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Response of the insulin-like growth factor-1 (Igf1) system to nutritional status and growth rate variation in olive rockfish (*Sebastes serranoides*)

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1 **Abstract**

2 Growth performance in vertebrates is regulated by environmental factors
3 including the quality and quantity of food, which influence growth via endocrine
4 pathways such as the growth hormone (GH) / insulin-like growth factor somatotropic
5 axis. In several teleost fishes, circulating concentrations of insulin-like growth factor-1
6 (Igf1) correlate positively with growth rate, and it has been proposed that plasma Igf1
7 levels may serve as an indicator of growth variation for fisheries and aquaculture
8 applications. This study tested whether plasma Igf1 concentrations might serve as an
9 indicator of somatic growth in olive rockfish (*Sebastes serranoides*), one species among
10 dozens of rockfishes important to commercial and recreational fisheries in the Northern
11 Pacific Ocean. Juvenile olive rockfish were reared under food ration treatments of 1% or
12 4% wet mass per d for 98 d to experimentally generate variation in growth. Juvenile
13 rockfish in the 4% ration grew 60% more quickly in mass and 22% faster in length than
14 fish in the 1% ration. Plasma Igf1 levels were elevated in rockfish under the 4% ration,
15 and individual Igf1 levels correlated positively with growth rate, as well as with
16 individual variation in hepatic *igf1* mRNA levels. Transcripts encoding the Igf binding
17 proteins (Igfbps) *igfbp1a* and *igfbp1b* were also at higher abundance in the liver of
18 rockfish in the 1% ration treatment, while mRNAs for *igfbp5a* and *igfbp5b* were elevated
19 in the skeletal muscle of 4% ration fish. These findings support the use of plasma Igf1 as
20 a physiological index of growth rate variation in rockfish.

1. INTRODUCTION

Nutritional stress in the form of reduced food quantity or quality affects growth performance in fish, which in turn can impact population-level processes such as recruitment success or reproductive output (Caselle et al., 2010; VenTresca et al., 1996). For populations of marine fishes, the lack of noninvasive metrics for determining spatial and temporal variation in growth performance has limited the ability to predict variation in fish stock reproduction and recruitment. The effects of nutritional stress on somatic growth in fishes, however, are regulated in part via changes in a variety of endocrine pathways including those involved in the somatotropic, thyroid, and glucocorticoid endocrine axes (Picha et al., 2008a; Power et al., 2001; Sadoul and Vijayan, 2016; Won and Borski, 2013), and indices of these endocrine pathways may be useful as biomarkers for assessing growth variation in wild fish stocks as well as in fish culture.

The growth hormone (GH)/insulin-like growth factor (Igf) system, in particular, has been shown to regulate somatic growth responses to variation in nutritional status (Fuentes et al., 2013; Picha et al., 2008a; Reindl and Sheridan, 2012). In most vertebrates, insulin-like growth factor-1 (Igf1) is the predominant mediator of growth following activation of the GH/Igf axis (e.g. Duan, 1997; Pérez-Sánchez and Le Bail, 1999; Reinecke et al., 2005). GH secreted from the anterior pituitary binds hepatic GH receptors to stimulate the production of Igf1, which regulates somatic tissue growth by binding Igf1 receptors in target tissues while also exerting negative feedback on pituitary GH production (Duan et al., 2010; Fuentes et al., 2013; Le Roith et al., 2001; Wood et al., 2005).

43 Teleost fishes have also evolved at least six types of Igf binding proteins (Igfbps),
44 which themselves evolved into multiple isoforms following gene duplication events in
45 some teleost lineages (Daza et al., 2011; de la Serrana and Macqueen, 2018; Shimizu and
46 Dickhoff, 2017). Igfbps modulate the activity of Igf1 and Igf2 by regulating the amount
47 of Igf hormone available to activate receptors, effectively inhibiting or prompting Igf
48 action. There is also evidence, however, that some Igfbps have their own biological
49 activity and can activate Igf1 receptors or other cell-surface or intranuclear proteins (e.g.,
50 transforming growth factor-β5 receptor, peroxisome proliferator-activated receptor γ)
51 independent of Igf hormone binding (Baxter, 2015; Chan et al., 2009; Clemmons, 2007,
52 2016; Duan and Xu, 2005; Huang et al., 2003; Jogie-Brahim et al., 2009).
53 For fish experiencing food limitation or deprivation, circulating concentrations of
54 Igf1 decrease while some Igfbps increase, ultimately contributing to reduced muscle and
55 skeletal growth (e.g., Kelley et al., 2001; Picha et al., 2008a; Reinecke, 2010; Shimizu
56 and Dickhoff, 2017). Such declines in circulating Igf1 with food restriction have been
57 observed in a wide variety of fishes, including Mozambique tilapia (*Oreochromis*
58 *mossambicus*, Breves et al., 2014; Uchida et al., 2003), Arctic charr (*Salvelinus alpinus*,
59 Cameron et al., 2007), gilthead sea bream (*Sparus aurata*, Pérez-Sánchez et al., 1995),
60 and several species of salmonids (Beckman et al., 2004a,b; Bower et al., 2008; Breves et
61 al., 2016; Kaneko et al., 2015; Pierce et al., 2005; Wilkinson et al., 2006). Igfbp
62 expression in fishes has also been found to be responsive to variation in food
63 consumption. Although the number of Igfbp isoforms that have evolved appears to differ
64 across teleost fishes, variation in food availability has been shown to influence expression
65 of the type 1 form of Igfbp (Igfbp1) in several taxa. For instance, Igfbp1 protein levels in

66 blood circulation have been observed to decline in post-smolt coho salmon,
67 *Oncorhynchus kisutch*, in the hours after feeding (Shimizu et al., 2009), and fasted coho
68 salmon have higher plasma Igfbp1 levels than fish not experiencing food restriction
69 (Shimizu et al., 2006). Mechanistically, at least some of these nutrition-associated
70 changes in Igfbp expression appear to be caused by inhibition of Igfbp1 gene expression.
71 This is supported by the observation of elevated mRNAs encoding the type 1 *igfbp* gene
72 in the liver of Atlantic salmon, *Salmo salar*, smolts under food restriction (Hevrøy et al.,
73 2011). Breves et al. (2016) likewise observed increased hepatic *igfbp1a1* mRNA levels
74 in Atlantic salmon parr fasted for 3 to 10 d.

75 In this study, the relationships between growth rate and the Igf1 system were
76 examined in juvenile olive rockfish, *Sebastes serranoides*, by regulating food ration to
77 rear fish with differing positive growth rates, and then exploring how growth differences
78 relate to variation in several components of Igf1 signaling. This experimental approach
79 was selected specifically to simulate variable diets and rates of positive growth, rather
80 than complete food deprivation (i.e., fasting), as has been the focus of many prior studies
81 on GH/Igf pathways in other fishes. Rockfishes of genus *Sebastes* are an important
82 component of commercial and recreational groundfish fisheries in the North Pacific
83 Ocean (e.g., Miller et al., 2014; Parker et al., 2000), and several species are being
84 explored for their economic viability and best rearing practices in mariculture (e.g., Lee,
85 2001; Son et al., 2014). Juvenile olive rockfish were reared for 98 d under differing ration
86 levels, and then examined for differences in growth rate, plasma Igf1, and the relative
87 abundance of gene transcripts encoding *igf1, igf2*, as well as isoforms of type 1, 2 and 5
88 *igfbps* in the liver and skeletal muscle. The link between somatic growth and transcript

89 expression levels was also examined for Igf1 receptors a (*igf1ra*) and b (*igf1rb*) in
90 skeletal muscle, to further assess how variation in nutritional status and growth alters
91 components of Igf axis signaling in this target tissue.
92

93 **2. MATERIALS AND METHODS**

94 ***2.1. Animals***

95 *2.1.1. Animal collection and husbandry*

96 Young-of-the-year juvenile (3-7 mo.) olive rockfish (*S. serranoides*) were
97 collected from San Luis Bay on the central coast of California, USA, between 5 May and
98 10 July 2016. Fish were collected using a Standard Monitoring Unit for the Recruitment
99 of Fishes (SMURF) (Ammann, 2004), which was deployed under California Polytechnic
100 State University's Center for Coastal Marine Sciences pier facility (35°10'12.3"N
101 120°44'27.2"W) in Avila Beach, California. The SMURF was positioned approximately
102 1 to 3 m below the surface for durations varying from 3 to 11 (4.35 ± 1.66, mean ± SD)
103 days.

104 Upon collection, juvenile rockfish were transferred to flow-through 340 L tanks in
105 captivity where they were maintained under ambient salinity (~33‰), temperature
106 (range: 12.4 – 18.9°C), and photoperiod conditions. Fish were fed *ad libitum* daily with
107 commercial fish pellet feed (BioPro2 pellets, 1.5 mm, BioOregon, Longview, WA, USA)
108 for at least 3 weeks prior to the start of the experiment. All procedures were approved by
109 the Institutional Animal Care and Use Committee of California Polytechnic State
110 University (Protocol # 1504).
111

112 *2.1.2. Species identification*

113 Young-of-the-year *S. serranoides* can be difficult to identify to the species level
114 using only morphological traits, especially from the sympatric yellowtail rockfish, *S.*
115 *flavidus*. Therefore a ~369 bp region of the mitochondrial DNA D-loop control region
116 was amplified and sequenced for each fish used in the experiment to confirm species
117 identity. PCR was performed using degenerate primers to the mtDNA D-loop region
118 developed by Hyde and Vetter (2007). Nucleotide sequences for those primers were as
119 follows: (forward) 5′-CCTGAAAATAGGAACCAAATGCCAG-3′, and (reverse) 5′-
120 GAGGAYAAAGCACTTGAATGAGC-3′. Genomic DNA was isolated from skeletal
121 muscle of each fish using the DNeasy® Cell and Tissue Kit (Qiagen, Valencia, CA,
122 USA), and the resulting genomic DNA was amplified in 50 μl PCR reactions containing
123 25 μl of GoTaq® Colorless Master Mix (Promega Corp., Madison, WI, USA), 18 μl
124 nuclease-free H_2O, 1 μl each of forward and reverse primer (10 mM), and 5 μl of
125 genomic DNA (69.5-154 ng/μl). All reactions were conducted using a thermal profile of
126 95°C for 5 min followed by 38 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 1
127 min, and then a 2 min final extension at 72°C. The resulting PCR products were
128 examined on 1.2% EtBr gels before being cleaned (QIAquick® PCR Purification Kit,
129 Qiagen) and Sanger sequenced (Molecular Cloning Labs, South San Francisco, CA,
130 USA). The resulting sequences were then aligned using Sequencher v5.1 software
131 (GeneCodes Corp., Ann Arbor, MI, USA) against existing mtDNA D-loop sequences
132 from GenBank to confirm species identity (*S. serranoides*, **DQ678575** and *S. flavidus,*
133 **DQ678548**).
134

