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Evaluation of growth status using endocrine growth indices, insulin-like growth factor (IGF)-I and IGF-binding protein-1b, in out-migrating juvenile chum salmon

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Abstract

This study aimed to utilize circulating insulin-like growth factor binding protein (IGFBP)-1b as a negative index of growth to evaluate the growth status of juvenile chum salmon (*Oncorhynchus keta*) in the ocean. First, rearing experiments using PIT-tagged juveniles were conducted to examine the relationship of circulating IGFBP-1b with growth rate of the fish in May and in June. The serum IGFBP-1b level negatively correlated with fish growth rate in both months, suggesting its utility as a negative index of growth. Next, the growth status of out-migrating juveniles in northeastern Hokkaido, Japan, was monitored for 3 years using the growth indices. Serum levels of IGF-I, a positive index of growth, in fish collected from the nearshore zone were low in May and high in June of all years. Levels of serum IGFBP-1b showed a trend opposite to that of serum IGF-I. However, the IGF-I/IGFBP-1b molar ratios well reflected the seasonal and regional trends. These findings suggest that the juveniles in June left the nearshore area under better growth conditions. The present study also suggests that the use of multiple growth indices would improve the sensitivity and accuracy to evaluate the current growth status of out-migrating juvenile chum salmon.

Keywords

Growth evaluation; Insulin-like growth factor binding protein-1b; Insulin-like growth factor-I; *Oncorhynchus keta*; Out-migrating juveniles

1. Introduction

Growth in animals is mainly regulated by the growth hormone (GH)-insulin-like growth factor-I (IGF-I) system (Daughaday and Retwein, 1989; Le Roith et al., 2001; Ohlsson et al., 2009). In this system, GH from the pituitary gland promotes growth mainly through stimulating hepatic production of IGF-I. IGF-I is released into the bloodstream and mediates many of the GH actions. The endocrine IGF-I in turn acts on the pituitary and hypothalamus to suppress the secretion of GH in a negative feedback loop. IGF-I circulates in the blood as a hormone at relatively high levels. This is due to the stabilization of IGF-I levels by IGF-binding proteins (IGFBPs). IGFBPs are important modulators of IGF-I activity by regulating its half-life and availability to the IGF-receptor on target tissues (Jones and Clemmons, 1995; Rajaram et al., 1997). In mammals, there are six types of IGFBPs in circulation and they each regulate the activity of IGF-I differently.

Recently, an approach to assess the recent/current growth status of fish using IGF-I has been proposed (Picha et al., 2008; Beckman, 2011). However, the plasma IGF-I level varies in response to feeding status: when fish were fed a high feed rate, IGF-I levels were high, and vice versa (Beckman et al., 2001, 2004a, 2004b). In addition, a positive relationship between plasma IGF-I and individual growth rate, measured at 2-week intervals, was found in post-smolt coho salmon (*Oncorhynchus kisutch*) (Beckman et al., 2001, 2004a, 2004b). Many laboratory studies using other salmon and fish species also reported that the level of circulating IGF-I responded to changes in individuals' nutritional status and reflected their recent growth rate. These findings support the notion that plasma/serum IGF-I is useful as a growth index (Picha et al., 2008; Beckman, 2011). Yet, despite the utility of circulating IGF-I as an index of fish growth, care should be taken in situations where the IGF-I–growth relationship is disrupted. The IGF-I–growth relationship has been found to be disrupted by changes in environmental factors, such as a rapid drop in water temperature, and/or by the physiological condition of individuals, such as precocious maturation (Beckman et al., 2004a, 2004b, 2004c). Thus, to make the assessment of growth status using IGF-I more reliable, it is necessary to develop new growth indices that show sensitivity to changes in nutritional and growth status, different to that of IGF-I.

IGFBP-1 is a candidate "negative" growth index since in mammals it increases under catabolic conditions and inhibits the anabolic action of IGF-I (Jones and Clemmons, 1995; Rajaram et al., 1997; Firth and Baxer, 2002). In serum/plasma of teleost fishes, three IGFBP bands are consistently detected at molecular ranges of 20–25, 28–32 and 40–45 kDa (Shimizu

and Dickhoff, 2017). In salmon, two low-molecular-weight IGFBPs have been identified: IGFBP-1a and IGFBP-1b (Shimizu et al., 2006, 2011). A radioimmunoassay for salmon IGFBP-1b has been developed and revealed that circulating IGFBP-1b increased under malnutritional status, such as with fasting or a reduced feeding ration (Shimizu et al., 2006, 2009). In addition, a negative correlation between circulating IGFBP-1b and individual growth rate was reported in post-smolt coho salmon (Shimizu et al., 2006). We recently established a time-resolved fluoroimmunoassay (TR-FIA) for salmon IGFBP-1b using the same components to those for radioimmunoassay (Fukuda et al., 2015). With this TR-FIA, we discovered that IGFBP-1b showed a negative relationship with growth rate in yearling and underyearling masu salmon (*O. masou*) (Kawaguchi et al., 2013; Fukuda et al., 2015). Thus, circulating IGFBP-1b may be useful as a negative growth index in juvenile salmon in the wild.

A large proportion of juvenile salmon die during the early stage of their ocean life because of size- and/or growth-dependent mortality, which determines the strength of stock recruitment (Healey, 1982). Beamish and Mahnken (2001) proposed a critical-size and critical-period hypothesis to explain this high mortality during early marine life. This hypothesis and others specify two critical periods: soon after sea entry, when death occurs mainly owing to maladaptation to a new environment and/or high predation pressure; and during fall to winter, when a shortage of energy reserves proves lethal for young salmon (Healey, 1982; Beamish and Mahnken, 2001; Beamish et al., 2004; Farley et al., 2007; Kocik et al., 2009). Although the precise timing and mechanism of mortality still need to be elucidated, the importance of fish size-gains and energy reserves for survival is recognized. Therefore, using endocrine growth indices for measuring and evaluating the growth status of salmon in the sea has great value for assessing the possibility of their survival.

Chum salmon (*O. keta*) is an important commercial fish along the Pacific Rim and a target of intensive hatchery releases in northern Japan (Miyakoshi et al., 2013). Several studies have noted that the rate of mortality during their early marine phase has a strong impact on stock recruitment (Bax, 1983; Fukuwaka and Suzuki, 2002; Wertheimer and Thrower, 2007). We recently reported on the usefulness of serum IGF-I levels for evaluating the growth status of juvenile chum salmon in the ocean (Kaneko et al., 2015; Taniyama et al., 2016). Out-migrating juveniles exhibiting a good growth condition were captured along the coast of northeastern Hokkaido in early to mid-June, while fish with a poor growth condition were consistently found in an estuary (Kaneko et al., 2015). Given that serum IGFBP-1b may be useful as a negative index of growth in salmon, we included its measurement in a field survey of juvenile chum

salmon. The present study first examined the relationship between circulating IGFBP-1b and growth rate in juvenile chum salmon under laboratory conditions, and next used levels of IGFBP-1b and IGF-I to evaluate the growth of out-migrating juveniles in the wild.

2. Materials and methods

2.1. Rearing experiment

Juvenile chum salmon were transferred from a local hatchery in northeastern Hokkaido (Kamisato Hatchery; Tsubetu, Abashiri-gun, Hokkaido, Japan; 43.6°N, 144.1°E) to an indoor rearing facility at the Faculty of Fisheries Sciences, Hokkaido University (Minato, Hakodate, Hokkaido, Japan; 41.8°N, 140.7°E). The fish were reared in 60-L freshwater glass aquariums (size 60 × 29.5 × 36 cm) in a temperature-controlled room (10°C), and each tank had a closed circulation system with filtration in the upper half. Until the beginning of the experiment, fish were fed to satiety once daily on a commercial diet (Marubeni Nisshin Feed Co. Ltd., Tokyo, Japan). In May and again in June 2015, fish were acclimated to artificial seawater (TetraMarin Salt Pro; Spectrum Brands Inc., Tokyo, Japan) by gradually increasing the salinity to 31–34 g kg^{-1} seawater over 1 week. In each month, fish were lightly anesthetized in 3% 2-phenoxyethanol (Kanto Chemical, Tokyo, Japan) and individually marked with PIT tags (size φ1.4 mm × 8.4 mm; Biomark, Boise, ID, USA) before being randomly placed into three 60-L seawater tanks (15 fish per tank). Average initial sizes (Ave. ± S.E) of the experimental fish in May and June were 5.7 ± 0.1 cm and 6.3 ± 0.1 cm, respectively. One group of juveniles was fed twice daily on the commercial diet, given at 3.0% of fish body weight/day for 10 days; a second group was fasted throughout the experimental period; a third group was fasted for 5 days and then refed for the following 5 days under the same condition as the fed group. Throughout the experiment, salinity was kept at 31–34 g kg^{-1} and the water temperature was maintained at 11.0–11.5°C. The experiment was carried out in accordance with the guidelines of the Hokkaido University Animal Care and Use Committee.

