# キンギョに対する3種の魚類用全身麻酔薬(2-フェノキシエタノール，MS-222，オイゲノール)の効果の比較


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# Comparison of the Mode of Action of Three Anesthetic Agents, 2-phenoxyethanol, MS-222, and Eugenol on Goldfish

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**Abstract:** The modes of action of three commonly used anesthetic agents, 2-phenoxyethanol (2-PE), MS-222, and eugenol, were compared on goldfish. Progressive stages of anesthesia induction were registered by measuring the time to induce six predetermined behavioral or physiological states. Dose dependency of 2-PE and MS-222 anesthesia was nearly identical in the lighter stages of anesthesia, whereas higher doses of MS-222 tended to induce a loss of ventilation. Compared with the other anesthetics, eugenol took relatively long time to achieve surgical anesthesia, whereas it induced the loss of ventilation soon thereafter. Eugenol anesthesia also required a longer recovery time compared with the other anesthetics. We suggest that, in goldfish, eugenol at low concentration is applicable for reducing short-term handling stress. The anesthetics 2-PE and MS-222 are preferable for use in cases involving surgical manipulations.

**Key words:** *Carassius auratus*; General anesthesia; Anesthetic agents

Appropriate anesthesia of fish is required for reducing handling and surgical stresses in many situations in aquaculture. Various chemicals have been reported to be effective for anesthetizing fish (*cf.* Ross and Ross 2008). The mode of induction and maintenance of general anesthesia has also known to be different among these anesthetic agents. Till date, considerable numbers of detailed reports on the behavioral and physiological effects of some specific major anesthetics are available (Ross and Ross 2008; Neiffer and Stamper 2009). Comparisons of the behavioral effects of different anesthetics on single fish species are available, including the rainbow trout *Oncorhynchus mykiss* (Tort et al. 2002), the cod *Gadus morhua* (Zahl et al. 2009), the gilthead sea bream *Sparus aurata* (Molinero and Gonzalez 1995), the sea bass *Centropristis striata* (King et al. 2005), the juvenile yellowfin tuna *Thunnus albacares* (Cano et al. 2014), and the carp *Cyprinus carpio* (Hikasa et al. 1986). However, little is known about differential responses to multiple anesthetics in one of the most widely used experimental fish, goldfish *Carassius auratus*, with the exception of the goldfish larvae (Massee et al. 1995). It is particularly important to have information of the different modes of anesthesia induction among anesthetic agents for appropriate choice and usage depending on cases.

A descriptive scheme for the progressive stages of anesthesia originally adapted by McFarland (1959) has been generally agreed (Ross and Ross 2008). In this scheme, the induction phase of anesthesia is divided into four stages: I, II, III, and IV. Stage I and II are further subdivided into two planes: I-1 (light sedation) and I-2 (deep sedation) for stage I, and II-1 (light anesthesia) and II-2 (deeper anesthesia) for stage II (McFarland 1959).

In the present study, we compared the behavioral impact of three commonly used anesthetics, 2-phenoxyethanol (2-PE), eugenol, and

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MS-222, on adult goldfish. We focused on the induction phase of, and the course of recovery from, the anesthesia because the mode of progress of anesthetic states is of practical importance. MS-222 and 2-PE have been used in many countries for fish anesthesia. Some fish farmers prefer 2-PE to MS-222 for anesthetizing fish because 2-PE has some favorable properties including the ease of preparation and low cost compared with MS-222 (Ortuño et al. 2002). However, 2-PE is not legally approved for use in aquaculture because of the uncertainty of its safety for humans (Hseu et al. 1998; Neiffer and Stamper 2009). However, MS-222 (tricaine methanesulfonate) is approved for use with food fish in the USA (U.S. Food and Drug Administration 1997), the European Union (Ross and Ross 2008; Velisek et al. 2011), and Canada (Health Canada 2010).

