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Physiological Responses of Dark-banded Rockfish *Sebastes inermis* to Anesthetization with Clove Oil

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In order to establish optimum anesthesia concentration, we tested the efficacy of clove oil at five different concentrations in large sized (mean SL 17.1±2.21 cm) and small sized (mean SL 0.6±0.06 cm) dark-banded rockfish *Sebastes inermis*. Optimal anesthesia concentration for dark-banded rockfish was 150 mgL^{-1} in both large and small sized fish. In general, fish exposed to higher anesthetic doses were rapidly induced but took longer to recover ($P<0.05$). Recovery time of small sized fish was longer than large sized fish in lower concentrations, while recovery time of large sized fish was longer than small sized fish in higher concentration ($P<0.05$). Using the established optimum aesthetic concentration, we evaluated the physiological response of dark-banded rockfish to clove oil by measuring plasma cortisol and glucose levels. Following administration of 150 mgL^{-1} clove oil at 20°C (optimum breeding temperature), plasma cortisol level was highest (42.2±11.318 μg/dL) after 0 hour, while plasma glucose level was highest (52.5±10.61 mg/dL) after 1 hour. Plasma cortisol and glucose concentrations required 6 and 2 hours, respectively, to return to pre-exposure levels.

Keywords: Anesthesia, clove oil, *Sebastes inermis*, plasma cortisol, plasma glucose

Introduction

Anesthesia can decrease stress levels when fish are subjected to blood sampling, immobilization, handling, injection of vaccines and antibacterial substances, medical treatment for diseases, artificial spawning, transport, and sorting (Park et al., 2008; Westerfield, 1993). In recent years, use of clove oil has become more popular in the aquaculture industry, based on the fact that it is safe, inexpensive, non-toxic to the environment, and does not require a withdrawal period compared to other synthetic-based anesthetics (Kang et al., 2005; Park et al., 2008). The effects of clove oil as an anesthetic has been studied in a number of fish species (Soto and Burhanuddin, 1995; Waterstrat, 1999; Woody et al., 2002; Seol et al., 2007).

Dark-banded rockfish *Sebastes inermis*, is an ovoviviparous teleost that lives in rocky areas of shallow coastal regions, and distributed widely across Korea and Japan (Choi et al., 2002). It has high commercial value and regarded as a productive and manageable species from an aquaculture perspective (Choi et al., 2005; Gwak and Park, 2006; Oh and Noh, 2006). However, no study has investigated the effects of anesthesia and possible physiological stresses on this species. The aim of this study was to establish optimum anesthetic concentration by investigating the effects of clove oil on large and small sized dark-banded rockfish at 20°C (optimum breeding temperature). Physiological responses were subsequently analyzed by measuring plasma cortisol and glucose levels.

Materials and methods

In April 2008, large and small sized dark-banded rockfish were obtained from the Gyeongsangnam-do Fisheries Resources Research Institute, Republic of Korea. Specimens were transported and reared in a recirculating culture system in the Fishery Genetics and Breeding Science Laboratory of Korea Maritime University. The recirculating culture system consisted of five 1100-L circular tanks, a 1100-L filtering tank, an aeration system and a temperature control system. Culture water was partially replaced with sand-filtered, aerated seawater (salinity 34±0.6 ppt, pH 7.6±0.5, dissolved oxygen 8.5±0.7 mgL^{-1}, ammonia 0.006 mgL^{-1}) every seven days.

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experiments began in July 2008. Fish were fasted for 24 hours before the start of experiments. Specimens used in experiments were measured using a digital vernier caliper (CD-20CP, Japan) and an electronic balance (JW-1, Republic of Korea). Average body length and weight of large sized dark-banded rockfish were found to be 17.1±2.21 cm and 136.0±49.30 g, respectively, while small sized fish were found to be 0.6±0.06 cm and 28.0±2.80 g, respectively.

