Clove oil, Quinaldine and AQUI-S as anesthetics for Yellow fin Seabream (*Acanthopagrus latus*) and blue fin Seabream (*Sparidentex hasta*)

Musaad Al-Roumi, Amani Al-Yaqout, Azad Ismail and Mohammed Al-Kandari

***Abstract*—**Anesthetics are used in fish husbandry and management to minimize stress during routine procedures, such as handling the fish. The tested doses of clove oil and quinaldine were 20, 40, 80 and 120 ppm, while the tested concentrations of AQUI-S were 20, 40 and 60 ppm. All the tested concentrations of the three drugs were effective on the tested fish from the two species to reach the total sleep and recover with no mortality. All the fish were observed for 96 h with no change in behavior or feeding.

***Keywords*—** Blue fin seabream, yellow fin seabream, quinaldine, clove oil, AQUI-S.

I. Introduction

HANDLING aquatic animals affects their physiology and behavior [1]-[2]. Stress-related cortisol releases in fish can suppress immunological capacity [3]-[4]. Anesthetics are agents used to induce, first, a calming effect, then successive loss of equilibrium, mobility, consciousness, and finally, reflex action in an organism exposed to higher concentrations of the drug, or exposed to longer time [5]. Anesthetics are used in fish husbandry and management to minimize stress during routine procedures, such as handling the fish during capturing, transportation, sorting, measurements or tagging [6]. The chemicals used as anesthetics in fish have generally been developed for purposes other than their use as anesthetics. For example, Quinaldine (2-methylquinoline) is one of the most widely anesthetics that is being used by marine biologists there are some questions about its safety because of reported associations between quinaldine and thyroid abnormalities in humans and mice [7]. Another important anesthetic used in fisheries is 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222). There are limitations in using MS-222 in the field as the US Food and Drug Administration (US FDA) requires that fish exposed to MS-222 must have a minimum of 21-day withdrawal period before they can be consumed by humans [8]-[9].

Organic farming of agricultural and horticultural crops is being used as a popular venture in the direction of sustained and eco-friendly food production activity [10].Organic farming looks for alternatives to those chemicals that are currently being used in aquaculture, and the anesthetics are one such important input. As a result, different chemical anesthetics were investigated to compare their effectiveness with a natural product, known as clove oil, on different species of fish. the effectiveness of clove oil as an anesthetic was recently studied and found to be a relatively safe, efficacious, and inexpensive anesthetic in a wide variety of marine fish species including rabbitfish [11]; goldfish [12], rainbow trout [13], Damselfish [14], coho salmon and White sturgeon [15], channel catfish [16] mollusks [17] and silver pomfret [18]. Due to the positive features reported on eugenol (clove oil) as anesthetic on aquatic animals, a new anesthetic compound (AQUI-S) was developed. AQUI-S was reported to contain 50% (540 g/l) isoeugenol (2-methoxy-4-propenylphenol) and 50% polysorbate 80. These materials are classified as GRAS by the FDA [2]. AQUI-S is approved for use without a withdrawal period in New Zealand, Australia, Chile and Faroe Islands, but still undergoing testing for approval by the FDA for use in the US [19]. The objective of this study was to conduct detailed investigations on the possibility of using clove oil and AQUI-S as natural alternatives to chemical anesthetics in fish. Their efficacy as anesthetic agents has been investigated against *Sparidentex hasta* and *Acantho pagruslatus*. Blue fin seabream (*Sparidentex hasta*) and and yellow fin seabream (*Acantho pagruslatus*) are important commercial marine fish species in the Gulf region.

Musaad Al-Roumi is with Kuwait Institute for Scientific Research, Environment and Life Sciences Research Center, Kuwait (e-mail: musaadm5@gmail.com).

Amani Al-Yaqout, is with Kuwait Institute for Scientific Research, Environment and Life Sciences Research Center, (corresponding author's phone: 965-99854579; e-mail: kisr.researcher@gmail.com).

Azad Ismail is with Kuwait Institute for Scientific Research, Environment and Life Sciences Research Center, Kuwait (e-mail: suruaz@gmail.com).

