Carbohydrate and Amino Acid Metabolism in the Liver of Masu Salmon *Oncorhynchus masou* in the Early Period of Seawater Migration

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About 100,000 individuals of masu salmon *Oncorhynchus masou* were released into Ukai River in Noto Peninsula. Then the released fish were collected in the river and coastal zone of the peninsula, and the hepatic enzyme activities were determined to investigate the metabolic response in the liver during the migration from river to sea. The activities of amino acid degrading enzymes (alanine aminotransferase and aspartate aminotransferase) and gluconeogenic enzyme (fructose-1,6-diphosphatase) were significantly higher in the fish caught in the sea than in those caught in the river, whereas no remarkable difference in the lipogenic and glycolytic enzyme activities was found between the fish caught in the river and those caught in the sea. These results suggest that amino acid degradation and gluconeogenesis in the liver of masu salmon were enhanced in the early period of seawater migration.

Key words: masu salmon, migration, hepatic enzyme, metabolism, seawater adaptation

Masu salmon occur in and around the Sea of Japan and they are an important fisheries resource. Therefore, hatchery enhancement programs of masu salmon have been conducted in Japan. In these programs, smolts are released to avoid competition for prey in river because smolts have a higher adaptability to seawater than parrs and rapidly migrate down river to sea.^{1,2)} When the fish migrate from river to sea, some hormones such as cortisol, growth hormone, and thyroid hormone play an important role in osmoregulation.¹⁻³⁾ These hormones are also involved in the intermediary metabolism in fish. Therefore, the osmoregulatory process is presumably accompanied by some metabolic changes. There are several studies on the effects of ambient salinity on protein, lipid, and carbohydrate metabolism in fish reared under laboratory conditions.⁴⁻⁶⁾ However, little information is available on the metabolic response to seawater under natural environment.

Artificially hatched masu salmon have been released into Ukai River in Noto Peninsula every year since 1986. All the released fish are marked by clipping the adipose fin to distinguish them from wild fish, and they are frequently caught by set-net fishery in the coastal waters around the peninsula. Therefore, this release program is useful for the investigation of the biochemical response of the released fish during migration. In the present study, the released fish were collected in the river and sea, and the activities of hepatic enzymes relating to carbohydrate, amino acid, and lipid metabolism were determined to study the metabolic response of the released fish to natural seawater environment.

Materials and Methods

Fish, Research Waters, and Sampling Methods

About 100,000 individuals of masu salmon *Oncorhynchus masou* averaging 36.9 ± 0.8 g in body weight and 15.7 ± 0.1 cm in fork length were released three kilometers upstream of the mouth of the Ukai River in Noto Peninsula

for three days from March 13, 1996. These fish had been reared for 17 months at the Ishikawa Prefectural Inland Water Fisheries Center, and their adipose fins had been clipped off as marking. Almost all fish were in the smolt stage when they were released. It has been previously suggested that the released fish migrate down the river to the sea and then move northward along the coastal zone of Noto Peninsula.⁷⁾ Therefore, the sampling was conducted along the migratory route (Fig. 1). The released fish were collected with a casting net at the release point, a seine at the river mouth, and two commercial set-nets (set-net A and B) in the sea. Set-net A and B were located near the river mouth and at the tip of the peninsula, respectively.

Fig. 1. Location of sampling stations.

The released fish caught by the set-net were numbered every day by fishermen. Water temperature was measured at four stations in the river on April 2 and 19. Water temperature in the river was between 6.1° and 9.2°C. According to the ocean observation conducted on March 21 and April 8, water temperature of the surface layer (≤ 20 m depth) of the coastal waters ranged from 9.7° to 10.4°C. Thus, water temperature was slightly higher in the sea than in the river.

Analytical Methods

The sampling was conducted on April 1 at the release point, on April 2 at the river mouth, on April 3 at set-net A, and on April 19 and 21 at set-net B. The fish were transported in an ice box to our laboratory, and the liver and skin with scales on one side of fish were removed and stored at -80°C until analyses.

