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MANAGEMENT BRIEF

# Induction, Recovery, and Hematological Responses of Pallid Sturgeon to Chemical and Electrical Sedation

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## Abstract

Currently, there are no sedatives approved by the U.S. Food and Drug Administration that are considered effective, safe to a broad range of fish species, practical to use, and allow sedated fish to be returned to public waters immediately upon recovery. Availability of such a sedative is critical for many field-based fisheries activities and research, particularly when working with federally listed threatened and endangered species such as Pallid Sturgeon *Scaphirhynchus albus*. Therefore, we conducted an experiment to quantitatively compare induction and recovery times of Pallid Sturgeon sedated using tricaine methanesulfonate (MS-222), eugenol, or electrosedation (pulsed DC) and assess the fish's hematological profile following sedation. Induction times varied significantly among the sedatives evaluated, of which electrosedation yielded the fastest induction times ( $0.2 \pm 0.04$  min, mean  $\pm$  SE) followed by MS-222 ( $1.8 \pm 0.19$  min) and eugenol ( $2.3 \pm 0.26$  min). Times to recovery of equilibrium and responsiveness to tactile stimuli also varied, ranging from  $1.4 \pm 0.1$  min for electrosedation to  $4.7 \pm 0.2$  min and  $6.4 \pm 0.7$  min for MS-222 and eugenol, respectively. Except for plasma osmolality, hematological variables (hematocrit, glucose, lactate, and cortisol) did not vary over a 6-h postsedation sampling period. Osmolality was lower in fish sedated with MS-222 and eugenol and higher in electrosedated fish compared with unsedated reference fish. Our results showed that all sedation protocols tested effectively sedated Pallid Sturgeon, all sedated fish recovered, and there was no delayed mortality associated with sedation.

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To efficiently collect data and manage fisheries populations, fisheries professionals need a safe and effective way to restrain fish during handling. Fish cannot be physically restrained in the

same manner as terrestrial animals without the risk of causing damage. If not properly sedated, epithelial damage or other physical injuries are likely to occur in fish (Ross and Ross 2008). Sedating fish before handling reduces the risk of injury to both the fish and the handler, and may also minimize the fish's stress response (Neiffer and Stamper 2009) and reduce the incidence or severity of stress-related complications after handling. Fish are commonly sedated for the collection of biometric data or tissue samples, or for tagging and other procedures.

Chemical sedatives are one option fisheries professionals have for sedating fish during handling. The only chemical sedative approved by the U.S. Food and Drug Administration (FDA) for use in fish is tricaine methanesulfonate (commonly referred to as MS-222; 99.5% tricaine methanesulfonate; Finquel, Argent Laboratories, Redmond, Washington [product no longer available]; Tricaine-S, Western Chemical, Ferndale, Washington). The sedative MS-222 is approved for the temporary immobilization of ictalurids, salmonids, esocids, percids, or hatchery and laboratory fish held at water temperatures above 10°C (CFR 2011). With few exceptions, fish sedated with MS-222 must be held for a 21-d withdrawal period before they are released or consumed. Another sedative option for fisheries professionals is carbon dioxide (CO<sub>2</sub>). Considered an unapproved, low regulatory priority drug, CO<sub>2</sub> can be used as an immediate-release sedative requiring no withdrawal period (FDA 2011). However, CO<sub>2</sub> can be unwieldy and unpredictable to use and is not effective on all species (Neiffer and Stamper 2009).

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There are means of sedating fish that may be more appropriate for field work. For example, a eugenol-based product, AQUI-S 20E (10% eugenol; AQUI-S New Zealand, Lower Hutt, New Zealand), is an investigational new animal drug (INAD) that may be used under the U.S. Fish and Wildlife Service (USFWS) compassionate INAD exemption authorization. Participants in the National INAD Program can use AQUI-S 20E as an immediate-release sedative for freshwater fish treated as a part of “field-based fishery management activities”; a 72-h withdrawal period applies to all other fish sedated with this product (UFRC 2014; FDA Center for Veterinary Medicine, 2012 memorandum to David Erdahl, U.S. Fish and Wildlife Service, on amended food-use authorization for fish treated with eugenol). Electricity is another option for sedating fishes. Fisheries professionals have used electricity as a means of immobilizing and capturing fish (i.e., electrofishing) for field surveys for decades. Recently, this technique has been modified for the specific purpose of sedating fish (Zydlewski et al. 2008; Trushenski et al. 2012a, 2012b, 2012c). Electroседation may offer several advantages over chemical sedatives in that it requires no withdrawal period, does not present any difficulties regarding chemical disposal, and may be easier to use, particularly in the field. Additionally, electroседation is not subject to FDA regulation as a drug and can be used by fisheries professionals for immediate-release sedation applications.

