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Title

Profiles of circulating insulin-like growth factor-I during smoltification of masu salmon reared under different conditions

Authors

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Abstract

We compared profiles of serum insulin-like growth factor (IGF)-I levels during smoltification of masu salmon reared under different environments, hatcheries and growth histories. Masu salmon from the Kenichi River in Hokkaido showed a sharp increase in serum IGF-I from April to May followed by a peak of gill Na^+,K^+-ATPase (NKA) activity. Fish at Kumaishi Hatchery had an IGF-I profile similar to that of the river fish while the increase in gill NKA was lower. At Shimamaki Hatchery, interval feeding during winter appeared to suppress the spring IGF-I peak. At Kumaishi Hatchery, a difference in size during smoltification affected IGF-I levels at release, but numbers of adult returned to the release site were not significantly different. In the following year, three release groups differing winter size and/or spring growth (Large-High, Large-Low and Small-High) were created. Large- and Small-High fish showed a higher IGF-I peak than Large-Low fish in April while smolt-to-adult return of Large-High fish was highest. These results suggest that in smolting masu salmon in freshwater, circulating IGF-I level alone is not a predictor of long-term survival in seawater. However, since growth history in freshwater affected the smolt-to-adult return, optimizing rearing conditions is a critical component of hatchery releases for masu salmon.

Keywords: masu salmon; smoltification; insulin-like growth factor-I; Na^+,K^+-ATPase; survival

Introduction

Masu salmon *Oncorhynchus masou* is an important target species of coastal fisheries off the northern Japan. However, its resource has been unstable and decreasing since 1970s [1, 2]. Hatchery program is commonly used to enhance resources of anadromous salmonid species and has been successful for Japanese chum salmon *O. keta* populations [3]. A large amount of effort has also been invested in hatchery releases for masu salmon, although it is far less successful [1, 2]. What makes hatchery releases for masu salmon difficult is its long residency in freshwater. In contrast to chum and pink salmon *O. gorbuscha* that migrate to the sea in their first spring, masu salmon reside in freshwater more than one year and need to go through the smoltification process before entering the ocean. Smoltification is a transition process by which the river-resident form of juvenile salmon (parr) transforms to the sea-run form (smolt) in freshwater. This process involves a suite of morphological, behavioral, ecological and physiological changes adaptive to ocean life [4-7].

In order for hatcheries to be successful in enhancing masu salmon resources, it is important to identify smolt traits that are advantageous to post-release migration and the ocean life, and such traits could be used to evaluate "smolt quality" of reared fish. Among several candidates proposed, seawater adaptability is the most widely used trait. Ability to maintain the internal osmotic pressure is essential for smolts to cope with the challenges of hypoosmotic environment and its failure results in immediate death or growth retardation in seawater [8]. Adaptation to seawater is achieved through coordination of the gills, kidney and intestine. The gills are important sites for extrusion of monovalent ions such as Na^+, K^+, Cl^- [9]. Extruding ions from the gills relies primarily on the activity of an ion pump, Na^+,K^+-ATPase (NKA), that is located in the basolateral membrane of ionocytes [10-12]. NKA pumps out three Na^+ from the cell and incorporates two K^+ into the cell using the energy from hydrolyzing an ATP. This ion gradient is further utilized by other ion cotransporters, channels and exchangers to regulate ion concentrations inside the body [10-12]. However, seawater adapatability may not be enough to secure smolts to return as adults since other environmental factors such as presence of predators, food availability and water temperature also affect their survival.

Another important trait affecting survival of post-smolt salmon is ability to grow in seawater. Several lines of evidence suggest that a majority of smolts die during the first year in the ocean due to growth-dependent mortality such as predation and nutritional deficiency [30-32]. Maximizing potential for growth in the ocean should improve smolt survival. There is evidence that bigger fish have higher gill NKA activity [34, 35] and the size at release positively affected smolt-to-adult return (SAR) in Chinook salmon *O. tshawytscha* [37]. In masu salmon, large smolts (32.6 g) had better adult recovery than small smolts (14.8 g) [38]. Based on these findings, bigger smolts are generally preferred for hatchery release.

However, some studies proposed that growth in spring rather than absolute size is more important for producing high quality smolts that return as adults [37, 39]. A rationale of this proposal is that enhanced spring growth promotes smolt-related traits such as gill NKA activity and migratory behavior [26, 40]. However, such rearing protocols have not been assessed for masu salmon. Moreover, the mechanism by which growth affects seawater adaptability is not fully understood.

The growth hormone (GH)-insulin-like growth factor (IGF)-I system plays a major role in promoting animal growth. In this endocrine system, IGF-I is mainly produced by the liver in response to GH stimulation, released into bloodstream as a hormone and mediates many of GH actions [21-23]. IGF-I is also produced by local tissues and acts through autocrine/paracrine pathways [22, 23]. In salmonids and other euryhaline fishes, the GH-IGF-I system also improves seawater adaptability by activating branchial NKA and inducing morphological development of the seawater-type ionocyte in concert with cortisol [13-17]. Circulating IGF-I often shows an increase during smoltification and is thought to activate NKA in the gills [26-29]. Thus, circulating IGF-I is suggested to be a critical molecule linking growth with seawater adaptation.

