**Title:**

Evaluation of circulating insulin-like growth factor (IGF)-I and IGF-binding proteins as growth indices in rainbow trout (*Oncorhynchus mykiss*)

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**Abstract**

Circulating insulin-like growth factor (IGF)-I has been proposed as a growth index in several teleosts, including salmonids, and its level in circulation is stabilized by multiple IGF-binding proteins (IGFBPs). Three IGFBPs, IGFBP-2b, -1a, and -1b, are consistently detected in salmonid blood and are suggested to be indices of positive or negative growth, although their applicability to rainbow trout (*Oncorhynchus mykiss*) is unclear. The present study examined the usefulness of IGFBPs along with IGF-I as a physiological indicator of growth rate in rainbow trout through a rearing experiment. Two groups of underyearling rainbow trout were pit-tagged and either fed or fasted for 33 days. A third group was fasted for 22 days, followed by refeeding for 11 days. Serum IGF-I levels were reduced after fasting for 22 days, but refeeding did not retore its levels to those of the fed control. Nevertheless, there was a positive relationship between serum IGF-I levels and individual growth rates over 33 days of experimentation, confirming its validity as a growth index. Ligand blotting using labeled human IGF-I revealed two IGFBP bands at 43 and 32 kDa, which corresponded to IGFBP-2b and an unidentified form, respectively. In contrast, bands corresponding to IGFBP-1a and -1b, which usually increase after fasting, were hardly detected, even in the fasted fish. The responses of circulating IGFBP-2b to fasting and refeeding were similar to those of circulating IGF-I and positively correlated with growth rate and IGF-I levels. The intensity of the serum 32-kDa IGFBP band was higher in constantly fed fish than in the fasted fish; however, its correlation with growth rate was weaker than those of IGF-I and IGFBP-2b. The present study shows that IGF-I and IGFBP-2b can be used as growth indices for rainbow trout. In contrast, circulating IGFBP-1a and -1b may not serve as negative growth indices in rainbow trout under regular aquaculture conditions because they are rarely detected by ligand blotting or respond to fasting/refeeding.

**Keywords**

aquaculture, compensatory growth, somatotropic axis, immunoassay, individual growth

## 1. Introduction

Insulin-like growth factor (IGF)-I is a 7.5 kDa polypeptide, which is structurally similar to proinsulin and plays an important role in cell proliferation, differentiation, and survival. IGF-I is produced in virtually all tissues and exerts its actions through endocrine, paracrine, and autocrine signaling in vertebrates (Daughaday and Rotwein, 1989; LeRoith et al., 2001; Wood et al., 2005; Ohlsson, 2009). Endocrine IGF-I is produced mainly by the liver upon growth hormone (GH) stimulus and mediates GH actions in various tissues (Daughaday and Rotwein, 1989; Ohlsson, 2009). Unlike insulin, circulating IGF-I levels are stabilized by the presence of IGF-binding proteins (IGFBPs), which prolong the half-life of IGF-I by protecting against glomerular filtration in the kidney and enzymatic degradation (Rajaram et al., 1997). They also regulate the availability of IGF-I to target tissues by either inhibiting or promoting the binding to the receptor (Firth and Baxter, 2002), making them important growth regulators in vertebrates.

Six IGFBPs have been identified and characterized in mammals (Shimasaki and Lin, 1991; Bach, 2018). Teleosts generally possess two paralogs of each member of the IGFBPs, except IGFBP-4 due to a third round of whole-genome duplication (WGD) that occurred after their divergence from other vertebrates (Ocampo Daza et al. 2011). Salmonids experienced an additional round of WGD and have up to four copies of each IGFBP, which resulted in the presence of 22 paralogous genes (Macqueen et al. 2013; de la Serrana and Macqueen, 2018). The retention of the most duplicated IGFBP paralogs suggests that the fine-tuning of IGF-I activity by IGFBPs had an adaptive value in salmonids and teleosts (Allard and Duan, 2018; de la Serrana and Macqueen, 2018).

In addition to understanding the roles and functions of IGF-I and IGFBPs in teleosts, there is an increasing interest in utilizing them as growth indices (Picha et al., 2008; Beckman, 2011). This is based on the findings that circulating IGF-I levels are generally positively correlated with individual growth rates in postsmolt coho salmon (*Oncorhynchus kisutch*; Beckman et al., 2004a,b,c). This relationship has also been observed in other salmonids and fish species, including masu salmon (*O. masou*: Kaneko et al., 2020), chum salmon (*O. keta*: Kaneko et al., 2015), Atlantic salmon (*Salmo salar*: Breves et al., 2020), olive rockfish (*Sebastes serranoides*: Hack et al., 2018), copper rockfish (*S. caurinus*: Hack et al., 2019), and cabezon (*Scorpaenichthys marmoratus*: Strobel et al., 2020). This characteristic makes circulating IGF-I a useful index for assessing and evaluating the growth status of fish under captivity and in the field.