The liver was homogenized with 9 volumes of cold water

by a Physcotron homogenizer (Nition Co., Ltd., NS-50) and centrifuged at 4,000 rpm for ten minutes at 4°C . The supernatant was used to analyze the following enzymes: aspartate aminotransferase (Asp-AT, EC 2.6.1.1); alanine aminotransferase (Ala-AT, EC 2.6.1.2); glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49); phosphogluconate dehydrogenase (PGDH, EC 1.1.1.44); phosphofructokinase (PFK, EC 2.7.1.11); and fructose-1,6-diphosphatase (FDPase, EC 3.1.3.11). G6PDH, PGDH, PFK, and FDPase activities were assayed by the methods described previously.^{8,9} Asp-AT and Ala-AT activities were determined by the Karman method.¹⁰ The enzyme reaction was performed at 25°C . The activity was expressed as units (μmoles of substrate or coenzyme converted per min) per liver per 100 g body weight. The guanine level in the skin was determined by the enzymatic method.¹¹ Results were represented as mean $\pm$ SEM. Statistical analyses of the results were done by Mann-Whitney *U*-test ($P < 0.05$).

Results

At set-net A, the released fish began to be caught on March 17, and they were caught until May 9 (Fig. 2). The peak day of the catch at set-net A was March 20. At set-net B, the released fish were caught from April 16 to May 16. The results indicate that the released fish migrated down to the sea immediately after the release and then moved to the tip of the peninsula after one month.

The biological data and skin guanine level of the fish used in the experiment are shown in Table 1. The ratio of male to female was higher in the river than in the sea. The means of body weight and fork length were higher in the fish caught in the sea (set-net A and B) than in those caught in the river (release point and river mouth). Hepatosomatic index was also high in the fish caught in the sea. The skin guanine level was low in the fish caught at the release point.

The results of the hepatic enzyme activities are shown in

Fig. 2. Number of released fish caught at set-net A and B.

Fig. 3. Hepatic enzyme activities of the released fish caught in the river (release point and river mouth) and sea (set-net A and B). Values are means $\pm$ SEM ($n = 15$). *Significantly ($P < 0.05$) different from the fish caught at the release point.

Table 1. Biological data and skin guanine level of fish used in the experiment

	Sampling date	Number of fish (male : female)	Body weight (g)	Fork length (cm)	HSI* ¹ (%)	Condition factor* ²	Guanine level ($\mu\text{mol/g skin}$)
Release point	1996/4/1	13 : 2	47.4 $\pm$ 3.2* ³	16.7 $\pm$ 0.4* ³	1.26 $\pm$ 0.10* ³	9.96 $\pm$ 0.14* ³	62 $\pm$ 4* ⁴
River mouth	1996/4/2	11 : 4	43.4 $\pm$ 3.4	16.4 $\pm$ 0.3	1.46 $\pm$ 0.06	9.52 $\pm$ 0.21	100 $\pm$ 10
Set-net A	1996/4/3	7 : 8	59.0 $\pm$ 3.8	18.0 $\pm$ 0.3	1.81 $\pm$ 0.04	9.94 $\pm$ 0.17	122 $\pm$ 8
Set-net B	1996/4/19,21	9 : 6	81.1 $\pm$ 5.5	19.6 $\pm$ 0.4	1.72 $\pm$ 0.06	10.5 $\pm$ 0.25	111 $\pm$ 5

*¹ Hepatosomatic index = (liver weight/body weight) $\times$ 100.*² Condition factor = (body weight/fork length³) $\times$ 1000.*³ Mean $\pm$ SEM (n = 15).*⁴ Mean $\pm$ SEM (n = 10). **Fig. 4.** Relationship between the skin guanine level and hepatic G6PDH and PGDH activities of the released fish (n = 40).

Fig. 3. Since no significant difference between male and female was detected in all the enzyme activities except FDPase activities of the fish caught at set-net A, the data from male and female were combined in the figure. Ala-AT and FDPase activities were significantly higher in the fish caught in the sea (set-net A and B) than in those caught in the river (release point and river mouth). Asp-AT activity was slightly high in the fish caught in the sea (set-net A and B). G6PDH and PGDH activities were significantly low in the fish caught at the river mouth, and PFK activity was significantly high in the fish caught at set-net A, while no remarkable difference in G6PDH, PGDH, and PFK activities was found between the fish caught in the river and those caught in the sea. In Fig. 4, G6PDH and PGDH activities are plotted against the skin guanine level. G6PDH and PGDH activities inversely correlated with skin guanine level, whereas the other enzyme activities did not correlate well with the guanine level (data not shown).

