Title

Utilization of an endocrine growth index, insulin-like growth factor binding protein (IGFBP)-1b, for postsmolt coho salmon in the Strait of Georgia, British Columbia, Canada

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Abstract

Monitoring the growth of salmon during their early marine phase provides insights into prey availability and growth rates may be linked to risks of size-dependent mortality. However, the measurement of growth rate is challenging for free-living salmon in the ocean. Insulin-like growth factor (IGF)-I is a growth-promoting hormone that is emerging as a useful index of growth in salmon. In addition, laboratory-based studies using coho salmon have shown that one of circulating IGF-binding proteins (IGFBPs), IGFBP-1b, was induced by fasting and thus could be used as an inverse index of growth and/or catabolic state in salmon. However, no study has measured plasma levels of IGFBP-1b in salmon in wild. We measured plasma IGFBP-1b levels for postsmolt coho salmon collected in the Strait of Georgia and surrounding waters, British Columbia, Canada, and compared regional differences in IGFBP-1b to ecological information such as seawater temperature and stomach fullness. Plasma IGFBP-1b levels were the highest in fish from Eastern Johnstone Strait and relatively high in Queen Charlotte Strait and Western Johnstone Strait, which was in good agreement with the poor ocean conditions for salmon hypothesized to occur in that region. The molar ratio of plasma IGF-I to IGFBP-1b, a theoretical parameter of IGF-I availability to the receptor, discriminated differences among regions better than IGF-I or IGFBP-1b alone. Our data suggest that plasma IGFBP-1b reflects catabolic status in postsmolt coho salmon, as highlighted in fish in Eastern Johnston Strait and is a useful tool to monitor negative aspects of salmon growth in the ocean.

Keywords

Insulin-like growth factor binding protein-1b; Inverse index of growth; Insulin-like growth factor-I; Endocrine growth indices; Coho salmon; Strait of Georgia

1. Introduction

Growth is a process intrinsic to successful development, long-term survival and subsequent maturation of animals. As such, individual measures of growth provide an indicator of animal fitness and its ability to cope with its environment. Thus, measures of growth for individuals from a population may provide an index of population viability and in turn reflect environmental or habitat suitability for that population. At its most basic level, growth is an increase in size which is accompanied with cellular changes. And, changes in size are easy to monitor for animals that can be serially sampled. However, re-capture of free-living and non-sessile organisms is difficult and nearly an impossibility for pelagic animals in the ocean. In these circumstances, investigators may use markers for past growth such as incremental banding in hard tissues such as scales or bone (Healey 1982; Campana and Thorrold 2001; Wells et al. 2003; Tomaro et al. 2012) or biochemical indices of current growth such as RNA/DNA ratio (Buckley 1984; Johnson et al. 2002; MacLean et al. 2008; Kawaguchi et al. 2013; Kaneko et al. 2015). While useful, these methods have some limitations.

The endocrine system is the primary driver of growth in vertebrates (Kostyo 1999). Component proteins of the endocrine growth system are attractive targets for the development of biological growth markers because the proteins are not merely correlated with growth but also induce growth. As such, a number of clinical assays in human medicine utilize endocrine markers of growth (Klauwer et al. 1997; Balic et al. 2008; Carter et al. 2014; Knacke et al. 2016). Insulin-like growth factor (IGF)-I is a peptide hormone that circulates in the blood and is an integral component of the endocrine growth system in fishes (Wood et al. 2005; Reinecke 2010). Recent laboratory work has

demonstrated that IGF-I is correlated to growth in fish (Beckman et al. 1998; Pérez-Sánchez and Le Bail 1999; Dyer et al. 2004; Picha et al. 2008a, b; Beckman 2011) and several field studies have demonstrated that IGF-I measures provide data relevant to ecological process affecting juvenile salmon in marine environments (Beckman et al. 2004a; Ferriss et al. 2014; Bond et al. 2014; Kaneko et al. 2015, 2019; Chamberlin et al. 2017; Wechter et al. 2017).

IGF binding proteins (IGFBPs) are a regulatory component of the endocrine growth axis. These molecules modulate the activity of IGF-I by potentiating or inhibiting the binding of IGF-I to IGF receptors (Rajaram et al. 1997; Wood et al. 2005; Kelley et al. 2006; Allard and Duan 2018). Three major IGFBPs have been detected in plasma of salmon and are termed IGFBP-1a, -1b and -2b, respectively (Shimizu et al. 2011a, b; Shimizu and Dickhoff 2017). Plasma IGFBP-1a and -1b levels increase under catabolic conditions such as fasting and stress, whereas plasma IGFBP-2b levels are high under anabolic conditions as is the case for IGF-I (Shimizu et al. 2003, 2006, 2011a; Beckman et al. 2004b, c; Shimizu and Dickhoff 2017). IGFBP-1b is of particular interest since it is sensitive to changes in feeding status, with levels increasing at reduced rations (Shimizu et al. 2006, 2009). The increased IGFBP-1b levels are presumably inhibiting IGF-I action by blocking coupling of IGF-I to the IGF receptor and thus limiting growth (Kajimura and Duan 2007; Wheatcroft and Kearney 2009).

We have previously measured salmon IGFBP-1b and revealed that it had an inverse relationship with individual growth rate in salmon (Shimizu et al. 2006; Kawaguchi et al. 2013; Kaneko et al. 2019). Thus, plasma IGFBP-1b may also be useful as an index of growth based on this inverse relationship between IGFBP-1b and growth

under laboratory conditions. However, the utility of IGFBP-1b as a growth index in the field has been reported only in one study for juvenile chum salmon (*Oncorhynchus keta*) out-migrating from the northeast coast of Hokkaido Island, Japan (Kaneko et al. 2019). One of difficulties in utilizing endocrine growth indices in filed surveys is to disentangle environmental factors, such as water temperature and salinity, that may affect the indices independent of growth modulation. In postsmolt coho salmon (*O. kisutch*) in freshwater, a rapid drop in water temperature from 11°C to 7°C changed plasma IGFBP-1b levels and decoupled its relationship with growth rate under laboratory settings (Shimizu et al. 2006). However, if environmental factors are measured and taken into account, measuring IGFBP-1b as well as IGF-I should inform us growth status of free-living salmon in the ocean. Therefore, field survey at regions where other oceanographic and ecological conditions are relatively well characterized is useful.

