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Use of AQUI-S®20E and BENZOAK® to Sedate Rainbow Trout, Cutthroat Trout, and Brown Trout 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, consciousness, 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 recovery from sedation.

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 two tricaine products available in the U.S. are TRICAINE-S (Western Chemical, Inc., Ferndale, Washington USA) and FINQUEL (Argent Laboratories, Redmond, Washington USA). Both are effective and widely used by fisheries professionals; however, a 21-day withdrawal period is required for fish entering the human food chain through stocking or slaughter. For many field applications, holding fish for 21 days postsedation is not practical and seriously compromises management or research activities.

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) and BENZOAK® (20% benzocaine; Europharma USA, Victoria, Minnesota USA) as immediate-release fish sedatives. Considerable research has shown that eugenol and benzocaine are efficacious for sedating freshwater and saltwater fishes to handleable (e.g., Trushenski et al. 2012a, 2012b, 2012c). However, FDA requires data that demonstrate a product is effective in its final formulation at its lowest proposed efficacious dose. To help obtain FDA approval of AQUI-S®20E and BENZOAK® for use on all freshwater-reared salmonids, we conducted three independent trials to evaluate the efficacy of these two sedatives for sedating subadult rainbow trout (RBT) *Onchoryhnchus mykiss,* adult cutthroat trout (CTT) *O. clarki*, and adult brown trout (BNT) *Salmo trutta* to handleable.

Methods

Trials 1 (RBT) and 2 (CTT) were conducted at the U.S. Fish and Wildlife Service's (USFWS) Bozeman Fish Technology Center, Bozeman, Montana USA in June 2011, and Trial 3 (BNT) was conducted at the U.S. Geological Survey's (USGS) Upper Midwest Environmental Sciences Center (UMESC), La Crosse, Wisconsin USA in September 2011. In each trial, fish were sedated to handleable with 25 mg per L eugenol (250 mg per L AQUI-S®20E), 40 mg per L benzocaine (200 mg per L BENZOAK®), or 80 mg per L tricaine (active control). We tested 25 mg per L eugenol and 40 mg per L benzocaine because these are the lowest efficacious doses that will be proposed for the respective product labels. 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 measured for length.

Ninety fish were used in each trial (30 fish individually sedated under static conditions with each of the three sedatives). Sedative solutions were prepared in bulk (e.g., 40 gal), and aliquots of these solutions were used to fill individual sedation containers. The contents of each sedation container were discarded after one fish had been sedated. When a fish was determined to be handleable, it was removed from the sedative solution, measured for length, and transferred to a container

supplied with fresh, flowing water. A fish was determined to be recovered when it regained equilibrium, resumed normal swimming behavior, and avoided obstacles (e.g., a net handle) placed in its 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 placed in a holding tank or raceway plumbed with fresh, flowing water and monitored for survival for 24 h.

In each trial, water temperature and dissolved oxygen (DO) concentration were measured in each sedation container before placing a fish into the solution. Sedative solution samples were collected from 30 randomly selected sedation containers (10 containers per sedative) and analyzed by spectrophotometry to verify concentrations of either eugenol or benzocaine. Water hardness, alkalinity, and pH were measured once in the source water used in each trial.

Results and Discussion

All fish became handleable within 3.3 min and recovered from sedation within 21.3 min (Table 1). Across all three trials, some fish exhibited headshaking when placed into a sedative solution; however, this behavior lasted only a few seconds and ceased as a fish became sedated. Behavior during recovery was characterized as normal, and there was no postsedation mortality. Mean water temperature and DO concentration ranged from 12.9 to 14.5°C and from 8.6 to 9.4 mg per L, respectively, across the three trials (Table 2). Hardness, alkalinity, and pH were within ranges suitable for rearing healthy, freshwater-reared salmonids (Table 2). Mean analytically verified concentrations of eugenol and benzocaine administered were within ±19.3% of their respective target concentrations (Table 3).

Based on these results, we concluded that AQUI-S®20E and BENZOAK® were effective in sedating subadult RBT, adult CTT, and adult BNT to handleable under the conditions tested. Many factors (e.g., sedative concentration, fish life stage, water temperature) can influence sedation and recovery times; however, it is likely that freshwater salmonids tested under conditions similar to those in our trials will become sedated and will recover within the time ranges we observed. Our results were accepted by FDA as providing evidence of efficacy for AQUI-S®20E and BENZOAK®.

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Table 1. Times to handleable and recovery.

Trial	Species	Mean time (minutes) to handleable (range)			Mean time (minutes) to recovery (range)		
		AQUI-S®20E	BENZOAK®	Tricaine	AQUI-S®20E	BENZOAK®	Tricaine
1	Rainbow trout	1.8 (1.4 – 2.2)	1.4 (1.0 – 1.8)	1.1 (0.8 – 1.4)	4.0 (2.6 – 6.1)	1.7 (0.9 – 3.1)	1.5 (1.8 – 2.5)
2	Cutthroat trout	2.1 (1.5 – 3.0)	2.1 (1.3 – 3.3)	1.8 (1.2 – 2.4)	11.1 (5.4 – 21.3)	8.8 (4.7 – 18.8)	7.1 (3.5 – 12.3)
3	Brown trout	1.8 (1.3 – 2.5)	2.1 (1.3 – 2.9)	1.5 (0.9 – 2.0)	7.1 (3.6 – 11.1)	6.0 (2.4 – 9.2)	4.6 (2.4 – 9.5)

Table 2. Water quality parameters measured.

Trial	Fish		Mean water quality parameters (range)				
	Species	Mean ± SD total length (cm)	Temperature (°C)	Dissolved oxygen concentration (mg per L)	Hardness (mg per L as $CaCO_3$)	Alkalinity (mg per L as $CaCO_3$)	pH
1	Rainbow trout	19.1 ± 2.8	14.5 (13.5 – 15.1)	8.6 (8.0 – 8.8)	244	169	8.2
2	Cutthroat trout	26.5 ± 3.0	14.0 (13.6 – 14.2)	8.6 (8.2 – 9.2)	212	168	8.0
3	Brown trout	30.9 ± 2.4	12.9 (12.1 – 14.5)	9.4 (8.3 – 10.5)	178	125	8.0

Table 3. Eugenol and benzocaine concentrations administered.

Trial	Species	Mean concentration administered (% difference from target)	
		Eugenol (mg per L)	Benzocaine (mg per L)
1	Rainbow trout	24.9 (-0.4)	34.2 (-14.5)
2	Cutthroat trout	27.3 (+9.2)	32.3 (-19.3)
3	Brown trout	27.1 (+8.4)	37.3 (-6.8)