Eugenol is relatively new as a fish anesthetic (Pirhonen and Schreck 2003). This substance is a major constituent of clove oil extracted from the clove tree *Eugenia aromatica* and is used as a food additive (Pirhonen and Schreck 2003). Therefore, eugenol is considered more attractive as an anesthetic in aquaculture than the other agents because of its availability, handler safety, and low cost (Keene et al. 1998; Detar and Mattingly 2004; Neiffer and Stamper 2009). This agent is solely approved for use in aquaculture in Japan. In contrast, eugenol has been noted for its narrow margin of safety (Sladky et al. 2001), and its effectiveness for surgical manipulations has yet to be established.

## Materials and Methods

*Animals and anesthetics*

Commercially obtained goldfish ($n$ = 45; body weight, 16.3–31.2 g; standard length, 8.2–10.3 cm; total length, 11.0–13.7 cm) were reared as a group in 200-$l$ plastic containers for one month before subjected to the experiment at 24–25℃ on a 14 h/10 h light/dark cycle. During the rearing period, goldfish were fed on commercially available pellets (Mini pellet, Kyorin, Himeji, Japan) twice a day. Individual goldfish were randomly assigned to the experiment to test the effect of either 2-PE (Wako, Osaka, Japan), eugenol (Nacalai tesque, Kyoto, Japan), or MS-222 (Nacalai tesque).

Freshly prepared anesthetics were dissolved in water taken from the rearing tank and used for anesthesia; three concentrations were used: 300 ppm, 500 ppm, and 700 ppm (v/v) for 2-PE; 20 ppm, 30 ppm, and 50 ppm (v/v) for eugenol; and 70 ppm, 100 ppm, and 150 ppm (w/v) for MS-222. The concentrations of the anesthetics were determined according to the preliminary experiment. Eugenol was dissolved in ethanol at the concentration of 10% before use. Bicarbonate (thrice the weight of MS-222) was added to the MS-222 solution to adjust the pH. Five goldfish were individually used for testing each concentration of each anesthetic. Since eugenol and MS-222 are commonly used with ethanol for a solubilizer and bicarbonate for pH adjuster, respectively, we didn't set ethanol alone and bicarbonate alone groups. We found no significant differences in the body size between the test groups (Steel–Dwass test, $P > 0.05$). Each fish was used once during the experiment. All animal experiments were conducted in accordance with the Guidelines for Animal Experimentation, Hiroshima University, during the light period.

*Measurements of the effects of anesthesia*

In the experiment, goldfish were transferred using a net to a cylindrical container (diameter, 160 mm) holding a 1-$l$ solution of anesthetic at 25℃ and the 30-min observation period was started immediately. The time taken to induce the following six states of anesthesia were measured: 1) the loss of balance, 2) the loss of righting ability, 3) suppressed ventilation with arrhythmic and weak opercular movement, 4) the loss of Mauthner reflex to tapping stimulation at 1-min intervals, 5) the loss of motor response to noxious stimulus produced by pinching the base of the tail with forceps at 1-min intervals, and 6) the loss of ventilatory movement. Soon after confirming the loss of ventilatory movement, the fish were transferred to another container containing 1-$l$ of fresh water for recovery. The response to noxious

stimulus and/or ventilation was not lost in some goldfish within the 30-min observation period in the anesthetic solutions. In these cases, the fish were kept in the anesthetic solution for 30-min and then allowed to recover when transferred to the fresh water. Recovery from the anesthesia was quantified by measuring the time taken to regain ventilation, righting ability, and normal swimming. The recovery observation period was set to 30 min. Recovered goldfish, as well as those that did not show complete recovery during the observation period, were transferred to another stock tank.

In accordance with the generally agreed descriptive scheme for the progressive stages of anesthesia adapted by McFarland (1959), behavioral responses in the present experiment were considered as signs for judging the anesthetic stage as follows: the loss of balance for stage II-1, the loss of righting ability and suppressed ventilation for stage II-2, the loss of Mauthner reflex and the loss of motor response to noxious stimulus for stage III, and the loss of ventilation for stage IV.

