

Effects of background color on seawater adaptation of masu salmon *Oncorhynchus masou*

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Abstract

The present study was undertaken to evaluate whether background color modulated the seawater adaptability of masu salmon. Juvenile masu salmon that were initially reared in fresh water were transferred to seawater tanks with either black or white backgrounds. Survival of the fish in these tanks was monitored over 30 days. Among 7 independent experiments, statistically lower survival rates were observed for fish in the black tanks compared to the white tanks in four out of the seven experiments. MSH is a typical peptide hormone whose release is facilitated against a black background in salmonid fishes. Next, the effects of synthetic MSH on the survival of masu salmon reared in a white tank containing seawater were examined. Implantation of a pellet containing the hormone caused death of all experimental fish within 56 days, whereas no mortality was observed in the control fish. These results suggest that environmental black backgrounds reduce the seawater adaptability of masu salmon.

Key words : Masu salmon; Black background; Melanocyte-stimulating hormone; Seawater adaptability

Introduction

In fish, neuroendocrine systems mediate changes in the photic environment as shown by the modifying effects of black and white backgrounds on the production of hormonal peptides and pigment migration and synthesis. For instance, a black background increases the plasma levels of α -melanocyte-stimulating hormone (α -MSH)¹⁻⁴⁾, which darkens the body color, whereas a white background increases the production of melanin-concentrating hormone (MCH), which causes the body color to become more pale⁵⁻⁹⁾. Thus, α -MSH and MCH, which are generated in the pituitary gland and hypothalamus, respectively, are representative hormones associated with body color changes in fish¹⁰⁾. In addition to body color changes, these peptides also participate in food intake as orexigenic and anorexigenic peptides, respectively, as has been shown in mammals¹¹⁻¹²⁾.

With regard to food intake, the roles of α -MSH and MCH are not yet fully understood in fish. However, some evidence suggests the possible involvement of MCH in food intake. Specifically, barfin flounder *Verasper moseri* reared in white tanks had higher *mch* expression and higher MCH levels in the brain and plasma¹³⁾, showed increased food intake¹⁴⁾, and grew faster¹⁵⁾ compared to conspecifics reared against a black background. These results suggest that the white background stimulated MCH production; elevated MCH levels then stimulated food intake, and finally, increased food intake resulted in somatic growth¹⁵⁾.

The stimulating effects of a white background on the growth of barfin flounder led us to investigate whether this phenomenon would also occur in other fish species. This assumption was assessed using anadromous masu salmon *Oncorhynchus masou*. In northern Japan, these fish spawn in rivers mainly from spring to summer. After remaining in their natal river for approximately

1.5 years, juvenile fish migrate downstream to the ocean¹⁶⁾. Based on the anadromous characters, the present study was originally undertaken to know if background color - white and black - would differentially influence masu salmon growth reared in fresh water and seawater. However, during the course of experiments, it appeared that background color could affect the survival of masu salmon. Thus the present paper reports the possible effects of background color on the seawater adaptation of masu salmon.

Materials and Methods

Fish

Masu salmon obtained from the Kamaishi Tobu Fisheries Cooperative Association (Kamaishi, Iwate, Japan) were used for fresh water to seawater transfer experiments at the Iwate Fisheries Technology Center (Kamaishi, Iwate, Japan) except for Experiment 1, which was done at the Association. Sea concentration was measured with a hand-held refractometer (IS/Mill-E, ATAGO Co., Ltd, Tokyo). Fish were fed a commercial pellet diet (Nosan Corporation, Yokohama, Japan). Measurement of fork length (FL) and body weight (BW) were performed under anesthesia with 0.05% 2-phenoxyethanol. FL and BW of the experimental fish and seawater temperature are shown in Table 1. All fish were reared under ambient photoperiod condition, i.e., these fish were reared indoor tanks and received daylight in combination with light from fluorescent lamps. Since we do not have a guideline for animal treatment in Iwate Fisheries Technology Center, all experiments were conducted according to the guideline for the care and use of animals which were used in Kitasato University where my partners shown in acknowledgement section belong to.

