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The Current Status for Application of Anesthesia to Aquatic Animals for Aquaculture in Republic of Korea

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ABSTRACT: Recently, the necessity of a kind of anesthesia for aquatic animals for aquaculture has emerged and its application is enlarging along with the development of aquaculture industry in Republic of Korea. The aim of this paper is to provide a review of studies on the anesthesia for aquatic animals in both seawater and fresh water including the those effects of anesthetics such as lidocaine-HCl and lidocaine-HCl/$NaHCO_3$, clove oil and derivatives, MS-222 and benzocaine, quinaldine, 2-phenoxyethanol, sodium bicarbonate ($NaHCO_3$), and hypothermia from 1988 to 2021 in Republic of Korea, in order to gather available data and to highlight the recent progress in the different fields of fishery anesthesia.

KEYWORKS: Anesthesia, Aquaculture, Aquatic animal, Republic of Korea

I. BACKGROUND

Sedation with the use of anaesthetics has been used in fish handling situations primarily when there is a need for rapid processing, such as transporting, inhibiting fish activities, culturing, tagging, measuring, injecting vaccines and antibacterial substances, medical treatment for diseases, artificial spawning, sorting, and preparation for trade [1-9]. These processing activities may result in negative behavioural and physiological effects such as decreased feeding, inhibition or enhancement of aggressive behaviour and susceptibility to disease [10]. The use of anaesthetics during the handling procedures provides a way to minimize these deleterious effects, to immobilize fish for some time, and to reduce physical damage that might occur during handling activities [6, 9-11]. Anaesthesia also has no harmful effect on mortality and morbidity [12]. Recently, the necessity of this kind of anesthesia for aquatic animals for aquaculture has emerged and its application is enlarging along with the development of aquaculture industry in Republic of Korea [13-19]. This review has organized the studies in Republic of Korea from 1988 to 2021 regarding the anesthesia for aquatic animals in seawater and fresh water including the those effects of anesthetics such as lidocaine-HCl and lidocaine-HCl/$NaHCO_3$, clove oil and derivatives, MS-222 and benzocaine, quinaldine, 2-phenoxyethanol, sodium bicarbonate ($NaHCO_3$), and hypothermia.

II. MAIN TEXT

Lidocaine-HCl and Lidocaine-HCl/$NaHCO_3$ (Appendix) The human anesthetic compound lidocaine-HCl; [2-(diethylamino)-N-(2,6-dimethylphenyl) acetimide hydrochloride], is also known as ^{TM3}Xylocaine. Lidocaine in freebase form is insoluble in water, but freely soluble in acetone or alcohol. It is generally used in the hydrochloride salt form (lidocaine-HCl) which is freely soluble in water [20]. Lidocaine-HCl, a white, water-soluble powder, is safe, inexpensive, non-toxic in the environment, and does not require a withdrawal period compared with other anesthetic chemicals. It was first administered to fish by Carrasco et al. [21]. Lidocaine-HCl, which has been safely used in dentistry, has been proven to be a safe anesthetic for some freshwater and marine fish in Republic of Korea [5, 11]. A number of studies have investigated its effectiveness, economic viability, reusability, toxicity, and side effects to ascertain its appropriateness as a fish anesthetic [22].

·**Marine animal: Finfish** Lidocaine as less toxic and more effective anaesthetics was tested for 11 commercially important marine fishes; spotty belly greenling (*Agrammus agrammus*), multicolor fin rainbowfish (*Halichoeres poecilepterus*), greenling (*Hexagrammos otakii*), perch (*Lateolebrax japonicus*), rock bream (*Oplegnathus fasciatus*), red seabream (*Pagrus major*), olive flounder (*Paralichyhys olivaceus*), dark-banded rock fish (*Sebastes inermis*), rabbit fish (*Siganus fuscescens*), file fish (*Stephanolepis cirrhifer*), and grass puffer (*Takifugu niphobles*) [23]. Park et al. [23] showed anaesthetic effects were clearly dose dependent and acute or chronic toxicities were not observed within clinical doses. The recovery time in the tested fish after anaesthetization was 3 to 4 minutes.

Anaesthetic effect of lidocaine hydrochloride-sodium bicarbonate mixture (lidocaine HCl/$NaHCO_3$) and tricaine methanesulfonate (MS-222) was tested for the greenling (*Hexagrammos otakii*) at three different temperature

regimes: 12℃, 8℃, and 24℃. Based on the exposure and recovery time, effective dose of lidocaine HCl/$NaHCO_3$ was 800 ppm (18℃) and 300 ppm (24℃) for greenling of 21.0±1.4 cm body length [24]. Park et al. [24] showed anaesthetic dose and temperature-dependent relationship in exposure and recovery time. Effective dose of MS-222 at 18℃ was proven to be 125 ppm and 150 ppm [24]. Combination of lidocaine HCl/$NaHCO_3$ and MS-222, considerably reduced the dosage of each anaesthetic required to give rapid, deep anaesthetic condition. Park et al. [24] reported in the dry exposure after anaesthetic, the control fish exhibition 22% mortality after dry exposure of 20 minutes; whereas, the anaesthetic condition with 800 ppm lidocaine HCl/$NaHCO_3$ for 1 minute exhibited delayed recoveries from the anaesthetic condition with mortalities of 20%, 41%, 78%, and 100% after dry exposures of 8, 12, 16, and 20 minutes, respectively.

Blood (hematocrit, hemoglobin and red blood cell) and plasma (cortisol, glucose, lactic acid, Na^+, Cl^-, K^+, osmolality, aspartate aminotransferase and alanine aminotransferase) factors in greenling (*Hexagrammos otakii*) after MS-222 and lidocaine-HCl anaesthetic were determined by Park et al. [25]. Greenling (mean body length: 25.8 ± 1.6 cm, mean body weight: 194.5 ± 33.8 g) were exposed to concentrations of 125 ppm MS-222 and 800 ppm lidocaine-HCl at 18°C [25]. Blood was collected from ten fish after anesthesia 30 minutes, 1, 3, 6, and 24 hours, respectively. After anesthesia, Park et al.[25] determined the concentrations of hematocrit, hemoglobin, red blood cell, cortisol, glucose, lactic acid, osmolality, Na^+, K^+, Cl^-, aspartate aminotransferase, and alanine aminotransferase. Park et al. [25] showed control and experimental group significant increases in plasma cortisol concentrations from their respective prestress levels at 30 minutes after anaesthetic. Concentrations of glucose were increased from 22.3 ng/mL to 28.5 ng/mL (30 minutes), 31.0 ng/mL (1 hour) in experimental group, respectively. Lactic acid concentrations for experimental group were lower than those for control group at 30 minutes, 1, and 3 hours. Park et al. [25] reported that in other items, control and experimental group did not show significant differences in this before and after anaesthetic.

The physiological response and the applicable concentration ranges of anesthetic clove oil and anesthetic lidocaine-HCl was determined, and the synergistic effect of a mixture of these two anesthetics on the in grass puffer (*Takifugu niphobles*) was investigated [26]. Gil et al. [26] showed the anesthesia times decreased and the recovery times increased with increasing concentrations of clove oil and lidocaine-HCl. Applicable concentration ranges for long-term transportation requiring more than 1 hour were 2 ppm for clove oil and 50 ppm for lidocaine-HCl. With mixtures of the two anesthetics, the anesthesia time decreased as the admixture concentration of clove oil and lidocaine-HCl increased. Anesthesia times of experimental groups with the combined anesthetics were shorter than those with the same concentrations of clove oil or lidocaine-HCl alone. Plasma cortisol concentrations were highest at 6 hours in all experimental groups anesthetized with the mixture of clove oil and lidocaine-HCl, while all groups with clove oil or lidocaine-HCl alone had the highest plasma cortisol concentrations at 12 hours. Plasma glucose concentrations were highest at 12 hours in experimental groups anesthetized with the mixture of clove oil and lidocaine-HCl, while groups with clove oil or lidocaine-HCl alone had the highest plasma glucose at 24 hours. Gil et al. [26] provided basic information about anesthetics and the synergistic effect of mixtures of anesthetics in this grass puffer species.

Anaesthetic effect of lidocaine hydrochloride-sodium bicarbonate mixture (lidocaine HCl/$NaHCO_3$) was tested for the winter flounder (*Pleuronectes americanus*) at five different temperature regimes: 3℃, 7℃, 11℃, 15℃, and 19℃ [15]. Anaesthetic dose and temperature-dependent relationship in exposure and recovery time were observed for the winter flounder of 17.2±0.1cm mean total length. Anaesthetic lidocaine HCl/$NaHCO_3$ showed rapid exposure time and rapid recovery time for winter flounder.

Simulated transportation of winter flounder (*Pleuronectes americanus*) fingerlings was carried out to study the effects of lidocaine-hydrochloride on water parameters [27]. Dissolved oxygen, ventilation rate, ammonia nitrogen, and pH of control group, sham control group, and lidocaine-hydrochloride treated groups of 5 ppm, 10 ppm, 20 ppm were tested at 1, 2, 3, 4, and 5 hours treatment duration [27]. Park et al. (27) presented lidocaine-hydrochloride treated groups, followed by sham control and control, were most effective in decreasing oxygen consumption and excretion of ammonia by fish. Lidocaine-hydrochloride dose-related decrease in oxygen consumption and excretion of ammonia were also established [27]. pH declines in lidocaine-hydrochloride and sham control groups were more rapid compared to the control group [27]. The results of [27] study revealed that lidocaine-hydrochloride is an effective sedative as a transportation mixture for winter flounder.

The effects of the different anesthetic MS-222 and lidocaine-HCl doses on the blood physiological responses, on the time required for anesthesia and recovery, and on the survival rates of black rockfish (*Sebastes schlegeli*) were reported [28]. Plasma cortisol was its highest levels (96.1±12.1 ng/mL) at 6 hours after the administration of 300

ppm of MS-222, and in all groups, plasma cortisol levels were higher than the initial levels during the anesthetic experiment. Kim et al. [28] showed fish receiving lidocaine-HCl also exhibited higher than initial plasma cortisol levels at almost experimental intervals. The middle size fish exhibited the highest glucose level (143.3±14.5 mg/dL) at 50 ppm of anesthesia after 1 hour, and every level was significantly higher than the initial level for at least 12 hours. Glucose levels in fish to which lidocaine-HCl was administered were comparable to the levels seen in conjunction with MS-222 treatment. Kim et al. [28] showed in fish anesthetized with MS-222, K^+ levels in the small size fish were significantly elevated after 1 hour, while Na^+ levels did not change in any of the groups throughout the experiment. Anesthetic time was significantly attenuated with increases in the concentrations of MS-222 and lidocaine-HCl. Kim et al. [28] also noted a correlation between anesthetic time and fish size, in that larger fish took a longer time, followed by the middle size and then the small size fish. The all fish size groups showed above 95% survival rates at every experimental concentration in MS-222 and 300-400 ppm in lidocaine-HCl. The results from Kim et al. [28] may indicate that 100-200 ppm MS-222 and 400 ppm lidocaine-HCl are the most effective doses as sedatives for the black rockfish and these doses could be used as the suitable anesthetics doses.

