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1 **Title**

2 Response of the salmon somatotropic axis to growth hormone administration under two  
3 different salinities

4

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20

21 **Key words**

22 insulin-like growth factor-I, growth hormone, seawater, salmon

23

**23 Abstract**

24 We compared the response of plasma insulin-like growth factor-I (IGF-I) to growth  
25 hormone (GH) administration under two different salinities to test the hypothesis that  
26 environmental salinity alters the “activity” of the GH-IGF-I axis. In July, postsmolt coho  
27 salmon reared in fresh water (FW) were transferred to either FW or half seawater (1/2  
28 SW) (15 ppt) tank. During the experiment, water temperature was maintained at 10°C  
29 for both salinities; photoperiod was adjusted to that of Seattle (48°N), and fish were not  
30 fed. Two days after transfer, fish were injected once with porcine GH (pGH) at a dose of  
31 2 or 8 µg/g body weight. Liver and blood samples were collected 1, 2 and 3 days after  
32 injection. Liver GH receptor (GHR) mRNA expression was analyzed by quantitative  
33 real-time RT-PCR, and plasma IGF-I, 41-kDa IGF-binding protein (main carrier of IGF-  
34 I) and pGH were quantified by radioimmunoassays. Transfer to 1/2 SW resulted in  
35 transient increases in basal levels of liver GHR mRNA and 41 kDa IGF-binding protein  
36 (IGFBP) but not IGF-I. The GH-injection increased liver GHR mRNA, plasma IGF-I  
37 and 41-kDa IGFBP in fish in both FW and 1/2 SW. However, the time course and  
38 magnitude of the response differed between salinities. Fish in FW receiving 8 µg/g pGH  
39 had the highest IGF-I levels ( $63.7 \pm 6.8$  ng/ml) one day after injection, whereas fish in  
40 1/2 SW showed a peak ( $88.8 \pm 14.3$  ng/ml) two days after injection of the same dose. It  
41 is speculated that the prolonged response to GH by fish in 1/2 SW may be due to slower  
42 disappearance of pGH from the circulation in fish in 1/2 SW. The transient increase in  
43 basal liver GHR mRNA may also contribute to a greater response for fish in 1/2 SW.  
44 These results suggest that salinity is capable of altering the “activity” of the GH-IGF-I  
45 axis in salmon.

46

## 46 **Introduction**

47 Smoltification in salmonids is a pre-adaptation to ocean life accompanied by a series of  
48 morphological, biochemical and behavioral changes (Hoar, 1988). One of the major  
49 achievements of smoltification is successful adaptation to seawater (SW). Salmon  
50 adapted to SW generally reach larger sizes than those in fresh water (FW). On the other  
51 hand, if juvenile salmon are transferred prematurely to SW, their growth is severely  
52 retarded and they become “stunts” (Folmar et al., 1982). These circumstances led  
53 scientists to hypothesize that salinity affects growth in salmonids, and successful SW  
54 adaptation results in an improved growth. However, the effect of salinity on growth in  
55 salmonids is inconsistent, probably due to varying experimental conditions such as water  
56 temperature, feeding ration, developmental stage, season and experimental period (Smith  
57 and Thorpe, 1976; McCormick et al., 1989; Morgan and Iwama, 1991; Usher et al., 1991;  
58 Duston, 1994; Handeland et al., 1998). On the other hand, the interaction between  
59 growth and salinity has been demonstrated in several fish species (for review, Bœuf and  
60 Payan, 2001). In tilapia (Oreochromis mossambicus), evidence indicates that SW rearing  
61 improves growth of this species (Kuwaye et al., 1993; Riley et al., 2002). In other marine  
62 fishes, optimal growth is sometimes seen at an intermediate salinity (Bœuf and Payan,  
63 2001). Improved growth at intermediate salinity may be explained by a reduction of the  
64 metabolic cost for osmoregulation, whereas appetite and/or the endocrine system may  
65 also play a role (Bœuf and Payan, 2001).

66 Growth hormone (GH) and insulin-like growth factor-I (IGF-I) are key hormones  
67 regulating growth of animals. In the classical somatomedin hypothesis, GH from the  
68 pituitary gland stimulates hepatic production of IGF-I via the GH receptor, and IGF-I

69 from the liver mediates many of the actions of GH (Daughaday and Rotwein, 1989).  
70 Recent findings have pointed out that GH has direct actions independent of IGF-I, and  
71 virtually all tissues express IGF-I that acts through autocrine and paracrine manners  
72 (Butler and LeRoith, 2001). Regardless of the site of production, the activity of IGF-I is  
73 modulated by a family of six IGF binding proteins (IGFBPs). In the circulation, IGFBPs  
74 prolong the half-life of IGF-I and deliver IGF-I to certain tissues (Rajaram et al., 1997).  
75 Increasing evidence indicates that the fish GH-IGF-I axis also plays an important role in  
76 osmoregulation as well as growth regulation (Sakamoto et al., 1993; Dickhoff et al.,  
77 1997; McCormick, 2001). However, how the GH-IGF-I axis operates these two  
78 processes simultaneously is poorly understood and the endocrine mechanism, if any, by  
79 which salinity influences growth is not known. In a study by Riley et al. (2002) on tilapia,  
80 the GH-IGF-I axis was “activated” by treatment with 17 $\alpha$ -methyltestosterone (MT) and  
81 SW rearing. The better growth of tilapia treated with MT and SW rearing was reflected  
82 by higher IGF-I levels (Riley et al., 2002). Circulating levels of IGF-I have been  
83 considered to be an index of growth in fish (Beckman et al., 1998; Uchida et al., 2003;  
84 Dyer et al., 2004). In coho salmon (Oncorhynchus kisutch), a series of experiment has  
85 shown that plasma IGF-I levels are positively correlated with growth rates of individuals  
86 in fresh water and seawater (Beckman et al., 2004a,b), implying that plasma IGF-I levels  
87 could be used to evaluate growth potential of salmon. In an attempt to understand how  
88 salinity might influence the "activity" of the GH-IGF-I axis and in turn growth in salmon,  
89 we examined response of the GH-IGF-I axis to GH administration under two different  
90 salinities.  
91

## 92 **Materials and Methods**

### 93 Fish rearing conditions

94 One-year-old postsmolt coho salmon were reared in FW at the Northwest Fisheries  
95 Science Center in Seattle, WA, USA. They were maintained in recirculated FW  
96 (dechlorinated city water that is buffered with sodium bicarbonate) in circular fiberglass  
97 tanks under natural photoperiod adjusted to that of Seattle, WA (48°N); flow rate was 25  
98 L/min; temperature ranged from 10.5°C to 13.0°C. Before fish were used for  
99 experiments, they were fed a ration of 1.25% body weight/day of a commercial diet  
100 (Biodiet Grower; Bioproducts Inc., Warrenton, OR, USA).