## Results

*Induction of anesthesia*

Figure 1 shows the progression of the induction phase of anesthesia induced by immersion in 2-PE (Fig. 1A), MS-222 (Fig. 1B), and eugenol (Fig. 1C). Dose dependency of 2-PE and MS-222 were almost identical to each other in the lighter stages of anesthesia (Fig. 1A, 1B). To achieve sufficient anesthesia for surgical manipulation, which requires the loss of response to noxious stimulus, within approximately 5 min, 700 ppm 2-PE and 150 ppm MS-222 were needed. However, progression to the deepest stage of anesthesia, i.e., the loss of ventilation, was different between these two anesthetics (Fig. 1A, 1B). MS-222 at a concentration of 150 ppm induced the loss of ventilation soon after the loss of response to noxious stimulus (Fig. 1B). Conversely, no goldfish showed a loss of ventilation during the 30-min observation period even at the highest concentration of 2-PE. For eugenol, transition from the loss of Mauthner reflex to the loss of nociceptive response appeared to take longer time than in the other anesthetics (Fig. 1C). Eugenol at higher concentrations also induced ventilation failure soon after the loss of response to tail pinching (Fig. 1C).

To compare the differences directly among the three anesthetics, we plotted the time/stage

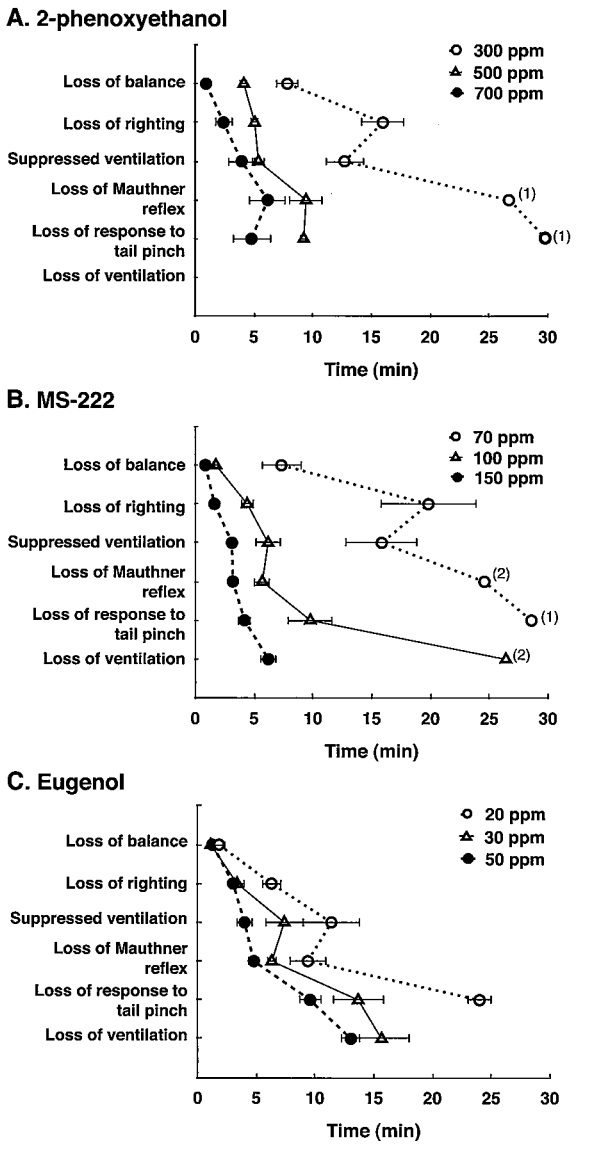


**Fig. 1.** Progressions of the induction phase of anesthesia achieved by immersion in the solutions of 2-phenoxyethanol ($n$ = 5; A), MS-222 ($n$ = 5; B), and eugenol ($n$ = 5; C). The average times to induce the six anesthetic states are shown. Horizontal bars denote SEM. In some cases, not all of the fish tested showed specific states of anesthesia. In those cases, the numbers of fish that show specific states of anesthesia are indicated at the side of the plots and error bars of corresponding data plots are not shown. No plots were shown when no fish showed specific states of anesthesia within 30-min observation period.