The effects of the anaesthetic agents, clove oil and mixture of clove oil with lidocaine-HCl on river puffer (*Takifugu obscurus*) and tiger puffer (*T. rubripes*) were evaluated by Park [18]. Anaesthesia times of clove oil were affected by water temperature (20, 24, and 28℃) and salinity (10, 20, and 30 ppt). Anaesthesia times of mixed samples were significantly similar with regard to exposure and recovery times, and all samples satisfied anaesthesia criteria (exposure time within 3 minutes and recovery time within 5 minutes) under the various temperatures and salinities, and the lowest to highest concentration of anaesthetics. Both species river puffer and tiger puffer had short exposure time with a high anaesthesia dose, high temperature (28℃) and intermediate salinity (20 ppt), and were highly affected by temperature and salinity. Park [18] showed the mixed anaesthetics had rapid exposure times and long recovery times in contrast to the effects of clove oil. Cortisol concentrations under the conditions of various clove oil dosages, salinity, and temperature for both species increased until 12 hours after recovery from anaesthesia. After 12 hours, cortisol concentrations decreased until after 48 hours. During the simulated transportation of both species, control and sedated clove oil groups (5 ppm) were measured for water parameters, dissolved oxygen (DO), CO_2, respiratory frequency, $NH^{4+,}$ and pH for 6 hours in 1 hour intervals, water parameters of sedated groups and controls were significantly different after 2 hours.

·**Marine animal: Shellfish** Choi et al. [29] reported the optimal concentration of lidocaine and MS-222 (tricaine methanesulfonate) for the exfoliation and recovery of abalone (*Haliotis discus hannai*) in different shell lengths, for the purpose of preventing the damage of shell and muscle. The response varied for different shell size groups (shell length 1, 2, and 3 cm). Choi et al. [29] suggested the result that the exfoliation and recovery time by lidocaine and MS-222 in shell length 1 cm group were more shorter than in 3 cm group. In shell length 1 cm group, the optimal concentrations of lidocaine and MS-222 for anaesthetic were 200 ppm and 100 ppm, respectively.

Park [17] investigated 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. At each group, as the concentration of anesthetics increased, the induction time decreased. For each anesthetic, the longer the shell length of six species in this experiment were, the more induction time increased. Park [17] reported 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 hours after recovery of anesthesia (RA) and cortisol concentrations of three anesthetics on each shellfish were highest at 6 hours after RA. At 6 hours 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 hours after RA was higher than that of the other shellfishes. At 6 hours after RA, cortisol concentrations of three anesthetics were decreased until 48 hours. Park [17] reported glucose concentrations of clove oil, lidocaine-HCl, and MS-222 on six seawater shellfish were increased until 12 hours after RA and glucose concentrations of three anesthetics on each shellfish were highest at 12 hours after RA. Park [17] reported that at 6 hours 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 hours after RA, glucose concentrations of three anesthetics were decreased.

·**Freshwater animal: Finfish** Lidocaine was evaluated as anaesthetics for seven species; crucian carp (*Carassius auratus*), oriental weatherfish (*Misgurnus anguillicaudatus*), Chinese weatherfish (*Misgurnus mizolepis*), channel

catfish (*Ictalurus punctatus*), Far Eastern catfish (*Silurus asotus*), Nile tilapia (*Oreochromis niloticus*), and rainbow trout (*Salmo gairdneri*) of fishes [30]. The response varied for seven test species [30]. Kim et al. [30] reported lidocaine was preferable to other conventional fish anaesthetics since it is cheap, safe and convenient to use.

Kim et al. [30] evaluated lidocaine as anaesthetics for seven species of fishes; crucian carp (*Carassius auratus*), oriental weatherfish (*Misgurnus anguillicaudatus*), Chinese weatherfish (*Misgurnus mizolepis*), channel catfish (*Ictalurus punctatus*), Far Eastern catfish (*Silurus asotus*), Nile tilapia (*Oreochromis niloticus*), and rainbow trout (*Salmo gairdneri*). The response varied for seven test species. Lidocaine was preferable to other conventional fish anaesthetics since it is cheap, safe and convenient to use.

Chung et al. [31] reported the effects of lidocaine on the parameters of haematology and blood chemistry in the carp (*Cyprinus carpio*). At low concentrations (100 and 200 ppm) of lidocaine, no significant changes in the parameters of haematology and blood chemistry were observed during recovery period from the treatment of lidocaine. However, at high concentrations (300 and 400 ppm) of lidocaine, red blood cell (RBC) and hematocrit (Ht) value. plasma glucose concentration, and the activity of lactate dehydrogenase (LDH) were markedly increased compared with the controls. These increases were maintained for up to 60 minutes. Based on these results, Chung et al. [31] suggested that lidocaine is generally not toxic to fish but has shown the physiological stress responses at high concentrations.

The effectiveness of lidocaine HCI (lidocaine HCl/sodium bicarbonate mixture) was tested as an anaesthetic for Chinese minnow (*Rhynchocypris oxycephalus*) and Amur minnow (*R. steindachneri*) at three different temperatures of 10℃, 15℃, and 20℃ [5]. Based on the exposure and recovery time, effective doses of lidocaine HCI were proven to be 300 ppm (20℃), 400 ppm (15℃), and 600 ppm (10℃) for Chinese minnow, and 400 ppm (20℃), 500 ppm (15℃), and 600 ppm (10℃) for Amur minnow respectively. Park et al. [5] reported anaesthetic dose and temperature-dependent relationship in exposure and recovery time were observed for these two *Rhynchocypris* spps. There were size-related increases of exposure time on Amur minnow in each dose of lidocaine HCI. However, dose-dependent increase of recovery time was found in only the large size group of *Rhynchocypris* spp.

The experimental transportation of Amur minnow (*Rhynchocypris steindachneri*) was carried out to study the effects of lidocaine-hydrochloride on water parameters [11]. The dissolved oxygen, ventilation rate, ammonia nitrogen, and pH of control group, sham control group, and lidocaine-hydrochloride treated groups of 2.5 ppm, 5 ppm, 10 ppm, and 20 ppm at time of 30 minutes, 60 minutes, 90 minutes, 120 minutes, 240 minutes, and 360 minutes after elapsed from treatment were tested. During the experiment time, Park et al. [11] found that lidocaine-hydrochloride treated groups were most effective, followed by sham control and control, in decreasing the oxygen consumption and the excretion of ammonia by the fish. There were lidocaine-hydrochloride dose-related decrease in oxygen consumption and the excretion of ammonia. Decreasing in pH value of lidocaine-hydrochloride groups and sham control group was much more higher than that of control group. Park et al. [11] indicated results from their study reveal lidocaine-hydrochloride is effective as sedative for transportation mixture in Amur minnow.

The efficacy of lidocaine hydrochloride and clove oil anaesthetics was evaluated in the Korean rose bitterling (*Rhodeus uyekii*) (Mori, 1935) and oily bitterling (*Acheilognathus koreensis*) at four different temperatures of 10, 15, 20, and 25℃ [32]. When complete anaesthesia was acquired less than 3 minutes and recovery was acquired less than 10 minutes, the optimal dose range of lidocain hydrochloride at 20℃ was 250~550 ppm in Korean rose bitterling, and 150~550 ppm in oily bitterling, respectively. In case of clove oi1, the optimal dose range at 20℃ was 40~200 ppm in Korean rose bitterling, and 80~240 ppm in oily bitterling, respectively. Kang et al. [32] reported that both of lidocaine hydrochloride and clove oi1 resulted in a negatively dose-dependent manner for anaesthesia induction time in these two species. Recovery times were more variable in relation to anaesthetic doses, but in general higher anaesthetic doses resulted in similar or longer recovery time.

To assess the effect of anaesthetic on stress response in cultured sweetfish (*Plecoglossus altivelis*) during transportation, Hur et al. [33] determined the levels of plasma cortisol, glucose, lactic acid, Na+, K+, Cl-, osmolality, and survival. Fish transportation was carried by car for 2 hours after anaesthesis with lidocaine-HCl/1,000 ppm $NaHCO_3$ in experiment. Mean plasma cortisol concentration before transportation was 170.7 ng/mL. After transportation, the levels of plasma cortisol increased to 518.5 ng/mL (control), 461.9 ng/mL (sham control), 369.4 ng/mL (20 ppm anaesthetic), 304.0 ng/mL (40 ppm anaesthetic), 405.7 ng/mL (80 ppm anaesthetic), and 499.1 ng/mL (160 ppm anaesthetic) in each experimental groups, respectively [33]. However

levels of glucose, lactic acid, Na^+, Cl^- and osmolality in 40 ppm anaesthetic group did not show significant differences in this before and after transportation.

The anesthetic effects of MS-222 (tricaine methanesulfonate), clove oil, 2-phenoxyethanol, $NaHCO_3$, lidocaine-HCl and lidocaine-HCl/$NaHCO_3$ in the glass catfish (*Kryptopterus vitreolus*) were investigated by Lee et al. [34] in 2017. Based on the efficacy criteria of complete anesthetic induction from 60 to 120 seconds, recovery within 300 seconds, the lowest effective concentrations at 24°C were determined to be 60 ppm (induction 82.8±17.6 seconds, recovery 80.2±34.7 seconds) for MS-222, 40 ppm (induction 70.5±8.2 seconds, recovery 83.4±17.7 seconds) for clove oil, 250 ppm (induction 64.3±24.0 seconds, recovery 62.8±15.6 seconds) for 2-phenoxyethanol, 300 ppm (induction 127.3±13.3 seconds, recovery 107.5±4.8 seconds) for lidocaine-HCl and 200/100 ppm (induction 81.2±17.2 seconds, recovery 98.3±19.7 seconds) for lidocaine-HCl/$NaHCO_3$.

Park et al. [35] evaluated the anesthetic effects (time required for anesthesia to take effect and recovery time) of two anesthetic agents, clove oil and lidocaine–HCl, on marine medaka (*Oryzias dancena*). Park et al. [35] anesthetized fish at different water temperatures (23, 26, and 29℃) and using different concentrations of clove oil (50, 75, 100, 125, 150, and 175 ppm) or lidocaine–HCl (300, 400, 500, 600, 700, and 800 ppm). The time required for anesthesia to take effect decreased significantly as both anesthetic concentration and water temperature increased for both clove oil and lidocaine–HCl. To anesthetize marine medaka within approximately 1 minute, the optimal concentrations for clove oil were 125 ppm at 23℃, 100 ppm at 26℃, and 75 ppm at 29℃, and for lidocaine–HCl were 800 ppm at 23℃, and 700 ppm at both 26℃ and 29℃. Park et al. [35] also compared anesthetic effects in marine medaka of different sizes. Both anesthetic exposure time and recovery time were significantly shorter for smaller fish than for larger fish.

Park et al.[6] determined the optimum concentrations of anesthetic clove oil and anesthetic lidocaine-HCl were determined for a species of adult marine medaka (*Oryzias dancena*), over a range of salinity conditions, and investigated in a transport simulation experiment by analyzing various water and physiological parameters. Research from Park et al. [6] indicated that the higher the concentration of anesthetic at each salinity, the shorter the anesthesia time at each salinity. Park et al. [6] showed that at each concentration, fish were anesthetized slower at water salinities over 10 ppt. Anesthesia time at 10 ppt was faster than any other salinity. In 10 ppt salinity, the dissolved oxygen (DO) concentrations and respiratory frequencies of the clove-oil-administered groups decreased until 48 hours, whereas the NH^{4+} and CO_2 concentrations increased until 48 hours. In same period, the DO, NH^{4+}, and CO concentrations and respiratory frequencies all decreased as the clove oil concentration increased. The trends in the DO, NH^{4+}, and CO_2 concentrations and respiratory frequencies in the lidocaine-HCl-administered groups were similar to those in the clove-oil-administered groups.