Some IGFBPs are also good markers of anabolic and catabolic conditions in fish and could be used as quantitative growth indices (Kelley et al., 2001; Kaneko et al., 2020). Three major IGFBP bands at 40–50, 25–30 and 20–25 kDa were consistently detected in the plasma/serum of several fish species by ligand blotting using labeled IGF-I (Kelley et al., 2001;

Shimizu and Dickhoff, 2017); these three IGFBPs have been identified as IGFBP-2b, -1a, and -1b, respectively (Shimizu et al., 2011a,b). Salmon IGFBP-2b is believed to be the main carrier of circulating IGF-I, and its level was positively correlated with individual growth rate as well as IGF-I levels (Shimizu et al., 2003; Beckman et al., 2004a). In contrast, fish IGFBP-1s have been shown to inhibit IGF-I actions in zebrafish (*Danio rerio*) and salmonids (Maures et al. 2001; Bauchat et al., 2001; Kamei et al., 2008; Tanaka et al., 2018; Hasegawa et al., 2020). In salmonids, IGFBP-1a and -1b levels increased by fasting and stress and negatively correlated with individual growth rates (Kaneko et al., 2020). These findings support the use of circulating IGFBPs in fish as positive or inverse growth indices.

Rainbow trout (*O. mykiss*) is an important species for aquaculture and is the second most aquacultured salmonid after Atlantic salmon (FAO, 2020). It is reared in a wide variety of salinity, temperature, photoperiod, rearing density, and water quality using open, semi-closed, or closed systems. Selective breeding has greatly improved the growth performance of rainbow trout under captivity (Leeds et al., 2016) and alternative feeds have been developed for sustainable aquaculture of this species and other fishes (Jalili et al., 2013; Hua et al., 2019). Therefore, evaluating the growth performance of rainbow trout under different environmental and feeding conditions is critical.

Despite its importance in aquaculture and fish physiology, the validation of circulating IGF-I and IGFBPs as growth indices in rainbow trout is incomplete. Taylor et al. (2005, 2008) first reported the relationship between plasma IGF-I and growth rate in rainbow trout. Rainbow trout is one of the first species to be reported for the presence of IGFBPs in fish (Niu et al., 1993). Multiple IGFBP bands were detected at 50, 42, 32, and 21 kDa by ligand blotting, and some of them responded to salinity change or handling stress (Shepherd et al., 2005, 2011). We have recently reported a positive correlation between serum IGF-I levels and serum IGFBP-2b band intensity in rainbow trout, suggesting that IGFBP-2b in trout is also a major carrier of circulating IGF-I (Cleveland et al., 2018, 2020). However, our initial screening of serum IGFBP-1a and -1b suggested that they might not be major circulating forms (Cleveland et al., 2018; 2020). Despite the information available for the regulation of circulating IGF-I and IGFBPs in rainbow trout, no study has comprehensively examined their relationships with feeding status and individual growth rates in this species. The objectives of the present study were to confirm the validity of serum IGF-I as a positive growth index in rainbow trout and to assess the use of circulating IGFBPs as growth indices through a fasting/refeeding experiment of individually tagged fish.

## 2. Materials and Methods

### *2.1. Fish and experimental design*

Fertilized eggs of rainbow trout were obtained from Troutlodge (Bonny Lake, WA) and

transferred to an indoor rearing facility in FRD Japan (Saitama, Japan). Juvenile trout were reared in 1 and 3-ton tanks connected to a recirculating aquaculture system (RAS) with nitrification and denitrification systems at 15 °C in 0.5 psu under a photoperiod regime of LD 24:0. Fish were fed a commercial diet containing 42% crude protein and 19% crude lipid (Nosan Corporation, Kanagawa, Japan) ad libitum twice daily. When fish exceeded 200 g, they were transferred to another rearing facility in FRD Japan (Chiba, Japan) and reared in a 7-ton indoor-pond installed with a RAS. Water temperature, photoperiod, and feeding conditions were the same as described above, with a salinity of 4 psu. On November 4, 2020, 48 eight-month-old rainbow trout with an average body length (BL), the length from the tip of the snout to the posterior end of the body covered with scales, of 24.1 ± 0.2 cm and body weight (BW) of 265.4 ± 7.8 g were anesthetized in 0.02% Eugenol (FA100, DS Pharma Animal Health Co., Ltd, Osaka, Japan) and individually marked with passive integrated transponder (PIT) tag (12.5 × 2.1 mm, Biomark, Boise, ID, USA). Sixteen fish were randomly placed into one of the three 200 L tanks (DAILITE, Tokyo, Japan) and assigned to the fed, fasted, or refed groups. The fed group was given a commercial diet daily to satiety, whereas the fasted group was fasted throughout the experimental period for 33 days. The refed group was fasted for first 22 days and then fed to satiety for the following 11 days. During the experimental period, four fish died and six fish lost their tag ID. Rearing and handling fish were carried out in accordance with the guidelines of the Hokkaido University Animal Care and Use Committee (#30-3).

*2.2. Sampling procedure*

At 0, 22, and 33 days after the beginning of the experiment, all individuals were anesthetized, read for PIT-tag, and measured for BL and BW. The condition factor (K) was calculated as follows: (BW (g)) × 100 / (BL (cm))$^3$. The specific growth rate (SGR) was calculated as follows: SGR (%/day) = ln ($s_2$ − $s_1$) × ($d_2$ − $d_1$)$^{-1}$ × 100, where $s_2$ is length or weight on day 2, $s_1$ is length or weight on day 1, and $d_2$ − $d_1$ is the number of days between measurements. Sixteen fish from each treatment group were divided into two categories: serial or single blood collection. Serial blood sampling was conducted for eight fish from each treatment on days 0, 22, and 33, whereas a single blood collection was performed for the other eight fish on day 33. Blood was withdrawn using a syringe from the caudal veins, allowed to clot overnight at 4 °C, and then centrifuged at 10,000 × *g* for 10 min. Serum was stored at -30 °C until use.