In the Mississippi and Missouri River basins, much effort is being put forth to study and manage populations of Pallid Sturgeon *Scaphirhynchus albus* (Quist et al. 2004; Colombo et al. 2007; Bettoli et al. 2009; Phelps et al. 2010). However, legal sedation options are limited when it comes to this endangered species, especially in the field; for example, MS-222 requires a 21-d withdrawal period and water must be buffered to minimize a shift in pH, whereas CO<sub>2</sub> sedation is not easily managed in this species. AQUI-S 20 E may be an effective immediate-release option; however, little information is available regarding the effectiveness of eugenol in terms of sedating sturgeon. Issues of legal use and effectiveness notwithstanding, chemical sedatives can be logistically difficult to use in the field; for example, accurately measuring water and sedative, disposal of treated water, and transportation and use of compressed gas cylinders or chemical solutions or premixes (i.e., for infusing or generating CO<sub>2</sub>) can be problematic under some field conditions. A safe, effective, and “field-friendly” method is needed for the sedation of finfishes, especially Pallid Sturgeon. Accordingly, we conducted a study to evaluate MS-222, eugenol, and pulsed-DC electricity as sedatives for juvenile Pallid Sturgeon in terms of sedative effectiveness and hematological responses of the fish to sedation.

## METHODS

**Sedation procedures.**—Pallid Sturgeon were obtained as age-0 individuals from Milford Fish Hatchery (Junction

City, Kansas) and Gavins Point National Fish Hatchery (Yankton, South Dakota) and were cultured at Southern Illinois University Carbondale until the time of the experiment. Triplicate groups of five sturgeon (20.6 ± 0.9 g, 20.9 ± 0.3 cm TL; grand mean ± SE measurements of individual fish) were transferred from a recirculating aquaculture system and placed into a sedation chamber (142-L cooler for electroседation, 114-L cooler for all others) prefilled with 70 L of culture water (water depth of ~8 cm for electroседation, ~10 cm for all others). Sedative chambers contained a chemical sedative solution or were equipped with an electroседation apparatus. Sturgeon were sedated according to the following protocols, selected based on our personal experience with sturgeon, FDA-concurred protocols for demonstrating the effectiveness of chemical sedatives in fishes, and previous work involving sedation of warm- and coolwater fishes (Trushenski and Bowker 2012; Trushenski et al. 2012a, 2012b, 2012c; Bowker et al. 2015):

1. Eugenol: 60 mg/L eugenol (600 mg/L AQUI-S 20E)
2. MS-222: 150 mg/L tricaine methanesulfonate (Finquel); no buffers were used as the source water was known to have an alkalinity in excess of 100 mg CaCO<sub>3</sub>/L; however, a pH decrease was still observed (see Table 1 for full details)
3. Electroседation: pulsed DC (200 V, 30 Hz, 25% duty cycle, 8-s exposure; settings are nominal values, current was not measured) delivered via Portable Electroanesthesia System (Smith-Root, Vancouver, Washington). Power density associated with electroседation was calculated as follows:

$$\text{Power density } (\mu\text{W}/\text{cm}^3) = \text{conductivity} \times (\text{voltage}/\text{distance between electrodes})^2$$

$$\text{Power density} = 2.87\mu\text{S}/\text{cm} \times (20\text{V}/77\text{cm})^2 = 19.4\mu\text{W}/\text{cm}^3$$

Aerated culture water was used to prepare the baths, and single baths were used throughout the study, i.e., baths were not exchanged between groups of fish. Water samples were collected from each sedative bath and the holding system, and the following were analyzed in duplicate: 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. All water quality variables were maintained within acceptable limits for *Scaphirhynchus* spp. (Table 1; Mims et al. 2002; South Dakota Department of Game Fish and Parks 2006).