An anesthetic protocol was optimized for microinjection-related handling of Siberian sturgeon (*Acipenser baerii*; Acipenseriformes) prolarvae, an extant primitive fish species commonly grown in aquaculture [36]. Comparative examinations of three selected anesthetics (clove oil, lidocaine, and MS-222) with a dosage regime of 50, 100, 200, and 400 mg/L indicated that MS-222 was the most efficient agent for Siberian sturgeon prolarvae, as evidenced by the fast induction of anesthesia with quick and uniform recovery [36]. Meanwhile, clove oil should be avoided, due to prolonged recovery times varying widely between individuals. None of the tested anesthetics significantly affected prolarval viability at any of the dosage regimes tested. Based on an analysis of the duration of an unconscious state in air, Kim and Nam [36] recommended a dose of 200 mg/L MS-222 for microinjection. Recovery time after use of this dose was influenced by the prolarval age and the development of gills, in which prolarvae older than 3 days after hatching required longer recovery times than did younger prolarvae. Post-recovery behavioral assessment showed no apparent difference between MS-222-anesthetized and non-anesthetized prolarvae in their swimming behavior and phototactic responses. Applicability of currently developed anesthetic protocol using MS-222 in larval microinjection was demonstrated with the injection of a visible dye to the anesthetized prolarvae, followed by the analysis of post-recovery viability. Kim and Nam [36] reported that the present anesthetic protocol based on 200 mg/L of MS-222 could provide researchers with practical usefulness with good safety margins for the micromanipulation and other related handlings of Siberian sturgeon prolarvae.

Goo et al. [37] determined the optimal dose of lidocaine-HCl for anesthetizing Siberian sturgeon (*Acipenser baerii*) to investigate the relationship between anesthetic effectiveness and fish size, and to analyze re-anesthetic effects and stress responses to lidocaine-HCl use. The anesthesia and recovery times were affected by the concentration of the anesthetic and fish body size. Anesthesia time decreased significantly as both the lidocaine-HCl concentration and body size increased, while recovery time decreased as the lidocaine-HCl concentration increased. Anesthesia time and recovery time decreased significantly as the lidocaine-HCl concentration and water

temperature increased. Goo et al. [38] pointed out plasma cortisol, plasma glucose, and lactic acid concentrations were indicative of stress reactions in this experiment. At 1-, 2-, and 3-day intervals, the anesthesia and recovery times increased as the number of anesthesia treatments increased but were not different between duplicate and triplicate. In 4-day interval groups, anesthesia and recovery times were not significantly different among the initial, duplicate, and triplicate treatments. Anesthesia and recovery times increased with the second anesthesia treatment. Anesthesia time decreased as the number of anesthesia treatments increased, but recovery times did not differ with the increase in number of anesthesia treatments. Goo et al. [37] presented lidocaine-HCl concentrations of 50 and 250 ppm in the larval and juvenile groups, respectively, showed an optimal anesthesia time of approximately 1 minute. The optimal anesthesia interval of lidocaine-HCl was 4 days, and frequent anesthesia resulted in negative effects by inhibiting sensitivity

·**Freshwater animal**: **Reptile** Attempts were made to understand how the different sizes (mean body weight of 4.1 ± 0.8 g for small and 182.6 ± 23.7 g for large) of the soft-shelled turtle (*Pelodiscus sinensis*) are affected by different temperature (25°C or 30°C), and different concentrations (700, 1000, and 1300 ppm) of anesthetic lidocaine hydrochloride–sodium bicarbonate [38]. Exposure time of the soft-shelled turtle was affected by all factors (temperature, concentration, and size). Exposure time of the soft-shelled turtle for anesthetizing decreased with increase in temperature and in concentration of lidocaine hydrochloride, and decrease in size. Recovery time for the soft-shelled turtle was also affected by all factors. Recovery time of the soft-shelled turtle increased with increase in temperature, concentration of lidocaine hydrochloride, and size. According to these results of Park et al. [38], lidocaine hydrochloride (1,000 ppm)–sodium bicarbonate seemed an effective anesthetic for sedating and handling the soft-shelled turtle.

Clove oil and Derivatives (Appendix) Clove oil has recently been suggested as an alternative aquatic animal anesthetic [35, 39-42]. Clove oil is a pale yellow liquid derived from the leaves, buds and stem of the clove tree (*Eugenia* sp.). Its active ingredients are eugenol (4-allyl-2-methoxyphenol) and iso-eugenol (4-propenyl-2-methoxyphenol), which can comprise 90-95% of clove oil by weight. Clove oil and eugenol are completely water soluble, particularly at cold temperatures. A 1:10 mixture of either in 95% ethanol yields a 100 mg/mL stock solution [43]. Clove oil has been used for many years as a food additive and a topical analgesic in dentistry, and is recognized as a GRAS (Generally Recognized As Safe) substance by the US FDA for use in humans [44]. ^{TM25}AQUI-S is a pharmaceutical derivative that contains 50% active ingredient and is registered for use with food fish in New Zealand and Australia with a nil withdrawal period [35, 44, 45]. However, neither anesthetic is approved for use with fish in North America. Both substances are safe to handle, but as with all chemical anesthetics, contact with eyes and mucous membranes should be avoided.

·**Marine animal: Finfish** Along with olive flounder (*Paralichthys olivaceus*), black rockfish (*Sebastes schlegeli*) is another very popular maricultured species in Republic of Korea [45]. As there is many difficulties in handlling live fish for aquaculturist, use of suitable anesthesia for proper handling of fish is very important in the field [45]. In this view, the effect of AQUI-S® has analysed for its use in the field. AQUI-S®, contains 50% isoeugenol, is a new anesthics for fish and zero-withdraw time required since it was approved as a safe additives of food [45]. Shin et al. [45] reported black rockfish adult exhibited sedation effect from 5 ppm at 10℃ and 15℃, and 7.5 ppm at 20℃, on the other hand, anesthesia was at least required 7.5 ppm at 10℃ and 15℃, and 10 ppm at 20℃. The fish was recovered from sedation and anesthesia after approximately 5 and 10 minutes, respectively. In case of black rockfish fry, sedation was recorded from 2.5 ppm at 20℃, and 5 ppm at 15℃ and 20℃. The least concentraion of anesthesia was 2.5 ppm at 10℃, 7.5 ppm at 15℃, and 5 ppm at 20℃. The acute toxic test showed that black rockfish adult and fry showed mortality above 12.5 and 15 ppm concentration of AQUI-S®, respectively.

The efficacy of clove oil as an anaesthetic and at producing a physiological response (plasma cortisol and glucose) was evaluated in the kelp grouper (*Epinephelus bruneus*) [46]. To acquire complete anaesthesia in less than 3 minutes and recovery in <10 minutes, three doses of clove oil were tested at 18, 22, and 26℃. Although higher anaesthetic doses resulted in shorter induction times and longer recovery times, and a lower temperature resulted in longer anaesthesia induction and slower recovery, Park et al. [46] found the optimal dose and administering temperature of clove oil to be 250-300 mg/L at water temperature of 18℃, 150-200 mg/L at water temperature of 22℃ and 50-100 mg/L at water temperature of 26℃ respectively. Following the administration of 150 mg/L of clove oil at 22℃, the plasma cortisol level was highest (4.24 ± 1.571 mg/dL) after 12 hours and the plasma glucose was highest (92.7 ± 9.61 mg/dL) after 2 hours.

Park et al. [41] tested the efficacy (e.g., induction time, recovery time) of clove oil as an anesthetic for rock bream (*Oplegnathus fasciatus*). In addition, Park et al. [41] 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 mg/L at 20℃, 100 to 125 mg /L at 24℃, and 50 to 75 mg /L at 28℃. Following the administration of 100 mg/L of clove oil at 24°C, the plasma cortisol level was highest (1.70 ± 0.148 μg/dL) after 1 hour while the plasma glucose level was highest (80.0 ± 1.41 mg/dL) after 2 hours. It took 2 days for the plasma cortisol and plasma glucose concentrations to return to pre-exposure levels.

In order to establish optimum anesthesia concentration, Park et al. [47] 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 mg/L in both large and small sized fish. In general, fish exposed to higher anesthetic doses were rapidly induced but took longer to recover. 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. Using the established optimum aesthetic concentration, Park et al. [48] evaluated the physiological response of dark-banded rockfish to clove oil by measuring plasma cortisol and glucose levels. Following administration of 150 mg/L clove oil at 20℃ (optimum breeding temperature), plasma cortisol level was highest (42.2 ± 11.318 mg/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.

Han et al. [48] evaluated the efficiency of clove oil, MS-222, and 2-phenoxyethanol as anesthetics in juvenile chub mackerel (*Scomber japonicus*). Stage A5 of anesthesia was assumed to be sufficient for conducting routine aquaculture procedures in less than 3 minutes, with recovery (stage R5) in less than 5 minutes. The lowest effective doses of the three anesthetics were 50 mg/L clove oil (anesthetic time of 71.3 seconds and recovery time of 167.0 seconds), 100 mg/L MS-222 (anesthetic time of 70.7 seconds and recovery time of 115.7 seconds), and 400 mg/L 2-phenoxyethanol (anesthetic time of 86.7 seconds and recovery time of 95.0 seconds). Anesthetic times decreased with increasing doses for all three anesthetic agents, and fish anesthetized with clove oil exhibited the longest recovery times. After 30 minutes, the highest plasma cortisol and lactate levels were detected with the use of clove oil, whereas the lowest values were observed with 2-phenoxyethanol. In addition, high glucose levels were maintained during recovery with clove oil, but the treatments did not differ.

The optimum concentrations of clove oil as an anesthetic for olive flounder (*Paralichthys olivaceus*) and the stress response of the fish to clove oil anesthesia were determined over a range of water temperatures, and investigated in a simulated transport experiment using analysis of various water and physiological parameters [9]. While the time for induction of anesthesia decreased as both the concentration of clove oil and water temperature increased, the recovery time increased. The plasma cortisol concentration in fish at each temperature increased up to 12 hours following exposure, then decreased to 48 hours. The DO dissolved oxygen concentrations, pH values, and the fish respiratory frequencies decreased over 6 hours following exposure to clove oil in all experimental groups, whereas the NH^{4+} and CO_2 concentrations in all experimental groups increased up to 6 hours. The pH values and DO concentrations increased with increasing clove oil concentration in the 6 hours following exposure, and the CO_2 and NH^{4+} concentrations and the respiratory frequencies decreased with increasing clove oil concentration. The results of Gil et al. [9] experiment suggest that clove oil reduced the metabolic activity of olive flounder, thus reducing NH^{4+} excretion and O_2 consumption.

The physiological response and the applicable concentration ranges of anesthetic clove oil and anesthetic lidocaine-HCl was determined, and the synergistic effect of a mixture of these two anesthetics on the in grass puffer *(Takifugu niphobles*) was investigated [26]. Gil et al. [26] showed the anesthesia times decreased and the recovery times increased with increasing concentrations of clove oil and lidocaine-HCl. Applicable concentration ranges for long-term transportation requiring more than 1 hour were 2 ppm for clove oil and 50 ppm for lidocaine-HCl. With mixtures of the two anesthetics, the anesthesia time decreased as the admixture concentration of clove oil and lidocaine-HCl increased. Anesthesia times of experimental groups with the combined anesthetics were shorter than those with the same concentrations of clove oil or lidocaine-HCl alone. Plasma cortisol concentrations were highest at 6 hours in all experimental groups anesthetized with the mixture of clove oil and lidocaine-HCl, while all groups with clove oil or lidocaine-HCl alone had the highest plasma cortisol concentrations at 12 hours. Plasma glucose concentrations were highest at 12 hours in experimental groups anesthetized with the mixture of clove oil and lidocaine-HCl, while groups with clove oil or lidocaine-HCl alone had the highest plasma glucose at 24 hours. Gil et al. [26] provided basic information about anesthetics and the synergistic effect of mixtures of anesthetics in this grass puffer species.