135 ***2.2. Sequencing of partial cDNAs linked to Igf signaling from olive rockfish***

136 *2.2.1. Total RNA isolation and reverse transcription*

137 Total RNA was extracted from the liver and skeletal muscle tissues of an olive

138 rockfish (86.8 mm standard length [SL], 12.77 g body mass) using TriReagent®

139 (Molecular Research Center, Inc., Cincinnati, OH, USA) and bromochloropropane for

140 phase separation. Extracted RNA was quantified by spectrophotometry (260:280 = 2.02;

141 P300 NanoPhotometer, Implen, Inc., Westlake Village, CA, USA) and DNase treated

142 (TURBO DNA-free kit, Life Technologies, Grand Island, NY, USA).

143 First strand cDNA was generated by reverse transcription (GoScript™ Reverse

144 Transcription System, Promega) in 20 µl reactions containing 4.88 µg total RNA

145 template (8 µl), 4 µl 5X buffer, 3 µl $MgCl_2$, 1 µl random primers, 1µl dNTPs, 0.5 µl

146 RNase inhibitor, 1 µl reverse transcriptase, and 1.5 µl nuclease-free water. Reactions

147 were incubated at 25 °C for 5 min, 42 °C for 1 h, followed by 70 °C for 15 min to

148 inactivate the reverse transcriptase enzyme.

149

150 *2.2.2. PCR amplification, sequencing, and assembly of partial cDNAs*

151 Degenerate primers were used to perform PCR to amplify partial cDNAs

152 encoding *igf1*, *igf2*, and the Igf1 receptors *igf1ra* and *igf1rb* as well as select *igfbps* from

153 olive rockfish. Degenerate primers were designed from consensus regions of these genes

154 identified by BLAST search of the genome assemblies for flag rockfish (*Sebastes*

155 *rubrivinctus,* **GCA_000475215**) and tiger rockfish (*Sebastes nigrocinctus,*

156 **GCA_000475235**), which were the only *Sebastes* taxa with genomes available at the time

157 of primer design. Partial cDNAs encoding *igf1* (accession no. **AF481856**), elongation

158 factor 1-alpha (*ef1α*, **KF430623**), and ribosomal protein L17 (*rpl17*, **KF430620**) from
159 Schlegel's black rockfish (*Sebastes schlegelii*), and *igf2* (**Y16643**) from shorthorn sculpin
160 (*Myoxocephalus scorpius*) were also used as part of the alignments for primer design.
161 The resulting partial sequences were aligned using Sequencher v5.1 software
162 (GeneCodes Corp.) to find consensus nucleotide regions, and degenerate primers were
163 synthesized by Eurofins Genomics (Louisville, KY, USA).
164 PCR was performed with degenerate primers in 50 µl reactions containing 25 µl
165 GoTaq® Polymerase Colorless Master Mix (Promega), 2 µl cDNA, 1 µl each of forward
166 and reverse primers (10-50 mM), and 21 µl nuclease-free H_2O. Reactions were amplified
167 under a thermal profile of 95 ˚C for 2 min, 37 cycles of 95 ˚C for 30 s, 53 ˚C for 30 s, and
168 72 ˚C for 1 min and 20 s, succeeded by 2 min of 72 ˚C. PCR products were then tested by
169 gel electrophoresis on 1.2% agrose gels. Any products that were of expected size were
170 cleaned (QIAQuick PCR Kit, Qiagen) and Sanger sequenced (Molecular Cloning Labs).
171

172 ***2.3. Food ration treatments***

173 Each olive rockfish was implanted intraperitoneally with a passive integrated
174 transponder (PIT) tag (7 mm, Loligo Systems, Inc., Viborg, Denmark) for individual
175 identification, which allowed for repeated measurements of standard length (SL, mm)
176 and body mass (g) from the same fish. Fish were then systematically assigned to one of
177 six 340 L tanks (0.97 m diameter x 0.48 m depth) to ensure each tank had the same
178 average wet body mass (2.95 ± 0.12 g, $F_{5,46} = 0.0827$, $p = 0.9946$) prior to commencing
179 food ration treatments. Tanks were randomly assigned to one of the following two food
180 rations: 1) high feed (4% mass of feed per fish wet mass; $n = 25$ fish), or 2) low feed (1%

181 mass of feed per fish wet mass; n = 27 fish) with three replicate tanks per treatment

182 group. The 4% ration was selected based on maximum daily food consumption by

183 juvenile olive rockfish in preliminary feeding trials. Each tank contained 7 to 10 fish to

184 allow for social interactions, and fish were fed daily by hand with 1.5 mm pellet feed

185 (BioPro2 pellets, Bio-Oregon®).

186 Fish standard lengths and weights were measured at day 0 (baseline) and then

187 again at time points of day 24, day 48, day 75, day 91, and day 98 of the experimental

188 treatments to quantify body size and growth variation. Feed mass was adjusted following

189 each measurement day to maintain the 1% and 4% ration amounts as fish grew during the

190 experiment. Lengths (SL) and weights were used to quantify body condition factor (K),

191 calculated as (mass/standard length^3) * 100 (e.g., Ricker, 1975; Lambert and Dutil, 1997),

192 as well as specific growth rate (SGR), calculated as follows (e.g., Lugert et al., 2014):

193 $SGR = \log(\frac{mass_{final} - mass_{initial}}{total\ days} \times 100)$.

194 After 98 days of the experimental ration treatments, fish were euthanized (tricaine

195 methanesulfonate, MS222, Argent Aquaculture, LLC, Redmond, WA, USA) and blood

196 was collected by severing the caudal peduncle. Blood was centrifuged at 3,000 x g for 10

197 min at 4°C, and the resulting plasma was stored at -80°C. Liver and skeletal muscle

198 tissues were also dissected from each fish, frozen immediately in liquid N_2, and kept at -

199 80°C until RNA extraction.

200

201 ***2.4. Plasma Igf1 quantification***

202 Plasma total (combined bound and unbound to Igfbps) Igf1 concentrations were

203 determined with a time-resolved fluoroimmunoassay (TR-FIA) method (Small and

204 Peterson, 2005), using dissociation enhanced lanthanide fluorescence immunoassay
205 reagents (DELFIA® reagents, Perkin-Elmer) and anti-recombinant Igf1 antiserum to
206 barramundi (*Lates calcarifer*) (GroPep BioReagents, Ltd., Thebarton, SA, Australia)
207 (Degger et al., 2000). This TR-FIA was modified from an RIA described by Shimizu and
208 colleagues (2000), and is described in detail in Ferriss et al. (2014). This TR-FIA was
209 validated previously for use in *Sebastes* rockfishes. All samples were run in duplicate.
210
211 ***2.5. Quantification of Igf system gene transcripts***
212 The relative abundance of gene transcripts for *igf1*, *igf2*, and isoforms of Igf
213 binding proteins type 1 (*igfbp1a,* and *-1b*)*,* type 2 (*igfbp2a,* and *-2b*)*,* and type 5 (*igfbp5a,*
214 and *-5b*) were quantified in the liver and skeletal muscle using real-time quantitative
215 reverse transcription PCR (qRT-PCR). In addition, the relative abundance of Igf1
216 receptors a (*igfra*) and b (*igfrb*) was also measured in skeletal muscle.
217 Total RNA was extracted from liver and muscle tissues using TriReagent®
218 (Molecular Research Center, Inc.) and bromochloropropane. RNA was subsequently
219 DNase treated (TURBO DNA-free kit, Life Technologies) and quantified by
220 spectrophotometry (260:280 ≥ 2.02, P300 NanoPhotometer, Implen, Inc.). RNA from
221 each fish was then diluted to 68.6 $ng{\cdot}\mu l^{-1}$ for liver and 20.8 $ng{\cdot}\mu l^{-1}$ for skeletal muscle to
222 standardize total RNA concentrations prior to reverse transcription. RNA was reverse
223 transcribed in 24 µl reactions with 2.86 µg (liver) or 0.87 µg (muscle) DNase-treated
224 RNA template (10 µl), 4.8 µl 5X buffer (GoScript™, Promega), 3.775 µl $MgCl_2$ (3.9 mM
225 concentration), 1.2 µl dNTPs (0.5 mM each dNTP), 1.2 µl random primers, 0.125 µl
226 RNase (Recombinant RNasin® Ribonuclease Inhibitor, Promega), 0.9 µl GoScript™

227 reverse transcriptase (Promega), and 2 µl nuclease-free water. Reverse transcription
228 reactions were conducted at 25˚C for 5 min, 42˚C for 60 min, and then completed with a
229 reverse transcriptase inactivation at 70˚C for 15 min.

230 Primers for SYBR Green qRT-PCR were designed to the protein coding regions
231 of each olive rockfish partial cDNA using the PrimerQuest tool of Integrated DNA
232 Technologies (Coralville, IA, USA). Primers were also designed to amplify *ef1a* and
233 *rpl17* for use as reference genes. All primers were synthesized by Eurofins Genomics
234 (Louisville, KY).

