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Interactions of long-term food ration variation and short-term fasting on insulin-like growth factor-1 (IGF-1) pathways in copper rockfish (*Sebastes caurinus*)

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ABSTRACT

Variation in food intake affects somatic growth by altering the expression of hormones in the somatotropic endocrine axis including insulin-like growth factor-1 (IGF-1). Here, we examined IGF-1 pathway responses to long- and short-term variation in food availability in copper rockfish (*Sebastes caurinus*), a nearshore Pacific rockfish important for commercial and recreational fisheries. Juvenile copper rockfish were raised under differing ration amounts (3% or 9% mass feed·g^{-1} fish wet mass·day^{-1}) for 140 d to simulate 'long-term' feeding variation, after which some fish from both rations were fasted for 12 d to generate 'short-term' conditions of food deprivation. Rockfish on the 9% ration treatment grew more quickly than those on the 3% ration and were larger in mass, length, and body condition (k) after 152 d. Fish on the 9% ration had higher blood glucose than those on the 3% ration, with fasting decreasing blood glucose in both ration treatments, indicating that both long-term and short-term feed treatments altered energy status. Plasma IGF-1 was higher in rockfish from the 9% ration than those in the 3% ration and was also higher in fed fish than fasted fish. Additionally, plasma IGF-1 related positively to individual variation in specific growth rate (SGR). The positive association between IGF-1 and SGR showed discordance in fish that had experienced different levels of food and growth over the long-term but not short-term, suggesting that long-term nutritional experience can influence the relationship between IGF-1 and growth in this species. Rockfish on the 3% ration showed a lower relative abundance of gene transcripts encoding *igf1* in the liver, but higher hepatic mRNAs for IGF binding proteins *igfbp1a* and *igfbp1b*. Fasting similarly decreased the abundance of *igf1* mRNAs in the liver of fish reared under both the 9% and 3% rations, while concurrently increasing mRNAs encoding the IGF binding proteins *igfbp1a*, *-1b*, and *-3a*. Hepatic mRNAs for *igfbp2b*, *-5a,* and *-5b* were lower with long-term ration variation (3% ration) and fasting. Fish that experienced long-term reduced rations also had higher mRNA levels for *igfbp3a*, *-3b*, and IGF receptors isoforms A (*igf1rA*) and B (*igf1rB*) in skeletal muscle, but lower mRNA levels for *igf1*. Fasting increased muscle mRNA abundance for *igfbp3a*, *igf1rA*, and *igf1rB*, and decreased levels for *igfbp2a* and *igf1*. These data show that a positive relationship between circulating IGF-1 and individual growth rate is maintained in copper rockfish even when that growth variation relates to differences in food consumption across varying time scales, but that long- and short-term variation in food quantity can shift basal concentrations of circulating IGF-1 in this species.

1. Introduction

Oceans are dynamic environments with temperature, surface currents, and vertical mixing varying on seasonal, interannual, or longer timescales (Deser et al., 2010). In the nearshore ocean, seasonal changes in wind intensity contribute to variation in upwelling intensity, which alters the abundance and quality of food resources for marine fishes (Beare and McKenzie, 1999; Logerwell et al., 2003). While the

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behaviors and life histories of marine fishes may be adapted to such seasonal variation, interannual variability in the timing and intensity of processes such as upwelling can generate spatial and temporal disparities in food availability (e.g., Bograd et al., 2009). Understanding the ecophysiological mechanisms by which food variability impacts somatic growth in marine fishes is crucial for predicting consequences for population processes including growth performance, reproductive output, and recruitment in marine fishes (Barth et al., 2007; Caselle et al., 2010; VenTresca et al., 1996; von Biela et al., 2015), as well as how fish populations will respond to future changes in climate conditions (Lefevre et al., 2017; Waples and Audzijonyte, 2016).

Food availability and nutritional composition influence somatic growth in fishes via several endocrine pathways including those involving thyroid hormones, glucocorticoids, and somatotropic hormones such as the growth hormone (GH)/insulin-like growth factor (IGF) axis (Picha et al., 2008a; Power et al., 2001; Sadoul and Vijayan, 2016; Won and Borski, 2013). The GH/IGF system, in particular, has been shown to regulate growth in response to both shorter- and longer-term variation in food availability (Fuentes et al., 2012a,b; Picha et al., 2008a; Reindl and Sheridan, 2012). In vertebrates, GH mediates growth directly by promoting certain metabolic processes in tissues (i.e., increasing lipolysis in adipose tissues, elevating triglyceride uptake by the liver, etc.) as well as indirectly by stimulating the synthesis and release of somatomedins including IGF-1 (Bergan-Roller and Sheridan, 2018), which promotes cell proliferation, cell differentiation and growth (Chen et al., 2000; Laviola et al., 2007; Reinecke et al., 2005; Wood et al., 2005). These effects of IGF-1 are influenced by IGF binding proteins (IGFBPs) that modulate the availability of IGF-1 (Butler and Le Roith, 2001; Duan and Xu, 2005). In addition to the hepatic production of IGF-1, the local production of IGF-1 in peripheral tissues also appears to be an important regulator of growth, as can be IGFBPs via their direct actions on metabolism, cell proliferation, and growth (e.g., Baxter, 2015; Chan et al., 2009; Clemmons, 2016; Jogie-Brahim et al., 2009).

Several components of the GH/IGF-1 system have been observed to respond to food availability and nutritional status in fishes. The majority of evidence for nutritional regulation of GH/IGF-1 signaling comes from experimental studies exposing teleost fishes to short-term conditions of complete food deprivation (i.e., fasting). Several studies have found that plasma GH concentrations in fish increase hours to weeks after beginning fasting (Fuentes et al., 2012a; Norbeck et al., 2007; Pierce et al., 2005; Salgin et al., 2012). Although fasting induces an elevation of circulating GH, plasma IGF-1 concentrations and growth typically decline in fasted fish (Norbeck et al., 2007; Pierce et al., 2007). Studies have also shown that liver *igf1* mRNA levels decline when fish experience complete food deprivation (Ayson et al., 2007; Breves et al., 2016; Duan and Plisetskaya, 1993; Fox et al., 2010; Kawaguchi et al., 2013; Larsen et al., 2001; Pedroso et al., 2006; Peterson and Waldbieser, 2009; Pierce et al., 2005; Vera Cruz et al., 2006; for reviews, see Beckman, 2011; Picha et al., 2008a), suggesting that GH stimulation of hepatic IGF-1 production is inhibited by fasting. This inhibition may occur via several interacting mechanisms including a downregulation of hepatic GH receptor expression that reduces liver sensitivity to GH (Gray et al., 1992; Norbeck et al., 2007; Peterson et al., 2009; Picha et al., 2008b; Saera-Vila et al., 2005; Small et al., 2006), the activation of pathways exerting inhibitory control on liver IGF-1 production (e.g., fibroblast growth factor 21 [FGF21]; see Beauloye et al., 2002; Inagaki et al., 2008), or a shift in GH action away from anabolism-promoting pathways in the liver and toward catabolic pathways such as hepatic lipolysis (Bergan-Roller and Sheridan, 2018).

Experimental studies in fishes and other vertebrates have found that short of complete food deprivation, small variations in nutrition can also alter plasma IGF-1. In rodents, long-term moderate caloric restriction without malnutrition reduces serum IGF-1 by ~40% (Breese et al., 1991; Dunn et al., 1997). Teleost fishes appear to show similar IGF-1 responses to moderate food restriction. For example, juvenile coho salmon (*Oncorhynchus kisutch*) reared under larger feed rations have been shown repeatedly to have higher circulating IGF-1 (Beckman et al., 2004a; Pierce et al., 2001). Individual IGF-1 concentrations in these salmon also associated positively with individual variation in growth rate (Beckman et al., 2004a; Pierce et al., 2001). A recent study with juvenile olive rockfish (*Sebastes serranoides*) also found lower plasma IGF-1 levels in fish raised on a reduced ration compared to fish under a higher ration allocation and higher somatic growth rate (Hack et al., 2018). In gilthead sea bream (*Sparus aurata*), fish fed a smaller ration showed both reduced GH binding capacity and lower IGF-1 immunoreactivity in the liver, suggesting that reduced food intake diminishes liver sensitivity to GH induction of IGF-1 production (Pérez-Sánchez et al., 1995).

Despite the large number of studies examining how food availability influences GH/IGF-1 signaling in fishes, it remains largely unclear whether prior food availability might alter the effects of fasting on IGF-1 production or peripheral tissue sensitivity to IGF-1. In some scenarios, animals have been observed to adjust later metabolic and growth responses to severe nutritional stresses based on prior experiences (e.g., Criscuolo et al., 2008; Krause et al., 2009), and adjustments to IGF-1 production or tissue sensitivity to IGF-1 could be contributing factors in such adjustments. In this study, we tested whether prior nutritional experience alters responses of circulating IGF-1 and IGF-1 signaling-associated gene expression in the liver and skeletal muscle of juvenile copper rockfish (*Sebastes caurinus*), a nearshore Pacific rockfish species important for both recreational and commercial fisheries. By feeding juvenile rockfish under a 9% or 3% food ration amount (% mass of feed per fish wet mass) over 140 d ('long-term' food ration variation), we raised fish to have higher or lower growth rates. After that 140 d period, some rockfish from both ration treatments were then fasted for 12 d while other fish continued to be fed ('short-term' food deprivation). We then examined the interactions of variation in 'long-term' ration amount on the responses of IGF-1 signaling pathways in liver and skeletal muscle to 'short-term' fasting.

2. Materials and methods

2.1. Animal collection

Young-of-the-year juvenile copper rockfish (*S. caurinus*) were collected during their coastal pelagic juvenile life phase using a Standard Monitoring Unit for the Recruitment of Fishes (SMURF) (e.g., Ammann, 2004; Wilson et al., 2008), which was positioned 1–2 m below the ocean surface at California Polytechnic State University's Center for Coastal Marine Sciences (CCMS) pier facility in Avila Beach, CA, USA (35°10′12.3″N 120°44′27.2″W). The SMURF was deployed between 5 May and 23 Sept 2016 for durations of 3 to 11 d (mean ± SD: 4.35 ± 1.66 d). All fish collected from the SMURF were maintained in captivity at the CCMS pier facility in flow-through 340 L tanks under ambient photoperiod, salinity (33‰) and temperature (range: 12.4 – 18.9 °C). Fish were fed *ad libitum* daily with commercial fish pellet feed (BioPro 2 pellets, BioOregon, Longview, WA, USA; BioPro 2 composition: 50% protein [min.], 22% lipid [min.], 8.5% moisture [max.], 1.0% fiber [max.], 13% ash [max.]) prior to beginning the experimental ration treatments (at least 5 months following collection). All experimental procedures were approved by the Animal Use and Care Committee of California Polytechnic State University, San Luis Obispo (Protocol # 1504).

2.2. Sequencing of partial cDNAs associated with IGF signaling in copper rockfish

2.2.1. RNA isolation and reverse transcription

In order to identify and sequence partial cDNAs for genes associated with IGF-1 signaling in *S. caurinus*, a single juvenile fish (101.2 mm SL, 24.54 g body mass) was euthanized using tricaine methanesulfonate (MS222, 300 mg/L; Argent Chemicals, Redmond, WA, USA). Total RNA

was extracted from the liver and skeletal muscle tissues using TRIreagent® (Molecular Research Center, Inc., Cincinnati, OH, USA), with bromochloropropane used for phase separation. Extracted RNA was then quantified by spectrophotometry (260:280 = 2.02; P300 NanoPhotometer, Implen, Inc., Westlake Village, CA, USA) and DNase treated (TURBO DNA-free Kit, Life Technologies, Grand Island, NY, USA).

First strand cDNA was synthesized in 20 µl reverse transcription reactions by incubating 2.85 µg total DNase-treated RNA template (8 µl) with 1 µl dNTPs (10 mM, Promega Corp., Madison, WI, USA), 1 µl oligo (dT) primers (500 µg/ml; Promega Corp.), 0.5 µl recombinant RNasin ribonuclease inhibitor (40 u/µl; Promega Corp.), 1.5 µl nuclease-free H_2O, 4 µl 5x buffer, 3 µl $MgCl_2$ (25 mM), and 1 µl GoScript™ reverse transcriptase (Promega Corp.). Reverse transcription reactions were run with a thermal profile of 25 °C for 5 min and 42 °C for 1 hr, followed by 70 °C for 15 min to inactivate the reverse transcriptase.

2.2.2. Amplification and sequencing of partial cDNA sequences

PCR was performed using degenerate primers designed from consensus regions of sequences for *igf1*, *igf2,* IGF-1 receptors A (*igf1rA*) and B (*igf1rB*), and IGFBPs identified from the genomes of the following rockfish species: flag rockfish, *Sebastes rubrivinctus* (**GCA_000475215**); tiger rockfish, *Sebastes nigrocinctus* (**GCA_000475235**), and rougheye rockfish, *Sebastes aleutianus* (**GCA_001910805**). Select cDNAs encoding the complete open reading frames for *igf1* (**AF481856**), elongation factor 1α (*ef1α*, **KF430623**), and 60S ribosomal protein L17 (*rpl17*, **KF430620**) from Schlegel's black rockfish (*S. schlegelii*) were also used for primer design. A detailed description of the design and nucleotide sequences for most of these degenerate primers is provided in Hack et al. (2018), and nucleotide sequences of degenerate primers for *igfbp3a* and *igfbp3b* are available in Supplementary Materials Table S1.

Complementary DNA was amplified in 50 µl reactions comprised of 25 µl of GoTaq® Colorless PCR Master Mix (Promega Corp.), 1 µl each of forward and reverse primer (10 µM for gene-specific primers, 50 µM for degenerate primers), 21 µl of RNase-free H_2O, and 2 µl of cDNA under a thermal profile of 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s and 52–55 °C for 1 min, and then 72 °C for 2 min. The resulting PCR products were then examined on 2% ethidium bromide gels. If needed, nested PCR reactions were performed to further amplify cDNA products. Resulting PCR products of predicted size were cleaned (QIAquick PCR Purification Kit, Qiagen) and Sanger sequenced (Molecular Cloning Lab, Inc., South San Francisco, CA, USA) using the same forward and reverse primers for the PCR reactions. The resulting sequences were assembled using Sequencher v5 software (Gene Codes Corp., Ann Arbor, MI, USA), and then compared for identity using the National Center for Biotechnology Information BLAST program (http://blast.ncbi.nlm.nih.gov/). Additional characterization and comparative analyses of the deduced polypeptide generated by the resulting IGFBP cDNAs was conducted by alignment using ClustalX software (Larkin et al., 2007) followed by phylogeny construction in MEGA v.7 software (Kumar et al., 2016), using the Neighbor-Joining method and a p-distance model for tree construction (Saitou and Nei, 1987). Positions containing alignment gaps were deleted only in pairwise comparisons (pairwise deletion of gaps) and uniform rates were assumed among sites. Confidence values for nodes were obtained by bootstrapping (1000 replicates).

