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The Effect of AQUI-S®20E Concentration on Sablefish Sedation and Recovery

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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, induced spawning, and transport. Sedating fish before handling can minimize stress and physical injury to the fish and help protect the handler. Ideally, a fish sedative is safe, effective, easy to administer, and inexpensive. In addition, it is desirable that the sedative have no withdrawal period so that treated fish may 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 ™ (Syndel, Ferndale, Washington, USA). Tricaine 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, 2015, 2017, 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) Aquatic Animal Drug Approval Program (AADAP) 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 juvenile Sablefish *Anoplopoma fimbria* to the handleable stage of anesthesia; first at 300, 450, and 600 mg/L AQUI-S®20E, then at the lowest proposed efficacious dose of 300 mg/L compared to 120 mg/L tricaine (active control). The results of these studies were then compared and combined with a previous AADAP efficacy study conducted on sablefish using 600 mg/L AQUI-S®20E (Bowker et al. 2016). These trials give a comprehensive view of the effect of AQUI-S®20E dosage on sedation time in Sablefish. This information is especially valuable since Sablefish are one of few non-salmonid cold-water marine species to be studied with this sedative.

Methods

Trials were conducted at the NOAA Manchester Research Station, Northwest Fisheries Science Center (Port Orchard, WA) on November 19 - 21, 2019. In the first trial, juvenile Sablefish were sedated to handleable with 300, 450, or 600 mg/L AQUI-S®20E (30, 45, or 60 mg/L eugenol, respectively). In the second trial, juvenile Sablefish were sedated to handleable with 300 mg/L AQUI-S®20E (30 mg/L eugenol) or 120 mg/L tricaine (active control). These data were then combined with a previous sablefish trial from 2016. In all cases, fish were determined to be handleable when they 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. Fish were considered recovered when they regained equilibrium, were swimming freely, and were able to avoid obstacles in their path. The first trial measured the sedation-to-handleable and recovery times of 10 individual juvenile Sablefish at each of the three

treatment concentrations. In this trial, the researchers were not blinded to the treatment concentration.
The second trial measured the sedation-to-handleable and recovery times of thirty individual juvenile Sablefish exposed to the lowest proposed efficacious AQUI-S®20E concentration and thirty individual juvenile fish exposed to the active control concentration. These test fish (n = 60) were split into four groups of fifteen fish each, which were randomly assigned to be sedated with either AQUI-S®20E or tricaine. The researchers determining the time-to-sedation and recovery of fish were blinded from the treatment sedative used during each round.

For both trials, working solutions of sedative for each round was prepared in bulk and tested for accuracy. Water samples from each bulk sedative solution were 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 (Meinertz & Hess 2014). Eugenol doses were considered acceptable if they were within ± 25% of the target dose. Fish were sedated in individual ~ 15 L (4 gal) static tubs with the sedative bath replaced between each fish. After sedation, each fish was weighed, measured, and moved to a static recovery bath. The water in each recovery bath was replaced between each fish. Fish were considered recovered when they regained equilibrium, were swimming freely, and were able to avoid an obstacle 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.

During both trials, water temperature and dissolved oxygen (DO) concentration were measured in each sedation container before placing fish in the solution. Salinity and pH were measured once in untreated source water.

The time-to-handleable and time-to-recovery data from trial 1 were statistically analyzed using Program R to determine differences between sedative concentrations. The data were checked for normality and homogeneity of variance using the Shapiro-Wilk test and Bartlett test, respectively. If these assumptions were met, a one-way Analysis of Variance (ANOVA) was used with a Tukey's post-hoc test. If the ANOVA assumptions were not met, the data was analyzed using a generalized linear model followed by a type II analysis of deviance and a Tukey style post-hoc test.

The time-to-handleable data from trial 2 was dichotomized with a success defined as a handleable time less than 5 min. The dichotomized data were then analyzed with a two-tailed, exact binomial test (SAS Proc Freq). An efficacious trial was defined as having a success rate (percentage of sedations less than 5 min) significantly greater than 80%.