Park et al. [49] provided anesthetic criteria of clove oil for an effective manipulation and transportation of red spotted grouper (*Epinephelus akaara*). When anesthesia temperature (20, 24, and 28℃) and concentration of clove oil (25, 50, and 75 ppm) were increased, the anesthesia and recovery time decreased and tended to be similar to each other between juvenile and adult. Also, as the temperature and concentration increased, the ratio of exposure time and recovery time between juvenile and adult were decreased. When plasma cortisol concentrations were compared for 48 hours after anesthesia with 50 ppm of clove oil, both the juvenile and adult fish grew up to 12 hours; however, thereafter decreased and there was no significant difference from control at 48 hours.

The effects of the anaesthetic agents, clove oil and mixture of clove oil with lidocaine-HCl on river puffer (*Takifugu obscurus*) and tiger puffer (*T. rubripes*) were evaluated by Park [18] Anaesthesia times of clove oil were affected by water temperature (20, 24, and 28℃) and salinity (10, 20, and 30 ppt). Anaesthesia Anaesthesia times of mixed samples were significantly similar with regard to exposure and recovery times, and all samples satisfied anaesthesia criteria (exposure time within 3 minutes and recovery time within 5 minutes) under the various temperatures and salinities, and the lowest to highest concentration of anaesthetics. Both species river puffer and tiger puffer had short exposure time with a high anaesthesia dose, high temperature (28℃) and intermediate salinity (20 ppt), and were highly affected by temperature and salinity. Park [18] showed the mixed anaesthetics had rapid exposure times and long recovery times in contrast to the effects of clove oil. Cortisol concentrations under the conditions of various clove oil dosages, salinity, and temperature for both species increased until 12 hours after recovery from anaesthesia. After 12 hours, cortisol concentrations decreased until after 48 hrs. During the simulated transportation of both species, control and sedated clove oil groups (5 ppm) were measured for water parameters, dissolved oxygen (DO), CO_2, respiratory frequency, NH^{4+}, and pH for 6 hours in 1 hour intervals, water parameters of sedated groups and controls were significantly different after 2 hours.

• **Marine animal: Shellfish** Park [17] investigated 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. At each group, as the concentration of anesthetics increased, the induction time decreased. For each anesthetic, the longer the shell length of six species in this experiment were, the more induction time increased. Park [17] reported 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 hours after recovery of anesthesia (RA) and cortisol concentrations of three anesthetics on each shellfish were highest at 6 hours after RA. At 6 hours 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 hours after RA was higher than that of the other shellfishes. At 6 hours after RA, cortisol concentrations of three anesthetics were decreased until 48 hours. Park [17] reported glucose concentrations of clove oil, lidocaine-HCl, and MS-222 on six seawater shellfish were increased until 12 hours after RA and glucose concentrations of three anesthetics on each shellfish were highest at 12 hours after RA. Park [17] reported that at 6 hours after RA, glucose concentrations of MS-222 on each shellfish were higher than that of clove oil and lidocaine-HCl and glucose concentration of granular ark was higher than that of the other shellfishes as well. From 12 to 48 hours after RA, glucose concentrations of three anesthetics were decreased.

·**Marine animal: Mollusca** Seol et al. [40] evaluated the anaesthetic effect of clove oil [2-methoxy-4-2-(2-propenyl)-phenol] on the common octopus (*Octopus minor*), in terms of the time required to become anaesthetized (‘anaesthetic time’) and recovery time. Seol et al. [40] used a factorial experimental design and administered clove oil at different temperatures (15, 20, and 25℃) and concentrations (50, 100, 150, 200, 250, and 300 mg/L). Seol et al. [40] observed the relationship between concentration and temperature, and each variable was effective. Anaesthetic time linearly decreased as the concentration and temperature increased. However, recovery time increased as the concentration increased and temperature decreased. There was no mortality. A concentration of 200 mg/L clove oil showed rapid anaesthetic and recovery times in the common octopus, indicating its suitability for this species.

·**Freshwater animal: Finfish** The efficacy of lidocaine hydrochloride and clove oil anaesthetics was evaluated in the Korean rose bitterling (*Rhodeus uyekii*) (Mori, 1935) and oily bitterling (*Acheilognathus koreensis*) at four different temperatures of 10, 15, 20, and 25℃ [32]. When complete anaesthesia was acquired less than 3 minutes

and recovery was acquired less than 10 minutes, the optimal dose range of lidocain hydrochloride at 20℃ was 250~550 ppm in Korean rose bitterling, and 150~550 ppm in oily bitterling, respectively. In case of clove oi1, the optimal dose range at 20℃ was 40~200 ppm in Korean rose bitterling, and 80~240 ppm in oily bitterling, respectively. Kang et al. [32] reported that both of lidocaine hydrochloride and clove oi1 resulted in a negatively dose-dependent manner for anaesthesia induction time in these two species. Recovery times were more variable in relation to anaesthetic doses, but in general higher anaesthetic doses resulted in similar or longer recovery time.

Lee et al. [34] investigated the anesthetic effects of MS-222 (tricaine methanesulfonate), clove oil, 2-phenoxyethanol, $NaHCO_3$, lidocaine-HCl and lidocaine-HCl/$NaHCO_3$ in the glass catfish (*Kryptopterus vitreolus*). Based on the efficacy criteria of complete anesthetic induction from 60 to 120 seconds, recovery within 300 seconds, the lowest effective concentrations at 24℃ were determined to be 60 ppm (induction 82.8 ± 17.6 seconds, recovery 80.2 ± 34.7 seconds) for MS-222, 40 ppm (induction 70.5 ± 8.2 seconds, recovery 83.4 ± 17.7 seconds) for clove oil, 250 ppm (induction 64.3 ± 24.0 seconds, recovery 62.8 ± 15.6 seconds) for 2-phenoxyethanol, 300 ppm (induction 127.3 ± 13.3 seconds, recovery 107.5 ± 4.8 seconds) for lidocaine-HCl, and 200/100 ppm (induction 81.2 ± 17.2 seconds, recovery 98.3 ± 19.7 seconds) for lidocaine-HCl/$NaHCO_3$. Thus, Lee et al. [34] presented 200/100 ppm of lidocaine-HCl/$NaHCO_3$, which was found to be an effective anesthetic agent.

Park et al. [35] evaluated the anesthetic effects (time required for anesthesia to take effect and recovery time) of two anesthetic agents, clove oil and lidocaine-HCl, on marine medaka (*Oryzias dancena*). Park et al. [35] anesthetized fish at different water temperatures (23℃, 26℃, and 29℃) and using different concentrations of clove oil (50 ppm, 75 ppm, 100 ppm, 125 ppm, 150 ppm, and 175 ppm) or lidocaine-HCl (300 ppm, 400 ppm, 500 ppm, 600 ppm, 700 ppm, and 800 ppm). The time required for anesthesia to take effect decreased significantly as both anesthetic concentration and water temperature increased for both clove oil and lidocaine-HCl. To anesthetize marine medaka within approximately 1 minute, the optimal concentrations for clove oil were 125 ppm at 23℃, 100 ppm at 26℃, 75 ppm at 29℃ and for lidocaine-HCl were 800 ppm at 23℃, and 700 ppm at both 26℃ and 29℃. Park et al. [35] also compared anesthetic effects in marine medaka of different sizes. Both anesthetic exposure time and recovery time were significantly shorter for smaller fish than for larger fish.

Park et al. [6] determined the optimum concentrations of anesthetic clove oil and anesthetic lidocaine-HCl for a species of adult marine medaka (*Oryzias dancena*), over a range of salinity conditions, and investigated in a transport simulation experiment by analyzing various water and physiological parameters. Research from Park et al.[6] indicated that the higher the concentration of anesthetic at each salinity, the shorter the anesthesia time at each salinity. Park et al.[6] showed at each concentration, fish were anesthetized slower at water salinities over 10 ppt. Anesthesia time at 10 ppt was faster than any other salinity. In 10 ppt salinity, the dissolved oxygen (DO) concentrations and respiratory frequencies of the clove-oil-administered groups decreased until 48 hours, whereas the NH^{4+} and CO_2 concentrations increased until 48 hours. In same period, the DO, NH^{4+}, and CO_2 concentrations and respiratory frequencies all decreased as the clove oil concentration increased. The trends in the DO, NH^{4+}, and CO_2 concentrations and respiratory frequencies in the lidocaine-HCl-administered groups were similar to those in the clove-oil-administered groups.

An anesthetic protocol was optimized for microinjection-related handling of Siberian sturgeon (*Acipenser baerii*; Acipenseriformes) prolarvae, an extant primitive fish species commonly grown in aquaculture [36]. Comparative examinations of three selected anesthetics (clove oil, lidocaine, and MS-222) with a dosage regime of 50, 100, 200, and 400 mg/L indicated that MS-222 was the most efficient agent for Siberian sturgeon prolarvae, as evidenced by the fast induction of anesthesia with quick and uniform recovery [36]. Meanwhile, clove oil should be avoided, due to prolonged recovery times varying widely between individuals. None of the tested anesthetics affected prolarval viability at any of the dosage regimes tested in study of Kim and Nam [36]. Based on an analysis of the duration of an unconscious state in air, Kim and Nam [36] recommended a dose of 200 mg/L MS-222 for microinjection. Recovery time after use of this dose was influenced by the prolarval age and the development of gills, in which prolarvae older than 3 days after hatching required longer recovery times than did younger prolarvae. Postrecovery behavioral assessment showed no apparent difference between MS-222-anesthetized and non-anesthetized prolarvae in their swimming behavior and phototactic responses. Applicability of currently developed anesthetic protocol using MS-222 in larval microinjection was demonstrated with the injection of a visible dye to the anesthetized prolarvae, followed by the analysis of post-recovery viability. Taken together, the anesthetic protocol of Kim and Nam [36] based on 200 mg/L of MS-222 could provide researchers with practical usefulness with good safety margins for the micromanipulation and other related handlings of Siberian sturgeon prolarvae.

Park [19] evaluated the anesthetic effects of clove oil and tricaine methanesulfonate (MS-222) on the Far Eastern catfish (*Silurus asotus*) by measuring the times to anesthesia and recovery. Each anesthetic effect of clove oil and MS-222 was tested in two groups of fish with different body sizes: a group of small fish (mean body length: 15.5±1.58 cm, mean body weight: 50.1±5.91 g, n=20) and a group of large fish (mean body length: 31.5±4.19 cm, mean body weight: 302.1±15.22 g, n=20). The anesthetics were used at concentrations of 200, 300, 400, 500, and 600 ppm. The results showed relationships between the concentration of the anesthetic and the body size of the fish. The time to anesthesia decreased linearly with increasing concentration in the large fish for both clove oil and MS-222. Based on an optimal anesthetic time of approximately 1 minute, the preferred concentrations of the anesthetics were 500 ppm for clove oil and 600 ppm for MS-222. Both the anesthetic time and the recovery time were shorter for the small fish than for the large fish. Park [19] showed that the smaller-sized Far Eastern catfish was more easily anesthetized and recovered more rapidly from anesthesia than the larger-sized fish.