Sturgeon were monitored during sedation to determine induction time, i.e., the length of time required to achieve

TABLE 1. Water quality variables measured in the study. Values represent means of composite samples from the sedative baths before and after use. Samples were analyzed in duplicate along with water samples collected from the holding recirculation system at the beginning and end of the study period.

Variable	Holding system	Sedative		
		Eugenol	MS-222	Electrosedation
Temperature (° C)	21.9	21.2	21.5	21.5
Dissolved oxygen (mg/L)	8.50	8.65	8.43	8.52
Total ammonia nitrogen (mg/L)	0	0.37	0.01	0.02
Nitrite-nitrogen (mg/L)	0.01	0.011	0.005	0.007
Nitrate-nitrogen (mg/L)	4.3	4.6	4.0	4.7
Alkalinity (mg CaCO <sub>3</sub> /L)	210	118	112	132
Hardness (mg CaCO <sub>3</sub> /L)	57.6	57.6	54.2	55.0
Salinity (‰)	1.44	1.30	1.41	1.48
Conductivity (µS/cm)	2.80	2.55	2.74	2.87
pH	8.52	8.40	7.09	8.56

stage IV anesthesia (Summerfelt and Smith 1990). Fish were considered sedated when they exhibited a total loss of equilibrium (unable to right themselves when turned upside down) and response to tactile stimuli (slight pressure, holding of the caudal peduncle to prevent fish from swimming away), but maintained a slow, steady opercular ventilation rate (observed, but not quantitatively measured). During electrosedation, tremors and opercular flaring were observed during electrical exposure. Even though sturgeon were not responsive when exhibiting these behaviors, induction was not considered complete until the tremors and/or flaring had stopped. After the group was induced, four sturgeon were returned to a tank in the holding system (supplied with flowing water and aeration) to evaluate recovery while a blood sample (see methods below) was taken from the remaining sturgeon. Fish in the recovery tank were observed to determine the recovery of equilibrium and responsiveness to tactile stimuli. Fish were considered fully recovered when all individuals had regained equilibrium and responded to tactile stimuli. The four sturgeon in the holding tank were subsequently sampled at 0.5, 1, 2, and 6 h after sedation (1 fish/group per time point, individuals were only sampled once) to determine blood chemistry.

Prior to blood sampling, all fish (including those sampled directly after sedation) were submerged in a bath of metomidate hydrochloride (Aquacalm, Western Chemical, Ferndale, Washington; ~3–5 mg/L for ~30 s) before sampling. Metomidate can suppress parts of the biochemical pathway leading to cortisol synthesis (Ross and Ross 2008). Although fish sampled immediately (0 h) after sedation could be easily handled, sedation was required to collect blood samples at later time points. Thus, for the sake of consistency in sampling protocols and to maintain compliance with in-house standards regarding animal welfare, all fish were exposed to the secondary sedative prior to handling and blood sample collection (same protocol used by Bowzer et al. 2012; Gause et al. 2012; Trushenski et al. 2012a, 2012b, 2012c). After exposure to the metomidate hydrochloride bath, fish were weighed (to the

nearest 0.1 g) and measured (to the nearest 0.5 cm TL). Blood samples were then collected from the caudal vasculature using heparinized, evacuated blood collection assemblies (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey). To minimize the possibility of effects from handling and blood sampling stressors, all samples were collected within 5 min of exposure to the metomidate hydrochloride bath. Fish were also sampled during the course of our experiment (2 fish/2-h interval, total of six fish) from a reference population of untreated fish that had not been handled or otherwise stressed for at least 48 h before sampling. Each fish was sampled once (i.e., no repeated blood sampling was conducted on any individual), and all fish were returned to the same holding system after handling and were monitored for 48 h for survival. Given the size of the fish involved, repeated blood sampling was not feasible; regardless, repeated sampling of the same individual would likely have resulted in substantially skewed results reflecting the effects of repeated handling, not the sedatives tested.

