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Article

Anaesthetic Tolerance of Juvenile Black Rockfish *Sebastess schlegeli*, Produced for Wild Stock Enhancement

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Abstract : 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 methanesulfonate (MS-222), and exposure to drying. The working dosage of MS-222 varied significantly 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. These results suggest 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.

Key words : anesthetic tolerance, tricaine methanesulfonate, wild stock enhancement, *Sebastes shclegeli*.

1. Introduction

The black rockfish, *Sebastes schlegeli*, is one of the major economic marine finfish cultured in Korea. Continuous, intense research on the species has resulted in advanced techniques for fish culture, with complete mastery of the rearing cycle. This great success in the aquaculture industry has drawn attention to replenishment of the decreasing wild stocks of this species by wild stock enhancement. The principle of hatchery-based stock enhancement was practiced in the later part of the 1800s and during the first 50 years of the 1900s (Barnabe 1990), but was discontinued in the 1950s, because no evidence was detected of increased yields resulting from the technique, after more than fifty years of practice (Shelbourne 1964). Consequently, it has been recommended that a new optimal release strategy be developed to improve the yield of wild stocks. This should consider size at release, timing of release, selection of healthy eggs, stocking densities, critical habitat assessment, and genetic impact assessment (Leber and Arce 1996; Leber *et al.* 1998; Tringali and Leber 1999).

Black rockfish and olive flounder are thought to be suitable for release for restocking purposes because they are essentially non-migratory. Hatchery-grown juveniles can differ from wild juveniles in behavior and other biological characteristics. However, little has been reported on the biological characteristics of juveniles intended for release.

An anesthetizing agent, tricaine methanesulfonate (MS-222), is used to reduce stress caused by a variety of

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activities, ranging from routine handling to biological and physiological research, surgery, and examinations of the health of aquatic organisms (Marking and Meyer 1985; Gilderhus and Marking 1987; Hunn and Greer 1992; Massee *et al.* 1995). MS-222 is useful for anesthetizing larval and juvenile fish (Hikasa *et al.* 1986; Chapman *et al.* 1988; Chatain and Corrao 1992; Rust *et al.* 1993; Massee *et al.* 1995). The effectiveness and safety of MS-222 vary with ambient environmental parameters, concentration and duration of treatment, and fish species and size (Schoettger and Julin 1967; Hikata *et al.* 1986; Gilderhus and Marking 1987; Mattson and Riple 1989; Satterfield and Flickinger 1995).

To evaluate the anesthetic tolerance of black rockfish produced in hatcheries for release, juvenile fish from different hatcheries were anesthetized using MS-222. The fish were further challenged by exposure to drying, to evaluate the strength of juveniles from different populations. This has implications for biological research, as well as examining the health of the fish.

2. Materials and methods

Fish

Wild and cultured juvenile black rockfish, Sebastes schlegeli, were collected, ranging from 4 to 10 cm in total length. The cultured fish were from two different types of culturing facility: land-based tanks, and ponds separated from the ocean by embankments. Once introduced into the laboratory, the fish were acclimated to captivity for a week before commencing the experiments. The test animals were maintained in filtered, aerated, flowing seawater, unless otherwise stated. The temperature of the rearing water was controlled at 20±0.5 °C.

Evaluation of anesthesia

Anesthesia and loss of equilibrium were determined by monitoring the 5 stages of anesthesia suggested by Massee *et al.* (1995) after reviewing previous studies (Schoettger and Julin 1967; Mattson and Riple 1989). In brief, in stage 3, fish lose their equilibrium and turn upside down, but are still able to swim; in stage 4, fish lose reflex activity and fail to respond to strong external stimuli; death occurs at stage 5, when respiratory movement ceases.

Anesthetization

Tricaine methanesulfonate (MS-222) was purchased from Sigma Co. To determine the working dosage of MS-222, each of 40 acclimated juvenile populations were evenly distributed between four duplicate containers (4 *l* carrying capacity) containing 4 different MS-222 solutions, 50, 100, 200, and 400 mg/*l*. Then, loss of equilibrium, time to anesthetization, and recovery were measured. For the recovery test, 5 fish each were anesthetized for 5, 10, 15, 20, and 25 minutes at an MS-222 concentration of 100 mg/*l*. The anesthetized fish were transferred to recovery containers supplied with filtered fresh seawater to measure the recovery time.

