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Use of AQUI-S®20E and BENZOAK® to Sedate Walleye, Yellow Perch, Common Carp, and Fathead Minnow 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 proposed lowest efficacious dose. To help obtain FDA approval of AQUI-S®20E and BENZOAK® for use on all freshwater-reared, coolwater finfish, we conducted four independent trials to evaluate the efficacy of these two sedatives for sedating fingerling walleye (WAE) *Sander vitreus*, yellow perch (YEP) *Perca flavescens*, common carp (CMC) *Cyprinus carpio*, and fathead minnow (FHM)*Pimphales promelas* to handleable.

Methods

All trials were conducted in September 2011 at the U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC), La Crosse, Wisconsin USA. In each trial, fish were sedated to handleable with 40 mg per L eugenol (400 mg per L AQUI-S®20E), 80 mg per L benzocaine (400 mg per L BENZOAK®), or 80 mg per L tricaine (active control). We tested 40 mg per L eugenol and 80 mg per L benzocaine because these are the lowest efficacious doses that will be proposed for coolwater finfish on 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 Trials 1 (WAE) and 2 (YEP), i.e., for each sedative, 30 fish were individually sedated under static conditions. Thirty fish were used in Trials 3 (CMC) and 4 (FHM), i.e., for each sedative, 10 fish were individually sedated under static conditions. 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 of 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 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 a fish was placed into the solution. Also, samples of sedative solution were collected and analyzed by spectrophotometry to verify concentrations of either eugenol or benzocaine. In Trials 1 and 2, sedative solution samples were collected from 30 randomly selected sedation containers (10 per sedative). In Trials 3 and 4, one sample was collected from the bulk tub of sedative solution. Water hardness, alkalinity, and pH were measured once in the source water used in each trial.

Results and Discussion

In all four trials, all fish became handleable within 5.0 min and recovered from handleable within 18.3 min (Table 1). There were no postsedation mortalities. In Trial 1, some (40%) of the WAE sedated with AQUI-S®20E exhibited "headshaking" or "gill coughing" behavior after being placed in the sedative solution; however, these behaviors ceased after a few seconds as the fish became sedated. In Trial 2, brief headshaking was also noted in some (33%) of the YEP sedated with AQUI-S®20E and some (7%) of the YEP sedated with BENZOAK®. Otherwise, no unusual behaviors were observed during sedation or recovery during any of the four trials. Across trials, mean water temperature and DO concentration ranged from 18.0 to 18.5°C and from 8.6 to 9.8 mg per L, respectively (Table 2). Source water hardness, alkalinity, and pH were within ranges suitable for rearing healthy coolwater finfish (Table 2). Mean analytically verified concentrations of the eugenol and benzocaine administered were within ±25% of their respective target concentrations (Table 3).

Based on these results, we concluded that AQUI-S®20E and BENZOAK® were effective in sedating fingerling WAE, YEP, CMC, and FHM to handleable under the conditions tested. Although many factors (e.g., sedative concentration, fish life stage, water temperature) can influence time to sedation and recovery, we speculate that other coolwater finfish tested under similar conditions will become sedated and recover in times similar to those observed in our trials. Results from these trials were submitted to and accepted by FDA as providing sufficient 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	Walleye	2.0 (1.5 – 3.8)	2.0 (1.3 – 3.0)	2.6 (1.4 – 4.1)	10.2 (4.6 – 14.3)	10.1 (6.1 – 18.3)	6.7 (3.5 – 10.2)
2	Yellow perch	3.0 (1.7 – 5.0)	2.1 (1.1 – 3.3)	3.5 (2.0 – 4.6)	9.7 (4.4 – 16.8)	7.9 (4.8 – 13.1)	6.9 (2.20 – 11.8)
3	Common carp	1.9 (1.5 – 2.4)	1.7 (1.1 – 2.3)	1.9 (1.5 – 2.3)	5.9 (4.6 – 7.6)	5.9 (3.5 – 8.8)	4.7 (3.4 – 6.3)
4	Fathead minnow	1.4 (1.0 – 2.0)	1.2 (0.8 – 1.7)	1.0 (0.8 – 1.5)	6.0 (4.4 – 7.7)	5.1 (2.8 – 8.2)	5.3 (3.8 – 6.5)

Table 2. Water quality parameters measured.

Trial	Fish		Mean water quality parameters (range)				
	Species	Mean ± SD total length (cm)	Temperature (ºC)	Dissolved oxygen concentration (mg per L)	Hardness (mg per L as $CaCO_3$)	Alkalinity (mg per L as $CaCO_3$)	pH
1	Walleye	13.5 ± 1.2	18.2 (18.0 – 18.5)	9.4 (9.1 – 9.5)	176	132	8.2
2	Yellow perch	10.5 ± 1.0	18.0 (17.9 – 18.1)	9.8 (9.5 – 10.0)	176	132	8.0
3	Common carp	7.7 ± 1.2	18.5 (18.2 – 18.6)	8.6 (8.5 – 8.8)	170	127	8.1
4	Fathead minnow	7.1 ± 0.7	18.5 (18.4 – 18.6)	8.7 (8.6 – 8.9)	170	127	8.1

Table 3. Eugenol and benzocaine concentrations administered.

Trial	Species	Mean concentration administered (% difference from target)	
		Eugenol (mg per L)	Benzocaine (mg per L)
1	Walleye	39.9 (-0.3)	78.1 (-2.4)
2	Yellow perch	44.7 (+11.8)	77.9 (-2.6)
3, 4	Common carp and Fathead minnow	49.8 (+24.5)	71.8 (-10.3)