Until analysis, blood samples were kept on wet ice (<6 h). Hematocrit (Statspin centrifuge, Fisher Scientific, Pittsburgh, Pennsylvania) and glucose (Freestyle Freedom Lite glucose meter, Abbott Laboratories, Abbott Park, Illinois) were immediately determined using whole blood samples. Samples were then centrifuged (3,000 × g, 4°C, 45 min), and the resulting plasma was stored at –80°C until further analysis. Lactate (Accutrend lactate meter, Roche, Mannheim, Germany), osmolality (Vapro 5520, Wescor, Logan, Utah), and cortisol (ELISA kit EIA 1887, DRG International, Mountainside, New Jersey) were determined using plasma samples. Although 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). The cortisol kit used has a range of 0–800 ng/mL with a sensitivity of 2.5 ng/mL for human samples, and this kit 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 all statistical analysis, sedation groups were used as the experimental unit ( $N = 3$ ), and differences were considered significant at  $P < 0.05$ . To determine whether differences in induction and recovery times among the sedative treatments were significant we used one-way ANOVA (PROC GLM) using the Statistical Analysis System, version 9.1 (SAS Institute, Cary, North Carolina). Although multiple blood samples were not collected from individual fish, multiple individuals were sampled over time from each sedation group. As a result, the associated data represented multiple observations made on the same experimental unit through time. Accordingly, blood chemistry data were analyzed using one-way, repeated measures ANOVA (PROC MIXED) with the Statistical Analysis System. When significant treatment and interaction effects were detected, we used post hoc Tukey's honestly significantly different (HSD) pairwise comparisons to determine where differences existed.

**RESULTS**

Induction times varied significantly (an asterisk indicates significant difference) among the sedative types (electrosedation\* < MS-222 = eugenol;  $F = 50.29$ ,  $df = 2$ ,  $P = 0.0002$ ; Figure 1). Pallid Sturgeon were induced most quickly using electrosedation ( $0.22 \pm 0.04$  min, mean  $\pm$  SE), whereas eugenol and MS-222 yielded mean induction times of  $2.33 \pm 0.26$  min and  $1.81 \pm 0.19$  min, respectively. Recovery of tactile response (electrosedation\* < MS-222 = eugenol;  $F = 62.74$ ,  $df = 2$ ,  $P < 0.0001$ ) and recovery of equilibrium (electrosedation\* < MS-222 = eugenol;  $F = 21.34$ ,  $df = 2$ ,  $P = 0.002$ ) also varied significantly among sedative treatments. In all treatments, fish regained tactile response before regaining equilibrium. Electro-sedated sturgeon regained tactile response ( $1.37 \pm 0.11$  min postsedation) and equilibrium ( $1.44 \pm 0.09$  min

postsedation) the quickest, followed by sturgeon treated with MS-222 (tactile =  $4.55 \pm 0.15$  min postsedation, equilibrium =  $4.71 \pm 0.16$  min postsedation) and fish treated with eugenol (tactile =  $5.42 \pm 0.25$  min postsedation, equilibrium =  $6.44 \pm 0.72$  min postsedation). Total handling time (induction time + total recovery time) also varied significantly among sedative treatments (electrosedation\* < MS-222 = eugenol). Total handling time was  $1.49 \pm 0.07$  min for electrosedation,  $4.71 \pm 0.16$  min for MS-222, and  $6.44 \pm 0.72$  min for eugenol.

Except for plasma osmolality, hematological variables (hematocrit, glucose, lactate, and cortisol) did not vary over time or among sedative treatments (Table 2; Figure 2). Fish sedated with MS-222 and eugenol had lower plasma

TABLE 2. Hematological responses of Pallid Sturgeon following sedation to stage IV of anesthesia using chemical sedatives or electrosedation. Values represent the mean  $\pm$  SE of triplicate samples at each time and treatment combination.  $P$ -values generated by the repeated-measures ANOVA are provided for each blood constituent, as are mean values for those of the reference population ( $n = 6$ ).

