Aquaculture 287 (2009) 427–430

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Short communication

Efficacy and physiological responses of rock bream, *Oplegnathus fasciatus* to anesthetization with clove oil

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ARTICLE INFO

Article history:
Received 4 March 2008
Received in revised form 18 October 2008
Accepted 26 October 2008

Keywords:
Anesthesia
Clove oil
Oplegnathus fasciatus
Plasma cortisol
Plasma glucose

ABSTRACT

We tested the efficacy (e.g., induction time, recovery time) of clove oil as an anesthetic for rock bream *Oplegnathus fasciatus*. In addition, we also evaluated the physiological response of fish to the anesthetic by measuring plasma cortisol and glucose. In general, fish exposed to higher anesthetic doses were rapidly induced but took longer to recover, while lower water temperatures resulted in longer induction and recovery times. Optimal anesthetic dose and water temperature were estimated to be 150 mgL^{-1} at 20 °C, 100 mgL^{-1} to 125 mgL^{-1} at 24 °C, and 50 mgL^{-1} to 75 mgL^{-1} at 28 °C. Following the administration of 100 mgL^{-1} of clove oil at 24 °C, the plasma cortisol level was highest (1.70±0.148 μg/dL) after 1 h while the plasma glucose level was highest (80.0±1.41 mg/dL) after 2 h. It took 2 days for the plasma cortisol and plasma glucose concentrations to return to pre-exposure levels.

1. Introduction

Farmed fish can be subjected to a range of stress factors including handling, confinement, transport, medication, water quality, and changes in water temperature and salinity (Singley and Chavin, 1971; Fryer, 1975; Donaldson, 1981; Wedemeyer and Mcleay, 1981). Anesthesia can decrease stress levels when fish are subjected to blood sampling, immobilization, handling, injection of vaccines and antibacterial substances, medical treatment for disease, artificial spawning, transport, and sorting (Westerfield, 1993).

In recent years, clove oil has been widely used in the aquaculture industry because it is evaluated that safe, inexpensive, non-toxic in the environment, and does not require a withdrawal period compared to other anesthetic chemicals (Kang et al., 2005). Clove oil has been studied as an anesthetic in a number of species (Endo et al., 1972; Hikasa et al., 1986; Soto and Burhanuddin, 1995; Keene et al., 1998; Waterstrat, 1999, 2005; Chanseau et al., 2002; Woody et al., 2002; Coyle et al., 2005; Seol et al., 2007).

Rock bream is raised in farms across the south coast Tongyeong, Geoje, Namhae and Yeosu in order to meet the high demand of this popular food-fish in Korea. Until now, no study has investigated the effects of anesthesia and possible physiological stresses on this species.

Therefore, the aim of this study was to determine the optimum concentration of anesthetic clove oil for rock bream over a range of temperature conditions. The physiological responses of plasma cortisol and plasma glucose were also subsequently investigated.

2. Materials and methods

In May 2005, cultured rock bream (*Oplegnathus fasciatus*) were obtained from the Gyeongsangnam-do Fisheries Resources Research Institute, Republic of Korea. Rock bream, *O. fasciatus* (Temminck et Schlegel) (Perciformes, Oplegnathidae), is a marine fish that lives in rock areas of shallow coastal regions, distributed across Korea, Japan, Taiwan and Hawaii (Choi et al., 2002). The fish were transported and reared in a recirculating culture system in the Fishery Genetics and Breeding Science Laboratory of the Korea Maritime University. The recirculating culture system consisted of five 1100-L circular tanks, one 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 weekend.

The anesthetic effect and blood physiological response experiment began in July 2007. Fish were fasted for 24 h before the start of the study. Rock bream used in the experiment were measured using a digital vernier caliper (CD-20CP, Japan) and an electronic balance (JW-1, Republic of Korea). Average body length and weight were found to be 14.8±1.66 cm and 139.5±39.24 g, respectively.

Five different concentrations of clove oil (50, 75, 100, 125, and 150 $mgL^{-}1$) were administered to rock bream, in three separate water temperature regimes (20, 24, and 28 °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

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doi:10.1016/j.aquaculture.2008.10.049

Table 1
Stage of anesthesia induction and recovery in clove oil efficacy tests performed in rock bream, *Oplegnathus fasciatus* (modified from Summerfelt and Smith, 1990; Woolsey et al., 2004)*

Stage	Characteristic behavior
Anesthesia	
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	
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

*Body weight and standard length were 139.5±39.24 g and 14.8±1.66 cm respectively in the experiment.

aquarium under temperature control system before one week of the start of the study, 10 fish at a time were exposed to different combinations of water temperature and anesthesia concentration. In total, 15 combinations of water temperature and anesthesia concentration were tested. Every experiment was conducted in triplicate. The anesthesia levels and recovery times of fish were measured in seconds using a stopwatch.

