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North American Journal of Fisheries Management

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/ujfm20>

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Published online: 10 Dec 2013.

To cite this article: Carlin M. Fenn, David C. Glover & Brian C. Small (2013) Efficacy of AQUI-S 20E as a Sedative for Handling and Cortisol Suppression in Pallid Sturgeon, North American Journal of Fisheries Management, 33:6, 1172-1178, DOI: [10.1080/02755947.2013.831000](https://doi.org/10.1080/02755947.2013.831000)

To link to this article: <http://dx.doi.org/10.1080/02755947.2013.831000>

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ARTICLE

Efficacy of AQUI-S 20E as a Sedative for Handling and Cortisol Suppression in Pallid Sturgeon

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Abstract

Challenges and regulations associated with handling fish during fisheries management activities have increased efforts to achieve U.S. Food and Drug Administration approval for an immediate-release sedative for fish. The objectives of this study were to (1) identify the target concentration of AQUI-S 20E for sedation of Pallid Sturgeon *Scaphirhynchus albus* to a handleable state followed by rapid recovery, and (2) to compare sedation and recovery times and cortisol stress response in sturgeon treated with the identified target concentration to those treated with MS-222. Juvenile Pallid Sturgeon from two size-classes were exposed to five concentrations of AQUI-S 20E: 70, 153, 364, 598, and 779 mg/L of water. The target concentrations of AQUI-S 20E for optimal sedation (<3 min) and recovery times (<5 min) identified for Pallid Sturgeon were 476 mg/L for small and 537 mg/L for large size-classes. Cortisol secretion in relation to presedation plasma cortisol levels was suppressed at the AQUI-S 20E target concentration of 500 mg/L but was not for MS-222. These data suggest an AQUI-S 20E concentration of 500 mg/L is efficacious while blocking the cortisol stress response for sedation to a handleable state in Pallid Sturgeon.

Both wild and hatchery-reared fish undergo stressors through practices common to fisheries management, such as capture, handling, transportation, tissue collection, tagging procedures, surgical operations, and artificial spawning. These stressors can be especially detrimental to biologically sensitive species such as Pallid Sturgeon *Scaphirhynchus albus* (Barton et al. 2000) and may lead to immune suppression, decreased survivorship, and reduced growth rates, among others. Activities involving handling and transportation of fish can be better facilitated through the immobilizing actions of aquatic sedatives, which also have the potential to reduce associated fish stress and tissue injuries (Iversen et al. 2003; Di Marco et al. 2011).

An ideal aquatic sedative induces rapid sedation to a handleable state (<3 min) and recovery (<5 min) and also minimizes hyperactivity and stress responses from treated fish (Marking and Meyer 1985). Additional variables to be considered when selecting an ideal aquatic sedative are its regulation status, commercial availability, delivery method, cost efficiency, environmental impact, and consumer safety (Akbulut et al.

2012). Currently, one of the most widely used chemical sedatives for aquatic species is tricaine methanesulfonate (MS-222; Marking and Meyer 1985; Popovic 2012), and it is the only U.S. Food and Drug Administration (FDA) approved chemical sedative for use with fish having the potential for human consumption. However, MS-222 has a mandatory 21-d withdrawal period for human consumption. Withdrawal periods make the use of sedatives often impossible for fisheries managers who often need to immediately release the fish with which they are working.

In addition to inducing rapid sedation and recovery, an ideal chemical sedative would suppress handling stress by blocking activation of the hypothalamic-pituitary-interrenal (HPI) axis, thus reducing the release of plasma cortisol, which is a blood indicator of physiological stress response in fish (Barton 2002; Small 2003, 2004; Webb et al. 2007). Following sedation, MS-222 has been shown to increase plasma cortisol levels in a variety of fish species, including Brown Trout *Salmo trutta* (Pickering et al. 1982), Channel Catfish *Ictalurus punctatus* (Small 2003), Striped Bass *Morone saxatilis* (Davis et al. 1982), Red Drum

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Received February 6, 2013; accepted July 24, 2013

Sciaenops ocellatus (Thomas and Robertson 1991), and Fathead Minnow *Pimephales promelas* (Palić et al. 2006). These findings suggest that MS-222 activates the HPI axis and the pathway by which cortisol is released (Barton 2002). Moreover, because of increased risk of mortality, MS-222 is not recommended for use in certain species, including Pallid Sturgeon (Wanner 2006).

