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Effectiveness of AQUI-S®20E (10% eugenol) to Lightly Sedate Rainbow Trout, Cutthroat Trout, and Chinook Salmon

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Fisheries professionals handle fish for a variety of reasons ranging from sorting to 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. Sedating fish before handling reduces the risk of injury to the fish, minimizes the fish's stress response (Neiffer and Stamper 2009), and reduces the incidence or severity of stress-related complications after handling (Ross and Ross 2008). Ideally, a fish sedative is safe, effective, easy to administer, inexpensive, and has no withdrawal period so that treated fish can be released into the wild after treatment.

Currently, the only product approved by the U.S. Food and Drug Administration (FDA) for the temporary immobilization of fish is Tricaine-STM (MS-222; Western Chemical, Inc., Ferndale, Washington, USA). In general, fish sedated with MS-222 must be held for 21 d before they can be released or consumed. There is a need in the U.S. for an immediate-release sedative where fish can be returned to public waters immediately after sedation. At present, AQUI-S®20E (10% eugenol; AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) is the only viable candidate for FDA approval as such.

AQUI-S®20E 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, the product can be used as an immediate-release sedative for fish treated as part of "field-based fishery management activities". The 'field use' authorization was originally restricted to freshwater fish, but was recently expanded to include marine fish. Although AQUI-S®20E has been shown to effectively sedate freshwater and marine fish to handleable, little-to-no information is available regarding its use to lightly sedate fish for purposes such as pre-transport loading, grading and sorting, staging broodstock, or transporting fish at low densities. As such, we conducted three trials to evaluate the effectiveness of AQUI-S®20E to lightly sedate fingerling Rainbow Trout *Oncorhynchus mykiss* (RBT), Cutthroat Trout *O. clarkii* (CTT), and Chinook Salmon *O. tshawytscha* (CHS) for up to 5 h with 30 mg/L AQUI-S®20E (3 mg/L eugenol).

Methods

RBT (6.7 ± 0.9 cm, mean ± SD) and CTT (8.7 ± 0.9 cm) trials were conducted at the USFWS Bozeman Fish Technology Center (Bozeman, Montana, USA) and the CHS (13.2 ± 0.9 cm) trial was conducted at the Idaho Department of Fish and Game Eagle Fish Health Laboratory (Eagle, Idaho, USA) in March-April 2017. Reference populations of about 500 fish were housed in either 84-cm diameter (total volume for RBT and CTT, 297 L) or 137-cm diameter (total volume for CHS, 491 L) tanks. Before sedation (1 d for RBT and CTT, 5 d for CHS), 30 fish were moved into each of 15 test tanks (46 cm diameter), water flow was set at 3.8 L/min with first pass water, and using completely randomized procedures, tanks were selected to be treated tanks (n=10) or control tanks (n=5). Fish were exposed to 30 mg/L AQUI-S®20E (3 mg/L eugenol) or a sham water blank and assessed for light sedation at 0, 5, 15, 30, 45, and 60 min and hourly thereafter for the next 4 h. Water temperature and dis-solved oxygen (DO) concentrations were measured hourly and supplemental aeration was initiated at the same time in all tanks (RBT – at 4 h; CTT – at 3 h, CHS – at 1 h) to maintain DO concentrations above 6 mg/L. Water samples

were collected hourly starting at 0 min from each tank for eugenol dose verification by spectrophotometry. Additional water samples were collected from each tank at the beginning, middle, and end of the 5 h light sedation period and measured for total ammonia and pH.

Light sedation was determined based on:

1. Fish swimming ability: fish were swimming/holding position or ≥ 1 lying on the tank bottom
2. Equilibrium: fish were dorsal side up or ≥ 1 lying on their side/ventral side up (total loss of equilibrium)
3. Catchability: number of fish able to be caught by hand (0-3 fish)
4. Position in water column: majority of fish dispersed throughout the tank or in the bottom half of the tank

The following criteria had to be met for treatment to be considered a success: (1) three fish caught by hand in at least 80% of the treated tanks, (2) no fish lost equilibrium in at least 80% of the treated tanks, (3) all fish were able to swim in at least 80% of the treated tanks, and (4) fish were spread throughout the water column in at least 80% of the treated tanks. Treatment failure comprised fish actively swimming and maintaining equilibrium, were positioned at the bottom half of the tank, and fewer than 3 fish could be caught be hand. At the end of the 5-h exposure period, water flow to each tank was restored and fish were monitored for 20-30 min to ensure that all recovered from light sedation. After fish were recovered and normal husbandry resumed, general and feeding behaviors were assessed daily for 2d.

At each time point, dichotomized light sedation data (fish were deemed lightly sedated or not) were statistically analyzed with the Fisher's Exact Test (PROC FREQ) using Statistical Analysis System (version 9.4, SAS Institute; Cary, North Carolina, USA) to detect significant differences ($P < 0.05$) in the occurrence of light sedation among treated and control tanks.

Results and Discussion

In all trials, fish in at least 80% of the treated tanks met the criteria of light sedation throughout the treatment period starting at 5 min post-exposure to AQUI-S®20E (Table 1). None of the control tanks met the criteria of light sedation at any time point, thus there was a significant difference between treated and control groups beginning at 5 min and persisting through the entire 5-h exposure period. The limiting factor precluding a tank of fish from being characterized as lightly sedated was the inability to catch 3 fish by hand. The most reliable indicator of light sedation turned out to be fish position in the water column: lightly sedated fish were always evenly dispersed throughout the water column, whereas unsedated fish were consistently near the bottom of the tank. No fish were observed to lose equilibrium or their ability to swim at any point in treated or control tanks in any trial.

Throughout all trials, mean water temperature and DO concentrations were maintained between 12.2 and 14.1°C and 6.1 and 8.4 mg/L, respectively. Mean eugenol concentrations ranged between 2.6 and 3.1 mg/L.

Based on results from these trials, 30 mg/L AQUI-S®20E (3 mg/L eugenol) was effective in lightly sedating a variety of fingerling salmonids in freshwater for up to 5 h.

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References

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Table 1. Summary of whether treatment success criteria were met, percent of treated tanks in which fish were characterized as lightly sedated, and mean hourly dose verification concentrations. Fish in control tanks did not meet the criteria of light sedation in any of the trials.

Time period	Rainbow Trout			Cutthroat Trout			Chinook Salmon		
	Treatment Success	% of Treated Tanks lightly sedated	Eugenol dose (mg/L)	Treatment Success	% of Treated Tanks lightly sedated	Eugenol dose (mg/L)	Treatment Success	% of Treated Tanks lightly sedated	Eugenol dose (mg/L)
0 min	No	0%	2.7	No	0%	2.9	No	0%	3.1
5 min	Yes	100%	---	Yes	90%	---	Yes	80%	---
15 min	Yes	100%	---	Yes	100%	---	Yes	100%	---
30 min	Yes	100%	---	Yes	100%	---	Yes	100%	---
45 min	Yes	100%	---	Yes	100%	---	Yes	100%	---
60 min	Yes	100%	2.8	Yes	100%	2.8	Yes	100%	2.8
2 h	Yes	100%	2.8	Yes	100%	2.8	Yes	100%	2.8
3 h	Yes	100%	2.6	Yes	100%	2.9	Yes	100%	2.9
4 h	Yes	100%	2.7	Yes	100%	2.8	Yes	100%	2.7
5 h	Yes	100%	2.8	Yes	100%	2.8	Yes	80%	2.8