Five different concentrations of clove oil (50, 100, 150, 200, and 250 mgL^{-1}) were administered to groups of 10 fish at 20°C. The stock solution of clove oil (Sigma, St Louis, MO, USA) was dissolved in 95% ethanol at a ratio of 1:10 (Cho and Heath, 2000). Fish were stocked in 12 L plastic tanks (quantity 10 L) in static condition of constant temperature using 500 L aquarium under temperature control system one week before of the start of study. Every experiment was conducted in triplicate. The anesthesia levels and recovery times of fish were measured in seconds using a stopwatch.

Anesthetic effect decision-based table (Table 1) was modified from data reported by Summerfelt and Smith (1990) and Woolsey et al. (2004). Anesthesia time was determined from when fish were stocked in anesthetized water to the time of stage A7, in which opercular movement ceased. Recovery time was determined from when fish were stocked in recovery water to the time of stage R6, in which normal swimming and responsiveness to visual stimulation recommenced.

Table 1. Stage of anesthesia induction and recovery in clove oil efficacy tests performed in dark-banded rockfish *Sebastes inermis* (modified from Summerfelt and Smith, 1990; Woolsey et al., 2004)

Anesthesia	
Stage	Characteristic behavior
A1	Normal swimming; opercular movement and normal general movement
A2	Swimming speed slowed; rolling from side to side
A3	Partial loss of equilibrium; swimming erratic
A4	Complete loss of equilibrium; swimming perfectly inside out; pectoral fin, pelvic fin and dorsal fin movement stop
A5	Little sedation; anal fin and tail fin movement stop
A6	Perfect sedation; only opercular movement
A7	Opercular movement ceased
Recovery	
Stage	Characteristic behavior
R1	Resume opercular movement
R2	Preferential movement of pectoral fin and tail fin
R3	Dorsal fin, pelvic fin and anal fin movement
R4	Swimming perfectly inside out
R5	Swimming erratic; redress the balance
R6	Normal swimming; responsiveness to visual stimuli

For this experiment, food supply was disrupted 24 hours prior to sampling. Blood physiological response was measured according to set time intervals after fish were anesthetized with clove oil concentration of 150 mgL^{-1} at a water temperature of 20°C, adopting middle values from the tested regimes. Blood samples were extracted from five randomly selected fish at control (before the initiation of the experiment), 0, 1, 2, 6, 12, 24, 48, and 72 hours post anesthesia. Fish used in this experiment were not involved in the anesthetic effect experiments. Blood was collected from the caudal vasculature using a disposable syringe (3 mL, Sung Shim Medical Co., Ltd, Bucheon, Republic of Korea) with heparin sodium (Shin Poong Pharm Co., Ltd, Ansan, Republic of Korea). Blood was extracted within 1 minute to minimize handling stress, and allowed to sit for 10 minutes at room temperature prior to centrifugation (Centrifuge Micro 17R, Hanil Science Industrial Co., Ltd, Incheon, Republic of Korea) for 10 minutes at 20,000 g. The collected plasma was transferred to another 1.5 mL microtube and kept at -70°C in a super low temperature refrigerator (CLN-50UW Nihon Freezer, Nihon Co., Japan) prior to analysis.

Plasma cortisol concentration was measured using 1470 WIZARD Automatic Gamma Counter (Cobra, Packard Co., Ramsey, MN, USA) after the antigen antibody response was derived using Coat-A-count TKCO Cortisol RIA Kit (DPC, Los Angeles, CA, USA) according to the methodology of Donaldson (1981). Plasma glucose concentration was analyzed according to methodology of Raabo and Terkildsen (1960; Kit 510, Sigma, St Louis, MO, USA), where production of H_2O_2 by glucose oxidase in the presence of *o*-dianisidine was evaluated as an absorbance increase at 450 nm. The experiment was performed twice, and results are reported as means±standard deviation (n=10) unless otherwise stated. Using the SPSS statistics package (SPSS 9.0, SPSS Inc., Chicago, IL, USA), one-and two-way analysis of variance (ANOVA) were carried out to test for statistical significance (P<0.05) between clove oil concentrations. Multiple comparisons were performed using Duncan's multiple range test (Duncan, 1955).