In May and in June, the fork length (FL) and body weight (BW) of all fish sampled were measured at the beginning of the experiment, and at 5 and 10 days after treatment. Condition factor (K) was calculated as: (BW (g)) × 100/(FL (cm))^3. Specific growth rate (SGR) was calculated as: 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. At the time of the initial sampling, eight fish were sampled for blood. On day 10, fish from each treatment were sampled for blood (Fed: n = 10 or 11; Fasted: n = 10; Refed: n = 10 or 11).

Blood was drawn from the caudal vein using a 10- or 20-µl plain glass tube (Microcap; Drummond Scientific Company, Broomall, PA, USA), allowed to clot overnight at 4°C, and finally centrifuged at 10,000 rpm for 15 min. Serum was collected and stored at −80°C until use.

2.2. Field survey

Field surveys were carried out around the Abashiri River and in the coastal waters of Hokkaido Island (Sea of Okhotsk), five times between mid-May and late June, from 2015 to 2017 (Fig. 1). The specific survey design and complete methods are detailed in Kaneko et al. (2015). Fish were caught every 10 days using a cast-net in the Abashiri River (44°00.778□N, 144°13.319□E), by drag-net in the estuary (44°01.376□N, 144°16.709□E), and by two-boat trawling within the port (44°00.674□N, 144°17.434□E; 600 m), along the coast (44°00.141□N, 144°17.903□E; 1,500 m) and in the nearshore zone (44°01.150□N, 144°20.284□E; 2,000 m). The trawl net (8-m-wide × 5-m-deep mouth, 18 m long, with wing nets 7 m long and a central bag with 5-mm mesh) was towed through the surface water (1–2 m deep) at 4–6 km h^{-1} in the morning (6:00–8:00). In 2017, fish were collected in the estuary using dip-nets at night under portable floodlights (30-min effort from 18:00–19:00). The sea surface temperature (SST) or river water temperature (WT) were recorded at each site before collection commenced. Fish from each site were sampled for the physiological analyses described above.

2.3. Sample analyses

For measuring IGF-I, serum was first extracted with an acid-ethanol, as described in Shimizu et al. (2000). IGF-I was quantified by TR-FIA, based on the method described in Small and Peterson (2005), using recombinant salmon/trout IGF-I (GroPep Bioreagents Pty Ltd, Adelaide, Australia) as a standard. Time-resolved fluorescence was measured using a Wallac ARVO SX or Wallac ARVO X4 multilabel counter (PerkinElmer, Waltham, MA, USA). In the present study, serum IGF-I levels in juvenile chum salmon caught in the wild were related to their size in 2015 (serum IGF-I = 21.5 × FL −78.6, $r^2 = 0.28$, $P < 0.0001$), 2016 (serum IGF-I = −12.5 × FL −111.5, $r^2 = 0.18$, $P < 0.0001$) and 2017 (serum IGF-I = 7.5 × FL −19.0, $r^2 = 0.35$, $P < 0.0001$), when the whole samples in each year were pooled and analyzed. To better understand current growth, as described above, we excluded the size-effect on IGF-I levels by standardizing the measured values to the mean lengths (Shimizu et al., 2009) using the following equation: Standardized hormone $value_1$ = hormone $value_1$ – [($length_1$ – mean length) × slope], where

hormone value$_1$ is the individual hormone level of a given fish, length$_1$ is the individual length of a given fish, mean length is the mean length of the juveniles caught in the field survey in 2015 (6.1 cm), 2016 (5.9 cm) and 2017 (6.0 cm), and slope is the hormone–length relation. Complete methods for the standardization are detailed in Shimizu et al. (2009) and Shimomura et al. (2012).

Serum IGFBP-1b levels were quantified by TR-FIA, as described in Fukuda et al. (2015). Briefly, a competitive method was employed by following a procedure for DELFIA immunoassays (PerkinElmer). Serum samples were first incubated with antiserum against purified salmon IGFBP-1b (Shimizu et al., 2006), overnight at 4°C, in a 96-well microtiter plate coated with goat anti-rabbit IgG (PerkinElmer). Biotinylated salmon IGFBP-1b was added to each well and incubated overnight at 4°C. After washing with DELFIA Wash Buffer (PerkinElmer), each well received europium-labeled streptavidin (PerkinElmer) followed by DELFIA Enhancement Solution (PerkinElmer). Time-resolved fluorescence was measured at 615 nm using a Wallac ARVO X4 multilabel counter (PerkinElmer). Our initial analysis using ligand blotting for IGFBPs suggested that trawling for 6 min followed by maintenance in a bucket for 1hr before sampling had no considerable effect on inducing IGFBP-1b in blood (data not shown).

Since salmon IGFBP-1b is a potential inhibitor of IGF-I action, the ratio of IGF-I to IGFBP-1b may reflect the fraction of circulating IGF-I available for promoting growth, which may reveal the balance of anabolism and catabolism in the individual. According to this hypothesis, we calculated the molar ratio of IGF-I (7.5 kDa) to IGFBP-1b (22 kDa) (IGF-I/BP-1b ratio) in the present study.

2.4. Statistical analyses

Results of the rearing experiments were first analyzed by two-way ANOVA (month × treatment) using JMP software (SAS Institute Inc., Cary, NC, USA). When significant effects were found, differences were further identified by one-way ANOVA followed by Tukey's honest significant difference (HSD) test. Simple regression analysis was also conducted using the JMP program and relationships were considered significant at $P < 0.05$. When analyzing the regression, circulating IGFBP-1b levels were transformed to natural logs to obtain the normal distribution. The field-survey data were grouped by month since each physiological parameter tended behave similarly within each month (Kaneko et al., 2015), and analyzed by one-way ANOVA (site). When significant effects were found, differences were further identified by one-

202 way ANOVA, followed by Tukey's HSD test. Differences between groups were considered to
203 be significant at $P < 0.05$.
204
205 **3. Results**
206 In the laboratory experiment, fasting for 10 days caused low and negative SGR in length and
207 weight, respectively, in both May and June (Fig. 2). Fish refed for 5 days showed an SGR that
208 was intermediate between that of the fed and fasted groups. Serum IGF-I levels in the fasted fish
209 were significantly low in both months (Fig. 3a), while serum IGFBP-1b levels were high in
210 June (Fig. 3b). IGF-I/BP-1b ratios in both months were lowest in the fasted fish and showed no
211 differences between the fed and refed groups (Fig. 3c). Serum IGF-I and natural-log-
212 transformed IGFBP-1b levels had positive and negative correlations with SGR, respectively, in
213 both months (Fig. 4a, b). Although regression coefficients of IGF-I with SGR were similar
214 between months (May: $r^2 = 0.58$, June: $r^2 = 0.59$), those of IGFBP-1b differed between months
215 (May: $r^2 = 0.62$, June: $r^2 = 0.41$). The IGF-I/BP-1b ratio showed positive but weaker correlation
216 with SGR in both May and June as compared with the levels of IGF-I and IGFBP-1b alone
217 (May: $r^2 = 0.41$, June: $r^2 = 0.37$; Fig. 4c). Serum IGF-I also correlated with FL, BW and K,
218 whereas the serum IGFBP-1b level and the IGF-I/BP-1b ratio had strong correlations with only
219 K in both months (Table 1). In addition, negative correlations were found between serum IGF-I
220 and IGFBP-1b levels. The morphological parameters are presented in Supplemental Table 1.
221 In the field survey, serum IGF-I levels in May of 2015 and 2016 were similarly high
222 in fish sampled from the river and slightly less toward the nearshore (Fig. 5a, c), while in June
223 of 2015 and 2017 the levels gradually increased in the samples between the river and the coast
224 (Fig. 5b, f). There was no significant difference in the levels of serum IGF-I among sites in May
225 of 2016, 2017 and in June of 2016 (Fig. 5d, e). Serum IGFBP-1b levels in May of 2015 and
226 2017 were consistently high in samples from the estuary and nearshore (Fig. 6a, e). Conversely,
227 serum IGFBP-1b in June among all years showed a gradual decrease between fish collected
228 from the river to the nearshore zone (Fig. 6b, d, f). The IGF-I/BP-1b ratio better represented
229 regional trends (Fig. 7). The ratios in May of 2015 and 2016 were high in fish from the river
230 and low in fish from the nearshore zone (Fig. 7a, c); in May of 2017 fish from the river showed
231 a low IGF-I/BP-1b ratio but fish from the coast had a high ratio (Fig. 7e). In June of all years, a
232 significant increase of the ratio was observed in fish collected in the coast or nearshore zone
233 (Fig. 7b, d, f).
234 Relationships between the serum IGF-I and IGFBP-1b levels in fish from each site

