Title:

Effects of feeding status and water temperature on swimming performance in juvenile chum salmon (*Oncorhynchus keta*)

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Abstract

We examined the effects of feeding status in freshwater and then subsequent seawater rearing temperature on growth, critical swimming speed (U_{crit}), and circulating insulin-like growth factor (IGF)-1 in juvenile chum salmon. Chum salmon fry weighing about 1.0 g were fed at 0, 1 or 3% body weight (BW) for 5 days in freshwater, acclimated to seawater at 4, 7 or 10 °C and then reared for 8 days with satiation feeding. Both freshwater feeding history and seawater rearing temperature affected fork length (FL), BW, IGF-1 levels and relative U_{crit} (FL/s) 8 days after seawater transfer. Relative U_{crit} positively correlated with FL and IGF-1 levels, suggesting an improvement in swimming ability attributed to growth. In a second experiment, we examined the effects of body size and growth on serum IGF-1, IGF-binding proteins (IGFBPs), and U_{crit}. The chum salmon fry were sorted into large (1.5 g) or small (1.2 g) groups. They were acclimated to seawater at 10 °C and fed at 1 or 4% BW for two months. Despite the differences in serum IGF-1 levels, there were no differences in relative U_{crit} among the groups. In contrast, absolute U_{crit} (cm/s) was correlated with FL and IGF-1 levels. Absolute U_{crit} negatively correlated with serum IGFBP-1b levels. The present study showed that poor feeding in freshwater followed by transfer to seawater at low temperature has profound effects on the growth and swimming ability of juvenile chum salmon, which may be linked to alterations in circulating IGF-1 and IGFBPs.

Keywords

Coastal temperature, Growth, Hormone, Mortality, Prolonged swimming

Introduction

Anadromous juvenile salmonids may suffer high mortality rates during the early stages of their marine life (Healey, 1982; Bax, 1983; Fukuwaka and Suzuki, 2002; Beamish et al., 2004; Wertheimer et al., 2007; Kocik et al., 2009). The critical size and critical period hypothesis states that an initial mortality phase occurs soon after sea entry due to predation, whereas a second mortality phase occurs during fall and winter, which is growth and condition-dependent (Beamish and Mahnken, 2001; Farley et al., 2007). While this hypothesis has been challenged (Beacham et al., 2017, 2018), Tucker et al. (2016) demonstrated size- and condition-selective predation of juvenile salmon by the seabird Rhinoceros Auklets (*Cerorhinca monocerata*) during their coastal migration. Moreover, smaller salmon fry released into rivers have been shown to be preferentially preyed upon by piscivorous salmonids (Hasegawa et al., 2021). The growth and condition-dependent mortality of juvenile salmon is likely mediated by their swimming ability because it directly affects the success of predator avoidance, capacity to find food, and dispersal potential (Martinez et al., 2003; Chícharo et al., 2011). Therefore, it is important to accurately evaluate the effects of environmental variation on the growth and swimming performance of juvenile salmon.

Swimming performance in fish is often evaluated using swim tunnels and can be categorized into three types: burst, prolonged, and sustained swimming. Burst and sustained swimming speeds reflect the short-term anaerobic and long-term aerobic capacities of fish, respectively, while prolonged swimming is an intermediate metabolic process (Beamish, 1978; Jones 1982; Hammer, 1995). Brett (1964) developed a method to accurately measure prolonged swimming performance, termed critical swimming speed (U_{crit}). Although the U_{crit} is measured in a laboratory setting, U_{crit} exploits the maximal aerobic swimming capacity of fish in a natural environment and has been widely used to evaluate swimming performance (Plaut, 2001). Fish body size is the most important factor determining U_{crit} (Beamish, 1978; Cano-Barbacil et al., 2020), but water temperature also has a significant influence (Kieffer et al., 1998; Claireaux et al., 2006; Zeng et al, 2009; McKenzie and Claireaux, 2010; Pang et al., 2016; Yu et al., 2018).

Chum salmon (*Oncorhynchus keta*) are the target of intensive hatchery releases in northern Japan. Approximately 1.8 billion juveniles per year have been released from hatcheries in northern Japan (Miyakoshi et al., 2013). The coastal catch of returning adults has decreased over the past several years. One reason for this may be the atypical fluctuations in seawater temperature along the coasts. Seki and Shimizu (1996) reported that return rates of marked juvenile chum salmon were higher when coastal water temperature at the time of release was above 5 °C. Along the coast of the northeast Hokkaido, out-migration of juvenile chum salmon starts when the coastal water temperature exceeds 8 °C and is accelerated above 13 °C (Nagata et al., 2007). Based on these findings, favorable coastal water temperature for juvenile chum salmon is considered to be between 7 and 13 °C (Nagata et al., 2016). We previously examined the effects of fasting on the

82 growth of juvenile chum salmon in freshwater and seawater and showed that a combination of
83 fasting in the river followed by entry into cold seawater resulted in reduced growth (Nakamura et
84 al., 2019). These results suggest that small size or/and growth retardation negatively affect
85 swimming ability and increases predation risk. In addition, a particle-tracking simulation
86 suggested that sustained swimming ability was important for juvenile chum salmon originated
87 from the Japanese coasts to reach their feeding area, the Sea of Okhotsk, while avoiding warmer
88 currents (Azumaya et al., unpublished data). However, the relationship between growth status and
89 swimming performance has not yet been evaluated.
90 The growth of vertebrates including fish is regulated by the growth hormone (GH)-
91 insulin-like growth factor (IGF)-1 system (Daughaday and Rotwein, 1989; Le Roith et al., 2001;
92 Wood et al., 2005; Ohlsson et al., 2009). In this system, GH from the pituitary gland promotes
93 growth either directly or indirectly by stimulating hepatic production of IGF-1 (Daughaday and
94 Rotwein, 1989). IGF-1 circulates in the blood at relatively high levels due to the stabilization of
95 IGF-1 levels by IGF-binding proteins (IGFBPs). IGFBPs are important modulators of IGF-1
96 activity that regulate its half-life and the availability of IGF-1 to IGF-receptors in target tissues
97 (Rajaram et al., 1997). In mammals, six types of IGFBPs are present in the circulation and regulate
98 the activity of IGF-1 differently (Rajaram et al., 1997; Bach 2018).
99 Circulating IGF-1 has been proposed as a growth index in both salmonids and several
100 other fishes, because levels increase under good feeding conditions and are positively correlated
101 with individual growth rates (Beckman et al., 2004a,b,c; Picha et al., 2008; Beckman, 2011; Hack
102 et al., 2017, 2018). Circulating IGFBPs in fish are also potential candidates for growth indices. In
103 salmonids, three to four IGFBPs have been detected in the circulatory system (Shimizu and
104 Dickhoff, 2017). Among these, IGFBP-2b is the major carrier of IGF-1 and its circulating levels
105 are positively correlated with individual growth rates (Beckman et al., 2004a,b; Shimizu et al.,
106 2011). In contrast, IGFBP-1a and -1b are induced by fasting or stress, and their circulating levels
107 are negatively correlated with individual growth rates (Shimizu et al., 2006; Kawaguchi et al.,
108 2013; Kaneko et al., 2020). Much less is known about the 32-kDa IGFBP but it is upregulated
109 under good feeding conditions and weakly but positively correlated with individual growth rates
110 at least in rainbow trout *(O. mykiss*; Izutsu et al., 2022 Although circulating levels of IGF-1 and
111 IGFBPs reflect the growth status of salmonids, their relationship with swimming performance
112 has not been examined. The present study examined the effects of feeding status and seawater
113 temperature on the growth and swimming performance of juvenile chum salmon and assessed
114 whether circulating IGF-1 or IGFBPs levels were linked with swimming performance.
115
116 **2. Materials and methods**
117 *2.1. Rearing experiment 1: Effects of feeding in freshwater and seawater temperature*

Fertilized chum salmon eggs from spawning adults that returned to the Chitose River, Hokkaido, Japan were used for rearing experiments. In mid April 2018, juveniles weighing approximately 1.0 g were assigned to one of three groups and reared in freshwater (9.5 °C) for 5 days at different feeding rates: 0% (fasted), 1%, or 3% body weight (BW) (Fig. 1a). On day 0, fish from each group were acclimated for 3 days to artificial seawater at low (4 °C), medium (7 °C), or high (10 °C) temperatures, resulting in nine treatments. Fish were fed during acclimation and reared for an additional 5 days with satiation feeding. All rearing protocols and experiments were performed in accordance with the guidelines for fish use by the Ichthyological Society of Japan.