2.3. Experimental growth rate treatments

All sexually-immature, juvenile copper rockfish were tagged intraperitoneally with passive integrated transponders (PIT tags) (7 mm, Loligo Systems, Inc., Viborg, Denmark), which permitted individual identification for repeated measurements of fish body size throughout the experiment. Tagged rockfish then were systematically assigned to one of eight 340 L tanks (0.97 m diameter × 0.48 m depth), with 9 fish per tank. Tanks were maintained with flow-through filtered seawater (~33 ppt) under ambient ocean water temperatures (12.45 ± 0.27 °C, mean ± SD) and photoperiod throughout the duration of the experiment. Rockfish were assigned to these 340 L tanks on the basis of body mass, so that each tank had a similar average body size at the beginning of the experiment.

On 10 Feb 2017, juvenile copper rockfish in the 340 L experimental tanks were weighed and measured for an initial (day 0) body size measurement prior to commencing experimental ration treatments. That day 0 measurement confirmed that the body mass (one-factor ANOVA: body mass, $F_{7,63} = 0.059$, $p = 0.9997$) and standard length ($F_{7,63} = 0.016$, $p = 0.9999$) of copper rockfish in the experimental tanks was similar prior to commencing the experimental ration treatments. On that same day, rockfish began to be fed one of two experimental food ration treatments: 3% or 9% mass of feed per fish wet mass. Four replicate tanks were used for each ration treatment, for a total sample size of $n = 35$–36 fish per ration level (due to the mortality of a single fish in one of the 9% ration tanks on day 21 of the experiment). Fish were fed these 3% or 9% rations for 140 d, with all fish weighed and measured on days 28, 58, 87, 120 and 140.

At the end of this 140 d period, rockfish in two of the 340 L tanks for each ration treatment (3% and 9%, $n = 18$ per treatment) were fasted for 12 d. Rockfish in the remaining two tanks for each food ration treatment, however, continued to be fed the 3% or 9% rations (% mass of feed per fish wet mass) daily over that 12 d period. This experimental design allowed examination of the effects on the GH/IGF-1 pathway of both different growth rates and of fasting on previous varying food availability.

At the end of this 12 d fasting or feeding period (day 152), all fish were euthanized using MS222, weighed, and measured. Blood collected by severing the tail was divided into two samples: one sample was centrifuged at 3000 × g for 10 min at 4 °C to obtain plasma, while the other sample was used for quantifying blood parameters including glucose levels, pH, and concentrations of several ions. Plasma was stored at −80 °C until analysis of IGF-1 hormone quantification. The liver and skeletal muscle tissue (fast-twitch or 'white' muscle from the caudal region) were also dissected, immediately frozen in liquid N_2, and stored at −80 °C until nucleic acid extraction.

2.4. Quantification of blood glucose, pH, Na^+, Cl^- and Ca^{+2}

Blood parameters including glucose (mmol l^{-1}) levels, pH, and concentrations of several ions (Na^+, Cl^- and Ca^{+2}) were measured using an ABL90 FLEX Blood Gas Analyzer (Radiometer America, Inc., Brea, CA, USA). To achieve sufficient blood volumes, samples from 2 to 3 fish from the same treatment tank were pooled to obtain a sample volume (65 µl) sufficient for blood parameter analysis, while still leaving enough blood from each separate fish to allow for plasma IGF-1 quantification. Sample sizes were reduced to $n = 4$–7 per treatment following blood pooling.

2.5. Plasma IGF-1 hormone quantification

Plasma IGF-1 concentrations were determined using a time-resolved fluoroimmunoassay (TR-FIA) method (Small and Peterson, 2005). This TR-FIA was modified from an RIA described by Shimizu and colleagues (2000) and is described in detail elsewhere (Ferriss et al., 2014). In brief, 20–35 µl volumes of plasma extract from rockfish were assayed using antiserum to recombinant barramundi (*Lates calcarifer*) IGF-1 (GroPep BioReagents, Ltd., Thebarton, SA, Australia) (Degger et al., 2000). Assays were run using dissociation enhanced lanthanide fluorescence immunoassay (DELFIA®, Perkin-Elmer) anti-rabbit IgG-coasted yellow 96-well plates and custom-labeled recombinant salmon IGF-1 (GroPep BioReagents, Ltd.). Extracts from all plasma samples were assayed in duplicate.

2.6. Measurement of RNA:DNA ratio

RNA/DNA ratio in the liver and muscle was measured using a spectrofluorimetric method described by Grémare and Vétion (1994), and modified by Kawaguchi and colleagues (2013). Frozen tissue was digested at 4 °C in a 20 mM phosphate buffered saline solution (with 0.15 M NaCl, pH 7.5) using Protease K (20 mg/ml; Invitrogen). After digestion, 56 µl of 0.1% sodium dodecyl sulfate (SDS) was added, and samples were incubated on ice for 15 min with mixing every 3 min. Samples were then centrifuged for 15 min at 4500 × g and 4 °C, and the resulting supernatant was used for measurement of RNA:DNA ratio.

DNA concentrations were measured spectrophotometrically using Hoechst 33,258 (Sigma-Aldrich), and total nucleic acid concentrations were measured using Thiazole orange (Sigma-Aldrich). DNA was quantified by combining 100 µl of supernatant with 50 µl of PBS buffer and 5 µl of Hoechst 33,258 solution (0.02 mg/ml). After incubation at 37 °C for 30 min, fluorescence was measured on a VICTOR X4 Multilabel Plate Reader (PerkinElmer, Waltham, MA, USA) at an excitation of 355 nm and emission of 460 nm. To quantify total nucleic acids, supernatant (100 µl) was combined with 50 µl of PBS buffer and 5 µl of Thiazole orange (4 µg/ml), and then assayed at 490 nm excitation and 545 nm emission. Standard curves for both assays were generated using purified DNA from the testes of chum salmon, *Oncorhynchus keta* (Sigma-Aldrich). Standards were assayed in triplicate, and all samples assayed in duplicate. The intra-assay % CV was 8.2% for the Hoechst 33,258 assay, and 4.7% for the Thiazole orange assay.

2.7. SYBR Green real-time quantitative RT-PCR assays

Total RNA was extracted from the liver and skeletal muscle using TriReagent (Molecular Research Center, Inc.) with bromochloropropane as the phase separation reagent. Total RNA was then DNase I treated (TURBO DNA-free Kit, Ambion), quantified by spectrophotometry (P300 NanoPhotometer, Implen; 260:280 ratios ≥ 1.95), and diluted to the same concentration before being reverse transcribed in 28 µl reactions containing 5.6 µl of 5× GoScript™ Buffer, 4.2 µl of $MgCl_2$ (25 mM), 1.4 µl dNTPs (10 mM, Promega Corp., Madison, WI, USA), 1.4 µl random hexamer primers (500 µg/ml; Promega Corp.), 0.11 µl recombinant RNasin ribonuclease inhibitor (40 u/µl; Promega Corp.), 0.25 µl nuclease-free H_2O, 1.05 µl GoScript™ reverse transcriptase (Promega Corp.), and 14 µl of RNA template (68 ng/µl for liver RNA, 35.3 ng/µl for muscle RNA) under a thermal profile of 25 °C for 5 min and 42 °C for 1 h, followed by 70 °C for 15 min.

Real-time quantitative PCR was then used to measure relative mRNA levels of genes involved in IGF signaling. Quantitative PCR reactions were run in 16 µl volumes containing 8 µl of iTaq™ Universal SYBR Green Supermix (BioRad Laboratories, Inc.), 4.5 µl of nuclease-free H_2O, 1.5 µl of cDNA, and 1 µl each of forward primer (10 µl) and reverse primer (10 µl). SYBR Green primers for each gene of interest were designed to the partial cDNAs isolated from copper rockfish as described above. Primers designed to *ef1α* and 60S ribosomal protein L17 (*rpl17*) from copper rockfish were used as endogenous reference genes. When possible, primers were designed to span an intron boundary. All primers were synthesized by Eurofins MWG Operon (Huntsville, AL, USA). Primer sequences are provided in Supplementary Materials Table S2. The specificity of each primer set was confirmed by cloning (TOPO® TA Cloning, Life Technologies) and Sanger sequencing select PCR products (Molecular Cloning Laboratories, South San Francisco, USA).

Quantitative real-time PCR (qRT-PCR) was performed in accordance with the guidelines of Bustin and coworkers (2009). All quantitative PCR reactions were run on a CFX96™ Real-Time PCR Detection System (BioRad Laboratories, Inc.) under a thermal profile of 95 °C for 2 min and 45 cycles of 95 °C for 10 s and 60 °C for 30 s, followed by a melt curve analysis. Standard curves for qRT-PCR were made for each tissue from RNA pooled from fish representing all treatment groups. Each standard was serially diluted and assayed in triplicate. DNA contamination was assessed by analyzing RNA samples that were not reverse-transcribed. Each qPCR run also included samples without cDNA as a further control. PCR efficiencies for each gene were calculated as % efficiency = $[10(^{1/slope}) - 1]\cdot 100$, and are provided for each set of primers in Supplementary Materials, Table S2. Correlation coefficients (r^2) were > 0.97 for the standard curve for each gene. For each tissue, the geometric mean value of *ef-1α* and *rpl17* was calculated and used to normalize the relative expression level of each mRNA in that given tissue. This geometric reference gene value did not vary with treatment conditions in the skeletal muscle ($p > 0.16$) but was affected by both food ration amount and fasting in the liver ($p < 0.0001$). The geometric reference gene value in liver was therefore normalized further to RNA:DNA ratio, as per the suggestion of Metzger and coworkers (2012) for the use of qRT-PCR approaches to measure liver mRNA levels in fasted teleost fishes. The RNA:DNA ratio in the liver varied among treatment groups (one-factor ANOVA, ration effect: $F_{1,66} = 16.091$, $p = 0.0002$), with fasted fish exhibiting lower RNA:DNA ratios than fed fish (Supplementary Materials, Fig. S4). In muscle, RNA:DNA ratio did not vary significantly among treatments ($F_{1,66} = 0.707$, $p = 0.5514$). Normalizing the reference gene value to RNA:DNA, however, resulted in similar hepatic reference gene values across all treatment groups ($p = 0.5429$). All gene transcript abundance data were therefore first normalized to RNA:DNA ratios and reference gene abundances for each tissue (Metzger et al., 2012), and then plotted as a relative level normalized to the mean value of that gene observed in rockfish from the 9% ration, fed treatment.

2.8. Statistical analyses

All data were examined for normality and equivalence of variances using both a Levene's test and a Bartlett test prior to treatment analyses. In cases where data failed to conform to normality, data was $\log(x + 1)$ transformed prior to analysis. All tests were two-tailed using an $\alpha = 0.05$, and were performed using JMP® Pro 12.2.0 software (SAS Institute Inc., Cary, NC, USA).

First, *t* tests were used to compare initial standard length, body mass, and body condition (k) between rockfish in the 3% and 9% ration treatment tanks at day 0, prior to commencing the different feeding amounts. Two-factor repeated measures ANOVA models with ration treatment (3% or 9%) and 'fed' or 'fasted' condition (representing the last 12 d) were then used to test for ration treatment effects on standard length (SL), body mass and condition factor between day 0 and day 152, immediately prior to commencing fasting of a subset of treatment tanks. Since rockfish in the 'fed' and 'fasted' categories from the same ration (3% or 9%) treatment showed no statistically significant differences in SL, mass or condition factor prior to commencing fasting on day 140, fish from these treatments were combined from day 0 to 140 in the data visualizations shown in Fig. 1. Specific growth rate (SGR) values as calculated from the change in body mass or length (SL) from day 0 to day 152 were compared using two-factor ANOVA models with 'ration' treatment, 'fed/fasted' treatment, and the interaction between these factors. Tukey HSD tests were used for pairwise comparisons of SGR among treatment groups.

Blood concentrations of glucose, Cl^-, and Ca^{+2} – as well as blood pH – were $\ln(x)$ transformed prior to comparison using a two-factor ANOVA model with 'ration' and 'fed/fasted' as main effects, followed by a Tukey HSD test for multiple comparisons. Plasma IGF-1 concentrations were compared using a two-factor ANOVA model with 'ration' treatment, 'fed/fasted' treatment, and the interaction between these factors. Post hoc comparisons between treatments was conducted using Tukey HSD tests. Linear regression models were used to test for relationships between individual variation in plasma IGF-1 and SGR (mass-specific and length-specific). The relative abundance of gene transcripts in the liver and muscle tissues were compared using two-

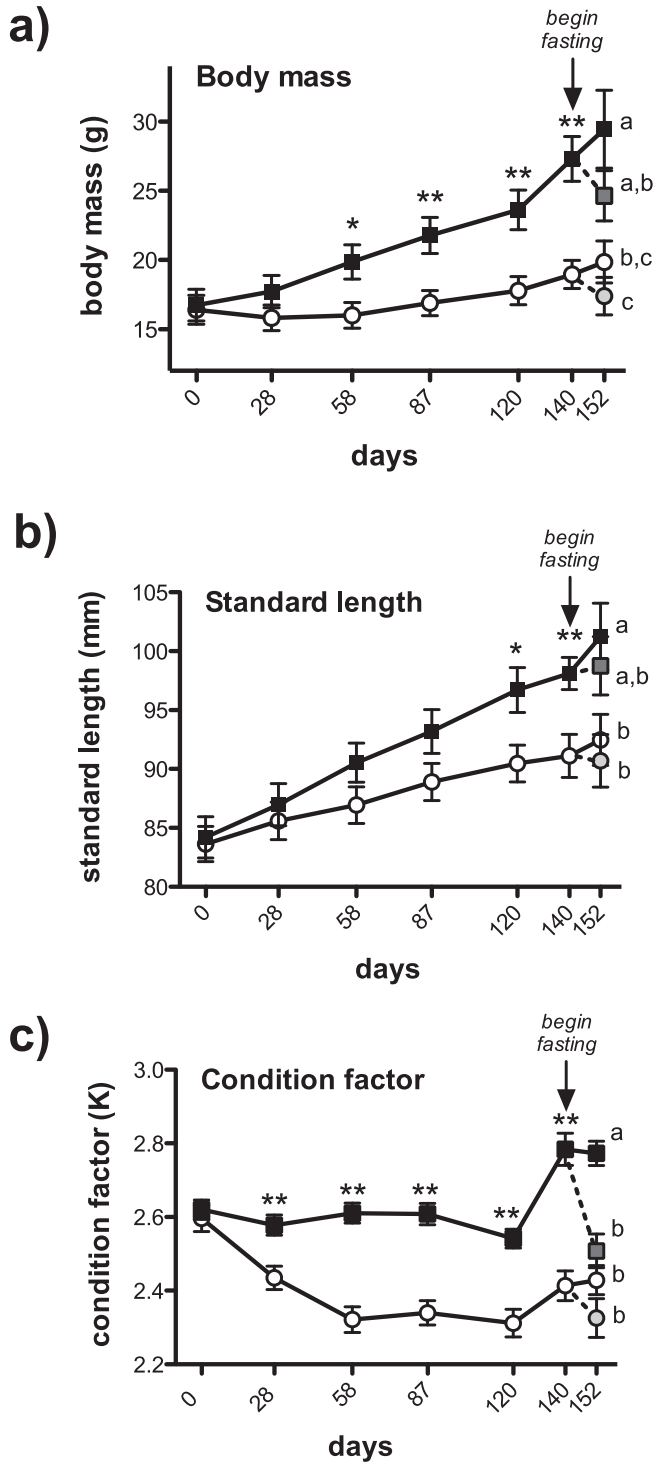

Fig. 1. Mean (± SEM) values of (a) body mass, (b) standard length, and (c) body condition factor (k) for juvenile copper rockfish reared under the 9% (dark squares) or 3% (light circles) ration (% wet wt.) treatments for 140 d, and then either fasted (dotted line) or continually fed for 12 d (days 140–152). Asterisks indicate difference between 3% and 9% ration treatments on that sampling day. Letters indicate pairwise differences among the four treatment combinations on day 152 (Tukey HSD tests).

factor ANOVA models with 'ration' and 'fed/fasted' as main effects and the interaction between these factors. Post hoc comparisons between treatments for each gene transcript were calculated using Tukey HSD tests.

