成長差がサケ幼魚(Oncorhynchus keta)の鰓のNa+,K+-ATPaseに与える影響

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Influence of Various Growth Rates on Development of Gill Na\(^+\), K\(^+\)-ATPase in Juvenile Chum Salmon, Oncorhynchus keta

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Abstract: Juvenile chum salmon, Oncorhynchus keta, were reared under various feeding conditions for three years. In 1992, fry which emerged in March were fed at 4% of body weight a day. In 1995, fry which emerged in February (95-a-class) and in March (95-b-class) were fed at 1% of body weight a day. In 1996, fry which emerged in February were fed at 3% of body weight a day. The instantaneous growth rate of the 92-class and the 96-class reached 0.0045 in March and February, respectively, followed by a decline below 0.0040. The 95-a-class and the 95-b-class stayed less than 0.0029 in their instantaneous growth rate throughout the experimental period. Significant differences in growth rate were observed between the 92-class and the 95-b-class in March, and between the 95-a-class and the 96-class in February. The gill Na\(^+\),K\(^+\)-ATPase activity of the 92-class and 96-class increased and peaked in May and June, respectively. However, both 95-a-class and 95-b-class did not show distinct increases in this enzyme activity. These results indicate that high growth for the first month after emergence may be a prerequisite for expressing gill Na\(^+\),K\(^+\)-ATPase of juvenile chum salmon.

Key words: Oncorhynchus keta; Chum salmon; Growth rate; Gill Na\(^+\),K\(^+\)-ATPase

Chum salmon, Oncorhynchus keta, is known for its unique early life stages among the genus Oncorhynchus. The fry of chum salmon migrate seaward soon after emergence from the gravel and this migration lasts from March to June, in Hokkaido, Japan 1,2). In nature, the landlocked form of this species is not known. Before entering the seashore area, there is an increase in hypo-osmoregulatory ability and a development of the seawater tolerance as pre-adaptation to the coming life at sea. The fish during the period can maintain stability of their internal ionic balance even upon direct transfer to seawater from fresh-water 3,4).

It has been well documented that the gill is a major site for active ion regulation by chloride cells in the marine teleost, including anadromous salmonids 5,6). In the specialized chloride cell of the gill filaments, Na\(^+\),K\(^+\)-ATPase is the enzymatic basis for active ion transport 5,7,8). It has been reported that the gill Na\(^+\),K\(^+\)-ATPase activity in chinook, O. tshawytscha, and coho salmon, O. kisutch and steelhead trout, O. mykiss, increases considerably during seaward migration 9). In addition, Nakano 10) showed that the gill Na\(^+\),K\(^+\)-ATPase activity in chum salmon was maximal when fry reached a size of 65 mm in fork length. This increase in enzyme activity is influenced by growth in brook trout, Salvelinus fontinalis, and chinook salmon 11,12). In chum salmon, however, the relationship between growth rate and gill Na\(^+\),K\(^+\)-ATPase activity has not been clear.

In this paper, the influence of growth rate on the expression of gill Na\(^+\),K\(^+\)-ATPase was examined during the early life stage in chum salmon.

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Materials and Methods

Fish, Collecting Schedule, and Rearing Condition
In 1992, 1995 and 1996, the examinations described below were conducted using juvenile chum salmon from the Ishikari River. The juvenile emerged from the gravel in the first week of February in 1995 (95-a-class) and in 1996 (95-b-class), and in the first week of March in 1992 (92-class) and in 1995 (95-b-class). The 92-class was maintained at 10°C during alevin and juvenile stages. The 95-a-class, 95-b-class and 96-class were kept at 8°C for alevin stage, then they were transferred to 10°C from the fry stage. These juveniles were fed commercial pellet at 4%, 1%, 3% of body weight a day in 1992, 1995, 1996, respectively. Fish was collected in the first week of each month from immediately after emergence to early June.

Comparison of Monthly Growth Rate and Calculation of Instantaneous Growth Rate
A group of 20-40 fish was caught randomly at each collecting time. After anesthetizing by tricaine methanesulfonate (MS222, Sankyo Co., 100 mg/l), fork length was measured. Based on these data, monthly growth rates of each class were compared statistically as mentioned below. Furthermore, instantaneous growth rates in fork length are estimated from the relationship:

\[ g(L) = \frac{\ln(L_t) - \ln(L_0)}{T} \]

where \( g(L) \) denotes the instantaneous growth rate in length during the time period \( T \) (the number of days from \( L_0 \) to \( L_t \)). \( L_0 \) denotes mean fork length at each collecting time, and \( L_t \) denotes mean fork length at the following collecting time.

