

Efficacy of Aqui-S as an Anesthetic for Market-Sized Striped Bass

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Abstract.—Market-sized 2-year-old striped bass *Morone saxatilis* (average weight, 645 g) were evaluated in three different concentrations of Aqui-S at one of three different temperatures. Time to stage 4 of anesthesia, recovery time, and plasma cortisol level as an indicator of acute stress response, were determined. We found a significant difference in the time to stage 4 between 25 mg/L and 35 or 45 mg/L. This difference was present at all three temperatures tested. There were also significant differences in recovery time and blood cortisol level, but these differences were not consistent across all temperatures. There was a significant interaction between temperature and dose when analyzing cortisol levels. For the range of concentrations evaluated, we recommend an Aqui-S dose of 35 mg/L when anesthetizing market-sized striped bass.

There are numerous situations in aquaculture (such as netting, crowding, and transport) that can cause primary stress responses in fish, including elevated plasma cortisol concentrations (Donaldson 1981). Repetitive stressors can lead to chronic stress and chronic elevation of plasma cortisol (Pickering 1992), which is considered undesirable because it has been associated with depressed growth (Pickering et al. 1991), reproduction (Campbell et al. 1992), and immune response (Pickering and Pottinger 1989). The use of an anesthetic during the handling or relocation of fish may not only protect the fish and the fish handler from physical trauma but also reduce the perception of the stressor and thus the magnitude of the stress response. A number of anesthetics have been tested for use with fish in the United States, though at present the only compound approved for use by the U.S. Food and Drug Administration (FDA) is Fingel (3-aminobenzoic acid ethyl ester methanesulfonate, also known as tricaine methanesulfonate or MS-222), which has a 21-d withdrawal period (Schnick et al. 1986) and is currently banned in Canada (Peake 1998). Carbon dioxide is generally recognized as safe by the FDA and has been used by aquaculturists (Anderson et al. 1997), but it typically has very long induction and recovery times (Gilderhus and Marking 1987), which can lead to increased and unnecessary stress for the animals. An efficacious fish anesthetic needs to be soluble in water and provide smooth, rapid induction and recovery from anesthesia (Brown 1993). Marking and Meyer (1985) described a number of characteristics of an efficacious anesthetic. These included (1) an induction time of less than 15 min, (2) nontoxicity to fish and humans, (3) no persistent effects on fish

physiology, and (4) no withdrawal time. A relatively new compound, Aqui-S, is approved as an anesthetic for food fish in Australia, New Zealand, and Chile (Small and Chatakondi 2005). The product, reported to contain 50% isoeugenol (2-methoxy-4-propenylphenol) and Polysorbate 80 as an inert surfactant, was recently evaluated for its effects as an anesthetic on the coldwater Atlantic salmon *Salmo salar* by Iverson et al. (2003). Small (2004) recently reported the effects of isoeugenol on the warmwater channel catfish *Ictalurus punctatus*. The FDA currently has Aqui-S under consideration as a food fish anesthetic (FDA 2002) and will ultimately determine whether there are toxicity issues and whether the product can be used with a zero withdrawal time. The times to induction and recovery and the stress response as determined by postexposure plasma cortisol levels were used to evaluate Aqui-S as an anesthetic for the temperate striped bass *Morone saxatilis*.

Methods

Source of fish and anesthetic.—All of the striped bass evaluated in this study were produced at the University of Maryland's Crane Aquaculture Facility. The market-sized striped bass used in this study averaged 645 g and 39.4 cm. Fish were acclimated for 2 weeks in 2-m-diameter tanks that were part of an indoor recirculation aquaculture system (RAS) that received ambient light. All tanks had a water turnover rate of 1 h. The RAS provided excellent water quality throughout the 11-week study (dissolved oxygen, 100% saturation; total ammonia, <0.01 mg/L; nitrite, <0.01 mg/L; calcium, >100 mg/L; and pH 7.8–8.2) and was used to keep water temperature to within 0.2°C of each experimental temperature. All fish were fasted 48 h before each efficacy trial. All of the experimental fish ($n = 24$) used in this study were approved for use at the anesthetic concentrations tested by the University

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of Maryland Institutional Animal Care and Use Committee (experimental protocol R-00-89). The anesthetic AQUI-S was provided by AQUI-S New Zealand (Lower Hutt, New Zealand).

Experimental approach to determine efficacy.—Market-sized striped bass were tested at three different concentrations (25, 35, and 45 mg/L) of AQUI-S. Each concentration was evaluated at three different temperatures (10, 15, and 20°C). A digital controller (Johnson and Johnson) was used to control a heat exchanger and bring the RAS water temperature to the desired experimental water temperature. No change in water temperature was required between initial, ambient holding conditions and those of the first experimental temperature trial at 20°C. Each of the two lower temperatures employed (15°C and 10°C) was established by evenly lowering the water temperature 5°C from the previous set of trials over 48 h. The fish were then allowed to acclimate to the upcoming trial temperature as well as to recover from the previous trial over an additional 12-d period. At a given experimental temperature, fish were randomly selected for testing over the course of 1 d. With an experimental population of 24, 8 replicate striped bass were tested at each AQUI-S concentration.