3. Results

3.1. Identification of partial cDNAs from copper rockfish

PCR and Sanger sequencing using degenerate or gene-specific primers amplified partial cDNAs encoding *igf1* (469 bp nucleotides, GenBank Accession no. **MH476242**) and *igf2* (419 bp, **MH476243**) from *S. caurinus*, as well as partial cDNAs for IGF binding proteins *igfbp1a* (676 bp, **MH476244**), *igfbp1b* (687 bp, **MH476245**), *igfbp2a* (515 bp, **MH476246**), *igfbp2b* (677 bp, **MH476247**), *igfbp3a* (157 bp, **MH476248**), *igfbp3b* (571 bp, **MH476249**), *igfbp5a* (200 bp, **MH476250**), and *igfbp5b* (696 bp, **MH476251**). Partial cDNAs for IGF-1 receptors a (426 bps; *igf1ra*: **MH476252**) and b (364 bps; *igf1rb*: **MH476253**) were also amplified and sequenced. Lastly, partial cDNAs encoding *ef1a* (725 bps; **MH476254**) and *rpl17* (458 bps; **MH476255**) were sequenced from *S. caurinus* for use as reference genes for qPCR analyses. BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analyses of the deduced amino acid sequences of these partial cDNAs was used to confirm their identities. Phylogenetic analyses of deduced amino acid sequences for the copper rockfish IGFBP cDNAs confirmed the identities of these binding proteins (Supplemental Materials, Fig. S1).

3.2. Ration-induced variation in body size, condition factor (k), and growth rate

At the beginning of the food ration treatments (day 0), copper rockfish in the four treatment groups (i.e., fed '3% or 9% rations' and then 'fed or fasted' for the last 12 d) were similar in body length (SL: $t = -0.077$, $p = 0.939$), body mass ($t = -0.284$, $p = 0.777$), and body condition ($t = -0.530$, $p = 0.598$). Across the 140 d of rearing in the ration treatments, however, rockfish in the 9% ration treatment grew to become larger in mass (ration·time interaction: $F_{6,65} = 23.594$, $p < 0.0001$) (Fig. 1a) and length (ration·time interaction: $F_{5,65} = 15.500$, $p < 0.0001$) (Fig. 1b) than fish in the 3% ration treatment. At day 140, rockfish in the 9% ration treatment thus exhibited larger body mass ($F_{3,67} = 6.485$, $p = 0.0006$) and length ($F_{3,67} = 4.085$, $p = 0.01$), as well as a greater condition factor ($F_{3,67} = 4.978$, $p = 0.0035$) (Fig. 1c), compared to fish in the 3% ration treatment. There were no differences in mass, length, or condition factor among the 'fed' and 'fasted' tank replicates within each ration treatment (3% or 9% rations) on day 140, prior to beginning the 12 d period of fasting (days 140–152 of the experiment). Following the 12 d period of fasting for some fish, juvenile rockfish reared under the 3% and 9% ration amounts continued to showed differences in length ($F_{1,67} = 12.931$, $p = 0.0006$) and mass ($F_{1,67} = 18.798$, $p < 0.0001$) (Fig. 1a,b). Despite fasting itself not resulting in any statistically significant differences in length ($p = 0.321$) or mass ($p = 0.067$) as measured on day 152, body condition of the juvenile rockfish on day 152 was lower in fish reared under the 3% ration than the 9% ration ($F_{1,67} = 24.309$, $p < 0.0001$), and was also reduced in fish from both ration treatments that experienced fasting for the final 12 d ($F_{1,67} = 15.159$, $p = 0.0002$) (Fig. 1c).

Feeding fish the differing 3% or 9% ration amounts coupled with either fasting or feeding for 12 d generated significant differences in both specific growth rates (SGRs) as calculated using mass or length. Prior to fasting, fish in the 3% and 9% rations already exhibited differences in both mass- and length-specific SGRs (Supplemental Materials, Fig. S2). At 152 d (after 12 d fasting), mass-specific SGR remained different between ration treatments ($F_{1,67} = 130.018$, $p < 0.0001$), and also differed between fasted and fed fish in the 9% ration treatment ($F_{1,67} = 13.525$, $p = 0.0005$) (Fig. 2a). Similarly, length-specific SGR was lower in fish experiencing reduced rations whether fed ($F_{1,67} = 81.088$, $p < 0.0001$) or fasted ($F_{1,67} = 5.755$, $p = 0.0192$) (Fig. 2b). No statistically significant interactions between ration amount and fasting/fed treatments were observed for either mass- or length-SGR.

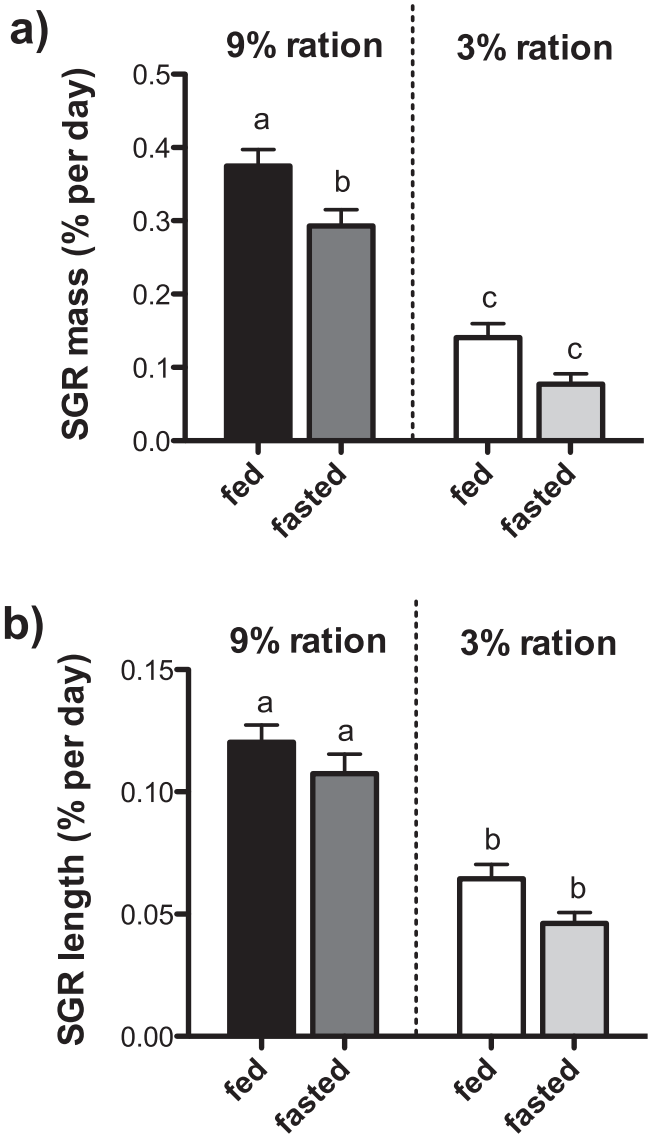

Fig. 2. Mean (± SEM) values of specific growth rate (SGR) on day 152 as calculated by (a) body mass or (b) length. Letters indicate pairwise differences among treatments (Tukey HSD tests).

3.3. Blood glucose, pH, and ion concentrations

Blood glucose concentrations were over twice as high in rockfish in the 9% ration treatment compared to fish in the 3% ration treatment ($F_{3,20} = 6.641$, $p = 0.018$). Fasted fish, however, exhibited significantly lower blood glucose concentrations in both ration treatments ($F_{3,20} = 35.060$, $p < 0.0001$) (Fig. 3). No interaction was observed between the effects of fasting and ration level; rockfish in the 3% ration experienced a 46.7% decline in blood glucose concentration, and fish in the 9% ration experienced a 49.8% decline, after 12 d of fasting.

Blood pH was measured at pH 7.01 ± 0.02 (mean ± SEM) and was similar across treatment groups. Likewise, blood concentrations of Na^+ (184.52 ± 1.10 mmol/L, mean ± SEM), Cl^- (150.36 ± 0.92 mmol/L), and Ca^{+2} (1.38 ± 0.12 mmol/L) did not differ across treatments, indicating that these blood parameters were not affected by feeding variation.

3.4. Plasma IGF-1 varied with nutritional status and growth rate

Plasma IGF-1 concentrations were lower in rockfish that were fasted

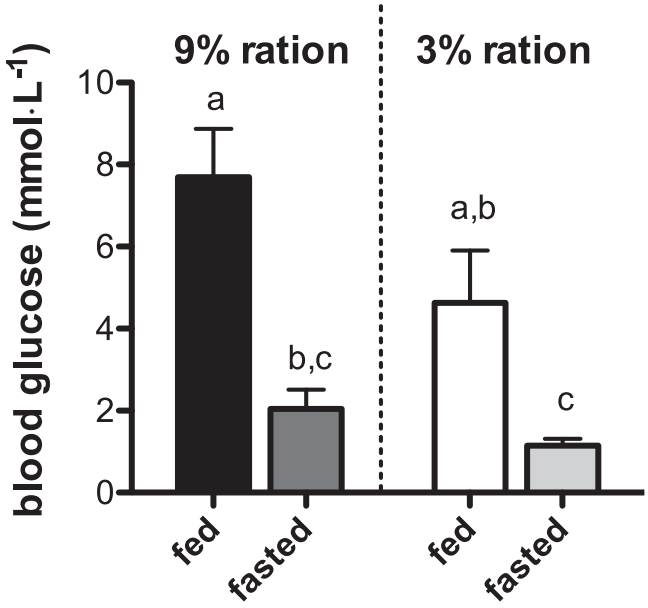

Fig. 3. Blood glucose concentration (mmol·L^{-1}) of juvenile copper rockfish in the 9% or 3% ration treatments that were fasted or continued to be fed for 12 d prior to sampling. Values are plotted as mean ± SEM values. Letters indicate pairwise differences among the treatments (Tukey HSD tests).

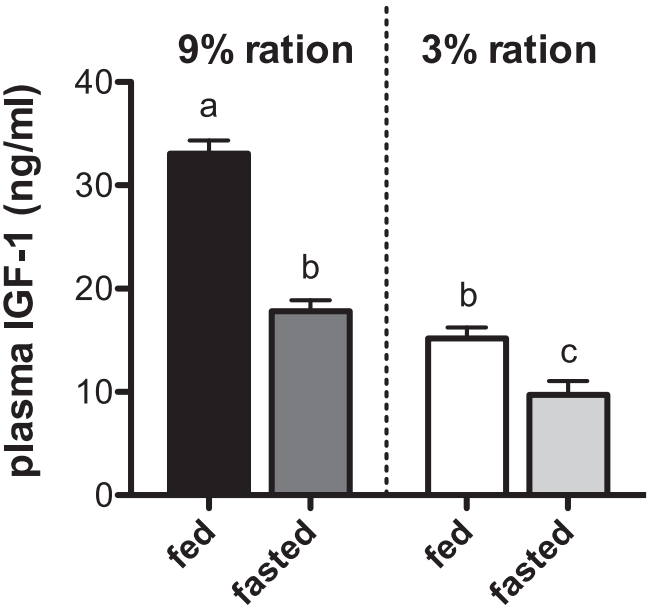

Fig. 4. Comparison of mean (± SEM) plasma IGF-1 concentrations in rockfish across ration and fast/fed treatments. Rockfish in the 9% ration had higher plasma IGF-1 than fish in the 3% ration, and fasted fish from both ration groups showed a reduction in plasma IGF-1, but the degree of that IGF-1 decline differed with prior nutritional experience (ration·fasting interaction; $F_{1,65} = 16.683$, $p = 0.0001$). Letters indicate pairwise differences among the treatments (Tukey HSD tests).

than those that continued to be fed for the last 12 d, but those differences also depended on prior ration treatment (ration·fasting interaction: $F_{1,65} = 16.683$, $p = 0.0001$) (Fig. 4). Fish in in the 9% ration that were fasted for the final 12 d had lower plasma IGF-1 than fish that continued to be fed. Similarly, fish in the 3% ration treatment that were fasted had lower IGF-1 than those on the 3% ration that continued to be fed. Rockfish reared on the 9% ration for the entire 152 d experimental period also had higher IGF-1 than those on the 3% ration, and fish from the 9% ration that were fasted had higher IGF-1 than those from the 3%

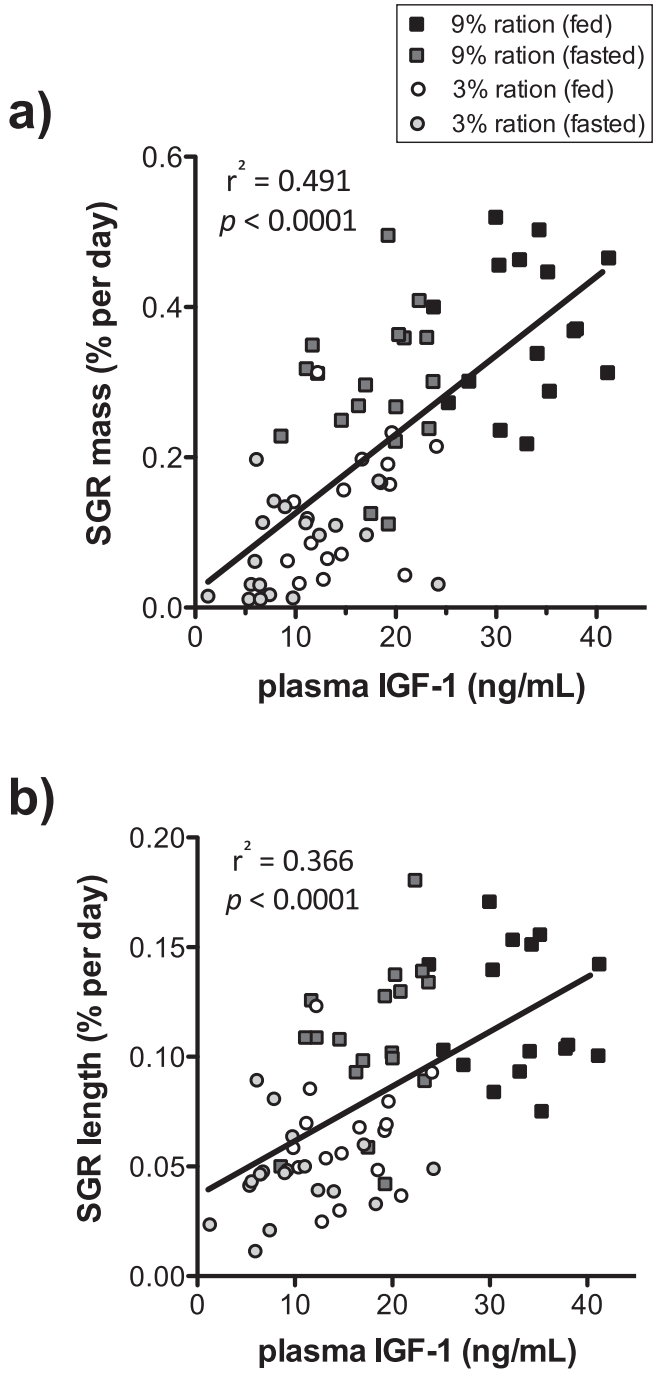

Fig. 5. Relationships between individual variation in plasma IGF-1 and specific growth rate (SGR) as calculated by change in (a) body mass or (b) body length (SL). Individual variation in both mass- and length-SGR, as calculated across the entire experimental period (152 d), associated positively with individual IGF-1 concentrations.

ration that were fasted.

