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Anesthetic Effects and Physiological Responses of Clove Oil, Lidocaine-HCl, and Tricaine Methanesulphonate on Seawater Shellfishes in Korea

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Clove oil, lidocaine-HCl 및 Tricaine Methanesulphonate에 대한 한국산 해산패류의 마취효과와 생리반응

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Abstract

This study aimed to investigate the effects of clove oil, lidocaine-HCl, and tricane methanesulfonate (MS-222) on scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*), and to compare the anesthetic effect among three anesthetics. Induction times of clove oil, lidocaine-HCl, and MS-222 were significantly affected by concentrations of anesthetics, and decreased drastically as the concentrations of anesthetics increased ($P < 0.05$). At each group, as the concentration of anesthetics increased, the induction time decreased ($P < 0.05$). For each anesthetic, the longer the shell length of six species in this experiment were, the more induction time increased ($P < 0.05$). Plasma cortisol and plasma glucose, which were measured to examine the stress response in seawater shellfishes in this experiment. Cortisol concentrations of clove oil, lidocaine-HCl, and MS-222 on six seawater shellfish were increased until 6 hrs after recovery of anesthesia (RA) and cortisol concentrations of three anesthetics on each shellfish were highest at 6 hrs after RA. At 6 hrs after RA, cortisol concentrations of MS-222 on each shellfish were higher than those of clove oil and lidocaine-HCl. Especially, cortisol concentration of granular ark at 6 hrs after RA was higher than that of the other shellfishes. At 6 hrs after RA, cortisol concentrations of three anesthetics were decreased until 48 hrs. Glucose concentrations of clove oil, lidocaine-HCl, and MS-222 on six seawater shellfish were increased until 12 hrs after RA and glucose concentrations of three anesthetics on each shellfish were highest at 12 hrs after RA. At 6 hrs after RA, glucose concentrations of MS-222 on each shellfish were higher than those of clove oil and lidocaine-HCl and glucose concentration of granular ark was higher than those of the other shellfishes as well. From 12 to 48 hrs after RA, glucose concentrations of three anesthetics were decreased.

Key words : Anesthetic (clove oil, lidocaine-HCl and MS-222), Physiological response, Seawater shellfish

Ⅰ. Introduction

Six species of shellfishes; scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa)*,

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and shortneked clam (*Ruditapes philippinarum*) inhabit the coasts of Korea(Yoo, 1988; Cho et al., 1995; Shin et al., 2006; Nam et al., 2012). Scallop is one of marine bivalve mollusks in the family Pectinidae. The scallop is a cold-tolerant species inhabiting the coastal waters of the Northern part of Korea and the Northern islands of Japan(Ito, 1997). This species is aqua-farmed in China, Korea, Japan, and Russia with more than 1,400,000 tones worth over US$ 373 million harvested in 2007(Nam et al., 2012). Ark shell belongs to the family Arcidae and is distributed in countries with the latitude similar to that of Korea, including Japan and China, where the species is cultivated commercially. The production of this species in Korea reached its peak in 1998. Yields have decreased gradually since 1998 with a current annual survival rate of 3-5%, necessitating urgent efforts to examine the causes of death and decrease in the productivity of this species(Shin et al., 2006). The surf clam is a widespread species found in the Northeast part of Korea. This species is aqua-farmed in China, Korea, Japan and cultivated commercially(Lee et al., 1997).

Blue mussel is one of the marine organisms that inhabit broadly in the coast of Korea, and it is also edible and greatly consumed in several countries. This species is studied because it can be used as reference materials in cultivation and processing industries with a view to investigating as proteinous resources(Cho et al., 1995). Granular ark is an intertidal infaunal bivalve belonging to the family Arcidae and this species inhabits the coasts of Korea. It is a small species with a shell length 3−4 cm and illustrates very little movement(Yoo, 1998). Shortneked clam is an aquaculture shellfish mainly distributed in the intertidal zone of East Asia. The shell color variation of this species is multicolorful and the morphological variation in shellfish has been reported to be related to the genetic distances between this species(Yokogawa, 1997).

Anesthesia can decrease stress levels when fish are subjected to blood sampling, immobilization, handling, vaccine and antibacterial substances injections, medical treatment for diseases, artificial spawning, transport, and sorting(Park et al., 2009). Capture, handling, crowding, confinement, and transport are all components of aquaculture, which influence the physiological stress response in various fishes and shellfishes(Park et al., 2009; Saydmohammed and Pal, 2009). It is generally accepted that animals respond to these stressors through a phenomenon called general adaptation syndrome (GAS)(Barton et al., 2000). In crustaceans, the primary effect of GAS involves the increased activation of crustacean hyperglycemic hormone under stressful conditions resulting in secondary changes (elevated glucose levels and changes in metabolic enzymes) and ultimately results in tertiary effects, which are manifested as gross changes in physiological performance of the whole body of the body of the animal(Saydmohammed and Pal, 2009).

As being potentially benefical for reducing the physiological effects of stress in prawns, anesthetic agents take on a great importance. Among the different anesthetics for aquaculture approved by U.S Food and Drug Administration (USFDA), tricane methanesulfonate (MS-222) is not effective in many crustaceans(Coyle et al., 2005). MS-222 is reported to cause occupational hazard (retinopathy) to the users and has a 21-day withdrawal period before the product can be consumed(Park et al., 2011). In recent years, however, it is a widespread truth that the use of clove oil and lidocaine-HCI

[2-(diethylamino)-N-(2, 6-dimethylphenyl) acetimeide hydrochloride] has become more popular in the aquaculture industry in that not only they are safe, inexpensive, non-toxic to the environment but also they do not require any withdrawal period compared to other synthetic-based anesthetics(Park et al., 2011). The effects of clove oil and lidocaine-HCl as anesthetics have been studied in a number of fish species(Park et al., 2011). The aim of this study was to establish optimum anesthetic by investigating the effects of MS-222, clove oil, and lidocaine-HCl on six species of Korean seawater shellfish and comparing the anesthetic effects among the three anesthetics. Physiological responses were subsequently analyzed by measuring plasma cortisol and glucose levels.

Ⅱ. Materials and methods

Experimental samples of six species; scallop (P*atinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked calm (*Ruditapes philippinarum*) were purchased from shellfish breeding farm (Tong young, Gyeongsangnam-do, Korea). 100 samples of each species were transported and reared in the Fishery Genetics and Breeding Sciences Laboratory of the Korea Maritime and Ocean University, Busan, Korea. Each of fish groups was adapted to the water temperature at 25±0.5℃. Samples of each species were measured using an electronic balance (Shimadzu, Japan) and vernier caliper (Mitutoyo, Japan).