Mohammed Al-Kandari is with Kuwait Institute for Scientific Research, Environment and Life Sciences Research Center, Kuwait (e-mail: m_alkandari@hotmail.com)

II. Methodology and Work Plan

A. Exponential Fish

Yellow fin sea bream (*Acanthopagrus latus*), and blue fin sea bream (*Sparidentex hasta*) cultured at Kuwait Institute for Scientific Research (KISR) facilitates were stocked in one-ton tanks for two weeks for acclimatization. Total of 20 fish have been used for each dose. The dose was considered effective if the fish lose their equilibrium into total sleep within 10 min and any behavioral changes in the fish were noted. Once anaesthetized, the fish were shifted to recovery tanks and their behavior was recorded. Furthermore, the fish were hold in normal rearing conditions for recording observations for four days to record any mortality or any change in feeding behavior. Fish were used only once and then, returned to the holding tanks.

B. In-Vitro Tests of the Stress Effect of Using Different Treatments on Fish.

For this test, the fish blood was collect using ethylenediaminetetraacetic acid (EDTA) as the anticoagulant. After that the red blood cells (RBC) were spinned down by centrifugation at 3000 rpm for 10 min at 4ºC, and the plasma was removed from the cells by drawing it off from the top. The purpose of conducting those experiments was to measure the stress level in the tested fish.

C. The Drugs

The tested concentrations of clove oil were 40, 80 and 120 ppm and 20, 40, 60 ppm of AQUI-S for Yellow fin Seabream. Clove oil was dissolve in ethanol with dilution factor 1:4.

D. Statistical Analysis

Data collected will be subjected to one-way analysis of variance (ANOVA) and Duncan's multiple range tests to arrive at the concentration and time effects in the order of efficacy per treatment.

III. Results

A. Anesthetic Effects of Different Treatments on Yellow Fin Sea bream

The fish weight range was 10.9-13.4 g, 10.8-13.3 g and 11.0-13.5 g for clove oil and AQUI-S experiments respectively. A total of 20 fish, were used for each concentration of the four treatments. The fish were exposed to the selected concentrations of the treatments and time of total sleep and complete recovery were recorded according to table 2. The data showed that all the used concentrations were effective and there was a correlation between concentration and time, as the concentration increased, time decreased (Fig. 1, 2 and 3). The DO in the anesthetic and recovery chambers was between 5.52 and 6.00 mg/l. There was no mortality observed in the concentrations between 20 to 60 ppm even after 96-hours post recovery.

TABLE I
Stages of Anaesthetization and Recovery in Fish Fingerlings
(Source: Keene et al., 1998)

Stage	Description	Behavior
1	Normal	Reactive to external stimuli; opercular rate and muscle tone normal.
2	Start of anesthetic induction	Partial loss of muscle tone; swimming erratic; increased opercular rate; reactive only to strong tactile and vibrational stimuli.
3	Total sleep	Total loss of reactivity; opercular movements slow and irregular; loss of all reflexes.
4	Start of recovery	Partial recovery of equilibrium with partial recovery of swimming motion.
5	Complete recovery	Reappearance of avoidance in swimming motion and reaction in response to external stimuli, but still, behavioral response is stolid.

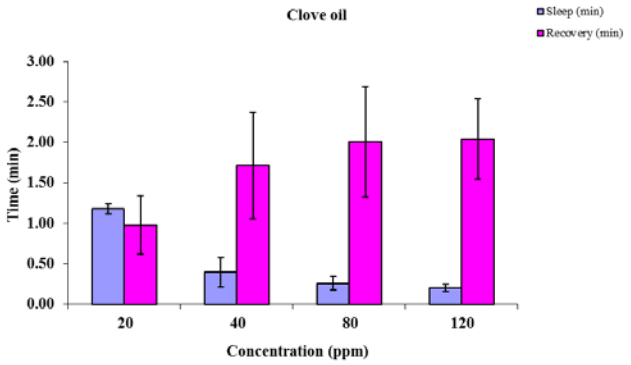

Fig.1 Effect of different concentrations of clove oil (ppm) on total anesthesia and recovery time of yellow fin sea bream (*Acanthopagrus latus*) fingerlings at 23 °C