relationships of the anesthetic effects of 2-PE at 700 ppm, MS-222 at 100 ppm, and eugenol at 50 ppm. We chose these concentrations since the periods to achieve surgical anesthesia (i.e., stage III), at which goldfish lost the Mauthner reflex and their response to noxious stimulus, were approximately 5–10 min for all of the anesthetic agents (Fig. 2). Anesthesia using 2-PE was the quickest to induce a loss of balance and that was significantly different from that using MS-222 (Steel–Dwass test, $P < 0.05$). The time taken to show the loss of response to the tail pinch in 2-PE also tended to be shorter than that in the other two anesthetics, although the difference was not significant (Steel–Dwass test, $P > 0.05$). The most prominent difference in the mode of anesthesia induction among three agents was at the deepest stage of anesthesia (stage IV; the loss of ventilation; Fig. 2). Eugenol at a concentration of 50 ppm was able to achieve stage II (the loss of balance and righting) and stage III anesthesia almost identical to that achieved by 700 ppm 2-PE and 100 ppm MS-222, but eugenol also induced a loss of ventilation soon after achieving stage III anesthesia, whereas the majority of fish in the other two anesthetics continued ventilatory movement throughout the observation period (Fig. 2).

*Recovery from anesthesia*

We also examined the recovery from anesthesia (Fig. 3). As long as ventilatory movements continued, individuals that experienced stage III anesthesia regained the righting ability and eventually started to swim within the 30-min recovery period (Fig. 3). All individuals that showed the loss of ventilatory movement regained ventilation quickly after transfer to the fresh water (Fig. 3). However, 9 of 10 individuals that stopped ventilatory movement within the 30-min immersion period in eugenol did not recover enough to show righting and swimming within the 30-min observation period after transferring the fish to the fresh water (Fig. 3C). In contrast, all goldfish that stopped ventilatory movement within the 30-min immersion period