Samples of each species were randomly selected for respective experiment to investigate the anesthetic of clove oil (contain 85% eugenol, Sigma, USA), lidocaine-HCl/1,000 ppm NaH_2CO_3 (Hongsung Chemical, Korea), and MS-222 (Sigma, USA). Five concentrations of clove oil (100, 150, 200, 250, and 300 ppm), lidocaine-HCl (300, 400, 500, 600, and 700 ppm), and MS-222 (300, 350, 400, 450, and 500 ppm) were set for the investigation and anesthetic water temperature was identical with recovery water temperature. Water temperature of each group was regulated to be the same as the anesthetic water temperature during the experiment. Until experiment termination, the water temperature was maintained at 25±0.5°C. Induction time was determined from the time when shellfish were stocked in anesthetized water to the time of final-stage state, in which shellfish perfectly opened their shell and showed no responsiveness to external stimuli. Recovery time was determined from the time when the shellfish were stocked in recovery water to the time of the final-stage state, in which normal breathing and responsiveness to visual stimulation were recommenced.

We conducted this experiment to observe the effects of anesthetics on the whole-body cortisol and glucose levels of each shellfish, and the stress responses of the experimental shellfish were measured at 0, 1, 2, 4, 6, 12, 24, and 48 hrs after recovery of anesthesia. Control groups were not anesthetized, but their cortisol levels were measured. For these measurements, 50 samples were used in each experimental group, and no distinction was made between male and female. We measured the whole-body cortisol and glucose levels of the control groups before the experiment. Individual samples from each experimental group were blotted onto paper towels to remove excess water, immediately frozen in liquid nitrogen for 10-30 s, and placed in individual 5.0-mL plastic screw cap centrifuge tubes. The samples were stored at -80℃

until we extracted the cortisol.

To determine the levels of whole-body cortisol and glucose of each shellfish, the whole frozen samples of each group were thawed, individually weighed, and placed in a glass test tube (15 × 85 mm). Each sample was homogenized in deionized water (0.5 mL) for 45–75 s using a Tissue-Tearor Homogenizer (model 985,370-04; BioSpec Products, Inc., Bartlesville, OK, USA). The homogenate on the Tissue Tearor J probe was rinsed into the sample tube with an additional 0.5 mL of deionized water. The homogenized samples were vortexed briefly and placed on ice. The probe was cleaned among the samples with distilled water followed by absolute ethanol, and then was rinsed again with distilled water. The homogenized contents of the glass test tube were transferred to a larger test tube (16 × 125 mm) to facilitate extraction. The original tube was rinsed with diethyl ether (0848-10; Mallinckrodt Baker, Inc., Paris, KY, USA), which was transferred to the larger tube for the subsequent extraction. Each sample was extracted twice with eight volumes (8 mL) of diethyl ether. For each extraction, the tube was vigorously vortexed with ether for 30 s and briefly centrifuged (400 ×g for 2–3 min) to separate aqueous and ether layers. The bottom layer (aqueous homogenate) was frozen in liquid nitrogen for 20 s, and the top ether layer was poured into a new test tube (16 × 100 mm) and dried in a Speed-Vac centrifuge (SVC100H; Savant Instruments, Inc., Holbrook, NY, USA). Greater extraction efficiency was made by adding tritiated cortisol (hydrocortisone, [1, 2, 6, 7-3H]-cortisol; #NET-396; Dupont NEN Research Products, Boston, MA, USA) to the homogenized samples (n=50) and by extracting the samples with the method described above.

After the second extraction, the samples were dried in a fume hood over night, reconstituted with 1 mL of phosphate-buffered saline containing 1% gelatin (Sigma, St. Louis, MO, USA), vortexed for 30 s, and stored at 4°C. The whole-body glucose level was analyzed according to the methodology of previous research(Raabo and Terkildsen, 1960), with kit 510 (Sigma, USA), in which the production of H_2O_2 by glucose oxidase in the presence of o-dianisidine was evaluated as the increase in absorbance at 450 nm. The experiment was performed triplicate and the results were reported were the means±standard deviations (n=50), unless otherwise stated. One and two-way analyses of variance (ANOVA) were used to test the significance ($P < 0.05$) of anesthetic effects on shellfish species according to the different concentrations of clove oil, lidocaine-HCl, and MS-222. The differences between the shellfish groups were analyzed by ANOVA using the SPSS statistics package (SPSS 24.0, SPSS Inc., Chicago, IL, USA) and multiple comparisons were performed using Duncan's multiple range test(Duncan, 1955).

Ⅲ. Results

The average shell length, shell height, shell width, and total weight of each species were shown in <Table 1>. As shown in [Fig. 1], the ranking of shell length on each group is presented in the order of scallop (P*atinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*). The ranking of shell height on each group is presented in the order of scallop, ark shell, blue mussel, surf clam, granular ark,and shortneked clam.

<Table 1> Average shell length, shell height, shell width, and total weight among scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*) used in this experiment*

	Scallop	Ark shell	Surf clam	Blue mussel	Granular ark	Shortneked clam
Shell length (mm)	85.8 ± 3.52	61.5 ± 4.11	43.9 ± 1.42	33.1 ± 5.91	31.0 ± 2.44	30.5 ± 1.12
Shell height (mm)	81.6 ± 4.12	57.1 ± 3.43	39.1 ± 1.52	63.2 ± 6.12	28.9 ± 3.33	23.7 ± 1.79
Shell width (mm)	34.1 ± 3.19	51.0 ± 3.19	51.4 ± 1.78	50.3 ± 4.32	45.1 ± 3.59	19.4 ± 4.77
Total body weight (g)**	28.9 ± 6.04	27.2 ± 6.21	21.5 ± 3.46	27.1 ± 8.7	19.2 ± 2.12	14.2 ± 3.16

*Each value is means ± SE (n=50). The ranking of shell length on each group is presented in the order of scallop, ark shell, surf clam, blue mussel, granular ark, and shortneked clam. The ranking of shell height on each group is presented in the order of scallop, ark shell, blue mussel, surf clam, granular ark, and shortneked clam.
**Total body weight (g)=shell weight (g)+body weight (g).