235 are shown in Figure 8. For both months, no relationship between the two levels was found in
236 fish collected in the river and in the port. Negative correlations were found for the values in fish
237 from the estuary and from the coast in June, and in fish from the nearshore zone in May;
238 conversely, a positive correlation was observed in fish captured at the coast in May.
239 Morphological parameters in the field survey are presented in Supplemental Table 2.
240
241 **4. Discussion**
242 The present study suggests that circulating IGFBP-1b is a useful tool to assess the degree of
243 growth retardation or stress in free-swimming fish. The value of IGFBPs as a stress marker was
244 originally proposed by Kelley et al. (2001, 2002, 2006). In fish, two low-molecular-weight
245 IGFBPs are often induced into circulation under catabolic conditions such as fasting and stress
246 (Kelley et al., 2001, 2002, 2006). These IGFBPs are considered useful to evaluate the effects of
247 catch-and-release or environmental pollution on short- and long-term growth performance of
248 fish in the wild (Kelley et al., 2006). The present study supports that notion and provides
249 quantitative data. Although our focus was on a commercial fish species and used a lethal
250 procedure, the techniques reported here might be applied to threatened or endangered species as
251 a non-lethal procedure, since a small volume of blood is sufficient for the analysis. Such an
252 evaluation of stress or growth retardation in wild fish is highly relevant to field/conservation
253 endocrinology (McCormick and Romero, 2017).
254 To utilize circulating IGFBP-1b as a negative index of growth for chum salmon, we
255 first examined its responses under conditions of fasting and refeeding using juveniles under
256 laboratory conditions. Circulating IGFBP-1 in mammals and fish generally increases in
257 response to fasting (Lee et al., 1993, 1997; Siharath et al., 1996; Kelley et al., 2001; Peterson
258 and Small, 2004; Shimizu et al., 2006, 2009; Kawaguchi et al., 2013; Fukuda et al., 2015). In
259 post-smolt coho salmon, plasma IGFBP-1b indeed responded to a period of fasting and to
260 changes in feeding ration (Shimizu et al., 2006, 2009). In masu salmon, a significant increase of
261 serum IGFBP-1b was seen in fish fasted for 4 weeks and these values remained high throughout
262 the experimental period (Kawaguchi et al., 2013). In this study, serum IGFBP-1b levels
263 increased when fish were fasted for 10 days and decreased to the basal levels after refeeding for
264 5 days, in both May and June. These results are in good agreement with previous studies of
265 coho and masu salmon.
266 A negative correlation between IGFBP-1b level and individual growth rate has been
267 reported for post-smolt coho and masu salmon (Shimizu et al., 2006; Kawaguchi et al., 2013;

Fukuda et al., 2015). However, the IGFBP-1b–growth relationship varied with fish conditions such as age and season. Kawaguchi et al. (2013) found a strong negative relationship in yearling masu salmon (r^2 = 0.71) between the serum IGFBP-1b level and growth rate. In contrast, the correlation was weak in underyearling fish (r^2 = 0.25) (Fukuda et al., 2015), suggesting the relationship may be influenced by age. In addition, plasma IGFBP-1b in post-smolt coho salmon showed significant correlation with growth rate during June to September, but regression coefficients varied among sampling dates at 2-week intervals from July to September (r^2 = 0.24-0.52) (Shimizu et al., 2006). In the present study, the level of circulating IGFBP-1b negatively correlated with fish growth rate, measured in both May and June. The slopes of the regression lines using May and June data did not significantly differ (ANCOVA; P = 0.1316). Thus, seasonal variation in the IGFBP-1b–growth relationship is apparently not great during the late period of downstream and coastal migration among juvenile chum salmon.

In the laboratory experiment, a negative correlation was observed between the levels of circulating IGFBP-1b and IGF-I in juvenile chum salmon. This agrees with findings for post-smolt masu salmon (Kawaguchi et al., 2013). This inverse relationship is not surprising since one action of IGFBP-1b is to sequester IGF-I from the blood circulation (Lee et al., 1993, 1997). In contrast, plasma IGFBP-1b levels showed no correlation with IGF-I in coho salmon (Shimizu et al., 2006, 2009). The lack of a relationship may reflect differences in the sensitivity of IGF-I and IGFBP-1b to nutritional input. Shimizu et al. (2009) compared postprandial changes of plasma IGF-I and IGFBP-1b and suggested that IGFBP-1b was more sensitive to food intake, quickly responding within hours, irrespective of fasting history. Thus, the balance between circulating IGF-I and IGFBP-1b can change within a short time after feeding, which may sometimes mask the relationship between IGFBP-1b and IGF-I.

IGF-I/BP-1b ratio is a theoretical parameter reflecting the amount of bioactive IGF-I for promoting growth. Mechanistically, when IGF-I is bound to IGFBP-1, it is removed from the circulation and does not promote growth. Indeed, the IGFBP-1 level in humans is inversely related to biologically active IGF-I (Frystyk et al., 1995, 2002; Skjærbæk et al., 2004). The molar ratio of IGF-I to IGFBP-1b may reflect the fraction of IGF-I that is actually delivered to target tissues, and thus directs the growth potential. We assumed that a higher correlation coefficient would occur for the IGF-I/BP-1b ratio with growth than would occur for the IGF-I or IGFBP-1b alone. However, the relationship uncovered between the IGF-I/BP-1b ratio and the growth rate did not support this. Even so, it is worth noting that the IGF-I/BP-1b ratio did relate to K. This finding suggests that combining measures of circulating IGF-I and IGFBP-1b reveals

301 the balance between anabolism and catabolism, and this warrants further validation of the ratio
302 as an integrative growth index.
303 We evaluated growth status of out-migrating juvenile chum salmon in the field using
304 multiple endocrine indices. As most hatchery-reared chum salmon juveniles in Japan are
305 released into rivers, it is important to know how growth status is altered under changing food
306 availability, salinity and water temperature, which would allow estimates of their chances of
307 survival during the critical period in the estuary, coastal and nearshore waters. During the
308 downstream migration of juvenile chum salmon, changes in salinity might have little effect on
309 IGFBP-1b; when juveniles were acclimated to full-strength seawater over 3 days, serum
310 IGFBP-1b levels did not change in either May or June (Nakamura et al., unpublished data). In
311 juvenile chinook salmon (*O. tshawytscha*), a direct transfer to full-strength seawater caused an
312 increase in plasma IGFBP-1b levels within 6 h, presumably owing to osmotic stress (Shimizu et
313 al., 2011). A gradual acclimation to 66% seawater among rainbow trout had no acute effect on
314 plasma IGFBP-1b up to 3 days after transfer (Shepherd et al., 2005). Although an effect from a
315 rapid change in salinity cannot be ruled out, juvenile chum salmon might locate a suitable
316 salinity by swimming vertically or horizontally. Thus, we assumed that the effect of salinity
317 change was not significant in our survey of juvenile chum salmon.
318 Water temperature is an important parameter to consider when growth status is to be
319 evaluated by IGFBP-1b. Shimizu et al. (2006) reported that a sudden drop in water temperature
320 within 1 day, from 11°C to 7°C, changed plasma IGFBP-1b levels in post-smolt coho salmon.
321 In contrast, a gradual decrease in water temperature over 3 days, from 10°C to 5°C, had no
322 influence on average serum IGFBP-1b levels in juvenile chum salmon, although their growth
323 rate was not measured (Nakamura et al., unpublished data). Thus, attention should be paid when
324 comparing IGFBP-1b levels between samples from regions with different water temperatures.
325 While taking into consideration the effect of water temperature, we attempted to
326 evaluate the growth status of juvenile chum salmon in the wild using IGFBP-1b together with
327 IGF-I. Serum IGFBP-1b levels were high in fish from the nearshore zone in May of 2015, and
328 this was accompanied by low IGF-I levels. The water temperature in the nearshore zone in May
329 (9.0 ± 0.1°C; Supplemental Table 3) was within the optimal range (8–13°C) for growth of
330 juvenile chum salmon (Nagata et al., 2007, 2016), and the water temperatures in the port and the
331 nearshore area did not differ. These data suggest that the high IGFBP-1b and low IGF-I levels
332 observed in fish in the nearshore area in May reflect poor growth conditions owing to poorer
333 nutritional conditions. In fact, the total wet weight of zooplankton per cubic meter in the survey

region in mid-May of 2015 was lower than that in other years (data not shown). In contrast, in June of all years, the serum IGFBP-1b levels were very low in fish from the nearshore zone. The low IGFBP-1b levels suggest that the juvenile salmon had good growth conditions owing simply to abundant food items. The water temperature in the nearshore zone in June of all years was within the optimal range (10.4–12.7°C), but in the port in 2015 it exceeded the optimal range (13.1 ± 0.8°C). Thus, juvenile salmon might need to move from the port into nearshore waters owing to the higher water temperatures regardless of food availability. However, the levels of both IGF-I and IGFBP-1b were low in fish from the nearshore zone in June of 2015—an atypical concordance that cannot be explained from the experimental data. Sorting out the mechanism and factors producing such a situation will be important for making the evaluation of growth status by IGF-I and IGFBP-1b more reliable.

As described above, the IGF-I/BP-1b ratio in fish may be a good indicator of their catabolic status and growth potential. In the present study, serum IGFBP-1b negatively correlated with IGF-I in fish under laboratory conditions; however, a negative correlation was not always found in fish sampled from the wild. This discordance in the IGF-I–IGFBP-1b relationship might be influenced by environmental factors. Although the exact significance of the ratio needs to be validated in future studies, two findings are worth mentioning. First, the IGF-I/BP-1b ratio better reflected regional and seasonal trends; the ratios in fish in May gradually decreased between fish sampled from the river to the nearshore zone, while the ratios increased in June. Second, the IGF-I/BP-1b ratio in fish captured in the nearshore zone showed higher variation, suggesting that some individuals were growing fairly well and others barely growing. These variances in "growth potential" while in the nearshore zone may affect their survival thereafter.