Analysis of Gill Na⁺, K⁺-ATPase Activity
The gill filaments dissected from the 10 anesthetized fish were rinsed in ice-cold homogenizing buffer (250 mM sucrose, 6 mM EDTA 2Na, 20 mM imidazole, pH 6.8), and kept frozen in this buffer (20 times w/v) at -40°C individually for later measurement of Na⁺,K⁺-ATPase activity. At the time of analysis, thawed gill filaments were homogenized in the homogenizing solution and centrifuged at 2,000 g, at 5°C for 5 min.

A 40μl of the supernatant was incubated either in 160μl of reaction mixture A (250 mM imidazole, 12.5 mM ATP2Na, 337.5 mM NaCl, 162.5 mM KCl, 50 mM MgCl₂) or 160μl of reaction mixture B (reaction mixture A and 2.5 mM ouabain) for 20 min at 37°C in a shaking incubator. The reaction was stopped by adding 4 ml of ice-cold iron TCA solution (610.1 mM TCA, 131.4 mM thiourea, 76.5 mM ferrous ammonium sulfate). Free inorganic phosphate (Pi) was measured according to the method of Goldenberg and Fernandez. Protein concentrations were determined by the method of Lowry et al. Protein activity is expressed as amount of Pi per milligram protein per hour (μmols Pi/mg pro./h).

Statistical Analysis of Monthly Growth Rate and Gill Na⁺, K⁺-ATPase Activity
The monthly growth rates were compared using ANCOVA to determine significant differences of each month between the 92-class and the 95-a-class, and between the 95-b-class and the 96-class. Gill Na⁺,K⁺-ATPase activity data were subjected to ANOVA followed by Student t-test or Cochran-cox test to determine significant differences.

Results

Growth of Fish
At the beginning of the experiment, mean fork length of all classes showed almost the same size between 36.3 mm and 37.3 mm. The mean fork length of each class varied from 56.6 mm to 78.0 mm in June, depending on feeding rate and rearing period (Table 1). The instantaneous growth rate was 0.0045 in March of 1992 and in February of 1996; however it was less than 0.0040 for the other months. The 95-a-class and the 95-b-class had instantaneous growth rates lower than 0.0029 throughout the experimental period. Significant differences (p < 0.001) in monthly growth rates were found between the 92-class and the 95-a-class in March, and between the 95-b-class and the 96-class in February.
Gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase Activity

The gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity of the 92-class was significantly increased (\(p < 0.001\)) from 11.0 \(0.7\) mols Pi/mg pro./h in March to 18.1 \(0.5\) mols Pi/mg pro./h in May, followed by a significant decrease (\(p < 0.001\)) to 11.0 \(0.9\) mols Pi/mg pro./h in June (Fig. 1). The 92-class and the 95-class showed a surge of gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity with growth and is maximal when the juvenile reaches a size of 65 mm in fork length. However, Salo\textsuperscript{17}) reported that chum salmon achieved maximum gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity at 48-55 mm in fork length. In the present study, the gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity of the 92-class and the 96-class peaked at 67.5 mm and 57.9 mm in fork length, respectively. From these data, it was expected that a surge of gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity would occur for chum salmon over about 50 mm in fork length. However, the 95-a-class and the 95-b-class of the present study did not show an increase in enzyme activity, despite their body size reaching 69.4 mm in June. These variations in fork length and enzyme activity indicate that development of gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is controlled by not only size but also other factors such as growth pattern. It seems that juvenile chum salmon with increased levels of gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is more likely to emigrate to sea successfully, similar to other salmonids\textsuperscript{9}).