Time (h)	Sedative		
	Eugenol	MS-222	Electrosedation
<b>Plasma cortisol (ng/mL; reference population average: 2)</b>			
$P_{\text{Sedative}} = 0.541, P_{\text{Time}} = 0.466, P_{\text{Sedative} \times \text{Time}} = 0.458$			
0	8 $\pm$ 5	20 $\pm$ 5	2 $\pm$ 5
0.5	3 $\pm$ 5	2 $\pm$ 5	8 $\pm$ 5
1	2 $\pm$ 5	5 $\pm$ 5	4 $\pm$ 5
2	2 $\pm$ 5	2 $\pm$ 5	8 $\pm$ 5
6	1 $\pm$ 5	1 $\pm$ 5	6 $\pm$ 5
<b>Hematocrit (%; reference population average: 17)</b>			
$P_{\text{Sedative}} = 0.962, P_{\text{Time}} = 0.887, P_{\text{Sedative} \times \text{Time}} = 0.257$			
0	16 $\pm$ 2	18 $\pm$ 2	18 $\pm$ 2
0.5	19 $\pm$ 2	16 $\pm$ 2	19 $\pm$ 2
1	16 $\pm$ 2	19 $\pm$ 2	20 $\pm$ 2
2	20 $\pm$ 2	19 $\pm$ 2	18 $\pm$ 2
6	20 $\pm$ 2	20 $\pm$ 2	15 $\pm$ 2
<b>Plasma osmolality (mOsm/kg; reference population average: 237)</b>			
$P_{\text{Sedative}} = 0.054, P_{\text{Time}} = 0.955, P_{\text{Sedative} \times \text{Time}} = 0.738$			
0	229 $\pm$ 4	231 $\pm$ 4	244 $\pm$ 4
0.5	228 $\pm$ 4	237 $\pm$ 4	244 $\pm$ 4
1	228 $\pm$ 4	236 $\pm$ 4	241 $\pm$ 4
2	231 $\pm$ 4	234 $\pm$ 4	239 $\pm$ 4
6	232 $\pm$ 4	237 $\pm$ 4	237 $\pm$ 4
<b>Blood glucose (mg/dL; reference population average: 41)</b>			
$P_{\text{Sedative}} = 0.905, P_{\text{Time}} = 0.270, P_{\text{Sedative} \times \text{Time}} = 0.043$			
0	42 $\pm$ 5.34	44 $\pm$ 5.34	52 $\pm$ 5.34
0.5	56 $\pm$ 5.34	58 $\pm$ 5.34	45 $\pm$ 5.34
1	64 $\pm$ 5.34	45 $\pm$ 5.34	52 $\pm$ 5.34
2	50 $\pm$ 5.34	50 $\pm$ 5.34	58 $\pm$ 5.34
6	51 $\pm$ 5.34	54 $\pm$ 5.34	51 $\pm$ 5.34

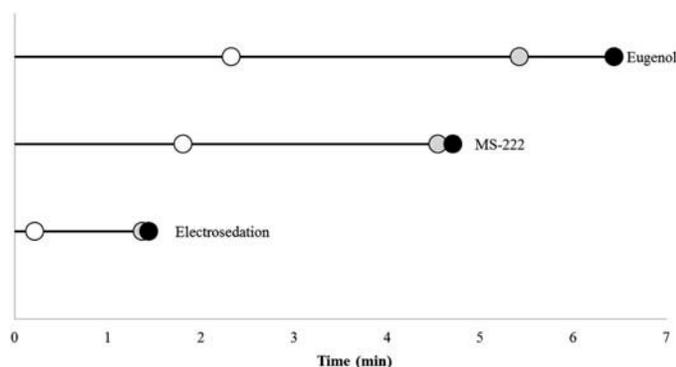


FIGURE 1. Schematic illustrating induction (white circles) of Pallid Sturgeon and recovery of responsiveness to tactile stimuli (gray circles) and equilibrium (black circles) following sedation with eugenol, tricaine methanesulfonate (MS-222), or electrosedation.

