Short paper

Assessment of nutritional conditions using kidney melano-macrophage density in hatchery-reared juvenile masu salmon *Oncorhynchus masou* released into a stream

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Abstract: Underyearling hatchery-reared masu salmon *Oncorhynchus masou* are released into streams in spring to increase the coastal masu salmon catch in Hokkaido. It is important to study the nutritional conditions of the juveniles after release in order to evaluate the hatchery program. Our study examined whether kidney melano-macrophage density (MMD) can monitor nutritional conditions of the juveniles after release. Significant negative correlations were found between MMD and liver triglyceride content, which reflects lipid storage as energy source, in recaptured juveniles and between the MMD and fish density in a stream from spring to summer. In conclusion, this study suggested that MMD was a negative indicator of nutritional conditions in the released juveniles. Meanwhile, the nutritional conditions of released juveniles worsened depending on fish density from spring to summer.

Key words: Masu salmon, Kidney, Nutritional condition, Melano-macrophage, Triglyceride

Anadromous masu salmon *Oncorhynchus masou* is one of the most important coastal fishery resources in Hokkaido of northern Japan. However, the coastal masu salmon catch has been in decline for the last 10 years (Nagata and Kaeriyama 2003). Therefore, a program to release underyearling hatchery-reared juvenile into streams in spring has been implemented in Hokkaido. It is significant to examine the nutritional conditions of the released juveniles in order to evaluate the program, since the success of the program depends on there being sufficient feed to sustain the growth of the juveniles in the stream. In general, growth and survival in stream salmonid juvenile are apparently density-dependent (Hume and Parkinson 1987). Misaka *et al.* (2007) revealed that there was a significant negative correlation between the mean triglyceride (TG) levels in the liver of juveniles and juvenile density. The levels of the liver TG, which is lipid storage as an energy source in fish, are appropriate as an index to evaluate nutritional conditions in wild and hatchery-reared juvenile masu salmon, since the decreased levels were induced by artificial starvation (Misaka *et al*. 2004). However, it is not sufficient to assess the nutritional conditions of the released juveniles using only the TG levels in the liver.

Melano-macrophage (MM), which contains the pigments hemosiderin, lipofuscin ceroid, and melanin and resembles macrophages ultrastructurally, is often observed in the kidney and spleen of teleosts. The aggregation of MMs in the kidney during starvation is considered to relate to humoral and inflammatory responses, the storage, destruction and detoxification of exogenous and endogenous substances, or iron recycling in the kidney (Agius and Roberts 1981). Increase in

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aggregation of MM during hypo-nutritional conditions originating from artificial starvation has been observed in juvenile rainbow trout *Oncorhynchus mykiss* (Agius and Roberts 1981) and juvenile masu salmon (Mizuno *et al*. 2002). Mizuno *et al*. (2002) reported that the MM density (MMD), the percentage of the area of melanin granules per cross-section area of kidney, which was calculated by image analysis using paraffin section of the kidney, reflected the nutritional conditions in wild and hatchery-reared juvenile masu salmon. In the present study, the relationship between liver TG content and kidney MMD and between the values of these two parameters and fish density were examined from spring to summer in hatchery-reared juvenile masu salmon released into a stream in spring in order to clarify whether MMD can be used as a marker to monitor the nutritional conditions of the released juveniles.

Utabetsu River is located in southern Hokkaido and flows into the Pacific Ocean (Fig. 1). The river is a year-round no-fishing area. In Kami-Utabetsu River, 8.2 km upstream from the mouth of Utabetsu River, there is a 550-m section between two dams that prevents wild masu salmon from migrating upstream (Fig. 1). In this section, six stations to examine the fish density and nutritional conditions of released juvenile were established at about 100- to 150-m spaces (Fig. 1). All stations that contain both pool and rapids were given a number in order from upstream to downstream. The average surface area and maximum depth of stations during the experiment were 34.91 to 100.97 m^2 and 0.45 to 1.27 m, respectively. Previous to this study, no juvenile masu salmon were found in this section, although a few yearling residual males, which originated from juvenile released in 2001, were observed. On May 1, 2002, ten thousand juveniles (fork length 5.31 ± 0.48 cm; mean ± standard deviation, n=50), which hatched and were reared in Erimo Research Branch of the Hokkaido Fish Hatchery (Present Institution: Utabetsu Hatchery of