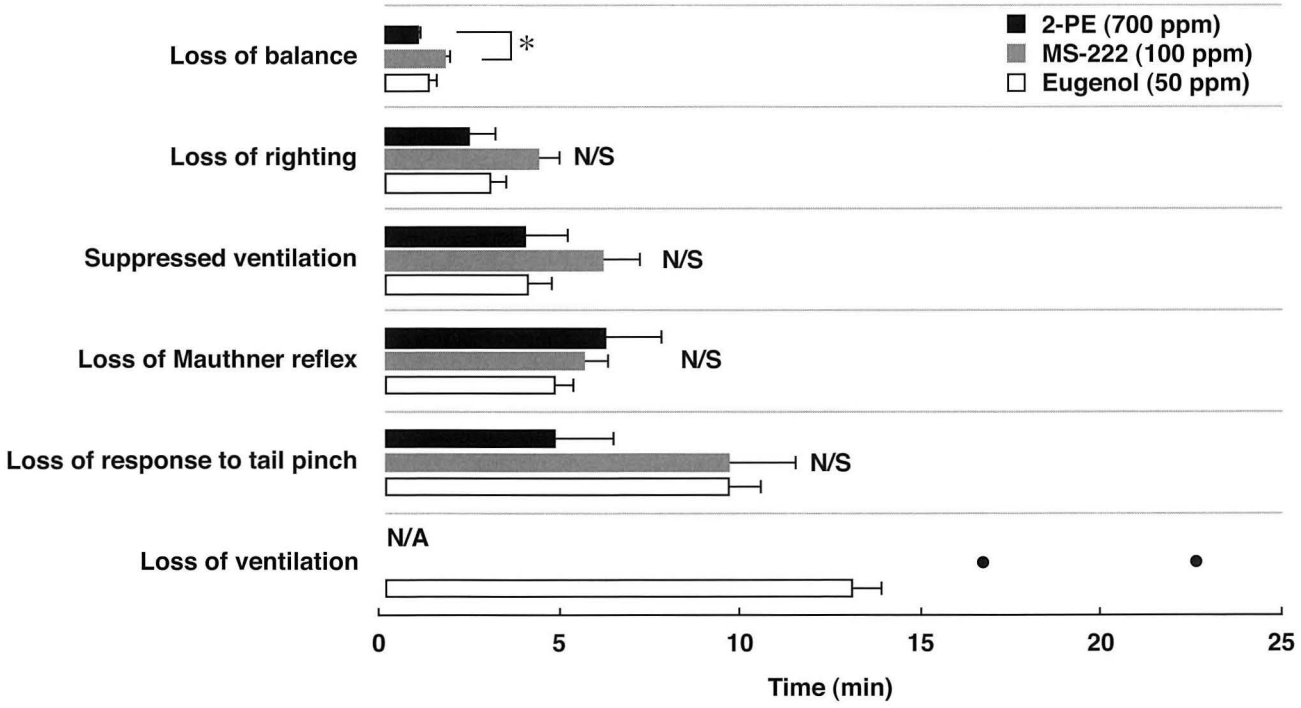


**Fig. 2.** Comparison of the effects of 2-phenoxyethanol (2-PE; 700 ppm; $n = 5$), MS-222 (100 ppm; $n = 5$), and eugenol (50 ppm; $n = 5$). For all anesthetics, time effective to induce surgical anesthesia (i.e., stage III, see Result for details) was approximately 5–10 min. The average times to induce the six anesthetic states are shown. Horizontal bars denote SEM. Asterisk denotes significant difference (Steel–Dwass test, $P < 0.05$). No goldfish immersed in 2-PE solution lost ventilation. Dots in the lowest case (loss of ventilation) denote the scores of two individuals that stopped ventilation with MS-222 anesthesia. N/S, not significant; N/A, not assigned.

in MS-222 showed righting and started to swim during the 30-min recovery period (Fig. 3B). For MS-222 anesthesia, the recovery period appeared to depend on the concentration of the anesthetic rather than the period of immersion. Goldfish treated with the higher MS-222 dose needed a longer period for recovery; however, they were in the anesthetic solution for a shorter period than those in the lower concentration solution (Fig. 3B). Similarly, goldfish that experienced the higher concentration of 2-PE tended to require a longer period for recovery (Fig. 3A), whereas the periods of immersion in the anesthetic were the same (30 min) for all concentrations of 2-PE.