The sedation and recovery times of the two trials were then combined with the data from AADAP's previous AQUI-S®20E sablefish trial (Bowker et al. 2016) and analyzed to compare the differences among the sedation concentrations using Program R. Again, the data were checked for normality and homogeneity of variance as above. If these assumptions were met, a one-way Analysis of Variance (ANOVA) was used with a Tukey's post-hoc test. If the ANOVA assumptions were not met, a non-parametric Kruskal-Wallis test was used with a built in post hoc test (package agricolae).

Results

Trial 1

In the first trial, AADAP researchers aimed to compare the higher end AQUI-S®20E dosage used in the previous trial (Bowker et al. 2016) with the low end dosage of 300 mg/L AQUI-S®20E used in the effectiveness trials of other marine species (Bowker et al. 2015, 2017). Mean time to handleable when sedated with 300, 450, and 600 mg/L AQUI-S®20E was 3.2 min (range, 2.0 – 4.4 min), 2.6 min (range, 2.0 – 3.4 min), and 2.0 min (range, 1.4 – 2.3 min), respectively. There were significant differences in the time it took for fish to become handleable among the different AQUI-S®20E concentrations ($P<0.001$, $F_{2,27}=16.1$), with all treatments being significantly different. Despite the longer time to handleable results of the 300 mg/L sedative dosage, none of the fish tested took longer than 5 min to reach the handleable stage.

Mean time to recover when sedated with 300, 450, and 600 mg/L AQUI-S®20E was 8.7 min (range, 5.5 – 11.0 min), 9.5 min (range, 7.6 – 11.7 min), and 10.1 min (range, 6.7 – 12.6 min), respectively. There were no significant differences among the resulting time to recovery among the AQUI-S®20E dosages ($P=0.25$, $F_{2,27}=1.44$).

All analytically verified eugenol doses were within 10% of their target dosages. The mean analytically verified eugenol dose of the 300 mg/L AQUI-S®20E treatment was 27.9 mg/L eugenol (92.9% of target). The eugenol concentrations of the 450 mg/L AQUI-S®20E bulk solutions were 43.1 mg/L (95.8% of target). The eugenol concentrations of the 600 mg/L AQUI-S20E bulk solutions were 54.8 mg/L (91.4% of target).

General fish behavior during sedation was characterized as mostly normal (80%) in the 300 mg/L treatment and mostly abnormal in the 450 and 600 mg/L treatment groups. Of the fish exposed to each of the higher AQUI-S®20E doses, 90 –

100% displayed head shake, gill cough, jumping, and/or slight agitation upon immersion in the sedative bath. However, this behavior lasted for only a short period of time. Behavior during recovery was predominately characterized as normal and no fish died during the study.

Mean water temperature and DO concentration of the buckets of sedative solutions were 11.2°C (range, 10.8 – 11.5°C) and 10.1 mg/L (range, 9.5 – 10.8 mg/L), respectively. Source water pH was 8.1.

Trial 2

Mean times to handleable and recovery for 30 fish sedated with 300 mg/L AQUI-S®20E were 4.25 min (range, 2.73 – 5.95 min) and 11.72 min (range, 7.82 – 15.97 min), respectively. Twenty-six fish (86.7%) became handleable within 5 min. This was not significantly greater than 80% (P = 0.5105). Four fish (13.3%) became handleable between 5.30 – 5.95 min. Mean times to handleable and recovery for fish sedated with 120 mg/L TRICAINE-S® were 3.51 min (range, 2.27 – 6.80 min) and 8.60 min (range, 5.90 – 12.22 min), respectively.