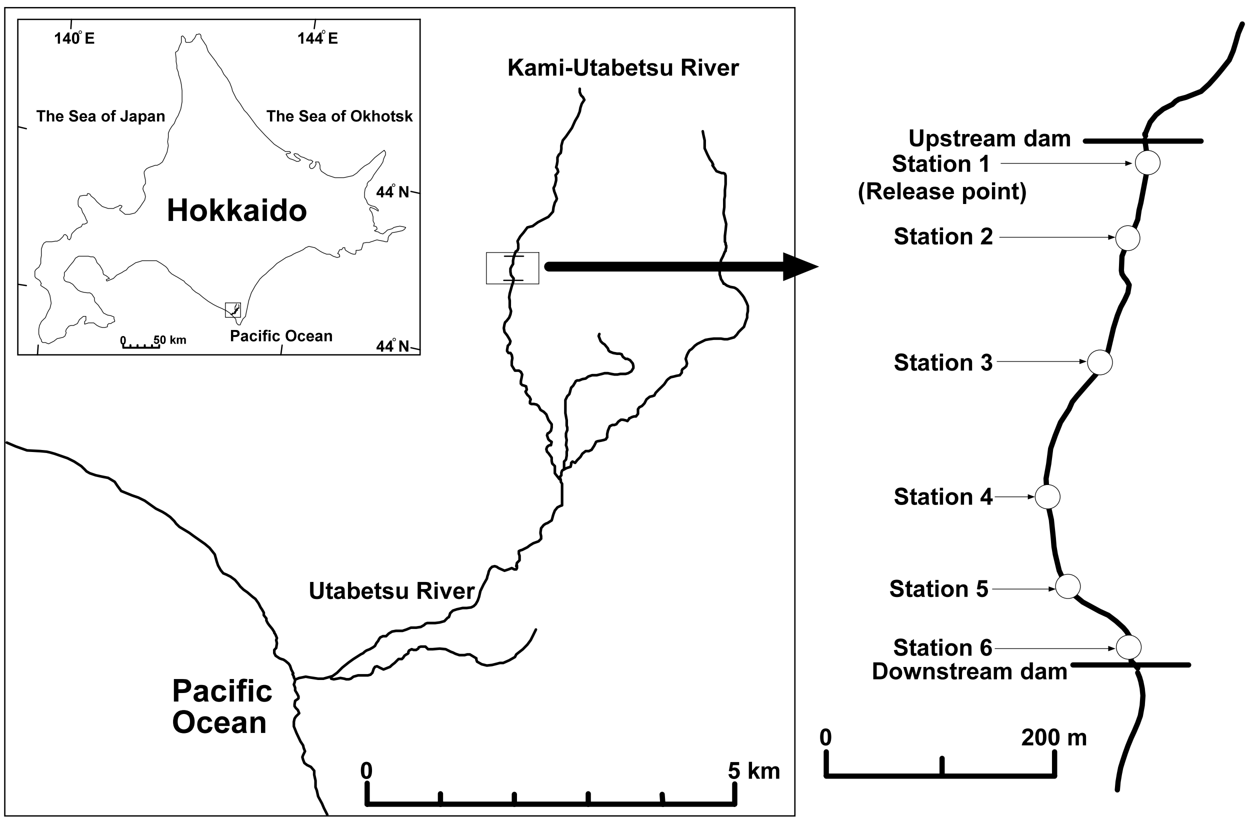

Fig. 1 Map showing Utabetsu River in southern Hokkaido and sampling stations in Kami-Utabetsu River.

Table 1 Total number and mean fork length of masu salmon captured in May and July, 2002

Sampling time	Underyearling fish		Yearling residual male	
	Number	Fork length (cm)	Number	Fork length (cm)
May, 30	404	6.88 ± 0.71	4	16.10 ± 1.24
July, 30	155	10.07 ± 1.23	2	18.5, 20.5

Data are expressed as mean ± standard deviation in all fish except for yearling residual male in July. The two numerals in yearling resident fish in July indicate surveyed values.