Sampling was conducted 3 and 8 days after transfer to seawater. Fork length (FL) and BW were measured at each sampling point (n = 8), and the condition factor was calculated as BW (g) × 100/FL (cm)^3. Blood was obtained by severing the tail and collecting it into a 10- or 20-μL plain glass tube (Microcap; Drummond Scientific Company, Broomall, PA, USA) and allowed to clot overnight at 4 °C. Serum was obtained after centrifugation at 9,730 ×g for 10 min and stored at –80 °C until further use. The other 10 fish from each treatment group were subjected to a swimming test, as described below.

2.2. Rearing experiment 2: Effect of size and feeding in seawater

Juveniles weighing approximately 1.0 g were obtained from the Kitami Salmon Enhancement Program Association. In mid-May 2021, the fish were sorted as either large (FL: 5.9–5.4 cm, BW: 1.8–1.2 g) or small groups (FL: 5.5–5.0 cm, BW:1.6–0.9 g). Fish were acclimated to artificial seawater for 2 days, and each size class of fish was fed either low (1% BW) or high (3% BW) rations for 55 days, resulting in four treatments: LargeLow, LargeHigh, SmallLow, and SmallHigh (Fig. 1b). Each treatment was reared in replicate tanks. Sampling was conducted 55 d after transfer to seawater, as described above.

2.3. Swimming performance

U_{crit} was measured using a swim tunnel with a swim chamber of 120 mm inner diameter and 600 mm length (volume: approximately 6.8 L; West Japan Fluid Engineering Laboratory, Nagasaki, Japan), as described by Urushiyama et al. (2024). The swim tunnel was placed in a thermostatic water tank (400 L FRP tank), and the water temperature was controlled using a cooling water circulator (CTP-3000, Tokyo Rikakikai, Tokyo, Japan). Prior to the trials, 10 individuals were randomly selected from each treatment group and measured for the FL of individual fish. These mean values were used to determine acclimation and stepwise water velocity of each trial. In Experiment 1, 10 juveniles were used for each trial and the water temperature of the swimming trial was corresponded to their rearing temperature (i.e., 4, 7, and 10 °C). At the start of each trial, the 10 fish were simultaneously placed in the one swim chamber and acclimated to an initial water

velocity 0.25 FL/s for 30 min. After acclimation, the water velocity was increased by 0.8 FL every 3 min. After the fish fatigued and were pinned on the mesh at the back of the chamber for 10 consecutive seconds, the elapsed time was recorded. In each trial, the time that 10 fish simultaneously placed in one swim chamber became fatigued and stuck to the mesh was recorded for each individual fish. Observations and recordings were made by two researchers: one judged the state of fatigue (whether or not the fish stuck to the mesh) and the other one recorded the time elapsed from the start of the experiment. The trial was finished after all 10 individuals in the chamber were stationary on the mesh. The FL and body weight of the chum salmon fry were measured after the trial. Relative U_{crit} (FL/s) was calculated using the equation described by Brett (1964): $U_{crit} = V_f + (T_f/T_p)\ V_p$, where V_f is the highest velocity (FL/s) attained for an entire 3 min interval, T_f is the time (min) taken until elimination during the final speed interval, T_p is the time interval (3 min), and V_p represents the speed increment (0.8 FL/s). U_{crit} of juvenile chum salmon in Experiment 2 was measured at ~10 °C in the same tunnel and same procedure except that the total of six individuals from each of two tanks were used for each trial due to their larger size. The absolute U_{crit} (cm/s) was calculated by multiplying the relative U_{crit} by the average FL.

2.4. Time-resolved fluoroimmunoassay (TR-FIA) for IGF-1

To measure IGF-1 levels, serum was first extracted using acid–ethanol, as described by Shimizu et al. (2000). Thereafter, IGF-1 was quantified using a TR-FIA, based on the method described by Small and Peterson (2005). Recombinant salmon/trout IGF-1 (GroPep Bioreagents Pty Ltd., Adelaide, Australia) was used as the standard. Time-resolved fluorescence was measured using a Wallac ARVO X4 Multilabel Counter (PerkinElmer, Waltham, MA, USA).

2.5. Ligand blotting for IGFBPs

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 3% stacking and 12.5% separating gels. Serum samples (2 µl equivalent) were treated with an equal volume of buffer containing 2% SDS and 10% glycerol at 85 °C for 5 min. The gels were then immersed in a solution of 50 mM Tris, 400 mM glycine, and 0.1% SDS, and electrophoresis was performed at 8 mA for the stacking gel and at 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-1 (DIG-hIGF-1) was performed according to previous protocol (Shimizu et al., 2000). Proteins separated by SDS-PAGE were electroblotted onto a nitrocellulose membrane (Bio-Rad, XX). The nitrocellulose membrane was incubated overnight with DIG-hIGF-1 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). IGFBPs were visualized by enhanced

chemiluminescence (ECL) western blotting (Amersham Life Science, Arlington Heights, IL, USA). The intensities of the serum IGFBP bands were semi-quantified using ImageJ version 1.440 (Schneider et al., 2012), normalized to human IGFBP-4 band intensity, and expressed as arbitrary density units (ADU).

2.6. Statistical analysis

The effects of feeding status in freshwater and subsequent seawater rearing temperature were analyzed by two-way analysis of variance (ANOVA) using JMP software. The effects of initial size and feeding ration were also tested using two-way ANOVA. When an interaction was found, the groups were further compared using Tukey's honest 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. Experiment 1: Effects of feeding in freshwater and seawater temperature

Fork length did not differ among feeding treatments or seawater rearing temperatures 3 days after transfer but fork length was affected by both low temperature ($P = 0.0026$) and low feeding ration ($P = 0.0132$) 8 days after transfer to seawater (Fig. 2a,b). BW was affected by feeding treatment and it was lower in the freshwater fasted group both 3 days ($P = 0.0280$) and 8 days ($P = 0.0015$) after transfer (Fig. 2c,d). BW was also affected by low temperature 8 days after transfer to seawater ($P < 0.0001$). At 3 days after seawater transfer, condition factor differed by freshwater feeding history ($P = 0.0002$) and there was an interaction with seawater rearing temperature at 10 °C ($P = 0.0244$), while both feeding history ($P = 0.0070$) and seawater rearing temperature ($P < 0.0001$) had significant effects on condition factor on day 8, being low in fasted fish and in fish at 7 or 4 °C (Fig. 2e,f).

Circulating IGF-1 levels in the freshwater fasted group were lower than those in the fed groups on day 3 ($P < 0.0001$) . Serum IGF-1 levels were higher in fish at lower seawater temperature on day 3 ($P = 0.0010$) while the opposite was the case on day 8 ($P < 0.0001$; Fig. 3a,b).

There was a main negative effect of low seawater temperature ($P < 0.0001$) on relative U_{crit} (FL/s; Fig. 3c, d). On day 8, relative U_{crit} was lower in the freshwater fasted groups than in the 3% fed groups ($P = 0.0211$) and in the low temperature groups ($P < 0.0001$; Fig. 3d). The responses of the absolute U_{crit} (cm/s) were essentially the same as those of the relative U_{crit} (data not shown).

A simple regression analysis was conducted to reveal the relationships between growth parameters and relative U_{crit} using the average value of 10 fish from each group. Nine average

values were used for each time point. The average relative U_{crit} 8 days after seawater transfer was positively correlated with the growth parameters of juvenile chum salmon (Fig. 4). In particular, a high correlation coefficient (r = 0.92) was seen between the average relative U_{crit} and average IGF-1 levels (Fig. 4d).

3.2. Experiment 2: Effects of size and feeding in seawater

Both initial size and ration had significant effects on FL (P < 0.0001 and < 0.0001), BW (P < 0.0001 and < 0.0001), and circulating IGF-1 levels (P = 0.0033 and 0.0002), whereas condition factor was only influenced by ration (P = 0.0004; Fig. 5). However, there were no differences in relative U_{crit} among the four groups (Fig. 6a). In contrast, absolute U_{crit} was lower in fish of small initial size (P = 0.0022) and in fish fed a low ration (P < 0.0001) (Fig. 6b). When the average values for each of the four groups (four average values from each treatment except IGF-1 with two average values) were used, the relative U_{crit} showed no correlation with FL, BW, condition factor or IGF-1 levels (Fig. 7). On the other hand, the average absolute U_{crit} was correlated with the average FL, BW, condition factor and IGF-1 levels (Fig. 8).