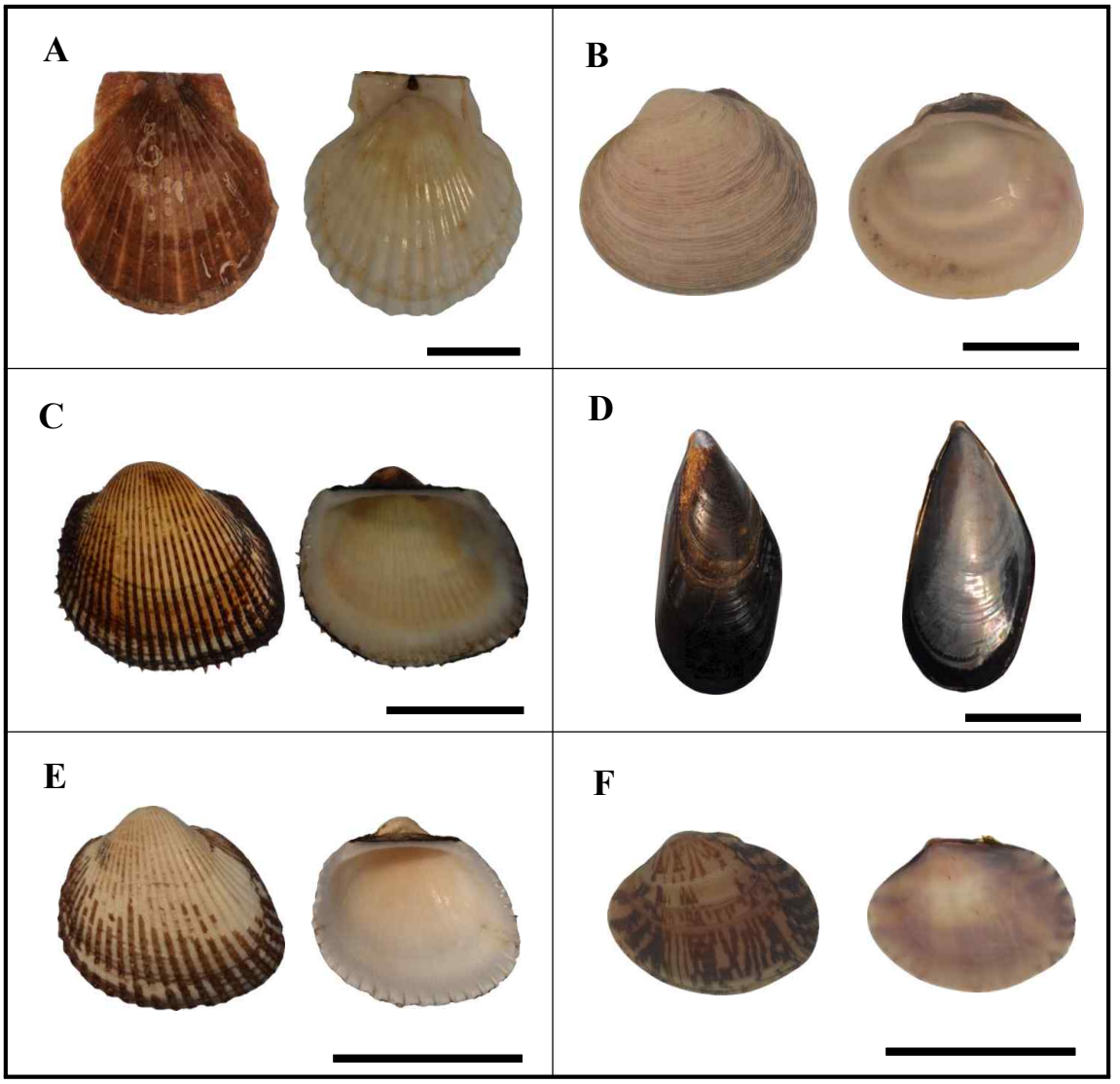
<Table 2> Effects of clove oil dose on anesthesia among scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*)*

Dose	Induction time (min)					
(mgL^{-1})	Scallop	Ark shell	Surf clam	Blue mussel	Granular ark	Shortneked clam
100	111 ± 6.9^{a}	98 ± 7.1^{a}	91 ± 9.1^{a}	75 ± 6.9^{a}	79 ± 2.5^{a}	73 ± 8.1^{a}
150	88 ± 5.8^{b}	87 ± 6.8^{a}	85 ± 8.5^{ab}	63 ± 6.7^{ab}	67 ± 3.3^{a}	69 ± 6.3^{a}
200	75 ± 6.7^{bc}	69 ± 3.1^{b}	66 ± 5.1^{bc}	50 ± 9.3^{bc}	49 ± 4.5^{b}	44 ± 5.1^{b}
250	64 ± 5.4^{cd}	58 ± 5.5^{bc}	54 ± 6.6^{cd}	37 ± 8.7^{c}	35 ± 6.1^{bc}	34 ± 4.7^{bc}
300	51 ± 3.0^{d}	44 ± 4.1^{c}	41 ± 4.8^{d}	27 ± 6.2^{c}	25 ± 4.2^{c}	22 ± 3.2^{c}

*The ranking of shell length on each group is presented in the order of scallop, ark shell, surf clam, blue mussel, granular ark, and shortneked clam. The ranking of shell height on each group is presented in the order of scallop, ark shell, blue mussel, surf clam, granular ark, and shortneked clam. Each value is means ± SE (n=50). Values in the same column not sharing common superscripts are significantly different ($P < 0.05$). The values are means of triplicate experiments.

During the anesthetic experiment, no sample died of the stress from anesthesia. <Table 2> and <Table 3> show the parameters associated with the effects on shellfish species of clove oil for each concentration. Induction time was significantly affected by clove oil concentrations, and decreased drastically as clove oil concentration increased ($P < 0.05$). At each group, as the concentration of clove oil increased, the Induction time decreased ($P < 0.05$). At each concentration of clove oil, as shell length increased, induction time increased as well (<Table 2>; $P < 0.05$). The ranking of induction time at all concentrations of clove oil is presented in the order of scallop, ark shell, surf clam, blue mussel, granular ark, and shortneked clam. But the induction time of blue mussel at 100 ppm and 150

ppm was shorter than that of granular ark and shortneked clam. In all experimental groups, as the concentration of clove oil increased, the recovery time decreased (<Table 3>; $P < 0.05$). As the shell length was increased, the recovery time of clove oil was decreased, that is, the recovery time was hardly affected by the clove oil concentration and the shell length ($P < 0.05$). The ranking of induction time at all concentrations of clove oil is presented in the order of scallop, ark shell, surf clam, blue mussel, granular ark, and shortneked clam.



[Fig. 1] Typical external morphology of scallop (*Patinopecten yessoensis*, A), ark shell (*Scapharca broughtonii*, B), surf clam (*Pseudocardium sachalinensis*, C), blue mussel (*Mytilus edulis*, D), granular ark (*Tegillarca granosa*, E), and shortneked clam (*Ruditapes philippinarum*, F). Left side: left valve; right side: right valve. Scale bars are 3 cm.