235 Quantitative real-time PCR assays were conducted in 16 µl reactions with 8 µl
236 iTaq™ Universal SYBR® Green Supermix (BioRad Laboratories, Inc., Hercules, CA,
237 USA), 1 µl of both forward and reverse primers (10 mM), 4.5 µl nuclease-free H_2O, and
238 1.5 µl cDNA template. All SYBR Green qRT-PCR reactions were run on a CFX
239 Connect™ Real-Time PCR System (Bio-Rad Laboratories, Inc.) under a thermal profile
240 of 95°C for 2 min, 42 cycles of 95 °C for 5 s and 60 °C for 30 s, followed by 65˚C for 15
241 s. For each gene, a standard curve was made using a pool of RNA extracted from tissues
242 of fish collected from both ration treatments. This pooled sample was serially diluted,
243 and each standard concentration was assayed in triplicate. Correlation coefficients (r^2) for
244 the standard curves were always greater than $r^2 = 0.96$. Melt curve analyses were also
245 performed to confirm amplification of a single product and the absence of primer–dimers
246 during each quantitative PCR run. PCR efficiencies for each gene were calculated using
247 the equation: $\%\ efficiency = [10^{(-1/slope)} - 1] \times 100$; mean efficiencies are
248 provided in **Supplemental Materials, Table S2**. For each gene, relative mRNA levels
249 were calculated using the standard curve and normalized to the geometric mean of *rpl17*

250 and *ef-1α* mRNA expression, which did not vary within a tissue between ration groups.
251 Abundance values of each gene of interest were then expressed as a relative level by
252 dividing the resulting values by the mean value of the low treatment group. Specificity of
253 the primer sets was also assessed by Sanger sequencing select PCR products for each
254 gene.
255
256 ***2.6. Statistical Analyses***
257 ANCOVA models were used to compare the length-mass body size relationships
258 between ration treatments at the start (day 0) and end (day 98) of the experiment.
259 Repeated-measures ANOVA models were then used to test for effects of ration treatment
260 on body mass, length, and condition factor (K) over the 98 d experimental period. There
261 were no within treatment tank effects, which was found by comparing ANOVA models
262 with and without 'tank' as a fixed effect, so 'tank' was not considered in subsequent
263 analyses. Student t-tests were then used to test for differences in mass, length, and
264 condition factor between the high and low food ration treatments on each measurement
265 day. Plasma Igf1 concentrations were compared using t tests, and Pearson's correlations
266 were used to examine relationships between Igf1 and both mass-specific SGR and length-
267 specific SGR for all fish combined. Associations between plasma Igf1 concentrations and
268 both hepatic and muscle *igf1* mRNA levels for all fish, as well as within treatments, were
269 tested using Pearson's correlations. All statistics were conducted using R v3.3.2 through
270 RStudio v1.0136.
271
272 **3. RESULTS**

273 ***3.1. Identification of partial cDNAs from olive rockfish***

274 Degenerate primer PCR amplified partial cDNAs encoding *igf1* (362 bp,
275 GenBank accession no. **MG366820**) and *igf2* (451 bp, **MG366821**) for olive rockfish, as
276 well as cDNAs for the following Igfbps: *igfbp1a* (679 bp, **MG366822**), *igfbp1b* (702 bp,
277 **MG366823**), *igfbp2a* (519 bp, **MG366824**), *igfbp2b* (678 bp, **MG366825**), *igfbp5a* (200
278 bp, **MG366826**), and *igfbp5b* (707 bp, **MG366827**). Partial cDNAs were also sequenced
279 for the Igf1 receptors a (*igf1ra,* 426 bp, **MG366828**) and b (*igf1rb,* 340 bp, **MG366829**).
280 BLAST analyses using GenBank (https://www.ncbi.nlm.nih.gov/) combined with
281 phylogenetic analysis of the deduced amino acid sequences for the partial cDNAs
282 encoding Igfbps confirmed the identity of these cDNAs.

283 Partial sequences encoding 416 bp of the cDNA for elongation factor 1-α (*ef1α*)
284 (**MG366830**) and 399 bp of a cDNA for 60S ribosomal protein L17 (*rpl17*) (**MG366831**)
285 were also amplified and sequenced from olive rockfish for use as internal reference genes
286 in real-time quantitative reverse-transcription PCR.

287

288 ***3.2. Ration differences generate variation in growth rate***

289 There were no significant differences in body size parameters (mass, standard
290 length, or condition factor) between rockfish in the two ration treatments at the start of
291 the experiment (day 0, $p \geq 0.24$), prior to commencing feeding. This equivalence of body
292 sizes between ration treatments on day 0 is illustrated as similarity in the length-mass
293 relationships between treatment groups (**Fig. 1**) (ANOVA model: treatment*length
294 interaction, $p = 0.842$). However, rockfish in the 4% ration treatment grew larger than
295 fish in the 1% ration over the course of the experiment, and by the end of the experiment

296 on day 98, rockfish reared under the 4% ration showed a different length-mass
297 relationship than fish under the 1% ration (**Fig. 1**) (ANOVA model: treatment*length
298 interaction, $F_{1,49} = 9.15$, $p = 0.0040$). Fish in the 4% ration were significantly larger in
299 mass ($F_{1,50} = 58.12$, $p < 0.001$) and body condition factor ($F_{1,50} = 28.65$, $p < 0.0001$) after
300 24 d, and were larger in SL ($F_{1,50} = 27.46$, $p < 0.0001$) after 48 d (**Fig. 2**), compared to
301 fish in the 1% ration treatment. Rockfish in the 4% ration treatment remained larger in
302 mass and length and also had a higher condition factor until the end of the experiment on
303 day 98. As expected by those body size differences, fish in the 4% ration treatment
304 ultimately exhibited greater mass-specific SGR ($F_{1,50} = 146.77$, $p < 0.0001$) and length-
305 specific SGR ($F_{1,48} = 59.37$, $p < 0.0001$) compared to fish in the 1% ration treatment (**Fig.
306 3**).
307
308 ***3.3. Effect of ration amount on plasma Igf1 concentrations***
309 Plasma Igf1 concentrations were significantly higher in rockfish from the 4%
310 ration treatment ($F_{1,48} = 9.509$, $p = 0.0034$) (**Fig. 4**). When looking at all fish, mass and
311 length specific SGR were both correlated positively to plasma Igf1 levels (**Fig. 5**),
312 whereas Igf1 concentrations showed no relationship with change in body condition ($r = -$
313 0.042, $p = 0.769$).
314 Mass-specific SGR amongst all fish was more strongly correlated than length-
315 specific SGR but both correlated to plasma Igf1 concentrations for multiple time periods
316 (**Table 1**). All time intervals for mass-specific SGR were correlated to plasma Igf1 except
317 the last 7 days (day 91-98, **Table 1**). Excluding the last 7 days, SGR for mass became
318 more strongly correlated to Igf1 at later time periods. Length-specific SGR was

319 significantly correlated to Igf1 concentrations at every time point. There were no
320 significant correlations within ration treatments.
321

322 ### *3.4. Regulation of mRNAs encoding Igfs and Igfbps in liver*

323 There was no difference between treatments for transcripts of *igf1* ($F_{1,45} = 1.663$, p
324 $= 0.2038$) or *igf2* ($F_{1,45} = 1.366$, $p = 0.2487$) in the liver (**Fig. 6A**). Of the binding
325 proteins, *igfbp1a* ($F_{1,44} = 11.63$, $p = 0.0014$) and *igfbp1b* ($F_{1,45} = 24.30$, $p < 0.0001$) had
326 the only differences between treatments with approximately 3 and 4-fold greater
327 abundance, respectively, in fish from the low ration treatment (**Fig. 6B**). Liver *igfbp2a,*
328 *igfbp2b, igfbp5a* and *igfbp5b* did not differ in relative mRNA abundance between ration
329 treatments.

330 Plasma Igf1 and hepatic *igf1* mRNA correlated strongly across all fish ($r =$
331 0.5447, $p = 0.0001$; **Fig. 7**). Within treatments, Igf1 also correlated to liver *igf1* with the
332 low ration having a stronger correlation ($r = 0.7031$, $p = 0.0005$; **Fig. S1A, Supplemental**
333 **Materials**) than the high ration ($r = 0.5537$, $p = 0.0050$; **Fig. S1B**). No significant
334 correlations were observed between plasma Igf1 and mRNA levels for *igfbp1a* ($r = -$
335 0.119, $p = 0.44$), *igfbp1b* ($r = -0.227$, $p = 0.14$), or any other Igfbp gene transcript in the
336 liver ($p = 0.50$-0.89). Several correlations were, however, observed in the liver between
337 mRNA levels for different transcripts involved in Igf1 signaling (**Table S3,**
338 **Supplemental Materials**).
339

340 ### *3.5. Ration effects on Igf system-associated mRNAs in skeletal muscle*

341 Transcripts encoding *igf1* were significantly more abundant in the skeletal muscle
342 of rockfish in the high ration treatment than in the low ration ($F_{1,44} = 30.50$, $p < 0.0001$)
343 but *igf2* did not differ between treatments ($F_{1,44} = 3.468$, $p = 0.0692$; **Fig. 8A**). While
344 *igfbp1a*, *igfbp1b*, *igfbp2a* and *igfbp2b* mRNA levels did not show any differences
345 between treatments, *igfbp5a* ($F_{1,44} = 5.963$, $p = 0.0187$) and *igfbp5b* ($F_{1,45} = 9.919$, $p =$
346 0.0029) levels were significantly higher in the high ration treatment compared to
347 respective low treatment fish (**Fig. 8B**). None of the examined Igf1 receptors showed any
348 difference in mRNA abundance between treatments (**Fig. 8C**).
349 Muscle *igf1* mRNA abundance correlated to plasma Igf1 when grouping all fish (r
350 $= 0.4880$, $p = 0.0007$; **Fig. 9**). This relationship appears driven largely by rockfish in the
351 4% ration treatment ($r = 0.4144$, $p = 0.0493$; **Fig. S2A, Supplemental Materials**), as the
352 relationship was not significant when fish in the low treatment were analyzed separately
353 ($r = 0.1487$, $p = 0.509$; **Fig. S2B**). Relatively few correlations were observed between
354 relative mRNA levels for different transcripts within the muscle tissue (**Table S4,**
355 **Supplemental Materials**).
356
357 **4. Discussion**
358 ***4.1. Relationship between plasma Igf1 and growth rate***
359 In the present study, the relationship between circulating Igf1 and growth rate was
360 examined in olive rockfish, one of several *Sebastes* rockfishes important as recreational
361 and commercial groundfish fisheries in the Northern Pacific Ocean. By feeding groups of
362 juvenile olive rockfish two different ration amounts (1% or 4% wet mass per d) for 98 d,