Making its Jurassic debut approximately 70 million years ago, the Pallid Sturgeon is a phylogenetically ancient bony fish species (Gardiner 1984; Krieger and Fuerst 2002) indigenous to the North American Mississippi and Missouri river systems. Anthropogenic activities spanning the past 100 years, including overfishing, pollution, impoundment, damming, and channelization of rivers, have dramatically altered their native habitats (Duffy et al. 1995; Mayden and Kuhajda 2002). Consequently, Pallid Sturgeon populations and catch rates declined, and the species was listed as federally endangered in 1990 (55 Federal Register 36641–36647). In response to this decline, recovery efforts were implemented through health-risk assessments, habitat and wild populations monitoring, supplemental stocking, and artificial propagation of wild and hatchery-reared fish (Webb et al. 2005). As previously mentioned, such activities can prove stressful to any fish but are particularly concerning for imperiled species. Given the limited available chemical sedative options, it is pertinent to evaluate a chemical sedative for use in Pallid Sturgeon and to identify the concentration that induces both rapid sedation and recovery, as well as suppresses the physiological stress reaction.

Identification of an ideal chemical sedative for Pallid Sturgeon could serve as a potential model for use among other sturgeon species. Because sturgeon populations are in critical decline throughout the Northern Hemisphere, many species are artificially propagated in hatcheries with the goal of increasing wild stocks (Webb et al. 2005). Additionally, as wild stocks continue to decrease, sturgeon are becoming more prevalent in culture conditions where they are reared for their prized meat and caviar (Wei et al. 2004; Di Marco et al. 2011). As such, commercial sturgeon operations would also benefit from an immediate-release chemical sedative alternative.

Both isoeugenol and eugenol, active ingredients found in clove oil, have been observed to sedate and suppress stress-related cortisol levels in some fish species (Iversen et al. 2003; Small and Chatakondi 2005; Roubach et al. 2005; Palić et al. 2006). A relatively new aquatic chemical sedative composed of 10% eugenol (active ingredient), AQUI-S 20E (AQUI-S New Zealand, Ltd.) has been authorized by the U.S. FDA as an immediate-release sedative for field use under U.S. Fish and Wildlife Service (USFWS) Investigational New Animal Drug (INAD) 11–741. Under this INAD, hatchery use requires a 72-h withdrawal period before treated fish can be harvested or released. With this authorization comes the need to establish specific guidelines for use. The objectives of this study were to (1) identify the target concentration of AQUI-S 20E for sedation of Pallid Sturgeon to a handleable state followed by rapid recovery, and (2) to compare sedation and recovery times

and stress response in sturgeon treated with the identified target concentration to those treated with MS-222. Plasma cortisol was measured to evaluate stress response because it is the primary glucocorticoid indicative of stress in sturgeon (Sangalang 1971; Webb et al. 2007).

METHODS

Test fish.—All Pallid Sturgeon were handled in accordance with approval from the Institutional Animal Care and Use Committee at Southern Illinois University Carbondale (SIUC) under Protocol 11–029. The sturgeon were obtained as fry from Gavins Point National Fish Hatchery (Yankton, South Dakota) and cultured for 12 months at the Center for Fisheries, Aquaculture, and Aquatic Sciences at SIUC. Three weeks before the experiment, 96 fish were graded into two weight-based size-classes (mean \pm SD), 71 \pm 24 g (small) and 274 \pm 61 g (large), and stocked into six 1,514-L tanks in a water recirculating aquaculture system. Size variation within cohorts is typical for this species under common culture conditions. Fish were then cultured under a 12 light : 12 dark photoperiod and fed a 50% protein starter feed (Aquamax Fry Starter, Purina Mills, LLC, Richmond, Indiana) once daily during the 3-week acclimation period. Feed was withheld 24-h before the experiment. Experimental fish were not used for other purposes before this study.

