampled from the reference population throughout the experiment.

benzocaine, it was less pronounced than that associated with CO_2 (not all fish exhibited hyperactive swimming and those that did were not as agitated as those exposed to CO_2). There were no mortalities during the two experiments, which involved the sedation and handling of 125 individuals.

DISCUSSION

Our results suggest that, despite taxonomic, biological, and physiological differences, cobias respond to chemo- and electrosedation in a manner broadly similar to that observed in largemouth bass *Micropterus salmoides* (Trushenski et al. 2012b), hybrid striped bass (white bass *Morone chrysops* × striped bass *M. saxatilis* [Trushenski et al. 2012a]), grass carp *Ctenopharyngodon idella* (Bowzer et al. 2012; Gause et al. 2012), and other species tested using similar sedation protocols (i.e., walleyes *Sander vitreus* [Bowker, unpublished data] and shovelnose sturgeon *Scaphirhynchus platorynchus* [Trushenski, unpublished data]). Although a relatively small number of individuals were involved in the present work ($n = 10$ for experiment 1, and $n = 3$ for experiment 2), the results are nonetheless compelling. The pattern of induction observed in our study was strikingly similar to the induction patterns observed for larger hybrid striped bass and largemouth bass ($\sim$500 g) sedated using similar sedative approaches (hybrid striped bass: 60 mg/L eugenol; 150 mg/L benzocaine; 150 mg/L MS-222; $\sim$400 mg/L CO_2; electrosedation = 60 V, 30 Hz, 25% duty cycle, 3-s exposure; largemouth bass: 60 mg/L eugenol; 150 mg/L benzocaine; 150 mg/L MS-222; $\sim$400 mg/L CO_2; electrosedation = 100 V, 30 Hz, 25% duty cycle, 3-s exposure): fish were sedated to stage IV in 0.2 min using electrosedation; in 1.3–1.9 min using eugenol, benzocaine, or MS-222; and in 2.5–3.6 min using CO_2 (Trushenski et al. 2012a, 2012b). Although the walleyes tested were smaller ($\sim$50 g), similar protocols (60 mg/L eugenol; 150 mg/L benzocaine; 150 mg/L MS-222; $\sim$400 mg/L CO_2; electrosedation = 100 V, 30 Hz, 25% duty cycle, 5-s exposure) yielded similar induction times for this species as well: fish were sedated to stage IV in 0.1 min using electrosedation; in 0.7–0.9 min using eugenol, benzocaine, or MS-222; and in 2.1 min using CO_2 (Bowker, unpublished data).

Similar times to induction to stage IV (referred to as "stage III" by the authors but equivalent to stage IV as defined by Summerfelt and Smith 1990) were observed in a study by Gullian and Villanueva (2009). In their study, two size-classes of juvenile cobia ($\sim$5 and $\sim$14 g) were sedated using various concentrations of clove oil (the product contained $\sim$88% eugenol; 20–100 mg/L) and MS-222 (40–120 mg/L). The authors found that, regardless of fish size, the time to induction with MS-222 ranged from 1.15 to 1.25 min and that with 60 mg/L clove oil from 1.70 to 2.22 min, the latter being slightly longer (0.49–1.01 min) than that observed in our study. The slower induction times observed by these authors may be attributable to the difference in eugenol purity between AQUI-S 20E and clove oil and the corresponding decrease in effective eugenol concentration ($\sim$53 mg/L versus 60 mg/L). Taken together, all of these experiments represent different fish sizes ($\sim$5–500 g), temperatures ($\sim$19–27°C), salinities ($\sim$0–39‰), and a broad taxonomic range (Rachycentridae, Centrarchidae, Moronidae, and Percidae). Thus, it would appear that the sedative approaches we investigated in cobias yield relatively consistent results in terms of induction to stage IV of sedation across a range of scenarios. The apparent consistency in safety and efficacy across a range of conditions and taxa is encouraging for fisheries professionals attempting to perform routine handling procedures such as those involved in collecting biometric data, tagging, or harvesting tissue noninvasively (e.g., fin clips and spines) in various research scenarios. Although sedative safety and efficacy have not been quantitatively demonstrated for all taxa, the data we generated assessing these sedative approaches in cobia and various other taxa suggest that with a modicum of experience researchers could apply the sedatives to most, if not all, fish without substantial risk of adverse effects. Nonetheless, when preparing to sedate an untested taxon or life stage, we advise researchers to conduct a preliminary test using a few individuals to determine appropriate sedation protocols.

