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| Author(s) | 崔, 聞達 |
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Utilization of circulating insulin-like growth factor-1 and its binding proteins
as physiological indices for hatchery release and aquaculture of salmonids
(増養殖業における生理学的指標としての血中インスリン様成長因子-1とその結合蛋白)

博士（環境科学） 氏 名 Cui Wenda（崔 聞達）

Summary

Salmonids are important economic species and targets of hatchery release and aquaculture in the world. Chum salmon (*Oncorhynchus keta*) populations in Japan are sustained by intensive hatchery releases. However, the number of returning adults has declined dramatically in last several years. There is a hypothesis that the decrease is related to a high mortality occurring in juveniles' early marine life, and the poor growth condition is the one of the immediate causes. Thus, monitoring the growth of juvenile chum salmon in down-migration and evaluating the process and degree of mortality after release are important. The otolith in fish is widely used to reconstruct the growth history and its elemental composition change could reflect the sea-entry timing. However, the otolith may not be suitable for estimating current growth status. Swimming ability of the juveniles presumably mediate their growth-dependent mortality because it affects their chance to avoid predation and to reach to the feeding area. Therefore, unraveling the link between growth and swimming performance is also important for improving hatchery release of juvenile chum salmon. For aquaculture, on the other hand, there is an increasing interest in culturing rainbow trout (*O. mykiss*) in seawater. Juveniles of anadromous salmonids generally go through a series of morphological and physiological changes called smoltification and acquire hypo-osmoregulatory ability before starting their marine life. These preparatory changes are developmentally and seasonally regulated. Photoperiod manipulation is a common technique to induce smoltification and enhance hypo-osmoregulatory ability in Atlantic salmon, however, it doesn't work well for rainbow trout. Moreover, body size has a stronger effect than photoperiod on the development of hypo-osmoregulatory ability in juvenile rainbow trout. However, the linkage among these parameters is still unclear.

Insulin-like growth factor (IGF) -1 is a growth-promoting hormone in vertebrates. It is mainly produced in the liver and its production is stimulated by growth hormone (GH). IGF-1 is secreted into bloodstream and delivered to target tissues. Then liver-derived IGF-1 mediates the growth-promoting action of GH. Circulating IGF-1 levels in fish including salmonids have been shown to be correlated with individual growth rates, and it respond well to changes of nutritional status. Thus, circulating IGF-1 level has been used for evaluating the growth condition of several fishes as growth index. Likewise, IGF-1 is also involved in the acquisition of hypo-osmoregulatory and potentially be its index. Circulating IGF-1 in salmonids is stabilized by three major subtypes of IGF-binding proteins (IGFBPs), IGFBP-1a, -1b and -2b. IGFBP-1s are inhibitors of IGF-1 action and their level in circulation are inversely correlated with individual growth rates. IGFBP-2b is believed to be a major carrier of circulating IGF-1 and possibly promotes the activity of IGF-1. Thus, these IGFBPs are also

candidates of growth indices. In addition to monitoring the growth of juvenile salmon, profiles of circulating IGF-1 and IGFBPs may be useful to interpret the relationship between growth and swimming ability or hypo-osmoregulatory ability. The goal of the present study was to expand the utility of circulating IGF-1 and IGFBPs as physiological indices for improving hatchery release and aquaculture of salmonids.

The present study first focused on the growth condition of out-migrating juvenile chum salmon on the northeastern coast of Hokkaido (Chapter 2). Besides body weight and fork length, growth status of juveniles was evaluated by measuring circulating IGF-1 level and analyzing the otolith increment. Fish were collected in the river, estuary, port and nearshore areas in Abashiri area during 2018-2020. Circulating IGF-1 levels were measured by time-resolved fluoroimmunoassay and growth rates after sea entry were estimated from radius of circuli on the otolith. Body weight and fork length of juvenile chum salmon were generally lower in the river and estuary than in coastal area. Profiles of circulating IGF-1 levels suggest growth of juvenile chum salmon was activated after the sea entry and further increased while moving toward offshore. Growth rate estimated by the otolith analysis also showed the similar trend. In addition, circulating IGF-1 level was positively correlated with the growth rate estimated from otolith analysis. However, the relationship was relatively weak and the relationships among growth indices and body size/condition at each location were not consistent between years. These results suggest the two indices have different sensitivities to growth alterations. Despite relatively low, the present study was the first to report a correlation between IGF-1 level and the growth rate from otolith analysis, suggesting that circulating IGF-1 and otolith analysis are good complements each other for the field survey on juvenile chum salmon.