Individual variation in plasma IGF-1 related positively to individual SGR values when SGR was calculated either by change in mass or length from day 0 to 152 (Fig. 5), with the strength of the relationship more robust for mass-specific SGR ($r^2 = 0.491$, $F_{1,67} = 64.631$, $p < 0.0001$) than for length-specific SGR ($r^2 = 0.366$, $F_{1,67} = 38.739$, $p < 0.0001$). Notable, plasma IGF-1 also associated positively with the variation in body size that was generated intentionally by rearing fish under different ration amounts (Supplemental Materials, Fig. S3). To verify that the relationship between individual IGF-1 and SGR resulted from growth variation and not merely variation in size, we calculated residual IGF-1 values from the IGF-1 regression to mass. Fish reared under the 3% ration amount had lower residual IGF-1 values, and mean residual IGF-1 was lower in fasted fish. Individual variation in residual IGF-1 values also associated positively with SGR (Supplemental Materials, Fig. S3), indicating that the observed relationship between circulating IGF-1 and SGR doesn't just emerge from variation in fish body size, but reflects a physiological association between plasma IGF-1 concentrations and growth.

Looking further at mass SGR only, the slope of the positive association with IGF-1 was consistent across all four treatment group combinations of ration-fed/fasting conditions ($F_{3,61} = 0.234$, $p = 0.8724$). However, the intercepts of the mass SGR association with IGF-1 concentrations varied across the four treatments ($F_{3,64} = 13.980$, $p < 0.0001$), suggesting that these particular food ration treatments generated consistently positive relationships between circulating IGF-1 and growth, but with some discordance among treatment groups (Beckman, 2011). Further examination of mass SGR and IGF-1 relationships revealed that long-term rearing under the 9% or 3% rations generated discordance in the elevation (i.e., intercept) of the mass-SGR and IGF-1 positive association (Fig. 6a) ($F_{1,66} = 40.266$, $p < 0.0001$), despite similar slopes for the relationships in both ration treatments ($p = 0.6980$). When examined separately by grouping fish from both rations into fasting/fed treatments only, mass SGR still associated positively with circulating IGF-1 concentration (Fig. 6b) ('fed' groups: $r = 0.779$, $p < 0.0001$; 'fasted' groups: $r = 0.592$, $p < 0.0001$); the relationships for 'fed' and 'fasted' fish in these 'fed' and 'fasted' groups did not differ in either slope ($p = 0.0845$) or intercept ($p = 0.0722$).

Similarly, the relationships between plasma IGF-1 and SGR calculated by length had similar slopes ($p = 0.3585$) but different intercepts ($F_{3,64} = 10.525$, $p < 0.0001$) for each of the four ration-fed/fasted treatment combinations. When grouped by ration treatment, the slopes of the association between length SGR and IGF-1 were found to be similar for fish in both the 9% and 3% rations ($p = 0.3585$), but had distinct intercepts (Fig. 6c) ($F_{3,64} = 10.525$, $p < 0.0001$). When grouped by fasting/fed treatments only, the length SGR associations with IGF-1 had similar slope ($p = 0.2036$) and intercept ($p = 0.0813$) parameters (Fig. 6d).

Since RNA:DNA in the liver varied with fasting (Supplemental Materials, Fig. S4a), individual RNA:DNA values were examined for correlation with an individual's SGR. Individual variation in liver RNA:DNA did not correlate significantly with mass-specific growth rate ($r = 0.210$, $p = 0.081$) or length-specific SGR ($r = 0.197$, $p = 0.103$) (Supplemental Materials, Fig. S5). Likewise, muscle RNA:DNA values for individual fish did not correlated with SGR as determined by change in either mass ($p = 0.761$) or length ($p = 0.519$) (Supplemental Materials, Fig. S6).

3.5. Food ration and fasting experience alter liver gene transcript abundance

The relative abundance of transcripts encoding *igf1* in the liver was lower in rockfish fed the 3% ration compared to the 9% ration amount (Fig. 7a) ($F_{1,66} = 29.220$, $p < 0.0001$), and also was reduced by fasting ($F_{1,66} = 14.694$, $p = 0.0003$). Individual variation in hepatic *igf1* mRNA level correlated positively with plasma IGF-1 concentrations (Fig. 7b) ($r = 0.707$, $p < 0.0001$), indicating that individual differences in circulating IGF-1 arose in part from differences in hepatic *igf1* expression. Each ration-fed/fasting treatment group showed positive associations between liver *igf1* and plasma IGF-1 that were similar in slope ($F_{3,61} = 0.187$, $p = 0.9049$) and intercept ($F_{3,64} = 0.366$, $p = 0.778$), indicating concordance in the hepatic *igf1* and plasma IGF-1 relationship across treatments experiencing differing nutritional conditions (Beckman, 2011).

Similar to *igf1* mRNAs, transcripts for *igf2* were lower in relative abundance under fasting, but only in rockfish reared on the 9% ration

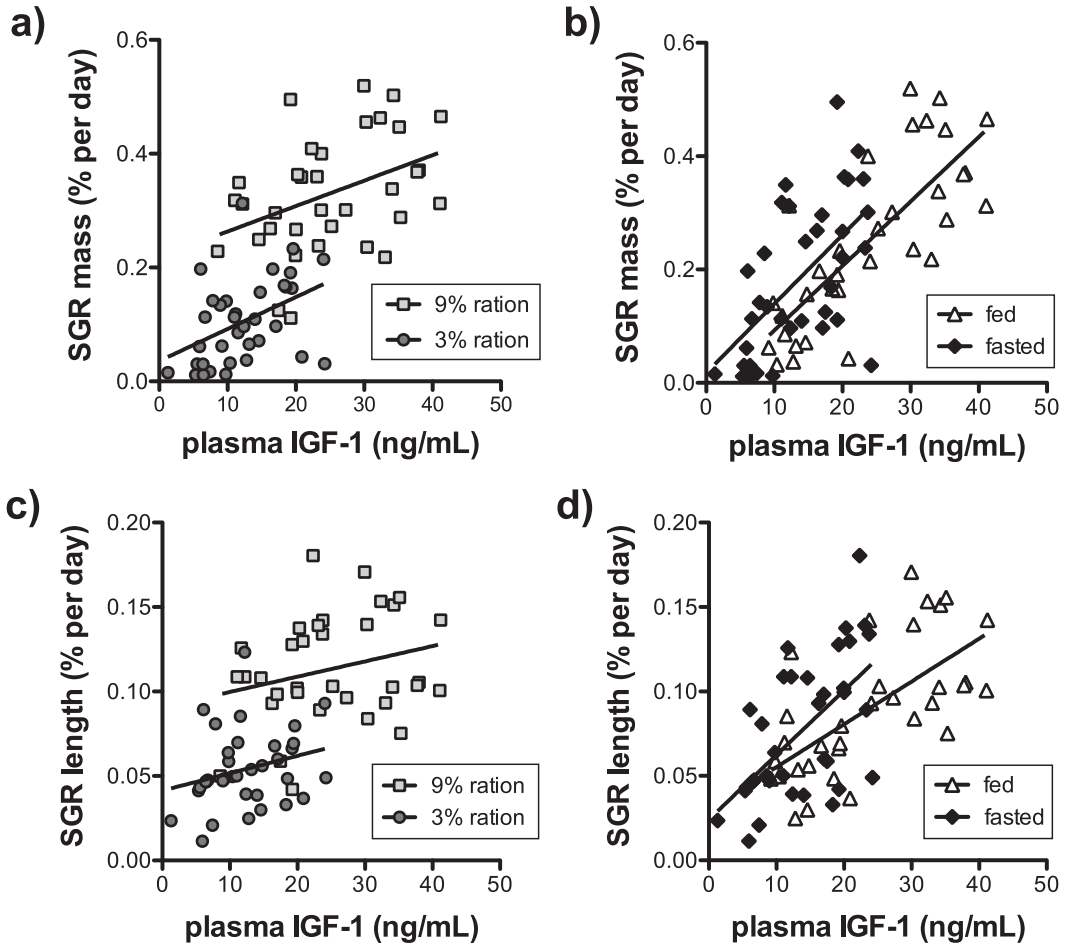

Fig. 6. Relationships between an individual's plasma IGF-1 concentration and specific growth rate (SGR) as calculated by grouping fish by long-term ration treatment (a and c) or fasting or continued feeding (b and d) for the final 12 d. Separate correlation analyses are shown for SGR calculated by change in body mass (a and b) or length (SL) (c and d). Relationships between SGR and IGF-1 in rockfish grouped by ration treatment had similar slopes but showed relationship discordance as differing intercepts, while relationships in fish grouped by recent feeding/fasting had similar slopes and intercepts.

amount (ration·fasting interaction: $F_{1,66} = 12.337$, $p = 0.0008$), and not in fish raised under the 3% ration, who already showed lower hepatic *igf2* mRNA abundance (Fig. 8).

The abundance of transcripts encoding IGF binding proteins *igfbp1a* (Fig. 9a) and *igfbp1b* (Fig. 9b) were elevated significantly in the liver of fasted fish in both ration treatments (fasting effect: *igfbp1a,* $F_{1,66} = 43.776$, $p < 0.0001$; *igfbp1b,* $F_{1,66} = 38.051$, $p < 0.0001$), and were also elevated in fish under the 3% ration compared to those under the 9% ration (ration effect: *igfbp1a,* $F_{1,66} = 8.894$, $p = 0.004$; *igfbp1b,* $F_{1,66} = 20.287$, $p < 0.0001$). While the hepatic abundance of *igfbp2a* mRNAs was not altered by either fasting or ration amount (Fig. 9c), *igfbp2b* mRNA levels were lower both in fish that were raised under the reduced 3% ration (Fig. 9d) ($F_{1,66} = 37.934$, $p < 0.0001$), and in fish that were fasted ($F_{1,66} = 13.721$, $p = 0.0004$). Liver transcript levels for the type 3 binding protein *igfbp3a* were also altered by both fasting and ration amount (Fig. 9e) (ration·fasting interaction: $F_{1,66} = 8.469$, $p = 0.0049$), with fasting increasing *igfbp3a* mRNA levels nearly 10-fold in rockfish raised under both the 9% ration and 3% ration. Additionally, unfasted fish in the 3% ration showed an *igfbp3a* mRNA level that was 3.5-fold higher than 9% ration unfasted fish. Transcripts encoding the other teleost type 3 IGF binding protein, *igfbp3b*, in the liver also were affected by ration amount, but showed a response to longer-term ration variation distinct from that of *igfbp3a*. Transcript abundance for *igfbp3b* was ~ 40% lower in rockfish reared under the 3% ration compared to those under the 9% ration (Fig. 9f) ($F_{1,66} = 13.651$, $p = 0.0004$), and were not affected by short-term fasting in either ration treatment. Transcripts encoding the type 5 binding proteins *igfbp5a* (Fig. 9g) and *igfbp5b* (Fig. 9h) showed similar changes in the liver, with rockfish raised under the 3% reduced ration exhibiting lower relative mRNA levels for both *igfbp5a* ($F_{1,66} = 20.144$, $p < 0.0001$) and *igfbp5b* ($F_{1,66} = 7.525$, $p = 0.0078$). Although not shown in the Tukey HSD pairwise comparison analyses, the ANOVA analyses indicated that short-term fasting decreased both liver *igfbp5a* ($F_{1,66} = 4.077$, $p = 0.0475$) and *igfbp5b* ($F_{1,66} = 8.404$, $p = 0.0051$) mRNA levels.

3.6. Food intake regulation of skeletal muscle mRNAs

Food ration and fasting altered gene transcript abundances for both IGF-1 receptors *igf1rA* and *igf1rB* in the skeletal muscle (Fig. 10). Transcripts for *igf1rA* in muscle were elevated in rockfish fed the 3% ration compared to the 9% ration ($F_{1,66} = 36.521$, $p < 0.0001$), and also were elevated in fasted fish in both ration treatments ($F_{1,66} = 41.645$, $p < 0.0001$). Similarly, transcripts for *igf1rB* were also observed to be at higher relative levels in the 3% ration rockfish compared to the 9% ration fish ($F_{1,66} = 35.950$, $p < 0.0001$), and were again elevated in fish from both rations that were fasted for the final 12 d ($F_{1,66} = 42.466$, $p < 0.0001$).