We previously reported that small-sized fish caught in the estuary had low IGF-I levels and were under poor growth conditions (Kaneko et al., 2015). In the present field surveys, however, fish from the estuary did not exhibit the lowest IGF-I levels. In contrast, the IGFBP-1b levels were relatively high in fish from the estuary. Furthermore, we calculated a low IGF-I/BP-1b ratio for fish in the estuary. This result supports our hypothesis that the growth status of fish in the estuary is relatively poor in the Abashiri area (Kaneko et al., 2015). Hence, the data from the estuary demonstrate one advantage of employing multiple growth indices.

In summary, the present study suggests that circulating IGFBP-1b can be used as a negative index of growth for juvenile chum salmon. Monitoring growth status using the levels of IGFBP-1b together with IGF-I suggested that juvenile chum salmon left the nearshore zone

under poor growth conditions in May, while juveniles in June left the nearshore zone through activating their growth. Although further validation on the sensitivity and stability of the multiple growth indices against environmental factors is needed, the use of these indices has the potential to contribute to fish stock assessments and field/conservation endocrinology as a way to monitor the balance between anabolism and catabolism.

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522
523

Figure legends

Fig. 1. Location of the survey area and sampling sites. Fish were caught in the river, estuary, port, coastal water and nearshore zone. Black circles and bars, respectively, indicate the collecting sites and trawling lines for juvenile chum salmon.

Fig. 2. Effects of fasting and refeeding on SGRs in length (a) and weight (b) on day 10 in May and in June. Individually tagged fish were either fed or fasted for 10 days, or fasted for 5 days and then refed for the following 5 days. Values are expressed as mean ± SE (n = 10 or 11). Symbols sharing the same letter indicate no significant difference.

Fig. 3. Effects of fasting and refeeding on serum IGF-I (a), IGFBP-1b level (b), and IGF-I/BP-1b ratio (c) on day 10 in May and in June. Individually tagged fish were either fed or fasted for 10 days, or fasted for 5 days and then refed for the following 5 days. Values are expressed as mean ± SE. The number in each group is shown under the corresponding bar. Symbols sharing the same letter indicate no significant difference.

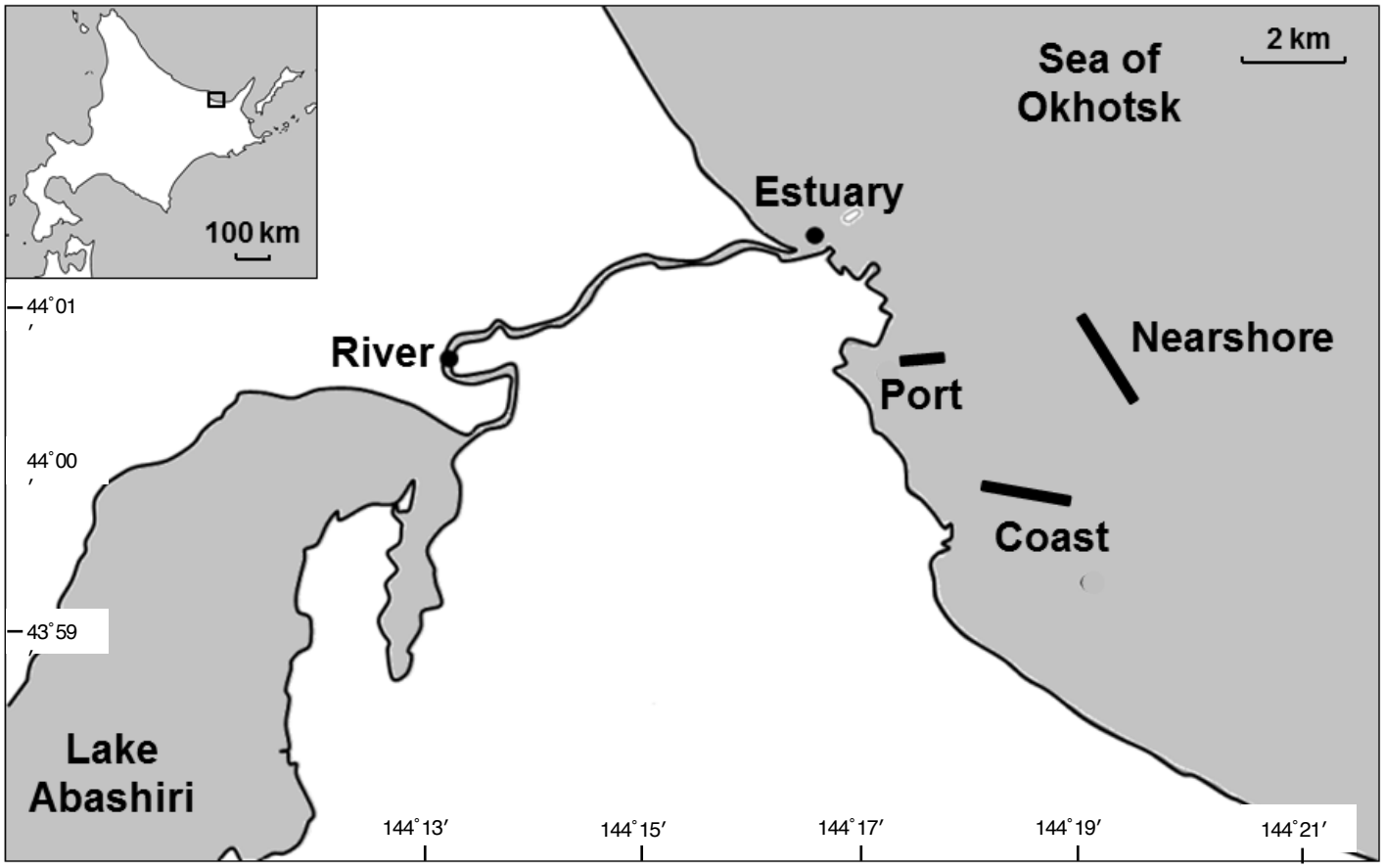
Fig. 4. Correlations of serum IGF-I (a), natural-log-transformed IGFBP-1b level (b), and IGF-I/BP-1b ratio (c) against SGR in weight of juvenile chum salmon in May (black circles) and June (while circles). Dots indicate data from the fed, fasted and refed fish (IGF-I: n = 29–32; IGFBP-1b: n = 23 or 24; IGF-I/BP-1b: n = 23 or 24).

Fig. 5. Regional variations of serum IGF-I level in May (light color: a, c, and e) and June (dark color: b, d, and f). Juvenile chum salmon were sampled from the river, estuary, port, coastal water and nearshore zone in 2015 (a, b), 2016 (c, d) and 2017 (e, f). Values are expressed as mean ± SE. The number in each group is shown under the corresponding bar. Symbols sharing the same letter indicate no significant difference.