Rearing masu salmon in white or black tanks

Rearing in fresh water (Experiment 1) : Effects of

background color on somatic growth of masu salmon were examined in this experiment. In August 2004, masu salmon in a gray tank were transferred to a black or white tank (500 *l* each) at the Kamaishi Tobu Fisheries Cooperative Association. Each tank contained 10 fish. The FL and BW of fish in the black tank were 12.5 ± 0.2 cm and 24.9 ± 1.6 g, and those of fish in the white tank were 12.5 ± 0.1 cm and 24.6 ± 0.9 g. Fish were reared for 6 weeks and fed twice daily (09:30 and 15:30) until satiety. The water temperature was 11–12°C.

Transfer from fresh water to seawater (Experiment 2):

Effects of background color on seawater adaptation of masu salmon were examined in this experiment at Iwate Fisheries Technology Center. In April 2005, the sea concentration of a blue tank (3,000 *l*) containing a mass of fish was increased from 0% to 30% over one day. On the following day, the sea concentration was increased to 70%. After 2 days, the sea concentration was increased to 100%, and the fish were reared for an additional 5 days at this concentration. Subsequently, 28 fish were randomly removed and divided into 2 groups, and then transferred to a black seawater tank (500 *l*) or to a white seawater tank (500 *l*), where they were reared for 30 days. During seawater adaptation in the blue tank, they were not fed. In the black and white tanks containing seawater, small amounts of pellets were given occasionally, because this rearing experiment addressed to clarify the effects of background color on survival, not on growth, in seawater. This feeding was also applied to the experiments described below.

Transfer from fresh water to seawater (Experiment 3):

Effects of background color on seawater adaptation of masu salmon were examined again in this experiment at Iwate Fisheries Technology Center. In May 2005, the sea concentration of a blue tank (3,000 *l*) containing a mass of fish was increased from 0% to 30% over 7 days. After 1 week, the sea concentration was increased to

65%, and the fish were reared at this concentration for a further 7 days. On the 8th day at 65% seawater, the concentration was increased to 80%, followed by 100% on the next day (9th day). On the 10th day, 60 fish were randomly removed and divided into 2 groups, and then transferred to a black or white seawater tank (500 *l*), in which they were reared for 30 days.

Transfer from fresh water to seawater (Experiments 4–8): Base on experiments 2 and 3, effects of background color on seawater adaptation of masu salmon were examined again in this experiment at Iwate Fisheries Technology Center. Initially, masu salmon reared in a blue freshwater tank (3,000 *l*) were randomly selected and transferred to a black or white tank (500 *l*) containing fresh water. Each tank contained 15 fish. Shortly after the transfer, the sea concentration of these tanks was increased to 70%, and the fish were reared for 3 days at this concentration. Subsequently, the concentration was increased to 100%, and the fish were reared for an additional 30 days. These experiments were conducted in April 2007 (experiment 4), May 2007 (experiment 5), October 2007 (experiment 6), January 2008 (experiment 7), and March 2008 (experiment 8), because we have limited number of experiment tank, so it was impossible to perform these five raising experiments simultaneously.

Administration of Des-Ac- α -MSH

Preparation of MSH pellet : Des-Ac- α -MSH was synthesized according to methods described previously¹⁷⁻¹⁸⁾. A cholesterol pellet containing Des-Ac- α -MSH (MSH pellet) was prepared according to the method of Lee et al. (1986)¹⁹⁾ with slight modifications. In brief, Des-Ac- α -MSH (13.4 mg) was dissolved in 3 *ml* of 70% ethanol and mixed completely with cholesterol powder (1 g; Wako, Osaka, Japan). After drying overnight, the resulting pellet was pulverized and then thoroughly mixed with 150 mg of cocoa butter. The resultant material was compressed into pellets weighing approximately 25 mg, which contained approximately 280 μ g of Des-Ac- α -MSH. It was demonstrated that this pellet was effective to enhance pigmentation of barfin flounder²⁰⁾.

Implantation of MSH pellet and rearing in seawater

Masu salmon were reared in a blue seawater tank (3,000 *l*) 4 months prior to implantation of MSH pellets. Fourteen fish were randomly selected; on the day of implantation in October 2005, the FL and BW were 16.0 \pm 0.5 cm and 51.2 \pm 5.1 g. These fish were divided into two groups. In one group ($n = 7$), the MSH pellet was implanted intramuscularly (one pellet/fish) using a Tag injector (K-Engineering, Tokyo, Japan) under anesthesia with 0.05% 2-phenoxyethanol. Control fish

Table 1. Experimental conditions and survival rate of masu salmon in seawater under white and black background.