The abundance of mRNAs for *igf1* (Fig. 11a) – but not those for *igf2* (Fig. 11b) – varied in skeletal muscle in patterns dependent on nutritional experience. Transcript abundance for *igf1* in muscle was lower under the 3% ration than the 9% ration and was also at lower relative levels in fasted fish from both treatments (ration·fasting interaction:

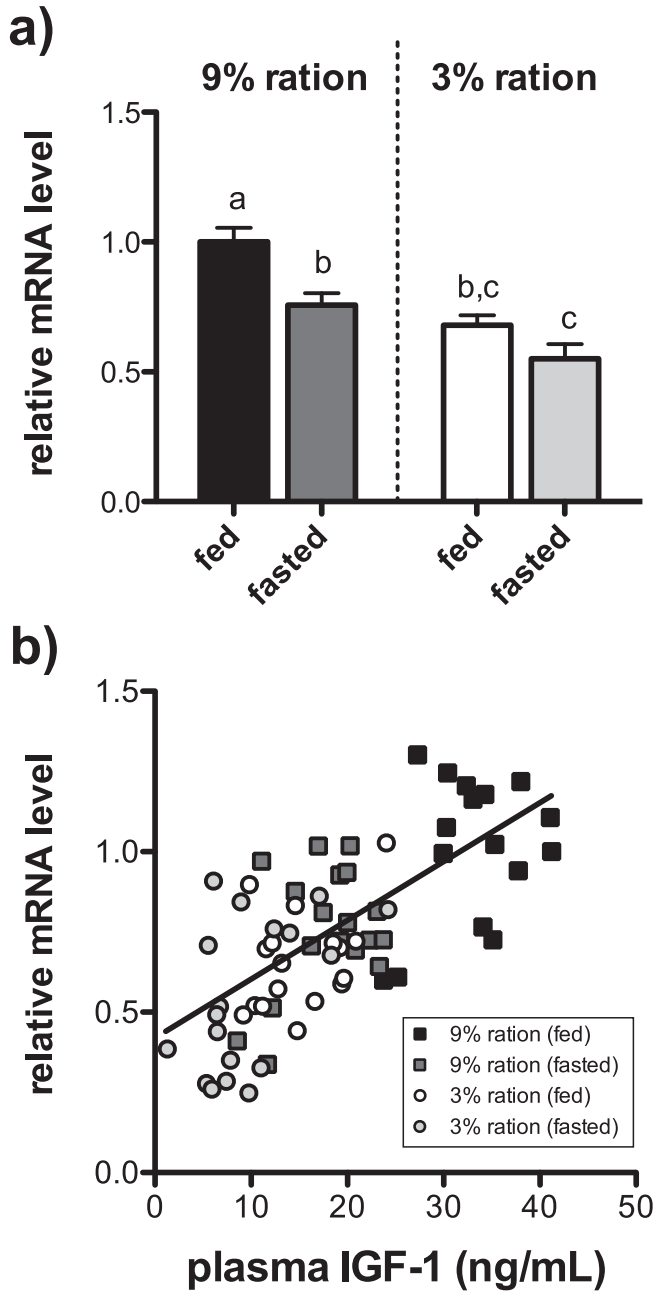

Fig. 7. Liver mRNA levels for *igf1* varied with nutritional experience. (a) Liver *igf1* mRNA levels were lower in rockfish reared on the 3% ration treatment than the 9% ration, and were also reduced by fasting. Data are plotted as mean ± SEM, and letters indicate pairwise differences among treatments (Tukey HSD tests). (b) Individual variation in liver *igf1* mRNA level correlated positively with plasma IGF-1 concentration (r = 0.707, $p < 0.0001$).

$F_{1,66} = 6.241$, $p = 0.0150$). While individual variation in muscle *igf1* mRNA levels correlated positively with individual variation in plasma IGF-1 when fish from all treatment groups were examined together (r = 0.5218, $p < 0.0001$), no such correlations were observed within each treatment separately, suggesting that an individual's plasma IGF-1 levels may not relate to their muscle *igf1* expression level directly via any causal mechanism, but rather may reflect broader changes in muscle tissue state induced by the different ration treatments.

Transcripts encoding several of the Igfbps in skeletal muscle also varied in relative abundance among rockfish with differing food availability (Fig. 12). While neither ration amount nor fasting influenced muscle mRNA levels for *igfbp1a* (Fig. 12a) or *igfbp1b* (Fig. 12b), short-term fasting reduced the relative abundance of mRNAs encoding *igfbp2a* in muscle (Fig. 12c) (fasting effect: $F_{1,66} = 14.575$, $p = 0.0003$), despite the long-term rations themselves not affecting muscle *igfpb2a* mRNA levels (ration effect: $F_{1,66} = 0.040$, $p = 0.8416$). Transcript abundance for *igfbp2b* in muscle was not affected by either the ration or fasting treatments (Fig. 12d).

The abundance of mRNAs encoding both rockfish type-3 IGFPBs (*igfbp3a* and *igfbp3b*) in muscle also varied with food intake. Ration amount affected the relative abundance of muscle *igfbp3a* gene transcripts with elevated mRNA levels in fish that experienced the reduced, 3% ration amount (Fig. 12e) (ration effect: $F_{1,66} = 26.756$, $p < 0.0001$). Fasted fish in both the 9% and 3% rations also showed elevated mRNA levels for *igfbp3a* in muscle (fasting effect: $F_{1,66} = 10.473$, $p = 0.0019$). Transcripts for the other type 3 Igfbp – *igfbp3b* – also were significantly elevated in fish under the 3% ration compared to the 9% ration (Fig. 12f) (ration effect: $F_{1,66} = 31.734$, $p < 0.0001$), and again showed a statistically significant – albeit smaller – increase in relative abundance in fasted fish (fasting effect: $F_{1,66} = 4.349$, $p = 0.0410$).

Transcript levels for *igfbp5a* in muscle also were altered by fasting, with the direction of change dependent on prior ration amount (Fig. 12g) (ration·fasting interaction: $F_{1,66} = 34.564$, $p < 0.0001$). While rockfish in the 9% and 3% rations that were not fasted had no differences in muscle *igfbp5a* mRNA levels, fasting led to decreased muscle *igfbp5a* mRNA abundance in fish from the 9% ration, but increased mRNA abundance in those from the 3% ration. While less distinct than the ration-induced changes in muscle *igfbp5a* mRNAs, *igfbp5b* mRNAs also occurred at a lower abundance in the muscle of rockfish from the 3% ration compared to those from the 9% ration (Fig. 12h) (ration effect: $F_{1,66} = 4.216$, $p = 0.0440$), although that ration effect appeared minor compared to the feeding-induced changes in muscle *igfbp5a* mRNA abundance.

4. Discussion

4.1. Modulation of the IGF-1 relation to growth by long-term food availability

GH/IGF-1 pathways of the somatotropic axis respond to food or macronutrient (i.e., protein) restriction in patterns suggestive of an adaptive response to shift energy allocation from cellular and somatic growth to essential cellular functions (e.g., Picha et al., 2008a; Reindl and Sheridan, 2012; Sonntag et al., 1999). Our results generally support those previous patterns of IGF-1 regulation by ration amount, but also point to differences in how long- versus short-term variation in food intake influences the relation of circulating IGF-1 to growth. Here, we tested for such differential effects by rearing juvenile copper rockfish on dissimilar food rations (9% and 3%) for a ~4.5 month period to mimic 'long-term' variation in feeding status. In doing so, we generated two groups of fish experiencing different rates of positive growth linked to ration variation. While the growth rates of copper rockfish in both the 3% and 9% ration treatments appear lower than growth rates observed for age-1 copper rockfish in the wild (e.g., Byerly, 2001), the variation in growth created by these rations still enabled analyses of how IGF-1 signaling related to positive growth rate variation. What is more, we also then fasted subsets of fish from those two ration groups for 12 d ('short-term') to examine whether differences in long-term food availability would affect how fish responded to short-term food deprivation.

Our data show that juvenile copper rockfish given a high ration amount, and therein showing faster growth, had a higher plasma IGF-1 concentration and liver *igf1* mRNA level than fish reared under conditions of reduced ration. Additionally, we found that fish which were fasted for 12 d had significantly lower plasma IGF-1 and hepatic *igf1* mRNA levels than conspecifics fed during that same 12 d period. Despite these treatment-induced differences in growth rate and plasma IGF-1, individual variation in plasma IGF-1 associated positively with

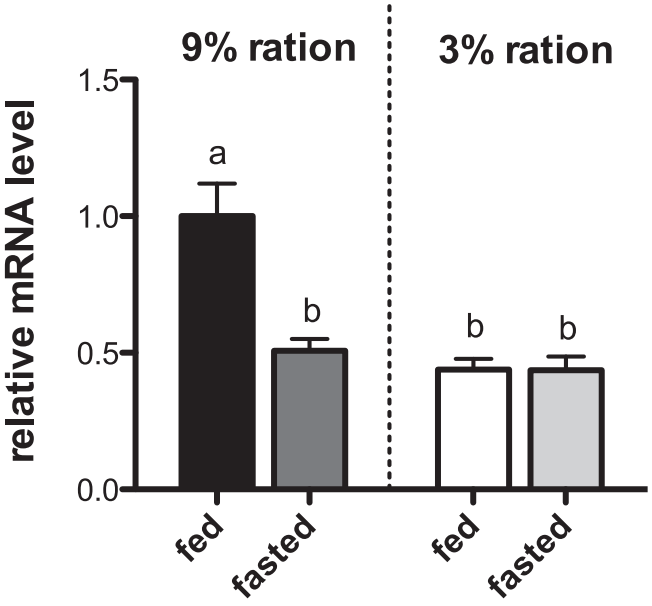

Fig. 8. Gene transcript abundance for *igf2* in the liver of copper rockfish reared on a 9% or 3% ration amount for 140 d, and then fed or fasted for an additional 12 d. Fasting resulted in lower liver *igf2* mRNA abundance in fish reared on the 9% ration, but not the 3% ration. Data are plotted as mean ± SEM. Letters indicate pairwise differences among treatments (Tukey HSD tests).

variation in growth rate under all ration and fed/fasting conditions. That relationship between growth rate and IGF-1 held even when circulating IGF-1 concentrations were corrected for body size differences, indicating that the observed nutrition-related variation in IGF-1 linked to growth variation, and not just body size variation. Fasting has been shown to reduce plasma IGF-1 concentrations in several fishes including sea bream (Pérez-Sánchez et al., 1995), tilapia (Breves et al., 2014; Uchida et al., 2003), as well as salmon and trout (Beckman et al., 2004a; Breves et al., 2016; Pierce et al., 2005; Wilkinson et al., 2006), and our observations here generally agree with the findings of those prior studies. Since several studies have observed hepatic *igf1* mRNA abundance to be at lower levels in fasted fish (e.g., Ayson et al., 2007; Kawanago et al., 2014; Montserrat et al., 2007; Pierce et al., 2005; Peterson and Waldbieser, 2009; Small et al., 2006; Vera Cruz et al., 2006), the fasting-induced reduction in circulating IGF-1 has assumed to be linked to reduced hepatic IGF-1 secretion.

As a whole, the consistency of the positive relationship between variation in circulating IGF-1 and growth in juvenile copper rockfish provides additional support for IGF-1 as a key endocrine regulator of growth in teleost fishes (Picha et al., 2008a; Beckman, 2011). A recent study with juvenile olive rockfish similarly found a positive correlation between individual variation in plasma IGF-1 and somatic growth rate (Hack et al., 2018), and IGF-1 has likewise been shown to relate positively with growth in coho salmon (Beckman et al., 2004a,b; Shimizu et al., 2009), Chinook salmon (*Oncorhynchus tshawytscha*; Beckman et al., 1998), masu salmon (*O. masou*; Kawaguchi et al., 2013), Atlantic cod (*Gadus morhua*; Davie et al., 2007), gilthead sea bream (Pérez-Sánchez et al., 1995; Mingarro et al., 2002), tilapia (*Oreochromis mossambicus*; Uchida et al., 2003), and several other fishes (e.g., Dyer et al., 2004; Picha et al., 2006). Taken together, our results here support the idea that IGF-1 can be used as a reliable growth index for juvenile copper rockfish in fisheries management and aquaculture applications (e.g., Picha et al., 2008a; Beckman, 2011).

However, our data also indicate that while the overall positive relationship between individual variation in plasma IGF-1 and growth may hold across a variety of nutritional conditions, differences in long-term differences in food intake can lead to discordance in the relation of IGF-1 to growth. This discordance was apparent as a difference in the elevation (i.e., intercept) of the positive relationship in rockfish between IGF-1 and SGR under the 3% and 9% ration treatments. Such discordance was observed between fish in the 3% and 9% treatments when SGR was calculated using change in mass or length. Discordance in the IGF-1 relationship with growth rate was not, however, observed under the short-term, 12 d fasting treatments, suggesting that persistent variation – but not short-term differences – in food availability may alter the relationship between IGF-1 and growth rate, even while still

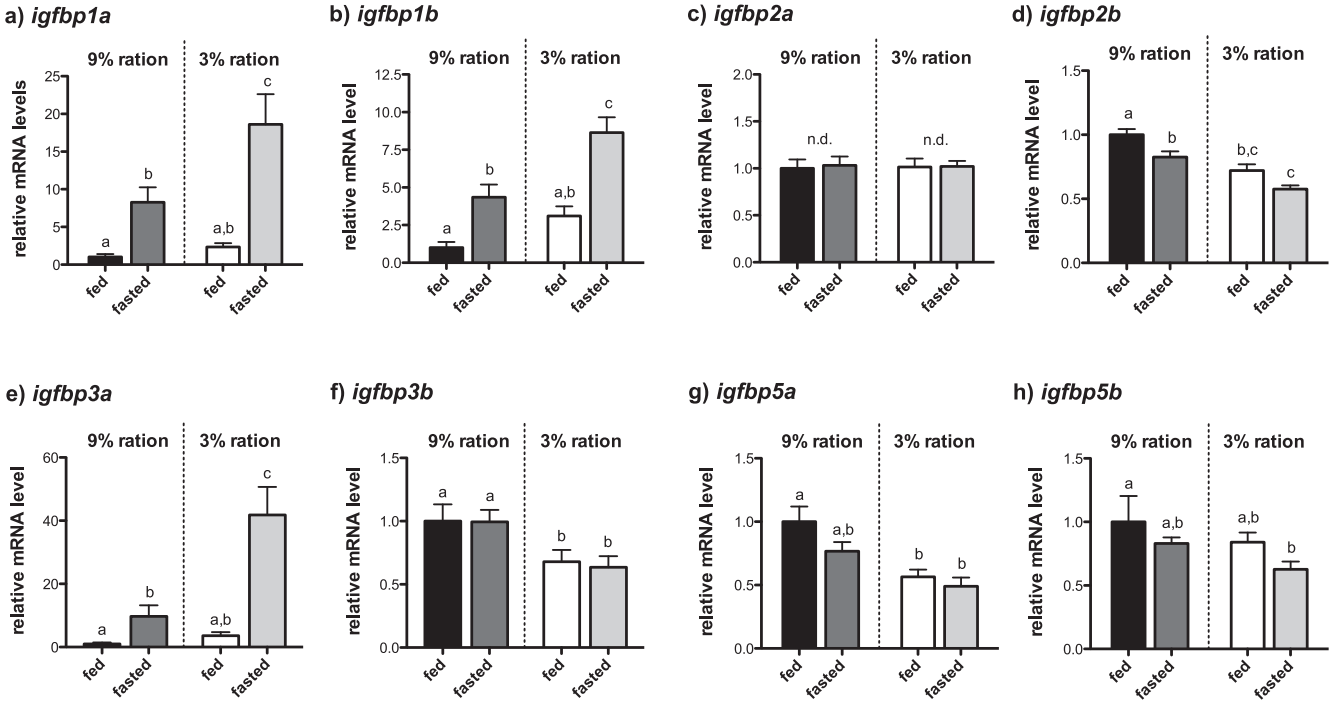

Fig. 9. Relative mRNA levels for IGF binding proteins (Igfbp) type 1, 2, 3 and 5 in the liver. Data are plotted as mean ± SEM. Letters indicate pairwise differences among treatments (Tukey HSD tests).