The Strait of Georgia is a marine fjord located between the Pacific coast of Canada and Vancouver Island, approximately 240 km long and 40 km wide. The Fraser River is a major freshwater source in the southern Strait of Georgia and there is strong tidal exchange through both the Strait of Juan de Fuca and Johnstone Strait, at the southern and northern ends of the Strait of Georgia, respectively. The Strait of Georgia has high primary productivity and thus supports a large biomass of zooplankton and pelagic fish such as Pacific herring (*Clupea pallasii*) (Hourston and Haegele 1980). The Strait of Georgia is an important coastal rearing area for juvenile Pacific salmon and growth conditions within the Strait of Georgia most likely influences year class strength (Beamish et al. 2008). After weeks to months of rearing in the Strait of Georgia, juvenile Pacific salmon migrate out of the Strait of Georgia, either through the Strait of Juan de

Fuca or Johnstone Strait into the North Pacific Ocean (Tucker et al. 2009; Beamish et al. 2012; Beacham et al. 2016).

Consistently low IGF-I levels have been found for juvenile salmon in Queen Charlotte Strait and/or Johnstone Strait respectively (Ferriss et al. 2014; Journey et al. 2017) in coastal waters of British Columbia. These regional variations in growth suggest that feeding conditions might be reduced in the Johnstone/Queen Charlotte Strait. Our hypothesis was that plasma IGFBP-1b levels in juvenile salmon would be highest in Johnstone Strait, based on the low IGF-I levels found there (Journey et al. 2017). Attention was focused on samples obtained from coho salmon since the relationships between IGF-I, IGFBP-1b and growth rate have been established under laboratory conditions in this species (Beckman et al. 2004a, b, c; Shimizu et al. 2006, 2009). The objective of the present study was to utilize circulating IGFBP-1b as an endocrine growth index of coho salmon postsmolt caught during a trawl survey of Strait of Georgia and surrounding waters in 2014 by Fisheries and Oceans Canada. We compared regional variation in IGFBP-1b levels to the regional variation in IGF-I levels reported in Journey et al. (2017) and correlated IGFBP-1b levels with seawater temperature, length, weight, condition factor and stomach fullness. We view these results as an important step towards establishing the measure of IGFBP-1b levels as an effective physiological index of feeding and growth for free-living fishes.

2. Materials and methods

2.1. Study location and fish sampling

Juvenile coho salmon were sampled in 12 regions (Fig. 1): Queen Charlotte Strait (QCST), West Johnstone Strait (WJS), East Johnstone Strait (EJS), Northern Discovery Islands (Ndisc), Discovery Islands (Disc), Desolation Sound (Des), Northern Strait of Georgia (NSOG), Malaspina Strait (Mala), Strait of Georgia (SOG), Southern Strait of Georgia (SSOG), Fraser River Plume (Plume), and Gulf Islands (Gulf). Juvenile coho salmon were captured via fishing trawls aboard the C.C.G.S. W.E. Ricker in late June and early July of 2014. Specifics of survey design and complete methods are detailed in Tucker et al. (2009) and Journey et al. (2017). Briefly, all fishing was conducted between 0600 and 2000 hours with a modified mid-water rope trawl (28–42 m wide × 12–18 m deep; cod end mesh, 0.6 cm; Cantrawl Pacific Ltd., Richmond British Columbia), with the mouth either at the surface (zero meter) or 15 meters deep, towed at average speed of 9.36 km h^{-1} (5 knots), for 30 min. Fish caught at each tow location and region (Fig. 1) were immediately identified and sorted species of juvenile salmon by morphological characteristics after the net was brought on board the ship and emptied, and measured for weight (BW) and fork length (FL) on board the research vessel. Condition factor (K) was calculated as follows: (BW (g)) × 1000/(FL (mm))^3. The numbers of fish sampled in each region were shown in the figure 3. A subset of the juvenile coho salmon was then bled via heparinized syringe within 30 minutes of being taken from the net. Fish were dead when retrieved from the net. Blood samples were immediately centrifuged, and the plasma was collected. The plasma samples were frozen at sea at –20°C and then transferred to a –80°C freezer in Seattle within 2 days following the end of the survey until analyzed. While the same vessel and trawling technique were used for the entire survey, stomachs were analyzed differently in QCST and WJS compared to the remainder

of the study area as the work was done by different personnel for different portions of the survey. For the majority of the study area stomach contents and stomach fullness were estimated visually for individual fish (% fullness vol/vol). In QCST and WJS stomach content was measured by weight, thus these stations are excluded from our stomach fullness analyses. Near surface average water temperature was measured in all regions via a temperature probe at mouth of trawl net. Near surface water temperature (four meters in depth) in QCST and WJS was measured with a SBE 911 plus CTD (Seabird Scientific, Bellevue, WA, USA).

2.2. Sample analyses

Plasma IGF-I levels were measured and reported in Journey (2015) or Journey et al. (2017). However, plasma from all individuals reported on in Journey et al. (2017) were not available for IGFBP-1b analysis due to limited volumes. Thus, IGF-I levels reported herein are a subset of those reported by Journey et al. (2017). Plasma IGFBP-1b levels were quantified by TR-FIA as described in Fukuda et al. (2015). Briefly, a competitive method was employed to measure IGFBP-1b by following a procedure for DELFIA immunoassays (PerkinElmer, Waltham, MA, USA). Plasma samples were first incubated with antiserum against purified IGFBP-1b from Chinook salmon (*O. tshawytscha*) serum (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 using Wallac ARVO SX multi-label counter (PerkinElmer) at 615 nm. All samples were normalized using inter-assay pools of coho salmon plasma at three known IGFBP-1b concentrations (low, mid and high), corresponding to approximately 75, 50 and 25% binding in the plate. The assay was validated for coho salmon by running serial dilution of plasma samples from juvenile coho salmon with differing physiological conditions (low or high IGF-I, low or high condition factor, immature or mature) and tested for parallelism with Chinook salmon IGFBP-1b standards (Shimizu et al. 2006). These plasma samples were selected from data generated by Journey et al. (2017). Slopes of the serial dilutions of plasma from coho salmon with differing physiological conditions, such as immature or precociously maturing (jack), low or high IGF-I and high or low condition factor were parallel with that of the standard (Fig. 2). These data demonstrate that the assay developed from Chinook salmon accurately reflects changes in IGFBP-1b levels in coho salmon.