*2.3. Time-resolved fluoroimmunoassay (TR-FIA) for IGF-I*

To measure IGF-I, serum was extracted with acid–ethanol as described by Shimizu et al. (2000). IGF-I was quantified by TR-FIA based on the method described by Small and Peterson (2005), using recombinant salmon/trout IGF-I (GroPep, Adelaide, SA, Australia) as the standard. Time-

resolved fluorescence was measured using a Wallac ARVO X4 (PerkinElmer, Waltham, MA, USA).

*2.4. Ligand blotting*

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a 3% stacking gel and 12.5% separating gel. Serum samples were treated with an equal volume of sample and buffer containing 2% SDS and 10% glycerol at 85 °C for 5 min. Gels were placed in a solution of 50 mM Tris, 400 mM glycine, and 0.1% SDS at 8 mA for the stacking gel and 12 mA for the separating gel until the bromophenol blue dye front reached the bottom of the gel.

Ligand blotting with digoxigenin-labeled human IGF-I (DIG-hIGF-I) was performed according to a previously described protocol (Shimizu et al. 2000). The nitrocellulose membranes were incubated overnight with DIG-hIGF-I and then incubated with antibodies against DIG conjugated horseradish peroxidase (Roche, Indianapolis, IN, USA) at a dilution of 1:1500–2500 for 1.5 h at room temperature (20–25 °C). IGFBP was visualized using enhanced chemiluminescence (ECL) western blotting reagents (Amersham Life Science, Arlington Heights, IL, USA). The intensities of serum IGFBP bands were semi-quantified using ImageJ version 1.440 (Schneider et al., 2012), normalized to the human IGFBP-4 band intensity, and expressed as an arbitrary density unit (ADU).

*2.5. Statistical analysis*

The effects of tank/feeding treatment and time were analyzed by two-way analysis of variance (ANOVA) using the JMP program (SAS Institute Inc., Cary, NC, USA). When significant effects were found, differences were further identified by one-way ANOVA followed by Tukey's honestly significant difference (HSD) test, with differences considered significant at $P < 0.05$. Simple regression analysis was also conducted using JMP software, and the relationships were considered significant at $P < 0.05$.

## 3. Results

*3.1. Body size*

There were main effects of tank/feeding treatment and time and their interaction on BL, BW and K (Fig. 1). Control-fed fish grew for 33 days of the experimental period (Fig. 1). Fasting for 22 days suppressed BW and K but not BL. Refeeding for 11 days had positive effects on BW compared to those of time-matched fasted group, whereas no difference was seen in BL and K. However, refeeding did not restore BW to values similar to those of fed fish. There were no effects of serial sampling on these parameters.

### *3.2. SGR*

SGRs in length and body weight (SGRL and SGRW) of the fasted group were lower than those of the fed group after 22 days of fasting (Table 1). Serial blood sampling negatively impacted SGRL in the fasted group ($P < 0.05$) which in turn resulted in a relatively low SGRL compared to the refed group despite the same fasting treatment during days 0-22. Refeeding for 11 days after 22 days of fasting increased the SGRW but not the SGRL. SGRs during 33 days in the refed group were higher than those in the fasted group but lower than those in the fed group.

### *3.3. Serum IGF-I*

There were main effects of tank/feeding treatment and time and their interaction on serum IGF-I (Fig. 2). Serum IGF-I levels were reduced after 22 days of fasting. Refeeding for 11 days had no effect on serum IGF-I levels. There was no effect of serial sampling on serum IGF-I (data not shown).

### *3.4. Serum IGFBPs*

Multiple IGFBP bands were visualized in the serum of rainbow trout by ligand blotting using DIG-labeled hIGF-I (Fig. 3). The IGFBP-2b bands at 41-45 kDa were consistently detected in all groups, whereas the IGFBP-1a and -1b bands at 28 and 22 kDa, respectively, were rarely detected. An unidentified IGFBP was detected at a molecular weight of 32 kDa. The 32-kDa IGFBP band was not detected in five out of 18 samples on day 22 and seven out of 38 samples on day 33 (data not shown).

The intensities of the bands of IGFBP-2b and 32 kDa IGFBP were semi-quantified and compared among the treatments (Fig. 4). There were main effects of tank/feeding treatment and time and their interaction on serum IGFBP-2b (Fig. 4a). The intensity of the IGFBP-2b band in fed fish increased over the experimental period of 33 days. Fish fasted for 33 days had a lower band intensity than that of fed fish. Refeeding for 11 days had no effect on the serum IGFBP-2b band intensity. There was a main effect of time on serum 32-kDa IGFBP where the intensity of the 32-kDa IGFBP band was reduced over the experimental period (Fig. 4b). Fasting nor refeeding had no significant effects on the serum 32-kDa IGFBP band intensity. There were no effects of serial sampling on the band intensity of IGFBP-2b and 32-kDa IGFBP (data not shown).