Preparation of anesthetic bath.—All anesthetic efficacy tests were conducted in a rectangular, portable plastic tank (67 × 90 × 50 cm) containing 300 L of water obtained from the RAS in which the experimental animals were maintained. The anesthetic bath was prepared according to the stepwise product label directions: (1) vigorously shake the container prior to withdrawal of the product; (2) withdraw the appropriate amount of product required to achieve the correct dosage given the relative volume of water of the anesthesia tank; (3) add the product to a 150–200-mL water sample taken from the anesthetic bath tank; (4) shake or vortex the mixture vigorously to allow the surfactant to uniformly mix with the anesthetic; and (5) add the anesthetic and water mixture directly to the anesthesia bath tank while vigorously aerating the water to uniformly distribute the anesthetic solution throughout the anesthesia tank water. The AQUI-S was aerated with a fine-bubble diffuser supplied with pure oxygen at a constant pressure of 276 kPa for exactly 3 min. At this time the oxygen diffuser was removed and an experimental fish was quickly netted from its maintenance tank and placed into the anesthetic bath. A Yellow Springs Instruments Model 55 oxygen meter was used to confirm that the oxygen level in the anesthesia water solution remained at or above saturation throughout each efficacy trial. A 40-W incandescent light was suspended above the surface of the anesthetic bath and the 2-m-diameter recovery tank.

TABLE 1.—Stages of anesthesia used to determine the efficacy of AQUI-S as an anesthetic for striped bass (modified from Summerfelt and Smith 1990).

Stage	Description
1	Slight loss of responsiveness to visual and tactile stimuli; equilibrium normal
2	Total loss of responsiveness to visual stimuli, and only strong tactile pressure causes a reaction; equilibrium normal
3	Reaction only to very strong tactile pressure; partial loss of equilibrium
4	No response to external stimuli; total loss of equilibrium

These lights generated approximately 200 lx at the water surface. This afforded the fish a light intensity similar to, albeit less variable than, that found over their maintenance tanks. The lights also afforded clear observations of the fish during the trials. For each trial, eight fish were randomly selected and individually tested, beginning with the lowest AQUI-S concentration (25 mg/L). Time (s) to stage 4 of anesthesia (equivalent to surgical plane; see Table 1) was recorded. Once the fish was anesthetized to stage 4, 1 mL of blood was collected from the caudal vasculature in heparinized syringes to determine the circulating level of cortisol. Blood was maintained on ice in syringes and then transferred to microfuge tubes containing 250 µg pf ammonium heparin (Sigma-Aldrich, St. Louis, Missouri). The plasma was collected by centrifugation (4°C) at 10,000 × gravity and stored at -20°C for later analysis. After the blood was collected, the fish was immediately placed into a 2-m tank in the same RAS system, where full recovery in isolation took place and the recovery time (RCT [s]) was recorded. When all eight striped bass evaluations were completed, the anesthetic bath was drained and replaced with fresh system water and the appropriate amount of AQUI-S to achieve the next, higher concentration. The new bath was then aerated with oxygen for 3 min before the first fish of the next group of eight striped bass was randomly selected for evaluation.

Cortisol analysis.—Cortisol was measured directly in plasma using ELISA kits (DRG Diagnostics, Mountainside, New Jersey). Serial dilutions of a striped bass plasma pool and ether extracts from the same pool diluted parallel to the cortisol standard curve. Reproducibility was previously verified in our laboratory (Wang et al. 2004) by assaying aliquots of a striped bass plasma pool within a single assay (coefficient of variation [CV; 100 × SD/mean] = 7.9%; n = 10) and between assays (CV = 9.5%; n = 10).

Statistical analyses.—All statistical analyses were conducted with the SAS 9.1 software system (SAS Institute, Cary, North Carolina). Data were subjected to

TABLE 2.—Mean time required to reach stage 4 of anesthesia, time to fully recover, and plasma cortisol level of striped bass exposed to three different doses of Aqui-S. Eight fish were tested in each trial. For a given temperature, values within a column without letters in common are significantly different ($P < 0.05$).