Results and discussion

Table 2 contains parameters associated with anesthetic effects of the five clove oil concentrations on large and small sized dark-banded rockfish. Anesthetic time was signifi-

Table 2. Size-specific effects of clove oil dose at 20°C among dark-banded rockfish *Sebastes inermis*

Dose (mgL^{-1})	Anesthetic time (sec)[1]		Recovery time (sec)[1]	
	Large size	Small size	Large size	Small size
50	220.4±19.11[a]	175.0±30.41[a]	244.6±37.52[e]	284.2±16.83[e]
100	183.8±17.28[b]	116.4±13.97[b]	273.0±18.79[d]	308.4±10.55[d]
150	107.2±35.98[c]	51.2±4.21[c]	339.6±73.62[c]	322.4±18.98[c]
200	87.8±7.95[d]	44.8±5.12[d]	407.2±39.64[b]	363.4±27.40[b]
250	69.6±9.40[e]	32.2±2.59[e]	470.2±106.42[a]	433.2±38.44[a]

Two-way ANOVA

	DF	Anova SS	Mean Square	F-value	P-value	DF	Anova SS	Mean Square	F-value	P-value
Size	1	31050.3	31050.3	94.63	< 0.0001	1	264.5	264.5	0.11	< 0.0001
Dose	4	156437.0	39109.3	119.20	< 0.0001	4	226491.0	56622.8	24.55	< 0.0001
Interaction	4	1418.9	354.7	1.08	< 0.0001	4	15747.0	3936.8	1.71	< 0.0001

[1]Each value is mean±standard deviation (n=10). Values in the same column not sharing common superscripts are significantly different (P<0.05).

cantly (P<0.05) affected by fish size and clove oil concentration, decreasing as clove oil concentration increased and increasing as fish size increased. Recovery time was significantly affected (P<0.05) by fish size and clove oil concentration. As anesthetic concentration increased, recovery time significantly increased (P<0.05). However, recovery time of small sized fish was longer than large sized fish in low concentrations, while recovery time of large sized fish was longer than small sized fish in higher concentrations (P<0.05).

Treatment with excessive anesthesia is very stressful to fish, causing abnormal metabolic rates, oxygen consumption, blood pressure, and blood physiological responses. Moreover, these optimum anesthetic concentration can minimize negative impacts and thus reduce stress in fish. Optimum anesthetic concentrations are usually expected to induce anesthesia within 3 minutes and allow recovery within 10 minutes (Gilderhus and Marking, 1987; Son et al., 2001; Park et al., 2003). Based on this requirement, we concluded that optimal anesthesia concentration for small and large dark-banded rockfish at 20°C was 150 mgL^{-1}. As such, this concentration can be regarded optimal across all size ranges.

In this study, dark-banded rockfish was shown to be sensitive to anesthetic effect of clove oil and fish size. Similar results were reported for goldfish *Carassius auratus*, muddy loach *Misgurnus anguillicaudatus*, Chinese minnow *Rhynchocypris oxycephalus*, Amur minnow *R. steindachneri* and Pacific cod *Gadus macrocephalus* (Mattson and Riple, 1989) in terms of clove oil concentrations, while our results also agreed with size-specific studies for white sea bream *Diplodus sargus*, and sharp snout sea bream *D. puntazzo* (Tsantilas et al., 2006).