101

### 102 Treatment of fish

103 In early July, 2002, after 24 hr of fasting, 156 fish were transferred directly to one of  
104 eight tanks containing fresh water or half seawater (15 ppt; 1/2 SW) made from artificial  
105 sea salt (Aquarium Systems Inc., Mentor, OH, USA). The average body length and  
106 weight of fish were  $15.6 \pm 0.2$  cm (mean  $\pm$  SE) and  $41.8 \pm 2.2$  g, respectively.  
107 Throughout the experiment, salinity was monitored daily; water temperature was kept at  
108 10°C for both salinities, and fish were not fed. Blood and liver samples from untreated  
109 fish were collected 1, 2 and 5 days after transfer (day 1, 2 and 5, respectively) as  
110 described below. Other fish were injected intraperitoneally with porcine GH (Sigma, St.  
111 Louis, MO, USA) in saline at a dose of 2 or 8  $\mu$ g/g body weight two days after transfer  
112 (day 4). Sham injected fish received saline only. Blood and liver samples were collected  
113 1, 2 and 3 days after the single injection (day 3, 4 and 5, respectively).

114

115 Sample collection

116 Fish were anesthetized in 0.05% tricane methanesulfonate (MS-222; Argent Chemical  
117 Laboratories, Redmond, WA, USA). Blood was withdrawn by cutting the caudal  
118 peduncle and letting blood flow into a heparinized glass tube. Plasma was collected after  
119 centrifugation at 700g for 15 min and stored at -80°C until use. Liver pieces were  
120 excised, immediately frozen in liquid nitrogen and stored at -80°C until use.

121

122 Sample analysis

123 Expression of growth hormone receptor mRNA in the liver was measured by real-time  
124 reverse transcript polymerase chain reaction (RT-PCR) as described in Fukada et al.  
125 (2004). Expression levels were normalized with an acidic ribosomal phosphoprotein P0  
126 (ARP). ARP is superior to 18S ribosomal RNA as a reference gene and has been adopted  
127 to RT-PCR for salmon IGF-I mRNA in the liver (Pierce et al., 2004). For measurement  
128 of IGF-I, plasma IGF-I was extracted with an acid-ethanol followed by cryoprecipitation  
129 as described in Breier et al. (1991) and quantified by RIA (Shimizu et al., 2000). Plasma  
130 41-kDa IGFBP levels were measured by RIA as described in Shimizu et al. (2003b).  
131 Porcine GH levels were measured by a commercial RIA kit (Linco Research Inc., St.  
132 Charles, MO). This RIA showed no cross reactivity with sham-operated salmon plasma  
133 (data not shown).

134

135 Statistical analysis

136 All measured values were not normally distributed and thus natural-log transformed  
137 before analyses to obtain normal distribution (D'Agostino and Pearson omnibus

138 normality test). Data sets for each dependent variable (liver GHR mRNA expression, and  
139 plasma IGF-I, 41-kDa IGFBP and porcine GH levels) were first analyzed by two- or  
140 three-way analysis of variance (ANOVA) by including GH treatment, salinity and time as  
141 factors. When significant effects were found, the data were further analyzed by one- or  
142 two-way ANOVA for each time point. Differences between groups were identified by  
143 Fisher's protected least-significant difference (PLSD) test. Differences between groups  
144 were considered to be significant at  $P < 0.05$ .

145

## 146 **Results**

147 Changes in the basal levels of liver GHR mRNA, plasma IGF-I and 41-kDa IGFBP in  
148 fish transferred to FW or 1/2 SW were compared (Fig. 1). There were overall effects of  
149 salinity and time on the liver GHR mRNA and plasma 41-kDa IGFBP levels, but no  
150 interaction was found (two-way ANOVA). Salinity and time had no significant effect on  
151 plasma IGF-I. Hepatic GHR mRNA and circulating 41-kDa IGFBP were significantly  
152 higher in the 1/2 SW group one day after transfer (day 1) (Fig. 1a,c) and the difference  
153 became insignificant thereafter. A similar trend of higher levels in 1/2 SW was seen in  
154 plasma IGF-I, although this was not statistically significant ( $P = 0.0551$ ).

155       There were overall main effects of GH treatment, salinity and time on the liver  
156 GHR mRNA expression (three-way ANOVA). An increase in liver GHR expression by  
157 the low dose of GH injection was evident in fish in both FW and 1/2 SW on day 3 (one  
158 day after injection) (Fig. 2a). On day 4 (two days after injection), the effect of GH was  
159 not seen in fish in FW but was seen in 1/2 SW (Fig. 2b). GHR mRNA levels became  
160 similar between the sham and treated groups on day 5 (three days after injection). When



161 fish received a high dose of GH, salinity enhanced the GH effect on the liver GHR  
162 expression one day after injection (Fig. 2b).

163 For plasma IGF-I, GH treatment, salinity and time had significant main effects,  
164 and an interactive effect was also seen (three-way ANOVA). Plasma IGF-I levels were  
165 increased by the low dose of GH treatment in both FW and 1/2 SW for two days (Fig. 3a).  
166 On day 3 (one day after injection), plasma IGF-I levels in the FW group with the low-  
167 dose GH injection were higher than those in 1/2 SW, and decreased gradually over time  
168 (Fig. 3a). When fish received the high dose of GH (8  $\mu\text{g/g}$ ), plasma IGF-I levels in the  
169 1/2 SW group showed a peak on day 4 (two days after injection) and were higher than  
170 those in the FW group (Fig. 3b).