2.3. Statistical analyses

IGFBP-1b, IGF-I and IGF-I/BP-1b ratios were first analyzed by one-way ANOVA (region) to determine regional differences using the JMP software (SAS Institute Inc., Cary, NC, USA). When significant effects were found, differences were further identified by Fisher's least significant difference (LSD) test. Differences among groups were considered to be significant at $P < 0.05$. Simple regression analyses of IGFBP-1b on FL, BW, K, water temperature, and stomach fullness were also conducted using JMP software, and relations were considered to be significant at $P < 0.05$. Only regions where stomach fullness was measured individually (all regions except QCST and WJS) were

used in analyses. Regression coefficients were generated after calculating mean value of each region or tow. Data from maturing male coho salmon were excluded from analyses because their plasma IGF-I concentration also reflects the stimulatory actions of gonadal steroids (Beckman et al. 2004b).

3. Results

Significant regional differences in plasma IGFBP-1b, IGF-I and IGF-I/BP-1b ratio were found (Fig. 3). The highest IGFBP-1b levels were found in EJS and levels were also relatively high in WJS and QCST (Fig. 3a). Plasma IGFBP-1b levels also tended to be high in fish captured in Gulf Islands, but they were low for fish found in the main SOG basin (Fraser River Plume to Northern Discovery Islands). Plasma IGF-I levels were generally low/high in regions where plasma IGFBP-1b levels were high/low (Fig. 3b). A notable exception to this inverse relationship was seen in fish from WJS and ESJ with moderate to high levels of both endocrine factors in each region. IGF-I/BP-1b ratios were generally similar to plasma IGF-I levels in main SOG basin, but the ratios discriminated differences between regions more clearly than plasma IGFBP-1b and IGF-I alone (Fig. 3c). Specifically, the ratios in QCST, WJS and EJS were low despite of the relatively higher plasma IGF-I level found in these regions.

Average plasma IGFBP-1b level in each region was plotted against average FL, BW, and K (Fig. 4). There was no clear trend in the relationship of IGFBP-1b with FL and BW (Fig. 4a, b). In contrast, there was a negative relationship between K values and IGFBP-1b across regions (Fig. 4c), and a significant negative correlation was found when the data were analyzed among tows (Table 1). Fish caught in WJS and QCST had low K

values (Fig. 4c). Relationships of plasma IGFBP-1b with plasma IGF-I level, water temperature, and stomach fullness were also analyzed (Fig. 5). No clear trend was seen between plasma IGFBP-1b and IGF-I levels due to relatively high IGF-I levels in fish caught in WJS and EJS (Fig. 5a). Overall, seawater temperature and stomach fullness showed trends of inverse relationships with plasma IGFBP-1b levels across regions (Fig. 5b, c), and significant negative correlations were found when the data were analyzed among tows (Table 1). In EJS, stomach fullness and seawater temperature were low and plasma IGFBP-1b levels were the highest (Fig. 5b, c). IGF-I/BP-1b ratio showed positive relationships with FL, BW, K, and water temperature when the data were analyzed among tows (Table 1).

4. Discussion

Plasma IGFBP-1b levels in juvenile coho salmon varied significantly among regions in coastal British Columbia in June of 2014, with the highest value in the EJS and relatively high values in the QCST, WJS and Gulf Islands. Salmon caught in these same regions also tended to have reduced plasma IGF-I levels, condition factor, and a reduced volume of food in their stomachs. Together, these data suggest that reduced feeding and growth of juvenile coho salmon collected in these same regions occurred. Furthermore, the combined use of the endocrine markers IGFBP-1b and IGF-I indicates that growth of juvenile coho salmon varies spatially across the Strait of Georgia and near-by coastal regions.

The potential effects of stress were a concern for utilizing IGFBP-1b as a growth index in field surveys. Capture by trawling might affect plasma IGFBP-1b levels

independent of growth/nutritional status. Several studies have demonstrated that plasma IGFBP-1b level may be increased by stress (Kelley et al. 2006; Shimizu and Dickhoff 2017). An acute 15-min confinement stress on sunshine bass (a hybrid between female white bass, *Morone chrysops* and male striped bass *M. saxatilis*) increased plasma 24- and 28-kDa IGFBPs in 2 h (Davis and Peterson 2006). In Jack mackerel (*Trachurus symmetricus*), a 30-kDa IGFBP was induced in response to a handling stress for 30-60 min (Kelley et al. 2006). However, Shepherd et al. (2011) assessed effects of acute stressor exposure (i.e. 5-min handling disturbance) on plasma IGFBPs in rainbow trout (*O. mykiss*) and found that the plasma levels of IGFBP-1a and -1b were unaffected. When juvenile chum salmon were sampled by trawling for 6 min and maintained in a bucket for 1 h post-capture, serum IGFBP-1b levels were not affected (16.2 ± 2.3 ng/mL without holding vs 14.1 ± 1.5 ng/mL after holding in a bucket; Kaneko et al. unpublished data). In the present study, fish were sampled within 30 min including time taken to process fish after the net came on-board. Although sampling by trawling might increase IGFBP-1b levels for some extent, sampling protocol was similar at all stations. Hence, it is unlikely that regional differences in IGFBP-1b are due to varying levels of stress. However, it has to be kept in mind that measured values might be the sum of the basal level and stress-induced level.