363 differences in SGR were generated, with rockfish given the 4% ration growing 60%
364 faster in mass and 22% more rapidly in length per day compared to fish in the 1% ration.
365 Juvenile olive rockfish experiencing faster growth under the 4% ration treatment
366 had higher plasma Igf1 concentrations than fish under the 1% ration. Similar differences
367 in circulating Igf1 linked to variation in food intake have been observed previously in
368 other fishes including tilapia (Breves et al., 2014; Uchida et al., 2003), sea bream (Pérez-
369 Sánchez et al., 1995), and salmonids (e.g., Beckman et al., 1998, 2004a; Breves et al.,
370 2016; Pierce et al., 2005; Wilkinson et al., 2006; for review, see Beckman, 2011). These
371 changes in circulating Igf1 appear to result from altered hepatic Igf1 production, as olive
372 rockfish displayed a positive correlation between plasma Igf1 and hepatic *igf1* mRNA
373 levels. In other studies, negative relationships between plasma GH and Igf1 levels have
374 been observed in food restricted fish, while positive correlations between these hormones
375 were present in fed individuals (e.g., Fox et al., 2006). Those shifting relationships
376 between circulating GH and Igf1 concentrations with differing food intake suggest that
377 nutrition-associated changes in plasma Igf1 result in part from changes in GH stimulation
378 of liver Igf1 production. Supporting that idea, fasting has been shown to reduce GH
379 binding in the liver, implying an increase in hepatic GH resistance in fish experiencing
380 food limitation (Gray et al., 1992; Pérez-Sánchez et al., 1994; see also Bergan-Roller and
381 Sheridan, 2018).
382 Individual variation in plasma Igf1 in olive rockfish also correlated positively
383 with individual variation in growth rate, supporting the previously proposed idea that
384 circulating Igf1 may serve as a physiological index of growth rate in fish (Beckman,
385 2011; Picha et al., 2008a). The strength of this correlation between plasma Igf1 and

386 growth rate in olive rockfish varied from approximately r = 0.29 to 0.43, depending on
387 whether SGR was measured using body length or mass, and generally was observed as
388 more robust with measurements of mass-specific SGR.

389 Similar to our findings here, Igf1 has previously been found to correlate positively
390 with growth rate across a variety of teleosts (e.g., Beckman, 2011; Beckman et al.,
391 2004a,b; Kaneko et al., 2015; Lankford and Weber, 2006; Uchida et al., 2003). In olive
392 rockfish, plasma Igf1 concentrations correlated over the last 7 d of growth to length-
393 specific SGR but not to mass-specific SGR, despite mass-specific SGR showing strongly
394 correlative relationships at all other measured time intervals (**Table 1**). In contrast, other
395 studies have observed Igf1 concentrations having the strongest correlation over the most
396 recent growth history (e.g., Beckman et al., 2004a). Given that somatic growth rates can
397 vary due to a variety of ecological factors (e.g., temperature, photoperiod, etc.; Beckman,
398 2011) in addition to food ration amount—and that the relative effects of such factors on
399 growth can vary among species (Beckman, 2011; Picha et al., 2008a)—any one of several
400 factors may have contributed to the weaker statistical relationship between mass-specific
401 SGR and plasma Igf1 during the last measurement time interval (day 91-98). Such
402 environmental influences are often linked to variation in feeding efficiency, which can
403 lead to changes in growth even with equivalent diet composition and food consumption
404 rates (Mingarro et al., 2002; Vera Cruz et al., 2006). In Pacific rockfishes, growth rates
405 have been demonstrated to change with fish age (Tsang et al., 2007), and both
406 temperature and body size can also impact growth in rockfishes (e.g., Boehlert and
407 Yoklavich, 1983; Kamimura et al., 2012). Due to changes in water temperature, growth
408 in fish typically slows in the fall season in conjunction with lowered plasma Igf1 levels

409 (Larson et al., 2001; Mingarro et al., 2002). Given that the last sampling date (day 98)
410 was 21 October 2016 – and that fish were reared under ambient photoperiod and ocean
411 temperatures – the absence of a significant correlation between Igf1 and mass-specific
412 SGR across the final 7 d of the experimental period may have resulted from a slowing of
413 growth with the transition into the fall season.
414
415 ***4.2. Growth-related variation in hepatic and muscle Igf1 and Igf2 gene expression***
416 Liver *igf1* mRNAs have been shown to decrease in fish experiencing restricted
417 food or complete food deprivation (fasting) in a variety of species including salmon (*O.*
418 *tshawytscha*, Pierce et al., 2005; *O. kisutch*, Duan and Plisetskaya, 1993), Nile tilapia (*O.*
419 *niloticus*, Vera Cruz et al., 2006), rainbow trout (*O. mykiss*, Monserrat et al., 2007),
420 grouper (*Epinephelus coioides*, Pedroso et al., 2006), channel catfish (*Ictalurus*
421 *punctatus*, Small et al., 2006; Peterson and Waldbieser, 2009), rabbitfish (*Siganus*
422 *guttatus,* Ayson et al., 2007), and yellowtail (*Seriola quinqueradiata*, Kawanago et al.,
423 2014). Studies in other fishes, such as Mozambique tilapia (Breves et al., 2014) and
424 Atlantic salmon (Breves et al., 2016), however, did not observe any change in hepatic
425 *igf1* mRNAs when food was withheld entirely. Hepatic *igf1* similarly failed to track
426 changes in plasma Igf1 in masu salmon (*O. masu*) experiencing a period of compensatory
427 growth (Kawaguchi et al., 2013). That observed variation in liver *igf1* mRNA responses
428 to feeding variation may arise from several interacting factors including taxonomic
429 variation, the duration and degree of food restriction, developmental or life stage
430 variation in GH induction of liver *igf1* expression, or interactions from other hormones
431 that can influence igf1 mRNA expression including cortisol, thyroid hormones, insulin,

432 and somatostatin (e.g., Butler and LeRoith, 2001; Schmid et al., 2003; Pierce et al., 2005;
433 Leung et al., 2008; Sheridan and Hagemeister, 2010). The present results with olive
434 rockfish correspond with those later studies, as liver *igf1* mRNA levels were unaffected
435 by the experimentally generated differences in ration amount and growth rate. Even so,
436 individual variation in hepatic *igf1* mRNA levels was found to correlate with plasma Igf1
437 in olive rockfish, as has been seen in other fish experiencing varied positive growth
438 (Gabillard et al., 2003; Pierce et al., 2005).
439 Several interacting mechanisms may underlie the nutrition-associate changes in
440 liver *igf1* mRNA levels observed here in rockfish. First, nutritional status can influence
441 GH receptor expression (Gray et al., 1992; Small et al., 2006; Norbeck et al., 2007),
442 which ultimately may contribute to reduced GH stimulation of liver Igf1 production and
443 release (e.g., Gabillard et al., 2006; Pierce et al., 2005). For instance, several fishes
444 undergoing fasting have been shown to have reduced hepatic GH receptor mRNA levels
445 (Norbeck et al., 2007; Peterson et al., 2009; Picha et al., 2008b; Saera-Vila et al., 2005;
446 Small et al., 2006), suggesting that that nutrition-associated declines in circulating Igf1
447 levels may be due in part to reduced GH stimulation of liver Igf1. In mammals, reduced
448 food intake has also been observed to increase liver production of fibroblast growth
449 factor 21 (FGF21) (Inagaki et al., 2007). FGF21 inhibits GH-mediated phosphorylation
450 of signal transducers and activators of transcription 5 (STAT5) regulatory proteins (e.g.,
451 Beauloye et al., 2002; Inagaki et al., 2008), which regulate the induction of *igf1* gene
452 expression following liver GH receptor activation (e.g., Herrington et al., 2000). While
453 the specific mechanisms whereby different planes of food availability altered plasma Igf1

454 and hepatic *igf1* gene expression were not examined in the present study in olive
455 rockfish, future work in fishes should explore those mechanisms in more detail.

456 Although the liver is the dominant tissue of Igf1 synthesis, peripheral Igf1
457 production also appears to be important for regulating growth via autocrine or paracrine
458 effects in some tissues (e.g., Firth and Baxter, 2002; Franz et al., 2016), and extrahepatic
459 *igf1* expression can be regulated in a tissue-specific pattern (Eppler et al., 2010; Fox et
460 al., 2010). Liver Igf1-knockout mice continue to exhibit normal growth (Le Roith et al.,
461 2001), suggesting that locally produced Igf1 may play an important, underappreciated
462 role in regulating somatic growth in vertebrates. Just as in the liver, extrahepatic *igf1*
463 mRNA abundance changes in fish undergoing food deprivation followed by refeeding
464 (e.g., Fox et al., 2010; Norbeck et al., 2007; Peterson and Waldbieser, 2009; Terova et al.,
465 2007).