We previously reported a profile of circulating IGF-I during smoltification of hatchery-reared masu salmon and found a positive relationship between IGF-I and gill NKA activity [29]. However, it is not known to what extent the IGF-I profile varies or how much it correlates with adult return. The aims of the present study were to compare circulating IGF-I profiles during smoltification of masu salmon reared under different conditions and examine if the level or profile of IGF-I could be used as an index of "smolt quality" that predicts adult return of hatchery-reared masu salmon.

Materials and methods

Fish

Naturally-reared yearling masu salmon were caught monthly (n = 6-9) from February to May 2008 at the Kenichi River (42°N, 140°E; Futami-gun, Hokkaido, Japan) by electric shocker (Model 12, Smith-Root Inc., Vancouver, WA, USA) and immediately transferred to the South Branch of Salmon and Freshwater Fisheries Institute, Hokkaido Research Organization (Kumaishi Hatchery) located by the river. Age-matched hatchery fish were also sampled (n = 7-10) on the same day. Fish were anesthetized by 3.3% 2-phenoxyethanol (Kanto Chemical, Tokyo, Japan) and measured for fork length and body weight. Condition factor was calculated as follows: (body weight) x 1000/(fork length)^3. Gill arches were excised and a block of gill filaments was immediately frozen on dry ice and stored at -80°C until analyzed for NKA activity. Blood was withdrawn by a syringe from the caudal vein, allowed to clot overnight at 4°C and centrifuged at 8,050g for 10 min. Serum was collected and stored at -30°C until use. The samplings

were carried out in accordance with the guideline of Hokkaido University Animal Care and Use Committees.

In November 2010, underyearling masu salmon reared at Kumaishi Hatchery were sorted by size (>9.5 cm) and visual inspected to remove precociously maturing males and potential non-smolting fish in the following spring. They were further divided into large (>11.5 cm) or small (9.5 - 11.5 cm) size categories. Fish from each group were marked by removing part of adipose, dorsal or pelvic fin in order to identify the treatment of returning adults and reared separately in outdoor ponds (24.6 x 3.5 m) using the river water. They were fed twice (November-February) or four times (March-June) a day on a commercial diet (Nippon Formula Feed Mfg, Kanagawa, Japan) with rations at 0.3-2.3%/body weight/day. Fish were released to the Kenichi River on May 13, 2011 by opening the gates of the ponds. From January 2011 to May 2011, seven fish were sampled monthly.

In November 2011, underyearling masu salmon reared at Kumaishi Hathery were sorted by size (>11.5 cm as large and 9.5 - 11.5 cm as small). They were further placed into one of three treatments: large size in winter and high feeding ration in spring (Large-High), large size in winter and low feeding ration in spring (Large-Low), and small size in winter and high feeding ration in spring (Small-High). We were unable to create the Small-Low group due to the limitation of space and labor since each group consisted of approximately 30,000 fish for release. Fish from each group were marked by removing part of adipose, dorsal or pelvic fin in order to identify the treatment of returning adults and reared separately in outdoor ponds (24.6 x 3.5 m) using the river water. The Large-High and Small-High groups were fed twice (November-February) or four times (March-May) a day on a commercial diet (Nippon Formula Feed Mfg) with rations at 0.3-2.3%/body weight/day. The Large-Low group received a restricted feeding ration at 0.2-1.9% depending on growth status. All fish were released to the Kenichi River on May 10, 2012 by opening the gates of the ponds.

Yearling masu salmon were also reared at Shimamaki Hatchery (42°N, 140°E; Shimamaki-gun, Hokkaido, Japan) during the smoltification period. Fish were maintained in the river water from the Chihase River in outdoor ponds. In 2012, fish were fed every three or four days on a commercial diet (Nippon Formula Feed Mfg) with rations at 0.3-0.4% (%/body weight/day as average) during January-early March, 1.0% during mid March-April and 1.3% in early May. In 2013, fish were fed daily with rations at 0.3-0.4% during January-early March, 1.2% during mid March-April and 1.3% in early May. Fish were sampled as described above, the tail was cut and blood was withdrawn from the caudal vein using a plain glass tube. Blood was allowed to clot overnight at 4°C and centrifuged at 8,050*g* for 10 min. Serum was collected and stored at -30°C until use.

Time-resolved fluoroimuunoassay (TR-FIA) for IGF-I

Prior to the assay, serum IGF-I was extracted with an acid-ethanol as described in Shimizu et al. [41]. IGF-I was quantified by TR-FIA based on the method described in Small and Peterson [42] using recombinant salmon/trout IGF-I (GroPep, Adelaide, SA, Australia) for standard and labeling with europium, and anti-barramundi IGF-I (GroPep) as a primary antiserum.

NKA activity assay

Gill NKA activity was measured according to Quabius et al. [43] with minor modifications. This method is based on the ability of NKA to hydrolyze ATP to give ADP and inorganic phosphorus with or without presence of ouabain at 37°C for 10 min. Liberated inorganic phosphorus reacted with ammonium molybdate was quantified by measuring absorbance at 630 nm using a spectrophotometer (Corona Electronic, Ibaraki, Japan). Protein concentration was measured by using BCA (bicinchoninic acid) Protein Assay Kit (Thermo Scientific, IL). The activity was expressed as Pi (μmol) per protein (mg) per period (h).