Five individuals from each population were anesthetized in 100 mg/*l* MS-222 for 2 minutes, and then removed from the containers and placed on wet tissue for 2-30 minutes, before being returned to the culture containers for recovery. Tolerance to drying exposure (for 3-30 minutes) was also measured using five individuals from each non-anesthetized population. All the experiments were duplicated.

Statistics

Data were analyzed for significance ($p<0.05$) using one-way ANOVA and Duncans multiple range test.

Table 1. Anesthetic tolerance of three populations of juvenile black rockfish, *Sebastes schlegeli* administrated with MS-222.

Population	Total length (cm)	MS-222 conc. (mg/*l*)	Loss of equilibrium (sec±SE)	Time to anesthetization (sec±SE)	Recovery (sec±SE)	Mortality (%)
Embank cultured	5.0±0.4	50	–	–	–	0
		100	106±14	216±31	102±12	0
		200	42±12	85±12	129±23	0
		400	19±3	38±6	505±57	0
Wild	5.2±0.4	50	–	–	–	0
		100	21±2	106±37	103±40	0
		200	17±4	39±7	119±53	0
		400	10±3	13±3	357±127	0
Land-based tank	4.5±0.5	50	–	–	–	0
		100	49±11	132±23	130±40	0
		200	14±1	32±1	118±16	0
		400	12±1	23±1	559±11	90

3. Results

Anesthetic concentration of MS-222 by size

Attempts were made to understand how juvenile fish with different growth histories are affected by the chemical anesthetic MS-222. Table 1 shows the anesthetization and recovery of juvenile black rockfish treated with 50, 100, 200, or 400 mg/*l* MS-222. None of the juveniles around 5 cm in total length showed any loss of equilibrium in 50 mg/*l* MS-222 for one hour. Concentrations exceeding 100 mg/*l* induced anesthesia in a concentration-dependent manner. At concentrations of 100-400 mg/*l*, the level of anesthesia differed significantly among fish populations. The juvenile fish from wild stocks were the most sensitive to the chemical, compared with those from land-based tanks and embanked populations.

The recovery time after anesthesia at 100 mg/*l* ranged from 103 to 130 seconds in all populations. It also differed with population ($p<0.05$). Wild fish were most sensitive to anesthesia at 100 mg/*l*. The juvenile fish from embanked populations showed the greatest resistance and fastest recovery.

Table 2. Anesthetic tolerance of different size of black rockfish, *Sebastes schlegeli* administrated with MS-222.

Size	MS-222 conc. (mg/*l*)	Time to anesthetization (sec)	Recovery (sec)	Mortality (%)
4 cm long	50	–	–	0
	100	56±14	49±44	0
	200	33±5	106±64	0
	400	15±1	213±110	70
6 cm long	50	–	–	0
	100	143±49	133±81	0
	200	40±7	181±62	0
	400	19±2	401±150	10
10 cm long	50	–	–	0
	100	218±22	87±22	0
	200	36±5	137±12	0
	400	19±0	315±21	0

Effect of fish size on anesthetic effect

Table 2 shows the size-dependent anesthetic effects of MS-222 for cultured black rockfish. None of the fish tested were anesthetized with exposure to 50 mg/*l* MS-222 for one hour. With increasing MS-222 concentration, smaller fish responded to the chemical before larger ones. For example, at 100 mg/*l*, MS-222 anesthesia required 56 seconds in 4-cm-long fish and 143 seconds in 6-cm-long fish. At higher concentrations, the rate of anesthesia was also size dependent. Once anesthetized, recovery was also size dependent. At the highest MS-222 concentration, 400 mg/*l*, all fish were anesthetized within 20 seconds, with varying mortality: 70, 10, and 0 % in the 4, 6, and 10-cm-long groups, respectively.

