# 学 位 論 文 内 容 の 要 旨

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## 学 位 論 文 題 名

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）

Salmonids are important economic species 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. This is likely to be related with the growth-dependent mortality occurring in juveniles' early marine life. Thus, monitoring the growth of juvenile chum salmon in down-migration may help to find the real drawbacks to their survivals. Elemental composition changes of the otolith record the sea-entry, however, its characteristic of growth estimation is not suitable for current growth. Besides, the growth-dependent mortality of juvenile salmon is presumably mediated by swimming ability since it affects their chance to avoid predation and to reach feeding area. Therefore, unraveling the link between their growth and swimming performance is also important for improving hatchery release. For aquaculture, on the other hand, there is an increasing interest in culturing rainbow trout (*O. mykiss*) in seawater. Juveniles of anadromous salmonids generally acquire hypo-osmoregulatory ability during smoltification, a series of preparatory changes for future marine life which are developmentally and seasonally regulated. However, inducing hypo-osmoregulatory ability in rainbow trout is an issue. Unlike Atlantic salmon, this species appears to be less sensitive to photoperiod manipulation and depends more on body size to develop hypo-osmoregulatory ability although their link is not clear.

Insulin-like growth factor (IGF) -1 is a growth-promoting hormone in vertebrates. It is mainly produced by the liver upon stimulation with growth hormone (GH) and delivered to target tissues through bloodstream, then mediate growth-promoting action of GH. Circulating IGF-1 levels in fish including salmonids have been shown to be correlated with individual growth rates. Thus, it is an emerging 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 whereas IGFBP-2b is a major carrier of circulating IGF-1 and may potentiate its action. They are inversely or positively correlated with growth rates and thus candidates of growth indices. Therefore, in addition to monitoring the growth of juvenile salmons, changes in circulating IGF-1 and IGFBPs are potentially helping 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.

Present study first compared profiles of circulating IGF-1 level and growth estimated from the otolith increment analysis in out-migrating juvenile chum salmon at northeastern coast of Hokkaido (Chapter 2). Fish were caught 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. Profiles of circulating IGF-1 levels suggested the

growth status of juvenile chum salmon was activated after the sea entry and further increased while moving offshore. Growth rate estimated by the otolith analysis supported similar suggestions. There were positive correlations between the two growth indices. However, the relationship was not constantly strong at all sampling sites, suggesting fluctuating sensitivities to growth alterations in both indices. Given the timeliness characteristics of the two indices, these results suggest that circulating IGF-1 and otolith analysis are good complements each other for the field survey on juvenile chum salmon.

Present study next examined the relationships of circulating IGF-1 and IGFBPs with swimming performance of juvenile chum salmon by rearing experiments (Chapter 3). First experiment examined effects of feeding and seawater temperature on growth and critical swimming speed ($U_{crit}$), the maximal sustainable swimming speed. Juvenile chum salmon about 1 g were first fasted, fed at 1% or 3%/body weight in freshwater, then transferred to seawater of different temperatures (4, 7 and 10 °C). Absolute $U_{crit}$, the swimming speed per second (cm/s), was measured and relative $U_{crit}$ was standardized by fork length (FL) of experimental fish (i.e. FL/s). Body size, serum IGF-1 levels and relative $U_{crit}$ were lower in fish transferred to 4 °C than in those to 10 °C, independent of feeding history in freshwater, with a few exceptions. These results showed that low seawater temperature had a profound effect on growth, serum IGF-1 levels and swimming ability of juvenile chum salmon. A positive correlation between serum IGF-1 level and relative $U_{crit}$ was seen 8 days after transfer to seawater, suggesting fish with high IGF-1 levels have relatively better swimming ability. In order to further test the links of circulating IGF-1 and IGFBPs to swimming ability, another experiment was conducted. 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. In this experiment, circulating IGFBPs were detected by ligand blotting using labeled IGF-1 and their band intensities were semi-quantified. Serum IGF-I and IGFBPs levels differed among treatments, however, neither of them was correlated with relative $U_{crit}$. While absolute $U_{crit}$ was positively and negatively correlated with serum IGF-1 and IGFBP levels, respectively. Although full reproducibility was not achieved, present results suggest that growth of juvenile chum salmon plays a crucial role in affecting swimming ability and circulating IGF-1 and IGFBPs may be linked with relative and/or absolute critical swimming speed under certain conditions.

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 examined 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, however, it was positively correlated with body weight, circulating IGF-1 and IGFBP-2b levels under SNP. These suggested the development of gill NKA activity in rainbow trout may be growth-related. In order to test this hypothesis, another experiment was conducted using pit-tagged yearling rainbow trout fed to satiation or at a restricted ration. In April, gill NKA activity as well as body size was higher in satiated fed fish and positively correlated with fork length, bodyweight and specific growth rate. These indicated a size-/growth-dependent activation in gill NKA activity. 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, which could be applied for the improvement of hatchery release and aquaculture of salmonids.