The 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 state, in which opercular movement ceased. Recovery time was determined from when fish were stocked in recovery water to the time of Stage R6 state, in which normal swimming and responsiveness to visual stimulation recommenced.

For this experiment, food supply was disrupted 24 h prior to sampling. The blood physiological response was measured with the passage of time after fish were anesthetized with a clove oil concentration of 100 mgL^{-1} at a water temperature of 24 °C by adding anesthetic directly to an aquarium, adopting middle values from the tested regimes. Blood samples were extracted from five fish at control, 0 (pre), 1, 2, 6, 12, 24, 48, and 72 h post anesthesia, respectively. The fish used in this experiment were not involved in the anesthetic effect experiment.

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). The blood was extracted within 1 min to minimize handling stress. Blood sat for 10 min at room temperature prior to centrifugation (Centrifuge Micro 17R, Hanil Science Industrial Co., Ltd, Incheon, Republic of Korea) for 10 min 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.

The 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 Donaldson (1981) method. The plasma glucose concentration was analyzed, according to Raabo and Terkildsen (1960; Kit 510, Sigma, St Louis, MO, USA), through, evaluating the production of H^2O^2 by glucose oxidase in the presence of *o*-dianisidine as an absorbance increase at 450 nm.

One- and two-way analyses of variance (ANOVA) were used to test for the significance ($P<0.05$) of the effects of temperature and clove oil concentration. The differences among groups were analyzed by ANOVA using the SPSS statistics package (SPSS 9.0, SPSS Inc., Chicago, IL, USA), and multiple comparisons were performed using Duncan's multiple range test (Duncan, 1955).

3. Results and discussion

Treatment with excessive anesthesia is very stressful to fish, causing abnormal metabolic rates, oxygen consumption, blood pressure, and blood physiological responses. Moreover, these side-effects can last for hours after fish recover from anesthesia (Summerfelt and Smith, 1990). Optimum anesthetic concentrations can minimize negative impacts and thus reduce stress in fish. Optimum anesthetic concentrations are usually expected to induce anesthesia within 3 min and allow recovery within 10 min (Gilderhus and Marking, 1987; Son et al., 2001; Park et al., 2003).

Table 2 contains the parameters associated with the effects of clove oil at each concentration and water temperature. Anesthetic time was significantly ($P<0.05$) affected by temperature and clove oil concentration, and decreased linearly as clove oil concentration and water temperature increased. At each water temperature, as the concentration of clove oil increased, the anesthetic time decreased. Furthermore, as water temperature increased, anesthetic time also decreased. The recovery time was significantly affected ($P<0.05$) by water temperature and clove oil concentration. The relationship between water temperature and clove oil dose was also significant ($P<0.05$) in relation to recovery time. As the concentration of the anesthetic increased, recovery time significantly increased ($P<0.05$). However, as the water temperature increased, recovery time significantly decreased ($P<0.05$).

Table 2
Effects of clove oil dose and starting water temperature on anesthesia among rock bream *Oplegnathus fasciatus*

Dose (mgL^{-1})	Anesthetic time (s)[1]			Recovery time (s)[1]		
	20 °C	24 °C	28 °C	20 °C	24 °C	28 °C
50	431±28.3^a	273±21.5^a	156±13.2^a	365±28.6^e	297±21.4^e	215±6.8^e
75	399±14.8^b	198±9.8^b	115±9.8^b	406±12.8^d	321±21.4^d	251±15.5^d
100	375±26.9^c	133±8.9^c	71±4.5^c	467±19.7^c	357±26.1^c	296±24.4^c
125	271±20.1^d	99±8.4^d	47±4.0^d	510±8.1^b	398±18.0^b	317±13.5^b
150	156±14.9^e	55±3.4^e	38±3.0^e	614±39.2^a	436±34.0^a	370±23.7^a

Two-way ANOVA

	DF	Anova SS	Mean square	*F*-value	*P*-value	DF	Anova SS	Mean square	*F*-value	*P*-value
Temperature	2	778,109.5	389,054.8	1716.12	<0.0001	2	424,089.0	212,044.5	414.17	<0.0001
Dose	4	384,843.4	96,210.9	424.38	<0.0001	4	300,467.2	75,116.8	146.72	<0.0001
Interaction	8	647,69.8	8096.2	35.71	<0.0001	8	21,815.7	2727.0	5.33	<0.0001

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