466 In the current study, rockfish from 4% ration treatments had elevated relative *igf1*
467 mRNA levels in skeletal muscle. While the functional significance of muscle Igf1
468 production in fishes is not clear, muscle *igf1* transcription has been shown to respond to
469 acute changes in food intake in Atlantic salmon, rainbow trout, and tilapia; in these
470 species, muscle *igf1* mRNA levels change within days after initiating fasting (Bower et
471 al., 2008; Breves et al., 2016; Fox et al., 2010; Montserrat et al., 2007; Picha et al.,
472 2008b) and refeeding (Chauvigné et al, 2003; Fuentes et al., 2012; Gabillard et al., 2006).
473 In fine flounder, *Paralichthys adspersus*, nutrition-related changes in muscle *igf1*
474 expression were shown to result in part from changes in GH stimulation (Fuentes et al.,
475 2012). In that work, Fuentes and colleagues (2012) found that nutrition regulates GH
476 receptor subtype expression in muscle, and that the ratio of full-length GH receptor to

477 truncated GH receptor subtypes in muscle shifts depending on nutritional status, therein
478 generating differences in GH-mediated *igf1* transcription. What is more, both Igf1 and
479 Igf2 have also been shown to increase *igf1* transcription in cultured myocytes from
480 gilthead sea bream (Azizi et al., 2016), so it's possible that the higher muscle *igf1* mRNA
481 levels observed in rockfish from the 4% ration may be a result of both greater GH
482 stimulation associated with the higher nutritional status of these fish (i.e., higher food
483 consumption) and enhanced Igf1 stimulation of muscle *igf1* gene expression.
484 While some studies in teleost fishes have observed notable responses of Igf2
485 signaling with nutritional deprivation (e.g., Gabillard et al., 2006), *igf2* mRNA levels in
486 olive rockfish did not vary in either liver or muscle when fish were reared under different
487 rations. Montserrat and colleagues (2007) similarly did not observe any changes in *igf2*
488 mRNA levels in the liver or muscle of rainbow trout experiencing fasting. Notably,
489 however, the majority of studies that examined *igf2* transcriptional responses to nutrition
490 have focused on extreme nutritional deprivation (i.e., fasting) followed by refeeding
491 (Bower et al, 2008; Chauvigné et al, 2003; Peterson and Waldbieser, 2009), and as such
492 are not entirely comparable to our current study that examined Igf signaling responses to
493 differing rates of positive growth.
494
495 ***4.3. Responses of Igfbp and Igf1 receptor mRNA abundance to ration amount***
496 Igfbps are critical for modulating the effects of Igf1 and Igf2, and the ratio of
497 Igf:Igfbp in circulation regulates hormone availability for receptor binding (Clemmons,
498 2016). The expression of Igfbp1a (regarded as the 28-32 kDa Igfbp protein in fishes) and
499 Igfbp1b (regarded as the 20-25kDa Igfbp) changes with nutritional status in some teleosts

500 (Shimizu et al., 2006; Picha et al., 2008a), with both Igfbp1a and 1b generally showing
501 elevated hepatic and plasma expression under nutritional restriction (Shimizu and
502 Dickhoff, 2017). Juvenile olive rockfish raised under the 1% ration treatment followed
503 that same pattern with a greater relative abundance of *igfbp1a* and *igfbp1b* transcripts in
504 the liver compared to fish in the 4% ration group. Those higher hepatic *igfbp1a* and *1b*
505 mRNAs agree with previous experimental findings in fishes that experienced complete
506 food deprivation (fasting) including Atlantic salmon (Breves et al., 2016), masu salmon
507 (Kawaguchi et al., 2013), and Mozambique tilapia (Breves et al., 2014).
508 Given the lack of response in the rockfish liver from all other Igf binding proteins,
509 *igfbp1a* and *igfbp1b* appear to be most sensitive to modulation by changes in nutritional
510 status, and therein may be contributing to differences in growth under food limitation. In
511 juvenile salmon, circulating Igfbp1a and Igfbp1b protein levels both correlate inversely
512 with growth rate (Kawaguchi et al., 2013). In light of that relationship, Kawaguchi and
513 coworkers (2013) proposed that plasma Igf1 may serve as a reliable, positive indicator of
514 growth, while plasma Igfbp1b may be a negative indicator. Despite not finding any
515 significant correlations between individual hepatic *igfbp1a* or *igfbp1b* mRNA levels and
516 individual growth rate in olive rockfish, it is still possible that *igfbp1a* and *igfbp1b* levels
517 are more sensitive to shifts in food consumption than plasma Igf1 under some conditions
518 of nutritional stress (e.g., Picha et al., 2008a), given the observed magnitude of hepatic
519 *igfbp1* mRNA responses to food limitation in rockfish.
520 Generally, transcripts encoding *igfbp1a* and *igfbp1b* are at lower abundance in
521 skeletal muscle than in liver (Bower et al., 2008; Bower and Johnston, 2010; Breves et
522 al., 2014; Fuentes et al., 2013; Safian et al., 2012). Because of this, *igfbp1* mRNAs are

523 less frequently measured in muscle tissues from teleosts exposed to food manipulation
524 experiments, and considerably less is known about the role of muscle Igfbp1 in regulating
525 growth of muscle tissue. In the current study in olive rockfish, muscle *igfbp1a* and
526 *igfbp1b* mRNA levels were unaffected by positive growth variation induced by
527 differences in food ration, even though both transcripts were detectable in the muscle.
528 Notably, there are conflicting findings concerning the regulation of *igfbp1* gene
529 expression in fish skeletal muscle, even across studies using the same species. In Atlantic
530 salmon, for instance, some studies have been unable to detect *igfbp1* mRNAs in
531 homogenized fast-twitch (white) muscle tissue or isolated, cultured myocytes (Bower et
532 al., 2008; Bower and Johnston, 2010). Other work with this species, however, not only
533 detected *igfbp1* transcript expression in this same tissue, but also observed expressional
534 regulation in response to temperature (Hevrøy et al., 2015). These incongruent findings
535 suggest that muscle *igfbp1* expression may vary with several factors besides nutritional
536 status, such as development age or ecological conditions (e.g., temperature, salinity,
537 season; see Shimizu and Dickhoff, 2017). Depending on the experimental testing
538 conditions, such environmental influences may interact to obscure clear patterns of *igfbp1*
539 regulation in muscle.
540 Similar to other teleosts, olive rockfish also transcribe two distinct *igfbp2* mRNAs
541 (*igfbp2a* and *igfbp2b*). In teleosts, *igfbp2* transcripts are found at the highest levels in
542 liver and white muscle (e.g., Safian et al., 2012), and Igfbp2 has been proposed to
543 function as the primary carrier of Igfs in blood circulation in salmonids (de la Serrana and
544 Macqueen, 2018; Shimizu and Dickhoff, 2017). In the rockfish studied here, liver and
545 muscle *igfbp2a* and *igfbp2b* mRNA levels were unaffected by food ration. In other

546 teleosts, however, regulation of both hepatic and muscle *igfbp2* transcripts has been
547 observed in fasting/refeeding experimental manipulations (Chen et al., 2014; Duan et al.,
548 1999; Gabillard et al., 2006; Safian et al., 2012). Hepatic *igfbp2* mRNA expression is
549 elevated by GH and suppressed by prolonged food deprivation (Duan et al., 1999; Chen
550 et al., 2014; Gabillard et al., 2006; Kelley et al., 2001; Safian et al., 2012), implying that
551 hepatic Igfbp2 is downregulated along with liver Igf1 production under severe nutritional
552 stresses, possibly to maintain the bound:unbound ratio of Igfs in plasma when fish
553 experience reduced food intake. Given that the experimental treatments with rockfish
554 involved differences in ration amount and not complete food deprivation/fasting, it
555 appears that the severity of nutritional stress in the 1% ration treatment was insufficient to
556 induce changes in Igfbp2 gene expression. Future studies, however, should examine
557 whether a more severe fasting stress would result in altered hepatic or muscle expression
558 of *igfbp2a* or *igfbp2b* mRNAs in rockfish, as has been observed in other fishes.
559 The Igf binding protein 5 (Igfbp5) is also expressed in many tissues and has been
560 linked to bone growth (Duan and Xu, 2005), skeletal muscle differentiation (Ren et al.,
561 2008; Safian et al., 2012), and juvenile development (Salih et al., 2004). Transcripts
562 encoding *igfbp5a* and *igfbp5b* are present in the liver of many teleost fishes (Breves et al.,
563 2014; Gabillard et al., 2006; Kamangar et al., 2006; Safian et al., 2012; Pedroso et al.,
564 2009; Zheng et al., 2017), but have generally not been shown to be sensitive in this tissue
565 to fasting stress (e.g., Breves et al., 2014; Gabillard et al., 2006). In skeletal muscle,
566 however, Igfbp5 gene transcription does appear to be regulated by nutrition (Bower et al.,
567 2008; Bower and Johnston, 2010; Gabillard et al., 2006; MacQueen et al., 2011; Zheng et
568 al., 2017; see Amaral & Johnston, 2011). For instance, in fine flounder, muscle *igfbp5*

569 mRNAs were downregulated while fasting and upregulated during refeeding (Safian et
570 al., 2012). Bower and Johnston (2010) also observed that an enhanced amino acid diet
571 increased *igfbp5* mRNAs in cultured myotube cells from Atlantic salmon, suggesting that
572 nutritional composition alone can influence muscle Igfbp5 expression. Olive rockfish
573 showed elevated mRNA levels for both *igfbp5a* and *igfbp5b* in muscle, supporting those
574 previously observed effects of food intake on Igfbp5 expression in this tissue in other
575 fishes. While the function and mechanism of action for Igfbp5 regulation in muscle
576 remains unclear, Igfbp5 proteins appear to be important for myogenesis and muscle
577 growth in teleost fishes (e.g., de la Serrana and Macqueen, 2018).
578 In target tissues such as skeletal muscle, the effects of Igf1 on cell proliferation,
579 differentiation, and migration occur via Igf receptors, which activate intracellular
580 transduction cascades including the phosphatidylinositol 3-kinase (PI3K)-Akt pathway
581 (Dupont and LeRoith, 2001). Partial cDNAs were amplified and sequenced encoding two
582 Igf1 receptors (*igf1ra* and *igf1rb*) from olive rockfish, and although juvenile rockfish
583 under differing food rations did not show altered levels of either Igf1 receptor mRNA in
584 liver or muscle, nutritional stresses such as fasting have been shown to influence Igf1r
585 transcript levels in other fishes (Bower et al., 2008; Chauvigné et al., 2003). Multiple Igf1
586 receptors have been detected in other fishes (Azizi et al., 2016; Chan et al., 1997; Escobar
587 et al., 2011; Greene and Chen, 1999), and different Igf1r forms can exhibit dissimilar
588 patterns of tissue expression in some contexts (e.g., Maures et al., 2002). In rainbow
589 trout, for instance, muscle *igf1ra* mRNA abundance increased under fasting and again
590 decreased during re-feeding, while *igf1rb* was unresponsive to these same changes
591 (Chauvigné et al., 2003). Azizi and coworkers (2016) recently found that Igf1