171 The response of 41-kDa IGFBP to GH treatment was essentially the same as that  
172 of IGF-I (Fig. 4). A positive effect of pGH injection lasted for two days. Salinity had a  
173 positive effect on the increase in 41-kDa IGFBP with the high-dose GH on day 4 (two  
174 days after transfer) (Fig. 4b).

175 The disappearance of exogenously injected pGH from the circulation was  
176 monitored by homologous RIA (Fig. 5). The pGH levels decreased rapidly after injection  
177 in both FW and 1/2 SW. However, the levels were always higher in fish in 1/2 SW than  
178 those in FW, showing that injected pGH was retained longer in the circulation in 1/2 SW.

179

## 180 **Discussion**

181 This study examined the effect of salinity on the basal levels of the GH-IGF-I axis  
182 components and their response to GH administration in postsmolt coho salmon. A  
183 relatively mild salinity change (FW to 1/2 SW) was chosen for the experiment as

184 transferring fish directly to full seawater (30-33 ppt) likely causes a stress response. A  
185 relatively short period of SW exposure (up to five days) was applied to the present study.  
186 Folmar and Dickhoff (1981) found that when yearling coho salmon were transferred to  
187 seawater, plasma ions (sodium, chloride and potassium) reached a steady state within a  
188 few days. On the other hand, Pierce et al. (2005) reported that fasting of coho salmon  
189 induced a decline of plasma IGF-I as early as day 4. For these reasons, fish were  
190 acclimated for two days prior to the GH administration in order to avoid the influence of  
191 the rapid physiological change during the initial phase of seawater adaptation and the  
192 negative effect of food deprivation on the GH-IGF-I axis. Under these experimental  
193 conditions, we evaluated the “activity” of the somatotropic axis by plasma IGF-I levels  
194 since several studies have revealed that circulating IGF-I levels are positively correlated  
195 with growth rates of fishes including salmon (Beckman et al., 1998, 2004a,b; Uchida et  
196 al., 2003; Dyer et al., 2004).

197         During seawater adaptation of salmonids, increases in secretion and metabolic  
198 clearance rate of GH, and occupancy and total number of liver GHR have been observed  
199 (Sakamoto et al.,1991; Sakamoto and Hirano, 1991, 1993). Plasma GH levels are also  
200 known to change during seawater adaptation of salmon (Björnsson et al., 1998). These  
201 changes in GH and its receptor support the concept that GH is a key hormone for  
202 seawater adaptation in salmonids. The action of GH in osmoregulation may be, at least  
203 partly, mediated by IGF-I. McCormick et al. (1991) demonstrated that IGF-I promotes  
204 osmoregulatory ability of salmon. Participation of the GH-IGF-I axis in osmoragulation  
205 has been also suggested in non-salmonid fishes (Mancera and McCormick, 1998).  
206 Despite the importance of the GH-IGF-I axis in osmoregulation, little is known about the

207 response of GHR mRNA, plasma IGF-I and its carrier protein (41-kDa IGFBP) levels  
208 during seawater adaptation in salmon. In the present study, liver GHR mRNA expression  
209 was transiently increased one day after 1/2 SW transfer. GHR expression tended to be  
210 higher in fish in 1/2 SW thereafter although it was not significantly different. This agrees  
211 with the finding by Sakamoto and Hirano (1991) that total GH binding sites in the liver of  
212 rainbow trout (Oncorhynchus mykiss) transferred to 80% SW tended to be high and  
213 became significantly higher two weeks after transfer. This suggests that GH binding  
214 capacity may be regulated at the transcriptional level. Average plasma IGF-I levels were  
215 similar between FW and 1/2 SW despite a tendency of higher IGF-I levels in 1/2 SW.  
216 There appears to be no negative impact of fasting for up to five days on the basal IGF-I  
217 levels because IGF-I levels were similar to those in fed fish (data not shown). Mixed  
218 results in the response of growth regulating hormones to salinity are seen in non-  
219 salmonid species. Transfer of tilapia from SW to FW for five months resulted in a  
220 decline in growth rate, increases in plasma GH and IGF-I, and decreases in pituitary GH  
221 and liver IGF-I mRNA levels (Riley et al., 2003). In black sea bream (Mylio  
222 macrocephalus) hepatic IGF-I increased in fish adapted to 1/3 SW and full SW after eight  
223 months (Deane et al., 2002). In the four spine-sculpin (Cottus kazika), adaptation to full  
224 SW for 44 days resulted in an elevation of hepatic IGF-I mRNA, but not pituitary GH  
225 mRNA (Inoue et al., 2003).

226         There is only one study examining the effect of salinity on circulating IGF-BPs  
227 (Shepherd et al., 2005). In their experiment, juvenile rainbow trout were gradually  
228 acclimated to 66% SW over five days. As a result, the intensity of four IGF-BP bands at  
229 21, 32, 42 and 50 kDa on ligand blotting was higher in fish at higher salinity. In the

230 present study, plasma 41-kDa IGFBP levels increased one day after 1/2 SW transfer  
231 while its increase lasted for just one day. This conflicts with the finding by Shepherd et  
232 al. (2005). As discussed above, differences in species and experimental conditions may  
233 contribute to the discrepancy. Overall, direct transfer of postsmolt coho salmon to 1/2  
234 SW had a transient, positive effect on liver GHR mRNA expression and 41 kDa IGFBP  
235 levels.