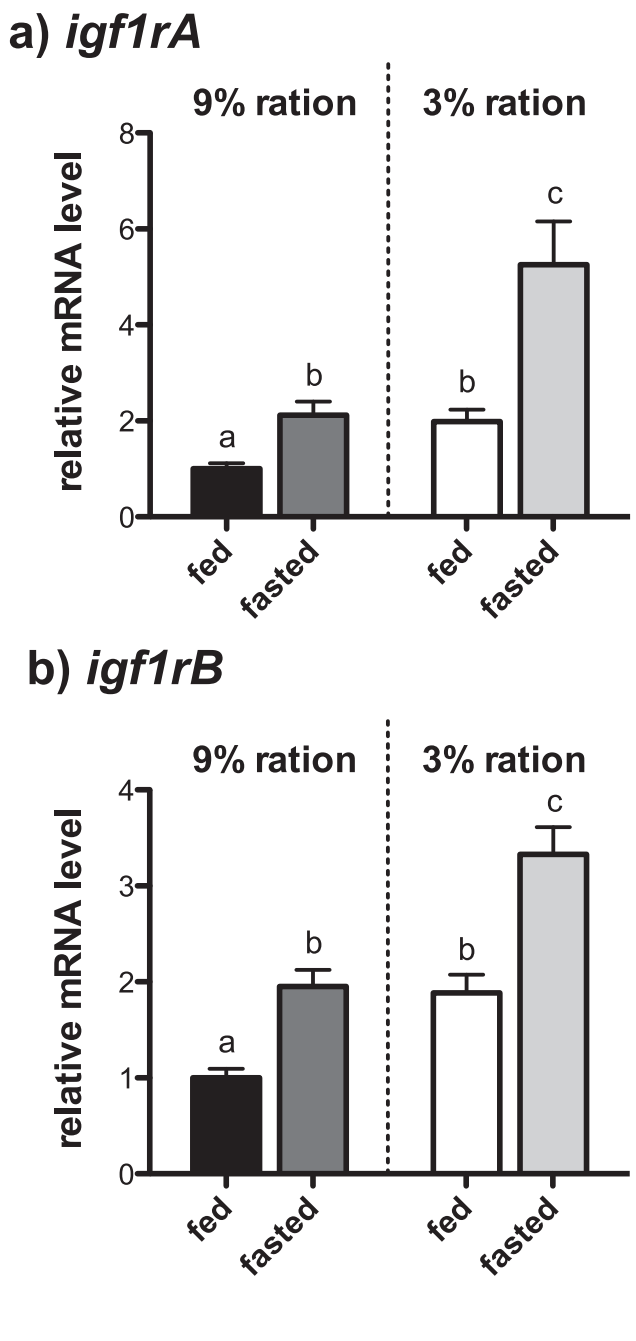

Fig. 10. Relative mRNA levels for IGF-1 receptors (a) *igf1rA* and (b) *igf1rB* in the skeletal muscle. Transcripts encoding both IGF-1 receptors were elevated in fish experiencing the reduced, 3% ration amount, and were also elevated in fish under fasting stress prior to sampling. Data are plotted as mean ± SEM. Letters indicate pairwise differences among treatments (Tukey HSD tests).

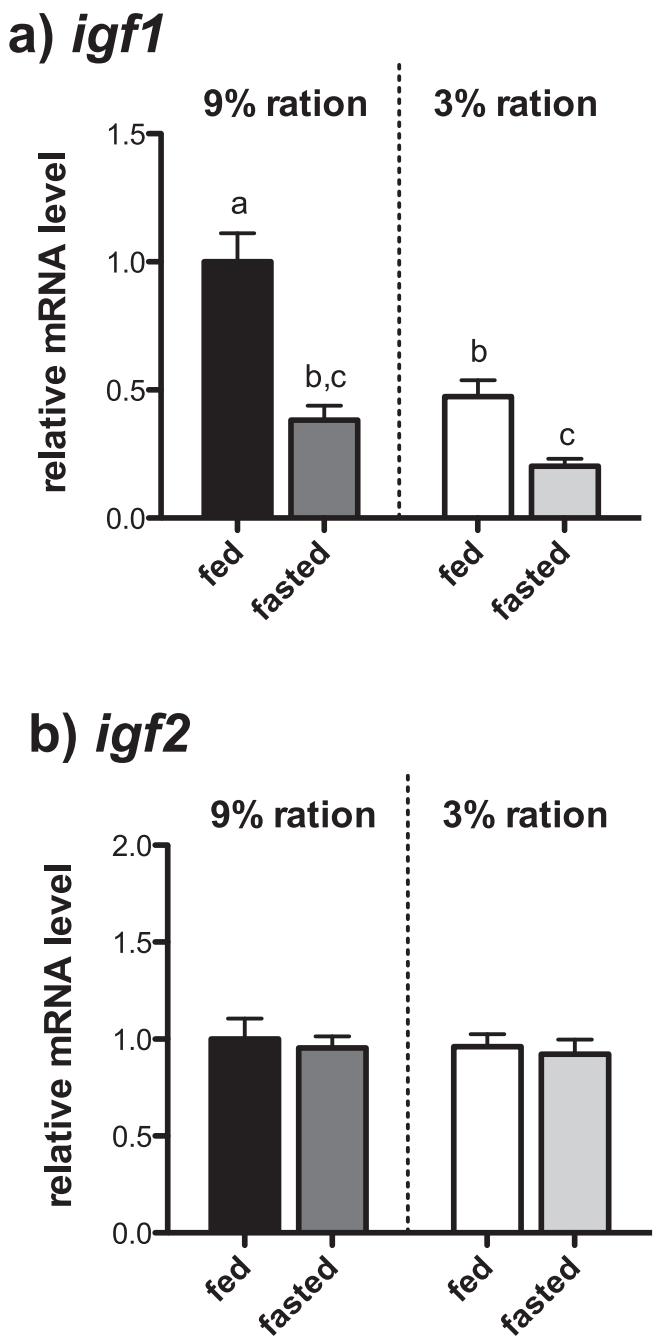

Fig. 11. Gene transcript abundance for (a) *igf1* and (b) *igf2* in the skeletal muscle. Muscle levels of *igf1* mRNA varied with nutritional experience and were observed to be at lower abundance in fish reared on the 3% ration compared to the 9% ration, and in fasted fish of both ration treatments. The abundance of *igf2* mRNAs, however, did not vary in muscle with nutritional experience. Data are shown as mean ± SEM values, and letters indicate pairwise differences among treatments (Tukey HSD tests).

supporting a positive relationship between the variables.

While it is unclear at present why differences in long-term food experience altered the IGF-1/growth relationship in rockfish, several possible factors may have contributed. Fish can show changes in basal metabolism, food consumption rates, feed utilization rate, and nutrient use and deposition efficiency as they grow in size (e.g., Paloheim and Dickie, 1966; Wurtsbaugh and Davis, 1977; Azevedo et al., 2004; Handeland et al., 2008; Van Leeuwen et al., 2012; Auer et al., 2015). While the cellular and molecular changes underlying such shifts are not fully known, changes in metabolic and feed efficiency parameters may be an adaptive response to maximize growth rates under varying conditions of food abundance and quality (Auer et al., 2015). Our observation here that rockfish reared on the 3% and 9% rations showed dissimilar elevation (i.e., intercepts) of the IGF-1 relationship to growth rate points to a possible role for the GH/IGF-1 axis in mediating adaptive growth flexibility under changing food availability

Beckman (2011) examined the possibility that certain environmental or physiological conditions could generate discordant relationships between IGF-1 and growth in fishes by looking at the available data on a multitude of demographic and ecological contextual factors. Based on Beckman's (2011) review of studies available at the time, many factors including diet composition, diel photoperiod variation,

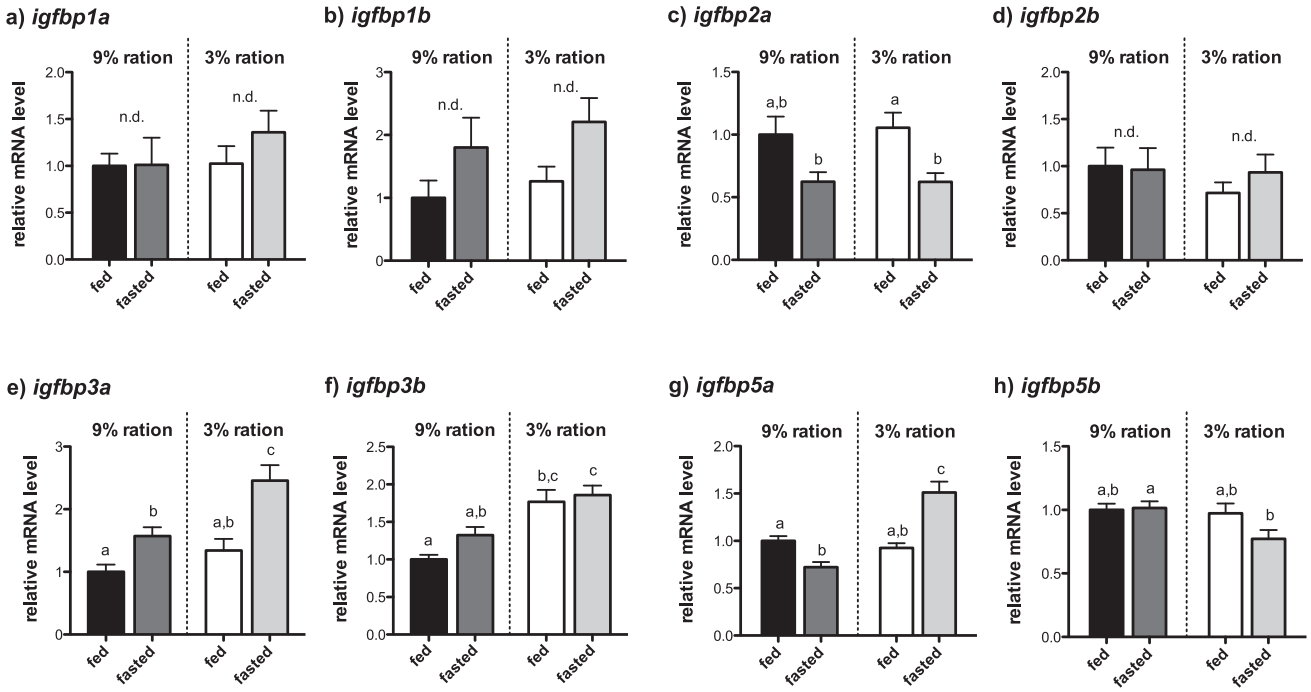

Fig. 12. Relative mRNA levels for IGF binding proteins (Igfbp) type 1, 2, 3 and 5 in the skeletal muscle. Data are plotted as mean ± SEM. Letters indicate pairwise differences among treatments (Tukey HSD tests).

and salinity did not appear to alter the IGF-1 relationship to growth. Juvenile Chinook salmon, for instance, reared on diets differing in protein/energy rations showed similar, concordant relationships between plasma IGF-1 and growth rate (Beckman et al., 2001). However, other factors including photoperiod/season, water temperature, and sexual maturation status (i.e., juvenile versus sexually mature) can alter the slope or intercept of associations between IGF-1 and growth under some circumstances (Beckman, 2011). For example, the relationship of plasma IGF-1 to SGR in coho salmon was found to be positive and similar in slope in juvenile male and female fish, but different from that of mature males, indicating that sexual maturation can alter the IGF-1/growth relationship (Beckman et al., 2004b). Photoperiod has also been observed to alter that relationship, as the IGF-1/growth relationship – while consistently positive – varied in slope in juvenile salmon sampled across a summer season from July to September (Beckman et al., 2004b).

Indeed, the effects of moderate food or macronutrient (e.g., protein) restriction on GH/IGF-1 pathways has been proposed as part of an adaptive response of the somatotropic endocrine axis to shift energy from growth to essential cellular functions such as tissue maintenance and repair when nutrients are limited (e.g., Sonntag et al., 1999). However, not all species respond similarly to caloric or macronutrient restriction. For instance, species can vary in how blood glucose levels are affected by food deprivation (Polakof et al., 2011). In some teleost fishes such as rainbow trout (*Oncorhynchus mykiss*) and warm-water carp and catfish, blood glucose levels remain stable even during several months of fasting (Hanna, 1962; Navarro and Gutiérrez, 1995). However, in juvenile sea bass (*Dicentrarchus labrax*) (Gutiérrez et al., 1991) and juvenile brown trout (*Salmo trutta*) (Navarro et al., 1993), glycemia declines within days of fasting. Our results here with juvenile copper rockfish are similar to those in juvenile bass and trout, and may reflect broader, age-dependent glycemia and growth modulation in teleosts, or differences in liver glucose metabolism related to different adaptive growth responses to dissimilar ecological foraging niches (e.g., Polakof et al., 2011). Such species variation in glucose responses to food deprivation may evolve if the shift of limited energy toward tissue maintenance and repair does not increase evolutionary fitness across all taxa—especially given likelihood for diverse ecological challenges and distinct life history tradeoffs across species (e.g., Shanley and Kirkwood, 2006). It might therefore also be expected that some species may also have evolved differences in IGF responses to nutritional deprivation. Studies to date in humans, for instance, have not observed reductions in serum IGF-1 under long-term caloric restriction as has been observed in other mammals (e.g., Fontana et al., 2008, 2016; Most et al., 2017). One way or another, future studies should explore not only how GH/IGF-1 pathways may act as adaptive modulators of growth under varying conditions of food availability, but also how differences in nutritionally-mediated growth modulation may have evolved among species experiencing divergent selective pressures.

4.2. Ration influences on hepatic gene expression for igf binding proteins

Other components of the somatotropic endocrine axis including IGFBPs and receptors play important roles in modulating IGF-1 availability and tissue sensitivity (e.g., Rajaram et al., 1997; Shimizu et al., 2009; Clemmons, 2016; Shimizu and Dickhoff, 2017; Bergan-Roller and Sheridan, 2018; Allard and Duan, 2018). These components can be regulated independently across individual tissues making examination of their regulation patterns crucial for understanding the phenotypic and fitness consequences of environmentally-induced changes in hormone level (e.g., Lema and Kitano, 2013).