Experimental design.—For the first objective, five concentrations of AQUI-S 20E (70, 153, 364, 598 and 779 mg/L of water) were evaluated to induce rapid sedation (<3 min) and recovery (<5 min) of the large and small Pallid Sturgeon size-classes. Solutions of AQUI-S 20E were prepared according to the USFWS Aquatic Animal Drug Approval Program's Suggested Guidelines for Preparing and Using AQUI-S 20E as a Sedative (<http://www.fws.gov/fisheries/aadap/AQUIS-E.HTM>), which is based on intended eugenol concentrations of 10, 20, 40, 60, and 80 mg/L of water. The general average found optimal for both size-classes, 500 mg/L of AQUI-S 20E, was further evaluated to address the second objective. In the second experiment, the plasma cortisol response of fish treated with 500 mg/L of AQUI-S 20E was compared with the response of fish treated with a solution of 100 mg/L MS-222 (TRICAINES; Western Chemical Inc., Ferndale, Washington) as a commercial control. In both experiments, a treatment group of eight fish from each size-class was randomly selected and sedated in groups of four (eight/size-class per treatment) in a well-aerated sedation chamber (8.55 mg/L dissolved oxygen; 22.5°C; YSI 550A dissolved oxygen meter; YSI Inc., Yellow Springs, Ohio). The treatment bath was prepared by dissolving the appropriate concentration of AQUI-S 20E or bicarbonate-buffered MS-222 (100 mg/L) into 37.8 L culture water (temperature, 22.5°C; pH, 8.1; alkalinity = 120 mg/L; total ammonia nitrogen, 0.02 mg/L; and nitrite nitrogen, 0.03 mg/L; LaMotte Company spectrophotometer and reagents; Chestertown, Maryland). Water samples were collected after each treatment group, and the actual concentration of eugenol was measured with a spectrophotometer

(Nanodrop 2000; Thermo Scientific, Wilmington, Delaware) according to Aquatic Animal Drug Approval Partnership SOP MISC 243.0. Regression analysis indicated AQUI-S 20E concentrations did not decrease over time ($P > 0.1$).

Sturgeon were netted from their holding tanks and within 2 min, 0.2 mL of blood was collected from the caudal vasculature. Each group of four fish was collected from a different holding tank to avoid the cumulative effects of stress that can occur due to capture avoidance during successive netting. Fish were then exposed to their respective sedative concentration for a maximum of 30 min to achieve a handleable state, which is defined as loss of balance and ability to self-right and respond to external stimuli (Summerfelt and Smith 1990). Because of the morphology and benthic nature of Pallid Sturgeon, fish were manually flipped over by the observer to assess their inability to maintain equilibrium. Once the fish were unable to self-right, they were considered sedated. To improve precision, one observer was responsible for evaluating handleability and recovery assessments. Time to induction was recorded, and fish were immediately bled after sedation to collect 0.2 mL of blood for postsedation plasma cortisol analysis.

After fish were bled, they were transferred into individual, aerated recovery tanks. Recovery time was defined as the time for individual fish to regain equilibrium and respond to external stimuli. Immediately following recovery, each fish was bled to quantify postrecovery plasma cortisol. Fish were then restocked into holding tanks and monitored for 24 h. There were no mortalities in experimental fish resulting from this study.

Plasma collection and cortisol analysis.—For all samples, blood was collected from the caudal vasculature into heparinized syringes and stored on ice (<3 h) until they could be processed. Blood was centrifuged ($3,000 \times g$, 10 min, 4°C), and the resultant plasma was stored at -20°C . Plasma cortisol was measured by fluoroimmunoassay (following Small and Davis 2002) and validated for Pallid Sturgeon. The displacement curve for cortisol standards was parallel to the displacement curve of serially diluted plasma from Pallid Sturgeon ($P > 0.05$). Intra-assay and inter-assay coefficients of variation were less than 10%. Accuracy, calculated as the percent of exogenous cortisol recovered from spiked plasma, was greater than 95%.