<Table 3> Effects of clove oil dose on recovery among scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*)*

Dose (mgL^{-1})	Recovery time (min)					
	Scallop	Ark shell	Surf clam	Blue mussel	Granular ark	Shortneked clam
100	201 ± 31.0[a]	197 ± 27.8[a]	191 ± 22.2[a]	179 ± 24.3[a]	178 ± 21.4[a]	176 ± 28.8[a]
150	188 ± 36.9[a]	186 ± 24.5[a]	180 ± 23.9[a]	162 ± 21.4[a]	161 ± 25.8[a]	160 ± 22.8[a]
200	178 ± 38.4[a]	164 ± 28.4[a]	161 ± 24.4[a]	152 ± 25.2[a]	147 ± 26.9[a]	145 ± 21.9[a]
250	161 ± 36.2[a]	158 ± 27.7[a]	153 ± 27.9[a]	141 ± 27.7[a]	138 ± 26.1[a]	132 ± 27.4[a]
300	154 ± 37.1[a]	144 ± 22.5[a]	142 ± 23.8[a]	123 ± 24.4[a]	121 ± 24.2[a]	120 ± 25.9[a]

*The ranking of shell length on each group is presented in the order of scallop, ark shell, surf clam, blue mussel, granular ark, and shortneked clam. The ranking of shell height on each group is presented in the order of scallop, ark shell, blue mussel, surf clam, granular ark, and shortneked clam. Each value is means± SE (n=50). Values in the same column not sharing common superscripts are significantly different ($P < 0.05$). The values are means of triplicate experiments.

<Table 4> and <Table 5> show the parameters associated with the effects on shellfish species of lidocaine-HCI for each concentration. Induction time was significantly affected by lidocaine-HCl concentrations, and as the concentration of lidocaine-HCl increased, the induction time decreased at each group ($P < 0.05$). Furthermore, at each concentration of lidocaine-HCl, as shell length increased, induction time increased as well (<Table 4>; $P < 0.05$). The ranking of induction time at all concentrations of lidocaine-HCl is silmilar to that of clove oil. However, the induction time of blue mussel at 300 ppm was shorter than that of granular ark and shortneked clam. In all experimental groups, as the concentration of lidocaine-HCl and shell length increased, the recovery time decreased (<Table 5>; $P < 0.05$).

<Table 4> Effects of lidocaine-HCl dose on anesthesia among scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*)*

Dose (mgL^{-1})	Induction time (min)					
	Scallop	Ark shell	Surf clam	Blue mussel	Granular ark	Shortneked clam
300	114 ± 7.9[a]	99 ± 9.1[a]	93 ± 8.8[a]	73 ± 8.1[a]	75 ± 6.7[a]	78 ± 8.3[a]
400	89 ± 8.8[b]	89 ± 8.8[ab]	86 ± 7.5[ab]	69 ± 8.8[a]	65 ± 6.3[a]	63 ± 7.2[ab]
500	73 ± 7.7[bc]	67 ± 7.4[bc]	65 ± 6.7[bc]	55 ± 7.5[ab]	59 ± 6.1[ab]	48 ± 6.9[bc]
600	67 ± 8.4[bc]	52 ± 6.7[c]	57 ± 6.3[c]	42 ± 7.7[bc]	40 ± 6.9[bc]	37 ± 6.7[cd]
700	58 ± 7.0[c]	48 ± 5.9[c]	44 ± 6.8[c]	29 ± 6.8[c]	26 ± 6.8[c]	23 ± 5.8[d]

*The ranking of shell length on each group is presented in the order of scallop, ark shell, surf clam, blue mussel, granular ark, and shortneked clam. The ranking of shell height on each group is presented in the order of scallop, ark shell, blue mussel, surf clam, granular ark, and shortneked clam. Each value is means ± SE (n=50). Values in the same column not sharing common superscripts are significantly different ($P < 0.05$). The values are means of triplicate experiments.

<Table 5> Effects of lidocaine-HCl dose on recovery among scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*)*

Dose	Recovery time (min)					
(mgL^{-1})	Scallop	Ark shell	Surf clam	Blue mussel	Granular ark	Shortneked clam
300	209 ± 39.0[a]	198 ± 29.8[a]	191 ± 25.2[a]	174 ± 27.3[a]	177 ± 25.4[a]	171 ± 24.8[a]
400	181 ± 34.9[a]	186 ± 21.5[a]	181 ± 23.9[a]	166 ± 24.4[a]	164 ± 28.8[a]	163 ± 21.8[a]
500	177 ± 37.4[a]	165 ± 27.4[a]	163 ± 29.4[a]	158 ± 22.2[a]	153 ± 21.9[a]	147 ± 26.9[a]
600	163 ± 33.2[a]	158 ± 24.7[a]	153 ± 21.9[a]	144 ± 21.7[a]	137 ± 25.1[a]	134 ± 22.4[a]
700	158 ± 32.1[a]	147 ± 27.5[a]	141 ± 28.8[a]	126 ± 27.4[a]	124 ± 24.2[a]	123 ± 26.9[a]

*The ranking of shell length on each group is presented in the order of scallop, ark shell, surf clam, blue mussel, granular ark, and shortneked clam. The ranking of shell height on each group is presented in the order of scallop, ark shell, blue mussel, surf clam, granular ark, and shortneked clam. Each value is means ± SE (n=50). Values in the same column not sharing common superscripts are significantly different ($P < 0.05$). The values are means of triplicate experiments.

That is, the recovery time was hardly affected by the lidocaine-HCl concentration and the shell length ($P < 0.05$). The ranking of recovery time at all concentrations of lidocaine-HCl is the same as that of clove oil.

<Table 6> and <Table 7> show the parameters associated with the effects on shellfish species of MS-222 for each concentration. Trends of induction times and recovery times on MS-222 were similar to clove oil and lidocaine-HCl. Induction time was significantly affected by MS-222 concentrations, and decreased drastically as the MS-222 concentration increased ($P < 0.05$). At each concentration of MS-222, as the shell length increased, induction time increased as well (<Table 6>; $P < 0.05$). The induction time of scallop at 350 ppm was shorter than that of ark shell and surf clam, and at the identical concentration, the induction time of granular ark was shorter than that of shortneked clam. At 400 ppm, the induction time of ark shell was shorter than that of surf clam. In all experimental groups, as the concentration of MS-222 and shell length increased, the recovery time decreased (<Table 7>; $P < 0.05$). The recovery time was affected by MS-222 concentration and the shell length ($P < 0.05$). However, at 300 ppm and 350 ppm, the recovery time of blue mussel was shorter than that of granular ark. The ranks of induction time and recovery time at all concentrations of MS-222 were the same as those of clove oil and lidocaine-HCl.

