KOREAN JOURNAL OF ICHTHYOLOGY, Vol. 35, No. 2, 67-74, May 2023
ISSN: 1225-8598 (Print), 2288-3371 (Online). DOI: https://doi.org/10.35399/ISK.35.2.1

Received: March 20, 2023
Revised: April 14, 2023
Accepted: April 21, 2023

# Effect of Water Temperature, Fish Age, and MS-222 Concentration on the Anesthetization of River Pufferfish, *Takifugu obscurus*

*By Seungyeon Lee[1,2,†], Phuong Thi Nguyen[1,2,†], Ho-Kyung Song[1,3], Sung Pyo Hur[4] and Jin-Hyoung Kim[1,2,*]*

[1]Division of Life Sciences, Korea Polar Research Institute, Incheon 21990, Republic of Korea
[2]Polar Science, University of Science and Technology, Incheon 21990, Republic of Korea
[3]Center for Ecology and Environmental Toxicology, Chungbuk National University, Cheongju 28644, Republic of Korea
[4]Department of Marine Life Science, Jeju National University, Jeju 63243, Republic of Korea

**ABSTRACT** **The river pufferfish (*Takifugu obscurus*) is a valuable species in aquaculture and genetic studies. Usage of fish anesthetics aids in the easier handling of fish during aquaculture. However, there are no studies on appropriate conditions required for effective anesthetization of pufferfish. This study aims to determine the optimal conditions (fish age, water temperature, anesthetic concentration) needed for the most common fish anesthesia, MS-222, to anesthetize *T. obscurus*. We tested three different water temperatures (20°C, 24°C, and 28°C), three different anesthetic concentrations (125 mg/L, 150 mg/L, and 175 mg/L), and two different fish ages (one- and two-year-old). Appropriate anesthetization conditions for *T. obscurus* ranged from 150 mg/L to 175 mg/L of MS-222 at 24°C to 28°C for one-year-old fish. For two-year-old fish, the appropriate conditions ranged from 150 mg/L to 175 mg/L of MS-222 at 28°C. However, to minimize side effects and risks, 150 mg/L of MS-222 at 24°C for one-year-old fish and 175 mg/L of MS-222 at 28°C for two-year-old fish are recommended for effective anesthetization.**

**Key words:** River pufferfish, *Takifugu obscurus*, anesthesia, recovery, MS-222

## INTRODUCTION

Fish anesthesia minimizes stress in fish by preventing physical injuries and delaying the activation of metabolic functions during measurement, blood collection, disease treatment, artificial seed production, transportation, and screening performed during aquaculture (Popovic *et al.*, 2012; Sneddon, 2012; Liu *et al.*, 2022). The chemical anesthetics used for fish are MS-222 (Tricaine methane-sulfonate, $C_{10}H_{15}NO_5S$), benzocaine ($C_9H_{11}NO_2$), carbon dioxide ($CO_2$), clove oil, AQUI-S, quinaldine ($C_{10}H_9N$), quinaldine sulfate ($C_{10}H_{11}NO_4S$), 2-phenoxyethanol ($C_8H_{10}O_2$), metomidate ($C_{13}H_{14}N_2O_2$), and etomidate ($C_{14}H_{16}N_2O_2$) (Marking and Meyer, 1985; Ross and Ross, 2009; Aydın *et al.*, 2015; Priborsky and Velisek, 2018). MS-222 has been the most common anesthetic agent for poikilotherms since 1967 (Daniel, 2009; Popovic *et al.*, 2012), because it has shown a high degree of effectiveness and safety in an aqueous solution state (Massee *et al.*, 1995; Popovic *et al.*, 2012). MS-222 is the only anesthetic approved by the US Food and Drug Administration (FDA) to be used in fish for human-consumption (Marking and Meyer, 1985; Popovic *et al.*, 2012). In the UK, Canada, Italy, Spain, and Norway, only registered veterinarians can use MS-222 for fish (Popovic *et al.*, 2012; Park, 2019b; Wang *et al.*, 2020).

The river pufferfish (*Takifugu obscurus*) (Tetraodontiformes: Tetraodontidae) is a euryhaline and anadromous species that can be adapted to a wide range of salinities (Kato *et al.*, 2005). *T. obscurus* prefers deep and clear in blackish water areas and freshwater for spawning; the fingerlings spend a few months in inland waters before

†Seungyeon Lee and Phuong Thi Nguyen equally contributed to this manuscript.
저자 직위: 이승연 (학생연구원), Phuong Thi Nguyen (학생연구원), 송호경 (박사후연구원), 허성표 (교수), 김진형 (책임연구원, 교수)
*Corresponding author: Jin-Hyoung Kim Tel: 82-32-760-5583, Fax: 82-32-760-5509, E-mail: kimjh@kopri.re.kr

swimming to the ocean for growth (Kato *et al*., 2005). *T. obscurus*, found in China, Japan, and Korea, is an important commercial aquaculture species because of its good taste and high market prices (Fisheries, 2013).