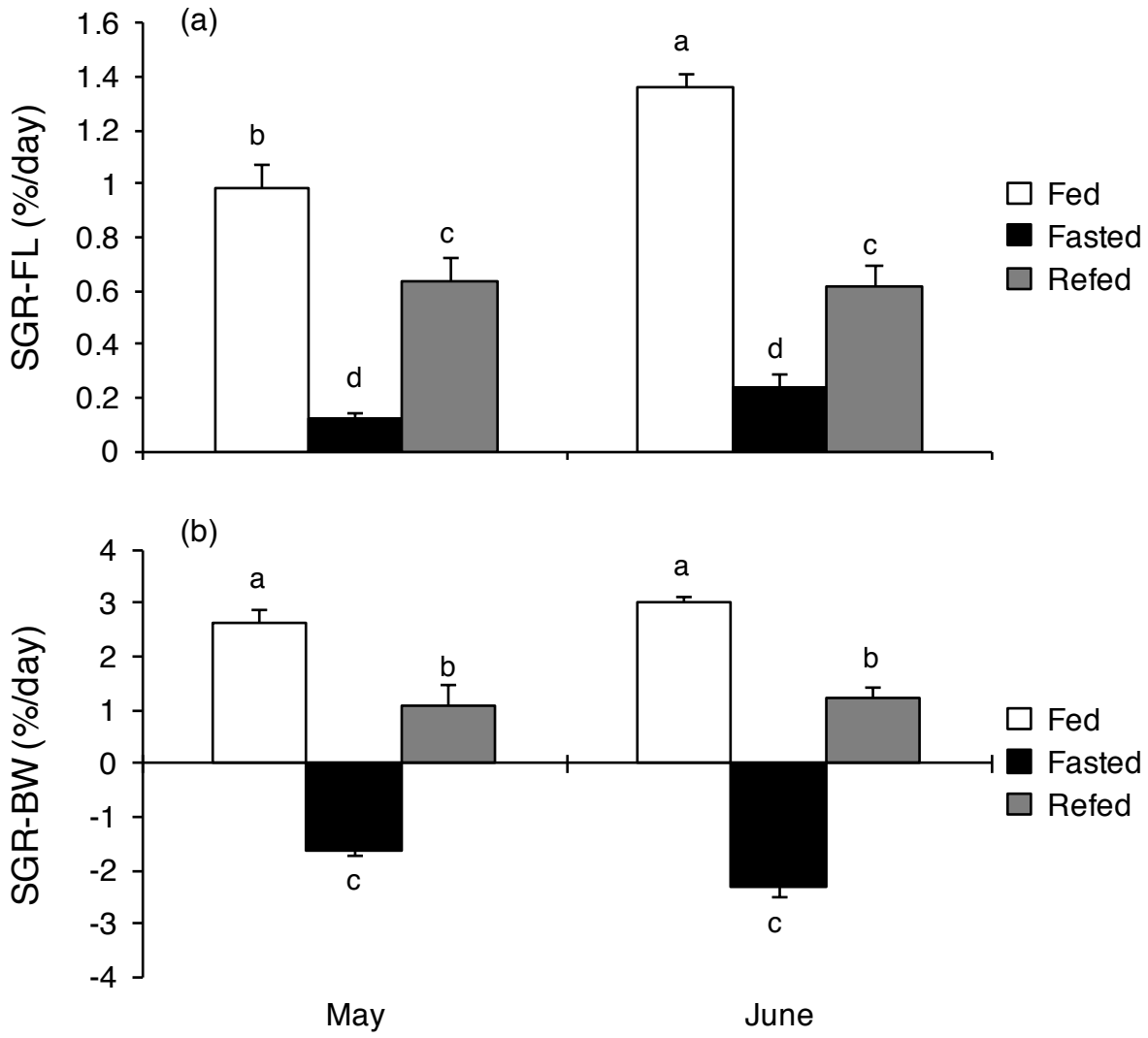
Fig. 6. Regional variations of serum IGFBP-1b level in May (light color: a, c, and e) and June (dark color: b, d, and f). Juvenile chum salmon were sampled from the river, estuary, port, coastal water and nearshore zone in 2015 (a, b), 2016 (c, d) and 2017 (e, f). Values are expressed as mean ± SE. The number in each group is shown under the corresponding bar. Symbols sharing the same letter indicate no significant difference.

557 Fig. 7. Regional variations in the IGF-I/BP-1b ratios in May (light color: a, c, and e) and June
558 (dark color: b, d, and f). Juvenile chum salmon were sampled from the river, estuary, port,
559 coastal water and nearshore zone in 2015 (a, b), 2016 (c, d) and 2017 (e, f). Values are
560 expressed as mean ± SE. The number in each group is shown under the corresponding bar.
561 Symbols sharing the same letter indicate no significant difference.
562
563 Fig. 8. Correlations between the serum IGF-I and IGFBP-1b levels of juvenile chum salmon
564 captured at each site in May (black circle) and June (while circle) in 2015. A correlation
565 coefficient in italic font indicates a positive relationship.
566