The band intensities of serum IGFBP were semi-quantified using ligand blotting. There was no effect of initial body size or feeding ration on the band intensities of serum IGFBP-2b and 32-kDa IGFBP (Fig. 9a,b). In contrast, feeding ration, but not initial size, affected the band intensities of serum IGFBP-1a (P = 0.0048) and -1b (P = 0.0015), which were higher in fish fed at 1% than in those fed at 4% (Fig. 8c,d). The band intensities of IGFBP-1a and 1b were negatively correlated with FL and BW (Table. 1). There were also negative correlations between the average IGFBP-1a/-1b band intensities and the average absolute U_{crit} (Fig. 10).

4. Discussion

In Experiment 1, feeding status in freshwater and subsequent seawater rearing temperature affected FL, BW, condition factors, and circulating IGF-1 levels in juvenile chum salmon 8 days after transfer to seawater. All fish were fed to satiation in seawater; however, there was still an effect of previous fasting or restricted feeding in freshwater after seawater transfer. This prolonged effect is consistent with the findings of Nakamura et al. (2019). In contrast, circulating IGF-1 levels were lower in fasted group in freshwater but caught up to the levels similar to fed group 8 days after feeding in seawater. Given the growth-promoting effect of IGF-1; FL and BW of fish with restricted feeding in freshwater would eventually catch up with those of fish with a 3% feeding ration in freshwater, if the experimental period of feeding in seawater was prolonged. Indeed, Oikawa et al. (2021) reported that when juvenile chum salmon were fasted for 5 days in freshwater, it took 20 days for all parameters to be restored. In contrast, baseline circulating IGF-1 levels appeared to be influenced by seawater temperature, which is in agreement with previous

findings (Beckman et al., 2004c). Thus, the treatments employed in the present study created fish with different body sizes/conditions and serum IGF-1 levels at the time of swimming performance assessment.

The swimming performance (U_{crit}) of juvenile chum salmon was also influenced by feeding status in freshwater and subsequent seawater rearing temperature. Cold seawater temperatures reduced the relative U_{crit} regardless of the feeding history in freshwater. Water temperature is an important factor affecting fish swimming performance (Kieffer et al., 1998; Claireaux et al., 2006; Zeng et al, 2009; McKenzie and Claireaux, 2010; Pang et al., 2016; Yu et al., 2018). Koumoundouros et al. (2002) reported a binomial relationship between relative U_{crit} and water temperature and suggested a range of 19.3–29.6 °C as an optimal temperature for the European sea bass (*Dicentrarchus labrax*). Although the seawater temperature range tested in the present study (i.e. 4, 7 or 10 °C) was too narrow to identify the optimal range, it provides evidence that a small difference in seawater temperature affects swimming performance of juvenile chum salmon, which would in turn influence their ability to escape from predators. The negative effect of fasting in freshwater on U_{crit} in fish transferred to 7 °C seawater was worth noting because it indicates the importance of feeding status in juveniles in the river after release from a hatchery even when coastal seawater temperature is optimal.

There were positive correlations between the average relative U_{crit} and the average body size/condition in Experiment 1. In the present study, average values were used for the correlation analyses because fish used to test swimming performance and fish sampled for physiological analysis were from the same tank but were different individuals. Although there were only a few data points, the relationships between relative U_{crit} and FL, BW, and the condition factor were strong, with r values ranging from 0.87–0.93. Body length is the primary factor determining the absolute U_{crit} (cm/s) in many fish species, and it is common to observe a positive relationship between these if the size range is sufficiently wide (Srean et al., 2017; Cano-Barbacil, 2020). In contrast, the relationship between body length and relative U_{crit} (BL/s) is generally negative, but can be positive or neutral depending on the species, developmental stage, season, and experimental settings (Mateus et al., 2008). In the present study, larger fish exhibited higher absolute and relative U_{crit} values and both parameters were positively correlated with FL. This may be due to the developmental stage of the juvenile chum salmon used in Experiment 1. The size range of the experimental fish was between 5.3–5.8 cm in FL and these fish could be classified as the pre-fingerlings at which bone ossification and body shaping progress is incomplete (Kaeriyama, 1986). Nobata et al. (2021) reported a significant correlation between burst swimming speed and condition factor in hatchery-reared but not naturally reared chum salmon fry. They suggested that fish with sufficient feeding tended to have better burst swimming abilities in early life stages. Juveniles in the present study were also fed to satiation after being

298 transferred to seawater. The condition factor values reported in Nobata et al. (2021) (0.54-0.61)
299 were lower than found in the present study (0.66–0.80). In addition, there was a positive
300 correlation between U_{crit} and the condition factor in the present study, suggesting that the juveniles
301 were small but not poor condition compared to wild fish after fasting for five days in freshwater,
302 followed by refeeding for 8 days.
303 This is the first study to report a positive relationship between U_{crit} and circulating IGF-
304 1 levels. This relationship may be due to IGf-1's association with body size because the circulating
305 IGF-1 levels were significantly correlated with individual body length/weight in Experiment 1 (r
306 = 0.32–0.52). However, the possibility that circulating IGF-1 directly affects swimming
307 performance cannot be ruled out because there was still a positive correlation between relative
308 U_{crit} (normalized by FL) and circulating IGF-1 levels. A relationship between IGF-1 and
309 swimming performance might be suggested from the perspective that swimming affects the GH-
310 IGF-1 system. Studies on gilthead sea bream (*Sparus aurata*) have reported swimming enhances
311 both growth and plasma IGF-1 levels (Sánchez-Gurmaches et al., 2013; Blasco et al., 2015). It
312 was suggested that swimming stimulates the GH-IGF-1 system, which in turn promotes muscle
313 growth and thus body growth (Vélez et al., 2017). However, the effect of circulating IGF-1 on
314 swimming performance has not been assessed.
315 Because large fish generally grow fast, it is not known if the growth rate is more
316 important for swimming performance than absolute size. To address these questions, Experiment
317 2 examined the effects of growth status and body size on U_{crit}. Large and small juvenile chum
318 salmon were subjected to high or low ration for approximately two months to create fish with
319 similar final sizes but different recent growth rates and serum IGF-1 levels (i.e., Large-Low and
320 Small-High groups). The treatment was successful in manipulating size and growth. On the other
321 hand, serum IGF-1 levels and absolute $\underline{U}_{crit}$ in the Small-High group tended to be higher than
322 those in the Large-Low and Small-High groups, they were not statistically different. Overall, the
323 relative U_{crit} (FL/s) and absolute U_{crit} (cm/s) values of the two groups (i.e., Large-Low and Small-
324 High) were similar. These results suggest that size but not growth rate is an important factor
325 affecting the swimming performance of juvenile chum salmon. However, because the serum IGF-
326 1 levels did not differ between the two groups, the possible direct effects of IGF-1 could not be
327 assessed.
328 In Experiment 2, despite the differences in size, growth, and IGF-1 levels, the relative
329 U_{crit} (FL/s) of juveniles was similar among the treatments, which contrasted with the results of
330 Experiment 1. One possible explanation for this discrepancy is the difference in the experimental
331 periods. Although both experiments used juvenile chum salmon with similar initial body sizes,
332 the experimental period was shorter in Experiment 1 (8 days after transfer to seawater) and longer
333 in Experiment 2 (55 days). Juveniles in the latter experiment were larger (7–10 cm) and at the

post-fingerling stage, so body formation was likely completed, and the relationship between swimming performance and body size may be fixed. In contrast, the absolute U_{crit} (cm/s) in the 1% fed small fish was significantly lower than that in the other three treatments. This result is consistent with the general tendency that larger fish have better swimming abilities (Beamish, 1978; Cano-Barbacil et al., 2020).

In Experiment 2, serum IGFBPs were analyzed for their responses to feeding manipulation and their relationship with U_{crit} in juvenile chum salmon. Four bands corresponding IGFBP-1a, -1b, -2b, and 32-kDa IGFBP were detected in the serum of juvenile chum salmon. The band intensities of serum IGFBP-1a and -1b were higher in fish with a 1% ration than in fish with a 4% ration. These responses are in good agreement with previous findings (Kaneko et al., 2019, 2020). In contrast, serum IGFBP-2b and 32-kDa IGFBP did not respond to restricted feeding, which conflicts with previous findings in postsmolt coho salmon, that report reduced levels with decreased ration (Beckman et al., 2004b). On the other hand, Hayashi et al. (2021) reported that fasting underyearling masu salmon did not affect circulating IGFBP-2b level. The sensitivity of IGFBP-2b to feeding may be influenced by the age, season, and species.