MS-222 and Benzocaine (Appendix) MS-222 (TMS, 3-aminobenzoic acidethyl ester methanesulfonate, [TM18]Finquel, tricaine, tricaine methanesulphonate, metacaine) and benzocaine (P-aminobenzoic acid ethyl ester, [TM1]Anesthesim, [TM14]Anesthone, [TM2]Americaine, ethylaminobenzoate, orthesin, parathesin) are the 2 most common anesthetic agents used in fish research studies and are also used in food fish production [44]. MS-222 is the most widely used fish anesthetic, and it is extremely effective for rapid induction of deep anesthesia. MS-222 is commonly used in research laboratories and has been registered by Health Canada for veterinary use with fish. It is a white crystalline powder that is easily dissolved in water, with a solubility of 1.25 g/mL water, at 20℃. Benzocaine is structurally similar to the chemical composition of MS-222; however, this agent has two forms: a crystalline salt with a water solubility of 0.4 g/L, or a freebase form which must be dissolved in ethyl alcohol first at 0.2 g/mL [20, 44], whereas MS-222 is soluble in water but has an acidic pH that requires addition of a buffer (i.e., sodium bicarbonate is usually added to obtain the desired pH).

·**Marine animal: Finfish** The strength of juvenile black rockfish (*Sebastes schlegeli*) raised in different hatcheries for wild stock enhancement was evaluated in terms of resistance to an anesthetizing agent, tricaine methane-sulfonate (MS-222), and exposure to drying [50]. The working dosage of MS-222 varied with fish size and hatchery population. Smaller fish were less resistant to the chemical than larger ones. MS-222 effects also differed with fish growth history. The fish cultured in embanked populations showed stronger resistance, earlier recovery, and lower mortality, compared to those cultured in land-based tanks or collected from wild stocks. Similar results were seen in juveniles challenged to dry exposure. According to their results, Son et al. [50] suggested that an embanked population of black rockfish is more resistant to anesthetic stress, expressed as anesthesia recovery and mortality, and that this population is healthier than others.

The effects of the different anesthetic MS-222 and lidocaine-HCl doses on the blood physiological responses, on the time required for anesthesia and recovery, and on the survival rates of black rockfish (*Sebastes schlegeli*) were reported [28]. Plasma cortisol was its highest levels (96.1±12.1 ng/mL) at 6 hours after the administration of 300 ppm of MS-222, and in all groups, plasma cortisol levels were higher than the initial levels during the anesthetic experiment. Kim et al. [28] showed fish receiving lidocaine-HCl also exhibited higher than initial plasma cortisol levels at almost experimental intervals. The middle size fish exhibited the highest glucose level (143.3±14.5 mg/dL) at 50 ppm of anesthesia after 1 hour, and every level was significantly higher than the initial level for at least 12 hours. Glucose levels in fish to which lidocaine-HCl was administered were comparable to the levels seen in conjunction with MS-222 treatment. Kim et al. [28] showed in fish anesthetized with MS-222, K^+ levels in the small size fish were significantly elevated after 1 hour, while Na^+ levels did not change in any of the groups throughout the experiment. Anesthetic time was significantly attenuated with increases in the concentrations of MS-222 and lidocaine-HCl. Kim et al. [28] also noted a correlation between anesthetic time and fish size, in that larger fish took a longer time, followed by the middle size and then the small size fish. The all fish size groups showed above 95% survival rates at every experimental concentration in MS-222 and 300-400 ppm in lidocaine-HCl. The results from Kim et al. [28] may indicate that 100-200 ppm MS-222 and 400 ppm lidocaine-HCl are the most effective doses as sedatives for the black rockfish and these doses could be used as the suitable anesthetics doses.

Han et al. [48] evaluated the efficiency of clove oil, MS-222, and 2-phenoxyethanol as anesthetics in juvenile (*Scomber japonicus*). Stage A5 of anesthesia was assumed to be sufficient for conducting routine aquaculture procedures in less than 3 minutes, with recovery (stage R5) in less than 5 minutes. The lowest effective doses of the three anesthetics were 50 mg/L clove oil (anesthetic time of 71.3 seconds and recovery time of 167.0 seconds), 100 mg/L MS-222 (anesthetic time of 70.7 seconds and recovery time of 115.7 seconds), and 400 mg/L 2-phenoxyethanol (anesthetic time of 86.7 seconds and recovery time of 95.0 seconds). Anesthetic times decreased with increasing doses for all three anesthetic agents, and fish anesthetized with clove oil exhibited the longest

recovery times. After 30 minutes, the highest plasma cortisol and lactate levels were detected with the use of clove oil, whereas the lowest values were observed with 2-phenoxyethanol. In addition, high glucose levels were maintained during recovery with clove oil, but the treatments did not significantly differ. The most effective of the three anesthetic agents was 2-phenoxyethanol, although all were considered acceptable for use in cultures of juvenile *Scomber japonicus* .

Blood (hematocrit, hemoglobin and red blood cell) and plasma (cortisol, glucose, lactic acid, Na^+, Cl^-, K^+, osmolality, aspartate aminotransferase, and alanine aminotransferase) factors in greenling (Hexagrammos otakii) after MS-222 and lidocaine-HCl anaesthetic were determined by Park et al. [25]. Greenling (mean body length: 25.8 ± 1.6 cm, mean body weight: 194.5 ± 33.8 g) were exposed to concentrations of 125 ppm MS-222 and 800 ppm lidocaine-HCl at 18℃ [25]. Blood was collected from ten fish after anesthesia 30 minutes, 1, 3, 6, and 24 hours, respectively. After anesthesia, Park et al. [25] determined the concentrations of hematocrit, hemoglobin, red blood cell, cortisol, glucose, lactic acid, osmolality, Na^+, K^+, Cl^-, aspartate aminotransferase, and alanine aminotransferase. Park et al. [25] showed control and experimental group displayed increases in plasma cortisol concentrations from their respective prestress levels at 30 minutes after anaesthetic. Concentrations of glucose were increased from 22.3 ng/ mL to 28.5 ng/mL (30 miuntes), 31.0 ng/mL (1 hour) in experimental group, respectively. Lactic acid concentrations for experimental group were lower than those for control group at 30 minutes, 1, and 3 hours. Park et al. [25] reported that in other items, control and experimental group did not show differences in this before and after anaesthetic.

The anaesthetic effect of tricaine methanesulfonate (MS-222) concentrations and water temperatures for longtooth grouper (*Epinephelus moara*) and hybrid grouper (*E. moara* ♀ × *E. lanceolatus* ♂) were investigated by Park et al. [51]. Anesthetic induction and recovery time were measured at 18, 22, 26, and 30°C of water temperature and 100, 150, 200, and 250 ppm of anesthetic concentrations. Anesthetic induction time tended to decrease with increasing concentration and water temperature. Recovery time was proportional to concentration, but inversely proportional to water temperature. However, Park et al. [51]. found that there was no significant differences in recovery time at 22°C or lower. The optimal anesthesia condition was 30°C and 100 ppm, which was the shortest recovery time for longtooth grouper, and 150 ppm at 30°C in the case of hybrid grouper because anesthetic time is significantly different with 100 ppm in spite of no significant differences with 100 ppm for recovery time. As a results of two-way ANOVA test, there was a significant difference between the species of longtooth and hybrid grouper. On the other hand, Park et al. [51] found that there was no interaction effect between concentration and species. Also, there was no interaction effect among species, concentration, and water temperature.

·**Marine animal: Shellfish** Choi et al. [52] investigated the optimal concentration of ethyl-p-aminobenzoate for the exfoliation and recovery of young abalone (*Haliotis discus hannai*) in according to different water temperatures, for the purpose of preventing the damage of shell and muscle to exfoliated from shelter. In the 14℃ water temperature, young abalones were exfoliated after 16, 35, 35, and 35 minutes in 150, 100, 75, and 50 ppm concentration of ethyl-p-aminobenzoate, and were recovered after 100, 60, 30, and 30 minutes, respectively. Exfoliation rate of abalone were 100% except for 50 ppm (80%) and recovery rate were 100% of all concentration. In the 18℃ water temperature, young abalones were exfoliated after 4, 4, 6, 8, 8, and 12 minutes in 300, 200, 150, 100, 75, and 50 ppm concentration of ethyl-p-aminobenzoate, and were recovered after 210, 180, 90, 60, 30, 20, and 20 minutes, respectively. Exfoliation rate of abalone were 100%, and recovery rate were 100% except for 200 and 300 ppm (90%). In the 24℃ water temperature, young abalones were exfoliated after 8, 10, 10, and 12 minutes in 150, 100, 75, and 50 ppm concentration of ethyl-p-aminobenzoate, and were recovered after 70, 50, 30, and 20 minutes, respectively. Exfoliation and recovery rate of abalone were 100%. In the 18℃ water temperature, exfoliation rate that treated with freshwater during 20 minutes were 80, 50, 30, and 5% in 100, 75, 50, and 25% of fresh water, and recovered after 60, 15, 10, and 2 minutes, respectively and recovery rate were 100% except for 100% freshwater [52]. In this study, Choi et al. [52] suggested the results that the exfoliation and recovery by ethyl-p-aminobenzoate were more effected in 18 and 24℃ of sea water temperature than those of 14℃. The optimal concentration of ethyl-p-aminobenzoate was 50 ppm at those water temperatures. Choi et al. [52] raised 20 individual of young abalones at water temperature of 16℃ in the l L of tank and checked the variation of dissolved oxygen (DO) by respiration of abalones that treated with 75 ppm of ethyl-p-aminobenzoate. Before anesthetizion, DO were 6.17~6.20 mg/L and slowly decreased. But after 60 minutes, DO decreasing were stopped in 5.42~5.46 mg/L. On the other hand, the control was continuously decreased and 5.27 mg/L after 60 minutes. The heartbeats of abalones were 33~45minutes in the water temperature of 18℃, but that treated with 100 ppm concentration of ethyl-p-aminobenzoate during 60 minutes, was 0 minute. And heartbeats of recovered abalones from anesthetizion were 29~43 minutes.

Choi et al. [29] reported the optimal concentration of lidocaine and MS-222 (tricaine methanesulfonate) for the exfoliation and recovery of abalone (*Haliotis discus hannai*) in different shell lengths, for the purpose of preventing the damage of shell and muscle. The response varied for different shell size groups (shell length 1, 2, and 3 cm). Choi et al. [29] suggested the result that the exfoliation and recovery time by lidocaine and MS-222 in shell length 1 cm group were more shorter than in 3 cm group. In shell length 1 cm group, the optimal concentrations of lidocaine and MS-222 for anaesthetic were 200 ppm and 100 ppm, respectively.

Park [17] investigated 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. At each group, as the concentration of anesthetics increased, the induction time decreased. For each anesthetic, the longer the shell length of six species in this experiment were, the more induction time increased. Park [17] reported 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 hours after recovery of anesthesia (RA) and cortisol concentrations of three anesthetics on each shellfish were highest at 6 hours after RA. At 6 hours 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 hours after RA was higher than that of the other shellfishes. At 6 hours after RA, cortisol concentrations of three anesthetics were decreased until 48 hours. Park [17] reported glucose concentrations of clove oil, lidocaine-HCl, and MS-222 on six seawater shellfish were increased until 12 hours after RA and glucose concentrations of three anesthetics on each shellfish were highest at 12 hours after RA. Park [17] reported that at 6 hours 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 that of the other shellfishes as well. From 12 to 48 hours after RA, glucose concentrations of three anesthetics were decreased.