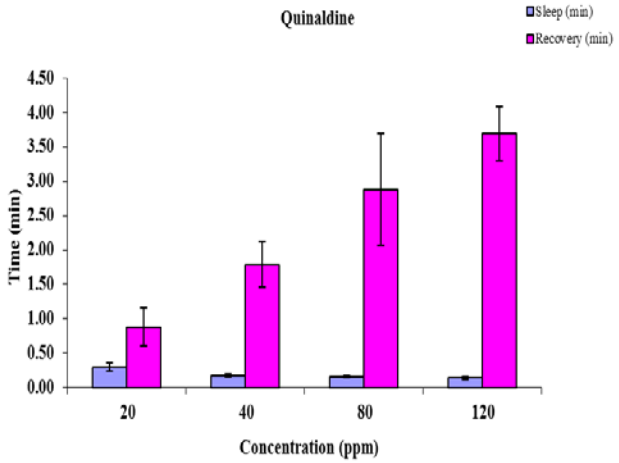

Fig. 2. Effect of different concentrations of quinaldine (ppm) on total anesthesia and recovery time of yellow fin sea bream (*Acanthopagrus latus*) fingerlings at 23 °C

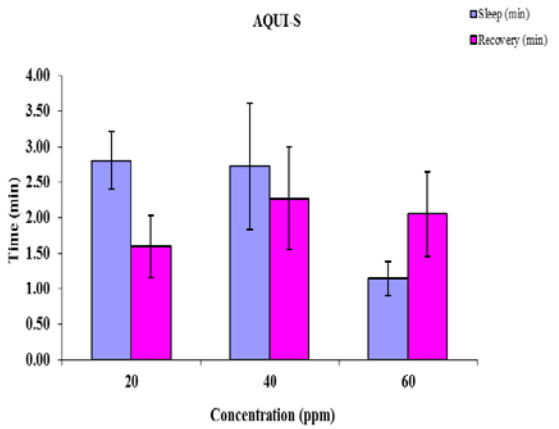

Fig. 3 Effect of different concentrations of AQUI-S (ppm) on total anesthesia and recovery time of yellow sea bream (Acanthopagrus latus) fingerlings at 23 °C.

B. Anesthetic Effects of Different Treatments on Blue fin Sea bream

Total number of fish used in this experiment was 300 of weight range of 10.9-13.4 g, 10.8-13.3 and 10.3-12.8 g for clove oil and AQUI-S experiments. A total of 20 fish, were used for each concentration of the four treatments. The fish were exposed to the selected concentrations of the treatments and time of total sleep and complete recovery were recorded according to table 2. The data showed that all the used concentrations were effective, and there was a correlation between concentration and time, as the concentration increased, time decreased (Fig. 4, 5 and 6). The DO in the anesthetic and recovery chambers was between 5.52 and 6.30 mg/l. There was no mortality observed in the concentrations between 20 to 120 ppm even after 96-hours post recovery.

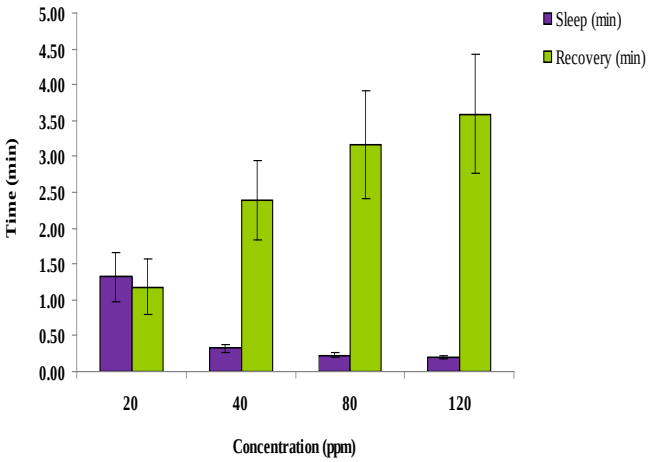

Fig. 4 Effect of different concentrations of clove oil (ppm) on total anesthesia and recovery time of blue fin sea bream (*Sparidentex hasta*) fingerlings at 23 °C.

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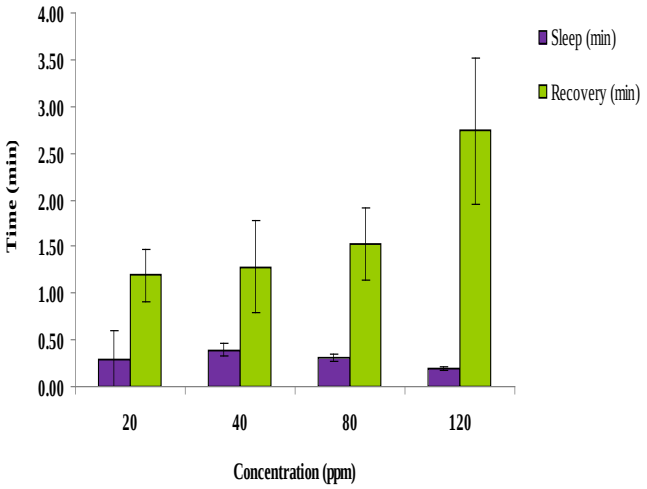

Fig. 5. Effect of different concentrations of quinaldine (ppm) on total anesthesia and recovery time of blue fin sea bream (*Sparidentex hasta*) fingerlings at 23 °C.

