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Use of AQUI-S®20E to Sedate Florida Pompano, Cobia, and Black Seabass to Handleable

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Sedatives are chemicals or physical agents that—with increasing treatment concentration and duration—first calm an animal and then cause successive loss of mobility, equilibrium, conscious- ness, and reflex action. Fisheries professionals routinely sedate fish for a variety of purposes including collection of samples or morphometric data, implantation of tags or tracking devices, and transport. Sedating fish before handling can minimize stress and physical injury to the fish and also help protect the handler. Ideally, a fish sedative is safe, effective, easy to administer, and inexpensive. Also, it is desirable that the sedative have no withdrawal period so that treated fish can be released into the wild immediately after treatment.

Currently, only tricaine methanesulfonate (tricaine or 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 tricaine product available in the U.S. is TRICAINE-S (Western Chemical, Inc., Ferndale, Washington USA). TRICAINE-S is effective and widely used by fisheries professionals; however, a 21-day withdrawal period is required for fishes entering the human food chain through stocking or slaughter and the product is not approved for marine fish species. For many field applications, holding fish for 21 days post- sedation is not practical and may seriously compromise management or research activities. Those working with marine fish species in a saltwater environment don't have many other suitable options.

In the U.S., efforts are underway to obtain FDA approval of AQUI-S®20E (10% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) as an immediate-release fish sedative. Considerable research has shown that eugenol is efficacious for sedating freshwater and marine fishes to handleable (e.g., Trushenski et al. 2012a, 2012b, 2013). However, FDA requires data to demonstrate a product is effective in its final formulation at its lowest proposed efficacious dose. Effectiveness and safety data have been generated by the U.S. Fish and Wildlife Service to support approval of AQUI-S®20E for use to sedate all freshwater finfish to handleable. Data are now needed to support a similar claim for use on marine fish in a saltwater environment. As such, we conducted four independent trials to evaluate the efficacy of AQUI-S®20E for sedating small and large fingerling Florida Pompano *Trachinotus carolinus*, fingerling Cobia *Rachycentron canadum*, and juvenile Black Seabass *Centropristis striata* to handleable.

Methods

Trials on Pompano and Cobia were conducted at the Virginia Seafood Agriculture Research and Extension Center (Hampton, VA, USA) in August and September 2015. The trial on Black Seabass was conducted at the Bradford Bay Farm (Quimby, VA, USA) in September 2015. In each trial, fish were sedated to handleable with 300 mg/L AQUI-S®20E (30 mg/L eugenol) or 120 mg/L tricaine (active control). We tested 30 mg/L eugenol because it is likely the lowest efficacious dose that might be used on marine fish reared or held in warm water (i.e., 25°C). A fish was determined to be 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.

Sixty fish were used in each trial: 30 fish were individually sedated under static conditions with each of the two sedatives. Aliquots of working sedative solutions prepared in bulk (80-gal) were used to fill individual sedation containers (2 gal in 5-gal plastic buckets). Contents of each sedation container were discarded after one fish had been become sedated and removed

from it. When a fish became handleable, it was removed from the sedative solution, measured for length and weight, and then transferred to a recovery tank of fresh, flowing (Pompano and Cobia) or static (Black Seabass) water. Static recovery baths were exchanged periodically so that dissolved oxygen (DO) concentration never fell below 7 mg/L. Fish were considered recovered when they regained equilibrium, resumed normal swimming behavior, and could avoid obstacles placed in their path. 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 fish holding tank plumbed with fresh, flowing water and monitored for survival for 24 h.

Water temperature and dissolved oxygen (DO) concentration were measured in each sedation container before placing fish in the solution. Water hardness, alkalinity, salinity, and pH were measured once in source water. In each trial, sedative solution samples were collected and analyzed to verify doses of eugenol by spectrophotometry from 20 randomly selected sedation containers.