### *3.5. Relationship with SGRs*

There were positive correlations between serum IGF-I levels at day 33 and SGRs over 33 days (Fig. 5a,b). The serum IGFBP-2b and 32-kDa IGFBP band intensities also positively correlated with both SGRL and SGRW, whereas the coefficient of correlation appeared to be higher with IGFBP-2b than with 32-kDa IGFBP (Fig.5c–f). When the correlations with SGRs during days

22–33 were compared among treatments, serum IGF-I, IGFBP-2b, and 32-kDa IGFBP showed positive correlations, except for the 32-kDa IGFBP with SGRL (Table 2). In the serially collected blood from fed fish, serum IGF-I levels on day 22 positively correlated with future SGRs during 22–33 days (SGRL: $r^2 = 0.49$, $P = 0.0117$; SGRW: $r^2 = 0.83$, $P < 0.0001$), which were comparable to those between serum IGF-I levels on day 33 and past SGRs during 22–33 days (SGRL: $r^2 = 0.65$, $P = 0.0016$; SGRW: $r^2 = 0.80$, $P < 0.0001$).

## 4. Discussion

In the present study, rainbow trout was subjected to fasting followed by refeeding to investigate responses of body conditions and the circulating IGF-I/IGFBP. After 22 days of fasting, BW, BL, and K in the fasted group were lower than those in the fed group, consistent with previous results (Gabillard et al., 2006; Medeirous et al., 2020). Refeeding for 11 days had positive effects on restoring BW and K but not BL when compared to the fasted group; however, none of the parameters matched those of the fed group, showing that 11 days of refeeding was insufficient to restore body size and conditions. Such slow recovery of growth from fasting has been previously reported in rainbow trout (Gabillard et al., 2006). Surprisingly, the SGRW of the refed group in the present study was not higher than that of the fed group as compensatory growth was often observed in fish after food restriction/deprivation followed by increasing the feeding ration (Ali et al., 2003). Despite the lack of compensatory growth in refed fish, the treatments in the present study (fed, fasted, and refed) resulted in different levels of SGR over 33 days.

Circulating IGF-I showed a response similar to that of BL, whose decreased levels after the 22 day fasting period did not increase even after 11 days of refeeding. The reduction in serum IGF-I levels after a few weeks of fasting is compatible with other studies on salmonids, including rainbow trout (Medeiros et al., 2020; Gabillard et al, 2006; Caldarone et al. 2016; Kaneko et al. 2019). However, the degree of recovery of circulating IGF-I after refeeding may depend on the strain/species, developmental stage and/or experimental setting. Gabillard et al. (2006) reported that plasma IGF-I levels in rainbow trout fasted for one month started increasing four days after refeeding and reached similar levels as the initial group after 14 days. In contrast, when rainbow trout were fasted for three weeks and refed for one week, there was no increase in serum IGF-I levels (Cleveland et al., 2020). In a study using yearling masu salmon that fasted for one month, two weeks of refeeding increased serum IGF-I levels, although the levels were still lower than those of fed controls (Kaneko et al., 2020). We previously reported that in coho salmon, continuously fed fish exhibited increased plasma IGF-I levels after a meal in 24 h, whereas fish previously fasted for three weeks did not (Shimizu et al., 2009). Thus, in addition to species differences, the fasting period also affects the response of circulating IGF-I to food intake. Further research to fully elucidate the underlying mechanism of growth after food deprivation in rainbow

trout needs analyses of the GH and IGF-I receptor abundance and signaling pathways.

Despite the relatively low recovery of average serum IGF-I levels after refeeding, there were positive relationships between serum IGF-I and SGRs in body length/weight during 22–33 days as well as during 0–33 days. Taylor et al. (2005, 2008) found a positive relationship between average plasma IGF-I levels and average SGR in adult rainbow trout. In contrast, Morro et al. (2019) reported that plasma IGF-I positively correlated with individual SGR of rainbow trout in seawater but not in freshwater, although the trout were exposed to different photoperiod regimes in freshwater. The results of the present study are similar to those of the study by Taylor et al. (2005, 2008) and confirm the positive relationship of individual fish under varying feeding conditions. Moreover, in the present study, half of the experimental fish were sequentially sampled for blood at days 0, 22, and 33. When fed and fasted groups were combined, serum IGF-I levels at day 22 and day 33 correlated with SGRL during days 22–33, showing that serum IGF-I levels projected future growth rate as long as feeding and other conditions were unaltered. Such links with both past and future growth rates have been suggested in coho salmon (Pierce et al., 2001) and is consistent with the growth-promoting action of IGF-I in salmon (McCormick et al., 1992). The results of the present study further strengthen the validity of circulating IGF-I as a "current" growth index in fish.

The response of serum IGFBP-2b was similar to that of serum IGF-I, and the intensity of IGFBP-2b band was positively correlated with serum IGF-I levels, supporting the notion that IGFBP-2b is a major carrier of circulating IGF-I (Shimizu et al., 2003; Shimizu and Dickhoff, 2017). However, IGFBP-2b not responding to refeeding for 11 days in rainbow trout was atypical. We previously showed that IGFBP-2b retained the ability to increase in response to a single meal after three weeks of fasting, whereas IGF-I did not in coho salmon (Shimizu et al., 2009). Low sensitivities of IGF-I and IGFBP-2b to refeeding may be a characteristic of rainbow trout. Thus, slow recovery of the endocrine parameters after fasting demands further studies on the mechanism of compensatory growth using rainbow trout.