Although *T. obscurus* is a valuable species for aquaculture and genetic studies, there are no studies on appropriate conditions to anesthetize it. This study aims to find the optimal water temperature and anesthetic concentration by fish age and the required time for anesthetization and recovery.

## MATERIALS AND METHODS

### 1. Ethics statement

All handling and experimental procedures followed the ethical guidelines regulated by the Animal Experimental Ethics Committee (KACC2201-002) established by KOPRI after completing training at the Foundation's Life Science Research Ethics Research Institute.

### 2. The fish and rearing conditions

*T. obscurus* were obtained from the Yangchon Fish Hatchery (Kimpo, Kyounggi-do, Korea) and transferred to the laboratory in Korea Polar Research Institute (KOPRI). At the institute, they were reared in the RAS (Recirculating Aquaculture System) for the water volume of 2,000 L. The water temperature was 24 ± 1°C. The dissolved oxygen was maintained at over 5.5 mg/L. The fish were fed twice a day, with total feed quantity proportional to 5% of their body weight.

### 3. Experimental procedure

The experimental conditions are shown in Table 1. This experiment was performed by adjusting three variables: water temperature (20°C, 24°C, and 28°C), anesthetic concentrations (125 mg/L, 150 mg/L, and 175 mg/L), and fish age (one- and two-year-old). While the average weight of the one-year-old fish was 3.13 ± 0.63 g, the average weight of the two-year-old fish was 33.60 ± 8.10 g. Fish were reared at 24°C of water before the experiment and were transferred to a tank at the same temperature just before the experiment. We prepared an experimental tank with 30 L volumetric size for one-year-old fish and filled it with 25 L of water. A 100 L tank was prepared and filled with 75 L of water for two-year-old fish. Heaters with 2.5 kW capacity were used in each experimental and recovery tanks, and the water temperature was automatically adjusted using a thermostat. We checked the water temperature regularly during the experiment to maintain a consistent temperature. The dissolved oxygen was maintained from 5.55 to 7.09 mg/L by using air diffuser strips and checking by YSI. A mesh was applied in the recovery tanks to divide the tank space into four parts to segregate individual fish.

We transferred ten fish from each group into the experimental/anesthetic tanks. The water temperature of the recovery tanks was set to be the same as that of the anesthetic tanks. The fish were placed in the anesthetic tanks one by one, and the time was measured using a stopwatch (± 1 sec). The anesthetized fish was taken out using a net individually, then the wet weight, total length, and standard length were measured. The recovery time was measured by a stopwatch that started when a fish was put into the recovery tank and stopped when the fish fully recovered. Time spent in anesthesia and recovery were determined following the standard criteria used in the previous experiments (Summerfelt *et al*., 1990; Woolsey *et al*., 2004; Park, 2019a). The anesthetic standard was established as the operculum movement of fewer than 10 times per minute (A6 stage). The recovery criteria were established as active self-swimming without any physical effect of aeration (R5 stage).

### 4. Statistical analyses

Three-way ANOVA was performed to compare the effect of three factors, water temperature, MS-222 concentrations, and fish age on anesthesia time and recovery time, using

**Table 1.** Experimental conditions

| Age | One-year-old fish | | | | | | | | | Two-year-old fish | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Water temperature (°C) | 20 | | | 24 | | | 28 | | | 20 | | | 24 | | | 28 | | |
| MS-222 concentration (mg/L) | 125 | 150 | 175 | 125 | 150 | 175 | 125 | 150 | 175 | 125 | 150 | 175 | 125 | 150 | 175 | 125 | 150 | 175 |
| Number of fishes | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Average wet weight (g) | 3.33 | 3.42 | 3.36 | 3.31 | 3.25 | 3.01 | 2.86 | 2.90 | 2.77 | 36.81 | 33.55 | 27.61 | 34.03 | 31.20 | 35.57 | 35.57 | 36.72 | 33.01 |

Robust statistical methods based on Wilcox' WRS functions in R package "WRS2" since the assumptions of normality and homogeneity were not satisfied. After determining the significant effects, within each factor, three-group comparisons were analyzed with the independent Kruskal-Wallis test ($P<0.05$), then two-group comparisons were made with a Mann-Whitney U-test, with $P$-value less than 0.0167 was considered significant, following the Bonferroni Correction Method.

## RESULTS

All results are summarized in Table 2. Overall, the anesthesia time and recovery time were affected by the water temperature and the concentration of MS-222 in both one- and two-year-old fish. The anesthesia induction time of $2.00 \pm 0.15$ min was the shortest in the one-year-old fish group, subjected to 175 mg/L anesthesia under 28°C. Under the same variables, the one-year-old fish recorded the shortest recovery time of $1.44 \pm 0.35$ min. Both the anesthesia time and recovery time of one-year-old fish were longest at 20°C.