Smolt-to-adult return (SAR)

From September through October in 2012 and in 2013, spawning adult masu salmon returned to the Kenichi River near the hatchery drainage were caught daily using by net, identified for treatment and counted. SAR was calculated as follows: SAR = number of tagged-adults returned x 100/ number of tagged-smolts released.

Statistical analysis

Values from precociously maturing males were not included in the analysis since those disturb IGF-I-growth relationships [44]. Results of the experiments were first analyzed by two-way ANOVA by including month and treatment as factors. When significant effects were found, the data were further examined by one-way ANOVA (between treatments within a month or between months within a treatment) followed by the Fisher's protected least significant difference (PLSD) test using the JMP program (SAS Institute Inc., Cary, NC). Differences among groups were considered to be significant at $P < 0.05$. Simple regression analysis was also conducted using JMP program and the relations were considered to be significant at $P < 0.05$.

SARs of groups in the same year were compared by χ^2 test using Stat View (SAS Institute). Differences among groups were considered to be significant at $P < 0.05$.

Results

Water temperature

Changes in water temperature at Kumaishi Hatchery using the Kenichi River water and Shimamaki Hatchery using the Chihase River water are shown in Figure 1. Overall, water temperature during January and February were similar in both hatcheries being < 4°C. It increased from March to May at Kumaishi Hatchery but stayed relatively low at Shimamaki Hatchery.

River and hatchery in 2008

FL, BW and condition factor of naturally- and hatchery-reared yearling masu salmon were similar in February and March (Fig. 2a,b,c). Condition factor was higher in naturally-reared fish from April and their FL and BW were also higher in May (Fig. 2c). Gill NKA activity was relatively low during February and April in naturally-reared fish but showed a sharp increase in May (Fig. 2d). In contrast, gill NKA activity in hatchery fish gradually, consistently increased from February to May. Serum IGF-I in wild fish was low in February and March and significantly increased in April through May, whereas hatchery fish showed a consistent increase in IGF-I from February to May (Fig. 2e). In both fish, serum IGF-I level positively correlated with body size (r^2 = 0.45-0.71) and gill NKA activity (r^2 = 0.27-0.36).

Kumaishi Hatchery in 2011 and 2012

In 2011, FL and BW were maintained low in Small-in-winter fish compared to Large-in-winter fish throughout the rearing period (January to May) except for BW in April (Fig. 3a,b,c). Condition factor was similar between treatments from January to May but became lower in Large-in-winter fish in May (Fig. 3c). Gill NKA activity in both groups were low during January and March but Small-in-winter fish showed a sharp increase in April (Fig. 3d). In contrast, Large-in-winter fish showed a gradual increase in gill NKA from March to May. Serum IGF-I in Large-in-winter fish gradually increased from January to April but tended to decrease in May (Fig. 3e). In contrast, small-in-winter fish showed a consistent increase in serum IGF-I from January to May and IGF-I levels in May was higher than those in Large-in-winter fish. In both groups, serum IGF-I level positively correlated with body size (r^2 = 0.39-0.73) and gill NKA activity (r^2 = 0.26-0.47).

In 2012, FL and BW were lower in the Small-High group from January to April but became insignificant in May (Fig. 4a,b,c). Condition factor was graded by the treatment throughout the experimental period being highest in Large-High fish and lowest in Large-Low fish (Fig. 4c). In all groups, gill NKA activity was gradually increased from February to April and showed a sharp increase in May (Fig. 4d). There were no significant differences in gill NKA among three groups during April and

May. Serum IGF-I in Large-High and Small-High fish consistently increased from February to April while that in Large-Low was significantly lower than two other groups during March and April (Fig. 4e). In May, IGF-I levels in all groups became as low as those seen in winter. Serum IGF-I level showed no significant relationships with body size or gill NKA activity.

Shimamaki Hatchery in 2012 and 2013

In both years, FL and BW were kept constant from January to April and increased in May (Fig. 5a,b). Condition factor was high in 2013 fish from February to April (Fig. 5c). It decreased in 2013 fish in May whereas increased in 2012 fish. Serum IGF-I levels stayed low from February to April but sharply increased in May in 2012 (Fig. 5d). In 2013, serum IGF-I significantly increased from February to March and further increased from April to May.

Smolt-to-adult return (SAR) at Kumaishi

Spawning adult masu salmon released from the hatchery in 2011 and 2012 were caught 16 months after release at the Kenichi River in 2012 and 2013, respectively. For the 2011-release fish caught in 2012, there was no significant difference in SAR between groups (Table 1). For the 2012-release fish caught in 2013, on the other hand, a significantly higher SAR was seen for Large-High fish (Table 1).