Correlation analyses revealed negative relationships between absolute U_{crit} (cm/s) and the band intensities of serum IGFBP-1a and -1b. It should be noted that although the fish tested for U_{crit} were identical to those used for the IGFBP analyses, the values were the averages of three individuals. This is because three fish were tested for U_{crit} and blood samples were collected thereafter. As is the case for IGF-1, their relationship may be via an association with body size because circulating IGFBP-1a and -1b levels are often negatively correlated with body size and growth rate (Shimizu et al., 2006; Kawaguchi et al., 2013). However, the possible functional link between serum IGFBP-1s and swimming performance should be addressed in future studies.

In summary, the present study showed that the swimming performance of juvenile chum salmon was affected by the combination of poor feeding status in freshwater and subsequent transfer to cold seawater. In addition, body size/condition factor may be more important than growth rate in the determination of swimming performance. These findings are particularly relevant for the production of robust juvenile chum salmon in hatcheries. The present study also reports, for the first time, the correlations between swimming performance and circulating levels of IGF-1 and IGFBP-1s, which invites future studies to seek their functional link with swimming performance.

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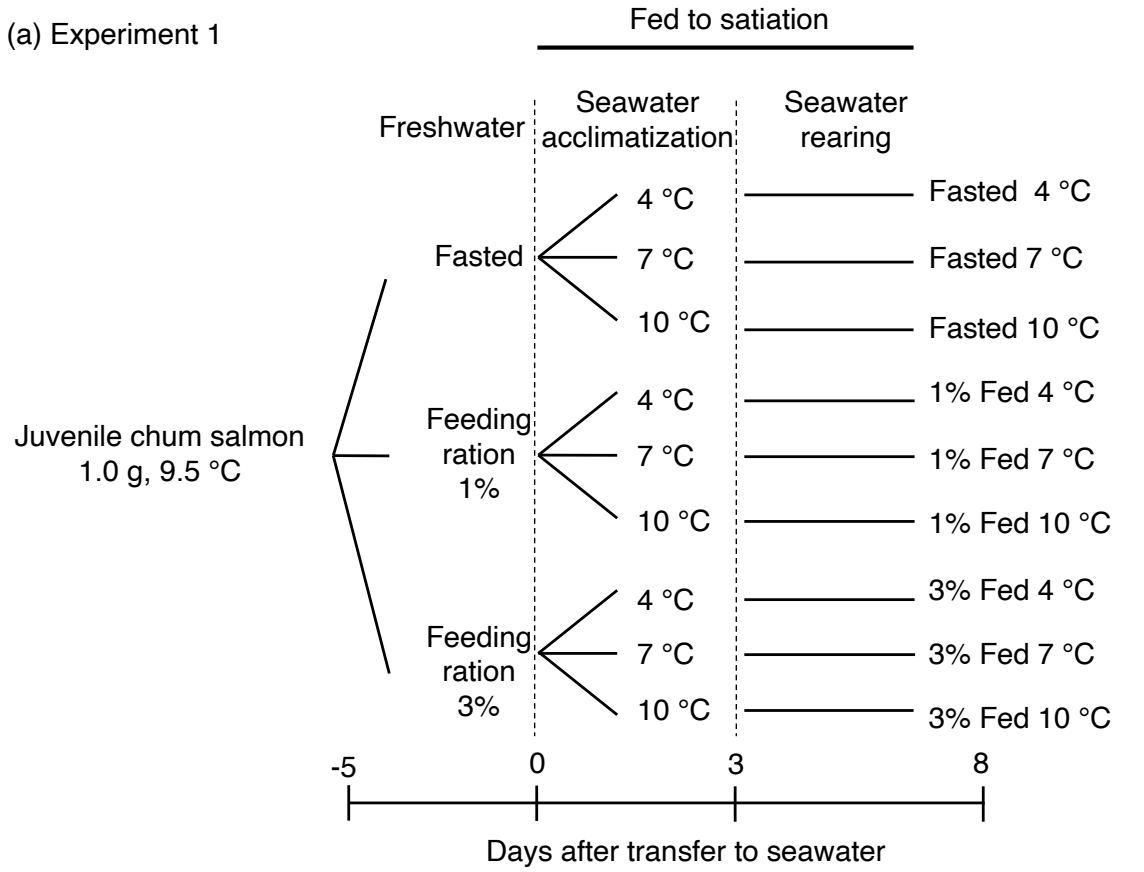
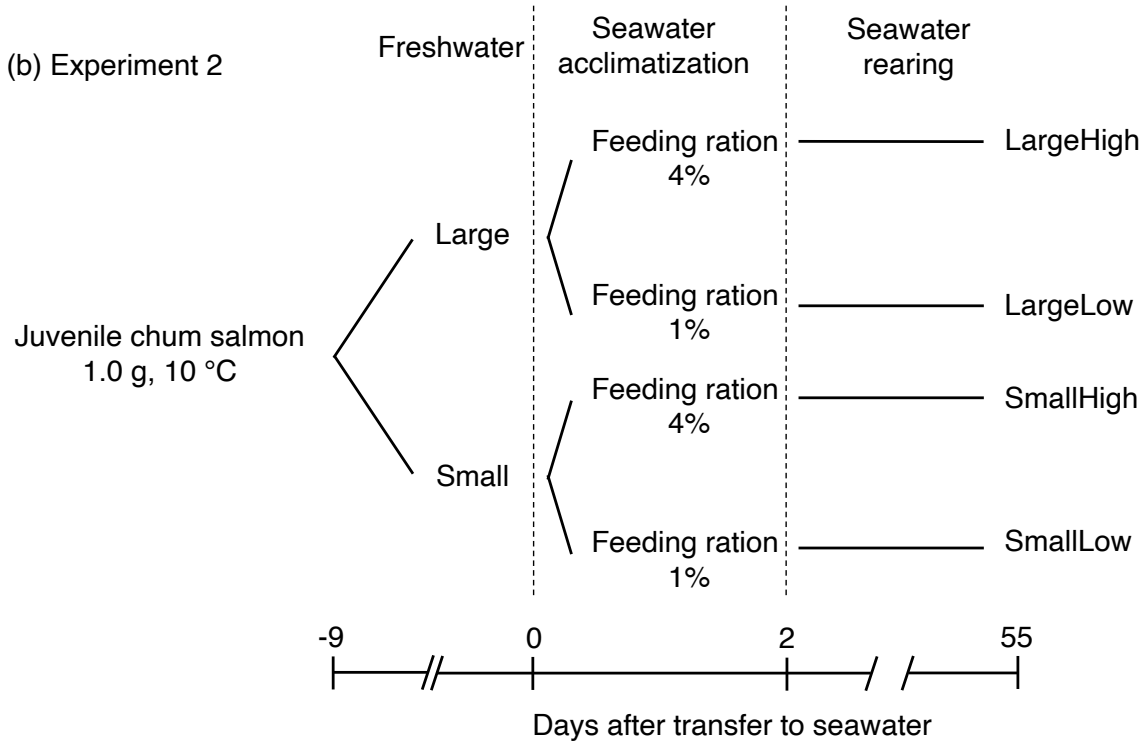
571

