

**Use of AQUI-S[®] 20E to Sedate California Yellowtail and White Seabass to Handleable**Jim Bowker^{1*}, Niccole Wandelaar¹, and Connie Silbernagel²¹*U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
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Sedatives are chemicals or physical agents that—with increasing treatment concentration and duration—calm an animal and cause successive loss of mobility, equilibrium, consciousness, and reflex action. Fisheries professionals routinely sedate fish for a variety of purposes, including collection of samples or morphometric data, fish health evaluations, 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) 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 have limited 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., Bowker et al. 2014, Trushenski et al. 2012a, 2012b, 2012c). 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 (USFWS) 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 two independent trials to evaluate the efficacy of AQUI-S[®] 20E for sedating fingerling California Yellowtail *Seriola dorsalis* and juvenile White Seabass *Atractoscion nobilis* to the handleable stage of anesthesia.

Methods

Trials were conducted at the Hubbs-SeaWorld Research Institute, Leon Raymond Hubbard, Jr., Hatchery Facility (HSWRI, Carlsbad, CA) on July 18 and 19, 2017. In each trial, either Yellowtail (mean total length and weight \pm 1 SD; 11.3 ± 1.1 cm and 15.4 ± 4.1 g) or Seabass (18.4 ± 1.3 cm and 61.1 ± 12.3 g) were sedated to handleable with 300 mg/L AQUI-S[®] 20E (30 mg/L eugenol) or 80 (Yellowtail) or 120 mg/L (Seabass) tricaine (active control). We tested 30 mg/L eugenol because, based on preliminary testing, it is likely the lowest efficacious dose that will be proposed by the sponsor for use on marine fish reared or held in warm water (i.e., 25°C) to consistently sedate such fish to handleable within 2 min. 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, whereby 30 fish were individually sedated under static conditions with each of the two sedatives. Working volumes of sedative solutions were prepared in bulk (two 30-gal batches for Yellowtail and four 30-gal batches for Seabass) and used to fill individual sedation containers (1.5 gal in 5-gal plastic buckets for Yellowtail; 2.5 gal in 5 gal buckets for Seabass). Contents of each sedation container were discarded after one fish had become sedated and removed from it. When a fish became handleable, it was removed from the sedative solution, measured for length and weight, and allowed to recover under static conditions in 19-gal tubs containing approximately 12 gal (45.4 L) of water. Static recovery baths were exchanged periodically so that dissolved oxygen (DO) concentrations never fell below 8 mg/L. Fish were considered recovered when they regained equilibrium, resumed normal swimming behavior, and could avoid a net handle placed in their path. Times to sedation and recovery were determined for each fish and general fish behavior was assessed qualitatively during sedation and recovery. Following recovery, fish were returned to a holding tank supplied with flowing water and monitored for survival for 24 h.

Water temperature and DO concentration were measured in each sedation container before placing fish in the solution. Salinity and pH were measured once in untreated source water. In each trial, 20 sedative solution samples were randomly collected and analyzed by UV/Vis spectrophotometry at 279 nm with a Genesys[®] 2 Spectrophotometer (Thermo Electron Scientific Corporation, Rochester, NY) to verify doses of eugenol. Eugenol doses were considered accurate if they were within \pm 25% of the 30 mg/L target dose (acceptable range; 22.5 to 37.5 mg/L).

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eugenol).

Results and Discussion

With AQUI-S®20E, mean times to handleable and recovery for Yellowtail and Seabass were ≤ 1.9 min and 5.0 min, respectively (Table 1). With tricaine, mean times to sedate fish were comparable whereas mean times to recovery were more rapid than that measured for AQUI-S®20E (Table 1). Elapsed time to sedation and then recovery was shorter for tricaine than for AQUI-S®20E (Figure 1). Abnormal fish behavior (e.g., agitation, head-shaking, piping) was observed infrequently among both species during exposure to AQUI-S®20E whereas it was observed more frequently among fish exposed to tricaine (Table 2). There was no post-sedation mortality. Seabass behavior was considered normal during sedation immediately after sedation with either sedative whereas Yellowtail behavior was considered mostly normal following sedation with AQUI-S®20E but abnormal following sedation with tricaine (Table 2).

Mean water temperature and DO concentration was approximately 25°C and > 8.0 mg/L during both trials. Salinity (35 ppt) and pH (8.2) were within ranges suitable for rearing healthy warm water marine finfish. Mean analytically verified eugenol doses were 30.5 mg/L for Yellowtail and 29.9 mg/L for Seabass.

Based on results from these trials, 300 mg/L AQUI-S®20E (30 mg/L eugenol) effectively sedated California Yellowtail and White Seabass to handleable under the conditions tested. Although there are many factors (e.g., sedative concentration, fish size and life-stage, water temperature) that might influence time to sedation and recovery, we speculate that similar marine finfish reared or held at similar water temperatures would become sedated and recover in comparable time periods. Results from these trials were submitted to FDA with a request that they be included in the body of evidence supporting treatment efficacy of AQUI-S®20E to marine finfish.

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Table 1. Mean total length and weight (±1 SD), and mean (range) times to handleable and recovery for California Yellowtail and White Seabass sedated to handleable with AQUI-S®20E or Tricaine-S.

Species	Fish Size		Mean Time (min) to Sedation (range)		Mean Time (min) to Recovery (range)	
	Total length (cm)	Weight (g)	AQUI-S20E	Tricaine	AQUI-S20E	Tricaine
Yellowtail	11.3 ± 1.1	15.4 ± 4.1	1.4 (1.0 – 2.5)	1.9 (1.2 – 2.6)	4.3 (2.7 – 8.5)	3.1 (1.1 – 7.2)
White Seabass	18.4 ± 1.3	61.1 ± 12.3	1.9 (1.2 – 2.5)	1.6 (0.8 – 2.5)	5.0 (3.8 – 6.6)	3.5 (2.3 – 7.5)

Table 2. Percentage of fish (out of 30) showing abnormal behavior (primarily slight agitation but also some instances of piping at the water surface and head-shaking) during sedative solution exposure and recovery from sedation.

Species	During Sedation		During Recovery	
	AQUI-S [®] 20E	Tricaine	AQUI-S [®] 20E	Tricaine
Yellowtail	17%	77%	3%	100%
White Seabass	10%	83%	0%	0%

Figure 1. Mean elapsed times (min) for fingerling California Yellowtail and juvenile White Seabass to become sedated to the handleable (H) stage of anesthesia with either AQUI-S[®]20E or Tricaine-S and recover (R) from sedation. X-axis = elapsed time in minutes.