Discussion

The final goal of our study is to establish indices of "smolt quality" that enable us to evaluate survival of released fish in seawater and ultimately return as adults. Gill NKA activity is routinely used as an index of the smolt quatily since the ability to adapt to hypoosmotic environment is essential for smolts to survive in the ocean. Indeed, gill NKA activity in spring had a positive relationship with SAR of Chinook salmon released to the Deschutes River, Oregon, USA [37]. However, seawater adaptability does not always guarantee long-term survival. Several studies suggest that high growth rate is the key to survive from predation pressure and nutritional deficiency during the first year in the ocean [30-32]. Thus, potential for growth after adapting to seawater is an important trait that affects and in turn predicts a long-term survival. Zydlewski and Zydlewski [45] compared growth rates of individually-tagged Altantic salmon *Salmo salar* smolts with varying degree of gill NKA and suggested that gill NKA activity of smolt in freshwater did not predict long-term growth in seawater. Therefore, there is a need to establish indices that predict long-term growth potential in seawater. Circulating IGF-I is a candidate for this purpose since it is generally positively correlated with individual growth rate in fish including masu salmon [44, 46-49]. In addition, circulating IGF-I levels in Chinook salmon smolts just before release

have been shown to relate to SAR [37].

There are a good number of studies looking at profiles of circulating IGF-I during smoltification of salmonids including masu salmon [29, 50]. However, the present study is the first to report the IGF-I profile in naturally-reared smolting masu salmon. This data is relevant for the hatchery programs since one of the major goals of the programs is to create hatchery fish that are physiologically equivalent to wild fish. Mizuno et al. [51] compared metabolic capability between naturally- and hatchery-reared masu salmon during smoltification and pointed out differences in some enzymes involved in carbohydrate metablosim, the citric acid cycle and the respiratory chain. In the present study, profiles of serum IGF-I as well as gill NKA activity of hatchery fish were compared to those of naturally-reared fish. Fish from the Kenichi River showed a sharp increase in gill NKA activity that was preceded by a significant increase in circulating IGF-I. Gill NKA activity and circulating IGF-I in hatchery fish showed trends similar to those in naturally-reared fish, although the magnitude of the changes was not as sharp as those in naturally-reared fish. Given that both fish experienced the same water temperature profile, the difference should be attributed to other factors such as nutritional status, physical rearing structure and rearing density. There may be a concern that sampling by electrofishing might cause stress response and in turn affect measured IGF-I levels in naturally-reared fish. Although this possibility cannot be completely ruled out, we think the effect of electrofishing was minimum since this method has been proven to be less stressful compared to cast net in ayu *Plecoglossus altivelis* [52]. In the present study, food quality and availability are likely reasons for the difference because the size of naturally-reared fish was similar to that of hatchery fish during February and March but became significantly larger and fatter in April through May. An increase in condition factor in naturally-reared fish in April might not be a typical response during smoltification but reflected an improvement of feeding status in the river. In both fish, serum IGF-I was positively correlated with body size and gill NKA activity. These results suggest that size or/and growth in spring is important for achieving high gill NKA activity and circulating IGF-I.

We next examined effect of winter size on gill NKA and serum IGF-I by sorting fish by fork length in November at Kumaishi Hatchery. Difference in size was retained throughout the smoltification period. Gill NKA activity was significantly higher in Small-in-winter fish in April. In contrast, there was no difference in serum IGF-I levels between groups in April but it was high in Small-in-winter fish in May. These patterns of gill NKA and serum IGF-I in Small-in-winter fish are in contrast to those found in the naturally-reared fish where IGF-I increased prior to activation of gill NKA. Local IGF-I expressed by the gill may be involved in the activation of NKA [29]. However, our unpublished data on gill *igf-1* mRNA levels in these fish showed that the relationship between gill *igf-1* and NKA was weak albeit significant (r^2 = 0.18; Shimomura et al., unpubl. data). We assume that the increase in gill NKA in the

small fish in April was secondary to the increase in fish size. Indeed, there was a positive relationship between fish size and gill NKA (r^2 = 0.38). Given that gill NKA activity is generally higher in large fish [34, 40], it is possible that IGF-I promoted growth of organs including the gill, which in turn resulted in higher NKA activity.

We further examined effects of winter size and spring growth on gill NKA and serum IGF-I in 2012. Profiles of gill NKA activity were similar among three groups showing a sharp increase from April to May. In contrast, IGF-I levels in Large-Low fish were significantly low in early spring (March-April), which might reflect restricted feeding ration during that period. It is of note that although IGF-I in Large-High and Small-High fish consistently increased from February to April, it dropped to the basal levels in May. This IGF-I profile is similar to that of naturally-reared fish in terms of an increase prior to the activation of gill NKA but differ in term of low values in May. Water temperature is known to affect the degree and/or timing of the spring IGF-I increase [53] and might also affect the IGF-I profile in the present study. However, there was little difference in rearing water temperature between 2011 and 2012 at Kumaishi Hatchery. Although a year-to-year variation of circulating IGF-I profile was also reported in Chinook salmon [37], the reason(s) for the variation is not known at present. It is also of note that despite the depressed IGF-I level, gill NKA activity was high in May in all groups and there was no relationship between them, which is in contrast to our previous study [29]. This finding doesn't discount the importance of IGF-I in regulating the development of seawater adaptability but suggests that circulating IGF-I does not correlate with gill NKA activity under certain conditions in masu salmon. Investigating this decoupling may be important to better understand the relative importance of circulating and local IGF-I in activating gill NKA.