Differences in IGFBP-1b levels between regions adjacent to Johnstone Strait might relate to differences in water temperature. Varying temperatures have been shown to affect the IGF-I/IGFBP system (Gabillard et al. 2005). We previously examined the effect of a drop in water temperature from 11°C to 7°C on plasma IGFBP-1b levels in postsmolt coho salmon. The effect was complex; a drop in water temperature decreased

plasma IGFBP-1b during the first 2 weeks, while there were increased levels during the 4th-7th week and no effect was seen at the 9th week (Shimizu et al. 2006). In the present study, there was an overall negative correlation between plasma IGFBP-1b and net temperature or near-surface (4 m) water temperature. This may suggest the higher IGFBP-1b levels for fish collected in QCST, WJS and Gulf Island might be due to the lower temperature in the region. However, there were only slight differences in IGFBP-1b for fish collected at temperatures ranging from 12^{o}C to 17^{o}C in all other SOG regions. EJS was 2^{o}C cooler (10^{o}C) than the adjacent region, yet IGFBP-1b levels were more than 50% greater than found in near-by areas. It is unlikely that a relatively small difference in temperature alone would produce a relatively large increase in IGFBP-1b. Thus we consider the higher IGFBP-1b levels found for coho salmon in EJS to be primarily due to other factors.

We assume poor feeding status was the major cause of the higher IGFBP-1b levels based on the IGF-I variation in addition to the results of our laboratory and field studies on IGFBP-1b (Shimizu et al. 2006; Kaneko et al. 2019). The results of this study are a sub-set of the data reported in Journey et al. (2017). They reported on IGF-I levels in five different species of juvenile salmon (coho, Chinook, sockeye, chum and pink salmon) collected across three years (2012 – 2014) in this same region and found lower IGF-I levels in fish from Johnstone Strait and Queen Charlotte Strait. Similarly, Ferriss et al. (2014) found lower IGF-I levels for juvenile salmon collected in Queen Charlotte Strait as compared to fish sampled in other coastal regions of British Columbia (coho, Chinook, sockeye and chum salmon; 2009 – 2011). Together these data provide partial support to the hypothesis presented in McKinnell et al. (2014), who suggested that the

strongly tidally mixed waters of Johnstone Strait and Queen Charlotte Strait would result in poor feeding conditions and subsequently reduced growth for juvenile salmon residing in these regions. The IGFBP-1b levels reported herein provide further evidence for relatively poor feeding and growth in Johnstone Strait and Queen Charlotte Strait.

Plasma IGFBP-1b and IGF-I levels may reflect differing physiological conditions and thus provide varying ecological information. Fasting postsmolt coho salmon for one or three weeks increased plasma IGFBP-1b in a time-dependent manner (Shimizu et al. 2009). A reduction of IGFBP-1b by food intake after fasting was relatively quick, with significant decreases within 10 h after a meal (Shimizu et al. 2009). In contrast, it takes several days of fasting to produce a significant decrease in IGF-I levels (Pierce et al. 2005). The consumption of a meal stimulates relatively modest, short-term increases in IGF-I level (several hours; Shimizu et al. 2009). The moderate IGF-I level but high IGFBP-1b level measured in fish from the QCST, WJS, EJS and Gulf Islands could reflect a relatively recent decrease in feeding (days to weeks), enough time to allow for the stimulation of IGFBP-1b levels due to reduced feeding but not long enough to result in a decrease in IGF-I levels related to a decrease in growth rate.

In this, and other studies in fish, a negative correlation between IGFBP-1b and body condition has been found (Shimizu et al. 2006; Kawaguchi et al. 2013; Fukuda et al. 2015; Kaneko et al. 2019) while IGFBP-1b levels are unrelated to body size. Conversely, Journey et al. (2017) found a positive correlation between IGF-I and body size in these data, similar as to what has been found in other studies (Beckman et al. 2004b; Shimizu et al. 2009; Kaneko et al. 2015, 2019). No correlation between IGF-I and condition factor has been reported (Beckman et al. 2004a; Ferriss et al. 2014; Fukuda et al. 2015). It is

worth noting that we found low condition in fish from the WJS, EJS, QCST and Gulf Islands also the regions with high IGFBP-1b and low IGF-I/BP-1b ratio. These data may suggest that feeding restriction for fish found in these areas has been for a relatively extended period.

The variation in information content between IGF-I and IGFBP-1b levels may be reflected in the IGF-I/BP-1b ratio. In humans, IGF-I/BP-1 ratio strongly associated with metabolic factors such as body mass index (BMI; body weight/height^2, which is similar to the condition factor in fish), circulating insulin, and glucose levels (Sandhu et al. 2004). On a qualitative scale, we can examine our results to assess if differences in the IGF-I/BP-1b ratio between regions might reflect differing physiological responses to feeding and growth that could provide useful ecological inferences. For instance, time course of change in response to a change in feeding or differing relationships with size or condition (Shimizu et al. 2009, 2011a; Kawaguchi et al. 2013). In contrast, if the differences in the ratio reflect differing physiological responses to varying temperature or the effects of capture stress, the ratio may provide lessor amounts of ecological insight.

There are advantages and disadvantages to the ecological approach we used to evaluate the utility of IGFBP-1b as an inverse indicator of growth. One of the greatest advantages is that one combines many possible sources of error, some of which are unknown, within a single exercise and then one simply evaluates if the results seem reasonable in relation to known physiological relationships and ecological conditions. Overall, our results are consistent with IGFBP-1b possessing qualities that might make it a useful ecological marker. However, there is also a clear lack of control and a clear lack of statistical power with respect to testing what environmental variables may affect

IGFBP-1b levels. Moreover, coho salmon caught in these regions were most likely mixtures of different origins (Beacham et al., 2016), which might have different baselines or responses of endocrine parameters to environmental factors as shown in two strains of smolting Atlantic salmon (Bernard et al., 2018). Most of the power to discern differences between regions in this study comes from samples in the Johnstone Strait and Queen Charlotte Strait. Water in these regions was colder and fish obtained there were less robust and had a smaller volume of food in their stomachs. We cannot discern individually which factor might have had the most effect on increased IGFBP-1b levels. However, even if we take into account that fish were mixtures of different origins, which might mask regional differences, we can infer that these fish (groups) were different than other fish (groups) captured in other regions due mainly to environmental conditions. Combined, IGFBP-1b and IGF-I levels allow us to make such distinction. Further laboratory work and more extended field surveys will allow us to discern specific environmental factors that may lead to increased IGFBP-1b levels.