Temperature °C	Dose (mg/L)	Cortisol (ng/mL)	Time (s) to	
			Stage 4	Recovery
20	25	147.5 z	528.4 z	335.4 y
	35	111.0 z	383.0 y	397.6 z
	45	143.3 z	362.0 y	372.9 yz
15	25	149.2 z	665.0 z	638.6 z
	35	82.4 y	466.5 y	619.8 z
	45	83.1 y	432.0 y	518.8 y
10	25	121.8 z	862.6 z	911.8 z
	35	95.6 zy	519.9 y	763.8 y
	45	61.8 y	487.0 y	802.6 y

analysis of variance (ANOVA) using mixed-model procedures. Pairwise contrasts were used to identify significant differences between the means at the 5% level. Data are reported as means \pm SEs. The probability of interaction between the Aqui-S concentration and temperature was estimated by mixed-model procedures. The assumptions of homogeneity of variance and normality of the data were tested, and the correlation procedure was used to estimate correlation coefficients.

Results

We found significant differences ($P < 0.05$) in the time required for striped bass to reach stage 4 of anesthesia among the concentrations tested, the time being greater at 25 mg/L than at 35 and 45 mg/L. This difference was significant and consistent for all three temperatures evaluated (Table 2). There were also statistically significant differences in RCT for all three concentrations of Aqui-S; however, these differences were not consistent across all temperatures. Time to

TABLE 3.—Pairwise correlation coefficients among time to stage 4 of anesthesia, recovery time (RCT), and cortisol level for the anesthetic Aqui-S at three temperatures.

Comparison	r	P
20° C		
Stage 4 versus RCT	0.516	<0.01
Stage 4 versus cortisol	0.372	>0.05
RCT versus cortisol	0.455	<0.05
15° C		
Stage 4 versus RCT	0.590	<0.01
Stage 4 versus cortisol	0.654	<0.001
RCT versus cortisol	0.323	>0.05
10° C		
Stage 4 versus RCT	0.493	<0.05
Stage 4 versus cortisol	0.456	<0.05
RCT versus cortisol	0.062	>0.05

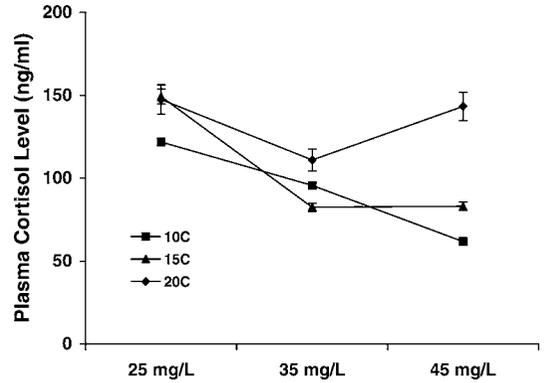


FIGURE 1.—Interactions between plasma cortisol level and temperature for striped bass exposed to three different concentrations of Aqui-S.

stage 4 and RCT both increased as temperature decreased (Table 2); they were significantly correlated at all of the concentrations tested and the differences were consistent (Table 3). Significant correlations were found between the time required to reach stage 4 and plasma cortisol level. There were also correlations between RCT and cortisol level. However, these correlations were not consistent across all temperatures and resulted in a significant interaction effect between cortisol and temperature (Figure 1).

Discussion

The efficacy of a fish anesthetic is most often associated with the time required to fully immobilize a fish (Gilderhus and Marking 1987; Small and Chatakondi 2005). More recently, anesthetics have been evaluated for their ability to elicit or inhibit a corticosteroid response apart from that which may be a result of handling or exposure to an acute stressor (Wagner et al. 2002; Iverson et al. 2003; Small 2004). The concentrations of Aqui-S examined in our study had a significant effect on the time required to take striped bass to surgical plane. The manufacturer's label recommended using 25 mg/L for salmonids, which required 8–14 min to anesthetize market-sized striped bass at temperatures between 10°C and 20°C. Even striped bass exposed to concentrations of 35 and 45 mg/L required 6 min or more to be fully immobilized. Cortisol levels were consistently highest (range, 120–150 ng/mL) at the lowest Aqui-S concentration examined at all three temperatures tested. However, at 20°C, cortisol levels were within the same range for all three Aqui-S concentrations. This may be due to increased metabolic activity associated with the test temperature, as suggested by Barton and Schreck (1987) for Chinook salmon *Oncorhynchus mykiss*, or

perhaps the warmer acclimation temperatures elicited a more profound stress response as, documented by Davis (2004) for hybrid striped bass (white bass *M. chrysops* × striped bass) and even more equivocally for striped bass (Davis and Parker (1990). Although the time required to immobilize the market-sized striped bass would not be considered rapid, it should be noted that the fish did not appear stressed or agitated as they were being anesthetized, as is often observed with compounds including quinaldine, carbon dioxide, and tricaine methanesulfonate (Davis and Griffin 2004). Both of the higher concentrations of examined resulted in low cortisol levels. Based on these results, we conclude that an Aqui-S dose of 35 mg/L is adequate to anesthetize market-sized striped bass.

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