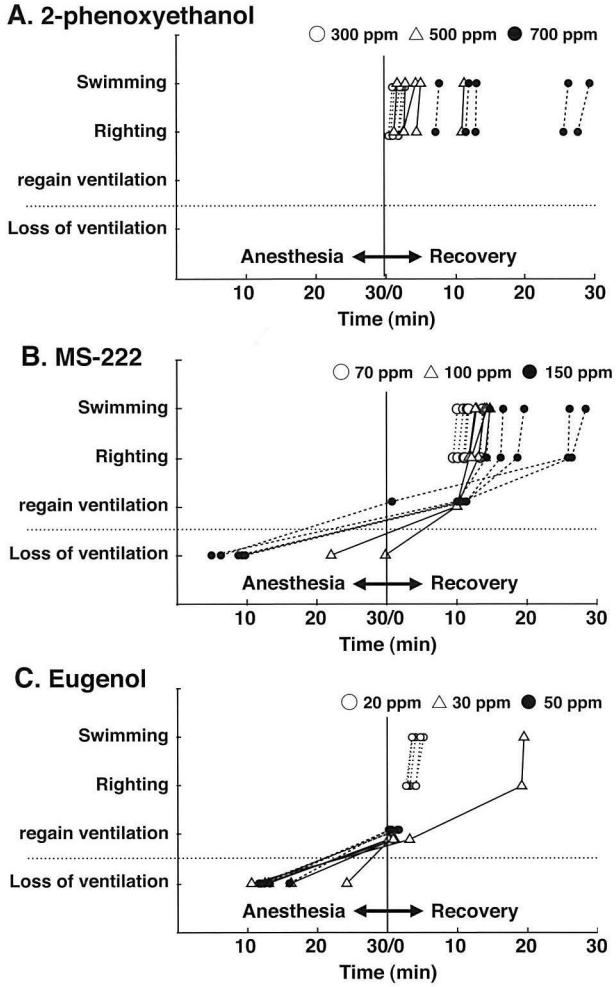


**Fig. 3.** Recovery from the anesthesia with 2-phenoxyethanol (2-PE; A), MS-222 (B), and eugenol (C). Recovery times required to regain ventilation, righting ability, and swimming activity are plotted for all individuals used in the experiment. Goldfish were transferred from the anesthetic solutions to the fresh water soon after confirming the cessation of ventilatory movement. In all cases for 2-PE and in lower concentrations for MS-222 and eugenol, the loss of ventilation was not induced during the 30-min immersion in the anesthetics. In these cases, goldfish were kept in the anesthetic solutions for 30 min, and then allowed to recover in the fresh water.

## Discussion

Exposure of red pacu *Piaractus brachypomus* to high concentrations of eugenol tends to cause ventilatory failure and rapid medullary collapse (Sladky et al. 2001). This relatively rapid effect of eugenol on medullary function was suggested to be due to the highly lipophilic property of this agent (Mylonas et al. 2005). Lipophilic eugenol may easily penetrate the gill epithelium and quickly be absorbed by the brain via the blood circulation (Summerfelt and Smith 1990; Mylonas et al. 2005). 2-PE is also lipophilic. In Atlantic cod *G. morhua*, the ventilatory movement ceased within a few minutes in 2-PE solution at the concentration of 500–600 ppm (Mattson and Riple 1989). However, we found that 2-PE did not induce the loss of ventilation in goldfish at the highest dose that induced stage III anesthesia as quickly as that induced by eugenol. The present results indicate that artificial irrigation of the gills is required when anesthetizing goldfish by immersion in eugenol solution at concentrations achieving stage III anesthesia. Conversely, goldfish can be kept at a state of surgical anesthesia with 700 ppm 2-PE for relatively long period without artificial gill irrigation.

For comparison of the mode of action among the anesthetics, the time courses of the anesthetic effects were examined at the concentration in which surgical anesthesia is achieved by each anesthetic in 5-10 minutes. Although eugenol is the only approved anesthetic agent for use in aquaculture in Japan, relatively quick loss of ventilation at doses inducing stage III anesthesia after approximately 5 min of immersion makes us cautious to use the anesthetic for long periods (>15 min in the present case). The present result suggests that eugenol has a more serious effect on the respiratory center in the medulla than that by 2-PE and MS-222. Considering this result, together

with the findings that goldfish anesthetized with eugenol needed more recovery time in fresh water, eugenol might not be suitable for use in cases involving surgical manipulations, which usually need deeper anesthesia (Cooke et al. 2004). The longer recovery time for eugenol anesthesia compared with MS-222 anesthesia was also reported in carp (Hikasa et al. 1986). The narrow safety margin and longer recovery time of eugenol anesthesia have also reported in some seawater fishes including skipjack *Trachurus japonicus*, purplish amberjack *Seriola dumerili*, striped jack *Pseudocaranx dentex*, and Japanese stingfish *Sebastes inermis* (Watanabe et al. 2006). In these fishes, effective doses were not able to be determined due to unacceptably inferior safety (Watanabe et al. 2006). However, it should be noted that induction to a light stage of anesthesia with eugenol was comparable to that with 2-PE and MS-222 (Fig. 2). Furthermore, elevation of plasma cortisol induced by eugenol anesthesia has been reported to be less than that by MS-222 anesthesia for some freshwater fish (Wagner et al. 2002; Small 2003). Thus, light eugenol anesthesia is possibly effective for reducing short-term handling stress (Iversen et al. 2003; Cooke et al. 2004).