Experiment	age	Folk length	Body weight	The number of fish	Seawater temperature (°C)	Date	Survival rate (%) at day15 in background		Survival rate (%) at day 30 in background	
		(cm)	(g)				white	black	white	black
2	0+	19.9 \pm 0.2	77.1 \pm 2.3	28	6.9–9.1	April 2005	100	29 ***	93	7 ****
3	1+	10.8 \pm 0.2	15.4 \pm 0.4	60	8.9–10.9	May 2005	57	27 *	57	20 **
4	0+	18.7 \pm 0.2	74.6 \pm 6.3	30	7.7–9.6	April 2007	67	20 *	53	20 (NS)
5	1+	19.7 \pm 0.3	84.4 \pm 5.2	30	9.4–10.9	May 2007	80	33 *	73	7 ***
6	1+	12.5 \pm 0.1	21.5 \pm 0.5	30	14.3–16.9	October 2007	33	13 (NS)	20	7 (NS)
7	1+	17.4 \pm 0.4	62.8 \pm 3.8	30	6.5–9.1	January 2008	73	33 (NS)	53	7 *
8	1+	19.0 \pm 0.4	81.0 \pm 4.6	30	4.6–5.6	March 2008	40	40 (NS)	20	27 (NS)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, Yates' chi-square test or Fisher's exact test. Statistical difference was analyzed on days 15 and 30.

received cholesterol pellets without De-Ac- α -MSH. Fish were reared in white indoor running seawater tanks (500 l) for 56 days. They were fed twice daily (8:30 and 16:00) until satiety. The seawater temperature during this experiment was 13.0–18.5°C.

Statistics

All data are shown as means \pm SEM. Statistical analyses were performed using StatView for Windows 5.0 (SAS Institute, Cary, NC, USA). Student's *t*-test was used for statistical analysis of the effect of tank color on FL and BW. Differences in survivability were determined by Yates' chi-square test when all expected values of the cases were >5 , or by Fisher's exact test when any expected values were <5 . Statistical significance was determined at the 5% level.

Results

Effects of tank color on somatic growth

Initially, the effects of tank color on the somatic growth of masu salmon in fresh water were examined (Experiment 1). After rearing in fresh water for 6 weeks, no significant difference was observed in both the FL and BW between the fish reared in white tank and black tank of the fish (FL; 13.9 ± 0.2 cm, BW; 29.4 ± 1.3 g ($n = 8$) for the fish in a white tank, and FL; 13.5 ± 0.2 cm, BW; 27.4 ± 1.5 g ($n = 10$) for the fish in a black tank). The effects of tank color on the somatic growth of masu salmon were also examined using fish in seawater (Experiment 2). Unexpectedly, most of the fish reared in the black tank died during the 30 days in seawater in experiment 2 (Fig. 1A). The survival rate in the black tank on days 15 and 30 were 29% and 7%, respectively, which was significantly lower ($P < 0.001$) than that (100% and 93%, respectively) of fish in the white tank (Table 1). A possible cause of this high mortality was that the acclimatizing procedure in experiment 2 was inappropriate to facilitate the survival of masu salmon

in seawater; therefore experiment 3 using a modified acclimatizing procedure was done in May. Subsequently, similar results were obtained as in experiment 2 (Fig. 1B), in which fish reared in the black tank showed lower survival rates than those in the white tank (Table 1).