IGFBPs have been shown to be critical for modulating the ratio of free:bound IGF-1 in blood circulation, therein reducing glomerular filtration of IGFs and altering the availability of IGF-1 to interact with receptors in target tissues (e.g., Guler et al., 1989; Rajaram et al., 1997; Clemmons, 2016; Allard and Duan, 2018). In mammals, only 1–5% of IGF-1 in blood circulation is free, and 95% of IGF-1 is bound to IGFBPs (Frystyk et al., 1994), with IGFBP3 being the most abundant binding protein for IGFs in circulation (Martin and Baxter, 1992; Allard and Duan, 2018). This also appears to be the case in teleost fish (e.g., Kelley et al., 1992; Shimizu et al., 1999). And yet, similar to mammals, teleost fishes possess at least six types of IGFBPs. In teleosts, however, these six IGFBP types have diverged further into multiple isoforms via gene duplication events (Daza et al., 2011; Garcia de la Serrana and

Macqueen, 2018), and as many as 22 *igfbp* genes have been identified in some teleost taxa (Allard and Duan, 2018). While IGFBPs are generally viewed as modulating IGF-1 and IGF-2 availability by binding these hormones in blood or extracellular fluid, some IGFBPs also can independently activate IGF receptors or other membrane or nuclear proteins (i.e., peroxisome proliferator-activated receptor γ, transforming growth factor-β5 receptor) to have cellular effects independent of the IGF hormones (Baxter, 2015; Chan et al., 2009; Clemmons, 2016; Jogie-Brahim et al., 2009; Allard and Duan, 2018).

Here, we examined how different food availabilities impacted gene transcript abundance for IGFBPs type-1, -2, -3, and -5 in the liver and skeletal muscle of rockfish, with transcriptional responses differing depending on the tissue and type of IGFBP. In the liver, rockfish maintained on the 3% ration treatment showed higher relative mRNA levels for *igfbp1a*, *-1b*, and *-3a* compared to fish on the 9% ration, with fasting significantly increasing transcript abundance of those IGFBPs in both ration groups. In contrast, transcripts encoding *igfbp3b* were lower in the liver of rockfish under the 3% ration than the 9% ration, and were unaffected by short-term food deprivation (fasting) in either ration treatment. Hepatic mRNA levels for the *igfbp2b*, *-5a*, and *-5b* isoforms were at lower relative abundance in fish maintained on the long-term 3% ration and were also lower in fasted fish in both treatment rations, while *igfbp2a* mRNA levels did not differ with either ration amount or fasting.

Taken as a whole, these results point to complex, independent patterns of IGFBP regulation in fish experiencing different durations of feeding variation. The functional implications of these isoform-specific transcriptional responses, however, are largely unknown, given that the functional roles of IGFBPs in fishes remain largely unexplored (Shimizu and Dickhoff, 2017; Garcia de la Serrana and Macqueen, 2018). Similar to our finding here in copper rockfish, IGFBP1a (which corresponds to the 28–32 kDa IGFBP in teleosts) and IGFBP1b (the 20–25 kDa IGFBP) expression in teleost liver is highly responsive to food deprivation, and several studies have shown that both mRNA and protein levels of Igfbp1a and -1b increase in the liver and serum of fish under complete food deprivation (Shimizu et al., 1999, 2006; Kelley et al., 2001; Peterson and Small, 2004; Picha et al., 2008a; Hevrøy et al., 2011; Kawaguchi et al., 2013; Breves et al., 2014, 2016). Given those increases in IGFBP-1a and -1b expression under catabolic conditions, as well as evidence that IGFBP1 can delay developmental rates in fish (Kajimura et al., 2005), IGFBP1a and -1b are thought to be primarily inhibitory for cell proliferation and growth (Shimizu and Dickhoff, 2017; Allard and Duan, 2018). Our results here showing elevated hepatic mRNA abundance for *igfbp1a* and *-1b* in copper rockfish – as well as in olive rockfish in a prior study (Hack et al., 2018) – experiencing lower food availability lend further support for the possible role of type 1 IGFBPs as inhibitors of IGF-1 availability and growth under conditions of food limitation.

Both long-term food limitation and short-term fasting also led to decreased hepatic *igfbp2b*, but not *-2a*, mRNA abundance in copper rockfish. In salmonid fishes, IGFBP2b protein (the salmon 41-kDa IGFBP) levels have been shown to decline in responses to food deprivation, and then increase upon refeeding (Shimizu et al., 2003, 2009; Beckman et al., 2004a,b; Pierce et al., 2005). Corresponding with these changes in IGFBP2b protein levels, several studies have shown liver transcript abundance for *igfbp2b* to be lower in fasted fish (Duan et al., 1999; Chen et al., 2014; Gabillard et al., 2006; Kelley et al., 2001; Safian et al., 2012), implying that the *igfbp2b* response that we observed here in rockfish may be a general response in teleost fishes. Shimizu and Dickhoff (2017) proposed that Igfbp2b in teleost fishes may be functionally analogous to mammalian Igfbp3 by acting as the primary carrier of IGF-1 in blood circulation and acting as a stimulator of IGF action in peripheral tissues. If accurate, the reduced hepatic *igfbp2b* mRNAs observed in copper rockfish implies a downregulation of IGF-1 promotion of cell proliferation and growth under both long- and short-term conditions of lower food availability.

In mammals, IGFBP3 serves as the major IGFBP by facilitating the IGF-1 transport and releasing the hormone in target tissues that express IGFBP proteases which degrade the binding protein (e.g., Martin and Baxter, 1992; Rajaram et al., 1997; Bunn and Fowlkes, 2003; Ranke, 2015). In fish, however, the function of IGFBP3 appears diverged from that in mammals despite retention of sequencing similarity (Shimizu and Dickhoff, 2017). GH has been shown to increase liver *igfbp3* mRNAs in some fishes (e.g., Cheng et al., 2002; Pedroso et al., 2009), and IGFBP3 has been shown to modulate development of zebrafish (*Danio rerio*) via mechanisms independent of IGF-1 (Li et al., 2005; Zhong et al., 2011).

While IGFBP5 has been demonstrated to regulate early development (Salih et al., 2004), bone growth (Duan and Xu, 2005), and muscle differentiation (Ren et al., 2008; Safian et al., 2012), the role of IGFBP5 isoforms in teleost fishes remains largely unknown. Gene transcripts for *igfbp5a* and *-5b* are present in the liver and a variety of other tissues in teleosts (e.g., Breves et al., 2014; Gabillard et al., 2006; Safian et al., 2012; Pedroso et al., 2009). Unlike what we observed here in copper rockfish, hepatic mRNAs encoding IGFBP5s were not responsive to fasting stress in trout or tilapia (Gabillard et al., 2006; Breves et al., 2014). While future studies are needed to better define the functional roles of the Igfbp3 and Igfbp5 isoforms in fishes, our observation of nutritional regulation of liver *igfbp3* and *-5* gene expression suggests that changes to hepatic production of IGFBP3 and IGFBP5 may play a role in regulating cell function and growth in fishes.

4.3. Nutritional effects on IGF signaling in skeletal muscle

Feeding amount effects on IGFBP gene transcript abundance in muscle had a distinct pattern from those in liver. Messenger RNAs encoding *igfbp2a* were lower in fish experiencing short-term fasting stress, but unaffected by long-term variation in food availability, while *igfbp1a*, *-1b,* and *-2b* mRNA levels were not affected by either long-term or short-term nutritional variation. Transcripts for *igfbp3a* and *-3b* were more abundant in the muscle of fish experiencing long-term feed restriction as well as in fish coping with short-term food deprivation. Transcripts encoding *igfbp5a* and *-5b* both changed with short-term fasting stress, although the direction of that change depended on prior, long-term food availability.

IGFBPs within skeletal muscle are thought to exert autocrine or paracrine actions by altering the local availability of IGFs. Such effects may occur via IGFBPs influencing the half-life of local IGFs, or via the sequestration of IGFs from IGF receptors (Garcia de la Serrana and Macqueen, 2018). In teleost fishes, IGFBP5 has been established as exerting a regulatory influence on myogenesis and muscle growth (Garcia de la Serrana et al., 2017). The role(s) of other IGFBPs in fish muscle, however, are much less clear. Similar to our results here, muscle *igfbp1a* and *igfbp1b* mRNA levels were unaffected by food ration amount in juvenile olive rockfish (Hack et al., 2018), but the functional significance muscle IGFBP1s are not known. Similarly, little information is available concerning the actions of IGFBP2 or IGFBP3 in peripheral tissues of teleosts, although fasting has been shown to reduce muscle *igfbp2* mRNA levels and increase muscle *igfbp3* mRNAs in fine flounder (*Paralichthys adspersus*) (Safian et al., 2012), a result similar to our findings here where fasted copper rockfish had lower muscle *igfbp2a* transcript abundance and higher muscle *igfbp3a* abundance.

Food restriction or deprivation has also been shown to alter IGFBP5 mRNAs in the skeletal muscle of several fish taxa (Bower et al., 2008; Bower and Johnston, 2010; Gabillard et al., 2006; Zheng et al., 2017; Hack et al., 2018). In juvenile olive rockfish, Hack and coworkers (2018) observed lower relative levels of *igfbp5a* and *igfbp5b* mRNAs in the skeletal muscle of fish reared under a reduced food ration amount for 98 d. Here, our data suggest a small reduction in *igfbp5b* mRNA abundance in the skeletal muscle of copper rockfish reared on reduced rations over ~4.5 mo., but also point to acute responses of muscle *igfbp5a* transcription in response to short-term fasting. Interestingly,

these fasting effects on *igfbp5a* were dependent on long-term ration experience such that fasting decreased muscle *igfbp5a* mRNA levels in rockfish reared under the 9% ration, but increased muscle *igfpb5a* mRNA abundance in fish reared on the reduced 3% ration. That observation points to a role for longer-term feeding experience in shaping how muscle *igfbp5* gene expression responds to more recent, short-term changes in food intake.

While prior research with olive rockfish by Hack and coworkers (2018) did not examine effects of fasting, muscle *igfbp5* mRNAs were observed to be at lower relative levels in fasted grass carp *Ctenopharyngodon idella* (Zheng et al., 2017), and downregulated by complete food deprivation and upregulated by refeeding in flounder (Safian et al., 2012). IGFBP5 proteins appear to have a role in modulating muscle growth and myogenesis in fishes (Garcia de la Serrana and Macqueen, 2018). In Atlantic salmon (*S. salar*), *igfbp5a* and *-5b* gene expression was observed to be at greatest levels in early-stage, cultured myoblasts but declined as these cells began exhibiting characteristics of myogenic differentiation (Bower and Johnston, 2010). Prior work has also documented in rainbow trout that IGFBP5 protein levels decline in atrophying fast-twitch muscle during the spawning season (Salem et al., 2010), again implying a role for IGFBP5 in the autocrine or paracrine regulation of muscle growth (e.g., Bower and Johnston, 2010; Garcia de la Serrana and Macqueen, 2018).

Our findings that the abundance of *igfbp5* mRNAs in rockfish muscle is dependent on long-term feeding experience may point to a role for plastic modulation of muscle IGFBP5 gene regulation as part of an adaptive response to maximize growth when food is limited (e.g., Sonntag et al., 1999; Auer et al., 2015). Such effects of prior, long-term feeding amount on IGFBP5 expression in muscle could be part of the mechanism for accelerated compensatory growth shown by fishes after a period of food deprivation (e.g., Ali et al., 2003). Under such a scenario, the divergent transcriptional responses of *igfbp5a* and *-5b* observed in rockfish might relate to context-dependent IGF signaling regulation under differing forms of stressors – specifically, in this case, nutritional stressors – as has been proposed as one hypothesis for why so many IGFBP forms evolved in some vertebrates (Allard and Duan, 2018). While such an adaptive role for muscle IGFBP5 is clearly speculative at this time, this should be examined further in future studies that test for interactions between long- and short-term nutritional stresses and IGF induction of muscle cell proliferation and growth.

Looking beyond changes in IGFBPs, we also found that the relative abundance of gene transcripts encoding the IGF receptor genes *igf1rA* and *igf1rB* were altered in skeletal muscle by ration amount. Specifically, we observed higher relative *igf1rA* and *igf1rB* mRNA levels in the muscle of copper rockfish on the 3% ration compared to those on the 9% ration, and also discerned that short-term fasting increased receptor mRNAs in both ration groups. While Hack and coworkers (2018) did not observe any differences in mRNA levels for either *igf1rA* or *igf1rB* in juvenile olive rockfish reared under differing food rations for 98 d, Chauvigné and colleagues (2003) detected increased mRNA levels for *igf1rA* – but not *igf1rB* – in the muscle of rainbow trout during fasting. Muscle IGF-1 receptor gene expression in fish can be regulated by a variety of factors including both IGF-1 and IGF-2 (Azizi et al., 2016), and tissue- and isoform-specific regulation of IGF-1 receptors may be common (Maures et al., 2002). In any case, our observation of increased IGF-1 receptor mRNA levels in rockfish muscle under long- and short-term food restriction implies a role for receptor expression changes as part of a compensatory response of peripheral GH/IGF-1 pathways when fish experience nutritional stresses (e.g., Bower et al., 2008).

4.4. Conclusions

Taken together, these findings with copper rockfish point to a role for prior food intake in shaping how the GH/IGF-1 axis responds to later conditions of food deprivation. More specifically, differences in long-term food ration amount appear to change the sensitivity of growth by rockfish to IGF-1, as indicated by the shift in elevation of how individual variation in circulating IGF-1 relates to growth rate in fish from the 3% and 9% ration treatments. The mechanism(s) by which nutritional experience alters the sensitivity of growth pathways to IGF-1 is not clear, but our data suggest that shifts in the sensitivity of muscle *igfbp5a* and *-5b* transcription to nutritional conditions may be contributing factors. Future work should explore the interactions between long- and short-term feeding experience on IGF-1 pathways further, and look in more detail at *igfbp5a* and *-5b* as possible contributors to what is likely to be a larger set of coordinated cellular and molecular mechanisms for altering skeletal muscle growth in fishes in response to long- or short-term conditions of poor food availability.