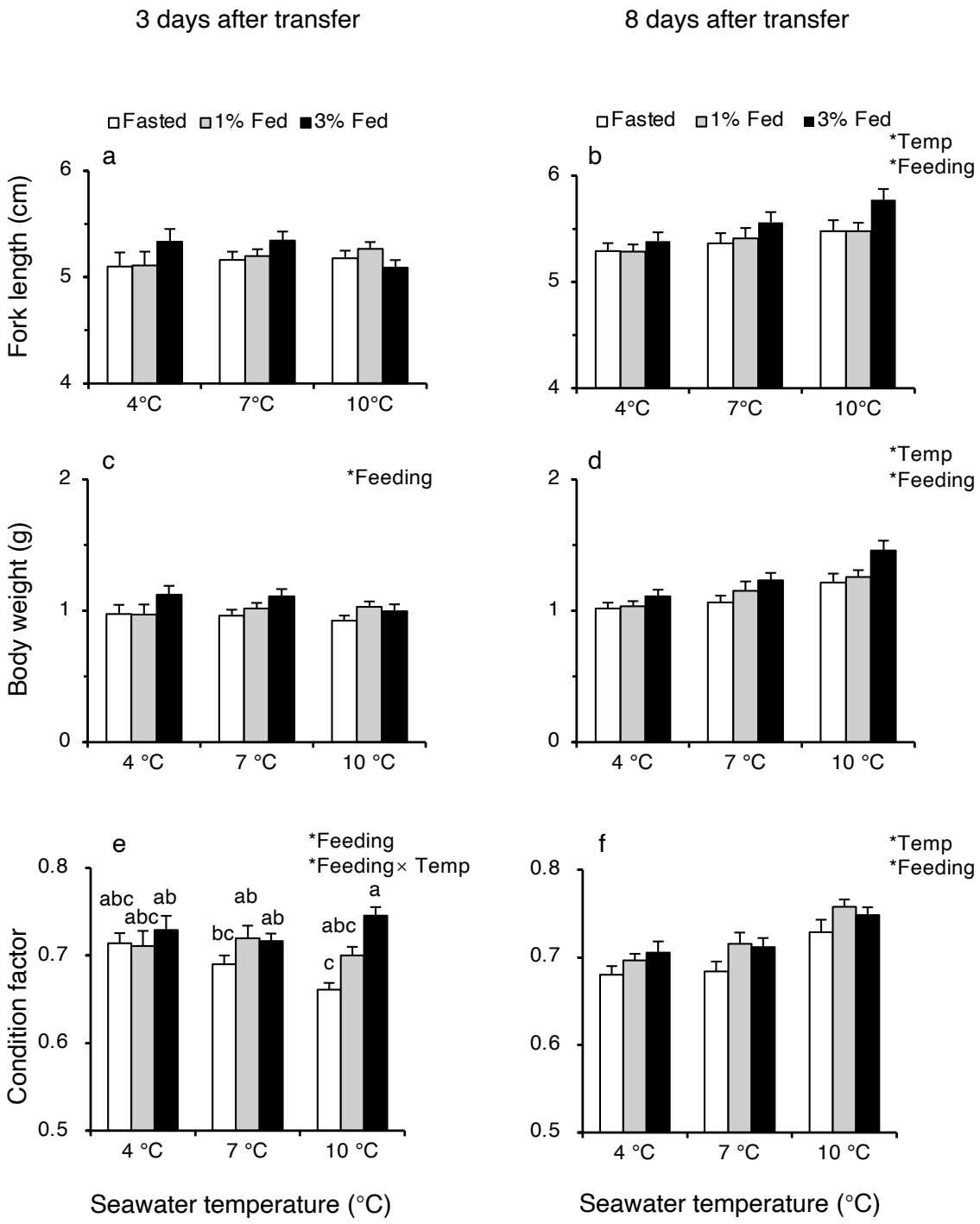
572 **Figure legends**

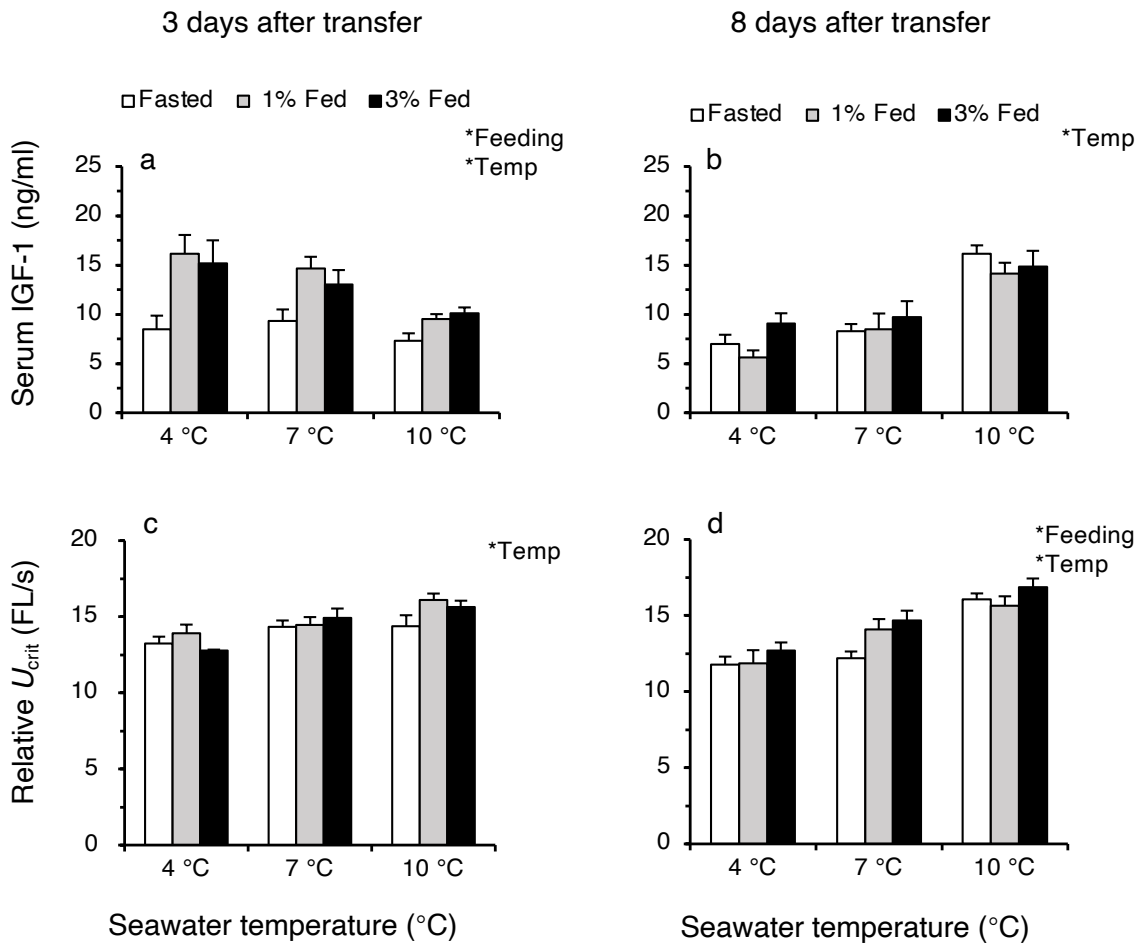
573 Fig. 1. Experimental designs to examine effects of feeding in freshwater and acclimated seawater
574 temperature (Experiment 1, a) and effects of size/growth in seawater (Experiment 2, b).
575
576 Fig. 2. Fork length (a,b), body weight (c,d) and condition factor (e,f) of juvenile chum salmon 3
577 days after transfer (left) and 8 days after transfer (right) in Experiment 1. Values are expressed as
578 means ± SE (n = 8). At a given temperature, groups sharing the same letters are not significantly
579 different from each other (Tukey's honest significant difference test, $P < 0.05$). Asterisks indicate
580 the factors have main effect or interaction in two-way ANOVA ($P < 0.05$).
581
582 Fig. 3. Serum IGF-1 levels (a,b) and relative U_{crit} (c,d) of juvenile chum salmon 3 days after
583 transfer (left); 8 days after transfer (right) in Experiment 1. Values are expressed as means ± SE
584 (n = 8). At a given temperature, groups sharing the same letters are not significantly different from
585 each other (Tukey's honest significant difference test, $P < 0.05$). Asterisks indicate the factors
586 have main effect in two-way ANOVA ($P < 0.05$).
587
588 Fig. 4. Correlations of swimming performance (relative U_{crit}) with fork length (a), body weight
589 (b), condition factor (c) and serum IGF-1 level (d) 8 days after seawater transfer in Experiment 1.
590 Feeding histories in freshwater are shown in different colors (fasted: white, 1% ration: grey, 3%
591 ration: black) and seawater rearing temperatures are shown in different shapes (4 °C: triangle,
592 7 °C: square, 10 °C: circle).
593
594 Fig. 5. Fork length (a), body weight (b), condition factor (c) and serum IGF-1 level (d) in juvenile
595 chum salmon in Experiment 2. Values are expressed as means ± SE (n = 12). Asterisks indicate
596 the factors have main effect in two-way ANOVA ($P < 0.05$).
597
598 Fig. 6. Relative U_{crit} (a) and absolute U_{crit} (b) in juvenile chum salmon in Experiment 2. Values
599 are expressed as means ± SE (n = 12). Groups without letters or shared same letters are not
600 significantly different from each other (Tukey's honest significant difference test, $P < 0.05$).
601 Asterisks indicate the factors have main effect in two-way ANOVA ($P < 0.05$).
602
603 Fig. 7. Correlations of swimming performance (relative U_{crit}) with, fork length (a), body weight
604 (b), condition factor (c) and serum IGF-1 level (d) after 55 days seawater rearing in Experiment
605 2. Intial sizes are shown in shapes (large: circule, small: triangle) and feeding rations are shown
606 in different colors (4%: black, 1%: grey).
607