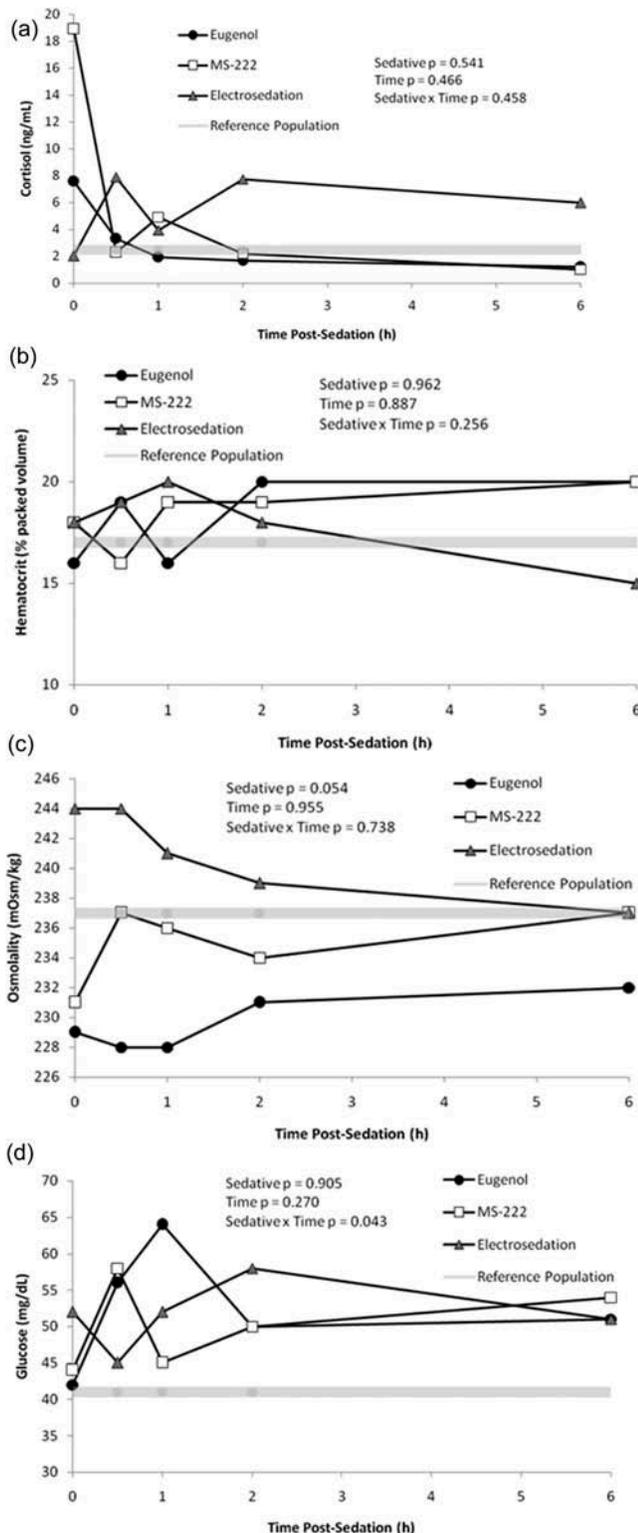


FIGURE 2. Time course of hematological responses—(a) cortisol, (b) hematocrit, (c) osmolality, and (d) glucose—of Pallid Sturgeon following sedation with eugenol, tricaine methanesulfonate (MS-222), or electrosedation. Points represent means reported in Table 2; gray reference bars represent means of values observed for sturgeon sampled from the reference population throughout the experiment.

osmolality concentrations whereas electrosedated fish had slightly elevated concentrations compared with the reference population. Regardless, these deviations were largely resolved within 6 h postsedation. Only plasma glucose was influenced by a significant interaction effect, indicating differences in the way sedative treatment affected this variable over time. Despite nonsignificant main effects, the interaction suggested a slight, transient increase in glucose after sedation among fish treated with eugenol and MS-222 and an opposite effect among electrosedated fish.

No mortalities were observed among the 51 Pallid Sturgeon (3 sedative treatments  $\times$  3 replicate groups/treatment  $\times$  5 fish/group = 45 experimental fish; 6 fish sampled from the reference population) used throughout this study.