[Fig. 2] shows the variations of whole-body cortisol concentrations after the recovery of clove oil, lidocaine-HCl, and MS-222 anesthesia on each shellfish. Cortisol concentrations of control group (no-anesthesia group) on scallop, ark shell surf clam, blue mussel, granular ark, and shortneked clam were 1.99, 1.59, 1.55, 1.50, 1.61, and 1.58 μg/dL, respectively. For 0 to 6 hrs after their recovery from anesthesia (RA), the cortisol concentrations of scallop, ark shell surf clam, blue mussel, granular ark, and shortneked clam on anesthesia group of clove oil were increased from 5.98, 5.68, 5.66, 5.67, 5.71, and 5.69 μg/dL, to 23.12, 23.79, 23.46, 24.75, 24.96, and 24.31 μg/dL, respectively. However, for 6 to 48 hrs after RA,

<Table 6> Effects of tricaine methanesulphonate (MS-222) dose on anesthesia among scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*)*

Dose	Induction time (min)					
(mgL^{-1})	Scallop	Ark shell	Surf clam	Blue mussel	Granular ark	Shortneked clam
300	117 ± 7.9^{a}	94 ± 6.1^{a}	91 ± 6.1^{a}	75 ± 6.2^{a}	74 ± 6.4^{a}	71 ± 7.1^{a}
350	83 ± 7.8^{b}	89 ± 5.8^{a}	85 ± 7.5^{ab}	69 ± 6.1^{ab}	64 ± 5.9^{ab}	67 ± 7.3^{a}
400	76 ± 8.7^{b}	63 ± 6.1^{b}	67 ± 6.1^{bc}	52 ± 5.8^{bc}	51 ± 5.2^{bc}	45 ± 6.1^{b}
450	60 ± 7.4^{b}	56 ± 6.5^{b}	55 ± 5.6^{cd}	47 ± 6.1^{cd}	33 ± 6.1^{cd}	32 ± 5.7^{b}
500	57 ± 8.0^{b}	44 ± 6.1^{b}	42 ± 6.8^{d}	29 ± 6.4^{d}	27 ± 5.2^{d}	25 ± 6.2^{b}

*The ranking of shell length on each group is presented in the order of scallop, ark shell, surf clam, blue mussel, granular ark, and shortneked clam. The ranking of shell height on each group is presented in the order of scallop, ark shell, blue mussel, surf clam, granular ark, and shortneked clam. Each value is means ± SE (n=50). Values in the same column not sharing common superscripts are significantly different ($P < 0.05$). The values are means of triplicate experiments.

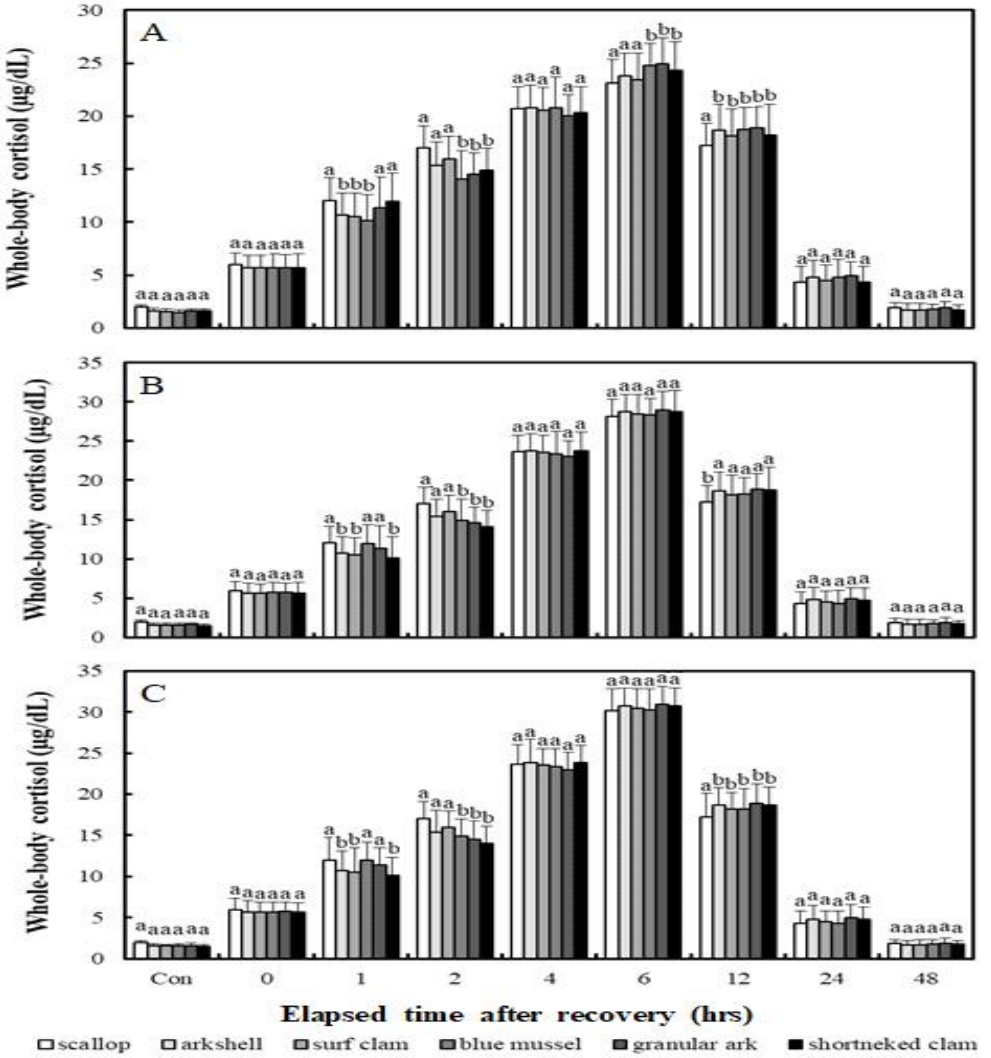
<Table 7> Effects of tricaine methanesulphonate (MS-222) dose on recovery among scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*)*

Dose	Recovery time (min)					
(mgL^{-1})	Scallop	Ark shell	Surf clam	Blue mussel	Granular ark	Shortneked clam
300	208 ± 39.0^{a}	198 ± 21.8^{a}	194 ± 28.2^{a}	176 ± 22.3^{a}	179 ± 29.4^{a}	174 ± 24.8^{a}
350	189 ± 34.9^{a}	181 ± 28.5^{a}	185 ± 26.9^{a}	164 ± 25.4^{a}	165 ± 22.8^{a}	162 ± 27.8^{a}
400	174 ± 37.4^{a}	165 ± 24.4^{a}	168 ± 21.4^{a}	148 ± 22.2^{a}	156 ± 24.9^{a}	147 ± 29.9^{a}
450	166 ± 32.2^{a}	157 ± 25.7^{a}	154 ± 29.9^{a}	133 ± 24.7^{a}	142 ± 27.1^{a}	134 ± 26.4^{a}
500	159 ± 34.1^{a}	143 ± 27.5^{a}	149 ± 24.8^{a}	125 ± 29.4^{a}	124 ± 29.2^{a}	123 ± 29.9^{a}