Despite the low sensitivity of serum IGFBP-2b to refeeding, it was positively correlated with SGR, similar to serum IGF-I. Our results are consistent with previous findings in coho salmon that plasma IGFBP-2b was also useful as a growth index (Beckman et al., 2004a,b,c). The present study also semi-quantified the intensity of IGFBP-2b band, which possesses IGF-binding ability (i.e. "intact" form), by ligand blotting, and previous studies quantified immunoreactive IGFBP-2b, which contained intact and fragmentated forms, by radioimmunoassay (RIA; Beckman et al., 2004b,c). Despite the difference in the detection principle, the two methods revealed similar relationships between IGFBP-2b and SGR, suggesting that most IGFBP-2b circulates in an intact form in immature rainbow trout. However, the degree of IGFBP-2b fragmentation in different stages, status, and species is important because the release of IGF-I

from IGFBP by enzymatic degradation is an important mechanism for delivering IGF-I to its receptor (Firth and Baxter, 2002). Although quantitative immunoassays such as RIA or TR-FIA are desirable for IGFBP-2b, the present study showed ligand blotting as a promising assay to show the robust relationship with SGRs under different feeding regimes and its utility as a growth index in rainbow trout.

The response of the 32-kDa IGFBP appeared to be more sensitive to feeding changes because it was reduced after fasting and restored by refeeding. The 32-kDa IGFBP is a fourth circulating form, along with IGFBP-1a, -1b and -1b, detected in the circulation of coho salmon, masu salmon, and rainbow trout (Shimizu and Dickhoff, 2017; Cleveland et al., 2018, 2020; Hayashi et al., in press). Our result that the 32-kDa IGFBP was positively influenced by feeding is consistent with that of Cleveland et al. (2020). However, although serum 32-kDa IGFBP responded to refeeding better than serum IGFBP-2b, its correlation with SGR was lower than that of IGFBP-2b. Based on these results, we hypothesize that the 32-kDa IGFBP has a higher sensitivity to feeding ration and plays a role in complementing with IGFBP-2b to protect IGF-I in the short-term, which will be addressed in a future study.

One of the significant findings from the present study is that IGFBP-1a and -1b were rarely detected in the serum of rainbow trout in all feeding treatments for up to one month. Several studies on salmon have shown that IGFBP-1a and/or -1b were useful as inverse growth indices in salmon (Shimizu et al., 2006; Kawaguchi et al. 2013; Kaneko et al. 2019; Kaneko et al. 2020), although this has not been demonstrated in rainbow trout. In the plasma/serum of rainbow trout, up to five IGFBP bands at 50, 42, 32, 30, and 21 kDa have been detected by ligand blotting (Niu and Le Bail, 1993; Bauchat et al., 2001; Shephered et al., 2005). The IGFBP bands at 30 and 21 kDa appeared to correspond to IGFBP-1a and -1b, respectively (Shimizu et al., 2011). Moreover, these IGFBPs are induced in the blood by cortisol (Shimizu et al., 2011a). Thus, two IGFBP-1s are present in the circulation of rainbow trout. Indeed, plasma IGFBP-1b levels in rainbow trout were measurable by TR-FIA and responded to changes in water temperature and seawater transfer (Hevrøy et al., 2015; Morro et al., 2020). However, in the present study, ligand blotting detected IGFBP-1a and -1b very rarely and weakly, which did not appear to respond to feeding status. This result contrasts with those of previous studies using masu salmon (Kawaguchi et al., 2013; Kaneko et al., 2020) but agrees with our previous study using rainbow trout, although its experimental design was incomplete (Cleveland et al., 2020). The poor reactivity of IGFBP-1s to nutritional change was also reported at the mRNA level. In an experiment using rainbow trout, *igfbp-1* mRNA was detected in both liver and muscle but did not respond to fasting for 30 days (Gabillard et al., 2006). The apparent lack of the IGFBP-1 response to fasting in rainbow trout might be due to genetic alterations after selective breeding for high growth in fish farms (Tymchuk and Devlin, 2005) where fish are fed to satiation and rarely experience long-term fasting.

Although the cause and significance are subjects of future study, IGFBP-1s play little role in regulating growth in domesticated rainbow trout under normal aquaculture conditions and are not useful as inverse growth indices unless fish are severely stressed.

In summary, the present study confirmed the validity of circulating IGF-I and IGFBP-2b as positive growth indices for rainbow trout. In contrast, IGFBP-1a and -1b are not reliable inverse growth indices under aquaculture conditions because of their low levels and low sensitivity to feeding status. The slow recovery of growth and IGF-I/IGFBP-2b from fasting and the apparent lack of growth regulation by IGFBP-1s make rainbow trout a unique comparative model to investigate the mechanism of growth regulation in fish.

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**Figure legends**

Fig. 1 . Effects of fasting and refeeding on body length (BL; a), body weight (BW; b) and condition factor (K; c). Values are expressed as means ± SE (day 0: $n$ = 48. day 22: $n$ = 40 and day 33: $n$ = 38). Groups sharing the same letters are not significantly different from each other (Tukey's HSD, $P < 0.05$).