Hidaka Salmon Propagation Association), were released at the Station 1 (Fig. 1). On May 30 and July 30, 2002, fish were caught twice over using both an electrofisher (Model 12-B Backpack Electrofisher, Smith-Root Inc., Vancouver, BC, Canada) and a cast net at all stations. After anesthesia of the fish using 2-phenoxyethanol, the fork length was measured. The fish numbers at each station were estimated by the double-pass removal method (Seber and LeCren 1967). The fish density at each station was calculated by dividing estimated number of juvenile and yearling residual males by surface area of the station. Ten to twenty juveniles per station were randomly sampled for analysis of MMD and TG levels and the rest were released again. The region above the right ventral fin of the kidney from 3 to 10 juveniles was dissected and placed in 4 % paraformaldehyde in 0.1 mol/l phosphate buffer for 24 h. After dehydration and paraffin-embedding of the sample, hematoxylin-eosin double staining was performed on 5 μm-thick paraffin sections. The image analysis method for analyzing MMD is described in Mizuno *et al*. (2002). The MMD was shown as a percentage of the area of melanin granules per cross-section area of kidney. The 7 to 10 juveniles were frozen on dry ice and stored at -80℃ until TG analysis. TG levels in the liver were analyzed according to the method described in Misaka *et al*. (2004). TG level in the liver was expressed as a percentage of TG weight to total liver weight. Correlation analysis between mean MMD and mean TG levels and between these parameters and fish density was performed by Spearman's rank correlation. Statistical significance was accepted when $P<0.05$.

Underyearling juveniles were recaptured at all stations in all sampling times except for Station 5 in July, while yearling residual males were found at Station 1 and 2 in May and Station 1 in July. Mean fork lengths of

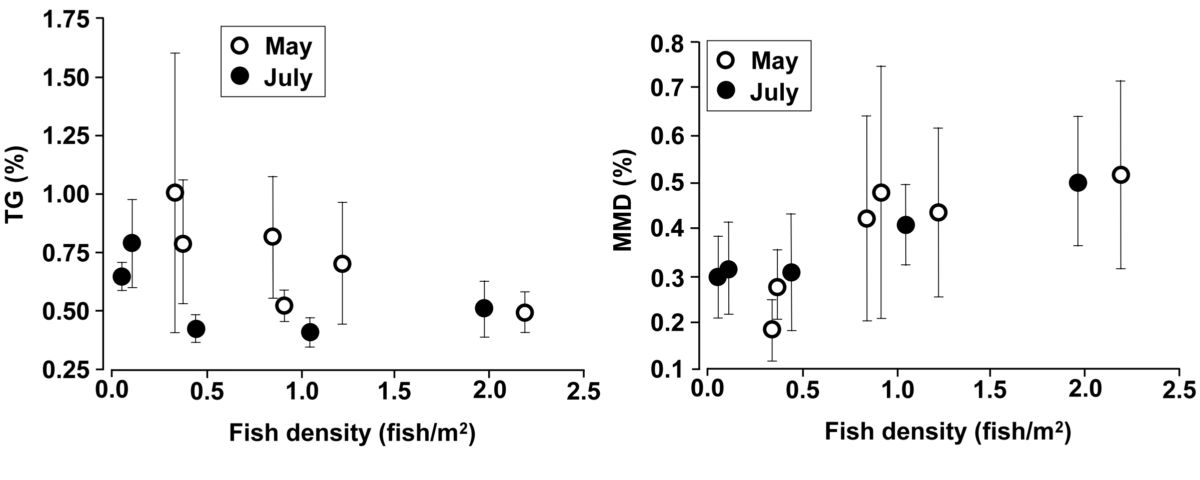

Fig. 2 Relationship between fish density and liver triglyceride (TG) level in juvenile masu salmon (left side) and between fish density and kidney melano-macrophage density (MMD) in juvenile masu salmon (right side). Values of TG levels and MMD are shown as mean standard deviation (TG level: n=7-10; MMD: n=3-10). Data at Station 5 in July are not indicated in this figure, since there were no fish at Station 5 in July.