Results and Discussion

All fish became handleable within 4.9 min (mean times ranged from 0.8 to 2.1 min) and recovered from sedation within 17 min (mean times ranged from 4.3 to 6.2 min; Table 1). During exposure to and recovery from AQUI-S®20E, fish behavior was considered mostly normal in Pompano, mostly abnormal in Cobia, and abnormal in some cases with Black Seabass (Table 2). Abnormal behavior was observed more frequently in Pompano and Black Seabass during and following exposure to MS222, whereas abnormal behavior of Cobia was similar to that observed among the group exposed to AQUI-S®20E. Abnormal behavior included fish piping at the surface, slight agitation, jumping, or head shaking. There was no post-sedation mortality. Mean water temperature and DO concentration ranged from 24.2 to 25.1°C and 7.7 to 8.7 mg/L, respectively, across the four trials (Table 3). Hardness, alkalinity, pH, and salinity were within ranges suitable for rearing healthy warmwater marine finfish (Table 3). Mean analytically verified doses of eugenol were within ±8% of target doses (Table 3).

Based on results from these trials, 300 mg/L AQUI-S®20E (30 mg/L eugenol) was shown to effectively sedate large and small fingerling Pompano, fingerling Cobia, and juvenile Black Seabass to handleable under the conditions tested. Although there are many factors (e.g., sedative concentration, fish life stage, water temperature) that might influence time to sedation and recovery, we speculate that warmwater marine finfish tested under similar conditions will become sedated and recover in comparable times. Results from these trials will be submitted to FDA with a request to be included in the body of evidence that supports treatment efficacy.

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Table 1. Fish species tested, mean total length and weight (± 1 SD), and mean (range) times to handleable and recovery for fish sedated to handleable with AQUI-S®20E (30 mg/L) or tricaine (120 mg/L).

Trial	Species	Fish size		Mean time (minutes) to sedation (range)		Mean Time (minutes) to recovery (range)	
		Total length (cm)	Weight (g)	AQUI-S®20E	Tricaine	AQUI-S®20E	Tricaine
1	Pompano small fingerling	9.9 ± 1.1	12.6 ± 4.0	0.9 (0.6 – 1.3)	1.1 (0.8 – 1.5)	4.3 (3.0 – 5.6)	3.1 (1.6 – 4.8)
2	Pompano large fingerling	15.7 ± 1.3	47.2 ± 11.0	1.1 (0.8 – 1.3)	1.3 (0.9 – 1.7)	5.0 (3.3 – 7.5)	3.6 (2.1 – 6.1)
3	Cobia	16.0 ± 1.2	19.9 ± 4.2	2.1 (1.0 – 4.9)	1.4 (1.0 – 2.0)	6.7 (4.6 – 9.1)	3.6 (2.4 – 4.8)
4	Black Seabass	19.6 ± 2.1	192.8 ± 66.1	0.8 (0.4 – 1.1)	1.0 (0.6 – 2.0)	5.7 (2.7 – 17.38)	2.9 (0.8-5.1)

Table 2. Relative number of fish (out of 30) showing abnormal behavior (i.e., slight agitation, piping at the water surface, jumping, head-shaking) during sedative solution exposure and recovery from sedation.

Trial	Species	During sedation		During recovery	
		AQUI-S®20E	Tricaine	AQUI-S®20E	Tricaine
1	Pompano small fingerling	0%	10%	0%	23%
2	Pompano large fingerling	7%	27%	0%	67%
3	Cobia	83%	73%	100%	100%
4	Black Seabass	30%	83%	13%	27%

Table 3. Water quality and eugenol dose verification results measured during the sedation trials.

Trial	Water quality parameters (mean ± SD where reported)						Eugenol Concentration (diff. from target dose)
	Temp (ºC)	Dissolved Oxygen (mg/L)	Hardness (mg/L$CaCO_3$)	Alkalinity (mg/L$CaCO_3$)	Salinity (ppt)	pH	
1	24.5 ± 0.2	8.2 ± 0.2	674	161	22.8	8.3	32.1 mg/L (+7.3%)
2	24.2 ± 0.4	8.7 ± 0.5					32.2 mg/L (+7.5%)
3	25.1 ± 0.5	7.7 ± 0.4	650	108	22.3	7.3	31.2 mg/L (+3.9%)
4	24.7 ± 0.2	8.0 ± 0.1	1080	159	37.6	8.3	32.3 mg/L (+7.8%)