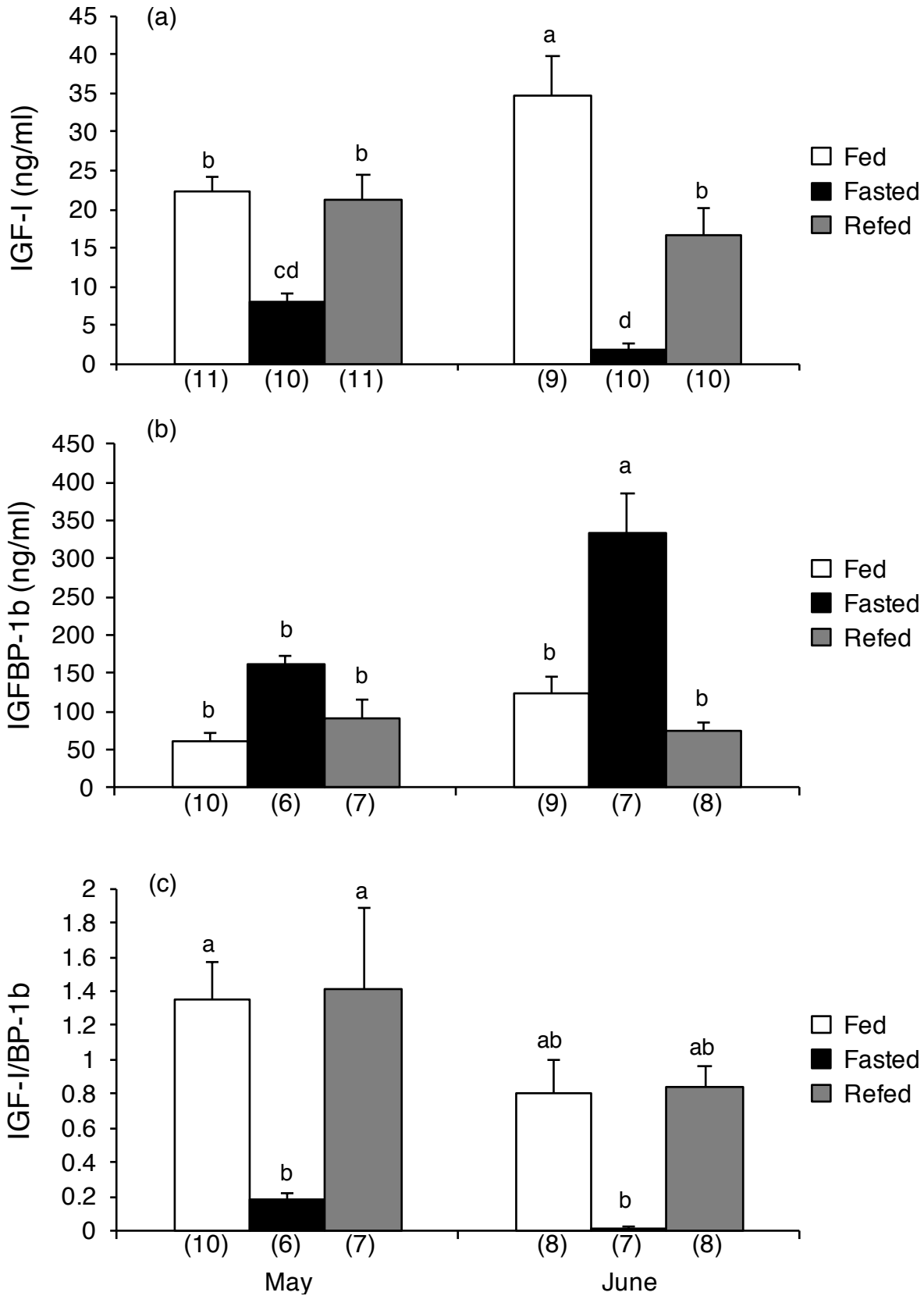
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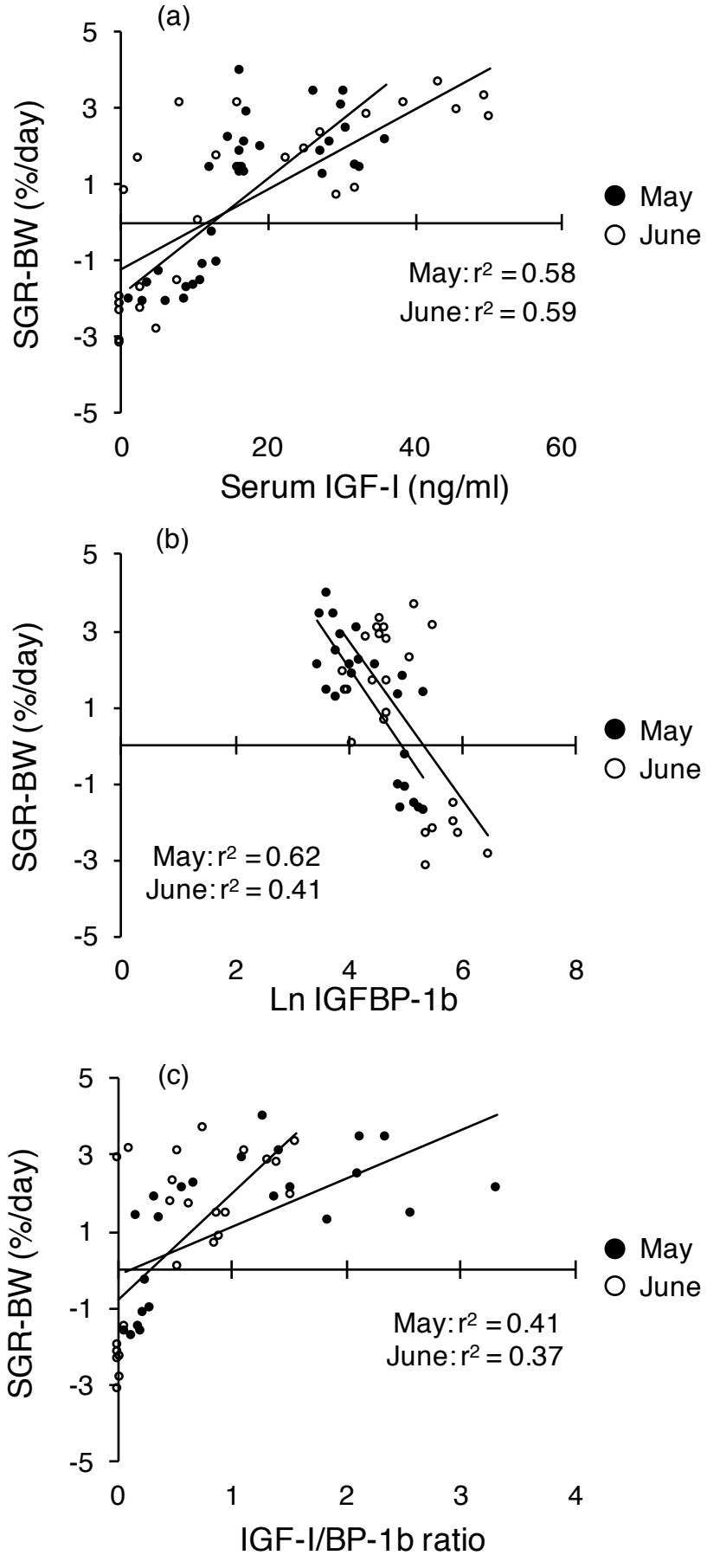
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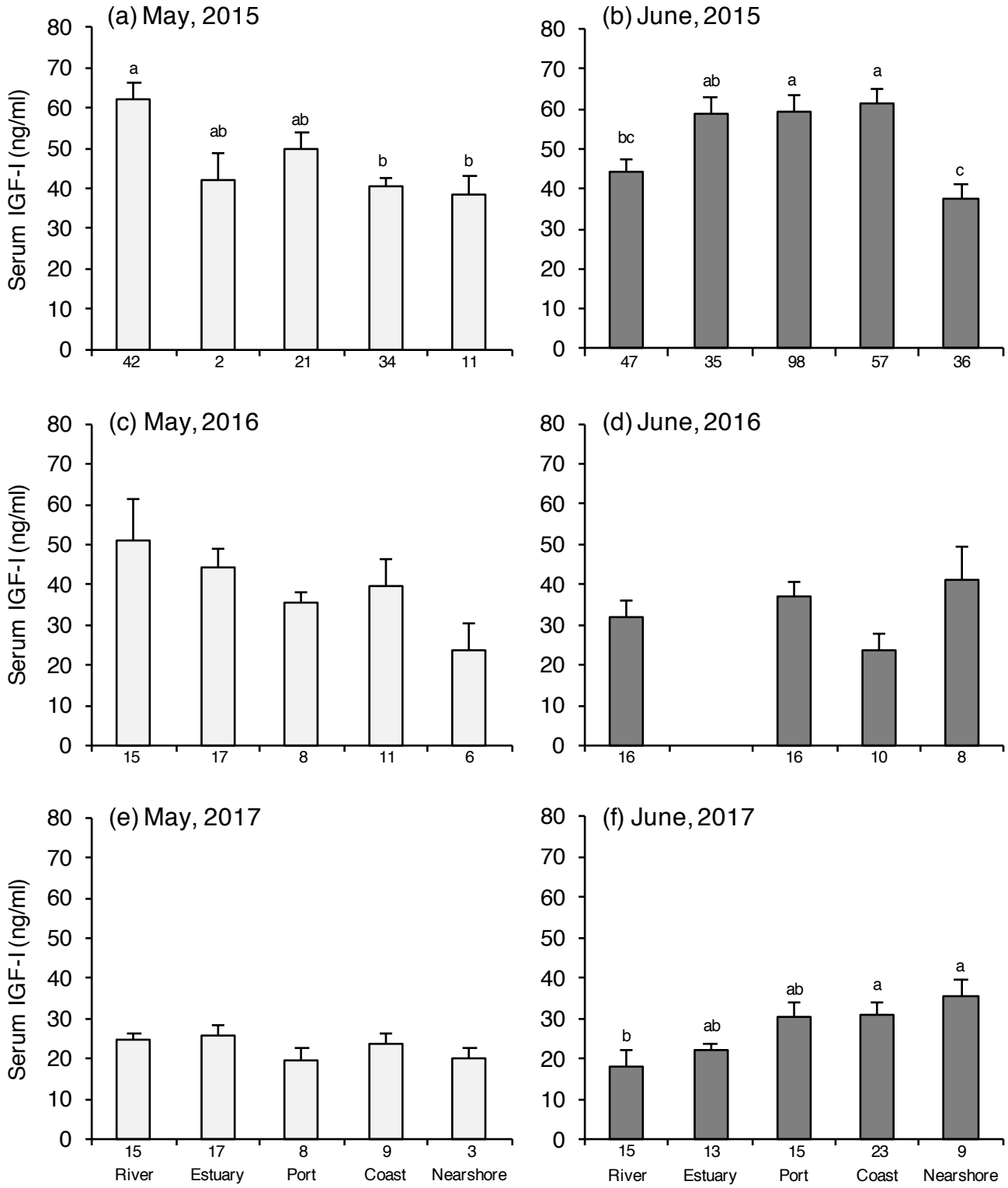
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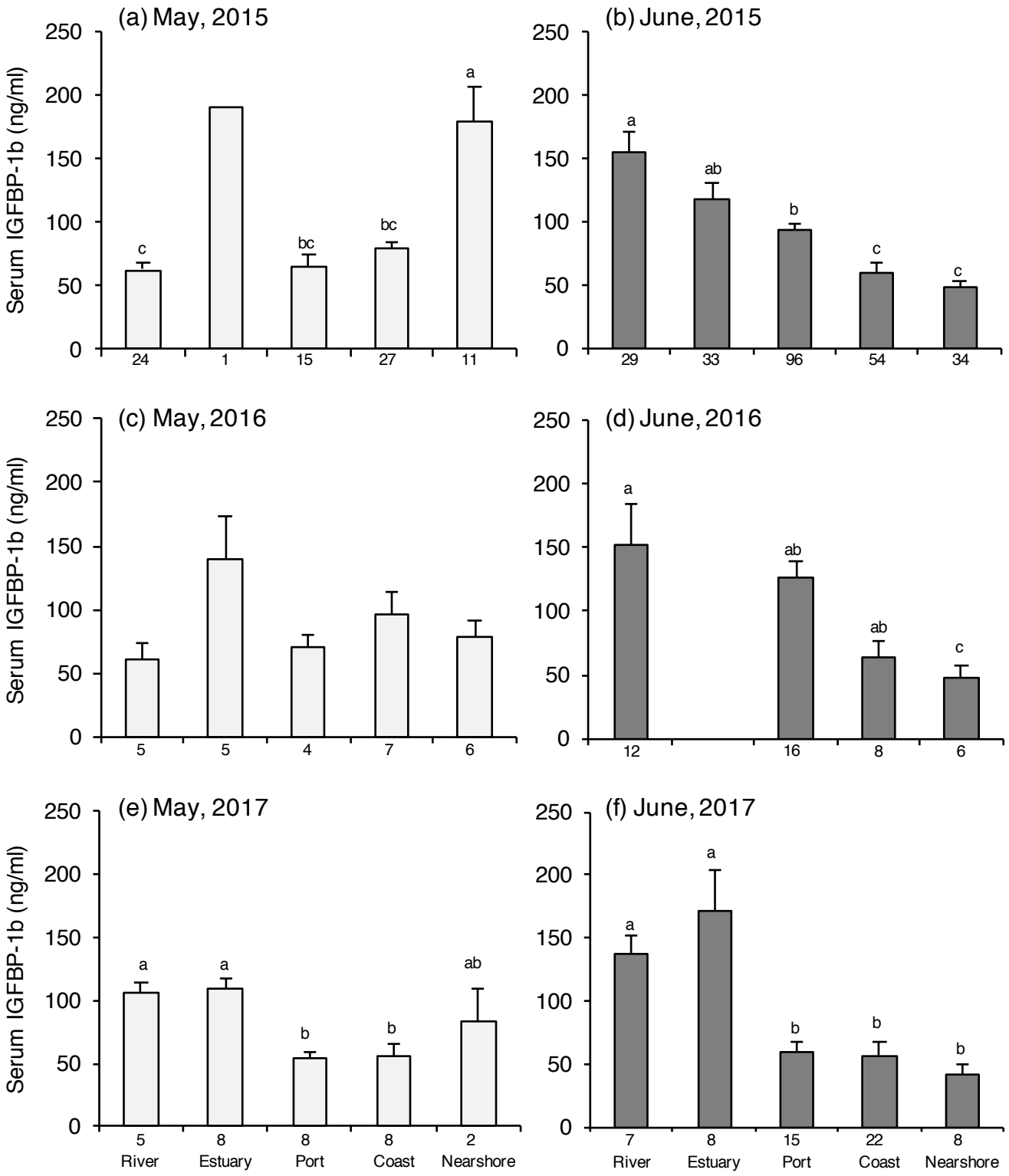
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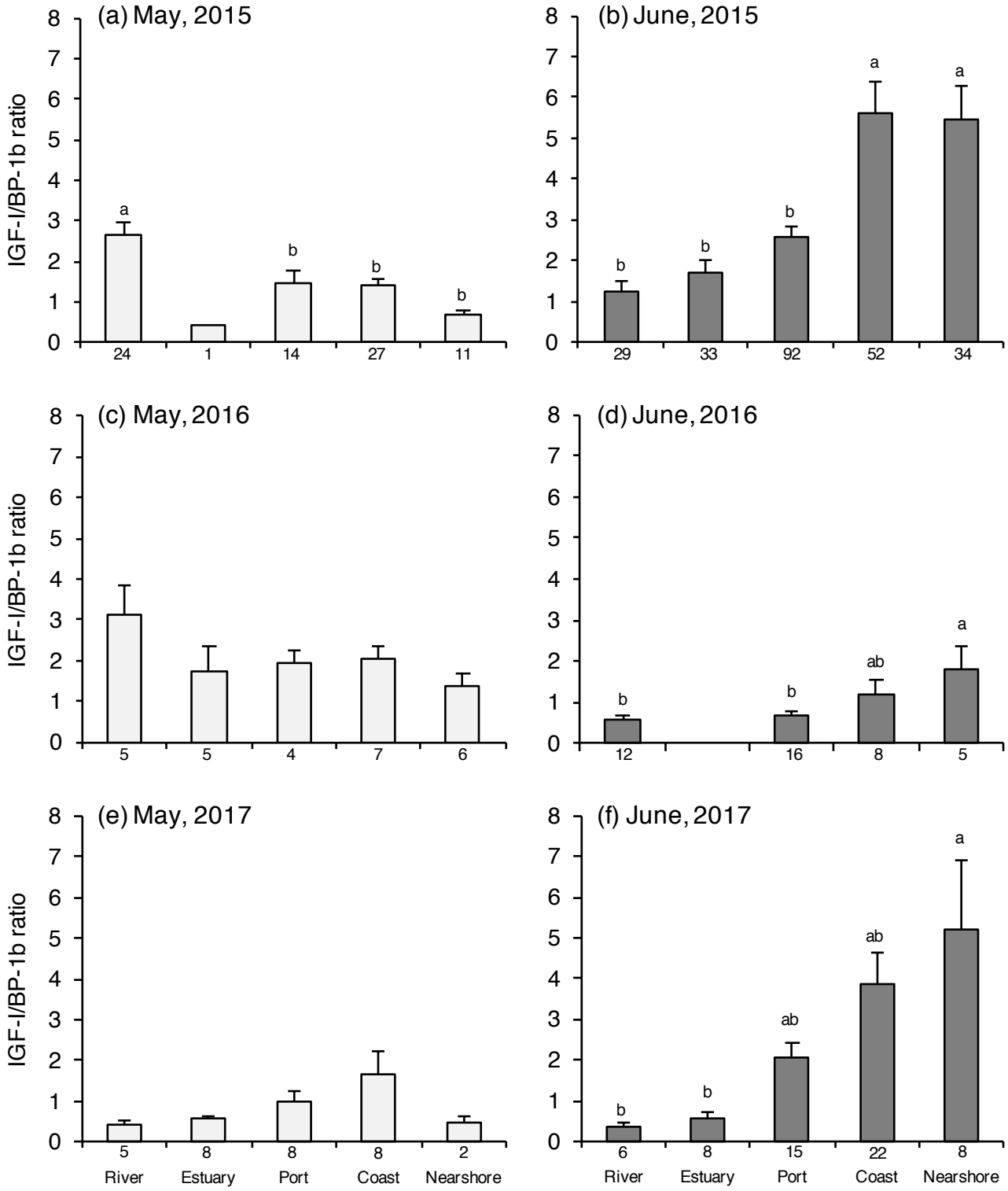
Kaneko et al., Fig. 5