592 downregulated both *igf1ra* and *igf1rb* transcripts while Igf2 upregulated only *igf1rb* in
593 culture myocytes from sea bream, indicating these Igf1 receptors may have different
594 mechanisms of hormonal regulation. Combined, such evidence suggests functional
595 specialization of the two teleost Igf1r types, at least in salmonids; what those different
596 functions might be, however, remains unclear at this time in teleost fishes.
597
598 **5. Conclusions**
599 Similar to other teleost fishes, individual variation in circulating Igf1
600 concentrations were found to correlate positively with variation in SGR in juvenile olive
601 rockfish. Depending on whether SGR was calculated from body mass or length,
602 correlation coefficients between Igf1 and SGR ranged from $r = 0.29$ to 0.43, and were
603 generally more robust when using mass-specific SGR measures than length-specific
604 measures. These results provide further evidence that nutritional limitation induces
605 hepatic *igfbp1a* and *igfbp1b* transcription in teleost fishes, reinforcing the proposed
606 functional role for these Igfbps in catabolism in fish (e.g., Shimizu and Dickhoff, 2017).
607 Taken as a whole, our finding of a positive correlation between individual
608 variation in SGR and plasma Igf1 supports the usefulness of measuring circulating Igf1
609 concentrations as an index for assessing relative growth in *Sebastes* rockfishes in
610 aquaculture or fishery management applications. Positive relationships between Igf1 and
611 SGR have now been established for many ray-finned fishes (reviewed by Beckman,
612 2011; Picha et al., 2008a), and measurements of Igf1 are beginning to be employed as a
613 non-lethal method for evaluating the growth ecology of wild fishes (e.g., Andrews et al.,
614 2011; Ferriss et al., 2014; Wechter et al., 2017). The application of this endocrine

615 approach has the potential to provide crucial information on growth variation in wild
616 fishes both among local spatial areas and across short-term time periods. Such
617 information has been challenging to obtain via other methods for quantifying growth
618 rates in wild fishes, and Igf1 measurements could serve as an important conservation tool
619 for identifying and protecting habitats critical for supporting fish growth, ultimately
620 enabling more effective management of wild fish stocks.
621

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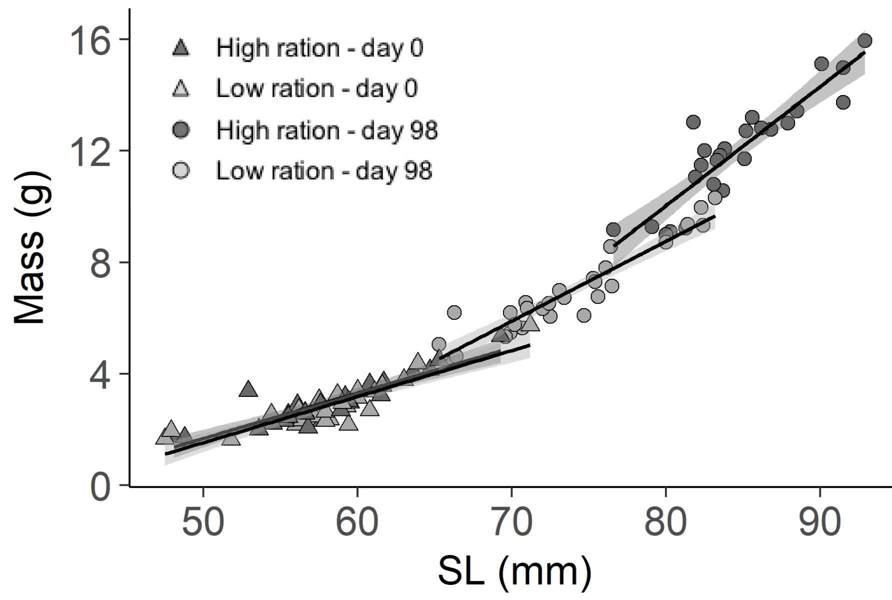
1108 **Figure Legends**
1109
1110 **Figure 1.** Changes in the relationship between standard length (mm) and body mass (g)
1111 for olive rockfish in the high ration (4% wet wt.) and low ration (1% wet wt.)
1112 treatments between day 0 (triangles) and day 98 (circles) of the 98 d experimental
1113 period.
1114
1115 **Figure 2**. Mean (±SEM) values of (**A**) mass and (**B**) standard length, and (**C**) condition
1116 factor for rockfish reared under high ration (4% wet wt.) or low ration (1% wet wt.)
1117 conditions. Mass and body condition factor differed between treatments beginning on
1118 day 24, while length differed beginning on day 48. Asterisks indicate difference
1119 between treatments on that sampling day.
1120
1121 **Figure 3**. High (4%) ration fish (dark gray bars, n = 25) showed a greater (**A**) mass-
1122 specific and (**B**) length-specific somatic growth rate (SGR) than low (1%) ration fish
1123 (light gray bars, n = 26). Bars represent group means (±SEM) of percent change per
1124 day with p-values from student t-tests.
1125
1126 **Figure 4**. Comparison of mean (±SEM) plasma Igf1 concentrations between high ration
1127 (4% wet wt.) and low ration (1% wet wt.) treatments. Rockfish in the high ration
1128 treatment (n = 24) had significantly higher plasma Igf1 than low ration fish (n = 26).
1129
1130 **Figure 5**. Individual (**A**) mass-specific somatic growth rate (SGR) and (**B**) length-
1131 specific SGR both correlated positively to plasma Igf1 concentration. SGR values
1132 shown are calculated from changes in body size across the entire experimental period
1133 (day 0 to 98). Lines represent Pearson correlation relationships for fish from both
1134 ration treatments combined (n = 50).
1135
1136 **Figure 6**. Relative hepatic mRNA levels from the high and low ration treatments. (**A**)
1137 Levels of *igf1* and *igf2* did not differ between treatments. (**B**) Transcripts encoding Igf
1138 binding proteins *igfbp1a* and *igfbp1b* were expressed at higher relative levels in
1139 rockfish reared under the low (1%) ration treatment. Data are shown as mean (±SEM)
1140 values. Lines indicate significant differences between treatments (Student's t-test: $*P$
1141 < 0.05, $**P < 0.01$, $***P < 0.0001$).
1142
1143 **Figure 7**. Individual variation in liver *igf1* mRNA levels correlated positively with
1144 plasma Igf1 concentrations. Correlation line shows the relationship for all fish
1145 combined (n = 50). Ration treatments are shown for reference.
1146
1147 **Figure 8**. Relative mRNA levels for *igf1*, *igf2*, and several genes encoding Igfbps in
1148 skeletal muscle of rockfish from the high (4%) and low (1%) ration treatments. (**A**)
1149 Transcript abundance for *igf1,* but not *igf2,* was higher in fish from the high ration
1150 treatment. (**B**) Only *igfbp5a* and *igfbp5b* mRNA levels in muscle differed between the
1151 two ration treatment groups. Data are plotted as mean (±SEM) values, and lines
1152 indicate significant differences between treatments (Student's t-test: $*P < 0.05$, $**P <$
1153 0.01, $***P < 0.0001$).

1154
1155 **Figure 9**. Correlation between plasma Igf1 and relative levels of mRNAs encoding *igf1*
1156 in skeletal muscle. The line represents the Pearson correlation relationship for all fish
1157 combined (n = 50). Treatment groups shown for reference.

Table 1. Mass and length-specific somatic growth rate (SGR) correlations to plasma Igf1 calculated across different time intervals.

	Day 0 – 98	**24 – 98**	**48 – 98**	**75 – 98**	**91 – 98**
Mass SGR	r = 0.4156 p = **0.0027**	r = 0.4171 p =**0.0026**	r = 0.4243 p = **0.0021**	r = 0.4266 p = **0.0020**	r = 0.2361 p = 0.0988
Length SGR	r = 0.3358 p = **0.0171**	r = 0.3181 p = **0.0244**	r = 0.3135 p = **0.0267**	r = 0.2895 p = **0.0414**	r = 0.3868 p = **0.0150**