Statistical analyses.—To determine the target AQUI-S 20E concentration for sedation of Pallid Sturgeon, ANCOVA was used to determine whether time to sedation and recovery were affected by AQUI-S 20E concentration and whether size-class (i.e., small and large) affected either the slope or elevation of this relationship. All continuous variables (i.e., time to sedation, time to recovery, and AQUI-S 20E concentration) were \log_e -transformed to meet the assumptions of ANCOVA and were then back-transformed for interpretation purposes. The AQUI-S 20E concentration required to achieve both a 3-min time to sedation and a 5-min recovery time following sedation, based on recommendations by Marking and Meyer (1985), was evaluated using contrast statements from the final models.

When determined, the efficacy of the target AQUI-S 20E concentration was compared with the standard concentration of MS-222 in terms of time to sedation, time to recovery, as well as postsedation and postrecovery plasma cortisol levels for small and large Pallid Sturgeon. The effect of anesthetic type, size-class, and the interaction between anesthetic type and size-class on time to sedation and time to recovery following sedation was analyzed using ANOVA. Time to sedation and time to recovery were \log_{10} -transformed to meet assumptions of ANOVA and were then back-transformed for interpretation purposes. Differences in cortisol responses between fish treated with AQUI-S 20E and MS-222 were determined by ANCOVA, controlling for differences in initial plasma cortisol levels (i.e., before treatments). Specifically, we tested whether the slope or elevation of the relationships between either postsedation or postrecovery plasma cortisol levels and initial cortisol levels depended on anesthetic type, size-class, or the interaction between anesthetic type and size-class. A backward model selection procedure was used to omit insignificant terms ($P > 0.05$) to determine the best model; only terms not contained in a higher-order interaction were considered for omission.

Individual fish were treated as the experiment unit in all analyses because there were no differences in (1) initial cortisol levels among tanks ($F_{1,24} = 0.50$, $P = 0.80$), (2) postsedation cortisol levels among tanks ($F_{6,16} = 0.51$, $P = 0.79$), (3) the relationships between postsedation cortisol levels and initial cortisol levels among tanks ($F_{6,16} = 0.52$, $P = 0.79$), (4) postrecovery cortisol levels among tanks ($F_{1,16} = 0.81$, $P = 0.57$), and (5) the relationships between postrecovery cortisol levels and initial cortisol levels ($F_{6,16} = 1.38$, $P = 0.28$). In terms of time to sedation and time to recovery, the design of the study made it impossible to separate the effect of tank from size. However, given the similarities among tanks with respect to cortisol levels throughout the study, we argue that treating each individual as the experimental unit is justified. Statistical analyses were performed using Statistical Analysis Systems software (SAS 2008) and significance was set at $\alpha = 0.05$ in all cases.

RESULTS

Target AQUI-S 20E Concentration

All Pallid Sturgeon, regardless of size-class, were sedated by AQUI-S 20E within 7.4 min at 364 mg/L, 3.1 min at 598 mg/L, and 2.8 min 779 mg/L. After 30-min exposure, neither large nor small sturgeon were sedated at an AQUI-S 20E concentration of 70 mg/L, and only 50% were sedated at 153 mg/L. After omitting fish that did not become sedated, ANCOVA indicated that the rate at which time to sedation (natural log scale) changed with AQUI-S 20E concentration was not different between size-classes ($F_{1,52} = 1.99$, $P = 0.16$); thus, the size-dependent slope effect was omitted from the model. The final model ($R^2 = 0.87$) indicated that \log_e time to sedation decreased with increasing concentrations of AQUI-S 20E ($F_{1,53} = 339.00$, $P < 0.001$); the slope of this relationship was common to both size-classes,