Recovery from MS-222 anesthesia

Table 3 shows recovery and mortality of three fish populations in different immersing times at 100 mg/*l* of MS-222. Increased immersion time induced delayed recoveries. In addition, the recoveries depended significantly upon the fish types($P<0.05$). Embanked fish immersed in 100 mg/*l* MS-222 for 5 minutes recovered by 75 seconds, while the fish from wild and land-based tank recovered by 110 and 147 seconds, respectively. Mortalities were also observed in the prolonged duration of immersion at the concentration. All the animals grown in the land-based tank died when the immersion duration reached 15 minutes, while no mortality was noted in the fish from embanked population. The wild fish population showed 60 % mortality in the immersion duration.

Tolerance against dry exposure

Three populations of juvenile black rockfish were exposed to air after MS-222 anesthesia to determine the recovery times and mortalities (Table 4). All the

Table 3. Effects of immersion time on recovery and mortality of black rockfish, *Sebastes schlegeli* at 100 m*l*/*l* MS-222.

Population		Immersion time (min)					
		5	10	15	20	25	30
Embank cultured	Recovery (sec±SE)	75±11	124±35	238±150	247±119	521±112	457±120
	Mortality (%)	0	0	0	60	90	90
Wild	Recovery (sec±SE)	110±42	160±77	197±67	-	-	-
	Mortality (%)	0	0	60	100	100	100
Land-based tank cultured	Recovery (sec±SE)	147±54	121±47	-	-	-	-
	Mortality (%)	0	0	100	100	100	100

Table 4. Recovery and mortality of juvenile black rockfish, *Sebastes schlegeli* exposed to air after anesthesia at 100 mg/*l* MS-222 for 2 minutes.

Population		Dry exposure after anesthesia (min)					
		2	5	10	15	20	30
Embank cultured	Recovery (sec ±SE)	21 ±12	32 ±9	30 ±17	42 ±20	66 ±12	69 ±22
	Mortality (%)	0	0	0	0	0	0
Wild	Recovery (sec)	58 ±33	55 ±31	70 ±59	99 ±23	351 ±307	152 ±91
	Mortality (%)	0	0	0	30	50	70
Land-based tank	Recovery (sec ±SE)	86 ±44	105 ±24	165 ±79	312 ±384	142 ±74	183 ±50
	Mortality (%)	0	0	40	50	30	70

Table 5. Recovery and mortality of juvenile black rockfish, *Sebastes schlegeli* exposed to air without anesthesia.

Population Item		Exposure period in the air (minute)					
		3	5	8	15	20	30
Embank cultured	Recovery (sec ±SE)	1±0	2±1	16±12	16±12	174±308	469±480
	Mortality (%)	0	0	0	0	0	30
Wild	Recovery (sec ±SE)	2±2	3±4	11±19	224±491	120±121	285±210
	Mortality (%)	0	0	0	30	70	90
Land-based tank cultured	Recovery (sec ±SE)	1±0	2±2	22±36	729±829	538±924	575±810
	Mortality (%)	0	0	0	50	70	70

anesthetized fish from embanked population recovered within 69 seconds after exposure to air in 30 minutes. The fish population from wild stock showed delayed recoveries from the anesthesia. Mortalities of wild stocks were marked by 30, 50, and 70 % after dry exposures for 15, 20, and 30 minutes, respectively. Ten-minute dry exposure caused 40 % mortality in the fish from land-based tank. Similar results were obtained when the three populations were solely exposed to air (Table 5).

4. Discussion

Mass releases of hatchery-raised valued species take a considerable part of recent marine aquaculture because of the stagnant growth of captured fisheries and confined farming area in Korea. The practice, however, has a drawback namely a "substantial initial reduction" after release. This might be from untimely releasing of the seeds (Furuta 1996; Tanaka *et al.* 1999), predation due to behavioral change (Furuta 1998), and growth retardation (Houde 1987). This drawback would much significant if unhealthy seed are released.

