Short Paper

Morphological changes in juxtaglomerular cells of the kidney during smoltification in masu salmon *Oncorhynchus masou*

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The masu salmon *Oncorhynchus masou* is an important coastal fishery resource in Hokkaido. To increase its number in the sea, many juveniles are released in rivers. However, the masu salmon catch in the sea does not always increase. To achieve this objective, it is necessary to provide seeds for release that are of good quality physiologically, behaviorally and nutritionally. One index of quality of salmonid seeds is seawater adaptability. It is thought that seed that has high seawater adaptability can survive migration from the river to the sea at higher rates. In general, the gill Na$^+$, K$^+$-ATPase activity or seawater transfer test has been used to judge seawater adaptability in salmonids. However, the method for analysis of gill Na$^+$, K$^+$-ATPase is not uniform and there may be variations in the interpretation of the results of the seawater transfer test according to individual judgment. Therefore, it is necessary to devise a reliable index of seawater adaptability.

Anadromous salmonids, including masu salmon, undergo smoltification, which is a series of physiological, behavioral, morphological and biochemical changes that transform a parr into a smolt before its migration to the sea. Smolts can maintain the osmolarity of their body fluid fairly constant in external water environments that have a wide range of osmolarity; hence, smolts of anadromous salmonids are able to adapt themselves to both fresh water and seawater environments. The kidney is one of the most important organs for maintaining osmotic homeostasis in teleosts. It is largely responsible for eliminating excess water, aiding the acid–base balance, and regulating some ions. In the kidney, juxtaglomerular cells (JGC) are thought to produce renin, which is the enzyme that converts angiotensinogen to angiotensin I. In the eel, it is known that dehydration during seawater adaptation is compensated by the accelerated drinking of water due to the action of angiotensin II produced from angiotensin I. Therefore, activation of the renin–angiotensin system has been thought to assist in the adaptation to seawater by teleosts. In addition, it is reported that the number of JGC present in seawater fish is greater than in fresh water fish and that the nuclear size of JGC decreases when euryhaline fish travel from seawater to fresh water. However, the morphological changes in the JGC of salmonids during smoltification are not known. In the present study, morphological changes in JGC during smoltification were examined by comparing smolting fish with precocious males, which were less adaptable to seawater. The purpose of the study was to determine whether the morphology of JGC can be used as an index of seawater adaptability.

Yearling smolting and precocious male masu salmon were captured monthly from the Utabetsu River, Erimo, southern Hokkaido, Japan, from January to May 2000 using either an electrofisher or a cast net. Distinguishing smolting fish from precocious males during the study was very easy because external phase differentiation between smolting fish and precocious males starts in late October and gonadal development of precocious males had already begun in January. Furthermore, almost all stream-resident parr that do not migrate to the sea as yearlings remain in southern Hokkaido as precocious males. Therefore, the odds

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that two yearling smolts never appear are a thousand to one.11

From each sampled fish, the gills and the body kidney right above the ventral fin were dissected. Na\(^+\), K\(^+\)-ATPase activity of the gill was measured according to the method described by Soyano et al. to estimate each fish’s adaptability to seawater.12 The body kidney was fixed with 4% paraformaldehyde in 0.1 M phosphate buffer at 4°C for 24 h. The tissues were dehydrated in ethanol, embedded in paraffin, and 5 μm paraffin sections, which were cut perpendicularly to the body axis, were prepared. Sections were then stained according to Bowie’s method,13 which is one of the methods available for detecting JGC in the kidneys of teleosts. Under a microscope, the area and number of JGC per each blood vessel with a diameter of 15 μm or smaller were recorded on an average of five individuals. The data were recorded using an NIH image analysis program (NIH Image, Vers. 1.57; National Institutes of Health, USA). To limit the number of blood vessel diameters being analyzed for the parameters of JGC, which would otherwise be endless, it was assumed that the blood vessel size was proportional to the number of JGC. Data were analyzed using one-way analysis of variance followed by the Student–Newman–Kuels multiple range test.

Figure 1 shows that gill Na\(^+\), K\(^+\)-ATPase activity increased significantly from March (pre-smolt) to May (full smolt) in the smolting fish (\(P<0.05\)), whereas it showed no change during the same period in precocious males. In May there was a significant difference in the values of smolting fish and precocious males (\(P<0.05\)). These data suggest that the ability of smolting fish to adapt to seawater progressed smoothly from March (pre-smolt) to May (full smolt), whereas it did not for precocious males during the same period.

Staining of JGC using Bowie’s method was detected in the small vessels seen at the juxtaglomerular regions of both smolting fish and precocious males (Fig. 2). These characteristics were consistent with studies of JGC in brook trout and rainbow trout.14 The present study shows that, like other salmonids, masu salmon also have JGC. In smolting fish, hypertrophy of JGC was observed during May (full smolt) compared to observations of kidney sections taken in January (parr) (Fig. 2a,b); however, this was not observed in precocious males (Fig. 2c,d).

Changes in the area and number of JGC of fish from January to May are shown in Fig. 3. In precocious males, the two parameters were kept constantly low from January (parr) through to May (full smolt). Conversely, for smolting fish, the area of JGC increased significantly from January (parr) to May (full smolt). During May, the value was significantly higher than that for the precocious males (\(P<0.05\)). The number of JGC in smolting fish did not change from January (parr) to March (pre-smolt). However, it increased significantly from March (pre-smolt) to May (full smolt) (\(P<0.05\)), and became significantly higher than that for pre-
seawater to fresh water,\textsuperscript{10} whereas the present study revealed that the area and number of JGC increased during smoltification while the fish were still in fresh water. It is worth noting that in the present study the increase in the number of JGC during smoltification occurred coincidentally with the increase in gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity. These results suggest that the number of JGC may serve as a good indicator for the development of seawater adaptability in masu salmon.

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