Recovery of equilibrium and tactile responsiveness also differed among the sedatives evaluated, with complete recovery occurring most rapidly among electrosedated fish, followed by fish sedated with benzocaine or MS-222 and fish sedated with eugenol or CO_2. With the exception of CO_2-treated fish, which progressively regained tactile responsiveness and then equilibrium (2.2 min elapsed between benchmarks), sedated cobias regained equilibrium first, followed quickly by tactile responsiveness (12 s elapsed between benchmarks). Despite differences in the process and pattern of recovery of equilibrium and tactile responsiveness, the differences in induction times were essentially repeated in terms of recovery and total handling time: induction and recovery were fastest among electrosedated fish and slowest among fish sedated with CO_2, with the other sedatives yielding intermediate times.

The present results are somewhat different from those observed in previous evaluations of these sedatives: the current and previous studies differ in terms of the range of handling times observed ($\sim$1–7 min, depending on the sedative and taxon), which sedative was associated with the longest total handling time (eugenol or CO_2, depending on the taxon), whether equilibrium or tactile responsiveness were regained first (variable among sedatives and taxa), and whether benchmarks of recovery were achieved slowly or in rapid succession (variable among sedatives and taxa; Trushenski et al. 2012a, 2012b). Despite relatively consistent results in terms of induction times, it seems there is considerable variability in the pattern and process of recovery among sedative types and among different fishes sedated using these approaches. This variation may be attributable to biological differences among taxa, differences in fish body size, or differences in abiotic factors such as water temperature or pH (Ross and Ross 2008). However, given the circumstances under which fish sedatives are most likely to be used (i.e., by experienced fisheries professionals familiar with what is

considered normal behavior for different fishes), it is likely that the variation among different taxa could be readily accommodated by adjusting the sedative dose and/or the amount of time allowed for recovery prior to release.

The transient changes in circulating cortisol, glucose, and lactate and the degree of osmolality that we observed indicate that cobias undergo an acute stress response following sedation. Depending on the sedative concentrations used, transient primary and secondary stress responses have been observed in fish following sedation with MS-222, CO_2, benzocaine, various clove derivatives, and pulsed-DC electricity (Davidson et al. 2000; Wagner et al. 2002; Davis and Griffin 2004; King et al. 2005; Bolasina 2006; Zahl et al. 2010; Trushenski et al. 2012a, 2012b). Although sedatives are often used with the intention of reducing handling stress (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), sedatives can elicit mild to moderate stress responses, particularly if their application is accompanied by changes in water chemistry (i.e., pH shifts associated with CO_2 and MS-222; Trushenski et al. 2012a). The increases in cortisol, glucose, lactate, and osmolality occurring 0.5–2 h postsedation are consistent with induction of the generalized stress response in fish, including both the primary (i.e., elevated cortisol) and secondary (i.e., elevated glucose, lactate, and osmolality) responses to stressor exposure (Mazeaud et al. 1977; Barton 2002). The time course of physiological responses is consistent with the responses of juvenile cobias exposed to other acute stressors, such as a 1-min air exposure challenge (Cnaani and McLean 2009; Trushenski et al. 2010) or 15-min low-water challenge (Trushenski et al. 2010); in both cases, the glucose and cortisol responses peaked within 2 h of stressor exposure and were largely resolved within 6 h. Additionally, the range of peak cortisol, glucose, lactate, and osmolality responses observed following sedation shows considerable overlap with the range of responses reported by Trushenski et al. (2010) in association with acute air exposure and low-water challenges (cortisol: ~190–450 versus ~130–230 ng/mL; glucose: ~50–190 versus ~130–190 mg/dL; lactate: ~4–13 versus ~1–9 mmol/L; and osmolality: ~420–450 versus ~400–450 mOsm/kg), though higher lactate responses were associated with CO_2 and higher cortisol responses were associated with CO_2, electrosedation, and eugenol.

Generally, the magnitude of the physiological stress response is considered indicative of stressor severity. Therefore, the greater magnitude and duration of the cortisol, glucose, lactate, and osmolality pulses observed among cobias sedated with CO_2 suggests that this drug is the most stressful of those we evaluated. This is also anecdotally supported by the observation of skin blanching in this treatment, which has been associated with stress in fish (Y. Iger and colleagues, abstract presented at the International Conference Aquaculture Europe, 2001). This is not surprising, as the pH of the CO_2 sedative baths was markedly lower than that of the culture water (8.2 versus 9.5). Induction times were also significantly longer for CO_2, and slower-acting