Anesthetics are essentially used for reducing stress which can be traced from a variety of activities ranging from routine handling to delicate surgical operations (Marking and Meyer 1985; Gilderhus and Marking 1987; Hunn and Greer 1992; Massee *et al.* 1995). MS-222 has been shown to be useful for anesthetizing larval fish (Hikasa *et al.* 1986; Chapman *et al.* 1988; Chatain and Corrao 1992; Rust *et al.* 1993; Massee *et al.* 1995). The optimum concentrations of anesthetics including MS-222 can be influenced by species, water temperature, and other ambient physical and chemical characteristics, such as total disolved organic matter and alkalinity (Schoettger and Julin 1967, 1969; Piper *et al.* 1982; Hikata *et al.* 1986; Schnick *et al.* 1986; Gilderhus and Marking 1987; Mattson and Riple 1989). Satterfield and Flickinger (1995) established an efficient concentration of MS-222 for anesthesia at 99 mg/*l* with time to immobilization of averaged 211 seconds for short-term storage of walleye *Stizostedion vitreum*. They also found the lower concentration of MS-222, 66 mg/*l*, worked but it was deemed too slow with average working time of 383 seconds. There was no significant difference in the working concentration of MS-222 between 99 and 132 mg/*l*, but the latter caused delayed recovery from the anesthesia. Murai and Catacutan (1981) suggested the ranged concentrations of MS-222 from 100 to 200 mg/*l* for anesthetization of milkfish fingerlings *Chanos chanos*. However, Alvarez-Lajonchere and Garcia-Moreno (1982) found that the concentrations also vary with the growth stages of the fish, in which optimum concentration of MS-222 for the fingerling milkfish was suggested 30 mg/*l*.

In the present study, the recovery time after anesthesia at 100 mg/*l* ranged from 103 to 130 seconds. As far as sensitivity to the chemical is concerned, wild fish were most sensitive to anesthesia at 100 mg/*l*. The juvenile fish from embanked population showed strongest resistance and fastest recovery against the chemical, reminiscent of the strength at least against the anesthetic. This was further supported by the findings in the higher concentration that the animals from pond culture showed extremely delayed recovery time and high mortality at concentration 400 mg/*l*, whereas no mortality was noticed at the concentration.

For most situations for fish anesthesia, MS-222 concentrations are ranging from 10 to 1,000 mg/*l*, depending upon species, size of same species, anesthetizing method, and ambient parameters (Schoettger and Julin 1967; Hikata *et al.* 1986, Schnick *et al.* 1986; Gilderhus and Marking 1987; Massee *et al.* 1995; Mattson and Riple 1989; Jennings and Looney 1998; Smith *et al.* 1999; Waterstrat 1999). Specimens from earlier life stages are sensitive over later stages. All fish tested were not anesthetized at 50 mg/*l* MS-222 in an hour. In the working concentrations, smaller fish were much more sensitive to the chemical than larger ones. In the highest MS-222 concentration, 70 % and 10 % of 6 cm and 10 cm long fish failed to recovery from the anesthesia, respectively. These results reveal that bigger black rockfish are stronger than smaller ones in the anesthetizing tolerance.

The fish were anesthetized in the MS-222 concnetration of 100 mg/*l* as was suggested in the Murai and Catacutan's work (1981). Increased immersion time induced delayed recoveries. This was also significantly dependent on the fish types. Embanked fish immersed for 5 minutes recovered by 75 seconds, while the fish from wild and land-based tank recovered by 110 and 147 seconds, respectively. All fish from land-based tank died when the immersion duration reached 15 minutes, while no mortality was noted in the fish from embanked population. The wild fish showed 60 % mortality in the immersion duration. These results explain that embanked black rockfish are more resistant against anesthetics among the three fish populations. In the dry exposure after anesthesia, all the anesthetized fish from embanked population recovered from the anesthesia within 69 seconds after exposure to air in 30 minutes. The fish population from wild stock showed delayed recoveries from the anesthesia with mortalities of 30, 50, and 70 % after dry exposures 15, 20, and 30 minutes, respectively. Ten-minute dry exposure caused 40 % mortality in the fish from land-based tank. Similar results were obtained when the three populations were solely exposed to air, supporting the previous results in which the embanked fish populations were healthier over the other two populations.

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