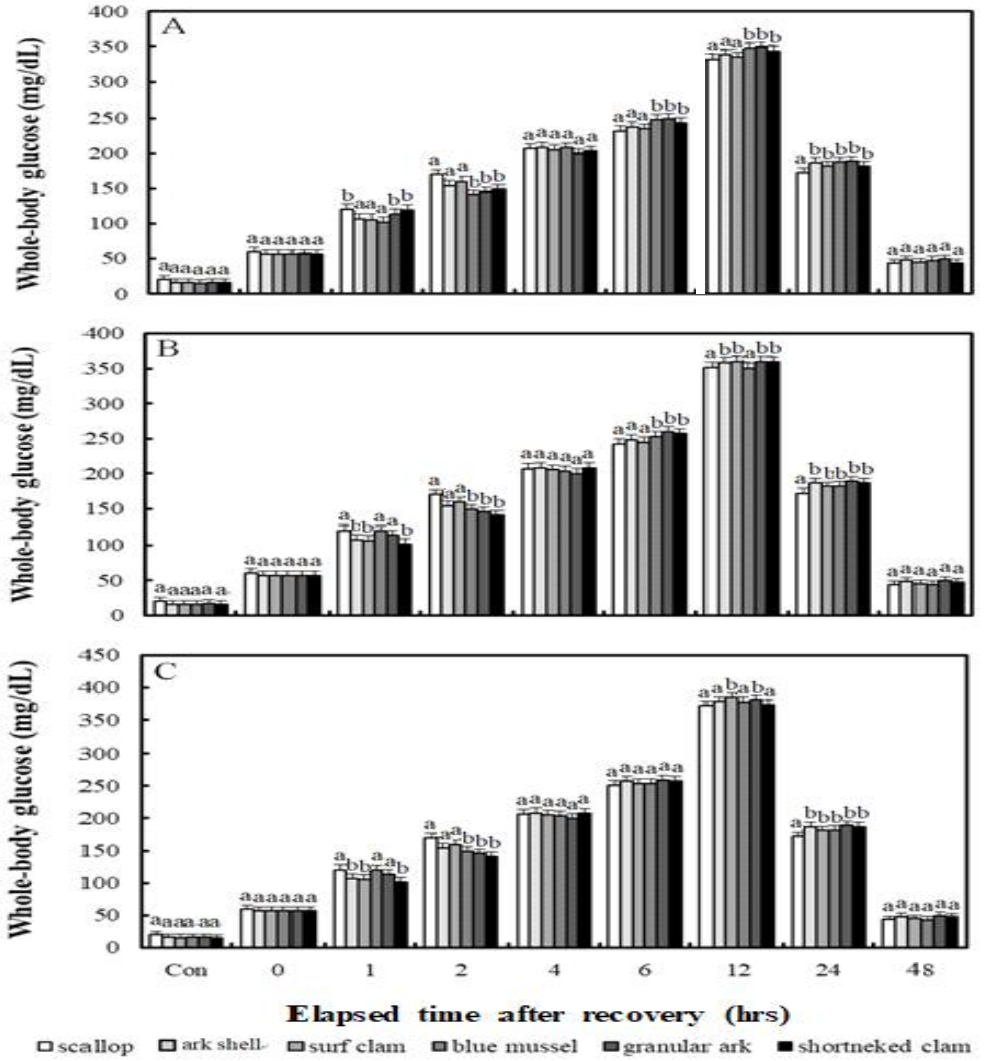
*The ranking of shell length on each group is presented in the order of scallop, ark shell, surf clam, blue mussel, granular ark, and shortneked clam. The ranking of shell height on each group is presented in the order of scallop, ark shell, blue mussel, surf clam, granular ark, and shortneked clam. Each value is means ± SE (n=50). Values in the same column not sharing common superscripts are significantly different ($P < 0.05$). The values are means of triplicate experiments.

the cortisol concentrations of each shellfish were significantly decreased from 23.12 μg/dL (scallop), 23.79 μg/dL (ark shell), 23.46 μg/dL (surf clam), 24.75 μg/dL (blue mussel), 24.96 μg/dL (granular ark), and 24.31 μg/dL (shortneked clam) to 1.88 μg/dL (scallop), 1.69 μg/dL (ark shell), 1.69 μg/dL (surf clam), 1.73 μg/dL (blue mussel), 1.89 μg/dL (granalar ark), and 1.72 μg/dL (shortmecked clam) respectively [Fig. 2A]. The cortisol concentration of each shellfish was highest at 6 hrs after RA. At 6 hrs after RA, the cortisol concentration of granular ark was higher than that of the other species, and

the cortisol concentration of scallop was lower than that of the other species. After RA, the trend of cortisol concentration on clove oil anesthesia of each shellfish was similar to that on lidocaine-HCl and MS-222 anesthesia of each shellfish [Fig. 2B and 2C]. At 6 hrs after RA, the cotisol concentrations of MS-222 on each shellfish were the highest in all experimental groups.



[Fig. 2] Effect of clove oil (A), lidocaine-HCl (B), and tricaine methanesulphonate (MS-222, C) anesthesia on variations of the whole-body cortisol concentrations in the blood plasma of scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis)*, granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*) during 48 hrs after recovery. Sample of each group was anesthetized by 300 ppm dose of each anesthetic. Values are means ± SE (n=50). Error bars represent the standard deviation of triplicate experiments ($P < 0.05$).



[Fig. 3] Effect of clove oil (A), lidocaine-HCl (B), and tricaine methane sulphonate (MS-222, C) anesthesia on variations of the whole-body glucose concentrations in the blood plasma of scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*) during 48 hrs after recovery. Sample of each group was anesthetized by 300 ppm dose of each anesthetic. Values are means ± SE (n=50). Error bars represent the standard deviation of triplicate experiments ($P < 0.05$).

[Fig. 3] shows the variations of whole-body glucose concentrations after the recovery of clove oil, lidocaine-HCl, and MS-222 anesthesia on each shellfish. Glucose concentrations of control group (no-anesthesia group) on scallop, ark shell surf clam, blue mussel, granular ark, and shortneked clam were 19.38, 18.43, 15.57, 15.03, 16.23, and 15.77 respectively. For 0 to 12 hrs after RA,

glucose concentrations of each shellfish on anesthesia group of clove oil were increased from 59.80 mg/dL (scallop), 57.12 mg/dL (ark shell), 56.62 mg/dL (surf clam), 56.54 mg/dL (blue mussel), 57.44 mg/dL (granular ark), and 56.84 mg/dL (shortneked clam) to 331.20 mg/dL (scallop), 337.91 mg/dL (ark shell), 334.69 mg/dL (surf clam), 347.11 mg/dL (blue mussel), 349.49 mg/dL (granular ark), and 343.10 mg/dL (shortneked clam) respectively. However, for 12 to 48 hrs after RA, the glucose concentrations of each shellfish were significantly decreased, and the glucose concentrations for each shellfish at 48 hrs after RA were higher than those of control group ($P < 0.05$), ([Fig. 3A]). Glucose concentrations of each shellfish were the highest at 12 hrs after RA. At 6 hrs after RA, the glucose concentration of granular ark was higher than that of the other species, and the glucose the concentration of scallop was lower than that of the other species. After RA, the trend of glucose concentration on clove oil anesthesia was similar to that of lidocaine-HCl and MS-222 anesthesia on each shellfish [Fig. 3B and 3C]. At 12 hrs after RA, glucose concentrations of MS-222 on each shellfish were higher than those of clove oil and lidocaine-HCl.