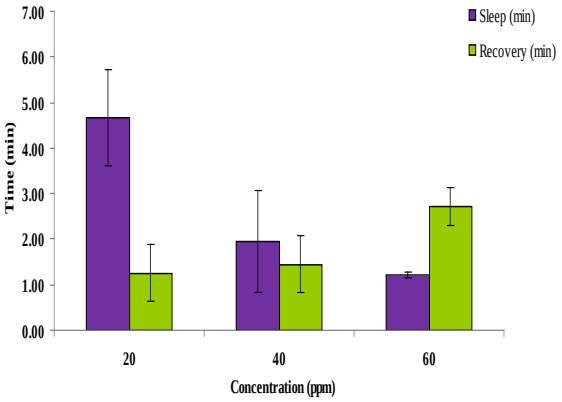

Fig. 6 Effect of different concentrations of AQUI-S (ppm) on total anesthesia and recovery time of blue fin sea bream (*Sparidentex hasta*) fingerlings at 23 °C.

C. Cortisol Level

Immediately post exposure to 20 ppm of EOs and chemicals, blood samples were collected from 10 fish, yellow fin sea bream and 10 fish of blue fin sea bream, for each concentration. Blood samples were analyzed at a private medical laboratory. Changes in the haematology (HGB, HCT, NEUT, LYMPH, MONO, EO, Cortisol and Glucose) of the tested fish were recorded. Different fish species showed different cortisol level post exposure to the treatments. The cortisol level is normally high in blue fin sea bream than yellow fin sea bream, and this is the reason that as they get affected by any slight external stimulation. After exposure of blue fin sea bream to the treatments, the cortisol level decreased, more than the natural level, in correlation to all treatments, but the lowest was recorded using AQUI-S (Fig. 7). Yellow fin sea bream showed lower cortisol level in the control than blue fin sea bream and showed as well decreasing in the level with all the tested treatment but, it was lowest using quinaldine and clove oil followed by thyme oil (Fig. 7).

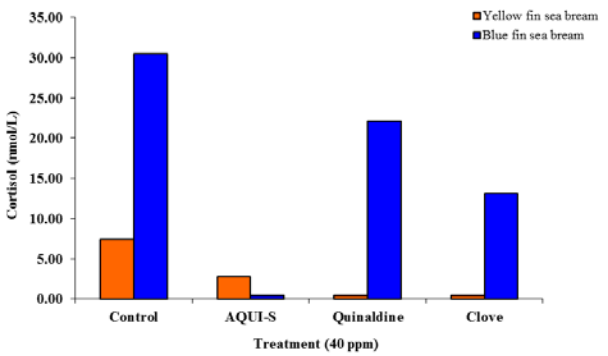

Fig.7 Effect of 20 ppm of the treatments for 1-2 min on the cortisol levels (nmol/l) in yellow fin sea bream and blue fin sea bream fish plasma.

IV. Discussion

The choice of anesthetics for field studies generally depends on several considerations as follows: availability; cost-effectiveness; ease of use; nature of the study; allow for the immediate release of the fish into the food chain; allow for swift induction of and recovery from anesthesia; not excessively disturb the physiological balance of the fish, which could reduce its chances of survival upon release; and safety for the user [20]. Fingerlings exposed to the treatments progressed sequentially through the various stages of anesthesia [21] and given in Table 1. Increases in the concentration of clove oil, AQUI-S and quinaldine, produced a reduction in the time required to lose equilibrium and an increase in the recovery time.

V. Conclusion

The recommended dose for yellow fin sea bream and blue fin sea bream fingerlings to elicit a complete anesthesia appeared to be 20 ppm of quinaldine, AQUI-S and clove oil. AQUI-S showed the lowest cortisol level in blue fin sea bream while quinalidine showed the lowest level with yellow fin sea bream. The natural products are such as clove oil and AQUI-S are recommended in aquaculture as they have less negative effect on humans as well as on the marine environment

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