Figure 1



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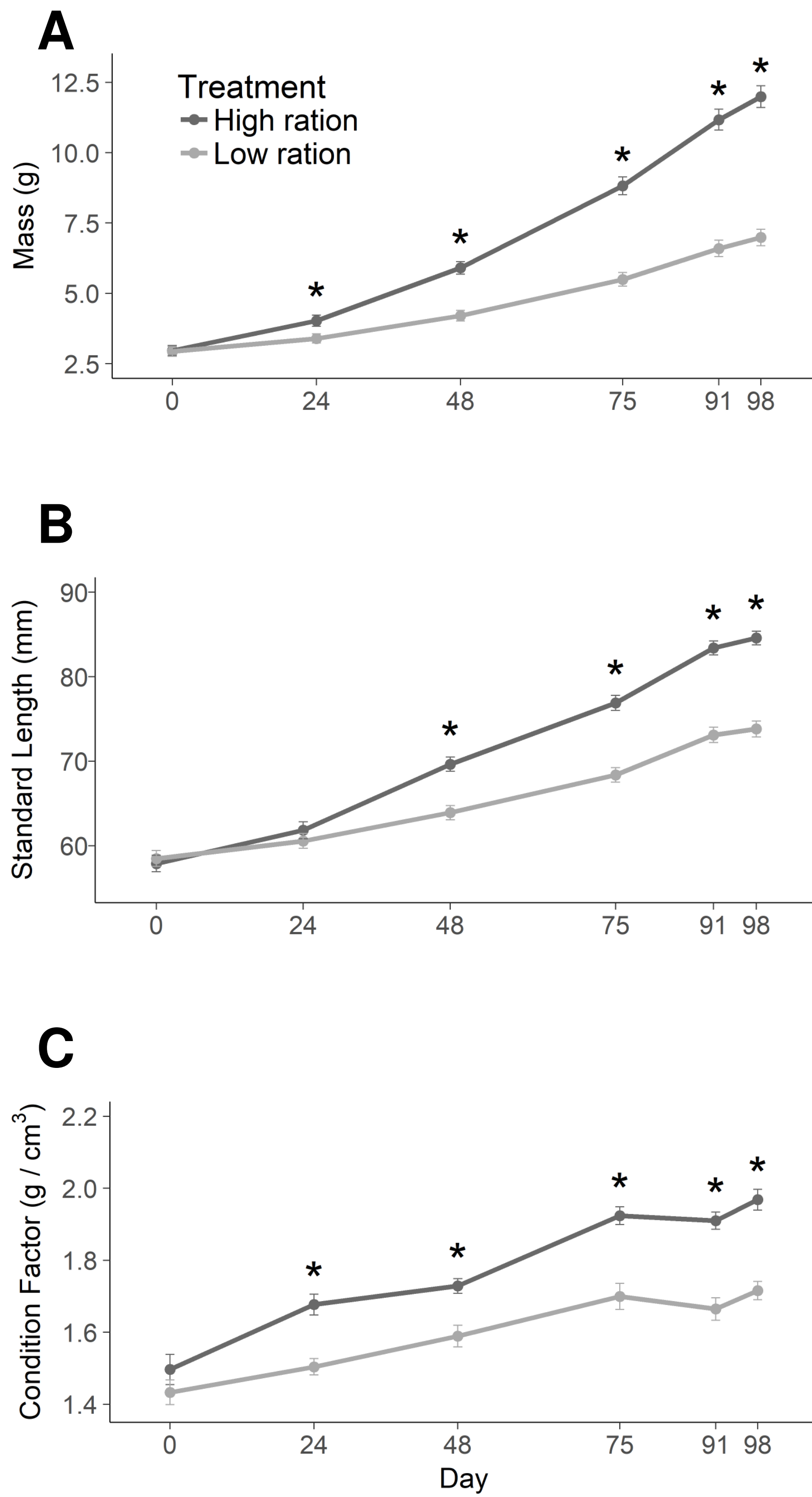

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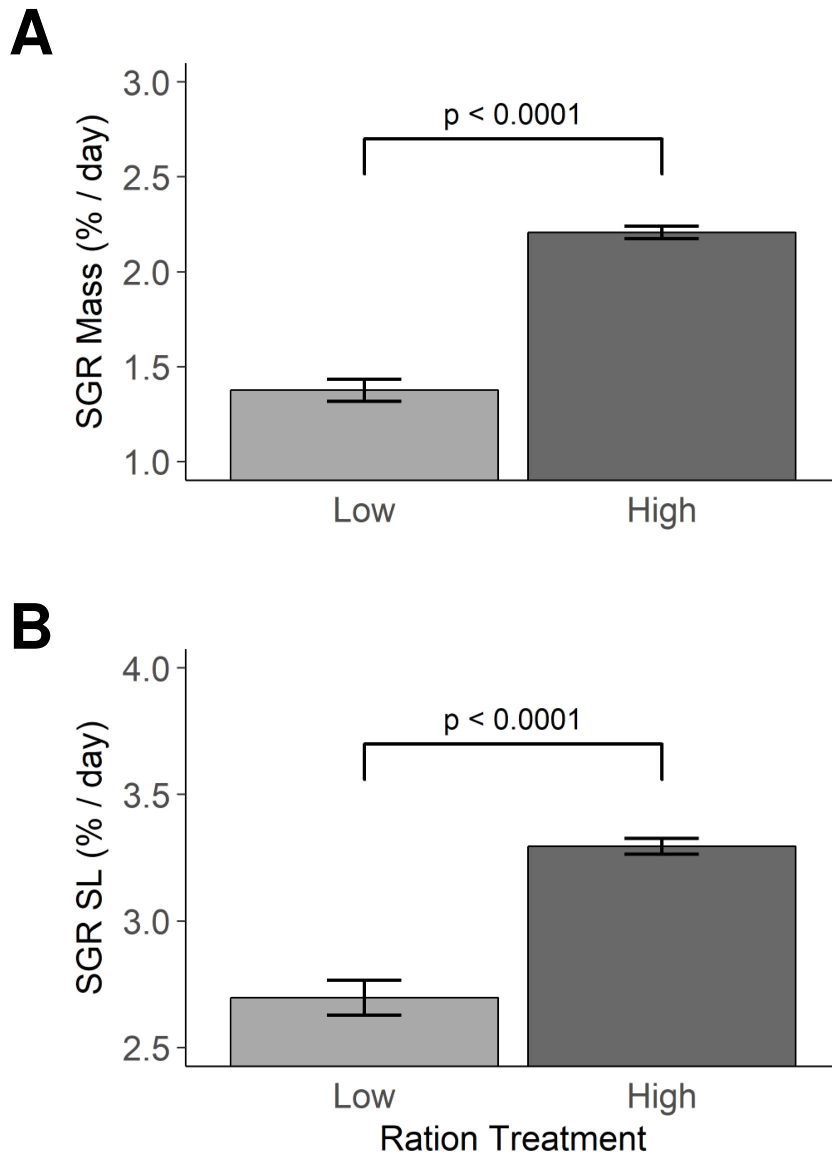

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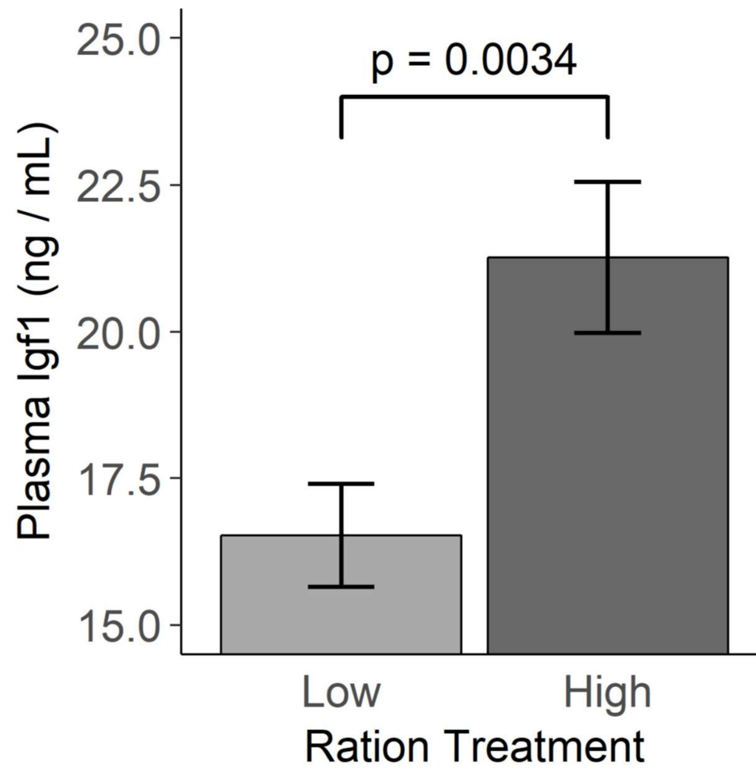

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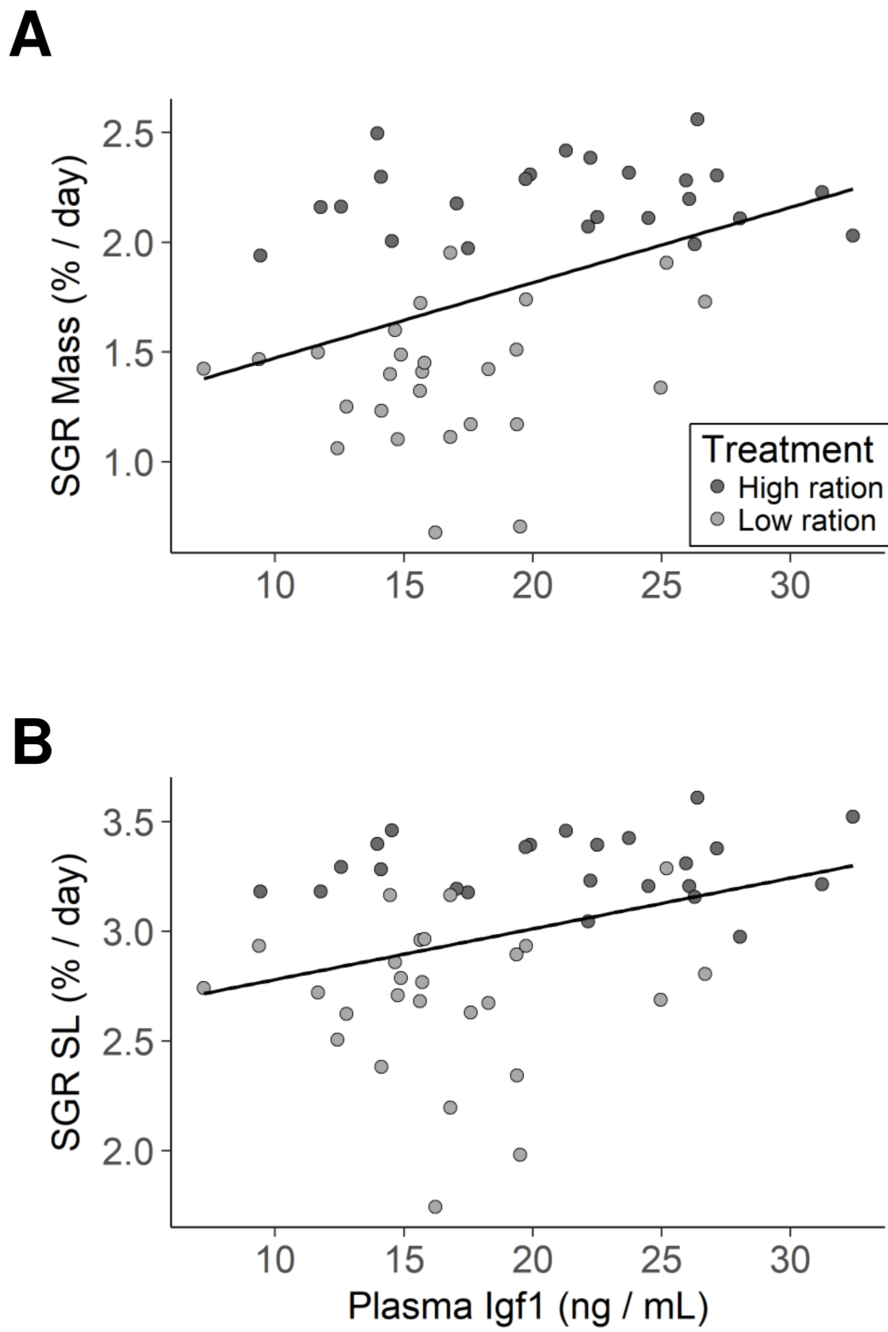