Ⅳ. Discussion

The previous study reported that the effect of magnesium sulphate, 2-phenoxyethanol, ethylene diaminetetraacetic acid disodium (EDTA), and procaine hydrochloride on abalone (*Haliotis midae*)(White et al., 1996). In the previous study, at each concentration of four anesthetics, as the shell length of abalone increased, induction time and recovery time of abalone increased(White et al., 1996). In this study, induction time and recovery times of clove oil, lidocaine-HCl, and MS-222 on six species were increased as the shell length of each species increased.

The patterns of clove oil, lidocaine-HCl and, MS-222 observed on scallop (*Patinopecten yessoensis*), ark shell (*Scapharca broughtonii*), surf clam (*Pseudocardium sachalinensis*), blue mussel (*Mytilus edulis*), granular ark (*Tegillarca granosa*), and shortneked clam (*Ruditapes philippinarum*) were similar to those of previous studies on other bony fishes such as sockeye salmon (*Oncorhynchus nerka*), greenling (*Hexagrammos otakii*), winter flounder (*Pleuronectes americanus*), rock bream (*Oplegnthus fasciatus*), and spiny-clawed prawn (*Macrobrachium rosenbergii*)(Woody et al., 2002; Park et al., 2003, 2004, 2009; Saydmohammed and Pal, 2009). The dose dependent response of six species to clove oil, lidocaine-HCl, and MS-222 showed a negative exponential curve with increased doses resulting in decreased time reaching the anesthetic state. In our experiments, induction times and recovery times of six species to clove oil, lidocaine-HCl, and MS-222 were much longer than those of seawater fish species to three anesthetics in the previous study. In general, optimum anesthticc concentrations in most fish are usually expected to be under anesthesia within 3 min and recover within 10 min but that kind of pattern is not true of six seawater shellfishes used in this study(Park et al., 2011).

As most anesthetics used on fishes and shellfishes are absorbed into tissues via gills, residues may come up(Marking and Meyer, 1985). Depuration is thought to reduce or eliminate these residues, ensuring that residues do not accumulate to the extent of toxic concentrations. The duration of depuration depends on the types of drug, target

species, dose rate, and route of administration, which may vary from a few hrs to several days or weeks(Booth, 1988). The previous study presents the data on accumulation and clearance of eugenol (clove oil) with the accumulation rate being 0% at 24 hrs after anesthesia and its residues from the muscle tissue of spiny-clawed prawn (*Macrobrachium rosenbergii*) at different time intervals after exposure to the anesthesia(Saydmohammed and Pal, 2009). Currently, tricaine methanesulfonate (MS-222) is most widely used to anesthetize fish, including food fish although it is considered mildly toxic as an edible anesthetic according to the US Food and Drug Administration (FDA). FDA has made guidelines that the fish treated with MS-222 should undergo an obligatory 21-day withdrawal period(Saydmohammed and Pal, 2009). Human anesthetic compound lidocaine-HCl is white powder soluble in water, which was first administered to fish in the previous study(Carrsco et al., 1984). Lidocaine-HCI, or a more effective and risk-free anesthetic, which has been safely used in the dentistry industry, was proven as a safe substitute for applying to various aquaculture-relevant fish species in Korea(Park et al., 2011). Clove oil is characterized by faster induction of anesthetic effects and prolonged recovery times in at least three teleost fishes(Park et al., 2011). Also, clove oil is evaluated to be safe, inexpensive and non-toxic to the environment, and besides, it does not require a withdrawal period compared to other anesthetic chemicals(Park et al., 2011). So clove oil and lidocaine-HCl are suitable for anesthetics of shellfish used in this study.

The term “whole-body cortisol” is used to describe the portion of corticosteroid which is extracted and measured with a cortisol-specific radioimmunoassay (RIA). However, the cortisol antibody may recognize other cortisol metabolites in the whole-body fraction that have not yet been identified(Pottinger et al., 1992). Nevertheless, according to the previous studies, it is certain that the majority of the corticosteroid recognized by the assay was cortisol. Therefore, We have chosen the term “whole-body cortisol” as the descriptor(Feist and Schreck, 2002). Whole-body cortisol was extracted through the modification of the method used for the eggs and embryos of the chinook salmon (*O. tshawytscha*)(Feist and Schreck, 2002).

Plasma cortisol and glucose levels after the recovery from anesthesia in red drum (*Sciaenops ocellatus*), simultaneously exposed to MS-222 and quinaldine anesthetic were reported to be elevated(Massee et al., 1995). The previous study stated that “Usually, the phenomenon that plasma cortisol concentration of fish rises from stress is first order reaction, the phenomenon that plasma glucose concentration rises from stress is the result of second-order reaction caused by hormone rise reaction from stress”(Barton and Iwama, 1991). This result has been reported in the kelp grouper (*Epinephelus bruneus*)(Park et al., 2008). The previous research suggested that the greater use of glucose for increased cell metabolism during early exposure to stress must have brought about the increase in blood glucose, even though glycogenolysis would have increased during this period(Martinez-Alvarez, 2002; Das et al., 2004). However, because of dysfunctional cell metabolism, the lower use of glucose later in the exposure period (after 48 hrs) resulted in an increase in blood glucose levels.

One of the most promising areas of aquaculture research is the development of the strategy to regulate the physiological response of fish by way of blocking the function of cortisol receptor as the

primary factor in stress response of fish(Pickering, 1992). However, in crustaceans, crustacean hyperglycemic hormone is responsible for eliciting the primary stress response. Therefore, cortisol receptor blockers used commonly in fish should prove to be insufficient as they usually fail to induce desired anesthetic response in shellfishes(Saydmohammed and Pal, 2009). Of the three anesthetics used in our experiment, clove oil is considered to impart mild anesthesia in mollusks. Considering anesthetic time, recovery time, safety, and physiological response among the three anesthetics, clove oil is most suitable for anesthetic of shellfish in this study. The results from this study will contribute to safe laboratory handlings of six seawater shellfishes, which are required by many researches and experiments.

References

Barton BA and Iwama GK(1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Rev Fish Dis 1, 3~26. https://doi.org/10.1016/0959-8030(91)90019-G

Barton BA, Bollig H, Hauskins BL and Jansen CR(2000). Juvenile pallid (*Scaphirhynchus albus*) and hybrid pallid × shovelnose (*S. albus* × *platorynchus*) sturgeons exhibit low physiological responses to acute handling and severe confinement. Comp Biochem Physiol A 126, 125~134. https://doi.org/10.1016/S1095-6433(00)00192-6

Booth NH(1988). Drug and chemical residues in the edible tissues of animals. In: Booth NH, McDonald LE (eds), 6th ed, Veterinary Pharmacology and Therapeutics, Iowa State Univ. Press, Iowa, pp 1149~1159.