All vertebrates have a functioning growth hormone/IGF system. Our techniques are not specific to juvenile salmon, in fact variation in IGF-I levels have been reported in a number of marine fishes (Terova et al. 2007; Picha et al. 2008b; Andrews et al. 2011; Beaudreau et al. 2011) as well as marine mammals (Richmond and Zinn 2009; du Dot et al. 2011) and terrestrial reptiles (Sparkman et al. 2010). Immunoassays are technical and require some expertise as well as adequate funding and are thus not universally available. However, the time course of change in these markers in relation to changes in feeding and growth (hours to days) makes them very useful for addressing ecological questions that require spatial and temporal specificity (a change in growth occurred at this place at this

time). We are encouraged by the additional information provided by IGFBP-1b levels beyond what is provided by IGF-I levels and intend to pursue an expanded palette of endocrine growth measures to provide ecological inference for free-living fishes.

In summary, we suggest coho salmon in the Johnstone Strait and Queen Charlotte Strait were under poor growth condition based on high IGFBP-1b levels in plasma. This finding is in good agreement with the previous ecological and physiological (eco-physiological) studies on coho salmon that pointed out low levels of plasma IGF-I due to poor biological productivity in the Johnstone Strait and Queen Charlotte Strait. Although more validation is necessary, the present study proposes that quantification of circulating IGF-I and IGFBP-1b would provide us the information on growth status of salmon and food availability in the regions.

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Compliance with Ethical Standards

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This study was carried out in accordance with all applicable international, national, and/or institutional guidelines for the care and use of animals.

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Figure legends

Fig. 1. Map of survey area in British Columbia, Canada and Strait of Georgia, USA. The tow locations in 2014 are shown and each color coordinated by region: Queen Charlotte Strait (QCST), Western Johnstone Strait (WJS), Eastern Johnstone Strait (EJS), Northern Discovery Islands (Ndisc), Discovery Islands (Disc), Desolation Sound (Des), Northern Strait of Georgia (NSOG), Malaspina Strait (Mala), Strait of Georgia (SOG), Southern Strait of Georgia (SSOG), Fraser River Plume (Plume) and Gulf Islands (Gulf). Sampling conducted in late June and early July.

Fig. 2. Displacement curves of the biotinylated-salmon IGFBP-1b with purified IGFBP-1b and plasma from coho salmon with different conditions. Jack: precocious maturing male, low/high IGF-I: fish with low or high IGF-I concentration, and low/high K: fish low or high K values (Journey et al. 2017). Binding (B/B_0) is expressed as a percentage of the specific binding.

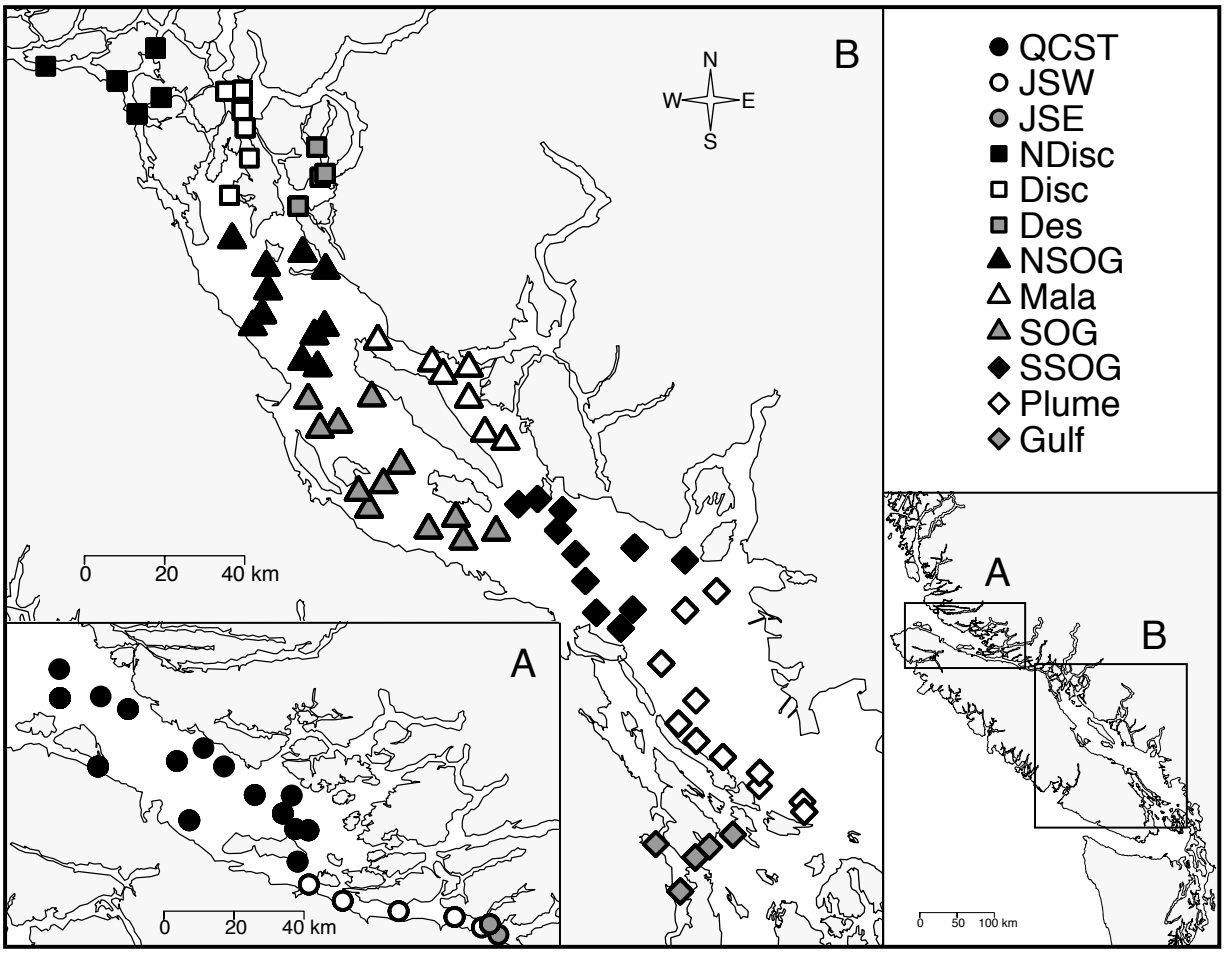
Fig. 3. Regional variations of plasma IGFBP-1b (a), IGF-I (b) and the molar ratio of IGF-I to IGFBP-1b (IGF-I/BP-1b; c). Values are expressed as mean ± SE. Number of fish analyzed in each region is shown under the corresponding bar. Symbols sharing the same letters are not significantly different from each other.