## DISCUSSION

Using the treatment protocols we evaluated, pulsed-DC electricity (delivered via the portable electroanesthesia system), eugenol (as AQUI-S 20E), and MS-222 (as Finquel) effectively sedated Pallid Sturgeon, and each of the individuals recovered successfully after sedation. In all treatments, electrosedation resulted in the fastest induction and recovery times, followed by MS-222 and eugenol. These differences resulted in total handling times of approximately 1.5 min or less for electrosedated fish versus ranges of 4.5–5 min or 5.5–8 min for those sedated with MS-222 or eugenol, respectively. Although these differences might be of practical relevance in some cases, most fisheries scientists would likely consider all observed induction, recovery, and total handling times acceptable under most circumstances.

Sedation methods and results have not been extensively reported for Pallid Sturgeon; however, the induction and recovery times we observed are broadly consistent with those reported for other species of sturgeon. Clove oil (containing eugenol and other constituents [isoeugenol and methyl-eugenol] with sedative properties in fish) yielded induction times ranging from 3 to 6 min in juvenile and subadult White Sturgeon *Acipenser transmontanus*; recovery times ranged from 3.0 to 3.5 min postsedation for the subadults and 4.5–7.0 min postsedation for the juveniles (Taylor and Roberts 1999). Persian sturgeon *A. persicus* exposed to a 200-mg/L solution of clove oil had induction times ranging from 2.3 to 2.8 min and recovery times ranging from 2.4 to 3.0 min postsedation (Imanpoor et al. 2010). Atlantic Sturgeon *A. oxyrinchus* became sufficiently sedated to allow for completion of surgical procedures within 4–8 min when exposed to a 100-mg/L solution of MS-222 and recovered within 3–8 min postsedation (Balazik et al. 2013). Some of these induction times are longer than those reported for a variety of teleosts sedated with chemical sedatives (Trushenski et al. 2012a, 2012b, 2012c; Bowker et al. 2015). However, induction and recovery times are strongly influenced by sedative dose and water temperature, not taxonomic grouping (Bowker et al.

2015). Faster sedation of Pallid Sturgeon is likely possible with higher concentrations of MS-222 and eugenol, though fisheries professionals should exercise due caution when using higher sedative doses as they may result in longer recovery periods and pose a greater mortality risk (Taylor and Roberts 1999; Imanpoor et al. 2010; Akbulut et al. 2011). Although electrosedation of sturgeons is not widely reported in the literature, our observations of faster induction and recovery times among electrosedated fish have been reported for a number of other taxa (Bowzer et al. 2012; Gause et al. 2012; Trushenski and Bowker 2012; Trushenski et al. 2012a, 2012b, 2012c), including Atlantic Sturgeon (Balazik et al. 2013). Though electrical exposure has been associated with mortality and other negative consequences under some circumstances (typically involving higher voltages or exposure durations), the absence of observations suggesting any such damage in the present work is encouraging.

Even though sedatives are commonly used to minimize stress in fish (Sandodden et al. 2001; Finstad et al. 2003; Wagner et al. 2003; Cooke et al. 2004; Small 2004; Palić et al. 2006), trials similar to the present one and others have shown that sedation itself can elicit a generalized stress response. Changes in cortisol levels (Davidson et al. 2000; Wagner et al. 2002; Davis and Griffin 2004; King et al. 2005; Zahl et al. 2010; Trushenski et al. 2012a, 2012b, 2012c), plasma glucose (Bourne 1984; Bernier and Randall 1998; Sladky et al. 2001; Cho and Heath 2000; Wagner et al. 2002; Trushenski et al. 2012a, 2012b, 2012c), hematocrit (Sladky et al. 2001; Cho and Heath 2000; Trushenski et al. 2012a, 2012b, 2012c), and osmolality (Bourne 1984; Trushenski et al. 2012a, 2012b, 2012c) have been noted following sedation. Our observations of Pallid Sturgeon blood chemistry following sedation are somewhat inconsistent with these reports. Few significant differences were observed, and the magnitude of these responses was comparatively small. Fish displaying lower stress responses are not necessarily “less stressed,” and comparing hematological responses among species has been cautioned against (Barton 2002). In particular, sturgeons can have lower physiological responses to stressors than teleosts, and Pallid Sturgeon have exhibited even lower responses than other sturgeon species (Barton et al. 2000; Kieffer et al. 2001; Baker et al. 2005; Webb et al. 2007). For example, in a study comparing the cortisol response of Atlantic Sturgeon to surgical procedures conducted with or without preoperative sedation or anesthesia, only surgery without sedation yielded a cortisol response that was significantly different from that of control fish, which were only handled and not subjected to any surgical procedure; statistical significance aside, the magnitude of the difference was nonetheless quite small (i.e., maximum response to surgery without sedation 1 h postoperation was ~12 ng/mL, response to handling only was ~2 ng/mL; Balazik et al. 2013). In a sense, perhaps our results are indeed consistent with the responses of other fishes to sedation, albeit in the “low responder” context of sturgeons.