sedatives have been linked to greater stress responses (Chiba et al. 2006; Trushenski et al. 2012a). Additionally, higher lactate responses have been previously linked to stressors that interfere with gas exchange (i.e., air exposure; Trushenski et al. 2010); given the inhibitory effects of environmental hypercapnia on CO_2 release and O_2 uptake at the gill, rapid transition to anaerobic respiration and lactate accumulation following sedation with CO_2 may be expected. Electrosedation was conducted in freshwater and was associated with the second highest cortisol response. It is possible that exposure to freshwater exacerbated the cortisol response to electrosedation in the same manner that low pH likely induced a greater response among fish sedated with CO_2. However, it is possible that the extremely short induction times associated with electrosedation limited the effects that freshwater exposure might have otherwise had on the secondary stress response parameters. A control treatment in which cobias were exposed to freshwater but not electrosedation would be necessary to parse the physiological response of these fish into the distinct effects of exposure to pulsed-DC electricity and freshwater. Regardless, it is important to note that all measured physiological perturbations, including the more marked responses associated with CO_2, were resolved within 6 h postsedation. Consequently, it seems unlikely that singular or periodic sedation of juvenile cobias using any of the approaches we evaluated would be sufficiently stressful to elicit the tertiary effects of stress (e.g., decreased growth, survival, or reproductive capacity) or other negative consequences in the near or long term.

One shortcoming of our study is that we did not assess fish for vertebral abnormalities or other internal lesions postsedation, which have been observed following exposure to pulsed-DC electrosedation in some (Gaikowski et al. 2001; Zydlewski et al. 2008) but not all fishes (Vandergoot et al. 2011). The occurrence of injuries such as vertebral compressions or fractures and hemorrhages appears to be highly dependent on the type and strength of the waveform used as well as the morphology and size of the fish involved. We cannot say whether such injuries occurred in the cobias we electrosedated. However, researchers who have reported injuries associated with pulsed-DC electrosedation have generally concluded that these injuries are relatively minor (e.g., occurring in a relatively small percentage of individuals or not resulting in delayed mortality) or may be avoided by modifying the electrosedation protocol to suit the circumstances. A second shortcoming is that we did not assess the behavior, physiological status, general performance, or survival of treated fish after completion of the 24-h observation period. To unequivocally demonstrate that these treatments do not negatively influence fish when used in an immediate-release context, it would be necessary to treat fish, release them, and monitor their performance poststocking. Given the withdrawal periods currently required for MS-222 (21 d), benzocaine, and eugenol (3 d), this was not readily possible. However, we anticipate that any adverse events associated with treatment will most likely occur immediately or shortly following sedation and that serious long-term effects are less likely. In previous studies, we

have held and observed fish for 2 d to several weeks following sedative treatment (Trushenski et al. 2012a, 2012b; Trushenski and Bowker, unpublished data). Excluding a few incidental mortalities, adverse effects of sedative treatment (e.g., abnormal behavior, histological pathologies, and mortality) were not generally observed in these studies, and in no case were they observed to develop or increase after 24 h. Based on this information, we think that using the sedatives tested in an immediate-release context is unlikely to yield long-term, adverse effects not quantified as part of our study.

Selecting an appropriate sedative can be challenging, particularly when several methods may be used to achieve the desired level of sedation. Choosing the appropriate sedative is generally a matter of the cost and logistics associated with the intended application. Although there are numerous scenarios in the fisheries profession requiring the use of sedatives, some generalizations can be made regarding the practical use of the sedatives described in this paper. For example, chemical sedatives are inexpensive in the short term compared with electrosedation, which requires a relatively high initial investment. However, purchasing an electrosedation unit is a one-time investment, and lower-cost alternatives to commercially available units may be an option for some users (Hudson et al. 2011). If large numbers of fish are being sedated regularly, an electrosedation unit may be more economical than chemical sedatives, but chemical sedatives may be more appropriate and cost-effective for small numbers of fish or infrequent sampling. Electrosedation is uniquely suited to field applications because it reliably and quickly sedates a variety of taxa without concerns about chemical disposal or withdrawal periods. Chemical sedatives may be more suited to research and hatchery facilities due to the need for proper chemical disposal and holding fish during the currently required withdrawal periods. By considering the effects of the different types of sedatives on the fish along with their costs and the intended application, fisheries professionals can make more informed decisions concerning which sedative to use. However, all of these generalizations are subject to change as costs change, new sedatives become available or are approved, or withdrawal periods are modified.