The two-year-old fish group took more time to reach the anesthesia and recovery standard than the one-year-old group under the age condition ($P<0.0167$). The MS-222 concentration, which required the longest time to anesthetize the fish, was 125 mg/L ($P<0.0167$, anesthesia time in Table 2).

The increase in water temperature from 20°C to 28°C resulted in decreased anesthesia time and recovery time in both age groups of fish ($P<0.05$). While the anesthesia time diminished steadily with the increase of water temperature from 20°C to 24°C and then to 28°C, the recovery time showed an insignificant change between 24°C and 28°C in one-year-old and two-year-old fish (Table 2).

The anesthesia time decreased when the MS-222 concentration was increased from 125 to 175 mg/L ($P<0.05$), whereas an exception occurred in anesthesia time at the concentration of 150 and 175 mg/L, where the decrease was not significant. The recovery time of one-year-old fish not significantly decreased when the MS-222 concentration increased. The opposite trend was found in the change of recovery time of two-year-old fish compared to the younger group: the time increased at, the higher concentration of anesthetics ($P<0.05$) by 3.32 min at 125 mg/L, 3.79 min at 150 mg/L, and 3.85 min at 175 mg/L, respectively, in an average of three temperatures.

When exposed to 20°C, two-year-old fish were anesthetized two times slower than the one-year-olds ($P<0.05$), and 1.5 times slower at 24°C ($P<0.05$) (Fig. 1a). Within the same age group, anesthesia time decreased significantly as the temperature increased for one- and two-year-old fish. When exposed to 20°C conditions, two-year-old fish recovered 1.27 times slower than one-year-old fish ($P<0.05$), 1.6 times slower at 24°C ($P<0.05$) and 2.13 times slower at 28°C ($P<0.05$) (Fig. 1b). The recovery time at 20°C was significantly slower within the same age group than at 24°C and 28°C ($P<0.05$). However, there was no significant difference in the recovery time at 24°C and 28°C. It showed the same tendency to decrease anesthesia and recovery time as the temperature increased.

**Table 2.** The anesthesia and recovery time at different temperatures and MS-222 concentrations

| Temperature (°C) | MS-222 concentration (mg/L) | Time (mins) | | | |
|---|---|---|---|---|---|
| | | One-year-old fish | | Two-year-old fish | |
| | | Anesthesia | Recovery | Anesthesia | Recovery |
| 20 | 125 | $5.82 \pm 2.10^{ad}$ | $3.71 \pm 1.37^{ad}$ | $12.63 \pm 3.73^{ad}$ | $4.06 \pm 1.85^{ad}$ |
| | 150 | $3.93 \pm 1.17^{bd}$ | $4.52 \pm 2.34^{ad}$ | $6.86 \pm 1.39^{bd}$ | $5.51 \pm 1.67^{abd}$ |
| | 175 | $3.09 \pm 1.08^{bd}$ | $3.52 \pm 1.23^{ad}$ | $5.55 \pm 0.90^{bd}$ | $5.32 \pm 1.99^{bd}$ |
| 24 | 125 | $3.46 \pm 1.37^{ae}$ | $2.39 \pm 1.72^{ae}$ | $6.50 \pm 0.69^{ae}$ | $3.21 \pm 0.96^{ae}$ |
| | 150 | $3.00 \pm 0.72^{be}$ | $1.51 \pm 0.38^{ae}$ | $4.41 \pm 0.97^{be}$ | $2.73 \pm 1.17^{abe}$ |
| | 175 | $2.96 \pm 0.58^{be}$ | $2.17 \pm 0.80^{ae}$ | $3.25 \pm 0.60^{be}$ | $3.78 \pm 0.94^{be}$ |
| 28 | 125 | $3.31 \pm 0.56^{af}$ | $1.62 \pm 0.63^{ae}$ | $3.66 \pm 0.92^{af}$ | $2.70 \pm 1.12^{ae}$ |
| | 150 | $2.21 \pm 0.47^{bf}$ | $1.64 \pm 1.03^{ae}$ | $2.69 \pm 0.43^{bf}$ | $3.16 \pm 2.02^{abe}$ |
| | 175 | $2.00 \pm 0.15^{bf}$ | $1.44 \pm 0.35^{ae}$ | $2.31 \pm 0.52^{bf}$ | $4.17 \pm 1.71^{be}$ |

Each value is the mean ± standard deviation of a data set (water temperature, MS-222 concentration, n = 10). In a column, different letters of (a, b, c) indicate significant differences for the different MS-222 doses at the same temperature, (d, e, f) for the different temperatures at the same MS-222 dose.