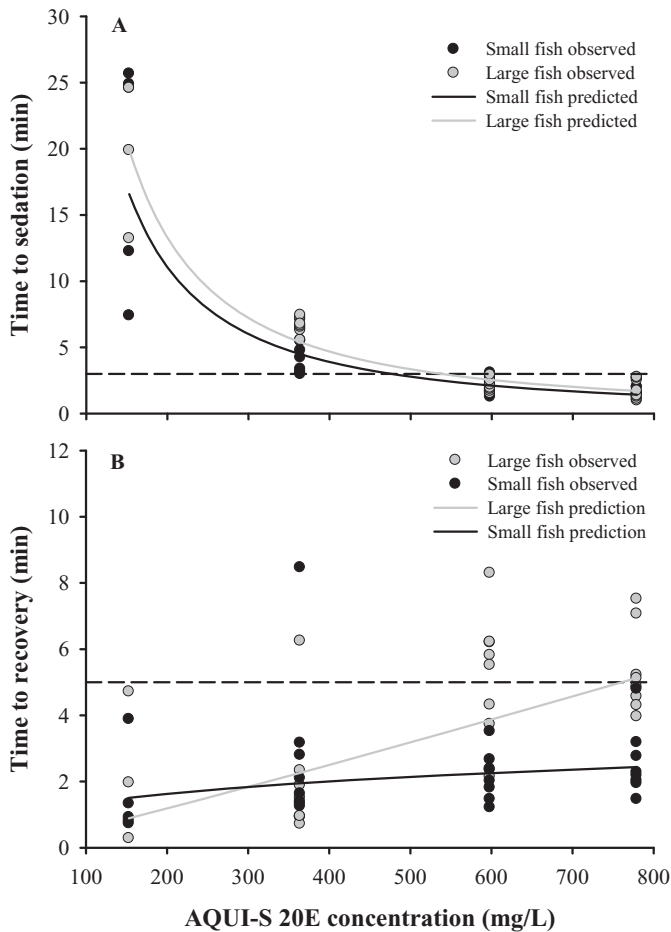


FIGURE 1. (A) Time to sedation (dashed line is the minimum time to sedation) and (B) time to recovery (dashed line is the maximum time to recovery) for small and large Pallid Sturgeon plotted as a function of AQUI-S 20E concentration. Fish that did not sedate within the 30-min trial period at 70 mg/L and 153 mg/L were not included.

as evidenced by the preceding test of parallelism. Although the relationship between \log_e time to sedation and AQUI-S 20E concentration was statistically parallel between the two size-classes, there were differences in the intercept values, or the elevation, between size classes ($F_{1,53} = 4.37, P = 0.04$; Figure 1A). Specifically, large fish required a slightly longer time to become sedated at any given AQUI-S 20E concentration ($t_{53} = 2.09, P < 0.05$). The target concentration required to, on average, sedate within 3 min was 476 mg/L (95% CI = 2.65–3.40 min) for small and 537 mg/L (CI = 2.64–3.41 min) and large Pallid Sturgeon.

Size-class also affected the recovery time following sedation ($F_{1,52} = 7.06, P = 0.01$), such that small Pallid Sturgeon were predicted to have a shorter recovery time than large fish at lower AQUI-S 20E concentrations and a longer recovery time at higher concentrations (Figure 1B). The AQUI-S 20E concentration predicted to sedate small and large Pallid Sturgeon within 3 min would also result in recovery times less than 5 min. Specif-

ically, small fish were predicted to recover within 3.01 min (95% CI = 2.41–3.77 min) following sedation at 476 mg/L and large fish to recover within 2.18 min (CI = 1.73–2.76 min) following sedation at 537 mg/L.

Comparisons of AQUI-S 20E and MS-222

Time to sedation and recovery.— \log_{10} time to sedation was different between fish treated with AQUI-S 20E and MS-222 ($F_{1,28} = 6.37, P = 0.02$), but the main effect of size-class and its interaction with treatment did not have an effect on time to sedation ($F_{1,28} \leq 3.15, P \leq 0.09$). Fish treated with AQUI-S 20E became handleable faster than those treated with MS-222 ($t_{28} = -2.52, P = 0.02$). The back-transformed mean time to sedation for fish treated was 2.33 min (SE = 1.06) for AQUI-S 20E and was 2.89 min (SE = 1.06) for MS-222.