Sixteen of the 30 fish (53%) exposed to AQUI-S®20E displayed abnormal behavior when placed into the exposure container. These behaviors included headshaking, slight agitation or excitement, and "gill cough" (an interruption in the normal ventilatory cycle). Eighteen of the 30 fish (60%) exposed to tricaine displayed the same abnormal behavior. Fish displayed abnormal behavior for approximately 20 sec or less, although the timing of each was not measured. General behavior of 29 of 30 fish recovering from sedation with AQUI-S®20E was characterized as normal. General behavior of 24 of 30 fish recovering from sedation with tricaine was characterized as normal. Abnormal behaviors during recovery consisted of either swimming in circles or slight agitation when confronted with an obstacle.

Overall mean water temperature of all sedative solutions (n = 60) during sedation was 10.8°C (range, 10.1 – 11.4°C) and overall mean DO concentration of all sedative solutions was 10.0 mg/L (range, 8.9 – 11.0 mg/L). Water pH of source water and a bulk solution of AQUI-S®20E was 8.05 and 7.53, respectively.

Overall, the mean analytically-verified concentration of eugenol (based on sedative solution samples collected from 10 randomly chosen exposure containers of AQUI-S®20E) was 28.2 mg/L, which was 94.1% of the target concentration.

Combined Data

After the data from the two new studies were combined with the previous study, the mean times to handleable for 300, 450, and 600 mg/L AQUI-S®20E were 4.00 ± 0.85, 2.57 ± 0.37, and 1.66 ± 0.29 min, respectively. There was a significant difference in sedation times among all the concentrations (Figure 1A, $X^2_2 = 69.9$, $P < 0.001$).

The combined mean times to recovery for 300, 450, and 600 mg/L AQUI-S®20E were 10.97± 2.17, 9.52 ± 1.49, and 7.99 ± 2.33 min, respectively. There was also a significant difference between the 300 and 600 mg/L treatments (Figure 1B, $F_{2,87} = 18.7$, $P < 0.001$).

Discussion and Conclusions

These three studies on the efficacy of AQUI-S®20E in Sablefish represent AADAP's only research on a non-salmonid, cold-water, marine species for this product. Sablefish is also the only marine species in which AADAP has conducted multiple efficacy studies for the same species. This has resulted in a relatively complete picture of the effects of different concentrations of AQUI-S®20E on sedation and recovery. Overall, the three trials indicate the efficacy of AQUI-S®20E for sablefish across a range of concentrations 300-600 mg/L AQUI-S®20E (30-60 mg/L eugenol).

The data from the three different dosages demonstrate a significant influence of AQUI-S®20E concentration on sedation time. At 300 mg/L AQUI-S®20E, the percentage of sedation times under 5 min was not significantly greater than 80%. Based on this criterion this dosage did not meet the effectiveness criterion, which was originally set forth in the FDA concurred protocol written in 2015. Despite this, the mean + SD of the combined sedation times at 300 mg/L AQUI-S®20E (4.85 min) was still less than the 5 min threshold. This suggests that this concentration of AQUI-S®20E will likely be acceptable by fisheries and aquaculture professionals as a minimum recommended dosage for this species. Practitioners could increase the dosage within the recommended limits to decrease the sedation time, if desired. The majority of the other saltwater efficacy studies conducted by AADAP utilized 300 mg/L AQUI-S®20E, and were mostly conducted on warmwater species (Bowker et al 2015, 2017). Sablefish are a deep-water benthic species with a relatively slow metabolism (Leeuwis et al. 2019), which may affect the sedation times of this species compared to warm water or salmonid species. Despite this, AQUI-S®20E remains an effective sedative option for this species.