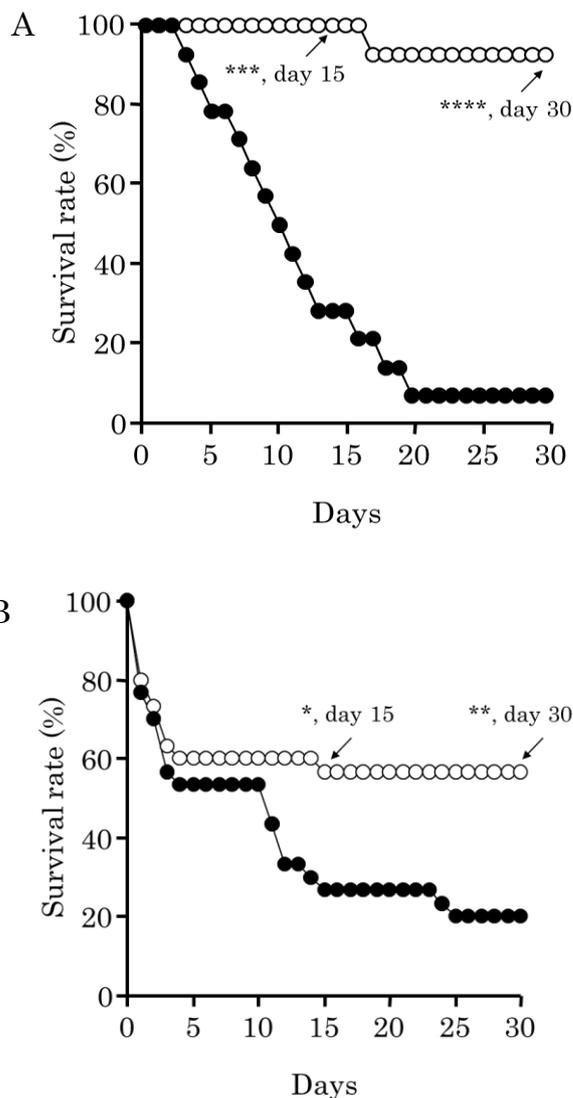


Fig. 1. Survival rate of masu salmon in black or white seawater tanks in experiment 2. Black and white circles show the survival rates in black and white tanks, respectively. Statistical analysis was done on days 15 and 30. (A) Initially, each tank contained 14 fish. Significant differences were observed between the black and white tanks on days 15 ($p < 0.001$) and 30 ($p < 0.0001$). (B) Initially, each tank contained 30 fish. Significant differences were observed between the black and white tanks on day 15 ($p < 0.05$) and 30 ($p < 0.01$). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