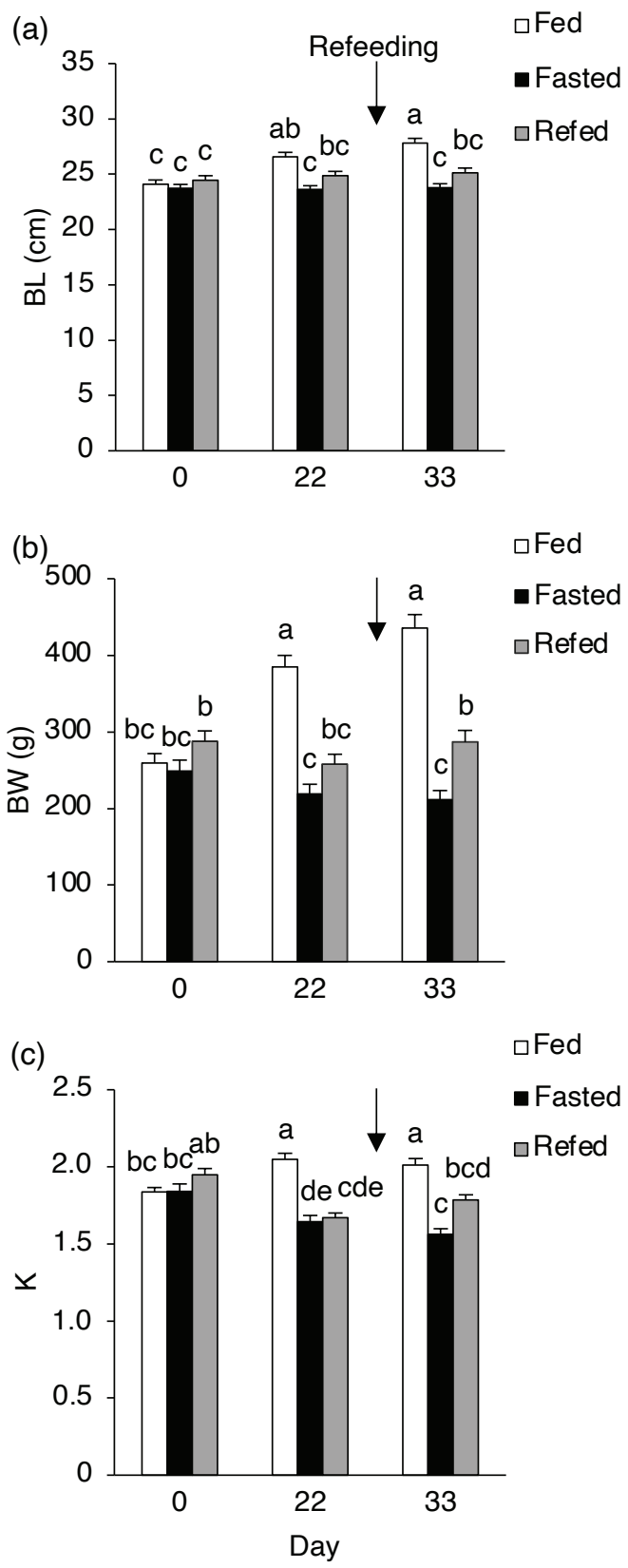
Fig. 2. Effects of fasting and refeeding on serum IGF-I levels. Values are expressed as means ± SE (day 0: $n$ = 22. day 22: $n$ = 18 and day 33: $n$ = 38). Groups sharing the same letters are not significantly different from each other (Tukey's HSD, $P < 0.05$).

Fig. 3. IGFBP patterns in serum of fed and fasted rainbow trout. Rainbow trout were fed or fasted for 33 days or were refed for 11 days after 22 days of fasting. Two microliters of serum was separated by 12.5% SDS-PAGE under non-reducing conditions, electroblotted onto a nitrocellulose membrane and subjected with ligand blotting using digoxigenin-labeled human IGF-I (50 ng) and antiserum against digoxigenin (1:20,000). Arrows indicate migration positions of human (left) NHS and trout (right) IGFBP bands. NHS: normal human serum.

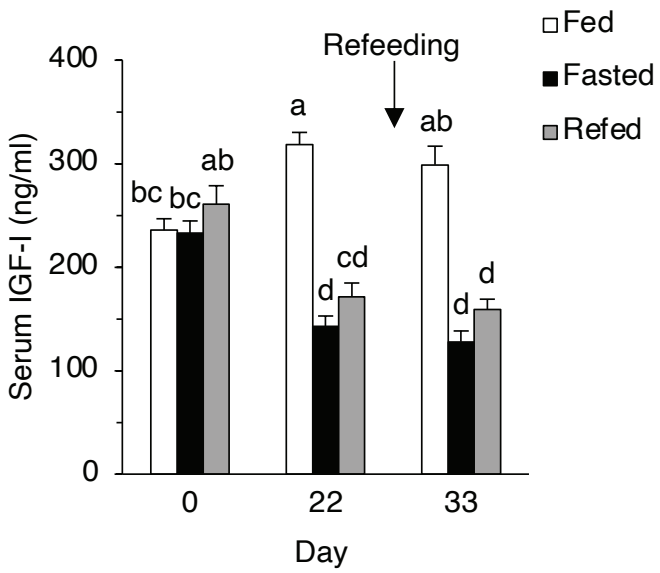
Fig. 4. Effects of fasting and refeeding on band intensities of serum IGFBP-2b (a) and 32-kDa IGFBP (b). The intensities of the IGFBP bands on ligand blotting were semi-quantified and expressed as arbitrary density unit (ADU). Values are expressed as means ± SE (day 0: $n$ = 22. day 22: $n$ = 18 and day 33: $n$ = 38). Groups sharing the same letters are not significantly different from each other (Tukey's HSD, $P < 0.05$).