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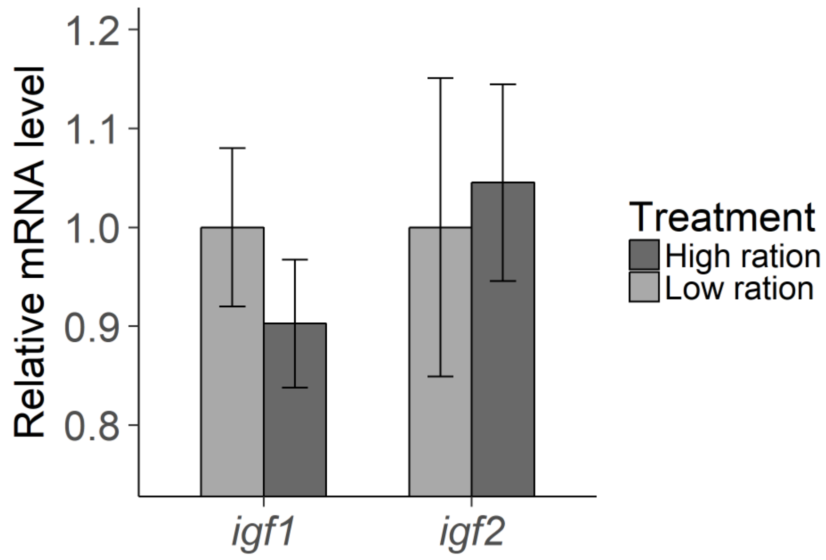

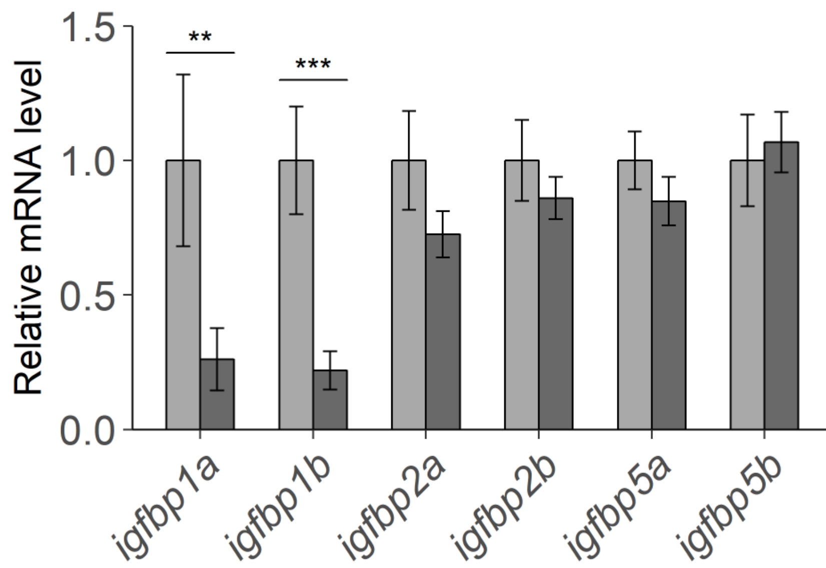

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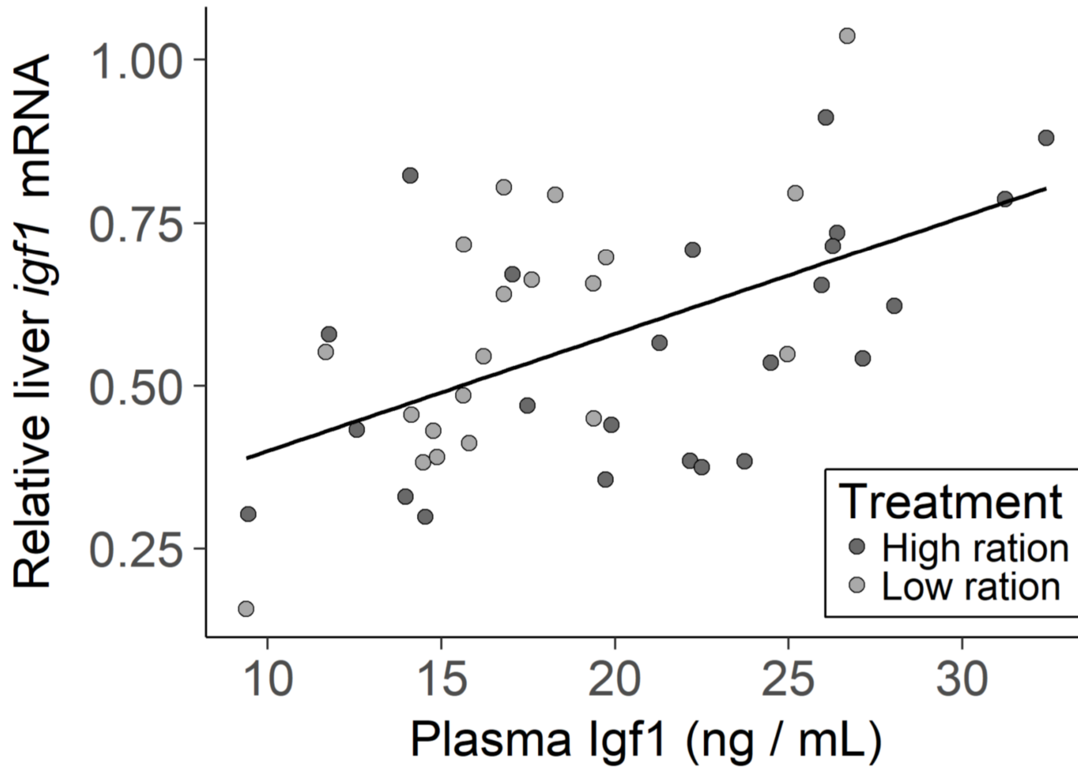

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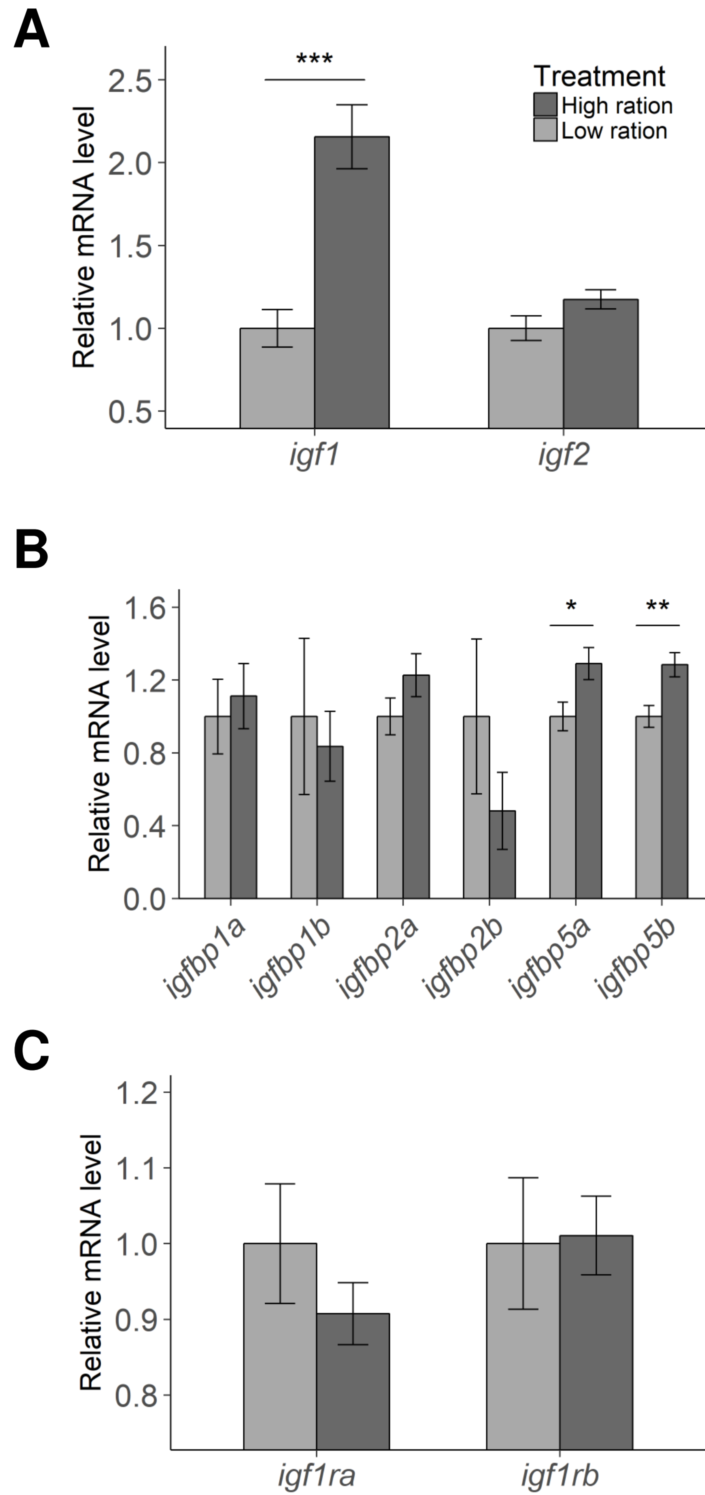

Figure 9