Kaneko et al., Fig. 6



Kaneko et al., Fig. 7



Kaneko et al., Fig. 8

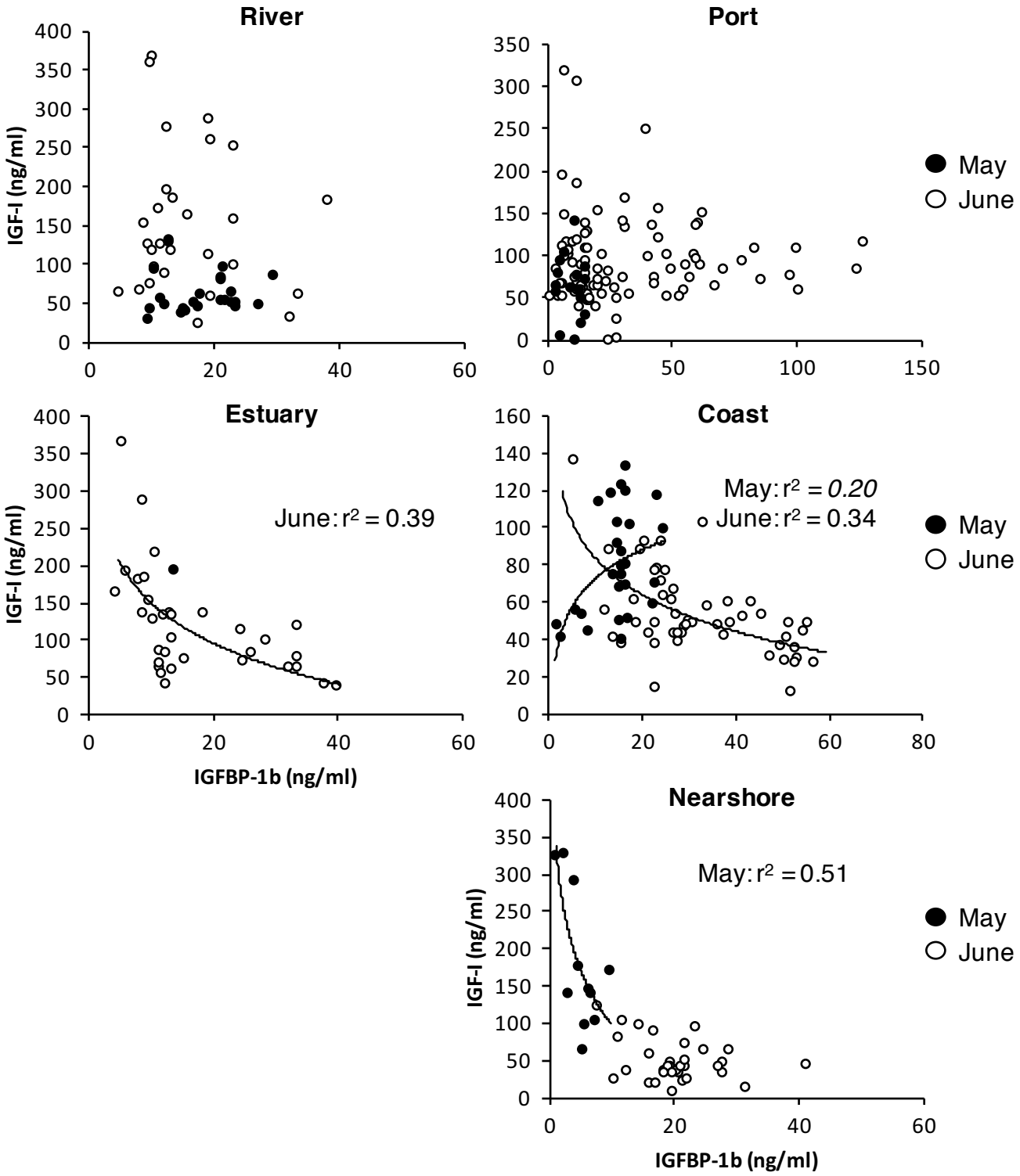


Table 1
Correlation coefficients (r) between physiological and morphological parameters on Day 10 in May and June

May	FL	BW	K	SGR-FL	SGR-BW	IGF-I
BW	0.93					
K	ns	0.52				
SGR-FL	0.47	0.62	0.59			
SGE-BW	0.52	0.68	0.64	0.95		
IGF-I	0.51	0.66	0.62	0.68	0.76	
IGFBP-1b	ns	ns	-0.67	-0.69	-0.76	-0.85
IGF-I/BP-1b	ns	ns	0.53	0.56	0.64	NA
June						
BW	0.97					
K	0.58	0.72				
SGR-FL	0.60	0.62	0.57			
SGE-BW	0.62	0.71	0.85	0.86		
IGF-I	0.60	0.65	0.65	0.75	0.77	
IGFBP-1b	ns	ns	-0.73	ns	-0.64	-0.48
IGF-I/BP-1b	ns	ns	0.61	ns	0.61	NA

IGFBP-1b values are transformed to natural-log. ns: not significant. NA: not analyzed.