Freshwater animal: Finfish Kim and Chun [53] compared anaesthetic effects (benzocaine) under various conditions temperature, concentration, pH, and body weight in Nile tilapia (*Oreochromis niloticus*). And Kim and Chun [53] compared actual anaesthetic effects at 50 ppm benzocaine at ambient temperature and pH for the grading of Nile tilapia. The results of Kim and Chun [53] study are this; 1. The effect of anaesthesia at 24℃ was better with low pH, that is 5.6 than high pH 6.6 and 7.6. 2. The anaesthetic effect was not different at different body weight form 11 to 1.350 g. 3. The fish were anaesthetized in 4~10 minutes at 50 ppm benzocaine at temperature 20~24℃ and pH 6.8-7.3 and recovered in 4-6 minutes when they were put back in the fresh water after 30 minutes anaesthesia. 4. Benzocaine was more sensitive at pH fluctuation than temperature. 5. Twenty four hour-TLm of benzocaine was 50 ppm at 24℃, pH 6.8 when the fish were put back in the fresh water alter 120 minutes.

An anesthetic protocol was optimized for microinjection-related handling of Siberian sturgeon (*Acipenser baerii*; Acipenseriformes) prolarvae, an extant primitive fish species commonly grown in aquaculture [36]. Comparative examinations of three selected anesthetics (clove oil, lidocaine, and MS-222) with a dosage regime of 50, 100, 200, and 400 mg/L indicated that MS-222 was the most efficient agent for Siberian sturgeon prolarvae, as evidenced by the fast induction of anesthesia with quick and uniform recovery [36]. Meanwhile, clove oil should be avoided, due to prolonged recovery times varying widely between individuals. None of the tested anesthetics affected prolarval viability at any of the dosage regimes tested in study of Kim and Nam [36]. Based on an analysis of the duration of an unconscious state in air, Kim and Nam [36] recommended a dose of 200 mg/L MS-222 for microinjection. Recovery time after use of this dose was influenced by the prolarval age and the development of gills, in which prolarvae older than 3 days after hatching required longer recovery times than did younger prolarvae. Post-recovery behavioral assessment showed no apparent difference between MS-222-anesthetized and non-anesthetized prolarvae in their swimming behavior and phototactic responses. Applicability of currently developed anesthetic protocol using MS-222 in larval microinjection was demonstrated with the injection of a visible dye to the anesthetized prolarvae, followed by the analysis of post-recovery viability. Taken together, the anesthetic protocol of [36] based on 200 mg/L of MS-222 could provide researchers with practical usefulness with good safety margins for the micromanipulation and other related handlings of Siberian sturgeon prolarvae.

Lee et al. [34] investigated the anesthetic effects of MS-222 (tricaine methanesulfonate), clove oil, 2-phenoxyethanol, $NaHCO_3$, lidocaine-HCl, and lidocaine-HCl/$NaHCO_3$ in the glass catfish (*Kryptopterus vitreolus*). Based on the efficacy criteria of complete anesthetic induction from 60 to 120 seconds, recovery within 300 seconds, the lowest effective concentrations at 24℃ were determined to be 60 ppm (induction 82.8 ± 17.6

seconds, recovery 80.2 ± 34.7 seconds) for MS-222, 40 ppm (induction 70.5 ± 8.2 seconds, recovery 83.4 ± 17.7 seconds) for clove oil, 250 ppm (induction 64.3 ± 24.0 seconds, recovery 62.8 ± 15.6 seconds) for 2-phenoxyethanol, 300 ppm (induction 127.3 ± 13.3 seconds, recovery 107.5 ± 4.8 seconds) for lidocaine-HCl, and 200/100 ppm (induction 81.2 ± 17.2 seconds, recovery 98.3 ± 19.7 seconds) for lidocaine-HCl/$NaHCO_3$. Lee et al. [34] showed that 200/100 ppm of lidocaine-HCl/$NaHCO_3$ was to be an effective anesthetic agent in this species.

Park [19] evaluated the anesthetic effects of clove oil and tricaine methanesulfonate (MS-222) on the Far Eastern catfish (*Silurus asotus*) by measuring the times to anesthesia and recovery. Each anesthetic effect of clove oil and MS-222 was tested in two groups of fish with different body sizes: a group of small fish (mean body length: 15.5±1.58 cm, mean body weight: 50.1±5.91 g, n=20) and a group of large fish (mean body length: 31.5±4.19 cm, mean body weight: 302.1±15.22 g, n=20). The anesthetics were used at concentrations of 200, 300, 400, 500, and 600 ppm. The results showed relationships between the concentration of the anesthetic and the body size of the fish. The time to anesthesia decreased linearly with increasing concentration in the large fish for both clove oil and MS-222. Based on an optimal anesthetic time of approximately 1 minute, the preferred concentrations of the anesthetics were 500 ppm for clove oil and 600 ppm for MS-222. Both the anesthetic time and the recovery time were shorter for the small fish than for the large fish. Park [19] showed that the smaller-sized Far Eastern catfish was more easily anesthetized and recovered more rapidly from anesthesia than the larger-sized fish.

· **Freshwater animal: Finfish** Seven species of fish in addition to Mozambique tilapia (*Tilapia mossambica*) were exposed to 250 ppm concentration of the anaesthetic quinaldine to determine the safe level for handling and transportation of these species [54]. The results obtained from the study of Yoon et al. [54] are as this; 1. The time taken to lose balance increased with a decrease on the concentration of the anaesthetic. 2. Anaesthetization must be carried out under temperature lower or higher rather than optimum temperature. 3. The longer the length of the fish, the longer the anaesthetization time and recovery time of fish. 4. Coefficient of recovery period and body length is 0.78. 5. At 10-15 minutes, after anaesthetization, the serum levels of glucose, ALP and SGOT were at peat. 6. LDH of the anaesthetized fish is much more increased than that of the unanaesthetized. 7 In the more 250 ppm treatment, the pyknosis of the brain and spleen tissue appeared.

2-Phenoxyethanol (Appendix) 2-Phenoxyethanol (2-PE) [1-hydroxy-2-phenotyethane, phenyl cellosolve, phenoxethol, phenoxetol, ethylene glycol monophenyl ether, beta-hydroxyethyl phenyl ether] is a colourless, oily, aromatic liquid with a burning taste, and has a solubility in water of 27 g/L at 20℃ [20]. It is often used as a topical anesthetic [20] widely used for sedation (particularly in fish transportation) and anesthesia [54-57] Advantages are low cost and the fact that no pH change occurs with the addition of 2-PE to seawater [58]. However, 2-PE produces hypoventilation and provides poor analgesia [59].

· **Marine animal : Finfish** Han et al. [48] evaluated the efficiency of clove oil, MS-222, and 2-phenoxyethanol as anesthetics in juvenile (*Scomber japonicus*). Stage A5 of anesthesia was assumed to be sufficient for conducting routine aquaculture procedures in less than 3 minutes, with recovery (stage R5) in less than 5 minutes. The lowest effective doses of the three anesthetics were 50 mg /L clove oil (anesthetic time of 71.3 seconds and recovery time of 167.0 seconds), 100 mg /L MS-222 (anesthetic time of 70.7 seconds and recovery time of 115.7 seconds), and 400 mg /L 2-phenoxyethanol (anesthetic time of 86.7 seconds and recovery time of 95.0 seconds). Anesthetic times decreased with increasing doses for all three anesthetic agents, and fish anesthetized with clove oil exhibited the longest recovery times. After 30 minutes, the highest plasma cortisol and lactate levels were detected with the use of clove oil, whereas the lowest values were observed with 2-phenoxyethanol. In addition, high glucose levels were maintained during recovery with clove oil, but the treatments did not differ.

· **Freshwater animal: Finfish** The anesthetic effects of MS-222 (tricaine methanesulfonate), clove oil, 2-phenoxyethanol, $NaHCO_3$, lidocaine-HCl, and lidocaine- HCl/$NaHCO_3$ in the glass catfish (*Kryptopterus vitreolus*) were investigated by Lee et al. [34] in 2017. Based on the efficacy criteria of complete anesthetic induction from 60 to 120 seconds, recovery within 300 seconds, the lowest effective concentrations at 24°C were determined to be 60 ppm (induction 82.8±17.6 seconds, recovery 80.2±34.7 seconds) for MS-222, 40 ppm (induction 70.5±8.2 seconds, recovery 83.4±17.7 seconds) for clove oil, 250 ppm (induction 64.3±24.0 seconds, recovery 62.8±15.6 seconds) for 2-phenoxyethanol, 300 ppm (induction 127.3±13.3 seconds, recovery 107.5±4.8 seconds) for lidocaine-HCl, and 200/100 ppm (induction 81.2±17.2 seconds, recovery 98.3±19.7 seconds) for lidocaine-HCl/$NaHCO_3$.

Sodium bicarbonate ($NaHCO_3$) When sodium bicarbonate ($NaHCO_3$) is the source of CO_2, carbonic acid, carbonic acid gas, and carbonic anhydride, the resulting anesthesia is sometimes called sodium bicarbonate anesthesia

which is available from grocery stores as baking soda [22]. Booke [60] determined that a 642 mg/L solution of $NaHCO_3$ at a pH of 6.5 was the most effective medium for causing rainbow trout (*Salmo gairdneri*), brook trout (*Salvelinus fontinalis*), and common carp (*Cyprinus carpio*) to cease swimming and to slow respiration within 5 minutes. They hypothesized that the mechanism was a pH-controlled release of carbon dioxide. $NaHCO_3$ at a dose of 900 mg/L for adult salmon which results in anesthesia in under 5 minutes, with a recovery time of 12.1 minutes [60]. There are obvious hazards in the use of concentrated sulfuric acid to release CO_2 from $NaHCO_3$ [60].

Freshwater animal: Finfish Lee et al. [34] investigated the anesthetic effects of MS-222 (tricaine methanesulfonate), clove oil, 2-phenoxyethanol, $NaHCO_3$, lidocaine-HCl, and lidocaine-HCl/$NaHCO_3$ in the glass catfish (*Kryptopterus vitreolus*). Based on the efficacy criteria of complete anesthetic induction from 60 to 120 seconds, recovery within 300 seconds, the lowest effective concentrations at 24℃ were determined to be 60 ppm (induction 82.8 ± 17.6 seconds, recovery 80.2 ± 34.7 seconds) for MS-222, 40 ppm (induction 70.5±8.2 seconds, recovery 83.4 ± 17.7 seconds) for clove oil, 250 ppm (induction 64.3 ± 24.0 seconds, recovery 62.8 ± 15.6 seconds) for 2-phenoxyethanol, 300 ppm (induction 127.3 ± 13.3 seconds, recovery 107.5 ± 4.8 seconds) for lidocaine-HCl, and 200/100 ppm (induction 81.2 ± 17.2 seconds, recovery 98.3 ± 19.7 seconds) for lidocaine-HCl/$NaHCO_3$.

Hypothermia (Appendix) Hypothermia is accomplished by lowering the ambient temperature of the fish with ice or cold water. Fish acclimated to higher temperatures may experience stress as a result of cold shock. Hypothermic anesthesia is more effective for fish acclimated to waters above 10℃, as sedative effects are not induced if acclimation temperatures are lower than this. In the latter case, an additional chemical anesthetic may be necessary to induce deep anesthesia [63]. Generally, hypothermic anesthesia has been induced in a variety of fishes by inducing a temperature change of about 10 to 25℃, or to near 0℃, by immersing them in crushed ice or ice water(see [22]). Hypothermia results in a slow, light anesthesia, which is characterized by an absence of motion, reduced power of exertion and diminished nerve sensitivity [1]. This is useful for transport, but it is not deep enough for any type of lengthy surgery. While this is not a common method of anesthesia today, it presents an alternative method when chemical anesthetics are not available or desirable.