Mean plasma cortisol concentration was 4.1±2.45 μg/dL before the experiment (Fig. 1a). Fish were moved to the recovery tank after administering 150 mgL^{-1} clove oil at in 20°C. Mean plasma cortisol concentration level of control group was 4.1±2.45 μg/dL, increasing to 42.2±11.31 μg/dL at 0 hr (P<0.05). Plasma cortisol concentration decreased

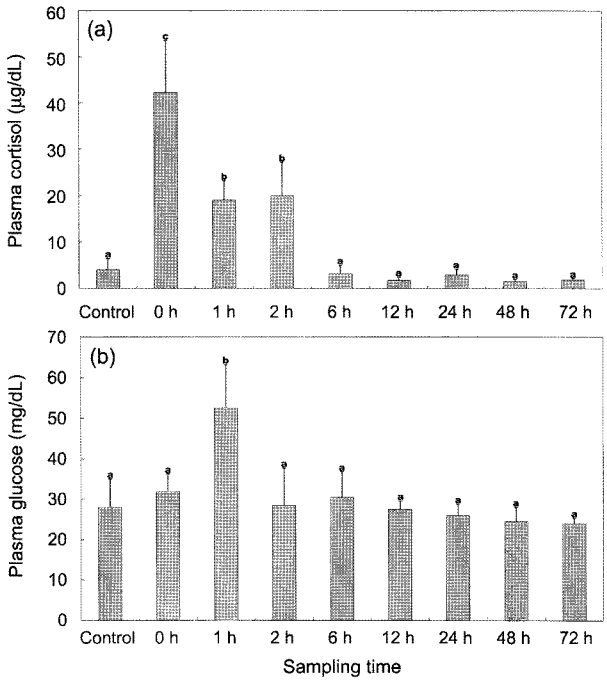

Fig. 1. Post-recovery physiological measurements (means±SD) of dark-banded rockfish *Sebastes inermis*: (a) plasma cortisol; (b) plasma glucose. Value are means±standard deviation (n=10) for experiments run on two occasions. Different letters on the bars indicate statistical significance between treatment and control groups at a sampling time (Duncan's multiple range test, P>0.05).

from 19.0±4.19 μg/dL at 1 hr of recovery to 20.0±7.16 μg/dL at 2 hrs. Plasma cortisol concentration after 6 hrs was found to be 3.1±1.90 μg/dL, similar to that of the control group (P>0.05).

Plasma glucose concentration was 28.0±7.07 mg/dL before the experiment (Fig. 1b). Fish were moved to the recovery tank after administering 150 mgL^{-1} clove oil at in 20°C. Mean plasma glucose concentration level of control group was 28.0±7.07 μg/dL. Initial mean recovery plasma glucose concentration was 32.0±4.24 mg/dL, similar to that of control group (P>0.05). Plasma glucose concentration increased to 52.5±10.61 mg/dL at 1 hr (P<0.05). Plasma glucose concentration after 2 hrs was found to be 28.5±9.19 ß¹/dL, similar to that of control group (P>0.05).

Plasma cortisol and plasma glucose are recognized as useful indicators of stress in fish (Schreck, 1982; Park et al., 2008). Plasma cortisol and glucose levels in red drum *Sciaenops ocellatus,* simultaneously exposed to MS-222 and Quinaldine anesthetic, were reported to be elevated (Massee et al., 1995). Barton and Iwama (1991) stated that "Usually, phenomenon that plasma cortisol concentration of fishes rises by stress is first order reaction, phenomenon that plasma glucose concentration rises is result of second-order first order reaction by hormone rise reaction by stress.". This trend has been reported in the gray mullet *Mugil cephalus* and kelp grouper *Epinephelus bruneus* (Chang and Hur, 1999; Park et al., 2008).

Our results show that plasma cortisol level increases faster than glucose concentrations. This result was similar to a study carried out by Chang and Hur (1999) and Park et al. (2008). The plasma cortisol concentration of anesthetized dark-banded rockfish returned nearest to control concentration after 6 hrs post-administration, while plasma glucose concentration returned nearest to control concentration after 2 hrs post-administration. Future investigations in dark-banded rockfish should focus on comparative physiological reactions induced by clove oil and other fish anesthetics.

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