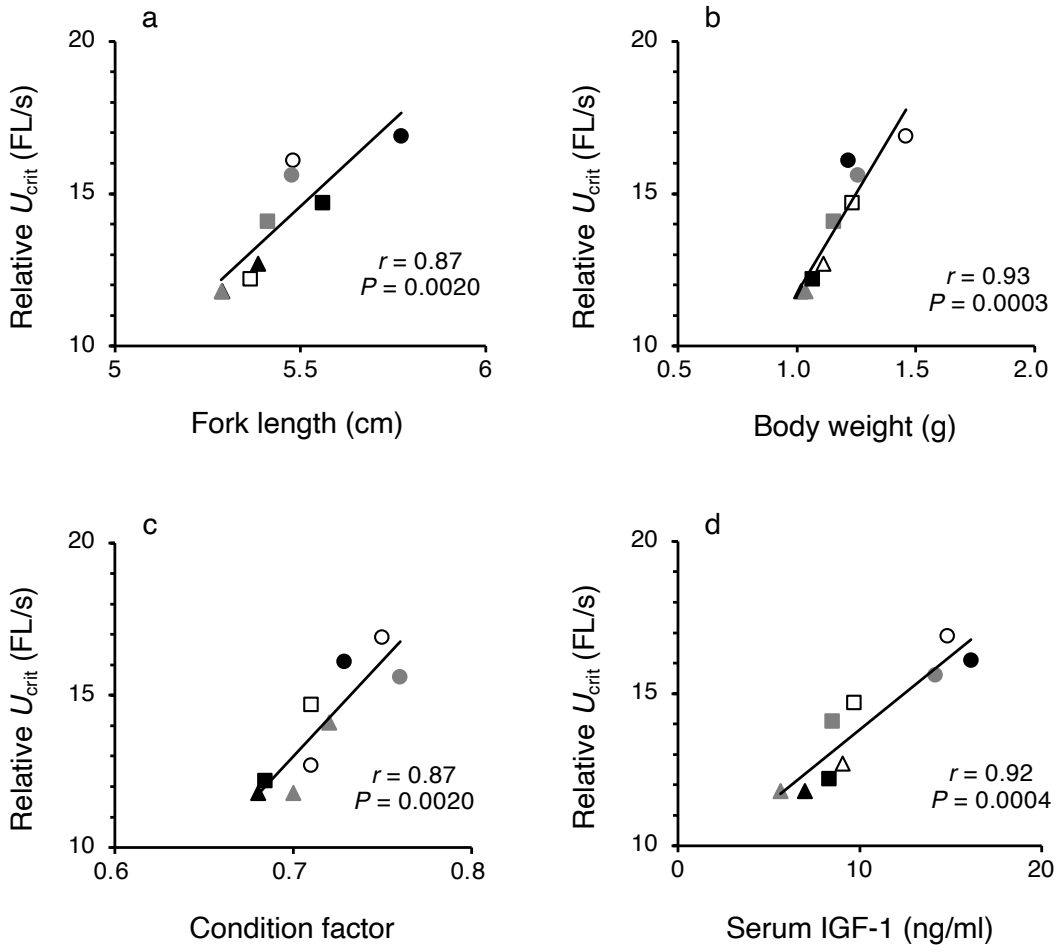
608 Fig. 8. Correlations of swimming performance (absolute U_{crit}) with, fork length (a), body weight
609 (b), condition factor (c) and serum IGF-1 level (d) after 55 days seawater rearing in Experiment
610 2. Intial sizes are shown in shapes (large: circule, small: triangle) and feeding rations are shown
611 in different colors (4%: black, 1%: grey).
612
613 Fig. 9. Band intensities of serum IGFBP-2b (a) , 32-kDa IGFBP (b), IGFBP-1a (c) and IGFBP-
614 1b (d) in juvenile chum salmon in Experiment 2. Values are expressed as means ± SE (n = 12).
615 Asterisks indicate the factors have main effect in two-way ANOVA ($P < 0.05$).
616
617 Fig. 10. Correlations of swimming performance (absolute U_{crit}) serum IGFBP-1a (a) and serum
618 IGFBP-1b (b) after 55 days seawater rearing in Experiment 2. Intial sizes are shown in shapes
619 (large: circule, small: triangle) and feeding rations are shown in different colors (4%: black, 1%:
620 grey).

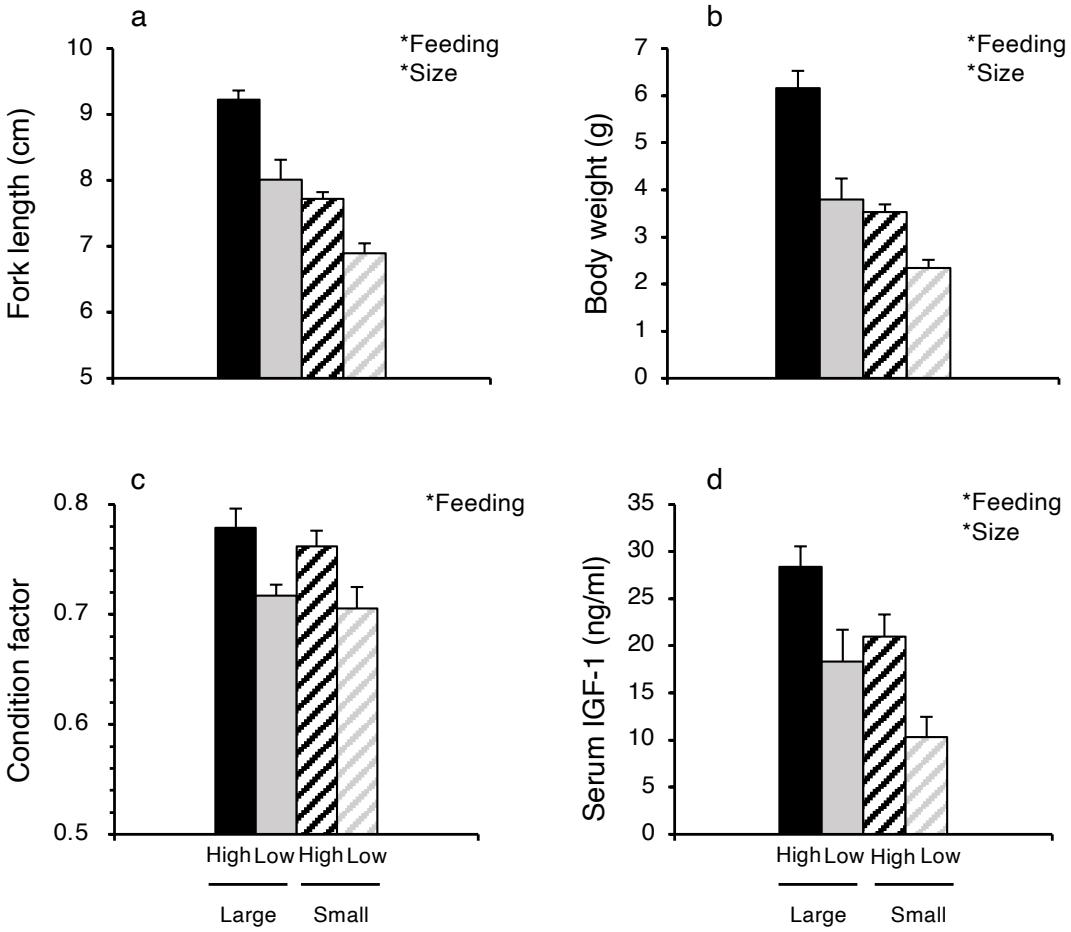

(a) Experiment 1
Fed to satiation
Freshwater
Seawater acclimatization
Seawater rearing
Juvenile chum salmon
1.0 g, 9.5 °C
Fasted
Feeding ration 1%
Feeding ration 3%
4 °C
7 °C
10 °C
Fasted 4 °C
Fasted 7 °C
Fasted 10 °C
1% Fed 4 °C
1% Fed 7 °C
1% Fed 10 °C
3% Fed 4 °C
3% Fed 7 °C
3% Fed 10 °C
-5
0
3
8
Days after transfer to seawater