Fig. 5. Correlations of serum IGF-I (a.b), IGFBP-2b (c,d) and 32-kDa IGFBP (e,f) with SGRs in length (a,c,e) and weight (b, d,f) during 33 days of the experimental period. Data are from fed, fasted and refed fish ($n$ = 38).

Izutsu et al., Fig. 1



Izutsu et al., Fig. 2



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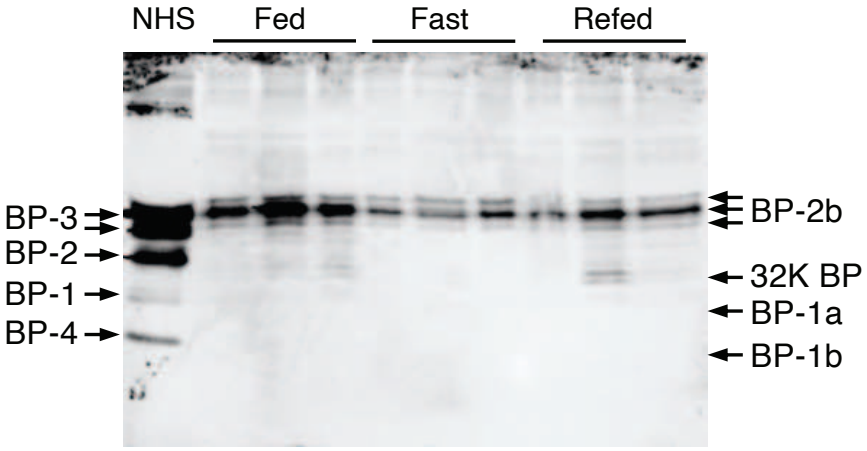

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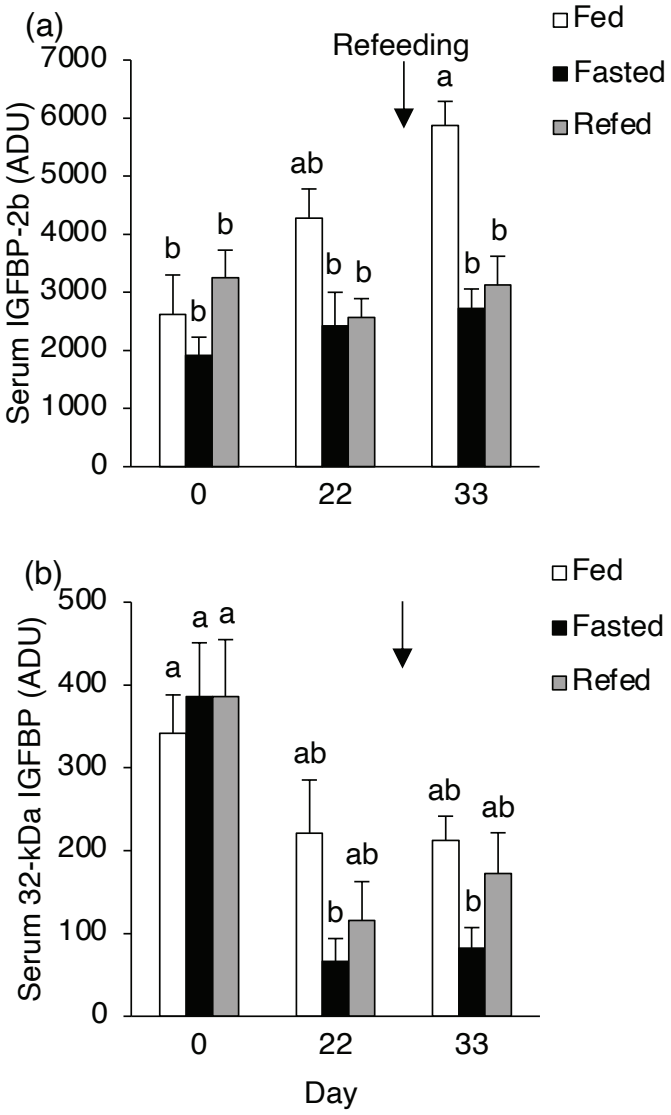