Discussion

There is few reports concerning gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity on chum salmon, despite the enzyme play a vital role in seawater tolerance in salmonids\textsuperscript{6,9,16}). According to Nakano et al.\textsuperscript{10}), gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity of chum salmon increases with growth and is maximal when the juvenile reaches a size of 65 mm in fork length. In the present study, the gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity of the 92-class and the 96-class peaked at 67.5 mm and 57.9 mm in fork length, respectively. From these data, it was expected that a surge of gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity would occur for chum salmon over about 50 mm in fork length. However, the 95-a-class and the 95-b-class of the present study did not show an increase in enzyme activity, despite their body size reaching 69.4 mm in June. These variations in fork length and enzyme activity indicate that development of gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is controlled by not only size but also other factors such as growth pattern. It seems that juvenile chum salmon with increased levels of gill Na\textsuperscript{+}, K\textsuperscript{+}-ATPase is more likely to emigrate to sea successfully, similar to other salmonids\textsuperscript{9}).

The four classes observed in this study appear to fall into two categories with respect...
to profiles of gill Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity. The first group had an evident surge of the enzyme activity as in the 92-class and the 96-class. The second did not show any fluctuation as in the 95-a-class and the 95-b-class. The first group was characterized by significantly higher growth rates than that of the second group for the first month after emergence. Wagner et al.\textsuperscript{18}) suggested that increased capacity to initiate osmoregulatory systems might result from increased size as well as growth rates in juvenile chinook salmon. Moreover, Ewing et al.\textsuperscript{11}) reported that low growth rates suppressed gill Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity in juvenile chinook salmon. The present data indicate that high growth rate for the first month after emergence is necessary for triggering expression of the gill Na\textsuperscript{+},K\textsuperscript{+}-ATPase in chum salmon.

The gill Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity of both the 92-class and the 96-class peaked after two months from emergence. Dickhoff et al.\textsuperscript{2} also found a significant correlation between high growth rate and smolt quality, including hypoosmoregulatory ability, in reared chinook salmon during the 1.5 to 2 months before release in spring. The Na\textsuperscript{+},K\textsuperscript{+}-ATPase in gills concentrate highly in the tubular system in the specialized chloride cells which are major sites of chloride and sodium excretion in seawater teleosts\textsuperscript{19,20}). Recently, Uchida and Kaneko\textsuperscript{21}) reported that chloride cells located in gill filaments were replaced by newly-differentiated chloride cells, and they speculated that this cellular turnover might activate the new chloride cells of chum salmon fry during seawater adaptation. It appears that chum salmon fry need about two months for structural and functional transformations in the gill, including activation of the Na\textsuperscript{+},K\textsuperscript{+}-ATPase as preparation for life in the sea. Furthermore, these processes are influenced by ambient temperature; higher water temperature increases the gill Na\textsuperscript{+},K\textsuperscript{+}-ATPase activity.

In conclusion, high growth for the first month after emergence is necessary for triggering expression of gill Na\textsuperscript{+},K\textsuperscript{+}-ATPase in chum salmon. Chum salmon fry seem to require about two months for structural and functional transformations in the gill, including activation of the Na\textsuperscript{+},K\textsuperscript{+}-ATPase as preparation for life in the sea.

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Growth Rates and Gill Na\textsuperscript{+},K\textsuperscript{+}-ATPase in Chum Salmon


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成長差がサケ幼魚（Oncorhynchus keta）の鰓の
Na+, K+-ATPaseに与える影響

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サケ幼魚（Oncorhynchus keta）を、様々な給餌条件下で飼育した。1992年3月に浮上した群（92年群）、1995年2月（95-a年群）および3月（95-b年群）に浮上した群、1996年2月に浮上した群（96年群）の給餌率は、実験期間を通じて各々1日体重当たり4%、1%、3%に設定した。92年群および96年群の鰓間成長係数は、各々3月と2月に0.0045以上を示したが、その後は0.0040以下になった。95-a年群と95-b年群の鰓間成長係数は、実験期間を通じて0.0029以下だった。浮上期が等しい年群間で成長率を比較した結果、92年群と95-b年群間の3月および95-a年群と96年群間の2月に有意差が認められた。92年群と96年群の鰓のNa⁺, K⁺-ATPase活性は浮上後上昇を続け、各々5月と6月に最高値に達した。しかし、95-a年群と95-b年群には、明確な酵素活性の上昇が認められなかった。これらの結果は、浮上から1カ月間の高成長が、サケ幼魚の鰓におけるNa⁺, K⁺-ATPaseの発達に重要であることを示している。