Although 2-PE is not approved for use in aquaculture in Japan and other countries, we found this agent is quite effective in inducing both light and deep anesthesia. The mode of action of 2-PE anesthesia observed in the present study was consistent with an earlier report on goldfish, in which the temperature dependency of 2-PE anesthesia and repeated exposure to this agent were examined (Weyl et al. 1996). Furthermore the present results regarding 2-PE and eugenol anesthesia are also consistent with a previous report for some commercially important seawater fish species in Japan including yellowtail *Seriola quinqueradiata*, red seabream *Pagrus major*, skipjack *T. japonicus*, Japanese flounder *Paralichthys olivaceus*, tiger puffer *Takifugu rubripes*, and Japanese stingfish *S. inermis* (Watanabe et al. 2006). In addition, in those fishes, recovery from 2-PE anesthesia has reported to be quicker than that from eugenol anesthesia even after relatively prolonged immersion period, suggesting the safety of 2-PE compared to eugenol (Watanabe et al. 2006). In addition to the satisfying effectiveness of 2-PE in inducing stage II and III anesthesia, we found the time transition to stage IV anesthesia, in which medullary collapse causes the loss of ventilation (McFarland 1959), was considerably larger than that in the other two anesthetics even at higher concentrations (Fig. 1). Contrary to these preferable aspects of 2-PE anesthesia, it has been reported that its use for anesthesia in rainbow trout results in a dramatic decrease in heart rate and blood pressure, whereas MS-222 produced only small cardiovascular alterations (Fredricks et al. 1993).

The mode of action of MS-222 on goldfish behavioral and physiological responses was similar to that of 2-PE (Fig. 1). MS-222 is known to have a good safety margin for fish and a relatively rapid excretion (Ross and Ross 2008), and approved for use in aquaculture in North America and the European Union. Although higher doses of MS-222 tended to induce deeper anesthesia including the loss of ventilation (Fig. 1B), goldfish rapidly recovered from the anesthesia, regained ventilation, and eventually started to swim (Fig. 3B).

It is apparent that for 2-PE, eugenol, and MS-222 anesthesia, recovery time primarily depends on the concentration of the anesthetic rather than on the duration of immersion. This is supported by the observation that the higher the dose of these agents the longer recovery time, whereas the immersion periods in the anesthetic solutions at higher concentrations were shorter than those in the solutions at lower concentrations. This result is consistent with the findings in two types of sea bream *Diplodus sargus* and *Diplodus puntazzo*, in which the time taken for the recovery from 2-PE anesthesia increased with increasing concentrations (Tsantilas et al. 2006). Contrary, it has been reported that recovery times decreased with increasing doses of 2-PE for Senegalese sole *Solea senegalensis* (Weber et al. 2009), and with increasing doses of 2-PE and clove oil for

European sea bass *Dicentrarchus labrax* and gilthead sea bream *S. aurata* (Mylonas et al. 2005). In these cases, the authors suggest that higher doses induced anesthesia more quickly and hence fish are in contact with the drug for shorter periods, thereby allowing the fish to recover faster (Mylonas et al. 2005; Weber et al. 2009). To determine the time and dose dependency of the recovery from the anesthesia, comparisons among different anesthetics in the situation of artificial gill irrigation by the anesthetic solutions are required in future studies.

The present findings may help one to determine an appropriate anesthetic agent and its concentration for goldfish according to the situation. It should be stressed that the modes of effect of anesthetic agents are highly specific to fish species and care should be taken in choosing anesthetics suitable for specific fish species (Summerfelt and Smith 1990).

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# キンギョに対する3種の魚類用全身麻酔薬（2-フェノキシエタノール，MS-222，オイゲノール）の効果の比較

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一般に広く用いられている3種の魚類用全身麻酔薬，2－フェノキシエタノール（2-PE），MS-222，オイゲノールのキンギョに対する効果を比較した。麻酔導入の進行状態はあらかじめ定めた6つの行動指標を示すまでの時間を測定することで判断した。2-PE と MS-222 は，浅い麻酔段階ではどちらもほぼ同様の用量依存性を示したが，高濃度で使用した場合 MS-222 では呼吸喪失を引き起こす傾向がみられた。オイゲノール麻酔では，手術麻酔段階まで達するのに比較的長時間要したが，その後速やかに呼吸が喪失した。またオイゲノールで麻酔した場合，他の麻酔薬よりも回復に時間がかかった。キンギョにおいて，オイゲノールは短時間のハンドリングストレス軽減を目的とした低濃度での使用が適切であり，2-PE と MS-222 は外科的操作を伴う状況での使用に適していることが示唆された。