Circulating IGF-I levels are affected by environmental factors such as feeding status, season, developmental stage, water temperature, photoperiod and stress. Larsen et al. [54, 55] examined effect of low temperature and fasting during winter on the physiological status of smolting coho salmon *O. kisutch*. Their study suggested that winter fasting did not impair the condition or smoltification of hatchery-reared fish [54, 55]. In the present study, a feeding protocol was tested at Shimamaki Hatchery in 2012 to give a compensatory increase of growth as well as IGF-I in spring by suppressing feeding frequency (fed every three or four days) during winter. As a result, growth and IGF-I were low during that period. From April to May when feeding frequency and ration were increased, FL, BW, condition factor and IGF-I were increased. However, IGF-I values were relatively low compared to those in Kumaishi fish. In 2013, on one hand, fish were fed daily at a slightly higher ration in early spring to test if an IGF-I profile resembling that in Kumaishi fish could be created by changing the feeding protocol. As a result, IGF-I levels started to increase in March and further elevated in May as seen in Kumaishi Hatchery. These

changes might reflect a combination of IGF-I response to increased feeding frequency and ration. In spite of the low rearing water temperature, which could suppress the IGF-I peak, at Shimamaki Hatchery, the current result suggests IGF-I profiles can be manipulated by changing feeding frequency or/and ration, although more work needs to be done to prove this hypothesis.

The most reliable way to evaluate the hatchery success is to look at SAR. In the present study, we estimated SAR of some rearing groups from Kumaishi Hatchery by catching spawning adults at the Kenichi River. In the 2011-release group, there were variations in growth history and profiles of serum IGF-I and gill NKA activity, but SARs were not significantly different between groups. In contrast, despite little variations in gill NKA and serum IGF-I at the time of release in 2012, growth history had a significant effect on SAR being highest in Large-High fish. Beckman et al. [37] reported SARs of Chinook salmon released from three hatcheries on the Deschutes River, Oregon, USA were different. Their study also revealed that gill NKA activity and plasma IGF-I level at the time of release showed significant relationships to SAR [37], which is in contrast to our finding. We have no good explanation for the lack of the relation between IGF-I and SAR in masu salmon but suspect that the ocean conditions and predation pressure had a stronger impact on survival than did the status of smolting at release. Ando et al. [56] reported that masu salmon released from Kumaishi Hatchery migrated to the Sea of Okhotsk through either along the Sea of Japan or Pacific Ocean side of Hokkaido Island. Although data is limited, conditions in the Sea of Okhotsk may be critical to their survival (Iijima et al. unpubl. data). As mentioned above, growth status in the early marine life is the key to survive for salmonids [30-32]. Filling a gap of information on the growth status of masu salmon from early ocean life through winter is neceassary. Monitoring growth of outmigrating postsmolts using IGF-I has been conducted for coho and Atlantic salmon to better estimate their survival and stock recruitment [46, 57, 58] and introducing such approach to masu salmon is important. The present study, however, provided evidence that growth history during freshwater residency affected long-term survival in the ocean in masu salmon.

In summary, the present study showed that profiles of circulating IGF-I during smoltification of masu salmon varied under different rearing conditions depending mainly on growth history. Although serum IGF-I may be important for both development of seawater adaptability and growth enhancement, serum IGF-I level alone was not a predictor of long-term survival after sea entry in masu salmon. However, differences in growth history had a significant effect on the adult returns. Thus, optimizing rearing conditions is a critical component for the success of hatchery release of masu salmon and development of physiological indices to evaluate the status of smolts is desired.

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487

Figure legends

Fig. 1. Changes in water temperature at Kumaishi Hatchery (a) and Shimamaki Hatchery (b). Kumaishi Hatchery and Shimamaki Hatchery used river waters from the Kenichi and Chihase Rivers, respectively.

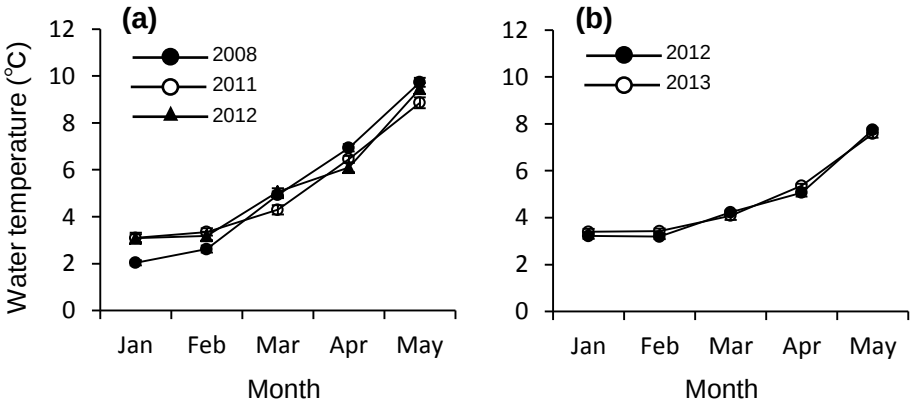
Fig. 2. Changes in FL (a), BW (b), condition factor (c), gill NKA activity (d) and serum IGF-I levels (e) during smoltification of naturally- and hatchery-reared masu salmon in 2008. Fish were caught monthly from the Kenichi River or reared at Kumaishi Hatchery. Values are expressed as means ± SE (n = 6-10). Symbols sharing the same letters are not significantly different from each other.