Fig. 4. Scatter plots of plasma IGFBP-1b levels against length (a), weight (b) and K (c). Regions were grouped based on the oceanographic characters and shown by the same symbols. Square: Queen Charlotte Strait (QCST), light triangle: Western Johnstone Strait

(WJS), dark triangle: Eastern Johnstone Strait (EJS), diamond: Gulf Islands (Gulf), and circles: all other regions of the Strait of Georgia.

Fig. 5. Scatter plots of plasma IGFBP-1b levels against plasma IGF-I levels (a), water temperature (b), and stomach fullness (c). Regions were grouped based on the oceanographic characters and shown by the same symbols. Square: Queen Charlotte Strait (QCST), light triangle: Western Johnstone Strait (WJS), dark triangle: Eastern Johnstone Strait (EJS), diamond: Gulf Islands (Gulf), and circles: all other regions of the Strait of Georgia.

Kaneko et al., Fig. 1



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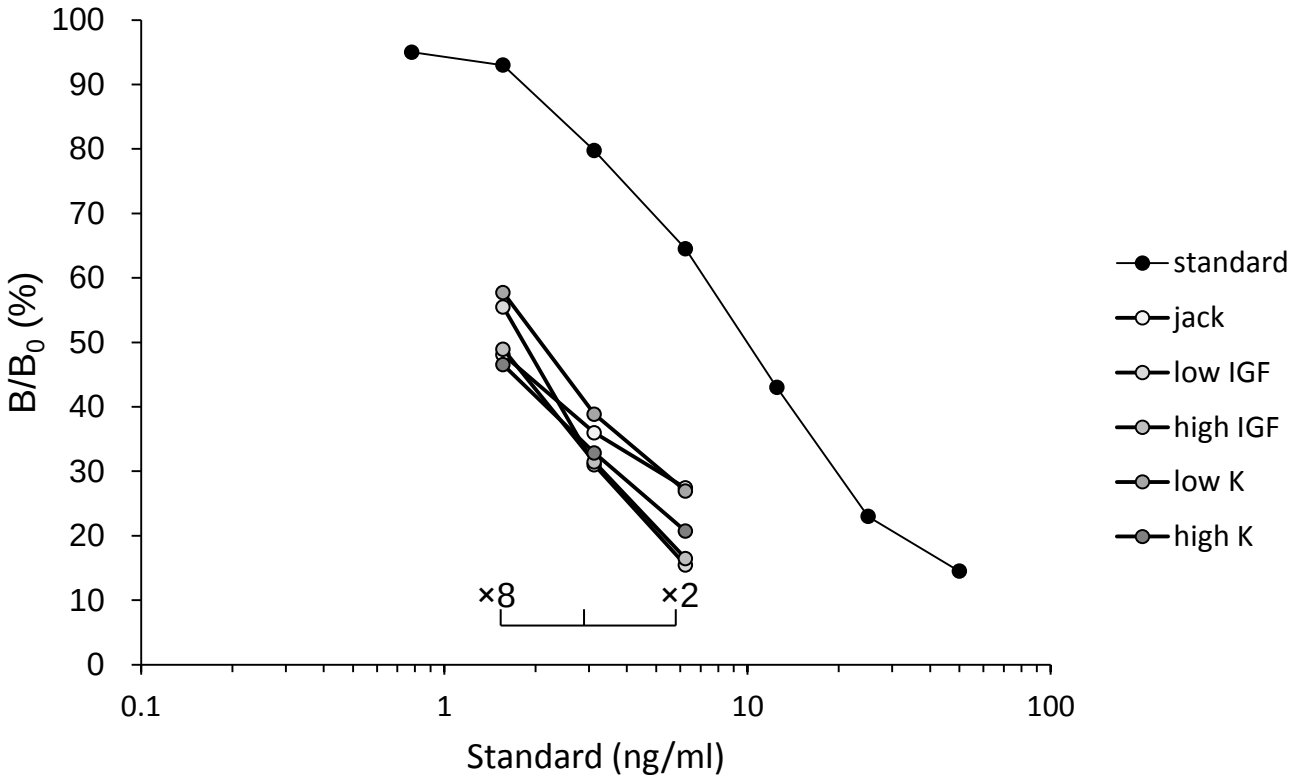

