U.S. Fish & Wildlife Service
Aquatic Animal Drug Approval Partnership

AADAP
Aquatic Animal Drug Approval Partnership Program

DRUG RESEARCH INFORMATION BULLETIN

DRIB No. 51
October 2016

Influence of Salinity on Sedation of Sunshine Bass with AQUI-S®20E (10% eugenol)

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Fisheries professionals handle fish for a variety of reasons ranging from simple weighing and measuring to invasive surgical procedures. Sedatives facilitate safe, easy handling of live fish and are essential tools for fish research, husbandry, and management. Fish cannot be physically restrained in the same manner as terrestrial animals without risking damage to epithelial tissue or other physical injuries (Ross and Ross 2008). Sedating fish before handling reduces the risk of injury to both the fish, and in some cases the handler, minimizes the fish's stress response (Neiffer and Stamper 2009), and reduces the incidence or severity of stress-related complications after handling.

Currently, only tricaine methanesulfonate (MS222) is approved by the U.S. Food and Drug Administration (FDA) for the temporary immobilization of fish and other aquatic, cold-blooded animals. The only MS222 product available in the U.S. is TRICAINE-S (Western Chemical, Inc., Ferndale, Washington, USA). In general, fish sedated with MS222 must be held for 21 d before they can be released or consumed. A wide range of sedatives have been tested by a number of researchers, but for a variety of reasons they are not considered viable candidates at this time for FDA approval as immediate-release sedatives.

AQUI-S®20E (10% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) is an investigational new animal drug (INAD) that is currently available for use under the U.S. Fish and Wildlife Service (USFWS) compassionate INAD exemption authorization. Under this authorization, AQUI-S®20E can be used as an immediate-release sedative for fish treated as part of "field-based fishery management activities"; whereas a 72 h withdrawal period applies to all other fish sedated with this product. Immediate-release authorization was originally restricted to field based fishery management based activities with freshwater fish, but the exemption authorization was recently expanded to include marine fish. Although AQUI-S®20E has been shown to effectively sedate freshwater and marine fish, little information is available on the influence differing salinities may have on sedation. As such, we conducted a series of trials to evaluate time to sedation and recovery of juvenile Sunshine Bass (Striped Bass *Morone saxatilis* ♂ × White Bass *M. chrysops* ♀) to handleable when exposed to AQUI-S®20E in freshwater or increasingly brackish salinities.

Methods

Trials on Sunshine Bass (52.2 g ± 1.3 g, mean ± SE) were conducted at the Center for Fisheries, Aquaculture, and Aquatic Sciences (CFAAS) at Southern Illinois University Carbondale (SIUC, Carbondale, Illinois, USA) in December 2013-January 2014. Trials were conducted with a reference population of 300 fish housed in 270-L polyethylene tanks in a recirculating aquaculture system (RAS) and were acclimated to holding conditions for approximately 2 weeks before the start of the first trial. Fish were sedated to "handleable" with 600 mg/L AQUI-S®20E (60 mg/L eugenol) at nominal salinities of 0, 5, 10, 15, 20, or 25 g/L and were sedated only one time. Initial salinity of the RAS was 0 g/L for the first trial. Thereafter, rock salt (NaCl) was added to the RAS to achieve the next highest salinity where fish were held for 1-2 d before sedation testing at that particular salinity. A fish was deemed handleable when it lost equilibrium and the ability to swim, could easily be caught by and held in hand, and did not struggle while being weighed or measured.

At each salinity, 30 individual fish (experimental unit) were sedated separately under static conditions in an appropriate sized tub filled with 18.9 L of AQUI-S®20E solution, monitored to determine when they became handleable, weighed to the nearest 0.1 g, transferred to a recovery tank (18.9 L), and monitored to determine when they recovered from sedation. Fish were considered recovered when they regained equilibrium, resumed normal swimming behavior, and could avoid obstacles placed in their path. Sedative baths were not aerated, but dissolved oxygen (DO) concentration did not decline below 8.0 mg/L in any bath; recovery baths were aerated. Sedative and recovery baths were

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used to test 15 individual fish and then replaced with fresh sedative solution or RAS water. Times to handleable and recovery were determined for each fish and general fish behavior was assessed during sedation and recovery. Following recovery, fish were returned to a holding tank in the RAS supplied with fresh, flowing water and monitored for survival for 24 h. Induction and recovery times were analyzed by one-way ANOVA (PROC GLIMMIX) using Statistical Analysis System (version 9.4, SAS Institute; Cary, North Carolina, USA) to detect significant differences among the nominal salinity treatments. In the event of significant treatment effects, post-hoc Tukey's HSD tests were used for pairwise comparisons of means.

Results and Discussion

Induction and recovery times of Sunshine Bass sedated with AQUI-S®20E were significantly affected by salinity, but the magnitude of these effects was relatively small (Table 1). Induction was shortest (0.90 min) among fish tested at 25 g/L salinity and longest (1.07 min) among those tested at 0 g/L salinity, but this was the only significant difference observed for induction time among the treatments. A similar pattern was observed for recovery times where shorter recovery times were observed among fish tested at higher salinities and longer recovery times observed among fish tested at lower salinities (range, 2.63-3.91 min).

Throughout all trials, water quality was maintained within ranges appropriate for Sunshine Bass (Kohler 2000; water temperature and DO concentration in sedative and recovery containers, 21.1°-23.0°C and 8.3-9.1 mg/L; pH, 8.3-8.5; alkalinity, 186-336; hardness, 74-165 mg $CaCO_3$/L; total ammonia N, 0.0-0.2 mg/L; nitrite N, 0.02-0.19 mg/L, and nitrate N, 2.9-10.0 mg/L). In each trial, sedative solution samples were collected from each exposure container and analyzed to verify doses of eugenol by spectrophotometry, and doses were within ±15% of the target dose of 60 mg/L eugenol.

Based on results from these trials, 600 mg/L AQUI-S®20E (60 mg/L eugenol) was shown to readily sedate Sunshine Bass to handleable, regardless of salinity.

For more information on this study, see the manuscript with the same title published in the North American Journal of Aquaculture

Acknowledgments

We thank Andrew Ciuris (CFAAS, SIUC) for his assistance with data collection and Tyson Fehringer (IDFG, Eagle Fish Health Lab) for his critical review of this bulletin.

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Table 1. Mean induction and recovery times for Sunshine Bass sedated with 600 AQUI-S®20E (60 mg/L eugenol) at different salinities. Means with common superscript letter labels were not significantly different ($P > 0.05$).

Time to event (min ± SD)	Salinity (g/L)					
	0	5	10	15	20	25
Handleable	1.07^{z} (0.20)	1.02^{yz} (0.26)	1.04^{yz} (0.17)	0.98^{yz} (0.23)	1.00^{yz} (0.16)	0.90^{y} (0.19)
Recovery	3.91^{z} (1.51)	3.76^{z} (0.97)	3.59^{yz} (0.96)	2.86^{x} (0.77)	2.99^{xy} (0.62)	2.63^{x} (0.66)