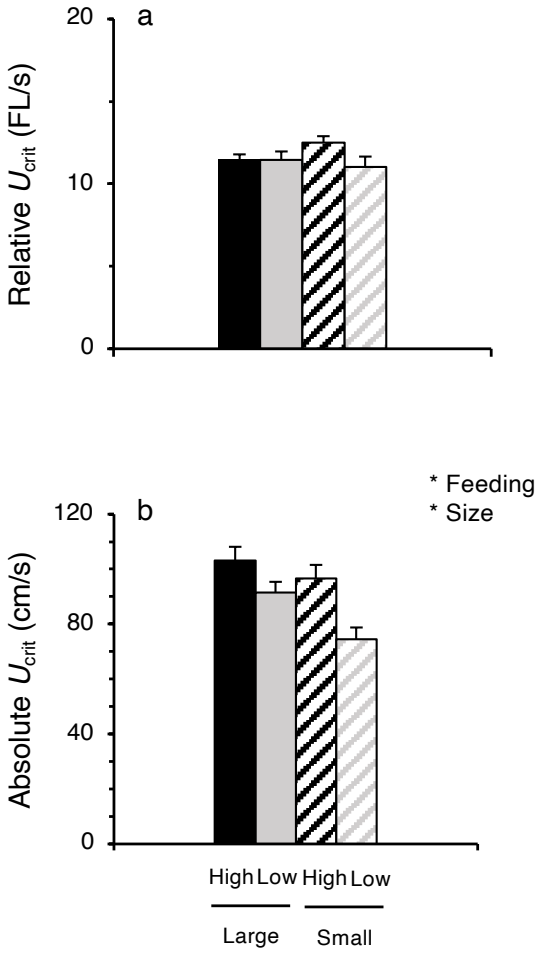

(b) Experiment 2
Freshwater
Seawater acclimatization
Seawater rearing
Juvenile chum salmon
1.0 g, 10 °C
Large
Small
Feeding ration 4%
Feeding ration 1%
Feeding ration 4%
Feeding ration 1%
LargeHigh
LargeLow
SmallHigh
SmallLow
-9
0
2
55
Days after transfer to seawater

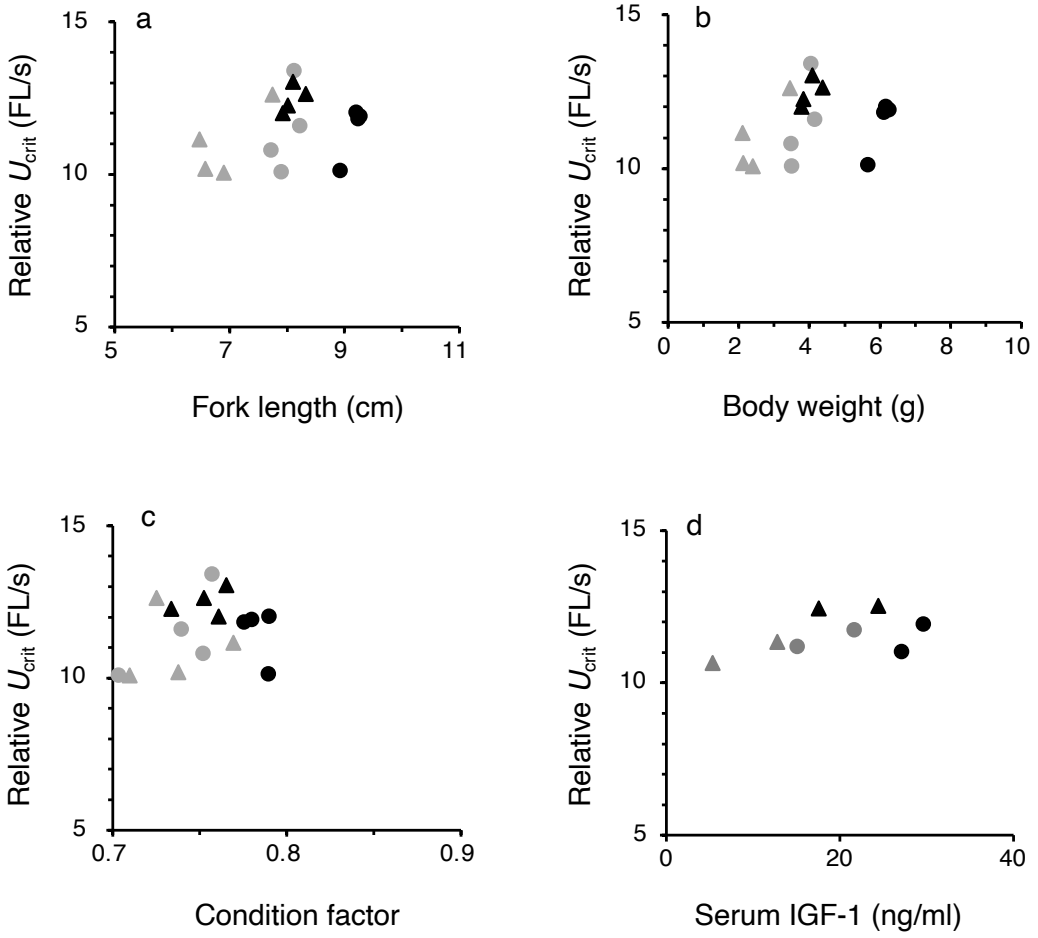

3 days after transfer
8 days after transfer
Fasted
1% Fed
3% Fed
a
b
c
d
e
f
Fork length (cm)
Body weight (g)
Condition factor
*Temp
*Feeding
*Feeding× Temp
4°C
7°C
10°C
4 °C
7 °C
10 °C
abc
abc
ab
bc
ab
ab
c
abc
a
Seawater temperature (°C)
Seawater temperature (°C)

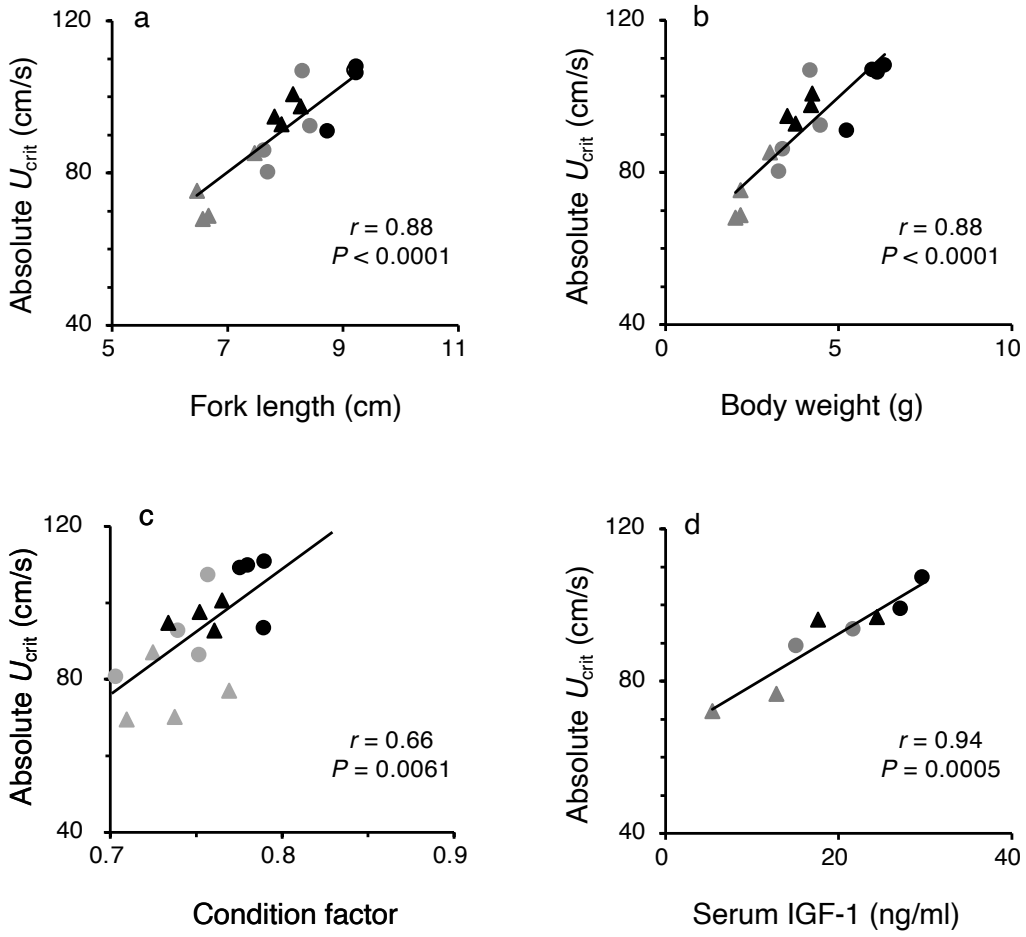

3 days after transfer
8 days after transfer
Fasted
1% Fed
3% Fed
*Feeding
*Temp
a
b
c
d
Serum IGF-1 (ng/ml)
Relative U_{crit} (FL/s)
4 °C
7 °C
10 °C
Seawater temperature (°C)