Carrsco S, Sumano H and Navarro-Fierro R(1984). The use of lidocaine‐sodium bicarbonate as anaesthetic in fish. Aquaculture. 41:395~398. https://doi.org/10.1016/0044-8486(84)90207-2

Cho YJ, Son BW and Choi HD(1995). The metabolites of a maine mollusk, *Mytilus edulis*: isolation of taurine and compositions of free fatty acids and free amino acids. Natur Prod Sci 4: 5~9. http://oceanrep.geomar.de/id/eprint/33556

Coyle SD, Dasgupta S, Tidweel JH, Beavers T, Bright LA and Yasharian DK(2005). Comparative efficacy of anesthetics for freshwater prawn *Macrobrachium rosenbergii*. J World Aquacult Soc 36, 282~290. https://doi.org/10.1111/j.1749-7345.2005.tb00332.

Das PC, Ayyappan S, Jena JK and Das BK(2004). Nitrite toxicity in *Cirrhinus mrigala* (Ham): acute toxicity and sub-lethal effect in selected haematological parameters. Aquaculture 235, 633~644. https://doi.org/10.1016/j.aquaculture.2004.01.020

Duncan DB(1955). Multiple range and multiple F tests. Biometrics 1, 1~42. https://doi.org/10.2307/3001478

Feist G and Schreck CB(2002). Ontogeny of the stress response in chinook salmon, *Oncorhynchus tshawytscha*. Fish Physiol Biochem 25, 31~40. https://doi.org/10.1023/A:1019709323520

Ito H(1997). *Patinopecten (Mizuhopecten) yessoensis*. In: Shumway SE (ed), Scallops: Biology, Ecology and Aquaculture. Elsevier, Amsterdam pp 1024~1055.

Lee JY, Chang YJ and Lee CS(1997). Reproductive cycle of surf clam, *Spisula sachalinensis*. J Kor Fish Soc 30, 132~138 (in Korean with English abstract).

Marking LL and Meyer FP(1985). Are better anesthetics needed in fisheries? Fisheries 10, 2~5. https://doi.org/10.1577/1548-8446(1985)010<0002:ABANIF>2.0.CO;2

Martinez-Alvarez RM, Hidalgo MC, Domezain A, Morales AE, Grarcia-Gallego M and Sanz A(2002). Physiological changes of sturgeon *Acipenser nacarii* caused by increasing environmental salinity. J Exp Biol 205, 3699~3706.

Massee KC, Rust MB, Hardy RW and Stickney RS(1995). The effectiveness of tricaine, quinaldine sulfate and metomidate as anesthetics for larval fish. Aquaculture 134, 315~359. https://doi.org/10.1016/0044-8486(95)00057-9

Nam M-M, Lee C, Moon TS and Huh MK(2012). Genetic diversity and population structure of the scallop, *Patinopecten yessoensis* in Korea, China, and Japan by random amplified polymorphic DNA markers. J Life Sci 22, 466~471. https://doi.org/10.5352/JLS.2012.22.4.466

Park I-S., Hur JW, Song YC, Im JH and Johnson SC(2004). Anaesthetic effect of lidocaine hydrochloride-sodium bicarbonate on the winter flounder (*Pleuronectes americanus*). Ocean Polar Res 26, 475~480. https://doi.org/10.4217/OPR.2004.26.3.475

Park I-S, Jo JH, Lee SJ, Kim YA, Kim KE, Hur JW, Yoo JS and Song YC(2003). Anaesthetic effect of lidocaine hydrochloride-sodium bicarbonate and MS-222 on the greenling (*Hexagrammos otakii*). J Kor Fish Soc 36, 449~453 (in Korean with an English abstract). https://doi.org/10.5657/kfas.2003.36.5.449

Park I-S, Park SJ, Gil HW, Nam YK and Kim DS(2011). Anesthetic effects of clove oil and lidocaine-HCl on marine medaka, *Oryzias dancena*. Lab Animal 40, 45~51. https://doi.org/10.1038/laban0211-45

Park MO, Hur WJ, Im S-Y, Seol D-W, Lee JH and Park I-S(2008). Anesthetic efficacy and physiological responses to clove oil-anaesthesized kelp grouper, *Epinephelus bruneus*. Aquacult Res 39, 877~884. https://doi.org/10.1111/j.1365-2109.2008.01941

Park MO, Im S-Y, Seol D-W and Park I-S(2009). Efficacy and physiological responses of rock bream (*Oplegnathus fasciatus*) to anesthetization with clove oil. Aquaculture 287, 427~430. https://doi.org/10.1016/j.aquaculture.2008.10.049

Pickering AD(1992). Rainbow trout husbandry: management of the stress response. Aquaculture 100, 125~139. https://doi.org/10.1016/0044-8486(92)90354-N

Pottinger TG, Moran TA and Cranwell PA(1992). The biliary accumulation of corticosteroids in rainbow trout, *Oncorhynchus mykiss*, during acute and chronic stress. Fish Physiol Biochem 10, 55~66. https://doi.org/10.1007/BF00004654

Raabo E and Terkildsen TC(1960). On the enzymatic determination of blood glucose. Scand J Clinic Lab Invest 12, 402~407. https://doi.org/10.3109/00365516009065404

Saydmohammed M and Pal AK(2009). Anesthetic effect of eugenol and menthol on handling stress in *Macrobrachium rosenbergii*. Aquaculture 298, 162~167. https://doi.org/10.1016/j.aquaculture.2009.10.020

Shin YK, Kim BH, Oh BS, Jung CG, Sohn SG and Lee JS(2006). Physiological responses of the ark shell, *Scapharca broughtonii* (Bivalva: Arcidae) to decreases in salinity. J Fish Sci Technol 9, 153~159. https://doi.org/10.5657/fas.2006.9.4.153

White HI, Hecht T and Potgieter B(1996). The effect of four anaesthetics on *Haliotis midae* and their suitability for application in commercial abalone culture. Aquaculture 140, 145~151. https://doi.org/10.1016/0044-8486(95)01185-4

Woody CA, Nelson J and Ramstad K(2002). Clove oil as an anesthetic for adult sockeye salmon (*Oncorhynchus nerka*): field trials. J Fish Biol 60, 340~347. https://doi.org/10.1111/j.1095-8649.2002.tb00284

Yokogawa K(1997). Morphological and genetic differences between Japanese and Chinese red ark shell, *Scapharca broughtonii*. Fisher Sci 63, 332~337. https://doi.org/10.2331/fishsci.63.332

Yoo JS(1988). Korean Shells in Color. Iljisa Publishing Co., Seoul p 196.

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