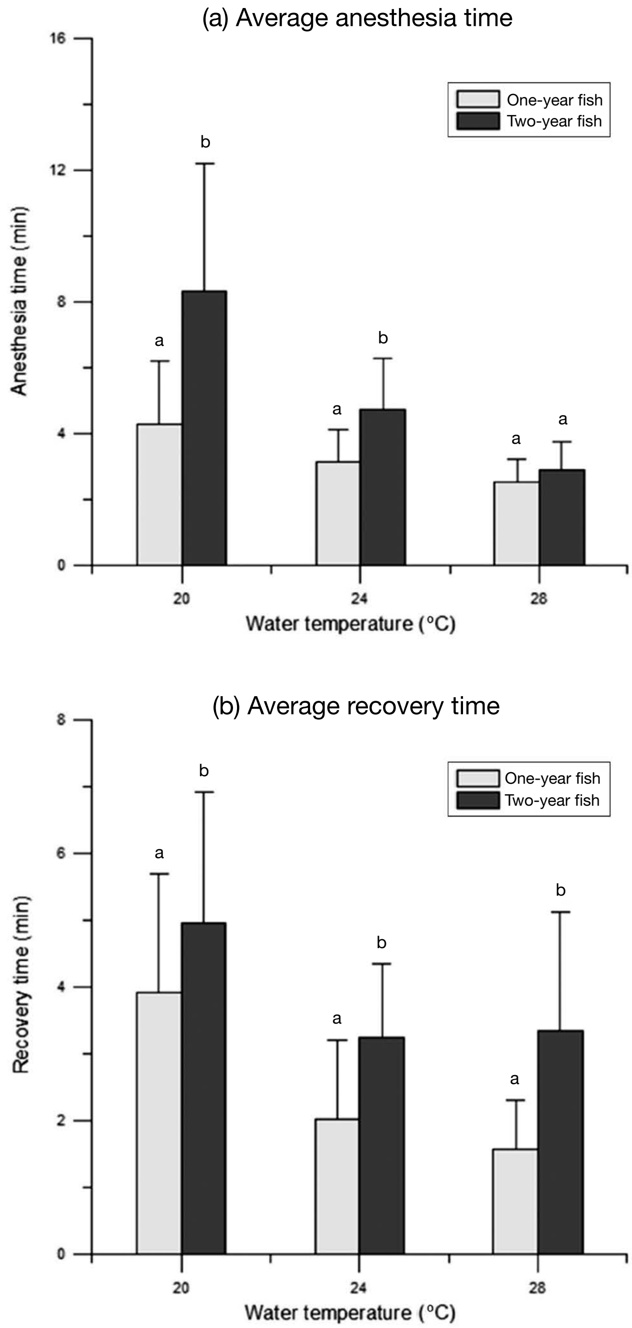


**Fig. 1.** The anesthesia time and recovery time of one- and two-year-old fish at different water temperatures. Different letters indicate the significant difference of these two groups ($P<0.05$).

## DISCUSSION

Although the effects of MS-222 for anesthetization of fish have been studied for a long time and in many species (Obradovic, 1986; Kidd and Banks, 1990; Wagner *et al.*, 2003; Holloway *et al.*, 2004; Feng *et al.*, 2011; Barbas *et al.*, 2017; Jacobsen *et al.*, 2019), there has been no study for *T. obscurus* so far. This is the first study on the effects of MS-222 concentration, water temperature, and fish age on the anesthesia and recovery time in the *T. obscurus*.

### 1. Effect of the concentration of anesthetics on anesthetization

For eastern catfish (*Silurus asotus*) with a mean body weight from 50.1±5.91 g to 302.1±15.22 g, the optimal anesthesia time of around 1 min was achieved with the preferred concentration of 600 mg/L (Park, 2019b). Lower anesthetic concentrations were recommended for smaller-sized fish. For example, the anesthetic concentration of 150 mg/L for pikeperch (*Sander lucioperca*) with a body-weight of 8.56 g to 52.91 g, denison barb (*Sahyadria denisonii*), and matrinxã (*Brycon cephalus*) induced anesthesia within three minutes without any mortality (Roubach *et al.*, 2001; Mercy *et al.*, 2013; Rożyński *et al.*, 2018). Anesthesia was effectively induced at even lower concentrations of 125 mg/L in black sea bass (*Centropristis striata*) and 100 mg/L in greater amberjack (*Seriola dumerilii*), respectively (King *et al.*, 2005; Maricchiolo and Genovese, 2011).

We effectively induced anesthesia in one-year-old river pufferfish using the lowest concentration of MS-222, 150 mg/L at 24°C, within 3.00±0.72 min. At the same anesthetic concentration, the two-year-old fish were anesthetized within 2.69±0.43 min at 28°C. As the appropriate concentration depends on species and size, there is a need to determine the appropriate anesthesia time for each species. The anesthesia induction within 3 min and complete recovery within 5 min has been recommended for the safety of the fish (Coyle *et al.*, 2004; Martins *et al.*, 2019).