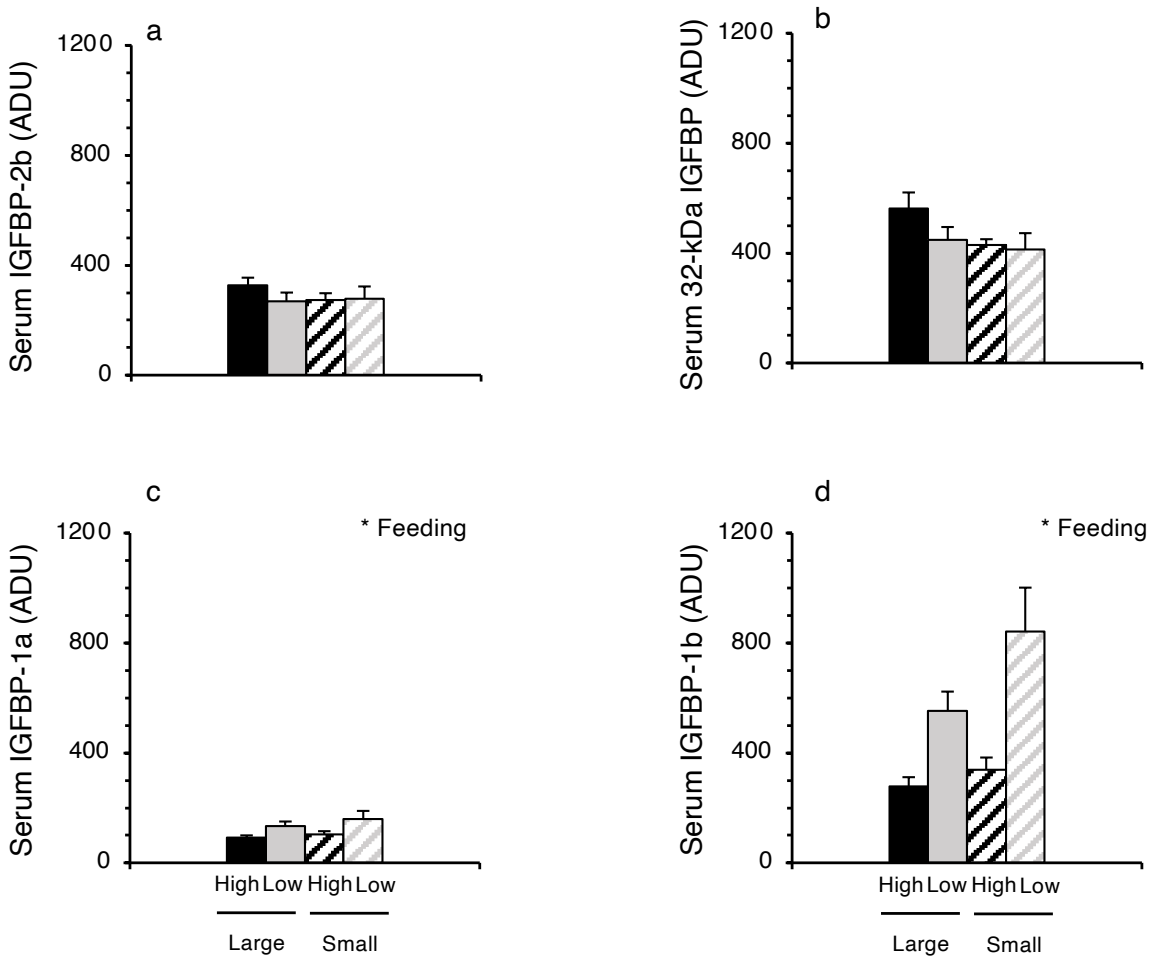

a
Relative U_{crit} (FL/s)
20
15
10
5
5.5
6
Fork length (cm)
r = 0.87
P = 0.0020
b
Relative U_{crit} (FL/s)
20
15
10
0.5
1.0
1.5
2.0
Body weight (g)
r = 0.93
P = 0.0003
c
Relative U_{crit} (FL/s)
20
15
10
0.6
0.7
0.8
Condition factor
r = 0.87
P = 0.0020
d
Relative U_{crit} (FL/s)
20
15
10
0
10
20
Serum IGF-1 (ng/ml)
r = 0.92
P = 0.0004

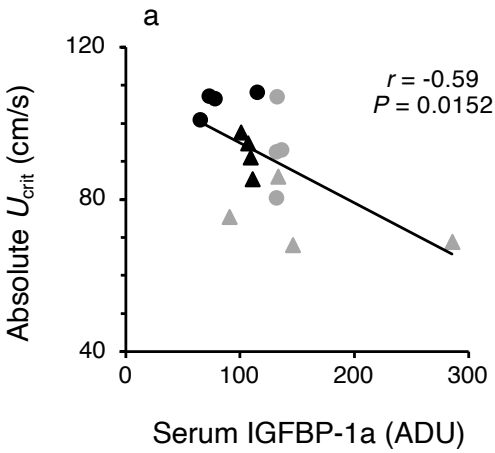

a
*Feeding
*Size
Fork length (cm)
10
9
8
7
6
5
b
*Feeding
*Size
Body weight (g)
7
6
5
4
3
2
1
0
c
*Feeding
Condition factor
0.8
0.7
0.6
0.5
High Low High Low
Large Small
d
*Feeding
*Size
Serum IGF-1 (ng/ml)
35
30
25
20
15
10
5
0
High Low High Low
Large Small

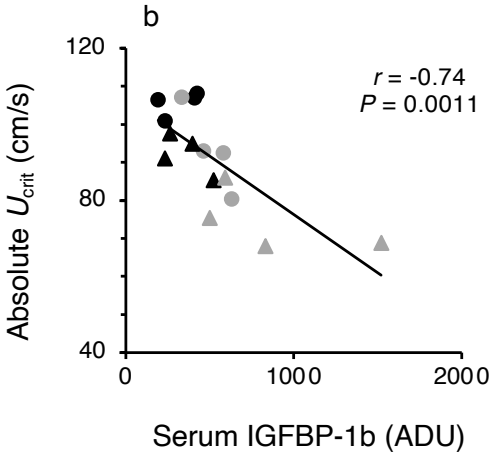

a
20
10
0
Relative U_{crit} (FL/s)
b
120
80
40
0
Absolute U_{crit} (cm/s)
* Feeding
* Size
High Low High Low
Large
Small


a
b
c
d
Relative U_{crit} (FL/s)
15
10
5
5
7
9
11
Fork length (cm)
0
2
4
6
8
10
Body weight (g)
0.7
0.8
0.9
Condition factor
0
20
40
Serum IGF-1 (ng/ml)


a
Absolute U_{crit} (cm/s)
120
80
40
5
7
9
11
Fork length (cm)
r = 0.88
P < 0.0001
b
Absolute U_{crit} (cm/s)
120
80
40
0
5
10
Body weight (g)
r = 0.88
P < 0.0001
c
Absolute U_{crit} (cm/s)
120
80
40
0.7
0.8
0.9
Condition factor
r = 0.66
P = 0.0061
d
Absolute U_{crit} (cm/s)
120
80
40
0
20
40
Serum IGF-1 (ng/ml)
r = 0.94
P = 0.0005


a
Serum IGFBP-2b (ADU)
1200
800
400
0
b
Serum 32-kDa IGFBP (ADU)
1200
800
400
0
c
* Feeding
Serum IGFBP-1a (ADU)
1200
800
400
0
High Low High Low
Large Small
d
* Feeding
Serum IGFBP-1b (ADU)
1200
800
400
0
High Low High Low
Large Small


a
120
80
40
0
100
200
300
r = -0.59
P = 0.0152
Absolute U_{crit} (cm/s)
Serum IGFBP-1a (ADU)


b
120
80
40
0
1000
2000
r = -0.74
P = 0.0011
Absolute U_{crit} (cm/s)
Serum IGFBP-1b (ADU)