236         Many of the components in the GH-IGF-I axis are regulated by their own control  
237 system. For example, GH induces production of IGF-I and IGFBP-3 in the liver, and  
238 IGF-I is capable of inhibiting GH synthesis and secretion by the pituitary (Le Roith et al.,  
239 2001). This regulatory mechanism appears to be operative in teleosts (Duan, 1997;  
240 Björnsson et al., 2002). The present study examined the effect of salinity on the potency  
241 of GH to stimulate the GH-IGF-I components (i.e. GHR mRNA, plasma IGF-I and 41-  
242 kDa IGFBP) in salmon. Biological actions of GH are mediated by a transmembrane GH  
243 receptor. GHR is composed of two subunits and forms a dimer with another GHR upon  
244 binding GH (Argetsinger and Cater-Su, 1996). It is not clear, however, if GHR  
245 expression is under control by GH. In mouse hepatocyte culture, GH alone had no effect  
246 on GHR mRNA cellular concentrations, whereas a synergistic effect with estrogen was  
247 seen (Contreras and Talamantes, 1999). In fish, no study has examined the effect of GH  
248 on GHR mRNA expression. The present study showed that liver GHR mRNA was  
249 increased one day after GH injection. When fish were held in FW, the GH effect  
250 diminished in two days, whereas the effect was still evident in fish in 1/2 SW. This  
251 difference is presumably attributed to relatively high levels of GHR mRNA in the sham-

252 operated group in FW on day 4 (two days after injection). However, it is clear that GH  
253 induces its own receptor in the liver in salmon.

254 Induction of circulating IGF-I by GH administration is a well-known response in  
255 salmonids (Moriyama et al., 1994; Moriyama, 1995). A new finding of this study is that  
256 the time course and magnitude of the IGF-I response differed between two salinities.  
257 Fish in FW showed a maximum response on day 3 (one day after injection) and IGF-I  
258 levels gradually decreased thereafter. In contrast, plasma IGF-I levels in fish in 1/2 SW  
259 peaked on day 4 (two days after injection). Moreover, when fish were injected at the  
260 high dose (8  $\mu\text{g/g}$ ), IGF-I levels were higher in fish in 1/2 SW than those in FW on the  
261 same date. It is possible that IGF-I levels in fish in FW reached a peak in less than 24 hr,  
262 although Moriyama (1995) reported that in trout in FW plasma IGF-I levels followed by  
263 GH injection continued to increase until 24 hr.

264 The 41-kDa IGFBP is one of three major circulating IGFBPs in salmon (Shimizu  
265 et al., 2003a,b). Although its identity is still not clear due to the lack of complete amino  
266 acid sequence data, several lines of evidence from physiological and biochemical studies  
267 suggest that it is physiologic equivalent of mammalian IGFBP-3 (Shimizu et al., 2003a,b;  
268 Beckman et al., 2004a,b). IGFBP-3 prolongs the half-life of IGF-I and therefore forms a  
269 large pool of IGF-I in the circulation (Rajaram et al., 1997). A similar IGFBP with a  
270 molecular mass of 40-50 kDa has been detected in other fish species and shown to be  
271 induced by GH, as is mammalian IGFBP-3 (Siharath et al., 1995; Park et al., 2000). The  
272 result from the present study is in good agreement with the previous findings in fish. It is  
273 worth noting that the response of the 41-kDa IGFBP to GH is almost identical to that of  
274 IGF-I. In addition, simple regression analysis confirmed that their levels are positively,

275 highly correlated (data not shown). These results further support our assumption that the  
276 41-kDa IGFBP is the main carrier of circulating IGF-I in salmon.

277         The present study showed that the response of circulating IGF-I to GH  
278 administration differed between two salinities. What caused the difference? An obvious  
279 possibility is a difference in the clearance of exogenous GH. Measurement of pGH by a  
280 specific RIA enabled us to monitor pGH levels after injection and revealed that pGH was  
281 retained longer in the circulation in fish in 1/2 SW. This may be due to a difference in  
282 the glomerular filtration rate at the kidney under different salinities. The kidney is one of  
283 the main sites of GH clearance in mammals, where 20-50% of circulating GH is cleared  
284 (Feld and Hirschberg, 1996). In salmonids, the glomerular filtration rate at the kidney is  
285 known to be reduced in a hyperosmotic environment (Brown et al., 1978). GH-binding  
286 protein (GHBP) might contribute to the slower disappearance of pGH from the  
287 circulation since GHBP has been shown to increase in SW in trout (Sohm et al., 1998).  
288 Another possible reason for the difference in the GH effect on IGF-I may be GHR. The  
289 transient increase in GHR mRNA levels in 1/2 SW might induce a greater response. The  
290 reason for the significantly higher IGF-I levels in fish that received 2  $\mu\text{g/g}$  GH in FW is  
291 not clear. It might be due to an inhibition of GH effect by GHBP increased in 1/2 SW. In  
292 mammals, GHBP is capable of inhibiting GH interaction with GHR (Barnard and Waters,  
293 1997). Levels of GHBP in fish in 1/2 SW might have been high enough to prevent the  
294 low dose of pGH, but not for the high dose of GH, from binding GHR. However, we  
295 have no data on GHBP levels.

296         The biological significance of the prolonged induction of IGF-I in SW observed  
297 in the present study is difficult to interpret; It is not known whether induced IGF-I would

298 be utilized for growth or osmoregulation. Collie et al. (1989) found that a two-day pre-  
299 adaptation of trout in 1/3 SW prior to transfer to 80% SW enhanced the plasma ion  
300 lowering effect of ovine GH. Although IGF-I levels were not measured in their study due  
301 to the lack of the immunoassay at that time, IGF-I was most likely induced in the  
302 circulation and might mediate the ion lowering action of GH. It is thus possible that in  
303 the present study the induced IGF-I might have an osmoregulatory action rather than a  
304 growth promoting action. To distinguish these two actions, a longer acclimation period  
305 in SW (with feeding) should be helpful in future work.

306 In summary, the present study suggests that salinity is capable of altering the  
307 “activity” of the GH-IGF-I axis. The prolonged response of plasma IGF-I in fish in 1/2  
308 SW may be due to a slower clearance of pGH. The transient increase in the basal liver  
309 GHR mRNA levels in fish in 1/2 SW may also contribute to the greater response.