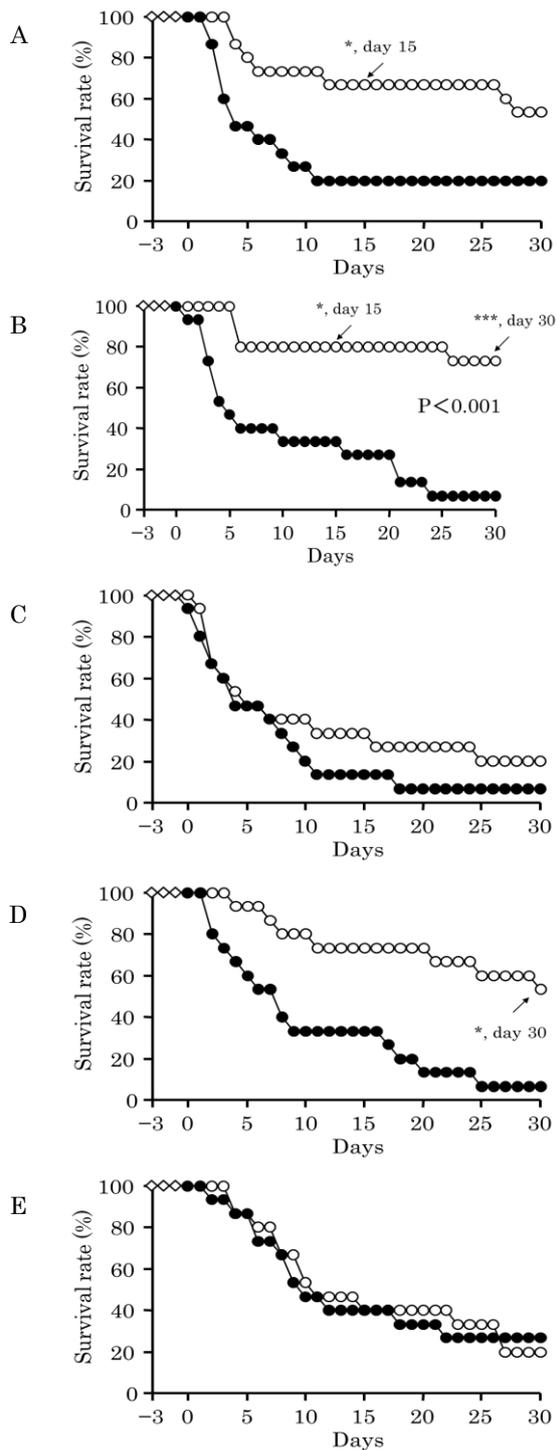


Fig. 2. Survival rate of masu salmon in black or white seawater tanks in experiments 4 (A), 5 (B), 6 (C), 7 (D), and 8 (E). Diamonds show the survival rate in 70% seawater. Black and white circles show the survival rates in black and white tanks, respectively. Statistical analysis was done on days 15 and 30. Initially, each tank contained 15 fish. Significant differences were observed on days 15 and 30 in experiments 4, 5 and 7. *: $p < 0.05$, ***: $p < 0.001$.

Effects of tank color on seawater adaptation

Fish were transferred from fresh water to seawater following the same procedures used for experiment 2 and 3. The major differences were the seasons during which the experiments were conducted and the body size of fish (Table 1). Excluding experiment 8 (Fig. 2E), which was performed in March, all the results of rearing fish in seawater showed lower survival rates among fish reared in black tanks than those reared in white tanks (Figs. 2A–D). Significant differences in the survival rates were observed in experiments 4 and 5 on day 15, and experiments 5 and 7 on day 30.

Effects of Des-Ac- α -MSH on seawater adaptation

After implanting MSH pellets, masu salmon were reared in a white tank containing running seawater. No deaths were observed over 56 days in control fish with implanted pellets containing no Des-Ac- α -MSH (Fig. 3).

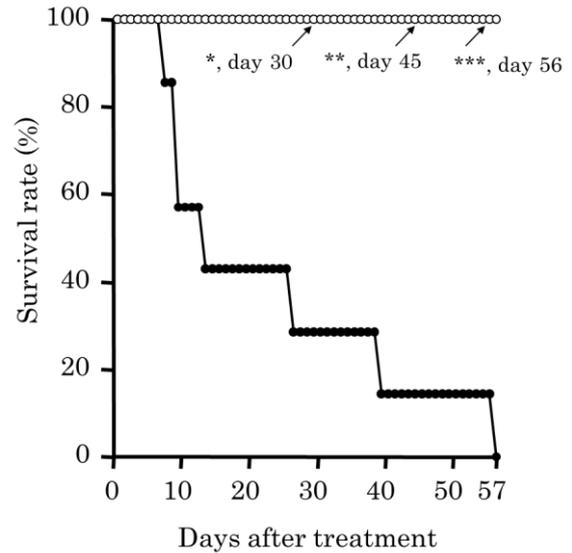


Fig. 3. Survival rate of masu salmon implanted with MSH pellets in a seawater tank with a white background. Black and white circles show the survival rates of MSH-treated and control fish, respectively. Statistical analysis was done on days 30, 45 and 56. Initially, each tank contained 7 fish. Significant differences were observed between the two groups on days 30 ($p < 0.05$), 45 ($p < 0.01$), and 56 ($p < 0.001$). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

In contrast, the fish with implanted MSH pellets were unable to survive in seawater. Fish died intermittently on days 7, 9, 13, 26, 39, and finally no fish survived. Statistical analyses done on days 30 ($P < 0.05$), 45 ($P < 0.01$) and 56 ($P < 0.001$) showed significant difference between MSH-implanted fish and control fish. These results indicated that Des-Ac- α -MSH had negative effects on the seawater adaptation of masu salmon.