Discussion

In the present study, the activities of hepatic Ala-AT, Asp-AT, and FDPase, which are involved in amino acid degradation and gluconeogenesis, were higher in the fish caught in the sea (set-net A and B) than in those caught in the river (release point and river mouth). This result suggests that amino acid degradation and gluconeogenesis in

the liver of the released fish were enhanced in the early period of seawater migration. Therefore, the requirement of the fish for protein as an energy source and gluconeogenic precursor was presumably increased by entry into seawater. In fact, it has been shown in rainbow trout that the fish reared in plasma-hyperosmotic water have a higher requirement for dietary protein than those reared in freshwater.^{12,13} On the other hand, at the mouth of Ukai River, saline wedge enters the river. According to the previous research,¹⁴ the bottom water at the river mouth is often full seawater, whereas the salinity of surface water is less than 14 ppt. In the present study, no significant increase in Ala-AT, Asp-AT, and FDPase activities was detected in the fish caught at the river mouth. Therefore, the released fish might be distributed in an upper layer of the river mouth.

The increase in the hepatic Ala-AT, Asp-AT, and FDPase activities may be explained in terms of the metabolic effects of cortisol, which promotes seawater tolerance of fish.³ Cortisol is generally thought to stimulate gluconeogenesis and amino acid degradation in fish as in mammals. It has been demonstrated that cortisol administration raises hepatic Ala-AT, Asp-AT, and FDPase activities of salmonids.^{15,16} Moreover, the plasma cortisol level in fish has been shown to increase after entry into seawater.^{2,17,18} Therefore, cortisol may be related to the activation of hepatic Ala-AT, Asp-AT, and FDPase of the released fish under natural seawater environment.

Nutrient composition of food is also an important factor affecting hepatic enzyme activities in fish. The activities of gluconeogenic and amino acid degrading enzymes have been shown to be high in the fish received a low carbohydrate/high protein diet.^{19,20} In regard to the prey of masu salmon, it has been shown that some aquatic insects such as ephemeroptera, plecoptera, and trichoptera occur in Ukai River.¹⁴ Moreover, according to the previous research of stomach contents, the released fish mainly preyed on pisces, amphipods, and euphausiids in the coastal waters (unpublished data). In the present study, proximate composition of the prey was not determined. However, the proximate composition of prey of pacific salmon has been reviewed in detail by Higgs *et al.*²¹ According to the review, the carbohydrate level in marine prey is generally lower than that in freshwater prey. Therefore, there is a possibility that the low carbohydrate level in marine prey affected the gluconeogenic and amino acid degrading enzyme activities. However, the enzymatic response to the nutrient composition of food is thought to depend on ration size.^{22,23} The ration size of the released

fish was unknown in the present study. Hence, the effects of nutrient composition of natural food on the enzyme activities could not be estimated. Besides, water temperature also affects the enzyme activities in fish. It has been shown that low water temperature causes the activation of several enzymes in fish.^{24,25)} In the present study, however, water temperature was higher in the sea than in the river. Therefore, the increase in the hepatic Ala-AT, Asp-AT, and FDPase activities can not be explained by the difference in water temperature.

In anadromous salmonids, smoltification is a complex phenomenon involving behavioral, morphological, and physiological changes, though it is visually characterized by body silvering due to the accumulation of guanine in the skin.²⁶⁾ On the other hand, it is well known that the body lipid level of several salmonids including masu salmon decreases during smoltification.^{2,27-29)} The decrease in body lipid level is thought to be due to the mobilization of body lipid.^{2,28)} In the present study, however, the activities of hepatic G6PDH and PGDH, which are involved in lipogenesis, decreased with an increasing level of skin guanine. This result suggests that low lipogenic activity is also associated with the reduction of body lipid level during smoltification.

In hatchery enhancement, it is of importance to evaluate the adaptability of artificially produced fish to natural environment. Recently, there are some attempts to use several biochemical parameters for evaluation of the adaptational status of released fish.^{30,31)} The present study showed that the hepatic Ala-AT, Asp-AT, and FDPase activities of the released fish were increased by entry into seawater. Therefore, the enzyme analysis is possibly useful in monitoring of the adaptational status of the released masu salmon to natural seawater environment.

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