310

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482

482 **Figure legends**

483 Fig. 1 Changes in liver GHR mRNA, plasma IGF-I and 41-kDa IGFBP levels after  
484 transfer to 1/2 SW.

485 Postsmolt coho salmon reared in FW were directly transferred to either FW or 1/2 SW .  
486 GHR mRNA was quantified by real-time RT-PCR using acidic ribosomal phosphoprotein  
487 P0 (ARP) as an internal control. Plasma levels of IGF-I and 41-kDa IGFBP were  
488 measured by radioimmunoassays. Values are mean  $\pm$  SEM (n = 6). For statistical  
489 analysis, values were natural-log transformed. Asterisks indicate significant difference  
490 between FW and 1/2 SW for a given time point (Fisher's PLSD,  $P < 0.05$ ).

491

492 Fig. 2 Effect of GH administration on liver GHR mRNA levels in postsmolts in FW and  
493 1/2 SW.

494 Fish were acclimated in FW or 1/2 SW for two days and injected once with 2  $\mu\text{g/g}$  body  
495 weight porcine GH (a) or 8  $\mu\text{g/g}$  (b). Sham fish received saline only. GHR mRNA was  
496 quantified by real-time RT-PCR using acidic ribosomal phosphoprotein P0 (ARP) as an  
497 internal control. Values are mean  $\pm$  SEM (n = 6). For statistical analysis, values were  
498 natural-log transformed. Symbols sharing the same letters are not significantly different  
499 from each other for a given time point (Fisher's PLSD,  $P < 0.05$ ).

500

501 Fig. 3 Effect of GH administration on plasma IGF-I levels in postsmolts in FW and 1/2  
502 SW.

503 Fish were acclimated in FW or 1/2 SW for two days and injected once with 2  $\mu\text{g/g}$  body  
504 weight porcine GH (a) or 8  $\mu\text{g/g}$  (b). Sham fish received saline only. Plasma levels of



505 IGF-I were measured by radioimmunoassay. Values are mean  $\pm$  SEM (n = 6). For  
506 statistical analysis, values were natural-log transformed. Symbols sharing the same  
507 letters are not significantly different from each other for a given time point (Fisher's  
508 PLSD, P < 0.05).

509

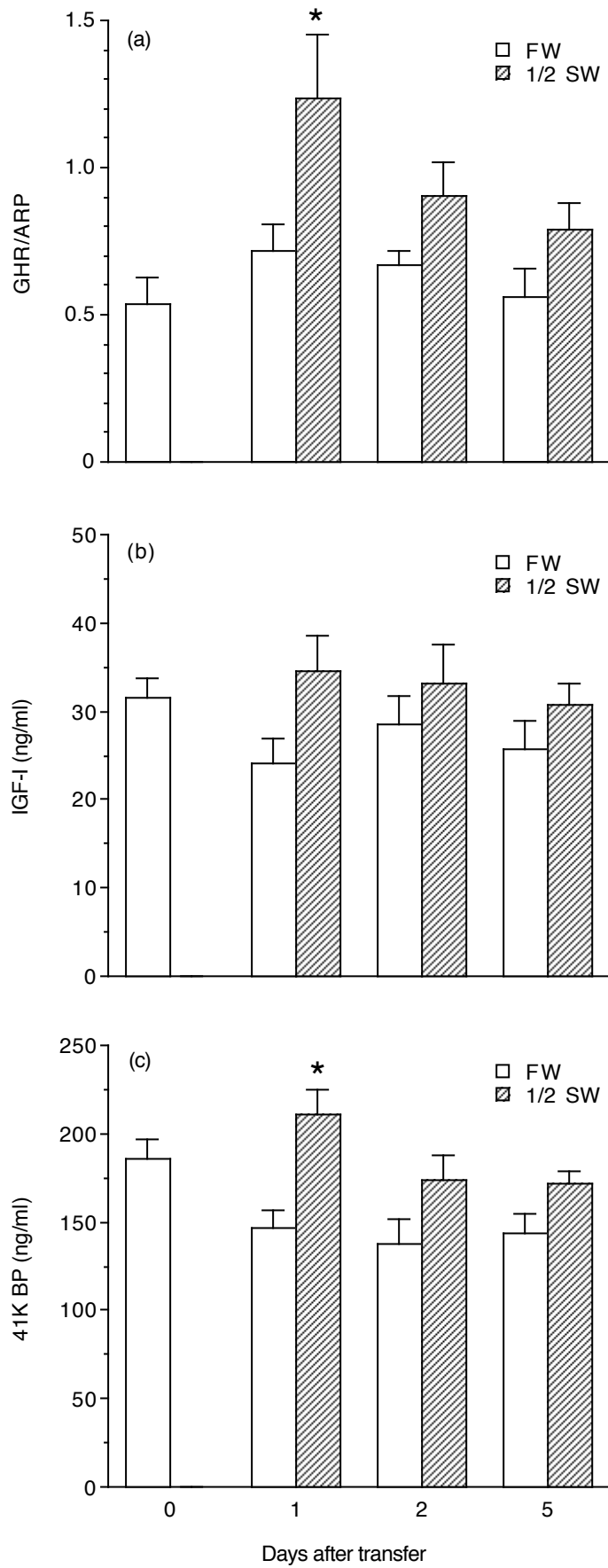
510 Fig. 4 Effect of GH administration on plasma 41-kDa IGFBP levels in postsmolts in FW  
511 and 1/2 SW.

512 Fish were acclimated in FW or 1/2 SW for two days and injected once with 2  $\mu$ g/g body  
513 weight porcine GH (a) or 8  $\mu$ g/g (b). Sham fish received saline only. Plasma levels of  
514 41-kDa IGFBP were measured by radioimmunoassay. Values are mean  $\pm$  SEM (n = 6).  
515 For statistical analysis, values were natural-log transformed. Symbols sharing the same  
516 letters are not significantly different from each other for a given time point (Fisher's  
517 PLSD, P < 0.05).