Fig. 3. Changes in FL (a), BW (b), condition factor (c), gill NKA activity (d) and serum IGF-I levels (e) during smoltification of masu salmon reared at Kumaishi Hatchery in 2011. Fish were divided by size in winter (Large: >11.5 cm, Small: 9.5-11.5 cm) and reared separately. Values are expressed as means ± SE (n = 6-7). Symbols sharing the same letters are not significantly different from each other.

Fig. 4. Changes in FL (a), BW (b), condition factor (c), gill NKA activity (d) and serum IGF-I levels (e) during smoltification of masu salmon reared at Kumaishi Hatchery in 2012. Fish were divided by size in winter (Large: >11.5 cm, Small: 9.5-11.5 cm) and fed at different rations to create high and low growth in spring (High and Low). Values are expressed as means ± SE (n = 6-7). Symbols sharing the same letters are not significantly different from each other.

Fig. 5. Changes in FL (a), BW (b), condition factor (c) and serum IGF-I levels (d) during smoltification of masu salmon reared at Shimamaki Hatchery in 2012 and 2013. Fish were fed every three or four days during early spring in 2012 whereas fed daily in 2013. Values are expressed as means ± SE (n = 6-7). Symbols sharing the same letters are not significantly different from each other.

Kaneko *et al*., Fig. 1



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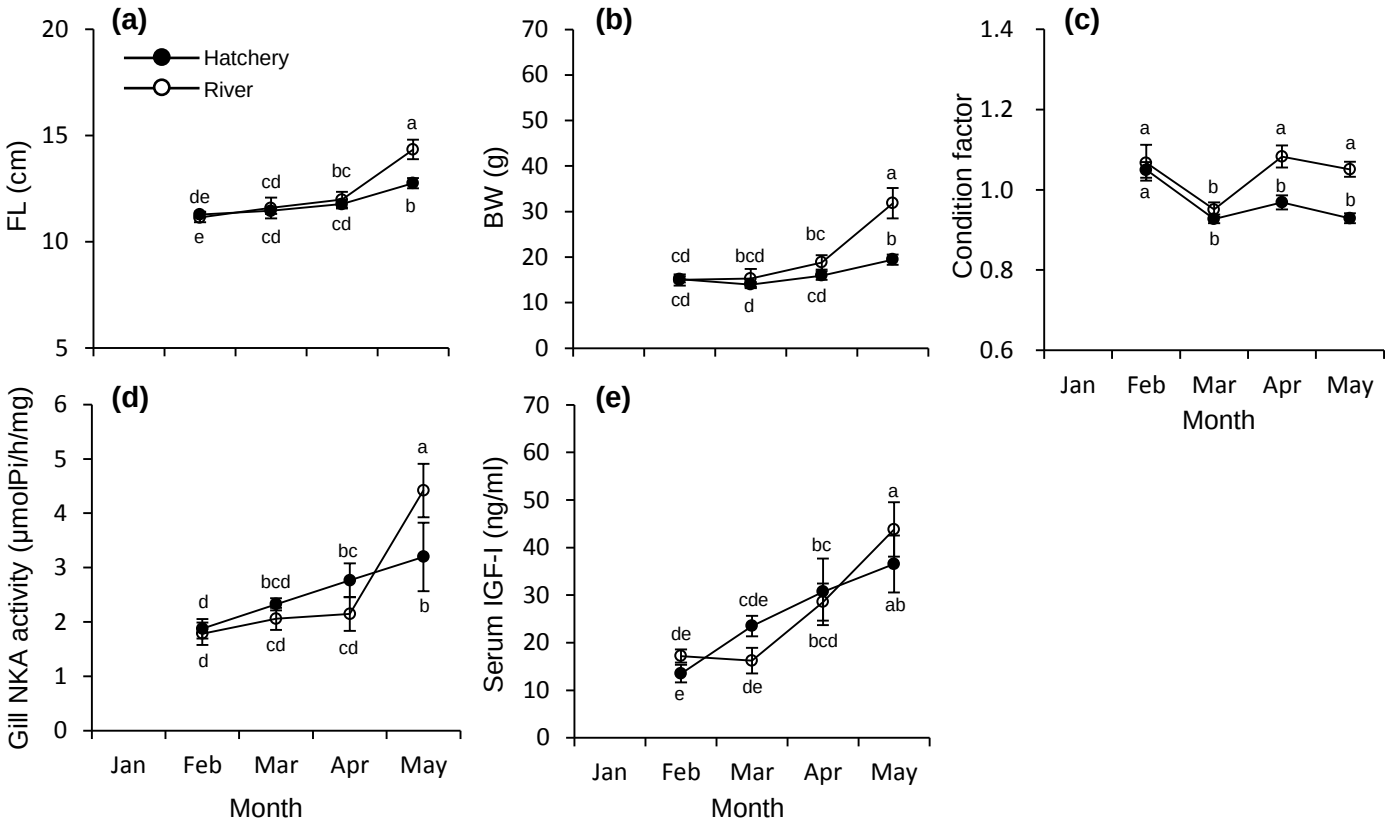