\log_{10} time to recovery was not different between fish treated with the target concentration of AQUI-S 20E and MS-222 ($F_{1,28} = 0.24, P = 0.63$), but there were differences in time to recovery between small and large fish ($F_{1,28} = 11.41, P = 0.002$). Specifically, small fish required 1.32 min longer to recover than large fish ($t_{28} = -3.38, P = 0.002$) and these differences were similar between AQUI-S 20E and MS-222 treatments ($F_{1,28} = 2.30, P = 0.14$). On average, small Pallid Sturgeon required 2.69 min (SE = 1.15) to recovery following sedation, and large fish required 1.37 min (SE = 1.15).

Initial cortisol level comparison.— \log_{10} initial cortisol levels were different between Pallid Sturgeon that were subsequently treated with the target concentration of AQUI-S 20E and MS-222 ($F_{1,30} = 61.65, P = 0.0003$); the main effect of size-class and its interaction with treatment did not have an effect on initial cortisol levels ($F_{1,28} \leq 2.01, P \geq 0.17$). As such, it was crucial to control for differences in initial cortisol levels in ANCOVA tests for differences in postsedation and postrecovery cortisol levels between treatments.

Postsedation and postrecovery cortisol levels.—The relationships between both postsedation and postrecovery cortisol levels and initial cortisol levels were different between treatments ($F_{1,28} \geq 9.97, P \leq 0.004$). Specifically, postsedation cortisol levels increased at a rate of 0.85 and postrecovery at 0.80 ng/mL per unit increase in initial cortisol levels for the MS-222 treatment ($t_{28} \geq 3.74, P \leq 0.0008$), whereas both the postsedation and postrecovery cortisol levels for the AQUI-S 20E treatment did not increase with initial cortisol levels ($t_{28} \leq 0.39, P \geq 0.70$). The average postsedation cortisol level for fish sedated using AQUI-S 20E was 1.70 ng/mL (SE = 0.14) and was 1.76 ng/mL (SE = 0.17) for postrecovery regardless of initial cortisol level. This suggests that AQUI-S 20E suppressed postsedation and postrecovery cortisol levels compared with initial cortisol levels, particularly for fish with higher initial cortisol levels (Figure 2).

The slopes between both postsedation and postrecovery cortisol levels and initial cortisol levels for MS-222 did not vary from a 1:1 relationship ($P < 0.05$), which suggests that MS-222 did not significantly reduce cortisol levels in Pallid Sturgeon following sedation or recovery. Additionally, the intercept value for