### 2. Effect of fish age (≒fish size) on anesthetization

Rożyński and Hopko (Rożyński *et al.*, 2018) reported that 100 mg/L of MS-222 concentration at 23°C anesthetized a small fish weighing under 10 g within 2.97 min, whereas a large one weighing over 40 g required 5.15 min anesthesia time, and both sizes recovered within 2 min. Park (2019b) stated that a smaller eastern catfish (*Silurus asotus*) needed a shorter time for both anesthesia and recovery in comparison to larger individuals ($P<0.05$). In *T. obscurus*, similar to previous studies, the two-year-old larger fish required significantly longer time for anesthesia and recovery compared to one-year-old smaller fish ($P<0.05$). We speculate that this difference arises due to the age of fish and sensitivity to anesthetics between the two sizes, which in turn might be influenced by the body weight, body composition, growth rate, and sexual maturity (Zahl *et al.*, 2012). The older fish with increas-

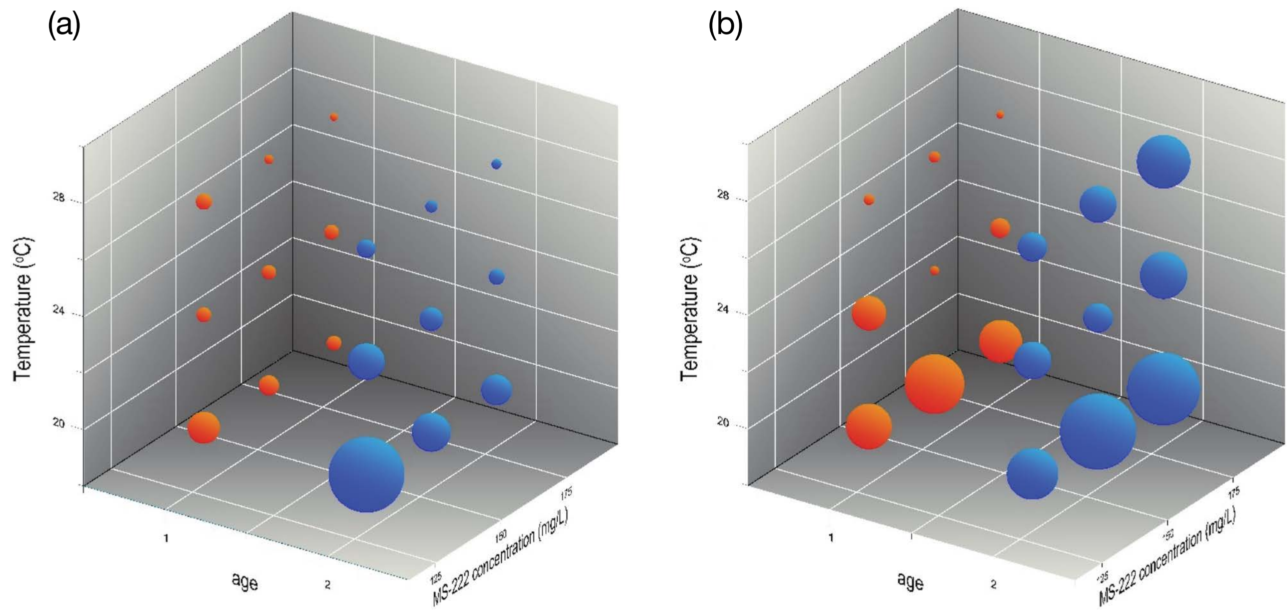


**Fig. 2.** The anesthesia time graph (a) and recovery time graph (b). The larger bubble, the longer it took. The overall trend in the 3D graph. First, in (a) graph, it can be seen that the more age, the lower the anesthetic concentration, the lower the temperature, and the longer the anesthesia time takes.

ing body size have lower basal metabolic rate and lower oxygen consumption than the younger (Schmidt-Nielsen and Knut, 1984; Clarke and Johnston, 1999). The effect of fish body weight on anesthesia is controversial, with few studies indicating no correlation between the two (Houston and Woods, 1972; Stehly and Gingerich, 1999) and others establishing a relationship between them (Rożyński *et al.*, 2018; Park, 2019b). In this study, we observed an exception, where no significant difference was found between the size of *T. obscurus* and the anesthesia time (Fig. 1) at 28°C. The longer recovery time in older fish could be explained by the high solubility of MS-222 in lipid, therefore, the anesthetic may last longer in large or gravid fish as the drug is removed from the reserved lipid and recovery proceeds slower (Coyle *et al.*, 2004). This weight-related recovery for MS-222 was reported in Senegalese sole (*Solea senegalensis*) (Weber *et al.*, 2009).