Persian Sturgeon sedated with clove “essence” and Adriatic Sturgeon *A. naccarii* sedated with MS-222 had mean glucose levels that ranged from 41 to 61 mg/dL (Cataldi et al. 1998; Imanpoor et al. 2010). Previous stress studies on Pallid and Shovelnose sturgeons have found glucose levels to range from 46 to 96 mg/dL in adults and 49–64 mg/dL in juveniles (Barton et al. 2000; Webb et al. 2007). Glucose levels in this study were within range of those previously reported values (Table 2; Figure 2). Cortisol levels of the Pallid Sturgeon were lower than levels in Adriatic Sturgeon sedated with MS-222 (range, 12–30 ng/mL; Cataldi et al. 1998) and comparable with those of stressed Pallid Sturgeon (range, 1–15 ng/mL; Barton et al. 2000; Webb et al. 2007; Haukenes et al. 2008; Figure 2a). “Typical” hematocrit and osmolality values have not been extensively reported in Pallid Sturgeon. Nonetheless, the ranges of these two blood variables (hematocrit range, 15–20%; osmolality range, 228–244 mOsm/kg; Figures 2b, c) were similar to those previously reported in other sturgeon species. In particular, juvenile Beluga *Huso huso* (also known as Great Sturgeon) have hematocrit levels of 14–32% (Falahatkar and Barton 2007; Bani et al. 2009), and juvenile Shortnose Sturgeon *A. brevirostrum* have hematocrit levels of 22–28% and osmolality levels of 232–295 mOsm/kg (Baker et al. 2005; Beyea et al. 2005; Knowles et al. 2006). Adult Lake Sturgeon *A. fulvescens* have hematocrit levels of 15–40% and osmolality concentrations of 207–318 mOsm/kg (Baker et al. 2008). Given the information available, it seems likely that sturgeons, including Pallid Sturgeon, exhibit an acute, transient stress response following sedation; however, the magnitude of this response is markedly smaller than that typically observed in teleosts following sedation. It is also possible that the response of sturgeons to stressors manifests differently than in teleosts and other variables may be better suited to quantifying their physiological responses.

Choosing the most appropriate sedative will depend on the circumstances (e.g., field versus laboratory setting, species and lifestage of fish to be sedated, ease of holding fish during withdrawal periods). In the case of Pallid Sturgeon, other considerations may apply. For example, the Pallid Sturgeon Recovery Team (<http://www.pallidsturgeon.org/>) advises against using MS-222 due to the possible adverse effects on Pallid Sturgeon (Wanner 2006). Given the allowed uses of chemical sedatives and the physiological effects, withdrawal periods, and induction and recovery times associated with these products, electrosedation may be a practical alternative to chemical sedatives for handling Pallid Sturgeon in the field. All of the sedative protocols we tested appeared safe to Pallid Sturgeon, although a definitive claim of safety to fish would entail, for example, testing higher doses and/or chronic exposures and a histological evaluation of target tissues. However, safety in a practical sense appears evident, given that no mortalities were observed and none of the sedatives evaluated induced osmoregulatory failure or elicited other responses one would typically associate with morbidity or delayed mortality.

Our hematological data suggest that stress responses occur in Pallid Sturgeon following sedation, but these, as in other fish, are acute and transient. Thus, issues of convenience aside, each of the sedative options evaluated appeared to be effective in sedating Pallid Sturgeon.

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