In conclusion, benzocaine, MS-222, eugenol, CO_2, and pulsed-DC electrosedation were all effective in sedating juvenile cobias to stage IV of sedation for the purposes of basic handling and morphometric measurement. Variations in induction and recovery times and physiological responses to sedation were observed. These differences can be reasonably accommodated within the context of typical field or laboratory research, though further research would be necessary to assess the relative suitability of the different sedatives for more invasive procedures. Although CO_2 and electrosedation may be tenable immediate-release options for some scenarios, these options may not be practical or advisable in other circumstances. We recommend that a greater range of immediate-release sedatives be made available to fisheries professionals so that they may select the sedative best suited to their application and collect the highest-quality data possible.

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APPENDIX: PHYSIOLOGICAL RESPONSES

TABLE A.1. Physiological responses of cobias following sedation to stage IV of anesthesia using various chemical sedatives or electrosedation. The values are least-squares means $\pm$ SEs of triplicate samples for each time point–treatment combination ($n = 3$). For given time points, means with different letters are significantly different ($P < 0.05$); means with common letters or no letters are not significantly different. The P-values generated by repeated-measures ANOVA are provided for each parameter.

		Sedative				
Parameter	Time (h)	Eugenol	Benzocaine	CO_2	MS-222	Electrosedation
Cortisol (ng/mL)	0	25 ± 54	43 ± 54	37 ± 54	80 ± 54	29 ± 54
	0.5	302 ± 54 yz	192 ± 54 yz	444 ± 54 z	143 ± 54 y	370 ± 54 yz
Sedative $P = 0.018$	1	52 ± 54 y	120 ± 54 y	450 ± 54 z	38 ± 54 y	75 ± 54 y
Time $P < 0.001$	2	56 ± 54	63 ± 54	145 ± 54	29 ± 54	43 ± 54
Sedative $\times$ Time $P = 0.002$	6	150 ± 54	111 ± 54	20 ± 54	21 ± 54	76 ± 54
Glucose (mg/dL)	0	15 ± 21	4 ± 21	17 ± 21	3 ± 21	20 ± 21
	0.5	142 ± 21	28 ± 21	101 ± 21	29 ± 21	75 ± 21
Sedative $P = 0.001$	1	102 ± 21 yz	52 ± 21 y	187 ± 21 z	70 ± 21 y	31 ± 21 y
Time $P < 0.001$	2	113 ± 21	51 ± 21	140 ± 21	62 ± 21	118 ± 21
Sedative $\times$ Time $P = 0.037$	6	37 ± 21	23 ± 21	54 ± 21	26 ± 21	49 ± 21
Hematocrit (%)	0	41 ± 4	27 ± 4	44 ± 4	46 ± 4	39 ± 4
	0.5	44 ± 4	41 ± 4	46 ± 4	35 ± 4	47 ± 4
Sedative $P = 0.523$	1	33 ± 4	34 ± 4	44 ± 4	28 ± 4	31 ± 4
Time $P = 0.001$	2	27 ± 4	32 ± 4	35 ± 4	37 ± 4	31 ± 4
Sedative $\times$ Time $P = 0.060$	6	37 ± 4	38 ± 4	27 ± 4	27 ± 4	34 ± 4
Osmolality (mOsm/kg)	0	412 ± 7	411 ± 7	402 ± 7	414 ± 7	412 ± 7
	0.5	430 ± 7	428 ± 7	447 ± 7	422 ± 7	434 ± 7
Sedative $P = 0.014$	1	409 ± 7 y	417 ± 7 y	455 ± 7 z	400 ± 7 y	407 ± 7 y
Time $P < 0.001$	2	409 ± 7	405 ± 7	421 ± 7	402 ± 7	410 ± 7
Sedative $\times$ Time $P = 0.015$	6	397 ± 7	398 ± 7	391 ± 7	388 ± 7	394 ± 7
Lactate (mmol/L)	0	2.5 ± 1.4	2.1 ± 1.4	2.3 ± 1.4	2.8 ± 1.4	3.2 ± 1.4
	0.5	4.0 ± 1.4	5.0 ± 1.4	10.5 ± 1.4	6.2 ± 1.4	6.7 ± 1.4
Sedative $P = 0.009$	1	2.4 ± 1.4 y	2.7 ± 1.4 y	13.5 ± 1.4 z	2.5 ± 1.4 y	3.9 ± 1.4 y
Time $P < 0.001$	2	1.9 ± 1.4 y	1.6 ± 1.4 y	9.8 ± 1.4 z	1.8 ± 1.4 y	3.8 ± 1.4 yz
Sedative $\times$ Time $P = 0.018$	6	1.4 ± 1.4	2.3 ± 1.4	1.1 ± 1.4	0.6 ± 1.4	1.9 ± 1.4