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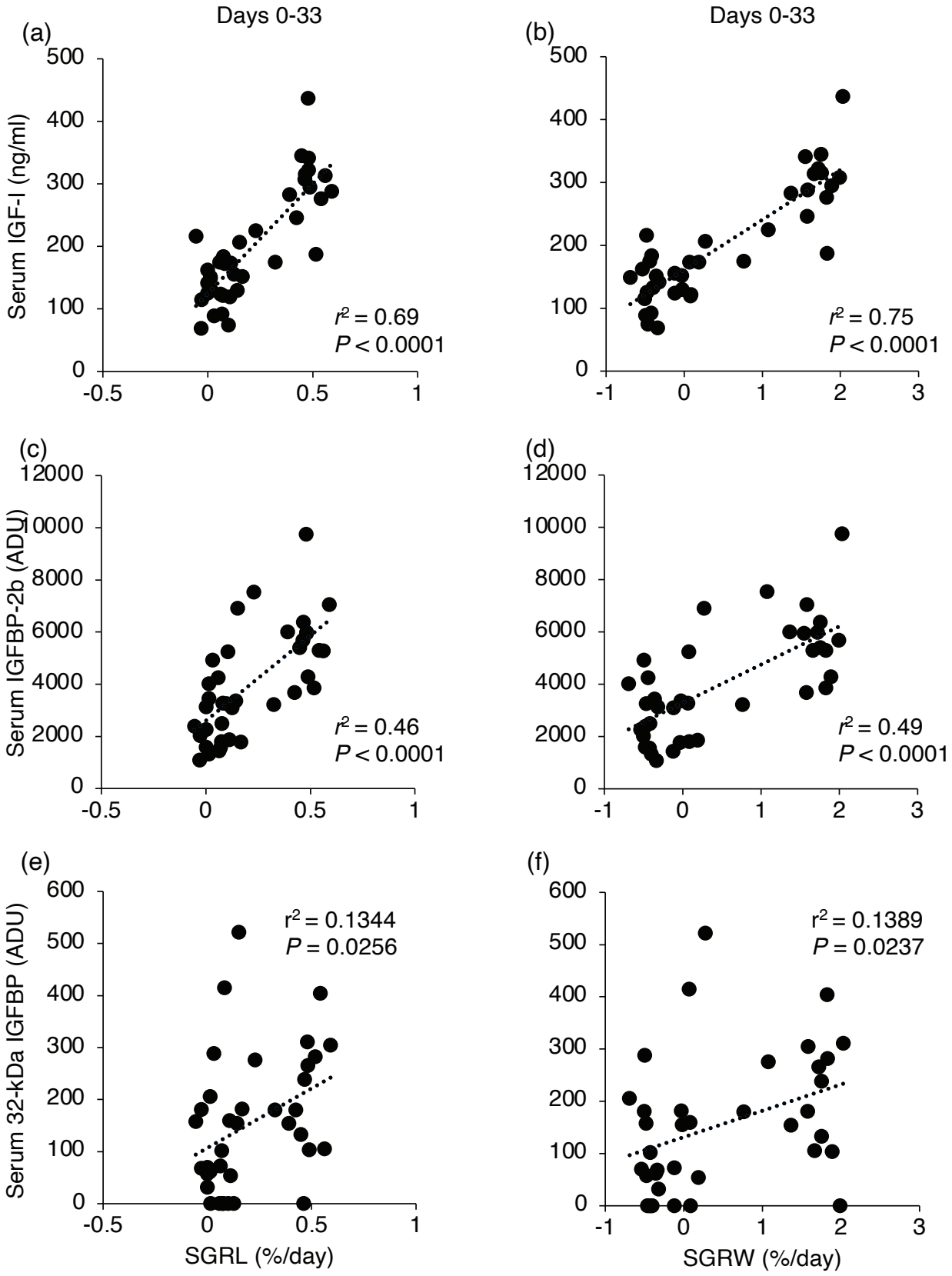

Table 1. Specific growth rates (SGR) in length (L) and body weight (W).

| | | Days 0–22 | Days 22–33 | Days 0–33 |
|---|---|---|---|---|
| SGRL (%/day) | Fed | $0.46 \pm 0.03^{a}$ | $0.47 \pm 0.05^{a}$ | $0.47 \pm 0.02^{A}$ |
| | Fasted | $0.01 \pm 0.01^{b}$ | $0.05 \pm 0.03^{b}$ | $0.02 \pm 0.01^{C}$ |
| | Refed | $0.10 \pm 0.02^{b}$ | $0.15 \pm 0.09^{b}$ | $0.12 \pm 0.03^{B}$ |
| SGRW (%/day) | Fed | $1.93 \pm 0.09^{a}$ | $1.26 \pm 0.07^{b}$ | $1.69 \pm 0.07^{A}$ |
| | Fasted | $-0.50 \pm 0.05^{c}$ | $-0.34 \pm 0.06^{c}$ | $-0.45 \pm 0.03^{C}$ |
| | Refed | $-0.51 \pm 0.04^{c}$ | $1.17 \pm 0.27^{b}$ | $0.06 \pm 0.09^{B}$ |

At a given time interval, groups without a letter or sharing the same letters are not significantly different from each other (Tukey's HSD, $P < 0.05$).

Table 2. Correlations between endocrine parameters and specific growth rates (SGR) in length (L) and weight (W) during days 22-33.

| | SGRL (22-33 days) | SGRW (22-33 days) |
|---|---|---|
| IGF-I | $r^2 = 0.39$ | $r^2 = 0.29$ |
| | $P < 0.0001$ | $P = 0.0006$ |
| IGFBP-2b | $r^2 = 0.34$ | $r^2 = 0.24$ |
| | $P < 0.0001$ | $P = 0.0021$ |
| 32-kDa IGFBP | – | $r^2 = 0.15$ |
| | ns | $P = 0.0184$ |

ns: not significant.