underyearling juveniles were 6.88 ± 0.71 cm in May and 10.07 ± 1.23 cm in July (Table 1). Estimated fish density ranged from 0.33 to 2.18 fish/m^2 in May and 0.04 to 1.96 fish/m^2 in July (Fig. 2). Growth increments in wild and released underyearling masu salmon juveniles are reported to decrease as population density increases (Nagata, 1989). Moreover, Hume and Parkinson (1987) reported that density at >0.7 fish/m^2 in the British Columbia stream in Canada resulted in increased mortality in rainbow trout, which may indicate that there are some juveniles with hypo-nutritional conditions in the present study. Mean MMD in the kidney was observed between 0.19 and 0.52% in May and between 0.30 and 0.50% in July (Fig. 2). On the other hand, mean TG levels in the liver showed between 0.49 and 1.02% in May and between 0.41 and 0.79% in July (Fig. 2). Significantly negative correlation was found between mean TG levels in the liver and mean MMD in the kidney (r=0.60, P=0.045) (Fig. 3). It was impossible to indicate an individual as a plot in Fig. 3, since an individual analyzed TG levels was different from one analyzed MMD. These results indicate that mean MMD in the kidney is a negative indicator to reflect nutritional conditions from spring to summer in juvenile masu salmon released into a stream and there are differences among location in nutritional conditions of juveniles in the same sampling time. In juvenile masu salmon, the borderline where dead fish is observed in the juvenile population in an artificial rearing environment is not only >0.5% in mean MMD in the kidney (Mizuno *et al*. 2002) but also <0.2% in mean TG level in the liver (Misaka *et al*. 2004). In the present study, some juvenile populations are on the verge of death in May and July according to the MMD borderline, although there are no dying populations according to the TG borderline. This discrepancy may originate from different effects between wild and artificial experimental environments on MMD and TG values. Consequently, dead juvenile might appear in the population in a wild environment when MMD would be >0.5%, even though mean TG level in the liver is >0.2%. The correlation between fish density and MMD was significant in May (r=0.50, P=0.0017) and July (r=0.59, P=0.0008). Also, the correlations between fish density and TG levels were significant both in May and July (May: r=0.57, P=0.0005; July: r=0.34, P=0.042). These results strongly suggest that the nutritional conditions of released juvenile masu salmon depend on fish density from spring to summer.

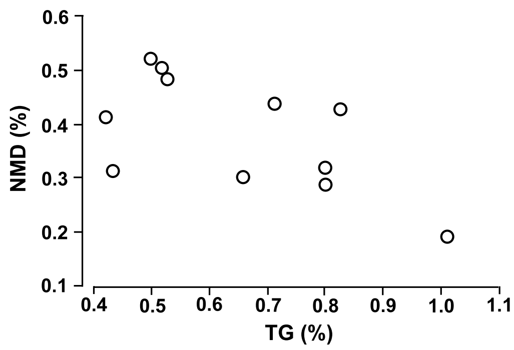

Fig. 3 Relationship between mean triglyceride (TG) levels in the liver and mean melano-macrophage density (MMD) in the kidney in juvenile masu salmon. The plots in this figure express the means of TG and MMD in the juvenile population at individual stations at individual times. This figure lacks of the data at Station 5 in July, because no juveniles were caught at Station 5 in July.

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和文摘要

腎臓メラノマクロファージの集積密度を活用した河川放流後の池産サクラマス幼魚の栄養状態評価

水野　伸也・三坂　尚行・宮腰　靖之

サクラマス増殖事業の１つの方策である当歳魚の春季河川放流の効果を評価する手段として，放流後の幼魚の栄養状態を調べることは重要である。本研究では，体液性免疫に関与する腎臓メラノマクロファージ（MMD）の集積密度を解析することにより，河川に放流された幼魚の栄養状態を春季から夏季の期間に評価できるかどうかを検討した。採捕された幼魚のMMDと肝臓中のトリグリセリド含量（TG）または採捕地点における幼魚の生息密度の間には有意な負の相関が観察された。TGは貯蔵エネルギー量を反映することから、MMD解析が放流後の幼魚の栄養状態評価に有効であると示唆された。また春季から夏季の期間中，放流後の幼魚の栄養状態は生息密度依存的に悪化すると考えられた。