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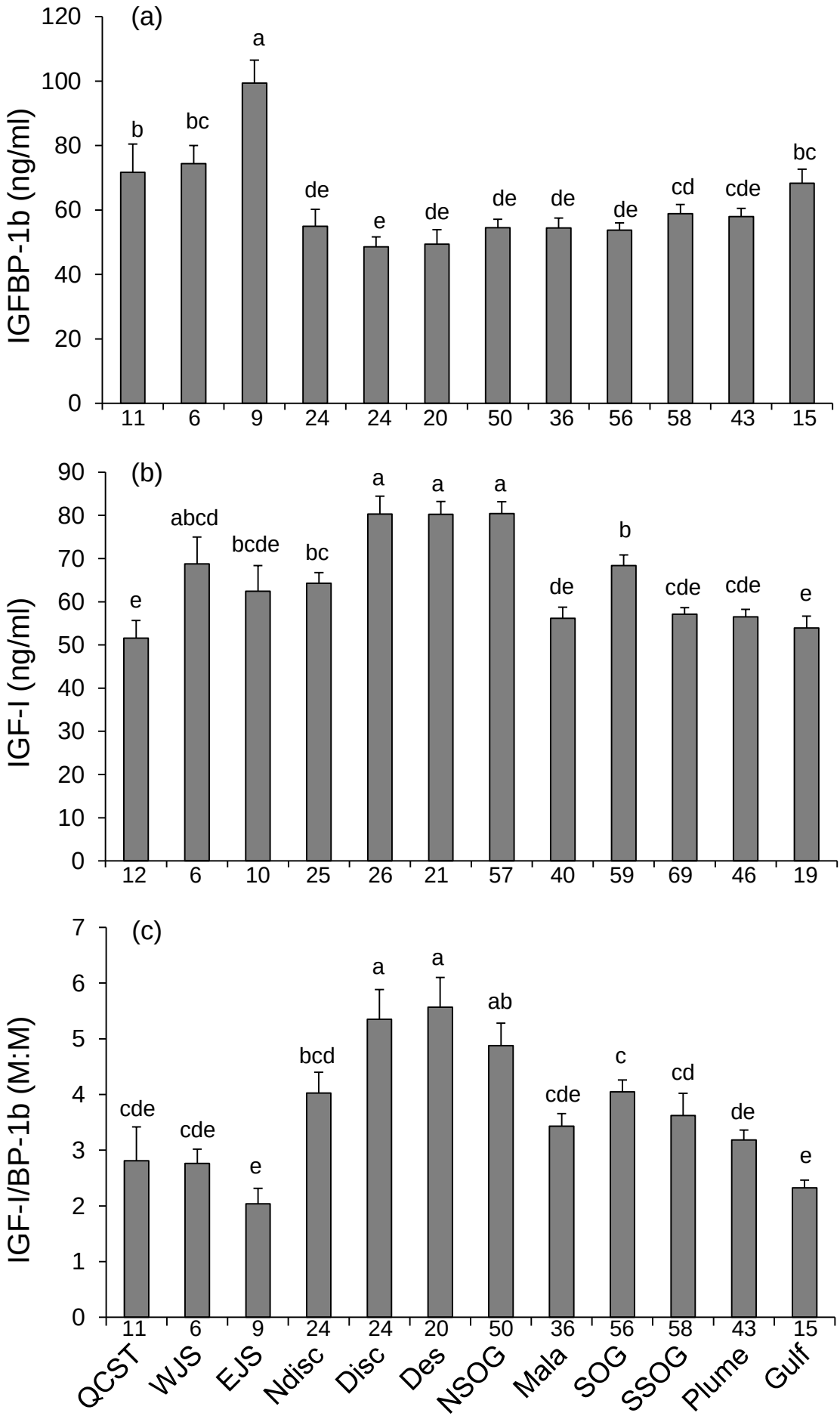

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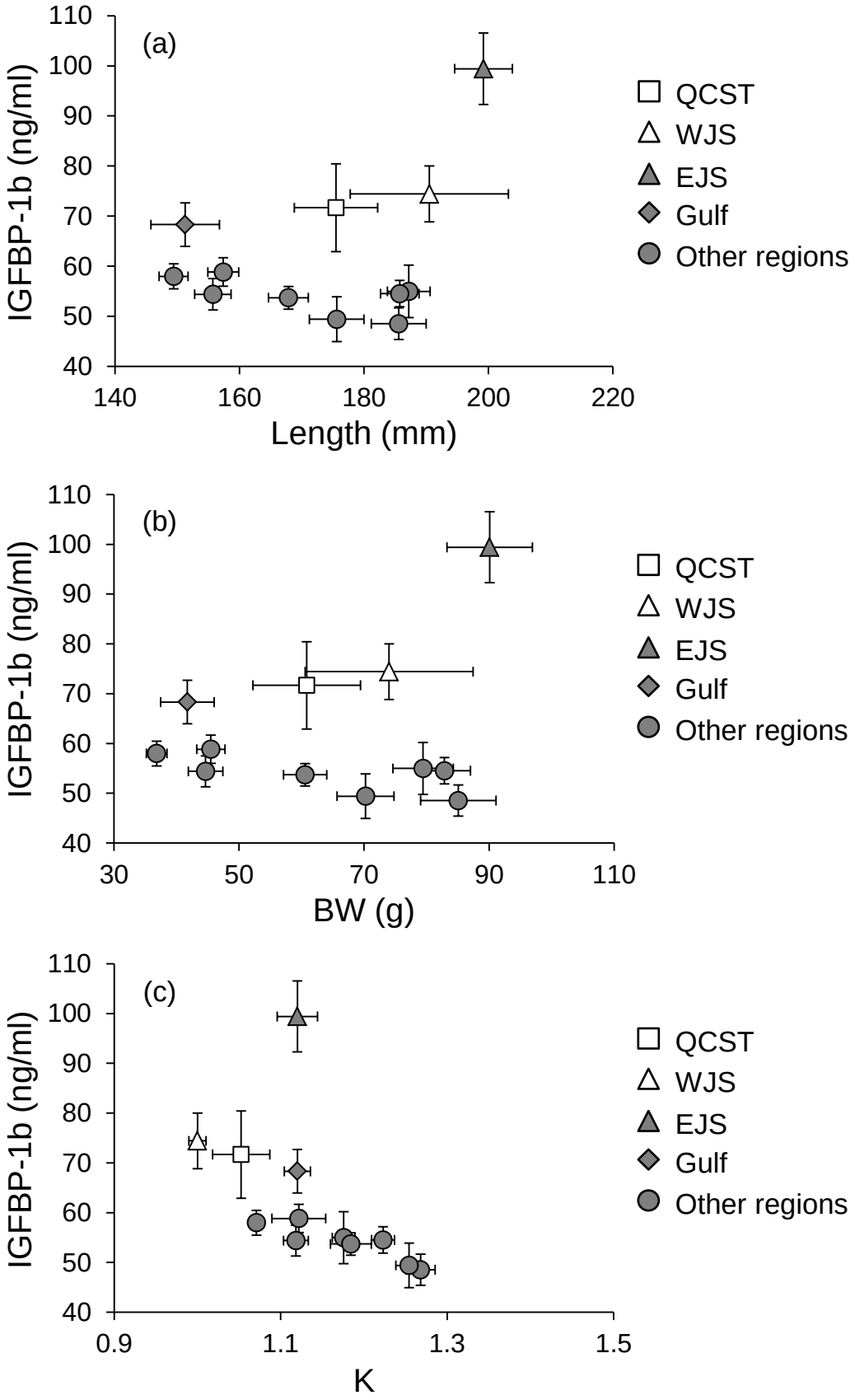