### 3. Effect of temperature on anesthetization

Higher temperatures decreased the anesthesia and recovery time in *T. obscurus* (Table 1). Similar findings have been reported in several fish species, such as Atlantic cod (*Gadus morhua*) (Zahl *et al.*, 2009), rainbow trout (*Salmo gairdneri*), common carp (*Cyprinus carpio*), fathead minnows (*Pimephales promelas*) (Sylvester and Holland, 1982; Hikasa *et al.*, 1986), and Atlantic halibut (*Hippoglossus hippoglossus*) (Zahl *et al.*, 2011). These results correspond to the strong influence of water temperature on the efficiency of anesthetics, particularly MS-222 in this study. The change could be recognized easily by the size of the bubbles in Fig. 2, which display the time consumed for anesthesia or recovery. These results allude that the body temperature in ectothermic fish depends on ambient temperature. Clarke and Johnston (1999) suggested a ratio that the basal metabolic rate in fish increases by two times with a 10°C rise in the ambient temperature (Q10), and this plays an important role in the processes relating to absorption and excretion of anesthetics in the body. Since a higher metabolic rate requires higher oxygen demand (Graham and Farrell, 1989), we recommend that sufficient oxygen should be provided to the fish during anesthetization to exclude stress.

The conditions tested in this study, including water temperature ranging from 20°C to 28°C and the MS-222 concentrations (125, 150, and 175 mg/L), are considered safe for various sizes of the *T. obscurus* as reported mortality in only one individual during the entire experiment. However, during long-term exposure, a range of side effects such as hypoxemia, change in the level of glucose, leukocyte, ammonia, triacylglycerol, and inorganic phosphate have been reported in fish (Kristan *et al.*, 2012; Sneddon, 2012). As the secondary effects differ between fish species, further studies on the physiological response will allow more benefit in developing anesthetic regiments for the pufferfish and other related species.

In conclusion, to achieve the recommended criteria of 3 min anesthesia time and 5 min recovery time in *T. obscurus*, we recommend an anesthetic concentration range from 150 mg/L to 175 mg/L at 24°C and 150 mg/L to 175 mg/L at 28°C for the one-year-old fish. The recommended conditions range from 150 mg/L to 175 mg/L for two-year-old fish at 28°C. Further, to minimize risk and side effects, the optimal anesthetization conditions are 150 mg/L of anesthetic concentration at 24°C for one-year-old fish and 175 mg/L concentration at 28°C for two-year-old fish.

## FUNDING

This research was supported the by Korea Polar Research Institute (KOPRI; grant number PE23160).

## REFERENCES

Aydın, İ., B. Akbulut, E. Küçük and M. Kumlu. 2015. Effects of temperature, fish size and dosage of clove oil on anaesthesia in turbot (*Psetta maxima* Linnaeus, 1758). Turkish J. Fish. Aquat. Sci., 15: 899-904.

Barbas, L.A.L., P.M. Pereira-Cardona, L.C. Maltez, L.O. Garcia, J.M. Monserrat and L.A. Sampaio. 2017. Anaesthesia and transport of juvenile tambaqui *Colossoma macropomum* (Cuvier, 1818) with tricaine methane-sulphonate: Implications on secondary and oxidative stress responses. J. Appl. Ichthyol., 33: 720-730. https://doi.org/10.1111/jai.13382.

Clarke, A. and N.M. Johnston. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. J. Anim. Ecol., 68: 893-905.

Coyle, S.D., R.M. Durborow and J.H. Tidwell. 2004. Anesthetics in Aquaculture. Southern Regional Aquaculture Center, 3900.

Daniel, P. 2009. Available chemotherapy in Mediterranean fish farming: use and needs. CIHEAM (Centre International de Hautes Etudes Agronomiques Méditerranéennes)/FAO (food and agriculture organization of the United Nations), Zaragoza.

Feng, G., P. Zhuang, L. Zhang, B. Kynard, X. Shi, M. Duan, J. Liu and X. Huang. 2011. Effect of anaesthetics MS-222 and clove oil on blood biochemical parameters of juvenile Siberian sturgeon (*Acipenser baerii*). J. Appl. Ichthyol., 27: 595-599. https://doi.org/10.1111/j.1439-0426.2011.01711.x.

Fisheries, M.o.O.a. 2013. Statistical Yearbook of Maritime Affairs & Fisheries, Sejong, Korea.

Graham, M. and A. Farrell. 1989. The effect of temperature acclimation and adrenaline on the performance of a perfused trout heart. Physiol. Zool., 62: 38-61.

Hikasa, Y., K. Takase, T. Ogasawara and S. Ogasawara. 1986. Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopental sodium in the carp, *Cyprinus carpio*. Jpn. J. Vet. Sci., 48: 341-351.

Holloway, A.C., J.L. Keene, D.G. Noakes and R.D. Moccia. 2004. Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. Aquac. Res., 35: 1025-1030. https://doi.org/10.1111/j.1365-2109.2004.01108.x.

Houston, A. and R. Woods. 1972. Blood concentrations of tricaine methane sulphonate in brook trout, *Salvelinus fontinalis*, during anesthetization, branchial irrigation, and recovery. J. Fish. Res. Board Can., 29: 1344-1346.