Supplemental table 1
Comparison of morphological parameters among treatments in chum salmon reared on May and June

May		Day 0	Day 10
FL	Fed	5.6 ± 0.1^{b}	6.1 ± 0.1^{a}
	Fasted	5.7 ± 0.1^{b}	5.8 ± 0.1^{ab}
	Refed	5.7 ± 0.1^{b}	6.0 ± 0.1^{ab}
BW	Fed	1.36 ± 0.05^{b}	1.76 ± 0.11^{a}
	Fasted	1.52 ± 0.07^{ab}	1.32 ± 0.09^{b}
	Refed	1.47 ± 0.06^{ab}	1.58 ± 0.10^{ab}
K	Fed	0.77 ± 0.02^{ab}	0.75 ± 0.02^{ab}
	Fasted	0.81 ± 0.01^{a}	0.67 ± 0.02^{c}
	Refed	0.79 ± 0.01^{ab}	0.74 ± 0.01^{bc}
June			
FL	Fed	6.4 ± 0.2^{b}	7.4 ± 0.2^{a}
	Fasted	6.3 ± 0.1^{b}	6.4 ± 0.2^{b}
	Refed	6.3 ± 0.2^{b}	6.8 ± 0.2^{ab}
BW	Fed	2.40 ± 0.18^{b}	3.29 ± 0.26^{a}
	Fasted	2.17 ± 0.15^{bc}	1.72 ± 0.18^{c}
	Refed	2.18 ± 0.16^{bc}	2.54 ± 0.24^{ab}
K	Fed	0.89 ± 0.01^{a}	0.79 ± 0.02^{c}
	Fasted	0.86 ± 0.01^{a}	0.63 ± 0.01^{d}
	Refed	0.85 ± 0.01^{ab}	0.79 ± 0.02^{bc}

Values are expressed as mean ± SE (Day 0: n = 16; Day 10: n = 10-11). Symbols sharing the same letters are not significantly different from each other.

Supplemental table 2
Comparison of morphological parameters in juvenile chum salmon caught at the river, estuary, port, coast and nearshore in May and June

	Month	Year	River	Estuary	Port	Coast	Nearshore
N	May	2015	42	2	21	34	11
		2016	16	17	8	11	10
		2017	16	18	8	9	3
	June	2015	48	35	101	58	3
		2016	16	1	16	10	8
		2017	16	13	16	24	9
	Month	Year	River	Estuary	Port	Coast	Nearshore
FL	May	2015	5.5 ± 0.1^{a}	5.2 ± 0.7^{ab}	5.0 ± 0.2^{b}	5.7 ± 0.1^{a}	5.7 ± 0.1^{a}
		2016	5.3 ± 0.1^{ab}	5.0 ± 0.1^{b}	5.1 ± 0.1^{b}	5.6 ± 0.1^{a}	5.2 ± 0.1^{ab}
		2017	5.1 ± 0.1^{bc}	5.0 ± 0.1^{c}	5.8 ± 0.2^{a}	5.5 ± 0.2^{ab}	5.1 ± 0.4^{abc}
	June	2015	5.8 ± 0.1^{d}	5.5 ± 0.1^{d}	6.5 ± 0.1^{c}	6.8 ± 0.1^{b}	7.2 ± 0.1^{a}
		2016	6.5 ± 0.2^{a}	5.0^{c}	6.9 ± 0.1^{a}	6.4 ± 0.1^{ab}	6.6 ± 0.1^{a}
		2017	5.9 ± 0.3^{b}	5.6 ± 0.2^{b}	6.6 ± 0.2^{ab}	7.1 ± 0.2^{b}	7.7 ± 0.5^{a}
	Month	Year	River	Estuary	Port	Coast	Nearshore
BW	May	2015	1.23 ± 0.04^{bc}	0.99 ± 0.36^{abc}	1.08 ± 0.09^{c}	1.50 ± 0.07^{a}	1.50 ± 0.08^{ab}
		2016	1.10 ± 0.04^{abc}	0.92 ± 0.04^{c}	0.97 ± 0.08^{bc}	1.30 ± 0.08^{a}	1.15 ± 0.07^{ab}
		2017	0.93 ± 0.03^{c}	0.80 ± 0.03^{c}	1.54 ± 0.13^{a}	1.48 ± 0.13^{ab}	1.05 ± 0.25^{bc}
	June	2015	1.42 ± 0.06^{d}	1.26 ± 0.04^{d}	2.14 ± 0.06^{c}	2.64 ± 0.14^{b}	3.11 ± 0.11^{a}
		2016	2.03 ± 0.17^{ab}	0.83^{b}	2.52 ± 0.13^{a}	2.34 ± 0.16^{ab}	2.48 ± 0.17^{ab}
		2017	1.76 ± 0.33^{cd}	1.32 ± 0.13^{d}	2.59 ± 0.21^{bc}	3.17 ± 0.22^{ab}	4.20 ± 0.73^{a}
	Month	Year	River	Estuary	Port	Coast	Nearshore
K	May	2015	0.74 ± 0.01^{b}	0.70 ± 0.01^{b}	0.81 ± 0.02^{a}	0.80 ± 0.02^{a}	0.79 ± 0.01^{ab}
		2016	0.73 ± 0.01^{b}	0.72 ± 0.01^{b}	0.73 ± 0.01^{b}	0.75 ± 0.01^{b}	0.82 ± 0.02^{a}
		2017	0.71 ± 0.01^{c}	0.65 ± 0.01^{d}	0.80 ± 0.02^{ab}	0.87 ± 0.02^{a}	0.77 ± 0.01^{bc}
	June	2015	0.71 ± 0.01^{d}	0.77 ± 0.01^{bc}	0.76 ± 0.01^{c}	0.80 ± 0.01^{ab}	0.83 ± 0.01^{a}
		2016	0.73 ± 0.02^{c}	0.68^{bc}	0.75 ± 0.01^{c}	0.89 ± 0.03^{a}	0.86 ± 0.03^{ab}
		2017	0.75 ± 0.02^{bc}	0.71 ± 0.02^{c}	0.87 ± 0.02^{a}	0.85 ± 0.02^{a}	0.83 ± 0.02^{ab}

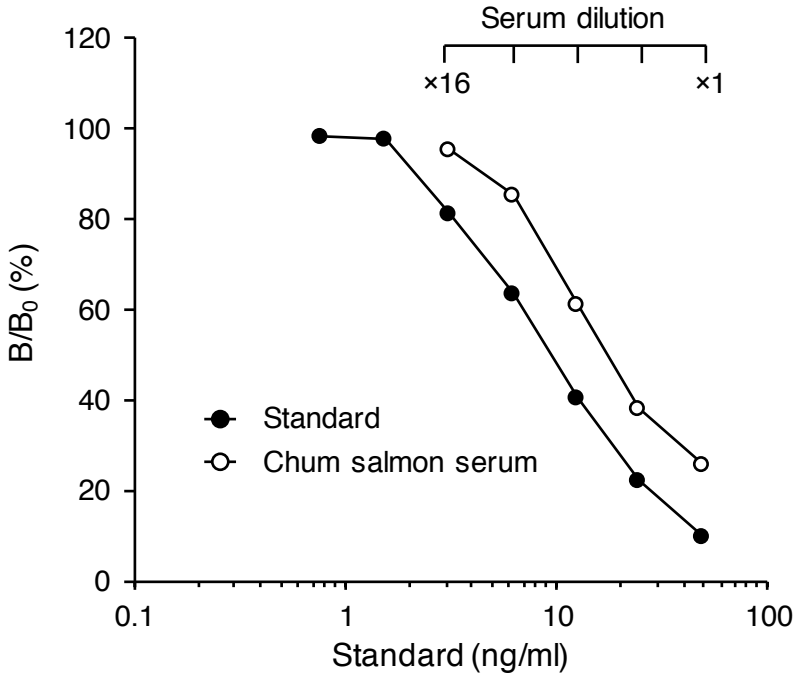
Values are expressed as mean ± SE. Symbols sharing the same letters are not significantly different from each other.

Supplemental table 3
Comparison of water temperature at the river and estuary or sea surface temperature at the port, coast and nearshore in May and June in all years.

WT/SST		River	Estuary	Port	Coast	Nearshore
May	2015	14.3 ± 0.9	12.9	8.2 ± 0.1	8.6 ± 0.4	9.0 ± 0.1
	2016	11.8 ± 1.4	11.2 ± 0.7	7.7 ± 1.9	6.8 ± 1.6	7.6 ± 2.3
	2017	12.6 ± 0.6	12.2 ± 0.9	7.1 ± 0.7	6.8 ± 0.7	7.6 ± 0.7
June	2015	17.7 ± 1.2	14.8 ± 0.3	13.1 ± 0.8	12.8 ± 0.7	11.5 ± 0.2
	2016	18.2 ± 2.3	14.8 ± 2.6	11.8 ± 2.4	11.9 ± 2.5	12.7 ± 2.9
	2017	17.2 ± 0.9	17.0 ± 1.4	10.8 ± 0.7	11.1 ± 1.4	10.4 ± 1.1

Values are expressed as mean ± SE.

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Suppl. Fig. 1. Displacement of biotinylated-salmon IGFBP-1b with the IGFBP-1b standard and serum from juvenile chum salmon. Sera of juvenile chum salmon fasted for 10 or 15 days were pooled and serially diluted to assess parallel displacement.