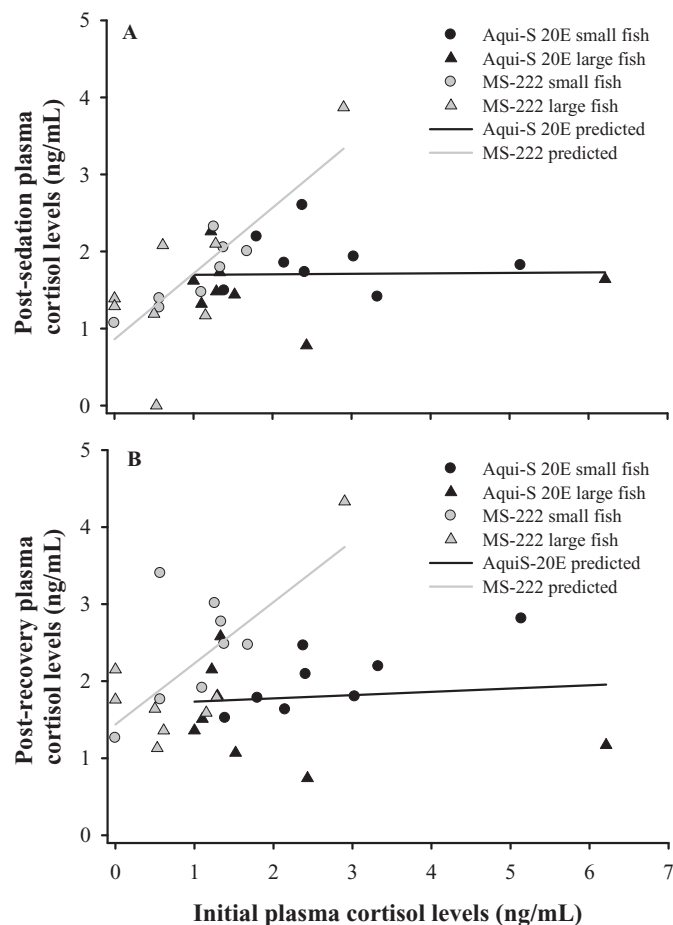


FIGURE 2. (A) Postsedation and (B) postrecovery plasma cortisol levels plotted as a function of initial plasma cortisol levels (i.e., presedation cortisol levels) for Pallid Sturgeon sedated with 500 mg/L AQUI-S 20E versus 100 mg/L MS-222. Small versus large size did not have an effect of cortisol levels.

the MS-222 treatment was >0 for the postsedation and postrecovery cortisol levels ($t_{28} \geq 5.70$, $P < 0.0001$), which indicated that fish having low initial cortisol levels that were sedated using MS-222 actually had higher postsedation and postrecovery cortisol levels than their initial level. We also found that the main effect of size-class and its two-way and three-way interactive effects with initial cortisol levels and treatment did not affect postsedation or postrecovery cortisol levels ($F_{1,25} \leq 3.60$, $P \geq 0.07$).

DISCUSSION

Despite the frequent use of chemical sedatives in hatchery and field management practices, their effects on Pallid Sturgeon and the resulting physiological stress responses following treatment have not been previously evaluated. The results of this study indicate AQUI-S 20E to be efficacious in sedating Pallid Sturgeon to handleable levels within the identified target concentrations of 476 mg/L for small and 537 mg/L for large fish. These concentrations resulted in the desired sedation

(<3 min) and recovery (<5 min) times that correspond to criteria for an ideal aquatic sedative, as defined by Marking and Meyer (1985). The lowest (70 mg/L) of the five evaluated concentrations of AQUI-S 20E evaluated is not recommended for use in Pallid Sturgeon because it was not successful in sedating any fish in either size-class within the allotted 30 min period. Furthermore, two other concentrations (153 and 364 mg/L) are also not suggested for use because only 50% of sturgeon were sedated in both size-classes and sedation periods longer than 5 min were required (7.4 min for 364 mg/L).

Though higher concentrations (598 and 779 mg/L) of AQUI-S 20E were found to induce sedation to handleable more rapidly in both size-classes, recovery and the abilities to respond to external stimuli took much longer. However, no mortalities were observed in any sturgeon among the different concentrations; therefore, it could be concluded that while these higher concentrations of AQUI-S 20E resulted in recovery >5 min, they are still considered safe for use in the size-classes evaluated.

Compared with MS-222, fish sedated with AQUI-S 20E became handleable more quickly, but the recovery time was the same for both chemicals. The ability of AQUI-S 20E to coat anatomical structures could result in shorter induction and longer recovery because the physical properties of eugenol allow it to persist on gill epithelia (Sladky et al. 2001). Shorter induction times for small fish may be related to greater body surface area and gill epithelia relative to total body volume, therefore becoming sensitive to the sedative more quickly than larger fish and, as a result of heightened sensitivity, requiring more time to recover (Treves-Brown 2000). These previous findings might account for the similarity in recovery times between AQUI-S 20E and MS-222, despite the shorter time to sedation with AQUI-S 20E.