·**Freshwater animal: Finfish** The study of Park et al. [64] demonstrated that heat- and cold-induced anesthesia can be used effectively to anesthetize the marine medaka (*Oryzias dancena*). The cold-anesthesia groups were treated at 4-10℃ and showed anesthesia times of 5.7-23.2 seconds and recovery times of 56.4-85.4 seconds, with all individuals surviving. The heat-anesthesia groups were treated at 36-42℃ and showed anesthesia times of 6.9-35.2 seconds and recovery times of 38.4-73.1 seconds with all individuals surviving. Park et al. [64] reported that when fish were anesthetized by heat and cold, the operculum movement number (OMN) greatly increased because the fish were attempting to overcome unfavorable conditions. The whole-body cortisol level of anesthetized marine medaka returned most closely to that of the control at 6 hours after recovery, whereas the whole-body glucose level returned most closely to the control level at 2 hours after recovery. Park et al. [64] reported that the results from their study extends previous researches and its results should contribute to the safe laboratory handling of marine medaka.

III. CONCLUSION

Commercially reared fish are subjected to confinement and handing including netting, weighing, sorting, vaccination, and transport. In research, fish may also undergo surgical procedures ranging from tagging, blood sampling, and small incisions for invasive procedures. In these situations, treatment with anesthetic agents may be necessary to ensure the welfare of the fish. In this paper, I provides information on a review of studies on the seven widely-used anesthetics which are effective and have been widely used for aquatic animals in both seawater and freshwater from 1988 to 2021 in Republic of Korea: lidocaine-HCl and lidocaine-HCl/$NaHCO_3$, clove oil and derivatives, MS-222 and benzocaine, quinaldine, 2-phenoxyethanol, sodium bicarbonate ($NaHCO_3$), and hypothermia.

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Appendix. Application of anesthesia to aquatic animals for aquaculture in Republic of Korea

Test aquatic animal	Anesthetic effect*	References
	Lidocaine-HCl and Lidocaine-HCl/$NaHCO_3$	
Marine animal		

Finfish		
Agrammus agrammus	220 ppm, 22°C, ET: 80	Park et al. (1988)[23]
Halichoeres poecilepterus	220 ppm, 22°C, ET: 59	Park et al. (1988)[23]
Hexagrammos otakii	220 ppm, 22°C, ET: 57	Park et al. (1988)[23]
	1,220 ppm, 12°C, ET: 172, RT: 222; 800 ppm, 18°C, ET: 62, RT: 237; 300 ppm, 24°C 66, RT: 208	Park et al. (2003)[24]
	1,200 ppm, 12°C, ,ET: 172, RT: 222; 800 ppm, 18°C, ET: 62, RT: 237; 300 ppm, 24°C 66, RT: 208	Park et al. (2015)[25]
Lateolebrax japonicus	500 ppm, 22°C, ET: 93	Park et al. (1988)[23]
Oplegnathus fasciatus	200 ppm, 22°C, ET: 48	Park et al. (1988)[23]
Pagrus major	50 ppm, 22°C, ET: 152, RT: 64	Park et al. (1988)[23]
Paralichthys olivaceus	200 ppm, 22°C, ET: 57	Park et al. (1988)[23]
Sebastes inermis	220 ppm, 22°C, ET: 46	Park et al. (1988)[23]
Siganus fuscescens	220 ppm, 22°C, ET: 74	Park et al. (1988)[23]
Stephanolepis cirrhifer	220 ppm, 22°C, ET: 65	Park et al. (1988)[23]
Takifugu niphobles	300 ppm, 22°C, ET: 54	Park et al. (1988)[23]
	1,000 ppm, 22°C, ET: 68, RT: 124	Gil et al. (2017)[26]
Pleuronectes americanus	1,300 ppm, 3°C, ET: 216, RT: 42; 1,300 ppm, 7°C, ET: 205, RT: 45; 1,300 ppm, 11°C 180, RT: 48; 1,300 ppm, 15°C, ET: 85, RT: 50; 500 ppm, 19°C, ET: 91, RT: 81	Park et al. (2004b)[15]
	5 ppm, 10 ppm, 20 ppm, 9°C; Simulated transportation lidocaine-HCI dose-related de in oxygen consumption, excretion of ammonia and PH by fish	Park et al. (2009a)[27]
Sebastes schlegeli	600 ppm, small size (14 cm TL, 15 g BW), 20°C, ET: 510, RT: 1,110; 600 ppm, midd (18 cm TL, 100 g BW), 20°C, ET: 630, RT: 780; Plasma cortisol, glucose, Na^+, H osmolality levels cluring 24 hrs after recovery were analyzed	Kim et al. (2005)[28]
Takifugu obscurus	600 ppm, lidocaine-HCl+45 ppm clove oil, 28°C, ET: 59, RT: 319	Park (2019b)[18]
Takifugu rubripes	600 ppm, lidocaine-HCl+45 ppm clove oil, 28°C, ET: 62, RT: 329	Park (2019b)[18]
Shellfish		
Haliotis discus hannai	200 ppm, 16°C, 1 cm shell length, ET: 1,200, RT: 2,400	Choi et al. (1998)[29]
Mytilus edulis	500 ppm, 25°C, ET: 55, RT: 158; Plasma cortisol and glucose levels during 48 hr recovery were analyzed	Park (2019a)[17]
Patinopecten yessoensis	700 ppm, 25°C, ET: 58, RT: 158; Plasma cortisol and glucose levels during 48 hr recovery were analyzed	Park (2019a)[17]
Pseudocardium sachalinensis	500 ppm, 25°C, ET: 65, RT: 163; Plasma cortisol and glucose levels during 48 hr recovery were analyzed	Park (2019a)[17]
Ruditapes philippimarum	400 ppm, 25°C, ET: 63, RT: 163; Plasma cortisol and glucose levels during 48 hr recovery were analyzed	Park (2019a)[17]
Scapharca broughtonii	500 ppm, 25°C, ET: 67, RT: 165; Plasma cortisol and glucose levels during 48 hr recovery were analyzed	Park (2019a)[17]
Tegillarca granosa	500 ppm, 25°C, ET: 59, RT: 153; Plasma cortisol and glucose levels during 48 hrs after recovery were analyzed	Park (2019a)[17]

Appendix. Continued

Test aquatic animal	Anesthetic effect*	References
	Lidocaine-HCl and Lidocaine-HCl/$NaHCO_3$	
Freshwater animal		

Finfish		
Carassius auratus	400 ppm, 24°C, ET: 56	Kim et al. (1988)[30]
Ictalurus punctatus	200 ppm, 24°C, ET: 64	Kim et al. (1988)[30]
Misgurmus anguillicaudatus	400 ppm, 24°C, ET: 59	Kim et al. (1988)[30]
Misgurmus mizolepis	300 ppm, 33°C, ET: 72	Kim et al. (1988)[30]
Oreochromis niloticus	400 ppm, 24°C, ET: 55	Kim et al. (1988)[30]
Salmo gairdneri	150 ppm, 12°C, ET: 59	Kim et al. (1988)[30]
Silurus asotus	75 ppm, 27°C, ET: 62	Kim et al. (1988)[30]
Cyprinus carpio	400 ppm, 22°C, PH 7.4, ET: 212, RT: 55; Increase in RBC count, hematocrit, glucose, activity in treated fish	Chung et al. (1994)[31]
Rhyn chocypris oxycephalus	300 ppm, 20°C, ET: 64, RT: 239; 400 ppm, 15°C, ET: 63, RT: 263; 600 ppm, 10°C, ET 229	Park et al. (1998b)[5]
Rhynchocypris steindachneri	400 ppm, 20°C, ET: 57, RT: 349; 500 ppm, 15°C, ET: 59, RT: 359; 600 ppm, 10°C, RT: 369; Stage sensitivity on ET and RT in trated fish	Park et al. (1998b)[5]
	20 ppm, 6hrs, 18°C; Simulated transportation decreased in oxygen consumption, excretion, ventilation, and PH in treated fish	Park et al. (1998a)[11]
Acheilognathus koreensis	250 ppm, 20°C, ET: 64, RT: 83; The lower temperature resulted in longer ET and RT	Kang et al. (2005)[32]
Plecoglossus altivelis	20, 40, 80, 160 ppm, 18°C, 2 hrs; Simulated transportation, results reveal lidocain effective as sedative for transportation mixture in this species	Hur et al. (2005)[33]
Rhodeus uyekii	450 ppm, 20°C, ET: 62, RT:99; The lower temperature resulted in longer ET and RT	Kang et al. (2005)[32]
Oryzias dancena	710 ppm, 23°C, ET:71, RT: 206; 700 ppm, 26°C, ET: 61, RT: 168, 700 ppm, 29°C, ET 124; Both ET and RT were shorter for smaller fish than for longer fish	Park et al. (2011)[35]
	700 ppm, 26°C, 10 ppt salinity, ET: 62, RT: 147; 800 ppm, 26°C, 0 ppt salinity, ET: 57, Simulated transportation, results (increase: DO. decrease: respiratory frequency, amm CO_2) reveal lidocaine-HCI is effective anesthetic, improving the transportation of the f	Park et al. (2017)[6]
Kryptopterus vitreolus	300 ppm, lidocaine-HCI, 25°C. PH 7.9, ET: 127, RT: 108; 200 ppm lidocaine-HCI/ $NaHCO_3$, 25°C, PH 7.9, ET: 81, RT: 98	Lee et al. (2017)[34]
Acipenser baerii	100 ppm, 20°C, ET: 67, RT: 680	Kim and Nam (2018)[36]
	500 ppm, 20°C, larvae, ET: 63, RT: 180; 250 ppm, 15°C, juvenile, ET: 57, RT: 170; 20°C, juvenile, ET: 60, RT: 129;. 100 ppm, 25°C, juvenile, ET: 57, RT: 122; 300 pp adult, ET: 110, RT: 165; Plasma cortisol, plasma glucose, and lactic acid concentrati indicative of stress response in this experiment. The optimal anesthesia interval of lidoca was 4 days, and frequent anesthesia resulted in negative effects by inhibiting sensitivity	Goo et al. (2019)[37]
Reptile		
Pelodiscus sinensis	1300 ppm, 25°C, large size, ET: 182, RT: 396, 1300 ppm, 25°C, small size, ET: 163, 1300 ppm, 30°C, large size, ET: 139, RT: 471, 1300 ppm, 30°C, small size, ET: 118, F	Park et al. (2006)[38]
Clove oil and Derivatives		
Marine animal		
Finfish		
Sebastes schlegeli	10 ppm, 10°C, adult, ET: 1200, RT: 540; 7.5 ppm, 15°C, adult, ET: 660, RT: 450; 15 pp adult, ET: 780, RT: 420; 2.5 ppm, 10°C, fry, ET: 450, RT: 1,100; 10 ppm, 15°C, fry, RT: 840; 10 ppm, 20°C, fry, ET: 150, RT: 180	Shin et al. (2006)[45]

Appendix. Continued

Test aquatic animal	Anesthetic effect*	References
Clove oil and Derivatives		
Marine animal		