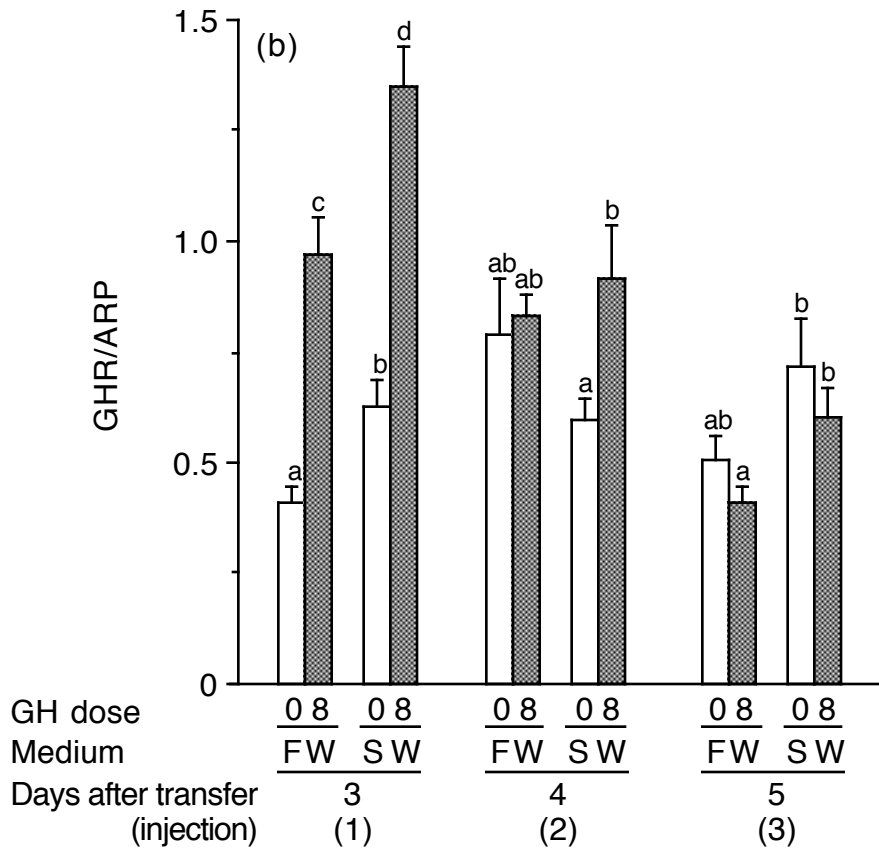
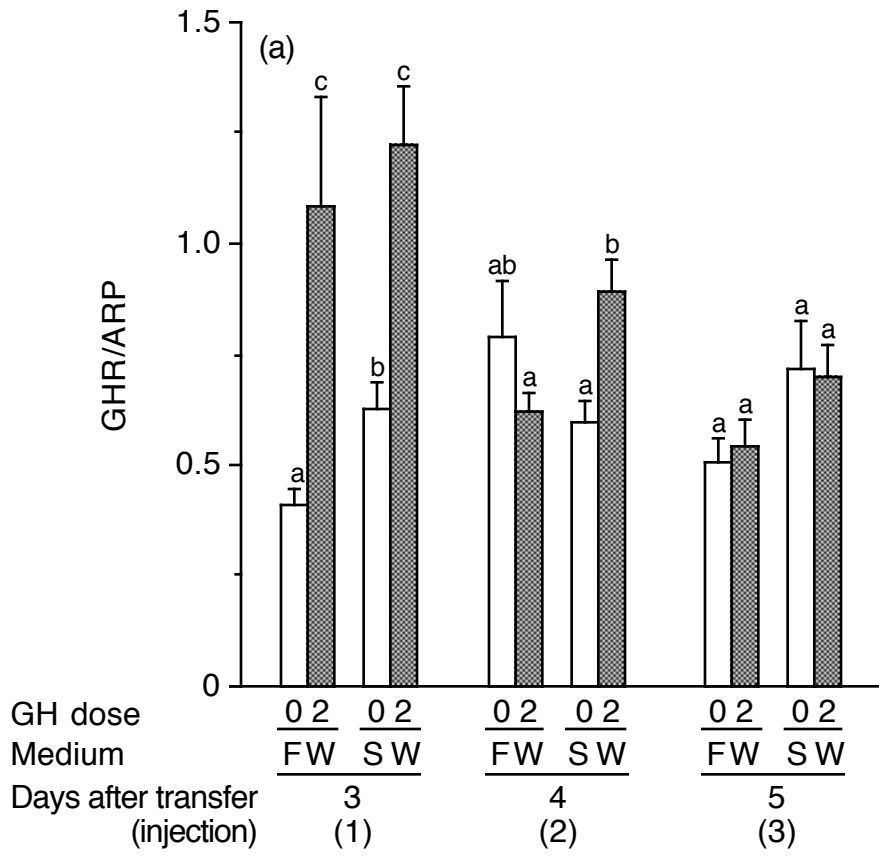
518