Jacobsen, J.V., K. Steen and K.J. Nilssen. 2019. Anaesthetic efficacy of Aqui-S, Benzoak, and MS-222 on lumpfish (*Cyclopterus lumpus*) fries. Impact from temperature, salinity, and fasting. PLos One, 14: e0211080. https://doi.org/10.1371/journal.pone.0211080.

Kato, A., H. Doi, T. Nakada, H. Sakai and S. Hirose. 2005. *Takifugu obscurus* is a euryhaline fugu species very close to *Takifugu rubripes* and suitable for studying osmoregulation. BMC Physiol., 5: 18. https://doi.org/10.1186/1472-6793-5-18.

Kidd, R.B. and G.D. Banks. 1990. Anesthetizing lake trout with Tricaine (Ms-222) administered from a spray bottle. Prog. Fish-Cult., 52: 272-273. https://doi.org/10.1577/1548-8640(1990)052<0272:Altwtm>2.3.Co;2.

King, W., B. Hooper, S. Hillsgrove, C. Benton and D.L. Berlinsky. 2005. The use of clove oil, metomidate, tricaine methanesulphonate and 2-phenoxyethanol for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.). Aquac. Res., 36: 1442-1449.

Kristan, J., A. Stara, J. Turek, T. Policar and J. Velisek. 2012. Comparison of the effects of four anaesthetics on haematological and blood biochemical profiles in pikeperch (*Sander lucioperca* L.). Neuro Endocrinol. Lett., 33: 66-71.

Liu, Y., X.-W. Zhou, H.-T. Ding, X.-J. Dong, J.-J. Zhang, Y.-C. Zheng, X.-N. Chen, H.-L. Cheng, Z.-J. Ding and J.-H. Xu. 2022. Effects of tricaine methanesulfonate (MS-222) on sedation and responses of yellow catfish (*Pelteobagrus fulvidraco*) subjected to simulated transportation stress. Aquaculture, 549: 737789.

Maricchiolo, G. and L. Genovese. 2011. Some contributions to knowledge of stress response in innovative species with particular focus on the use of the anaesthetics. Open Mar. Biol. J., 5: 24-33.

Marking, L.L. and F.P. Meyer. 1985. Are better anesthetics needed in fisheries?. Fisheries, 10: 2-5.

Martins, T., A. Valentim, N. Pereira and L.M. Antunes. 2019. Anaesthetics and analgesics used in adult fish for research: A review. Lab. Anim., 53: 325-341. https://doi.org/10.1177/0023677218815199.

Massee, K.C., M.B. Rust, R.W. Hardy and R.R. Stickney. 1995. The effectiveness of tricaine, quinaldine sulfate and metomidate as anesthetics for larval fish. Aquaculture, 134: 351-359.

Mercy, T.A., V. Malika and S. Sajan. 2013. Use of tricaine methanesulfonate (MS-222) to induce anaesthesia in *Puntius denisonii* (Day, 1865) (Teleostei: Cypriniformes: Cyprinidae), a threatened barb of the Western Ghats, India. J. Threat. Taxa, 5: 4414-4419.

Obradovic, J. 1986. Effects of anesthetics (halothane and Ms-222) on crayfish, *Astacus-astacus*. Aquaculture, 52: 213-217. https://doi.org/10.1016/0044-8486(86)90146-8.

Park, I.S. 2019a. Anaesthetic efficacy and physiological response of clove oil and lidocaine-HCL on river puffer, *Takifugu obscurus* and tiger puffer, *T. rubripes*. Dev. Reprod., 23: 21-34. https://doi.org/10.12717/DR.2019.23.1.021.

Park, I.S. 2019b. The anesthetic effects of clove oil and MS-222 on Far Eastern catfish, *Silurus asotus*. Dev. Reprod., 23: 183-191. https://doi.org/10.12717/DR.2019.23.2.183.

Popovic, N.T., I. Strunjak-Perovic, R. Coz-Rakovac, J. Barisic, M. Jadan, A.P. Berakovic and R.S. Klobucar. 2012. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. J. Appl. Ichthyol., 28: 553-564. https://doi.org/10.1111/j.1439-0426.2012.01950.x.

Priborsky, J. and J. Velisek. 2018. A review of three commonly used fish anesthetics. Rev. Fish. Sci. Aquac., 26: 417-442.

Ross, L.G. and B. Ross. 2009. Anaesthetic and sedative techniques for aquatic animals. John Wiley & Sons.

Roubach, R., L.d.C. Gomes and A.L. Val. 2001. Safest level of tricaine methanesulfonate (MS-222) to induce anesthesia in juveniles of matrinxã, *Brycon cephalus*. Acta Amazon., 31: 159-163.

Rożyński, M., M. Hopko, K. Stawecki and Z. Zakęś. 2018. Impact of fish size, water temperature, and MS-222 concentration on inducing general anesthesia in pikeperch (*Sander lucioperca*). Aquac. Res., 49: 2774-2781. https://doi.org/10.1111/are.13738.