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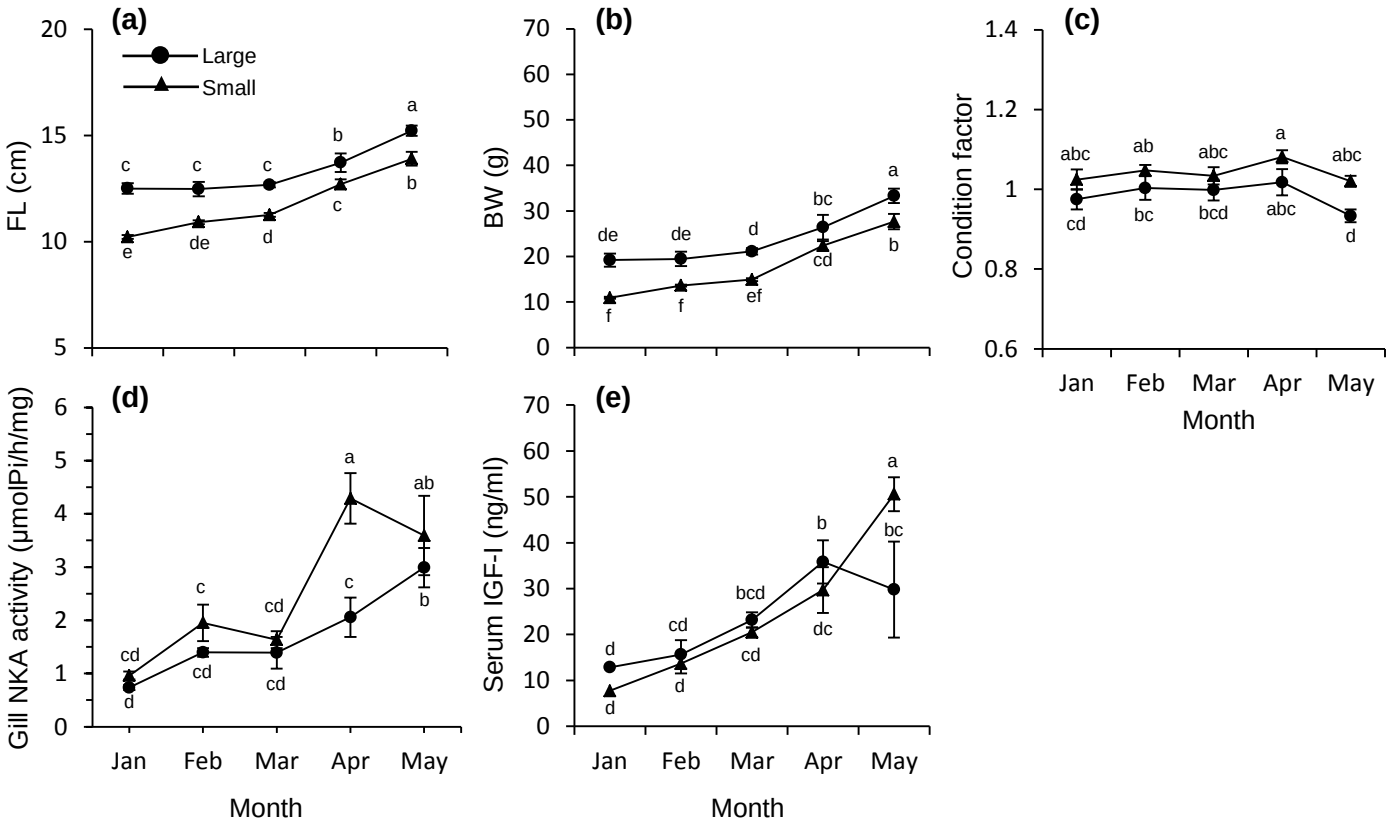