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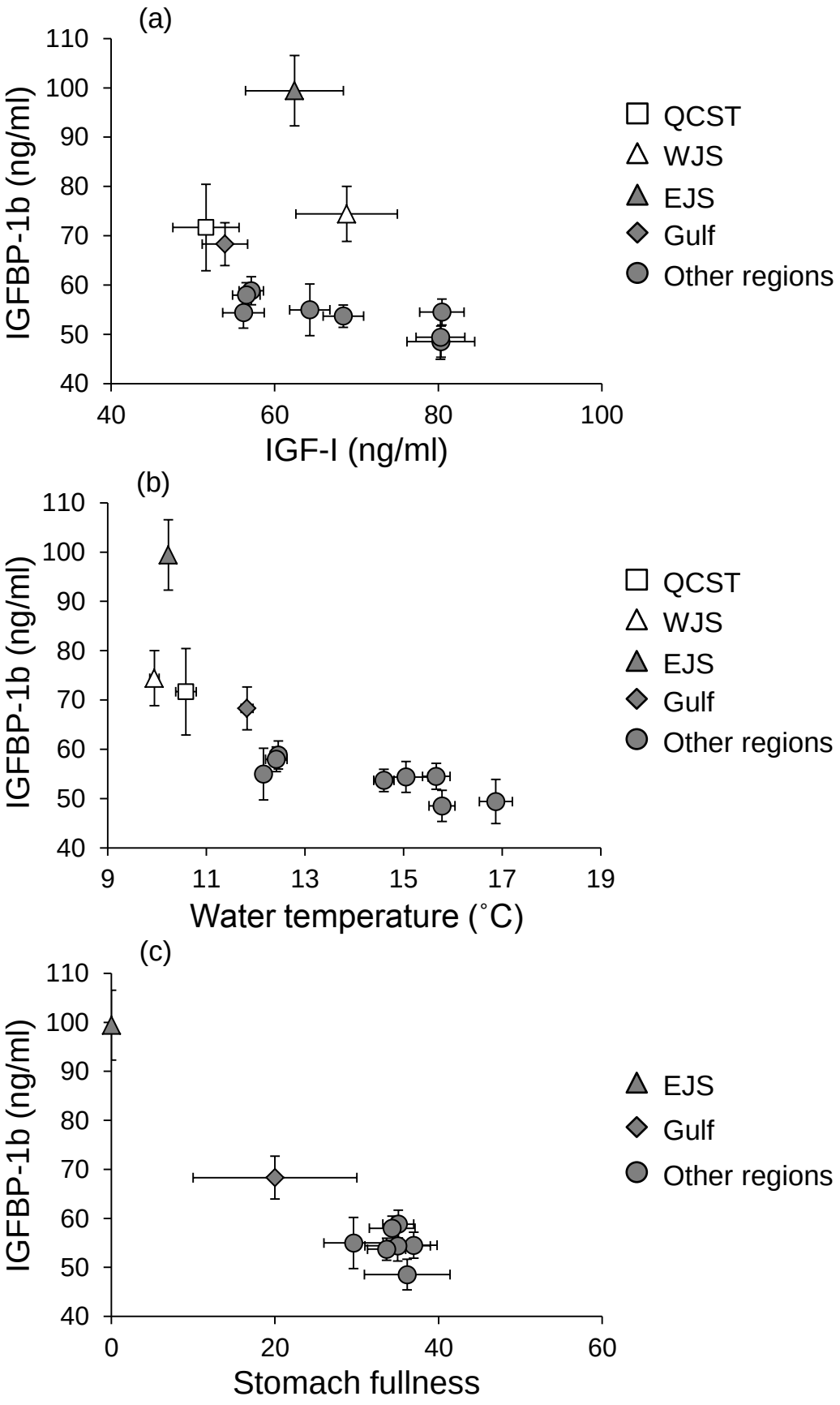

Table 1
Correlation coefficients (r) of mean plasma IGFBP-1b levels and IGF-I/BP-1b ratio with morphological parameters and ecological factors by tow.

		IGFBP-1b		IGF-I/BP-1b	
	N	**r**	***P* value**	**r**	***P* value**
Length	71	ns	0.2260	0.41	0.0004
Weight	70	ns	0.0996	0.47	<.0001
K	70	-0.34	0.0042	0.60	<.0001
Water temperature	70	-0.40	0.0006	0.50	<.0001
Stomach fullness	52	-0.32	0.0192	ns	0.1574

There were no values of stomach content for QCST or WJS. IGFBP-1b values were natural-log transformed prior to analysis. ns: not significant.

Supplemental table 1
Correlation coefficients (r) of mean plasma IGFBP-1b levels and IGF-I/BP-1b ratio with morphological parameters and ecological factors by individual fish.

		IGFBP-1b		IGF-I/BP-1b	
	N	**r**	***P* value**	**r**	***P* value**
Length	326	ns	0.9991	0.25	<.0001
Weight	321	ns	0.3562	0.30	<.0001
K	321	-0.18	0.0009	0.25	<.0001
Water temperature	322	-0.15	0.0056	0.26	<.0001
Stomach fullness	244	ns	0.0964	ns	0.2407

There were no values of stomach content for QCST or WJS. IGFBP-1b values were natural-log transformed prior to analysis. ns: not significant.