Schmidt-Nielsen, K. and S.-N. Knut. 1984. Scaling: why is animal size so important? Cambridge University Press.

Sneddon, L.U. 2012. Clinical anesthesia and analgesia in fish. J. Exot. Pet Med., 21: 32-43.

Stehly, G. and W. Gingerich. 1999. Evaluation of AQUI-STM (efficacy and minimum toxic concentration) as a fish anaesthetic/sedative for public aquaculture in the United States. Aquac. Res., 30: 365-372.

Summerfelt, R., L. Smith, C. Schreck and P. Moyle. 1990. Methods for fish biology. Anaesthesia, surgery and related techniques. American Fisheries Society, Bethesda, 213-272.

Sylvester, J. and L. Holland. 1982. Influence of temperature, water hardness, and stocking density on MS-222 response in three species of fish. Prog. Fish-Cult., 44: 138-141.

Wagner, G.N., T.D. Singer and R.S. McKinley. 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquac. Res., 34: 1139-1146. https://doi.org/10.1046/j.1365-2109.2003.00916.x.

Wang, W.H., H.B. Dong, Y.X. Sun, C.Y. Sun, Y.F. Duan, Q.H. Gu, Y. Li, M.J. Xie and J.S. Zhang. 2020. Immune and physiological responses of juvenile Chinese sea bass (*Lateolabrax maculatus*) to eugenol and tricaine methanesulfonate (MS-222) in gills. Aquac. Rep., 18: 100554. https://doi.org/10.1016/j.aqrep.2020.100554.

Weber, R., J. Peleteiro, L.G. Martín and M. Aldegunde. 2009. The efficacy of 2-phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agents in the Senegalese sole (*Solea senegalensis* Kaup 1858). Aquaculture, 288: 147-150.

Woolsey, J., M. Holcomb and R. Ingermann. 2004. Effect of temperature on clove oil anesthesia in steelhead fry. N. Am. J. Aquac., 66: 35-41.

Zahl, I.H., A. Kiessling, O.B. Samuelsen and M.K. Hansen. 2009. Anaesthesia of Atlantic cod (*Gadus morhua*) - Effect of pre-anaesthetic sedation, and importance of body weight, temperature and stress. Aquaculture, 295: 52-59.

Zahl, I.H., A. Kiessling, O.B. Samuelsen and M.K. Hansen. 2011. Anaesthesia of Atlantic halibut (*Hippoglossus hippoglossus*) Effect of pre-anaesthetic sedation, and importance of body weight and water temperature. Aquac. Res., 42: 1235-1245.

Zahl, I.H., O. Samuelsen and A. Kiessling. 2012. Anaesthesia of farmed fish: implications for welfare. Fish Physiol. Biochem., 38: 201-218. https://doi.org/10.1007/s10695-011-9565-1.

# 황복의 마취에 미치는 수온 및 연령과 MS-222 농도의 영향

*이승연*[1,2] · *Phuong Thi Nguyen*[1,2] · *송호경*[1,3] · *허성표*[4] · *김진형*[1,2]

[1]극지연구소 생명과학연구본부, [2]과학기술연합대학원 극지과학전공, [3]충북대학교 생태환경 독성센터, [4]제주대학교 해양생명과학과

**요 약 :** 황복(*Takifugu obscurus*)은 양식 어종 중 하나이며, 유전체 연구에서도 의미 있는 종이다. 양식 어종의 경우 어류 마취제를 사용하면 양식 과정에 있어서 어류를 좀 더 쉽게 다룰 수 있다. 그러나, 지금까지 황복의 효과적인 마취와 회복을 위해 필요한 적절한 조건에 대한 연구는 없었다. 본 연구는 가장 일반적인 어류 마취제인 MS-222를 이용하여 황복을 마취하는 데 필요한 연령, 수온 및 마취 농도 등 최적의 조건을 파악하는 데 그 목적이 있다. 실험에서는 세 가지 다른 수온(20°C, 24°C, 28°C) 및 마취 농도(125 mg/L, 150 mg/L, 175 mg/L)와 두 가지 다른 어류 연령(1년생과 2년생)을 테스트하였다. 실험 결과 황복의 적절한 마취 조건은 24°C와 28°C에서 MS-222 150 mg/L에서 175 mg/L까지의 범위였다. 2년생 어류의 경우도 28°C에서 MS-222 150 mg/L에서 175 mg/L의 범위를 보였다. 부작용과 위험을 최소화하기 위한 효과적인 마취 농도는 1년생 어류의 경우 24°C에서 MS-222 150 mg/L, 2년생 어류의 경우 28°C에서 MS-222 175 mg/L가 권장된다.

**찾아보기 낱말 :** 황복, *Takifugu obscurus*, 마취, 회복, MS-222