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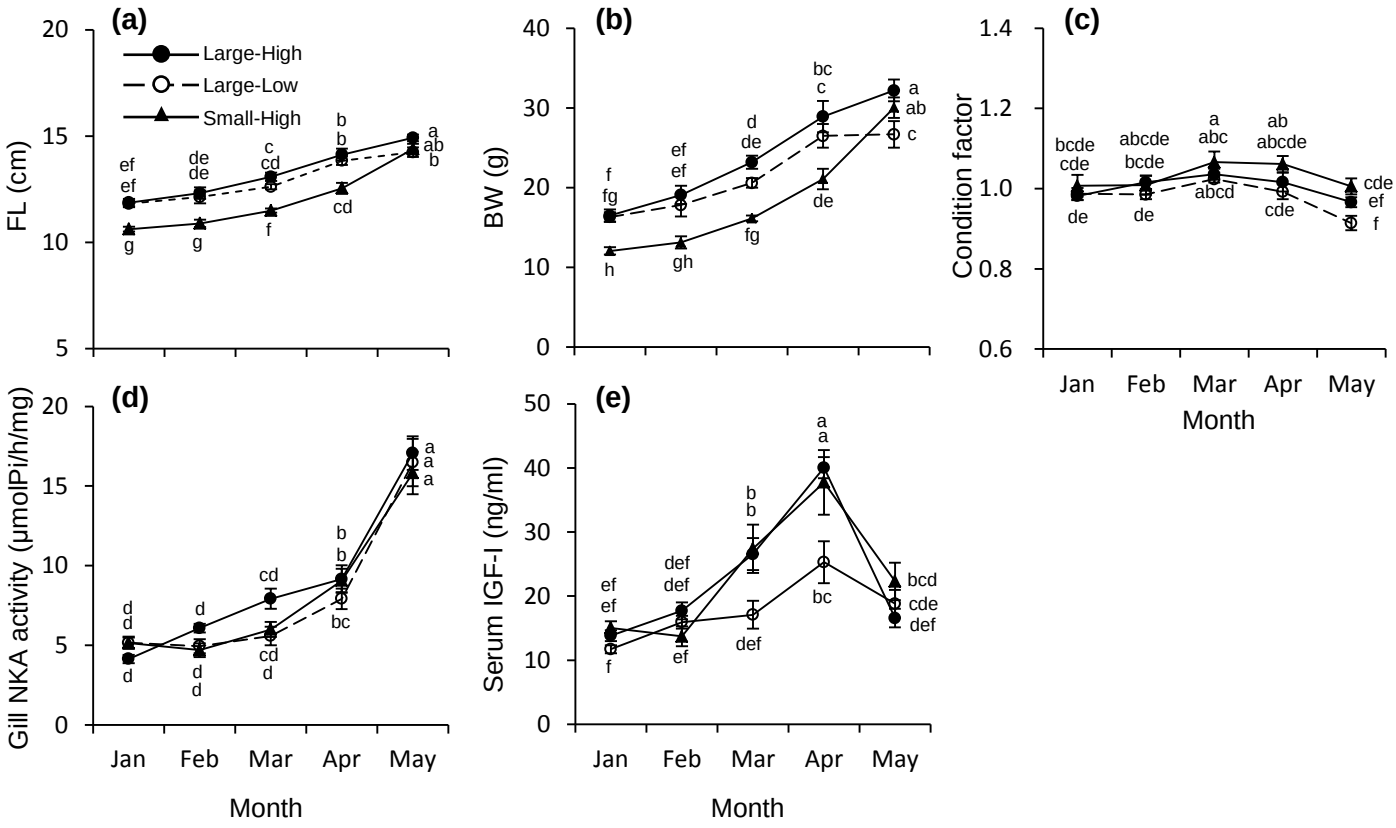

Kaneko *et al*., Fig. 5

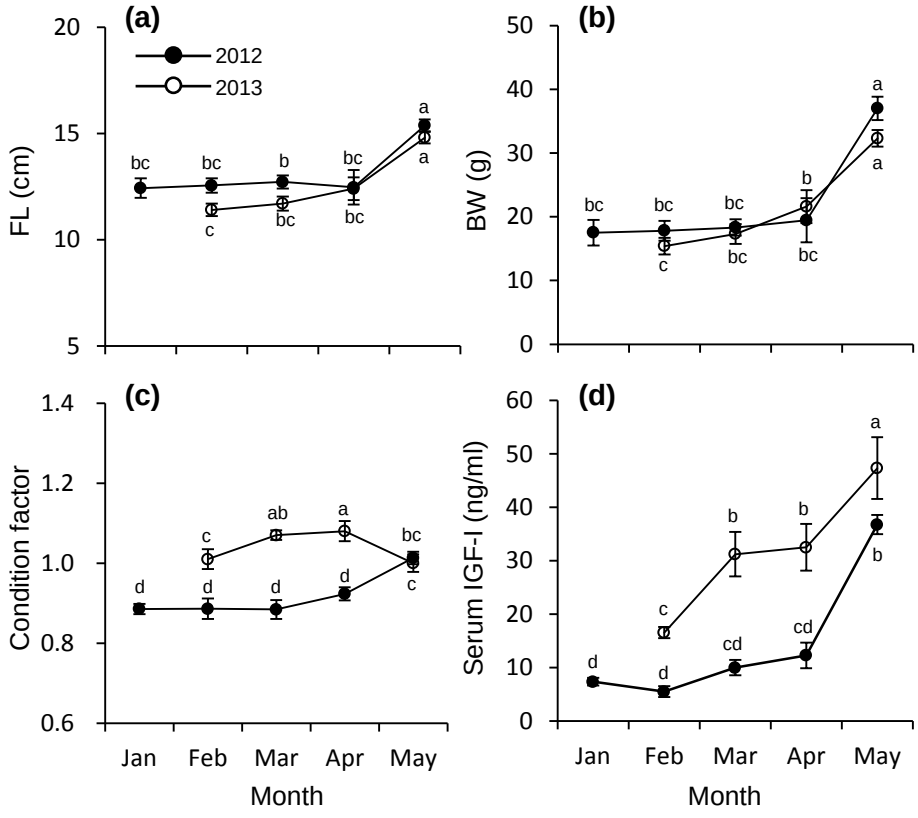

Table 1
SAR of masu salmon released from Kumaishi Hatchery in 2011 and 2012

		Smolts released	Adults caught in river	Return rate (%)
2011	Large	30,060	45	0.150
	Small	25,459	47	0.185
2012	Large-High	28,964	209	0.722*
	Large-Low	27,912	107	0.383
	Small-High	28,641	111	0.388

Large: FL > 11.5 cm in November, Small: FL 9.5-11.5 cm in November; High: high growth in spring, Low: low growth in spring. An asterisk indicates significant difference among groups in the same year.