519 Fig. 5 Disappearance of injected porcine GH from the circulation of postsmolts in FW  
520 and 1/2 SW

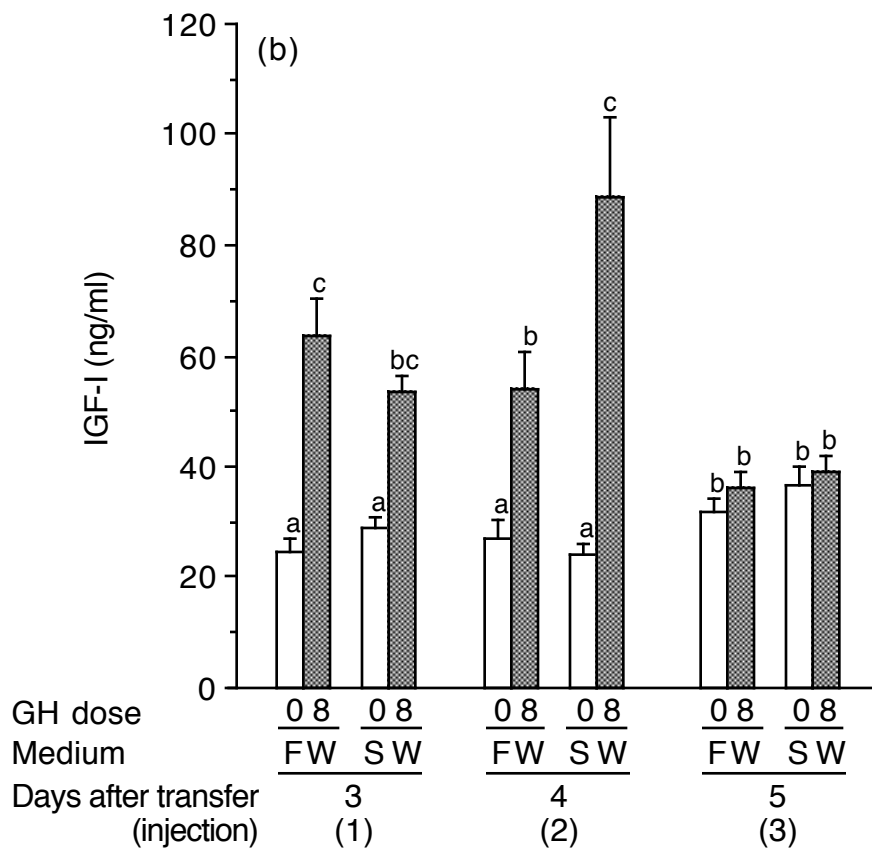
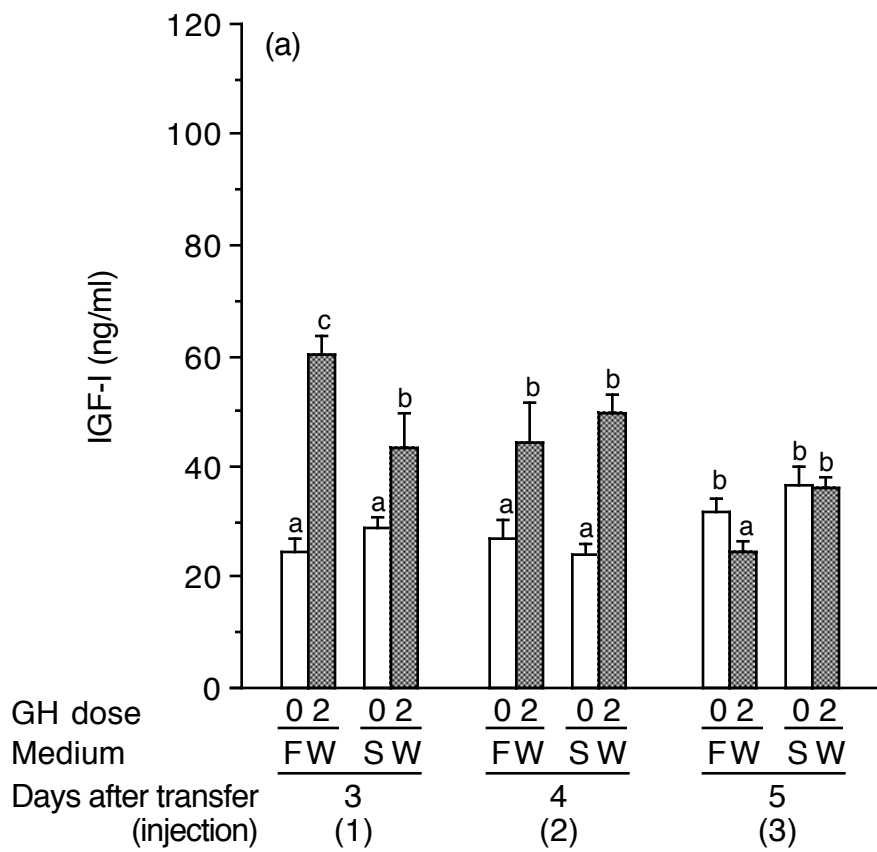
521 Fish were acclimated in FW or 1/2 SW for two days and injected once with 2  $\mu$ g/g body  
522 weight porcine GH (a) or 8  $\mu$ g/g (b). Plasma levels of porcine GH were measured by a  
523 specific radioimmunoassay. Values are mean  $\pm$  SEM (n = 6). For statistical analysis,  
524 values were natural-log transformed. Symbols sharing the same letters are not  
525 significantly different from each other for a given time point (Fisher's PLSD, P < 0.05).