The combined data also indicates a significant difference in recovery time between 300 and 600 mg/L AQUI-S®20E. This difference was not detected in trial 1 alone, which actually showed the opposite trend, though it was not significant. However, with the extra data from trial 2 and the previous study (recovery time from 600 mg/L: 7.3 ± 2.0 min) serving to increase the power of the statistics, a difference was detected. Shorter recovery times at the 600 mg/L treatment were witnessed in the previous experiment than in trial one. This could be an artifact of the different researchers or different conditions of the previous study. However, the overall significantly shorter time to recovery could be considered evidence that 600 mg/L may be the preferred dosage in some instances. However, 100% of fish exposed to 600 mg/L AQUI-S®20E displayed some form of abnormal behavior and only 45% of fish exposed to 300 mg/L AQUI-S®20E displayed any abnormal behaviors. These abnormal behaviors may not have much effect on fish health, but may want to be considered if working with a sensitive or threatened/endangered species.

Overall, we believe this data supports 300 mg/L AQUI-S®20E as the minimum effective dose for Sablefish. Results from these trials were submitted to FDA with a request to be included in the body of evidence supporting treatment efficacy of AQUI-S®20E.

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Figures

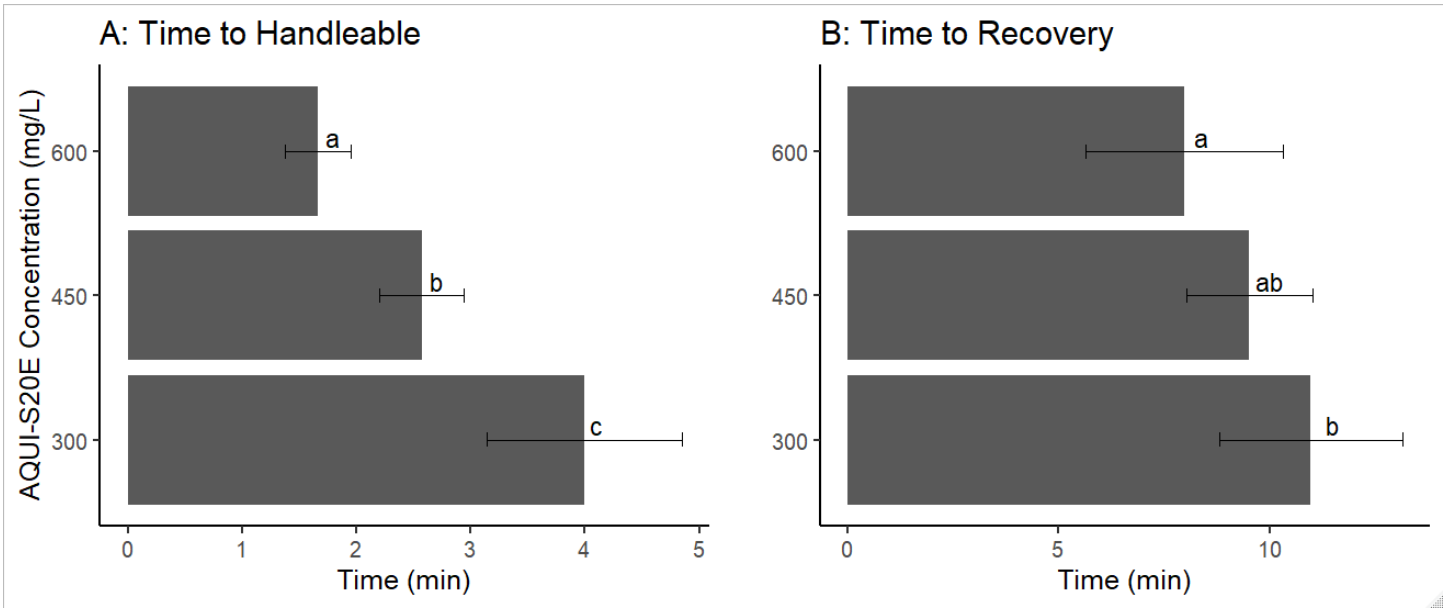

Figure 1: Mean ± SD of the (A) time to handleable and the (B) time to recovery data of Sablefish after combining the data from three studies conducted by AADAP covering three AQUI-S®20E concentrations.

Figure 2: Photograph of a juvenile Sablefish being measured after being sedated.