Discussion

The present study reports the effects of tank color (black or white) on the somatic growth and seawater adaptability of masu salmon. In fresh water, difference in tank color (white or black) did not affect the growth of masu salmon during 6 week raising. These results are different from previous results obtained using barfin flounder¹⁵⁾, in which barfin flounder reared in white tank grew faster than those reared in black tank. Although experiment 2 and 3 were performed to compare the effects of background color on the growth of masu salmon in seawater, it was unable to accomplish these because of the remarkably high mortality of fish in the black tank. However, these results did suggest the alternative possibility that background color may affect seawater adaptation of masu salmon. Both in the experiment 2 (Fig. 2A) and 3 (Fig.2 B), masu salmon reared in white tank showed higher survival rates than those reared in black tank. In other words, black background could reduce survival rates of masu salmon compared to the fish reared in white tank. Thus, similar examinations were repeated an additional 5 times to evaluate the effects of tank color on fish survival.

A series of data showed that the survival rates of fish reared in black tanks were relatively low compared to those reared in white tanks, although the results obtained from all experiments did not always show statistically significant differences in the survival rates between the fish reared in black tanks and white tanks.

Seasonal differences related to the timing of seaward migration may be related to this inconsistency. For example, in southern Hokkaido, juvenile masu salmon begin to develop the characteristic silver and white coloration of smolts in March and rapidly turns into smolts in April¹⁶⁾. According to this morphological changes, juvenile masu salmon may have better ability of seawater adaptation in spring than other seasons. The present study indicate white background well contribute to seawater adaption of masu salmon in April and May (Table 1), which may partially be related to timing of smoltification. Nevertheless, the black background color showed negative effects on the seawater adaptability of masu salmon in all experiment points.

α -MSH is a typical peptide hormone whose release is facilitated against a black background in salmonid fishes¹⁻⁴⁾, suggesting that this hormone may be associated with relatively high mortality of masu salmon in black tank; therefore, the effects of α -MSH on the seawater adaptability of masu salmon was examined in the present study. Des-Ac- α -MSH was used because MSH pellets transplanted into barfin flounder were previously shown to work well²⁰⁾ and amino acid sequence of the peptide is well conserved among teleosts²¹⁾. The result—high mortality of the fish received exogenous MSH—suggests that a black background color possibly inhibits the seawater adaptation of masu salmon by way of Des-Ac- α -MSH.

Des-Ac- α -MSH is generated from the precursor protein proopiomelanocortin (POMC) in the pituitary gland^{10, 22)}. In addition to Des-Ac- α -MSH, which has no acetyl group, α -MSH and diacetyl (Di-Ac)- α -MSH, which have one and two acetyl groups at the N-terminal, respectively, are also generated in teleost fishes^{18, 23-25)}. These peptides exhibit their activities via melanocortin receptors, which are widely distributed throughout the body^{21, 26, 27)}, suggesting that Des-Ac- α -MSH as well as α -MSH and Di-Ac- α -MSH could modulate the functions

of cells in the tissues that participate in ion and water movement, although, to my knowledge, no reports demonstrating relationship between MSH and osmoregulation have been published. Additional interpretation could be proposed for the effects of Des-Ac- α -MSH. In this experiment, all of the experimental fish were reared in white background color, and hence these fish probably increased MCH levels. Thereafter, MSH levels were artificially increased by the implantation. Thus it is speculated that the fish had high levels of both MCH and MSH, and such unnatural hormone levels influenced the survival of the fish. In this context, MSH alone is not the mediator for the death of fish in the seawater. Further experiments are necessary to confirm the function of MSH.

The present investigation suggests that background color may modulate the seawater adaptability of masu salmon. Specifically, the survival rates of masu salmon reared in a black tank containing seawater were low compared to those of fish reared in a white tank. When Des-Ac- α -MSH was administered via implantation of an MSH pellet to masu salmon reared in a white tank containing seawater, all fish died within 57 days. This result suggests that the peptide lowered the ability of the fish to adapt to seawater by directly or indirectly modulating the functions of tissues or cells associated with ion and water movement.

Acknowledgments

I am deeply grateful to Prof. Akiyoshi Takahashi, Prof. Masafumi Amano, Prof. Hiroaki Chiba, and Prof. Kanta Mizusawa of Kitasato University for their generous and patient guidance over many years, which has been invaluable in shaping my research foundations. I record my sincere appreciation here with the utmost respect. I also acknowledge Mr Daisuke Kato, Iwate Fisheries Technology Center, and Mr Toshiki Satodate, Kamaishi Tobu Fisheries Cooperative Association, for their

technical assistance.

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