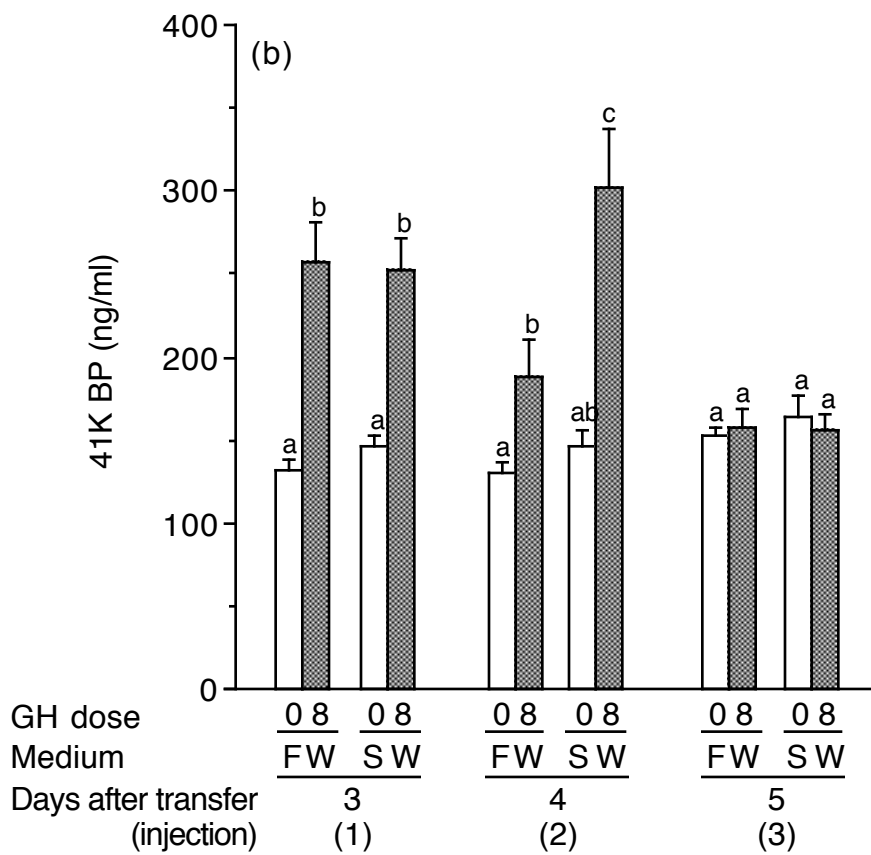
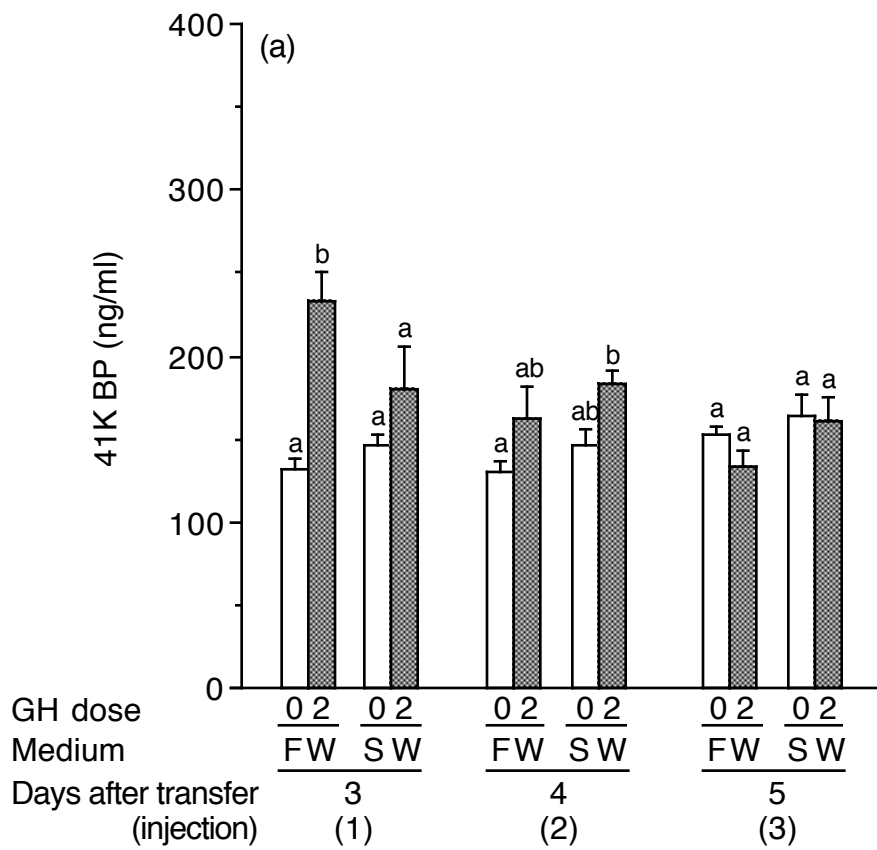
Shimizu et al., Fig. 1



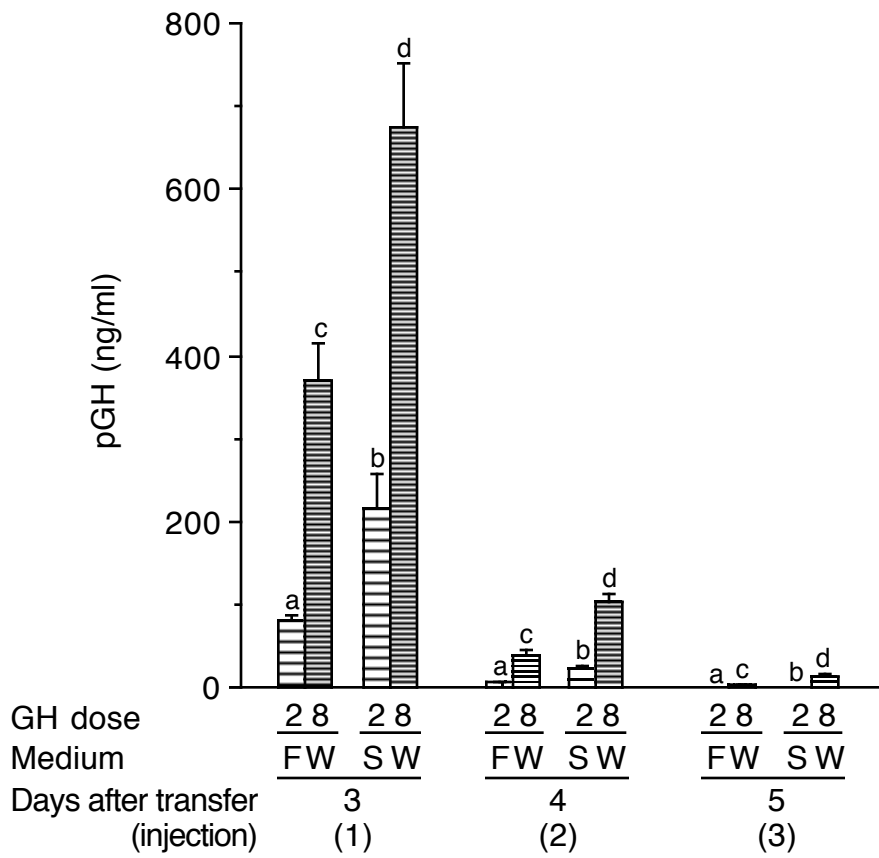
Shimizu et al., Fig. 2



Shimizu et al., Fig. 3



Shimizu et al., Fig. 4



Shimizu et al., Fig. 5