Many biotic and abiotic factors can affect sedative efficacy: fish species, life stage, health, nutritional status, seasonal changes in physiology, and water quality (Sylvester et al. 1982; Josa et al. 1992; Tsantilas et al. 2006). Results of our study indicate differences between size-classes; larger fish generally require more time to achieve handleability, and smaller fish treated with AQUI-S 20E and MS-222 require more time to recover. These findings are consistent with past studies evaluating the differences of chemicals on induction and recovery times among different size-classes of fish (Small 2003; Zahl et al. 2011).

Although rapid induction and recovery are key components of an ideal sedative, stress suppression is an important additional factor to consider when evaluating an appropriate chemical sedative. After correcting for any differences relative to initial plasma cortisol levels between the AQUI-S 20E and MS-222 treatments resulting from biological variability, the present results indicate AQUI-S 20E suppressed the acute stress response in Pallid Sturgeon because postsedation and postrecovery cortisol levels did not increase from initial cortisol levels. Conversely, MS-222 increased cortisol levels postsedation and postrecovery, even in fish with low initial cortisol levels. Past

studies evaluating the cortisol response following sedation of sturgeon with MS-222 further supports these findings (Di Marco et al. 2011; Feng et al. 2011; Matsche 2011). Identifying the physiological changes that occur following sedation is especially imperative when sedating valuable and endangered fish species or when the procedure requiring sedation is particularly invasive, such as surgery (Matsche 2011).

Because AQUI-S 20E is a relatively new chemical sedative, there are few studies regarding the efficacy to sedate and suppress stress in fish. Published findings are starting to emerge regarding target concentration identification and are expected to increase, especially following the advent of the FDA-authorization for field use as an immediate-release sedative under USFWS INAD 11-741. Gause et al. (2012) observed that, although Grass Carp *Ctenopharyngodon idella* sedated with AQUI-S 20E experienced a greater plasma cortisol response than the reference population, acute (<0.5 h) response of plasma cortisol was much less (<75 ng/mL) than fish treated with MS-222 (>150 ng/mL). Further research is needed to address cortisol suppression effects in other species.

Elevated Pallid Sturgeon plasma cortisol levels in our study were mostly <4.0 ng/mL. The physiological stress response of Pallid Sturgeon is considered to be lower than for other sturgeon species and teleosts (Barton 2002; Barton et al. 2000; Webb et al. 2007). For example, resting levels of plasma cortisol in Pallid Sturgeon have been identified in the range of 0.67–3.0 ng/mL (Barton et al. 2000, Webb et al. 2007). The initial levels observed in our study fall in the middle of this range, with increased levels in MS-222 treated fish. Plasma cortisol levels of stressed Pallid Sturgeon have been observed around 3.0 ng/mL at 1 h following 30 s of handling and at 10.7 ng/mL following 6 h handling and 6 h confinement (Barton et al. 2000; Webb et al. 2007). In contrast, most teleost species generally exhibit a maximum cortisol response of 100–200 ng/mL (Barton 2002), although some have provided exceptions, such as Atlantic Cod *Gadus morhua* (<15 ng/mL; Hemre et al. 1991) and Walleye *Sander vitreus* (>229 ng/mL; Barton 2002).

In summary, AQUI-S-20 E was observed to be an efficacious chemical sedative alternative to MS-222, offering rapid sedation and recovery times in Pallid Sturgeon. Desirable concentrations of AQUI-S 20-E to induce handleability in <3 min and recovery in <5 min were determined to be 476 mg/L for small and 537 mg/L for large Pallid Sturgeon. Sedation achieved with AQUI-S 20 E at a concentration of 500 mg/L suppressed the cortisol stress response in Pallid Sturgeon and is recommended for procedures requiring stress suppression.

ACKNOWLEDGMENTS

The authors wish to sincerely thank Elliott C. Kittel and Lucas R. Nelson for their assistance with data collection and fish husbandry. Gratitude is also extended to the Center for Fisheries, Aquaculture, and Aquatic Sciences of Southern Illinois University Carbondale for supplying the facilities necessary for this

research. Funds for this work were provided, in part, to BCS by the Illinois Soybean Association, Grant 10-ISA-36-443-3.

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