Finfish		
Epinephelus bruneus	250 ppm, 18°C, ET: 56, RT: 63; 100 ppm, 22°C, ET: 59, RT: 26; 50 ppm, 26°C, ET: Post-recovery physiological measurements (plasma cortisol and plasma glucose)	Park et al. (2008)[46]
Oplegnathus fasciatus	150 ppm, 20°C, ET: 156, RT: 614; 150 ppm, 24°C, ET: 55, RT: 436; 100 ppm, 28°C, 296; Post-recovery, physiological measurements (plasma cortisol and plasma glucose)	Park et al. (2009c)[41]
Sebastes inermis	250 ppm, 20°C, large size, ET: 70, RT: 470; 150 ppm, 20°C, small size, ET: 51, RT recovery, physiological measurements (plasma cortisol and plasma glucose)	Park et al. (2009b)[47]
Scomber japonicus	100 ppm, 26°C, ET: 53, RT: 283; Post-recovery, physiological measurements (plas plasma glucose and plasma latic acid)	Han et al. (2011)[48]
Paralichthys olivaceus	150 ppm, 15°C, ET: 60, RT: 960; 90 ppm, 20°C, ET: 63, RT: 435; 90 ppm, 25°C, ET: 5 Physiological measurements (plasma cortisol); Clove oil reduced the metabolic activ flounder, thus reducing NH_4^+ excretion and O_2 consumption	Gil et al. (2016)[9]
Takifugu niphobles	50 ppm, 20°C, ET: 72, RT: 242	Gil et al. (2017)[26]
Epinephelus akarra	75 ppm, 20°C, ET: 75, RT: 107; 75 ppm, 24°C, ET: 67, RT: 88; 75 ppm, 28°C, ET: Post-recovery physiological measurement (plasma cortisol)	Park et al. (2018)[49]
Takifugu obscurus	60 ppm, 20 ppt salinity, 28°C, ET: 66, RT: 194; 45 ppm clove oil + 600 ppm lidocaine ET: 59, RT: 319; 5 ppm, 28°C, simulated transportation, clove oil elapced time-relate DO, CO_2, and respiratory frequency by fish	Park (2019b)[18]
Takifugu rubripes	60 ppm, 20 ppt salinity, 28°C, ET: 71, RT: 198; 45 ppm clove oil + 600 ppm lidocaine ET: 62, RT: 329; 5 ppm, 28°C, simulated transportation, clove oil elapced time-relate DO, CO_2, and respiratory frequency by fish	Park (2019b)[18]
Shellfish		
Mytilus edulis	150 ppm, 25°C, ET: 63, RT: 162; Plasma cortisol and glucose levels during 48 hrs af were analyzed	Park (2019a)[17]
Patinopecten yessoensis	250 ppm, 25°C, ET: 64, RT: 161; Plasma cortisol and glucose levels during 48 hrs af were analyzed	Park (2019a)[17]
Psedocardium sachalinensis	200 ppm, 25°C, ET: 66, RT: 161; Plasma cortisol and glucose levels during 48 hrs af were analyzed	Park (2019a)[17]
Ruditapes philippinarum	150 ppm, 25°C, ET: 69, RT: 160; Plasma cortisol and glucose levels during 48 hrs af were analyzed	Park (2019a)[17]
Scapharca broughtonii	250 ppm, 25°C, ET: 58, RT: 158; Plasma cortisol and glucose levels during 48 hrs af were analyzed	Park (2019a)[17]
Tegillarca granosa	150 ppm, 25°C, ET: 67, RT: 161; Plasma cortisol and glucose levels during 48 hrs af were analyzed	Park (2019a)[17]
Mollusca		
Octopus minor	300 ppm, 15°C, ET: 225, RT: 1,777; 300 ppm, 20°C. ET: 223, RT: 1,421; 300 ppm, 25° RT: 964	Seol et al. (2007)[40]
Freshwater animal		
Finfish		
Acheileganthus koreensis	160 ppm, 20°C, ET: 63, RT: 190; The lower temperature resulted in longer ET and RT	Kang et al. (2005)[32]
Rhodeus uyekii	120 ppm, 20°C, ET: 42, RT: 88; The lower temperature resulted in longer ET and RT	Kang et al. (2005)[32]
Oryzias dancena	125 ppm, 23℃, ET: 62, RT: 173; 100 ppm, 26℃, ET: 56, RT: 129; 75 ppm, 29℃, ET: 5 Both ET and RT were shorter for smaller fish than for longer fish	Park et al. (2011)[35]
	100 ppm, 26℃, 10 ppt salinity, ET: 62, RT: 117; 125 ppm, 26℃, 10 ppt salinity, ET: 5 150 ppm, 26℃, 30 ppt salinity, ET: 63, RT: 151; Simulated transportation, results reve is effective anesthetic, improving the transportation of the fish (increase: DO; decrease frequency, ammonium and CO_2)	Park et al. (2017)[6]

Appendix. Continued

Test aquatic animal	Anesthetic effect*	References
	Clove oil and Derivatives	
Freshwater animal		

Finfish		
Kryptopterus vitreolus	40 ppm, 25℃, PH 7.9, ET: 71, RT: 83	Lee et al. (2017)[34]
Acipenser baerii	200 ppm, 20℃, ET: 65, RT: 1,030	Kim and Nam (2018)[36]
Silurus asotus	500 ppm, large size, ET: 62, RT: 173; 300 ppm, small size, ET: 57, RT: 131	Park (2019c)[19]
	MS-222 and Benzocaine	
Marine animal		
Finfish		
Pagruns major	250 ppm, 22℃, ET: 45, RT: 50	Park et al. (1988)[23]
Sebastess schlegeli	400 ppm, 20℃, embank cultured, ET: 38, RT: 505; 200 ppm, 20℃, wild, ET: 39, RT: 119; 200 ppm, 20℃, land-based tank, ET: 32, RT: 118; 100 ppm, 20℃, 4 cm long, ET: 56, RT: 49; 200 ppm, 20℃, 6 cm long, ET: 40, RT: 181; 200 ppm, 20℃, 10 cm long, ET: 36, RT: 137; 50 ppm, small size (14 cm, TL, 15 g BW), 20℃, ET: 19, RT: 11; 50 ppm, middle size (18 cm TL, 100 g BW), 20℃, ET: 20, RT: 5)	Son et al. (2001)[50]
	200 ppm, small size (14 cm TL, 15 g BW), 20℃, ET: 60, RT: 540; 200 ppm, middle size (18 cm TL, 100 g BW), 20℃, ET: 120, RT: 498; Plasma cortisol, glucose Na^+, K^+, and osmolality levels during 24 hrs after recovery were analyzed	Kim et al. (2005)[28]
Hexagrammos otakii	125 ppm, 18℃, ET: 58, RT: 103	Park et al. (2003)[24]
	125 ppm, 18℃, ET: 58, RT: 103	Park et al. (2015)[25]
Scomber japonicus	200 ppm, 26℃, ET: 50, RT: 182; Post-recovery physiological measurements (plasma cortisol, plasma glucose, and plasma latic acid)	Han et al. (2011)[48]
Epinephelus moara	150 ppm, 18℃, ET: 43, RT: 117; 150 ppm, 22℃, ET: 63, RT: 131; 100 ppm, 26℃, ET: 54, RT: 66; 100 ppm, 30℃, ET: 24, RT: 62	Park et al. (2019)[51]
Epinephelus moara (♀) × *E. lanceolatus* (♂)	200 ppm, 18℃, ET: 57, RT: 203; 200 ppm, 22℃, ET: 54, RT: 157; 150 ppm, 26℃, ET: 57, RT: 108; 150 ppm, 30℃, ET: 31, RT: 36	Park et al. (2019)[51]
Shellfish		
Haliotis discus hannai	150 ppm, 14℃, ET: 960, RT: 9,900; 200 ppm, 18℃, ET: 240, RT: 5,700; 75 ppm, 24℃, ET: 600, RT: 1,800	Choi et al. (1997)[52]
	100 ppm, 16℃, 1 cm shell legth, ET: 20, RT: 30	Choi et al. (1998)[29]
Mytilus edulis	400 ppm, 25℃, ET: 52, RT: 148; Plasma cortisol and glucose levels during 48hrs after recovery were analyzed	Park (2019a)[17]
Patinopecten yessoensis	450 ppm, 25℃, ET: 60, RT: 166; Plasma cortisol and glucose levels during 48hrs after recovery were analyzed	Park (2019a)[17]
Pseudocardium sachalinensis	450 ppm, 25℃, ET: 55, RT: 154; Plasma cortisol and glucose levels during 48hrs after recovery were analyzed	Park (2019a)[17]
Ruditapes philippnarum	350 ppm, 25℃, ET: 67, RT: 162; Plasma cortisol and glucose levels during 48hrs after recovery were analyzed	Park (2019a)[17]
Scapharca broughtonii	400 ppm, 25℃, ET: 63, RT: 165; Plasma cortisol and glucose levels during 48hrs after recovery were analyzed	Park (2019a)[17]
Tegillarca granosa	350 ppm, 25℃, ET: 64, RT: 165; Plasma cortisol and glucose levels during 48hrs after recovery were analyzed	Park (2019a)[17]

Appendix. Continued

Test aquatic animal	Anesthetic effect*	References
	MS-222 and Benzocaine	
Freshwater animal		

Finfish		
Oreochromis niloticus	200 ppm, 24℃, ET: 49, RT: 90; 100 ppm, 30℃, ET: 82, RT: 73; 75 ppm, 30℃, ET: 147, RT: 68; 50 ppm, 30℃, ET: 424, RT: 60	Kim and Chun (1989)[53]
Kryptopterus vitreolus	60 ppm, 25℃, PH 7.9, ET: 83, RT: 80	Lee et al. (2017)[34]
Acipenser baerii	100 ppm, 20℃, PH 7.2-7.4, ET: 68, RT: 110. 200 ppm of MS-222 could provide researchers with practical usefulness with good safety margins for the micromanipulation and other related handlings of Siberian sturgeon prolarvae	Kim and Nam (2018)[36]
Silurus asotus	600 ppm, large size, ET: 61, RT: 204; 400 ppm, small size, ET: 56, RT: 109	Park (2019c)[19]
Quinaldine		
Freshwater animal		
Finfish		
Carassius auratus	250 ppm, 25℃, PH 7.2, ET: 139, RT: 330	Yoon et al. (1989)[54]
Carassius carassius	250 ppm, 25℃, PH 7.2, ET: 204, RT: 368	Yoon et al. (1989)[54]
Cyprinus carpio	250 ppm, 25℃, PH 7.2, ET: 39, RT: 372	Yoon et al. (1989)[54]
F1 (Israeli cap×colored carp)	250 ppm, 25℃, PH 7.2, ET: 21, RT: 699	Yoon et al. (1989)[54]
Misgurmus anguillicaudatus	250 ppm, 25℃, PH 7.2, ET: 25, RT: 704	Yoon et al. (1989)[54]
Poecilia reticulata	250 ppm, 25℃, PH 7.2, ET: 30, RT: 360	Yoon et al. (1989)[54]
Tilapia mossambica	250 ppm, 25℃, PH 7.2, ET: 105, RT: 720	Yoon et al. (1989)[54]
2-Phenoxyethanol		
Marine animal		
Finfish		
Scomber japonicus	600 ppm, 26℃, ET: 57, RT: 155; Post-recovery physiological measurements (plasma cortisol, plasma glucose, plasma latic acid)	Han et al. (2011)[48]
Freshwater animal		
Finfish		
Kryptopterus vitreolus	250 ppm, 25℃, PH 7.9, ET: 64, RT: 63	Lee et al. (2017)[34]
Hypothermia		
Freshwater animal		
Finfish		
Oryzias dancena	36℃, ET: 35, RT: 37; 10℃, ET: 23, RT: 56; Plasma cortisol and glucose levels during 72 hrs after recovery were analyzed; Operculum movement during 48 hrs after recovery was checked	Park et al. (2014)[61]

*ET: exposure time (sec), RT: recovery time (sec).