The present study next examined the effects of feeding and water temperature on swimming performance in juvenile chum salmon by rearing experiments (Chapter 3). This series of experiments were carried out at Hokkaido research organization as collaboration. In 2018, the first experiment was carried out. Juvenile chum salmon about 1 g were first fasted, fed at 1% or 3%/body weight in freshwater and then transferred to seawater of different temperatures (4, 7 and 10 °C). Swimming ability was evaluated by critical swimming speed ($U_{crit}$), the maximal sustainable swimming speed. Absolute $U_{crit}$, the swimming speed per second (cm/s), was measured by swimming test in a stamina channel. In order to exclude the effect of body size on the swimming ability, relative $U_{crit}$, which was standardized by fork length (FL) of experimental fish, was used in the first experiment. Body size and circulating IGF-1 levels were lower in fish in 4°C seawater than those in 10°C. On the other hand, pre-fasted fish also showed lower growth than fish fed at 3% in some sampling points. Similarly, relative $U_{crit}$ of fish in 4 °C seawater was lower than that in 10 °C and pre-fasted fish showed lower relative $U_{crit}$ than fish fed at 3% in 7 °C, after 8 days sea transfer. These results show that low seawater temperature has a profound effect on growth, serum IGF-1 levels and swimming ability of juvenile chum salmon. In addition, average relative Ucrit was positively correlated with average values of growth parameters, including circulating IGF-1 level. The $r^2$ value of a linear regression of circulating IGF-1 versus relative $U_{crit}$ was as high as 0.85. This suggests that swimming ability of juvenile chum salmon is positively related with the growth status of fish and circulating IGF-1 levels may be linked with individuals'

swimming ability. To further test the links of growth and circulating IGF-1 with swimming ability, another experiment was conducted in 2020. Juvenile chum salmon were first divided into large and small groups and each group was fed either a high or low ration for about two months. As expected, large fish fed with high ration showed the highest body size and circulating IGF-1 level and small fish fed with low ration was the lowest. However, there was no significant difference in relative $U_{crit}$ among four groups. In this experiment, circulating IGFBPs were detected by ligand blotting using labeled IGF-1 and their band intensities were semi-quantified. IGFBP-1a and -1b levels in the circulation were significantly higher in fish fed with low ration, however, neither IGF-1 nor IGFBPs was correlated with relative $U_{crit}$. When absolute $U_{crit}$, expressed as cm/s, was calculated, the small fish fed with low ration had a lower absolute $U_{crit}$ than other treatments. This indicates the swimming ability of large individual is less affected by feeding conditions. On the other hand, absolute $U_{crit}$ was positively and negatively correlated with serum IGF-1 and IGFBP-1a/-1b levels, respectively. Although full reproducibility was not achieved, the present results suggest that growth of juvenile chum salmon plays a crucial role in affecting swimming ability. Meanwhile, the present study is also the first to find the potential linkage of circulating IGF-1 and IGFBPs with relative and/or absolute critical swimming speed under certain conditions.

The present study also examined the potential utility of circulating IGF-1 and IGFBPs as indices of the acquisition of hypo-osmoregulatory ability or the degree of smoltification in rainbow trout by rearing experiments (Chapter 4). First experiment was collaborated with University of Bergen, Norway, in 2016, examining the effects of photoperiod regimes on the activity of gill $Na^+$, $K^+$-ATPase (NKA), an ion pump essential for hypo-osmoregulation, in yearling rainbow trout. Fish were exposed to four photoperiod regimes: Simulate natural photoperiod (SNP), Constant light (LL), Advanced photoperiod (APP) and Delayed photoperiod (DPP). Gill NKA activity was not activated by photoperiod manipulations. In addition, neither circulating IGF-1 nor IGFBP-2b levels responded. However, gill NKA activity of yearling rainbow trout was positively correlated with body weight, circulating IGF-1 and IGFBP-2b levels under the SNP condition. These suggest the development of gill NKA activity in rainbow trout is related with body size and/or growth. To test this hypothesis, another experiment was conducted at Nanae Fresh-Water Station in 2020. Yearling rainbow trout were pit-tagged and divided into feeding to satiation or at a restricted ration from April to July. As expected, fish fed to satiation had larger body weight and forklength and circulating IGF-1 and circulating IGFBP-2b levels were high. Gill NKA activity in April was higher in satiated fed fish than restricted fish and it was positively correlated with fork length, body weight and specific growth rate. These indicate the size-/growth-dependent activation in gill NKA activity of rainbow trout. On the other hand, circulating IGF-1 and IGFBP-2b levels were positively related with growth parameters but only IGFBP-2b was correlated with gill NKA activity. These results suggest that circulating IGF-1 and/or IGFBP-2b mediate the size-dependent activation of gill NKA activity in yearling rainbow trout during spring and may be used to evaluate the acquisition of hypo-osmoregulatory ability.

In summary, the present study has expanded the utility of circulating IGF-1 and IGFBPs as physiological

indices of growth, swimming performance and hypo-osmoregulatory ability. The major achievement of the present study is three folds. First, the present study reports the circulating IGF-1 level is positively correlated with the growth rate estimated from otolith increment analysis. Second, the present study has revealed swimming ability of juvenile chum salmon is related with growth and circulating levels of IGF-1 and IGFBP-1s are correlated with critical swimming speed in juvenile salmonids. Third, the present study suggests that osmoregulatory ability in rainbow trout is related with growth and positive correlations are present among circulating IGF-1 level, IGFBP-2b level and gill NKA activity. The findings of the present study warrant further validation of IGF-1 and IGFBPs for physiological indices, which should provide a rational basis to improve growth performance of target fish, and in turn increase production in aquaculture and marine survival in resource enhancement.