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Appendix A. Supplementary data

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References

Ali, M., Nicieza, A., Wootton, R.J., 2003. Compensatory growth in fishes: a response to growth depression. Fish Fish. 4, 147–190.
Allard, J.B., Duan, C., 2018. IGF-binding proteins: why do they exist and why are there so many? Front. Endocrinol. 9, 117.
Ammann, A.J., 2004. SMURFs: standard monitoring units for the recruitment of temperate reef fishes. J. Exp. Mar. Biol. Ecol. 299, 135–154.
Auer, S.K., Salin, K., Rudolf, A.M., Anderson, G.J., Metcalfe, N.B., 2015. Flexibility in metabolic rate confers a growth advantage under changing food availability. J. Anim. Ecol. 84, 1405–1411.
Ayson, F.G., de Jesus-Arson, E.G.T., Takemura, A., 2007. mRNA expression patterns for GH, PRL, SL, IGF-I and IGF-II during altered feeding status in rabbitfish, *Siganus guttatus*. Gen. Comp. Endocrinol. 150, 196–204.
Azevedo, P.A., Leeso, N.S., Cho, C.Y., Bureau, D.P., 2004. Growth, nitrogen and energy utilization of juveniles from four salmonid species: diet, species and size effects. Aquaculture 234, 393–414.
Azizi, S., Nematollahi, M.A., Amiri, B.M., Vélez, E.J., Salmerón, C., Chan, S.J., et al., 2016. IGF-I and IGF-II effects on local IGF system and signaling pathways in gilthead sea bream (*Sparus aurata*) cultured myocytes. Gen. Comp. Endocrinol. 232, 7–16.
Barth, J.A., Menge, B.A., Lubchenco, J., Chan, F., Bane, J.M., Kirincich, A.R., et al., 2007. Delayed upwelling alters nearshore coastal ocean ecosystems in the northern California current. Proc. Natl. Acad. Sci. USA 104, 3719–3724.
Baxter, R.C., 2015. Nuclear actions of insulin-like growth factor binding protein-3. Gene 569, 7–13.
Beare, D.J., McKenzie, E., 1999. Connecting ecological and physical time series: The potential role of changing seasonality. Mar. Ecol. Prog. Ser. 78, 307–309.
Beauloye, V., Willems, B., de Coninck, V., Frank, S.J., Edery, M., Thissen, J.-P., 2002. Impairment of liver GH receptor signaling by fasting. Endocrinology 143, 792–800.
Beckman, B.R., Larsen, D.A., Moriyama, S., Lee-Pawlak, B., Dickhoff, W.W., 1998. Insulin-

like growth factor-I and environmental modulation of growth during smoltification of spring Chinook salmon (*Oncorhynchus tshawytscha*). Gen. Comp. Endocrinol. 109, 325–335.
Beckman, B.R., Shearer, K.D., Cooper, K.A., Dickhoff, W.W., 2001. Relationship of insulin-like growth factor-I and insulin to size and adiposity of under-yearling chinook salmon. Comp. Biochem. Physiol. A 129, 585–593.
Beckman, B.R., Shimizu, M., Gadberry, B.A., Cooper, K.A., 2004a. Response of the somatotropic axis of juvenile coho salmon to alterations in plane of nutrition with an analysis of the relationships among growth rate and circulating IGF-I and 41 kDa IGFBP. Gen. Comp. Endocrinol. 135, 334–344.
Beckman, B.R., Fairgrieve, W., Cooper, K.A., Mahnken, C.V.W., Beamish, R.J., 2004b. Evaluation of endocrine indices and growth in individual postsmolt coho salmon. Trans. Am. Fish. Soc. 133, 1057–1067.
Beckman, B.R., 2011. Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. Gen. Comp. Endocrinol. 170, 233–252.
Bergan-Roller, H.E., Sheridan, M.A., 2018. The growth hormone signaling system: insights into coordinating the anabolic and catabolic actions of growth hormone. Gen. Comp. Endocrinol. 258, 119–133.
Bograd, S.J., Schroeder, I., Sarkar, N., Qiu, X., Sydeman, W.J., Schwing, F.B., 2009. Phenology of coastal upwelling in the California Current. Geophys. Res. Lett. 36, L01602.
Bower, N.I., Li, X., Taylor, R., Johnston, I.A., 2008. Switching to fast growth: the insulin-like growth factor (IGF) system in skeletal muscle of Atlantic salmon. J. Exp. Biol. 211, 3859–3870.
Bower, N.I., Johnston, I.A., 2010. Transcriptional regulation of the IGF signaling pathway by amino acids and insulin-like growth factors during mysogenesis in Atlantic salmon. PLoS ONE 5, e11100.
Breese, C.R., Ingram, R.L., Sonntag, W.E., 1991. Influence of age and long-term dietary restriction on plasma insulin-like growth factor-1 (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins. J. Gerontol. 46, B180–B187.
Breves, J.P., Tipsmark, C.K., Stough, B.A., Seale, A.P., Flack, B.R., Moorman, B.P., Lerner, D.T., Grau, E.G., 2014. Nutritional status and growth hormone regulate insulin-like growth factor binding protein (igfbp) transcripts in Mozambique tilapia. Gen. Comp. Endocrinol. 207, 66–73.
Breves, J.P., Phipps-Costin, S.K., Fujimoto, C.K., Einarsdottir, I.E., Regish, A.M., Björnsson, B.T., McCormick, S.D., 2016. Hepatic insulin-like growth-factor binding protein (igfbp) responses to food restriction in Atlantic salmon smolts. Gen. Comp. Endocrinol. 233, 79–87.
Bunn, R.C., Fowlkes, J.L., 2003. Insulin-like growth factor binding protein proteolysis. Trends Endocrinol. Met. 14, 176–181.
Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., et al., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55, 611–622.
Butler, A.A., Le Roith, D., 2001. Minireview: tissue-specific versus generalized gene targeting of the igf1 and igf1r genes and their roles in insulin-like growth factor physiology. Endocrinology 142, 1685–1688.
Byerly, M.M., 2001. The ecology of age-1 copper rockfish (*Sebastes caurinus*) in vegetated habitats of Sitka Sound, Alaska. M.S. Thesis. University of Alaska, Fairbanks.
Caselle, J.E., Kinlan, B.P., Warner, R.R., 2010. Temporal and spatial scales of influence on nearshore fish settlement in the Southern California Bight. Bull. Mar. Sci. 86, 355–385.
Chan, S.S.Y., Schedlich, L.J., Twigg, S.M., Baxter, R.C., 2009. Inhibition of adipocyte differentiation by insulin-like growth factor-binding protein-3. Am. J. Physiol. Endocrinol. Metabol. 296, E654–E663.
Chauvigné, F., Gabillard, J.C., Weil, C., Rescan, P.Y., 2003. Effect of refeeding on IGFI, IGFII, IGF receptors, FGF2, FGF6, and myostatin mRNA expression in rainbow trout myotomal muscle. Gen. Comp. Endocrinol. 132, 209–215.
Chen, J.-Y., Chen, J.-C., Chang, C.-Y., Shen, S.-C., Chen, M.-S., Wu, J.-L., 2000. Expression of recombinant tilapia insulin-like growth factor-I and stimulation of juvenile tilapia growth by injection of recombinant IGFs polypeptides. Aquaculture 181, 347–360.
Chen, W., Li, W., Zhang, Z., Jiang, X., Li, M., 2014. Cloning, molecular characterization and expression analysis of insulin-like growth factor binding protein-2 (IGFBP-2) cDNA in goldfish, *Carassius auratus*. Fish Physiol. Biochem. 40, 1669–1681.
Cheng, R., Chang, K.-M., Wu, J.-L., 2002. Different temporal expressions of tilapia (*Oreochromis mossambicus*) insulin-like growth factor-I and IGF binding protein-3 after growth hormone induction. Mar. Biotechnol. 4, 218–225.
Clemmons, D.R., 2016. Role of IGF binding proteins in regulating metabolism. Trends Endocrinol. Metabol. 27, 375–391.
Criscuolo, F., Monaghan, P., Nasir, L., Metcalfe, N.B., 2008. Early nutrition and phenotypic development: 'catch-up' growth leads to elevated metabolic rate in adulthood. Proc. R. Soc. Lond. B 275, 1565–1570.
Davie, A., Porter, M.J.R., Bromage, N.R., Migaud, H., 2007. The role of seasonally altering photoperiod in regulating physiology in Atlantic cod (*Gadus morhua*). Part II. Somatic growth. Can. J. Fish. Aquat. Sci. 64, 98–112.
Daza, D.O., Sundström, G., Bergqvist, C.A., Duan, C., Larhammar, D., 2011. Evolution of the insulin-like growth factor binding protein (IGFBP) family. Endocrinology 152, 2278–2289.
Degger, B., Upton, Z., Soole, K., Collet, C., Richardson, N., 2000. Comparison of recombinant barramundi and human insulin-like growth factor (IGF)-I in juvenile barramundi (*Lates calcarifer*): in vivo metabolic effects, association with circulating IGF-binding proteins, and tissue distribution. Gen. Comp. Endocrinol. 117, 395–403.
Deser, C., Alexander, M.A., Xie, S.P., Phillips, A.S., 2010. Sea surface temperature variability: patterns and mechanisms. Ann. Rev. Mar. Sci. 2, 115–143.
Duan, C., Plisetskaya, E.M., 1993. Nutritional regulation of insulin-like growth factor-I mRNA expression in salmon tissues. J. Endocrinol. 139, 243–252.
Duan, C., Ding, J., Li, W., Tsai, W., Pozios, K., 1999. Insulin-like growth factor binding protein 2 is a growth inhibitory protein conserved in zebrafish. Proc. Natl. Acad. Sci. USA 96, 15274–15279.
Duan, C., Xu, Q., 2005. Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. Gen. Comp. Endocrinol. 142, 44–52.
Dunn, S.E., Kari, F.W., French, J., Leininger, J.R., Travlos, G., Wilson, R., Barrett, J.C., 1997. Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. Cancer Res. 57, 4667–4672.
Dyer, A.R., Barlow, C.G., Bransden, M.P., Carter, C.G., Glencross, B.D., Richardson, N., Thomas, P.M., Williams, K.C., Carragher, J.F., 2004. Correlation of plasma IGF-I concentrations and growth rate in aquacultured finfish: a tool for assessing the potential of new diets. Aquaculture 236, 583–592.
Ferriss, B.E., Trudel, M., Beckman, B.R., 2014. Regional and inter-annual trends in marine growth of juvenile salmon in coastal pelagic ecosystems of British Columbia. Canada. Mar. Ecol. Prog. Ser. 503, 247–261.
Fontana, L., Weiss, E.P., Villareal, D.T., Klein, S., Holloszy, J.O., 2008. Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentrations in humans. Aging Cell 7, 681–687.
Fontana, L., Villareal, D.T., Das, S.K., Smith, S.R., Meydani, S.N., Pittas, A.G., et al., 2016. Effects of 2-year calorie restriction on circulating levels of IGF-1, IGF-binding proteins and cortisol in nonobese men and women: a randomized clinical trial. Aging Cell 15, 22–27.
Fox, B.K., Breves, J.P., Davis, L.K., Pierce, A.L., Hirano, T., Grau, E.G., 2010. Tissue-specific regulation of the growth hormone/insulin-like growth factor axis during fasting and re-feeding: importance of muscle expression of IGF-I and IGF-II mRNA in the tilapia. Gen. Comp. Endocrinol. 166, 573–580.
Frystyk, J., Skjaerbaek, C., Dinesen, B., Orskov, H., 1994. Free insulin-like growth factors (IGF-I and IGF-II) in human serum. FEBS Lett. 348, 185–191.
Fuentes, E.N., Kling, P., Einarsdottir, I.E., Alvarez, M., Valdés, J.A., Molina, A., Björnsson, B.T., 2012a. Plasma leptin and growth hormone levels in the fine flounder (*Paralichthys adspersus*) increase gradually during fasting and decline rapidly after refeeding. Gen. Comp. Endocrinol. 177, 120–127.
Fuentes, E.N., Einarsdottir, I.E., Valdes, J.A., Alvarez, M., Molina, A., Björnsson, B.T., 2012b. Inherent growth hormone resistance in the skeletal muscle of the fine flounder is modulated by nutritional status and is characterized by high contents of truncated GHR, impairment in the JAK2/STAT5 signaling pathway, and low IGF-I expression. Endocrinology 153, 283–294.
Gabillard, J.C., Kamangar, B.B., Montserrat, N., 2006. Coordinated regulation of the GH/IGF system genes during refeeding in rainbow trout (*Oncorhynchus mykiss*). J. Endocrinol. 191, 15–24.
Garcia de la Serrana, D., Macqueen, D.J., 2018. Insulin-like growth factor-binding proteins of teleost fishes. Front. Endocrinol. 9, 80.
Garcia de la Serrana, D., Fuentes, E.N., Martin, S.A.M., Johnston, I.A., Macqueen, D.J., 2017. Divergent regulation of insulin-like growth factor binding protein genes in cultured Atlantic salmon myotubes under different models of catabolism and anabolism. Gen. Comp. Endocrinol. 247, 53–65.
Gray, E.S., Kelley, K.M., Law, S., Tasi, R., Young, G., Bern, H.A., 1992. Regulation of hepatic growth hormone receptors in coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 88, 243–252.
Grémare, A., Vétion, G., 1994. Comparison of several spectrofluorimetric methods for measuring RNA and DNA concentrations in the deposit-feeding bivalve *Abra ovata*. Comp. Biochem. Physiol. B 107, 297–308.
Guler, H.P., Zapf, J., Schmid, C., Froesch, E.R., 1989. Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. Acta Endocrinol. 121, 753–758.
Gutiérrez, J., Pérez, J., Navarro, I., Zanuy, S., Carrillo, M., 1991. Changes in plasma glucogon and insulin associated with fasting in sea bass (*Dicentrarchus labrax*). Fish Physiol. Biochem. 9, 107–112.
Hack, N.L., Strobel, J.S., Journey, M.L., Beckman, B.R., Lema, S.C., 2018. Response of the insulin-like growth factor-1 (Igf1) system to nutritional status and growth rate variation in olive rockfish (*Sebastes serranoides*). Comp. Biochem. Physiol. A 224, 42–52.
Handeland, S.O., Imsland, A.K., Stefansson, S.O., 2008. The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. Aquaculture 283, 36–42.
Hanna, M.Y., 1962. Effect of starvation on carbohydrate content of tissues and on relative weights of organs of *Clarias lazera*. Z. Vergl. Physiol. 45, 315–321.
Hevrøy, E.M., Azpeleta, C., Shimizu, M., Lanzén, A., Kaiya, H., Espe, M., Olsvik, P.A., 2011. Effects of short-term starvation on ghrelin, GH-IGF system, and IGF-binding proteins in Atlantic salmon. Fish Physiol. Biochem. 37, 217–232.
Inagaki, T., Lin, V.Y., Goetz, R., Mohammadi, M., Mangelsdorf, D.J., Kliewer, S.A., 2008. Inhibition of growth hormone signaling by the fasting-induced hormone FGF21. Cell Metabol. 8, 77–83.
Jogie-Brahim, S., Feldman, D., Oh, Y., 2009. Unraveling insulin-like growth factor binding protein-3 actions in human disease. Endocr. Rev. 30, 417–437.
Kajimura, S., Aida, K., Duan, C., 2005. Insulin-like growth factor-binding protein-1 (IGFBP-1) mediates hypoxia-induced embryonic growth and developmental retardation. Proc. Natl. Acad. Sci. U.S.A. 102, 1240–1245.
Kawaguchi, K., Kaneko, N., Fukuda, M., Nakano, Y., Kimura, S., Hara, A., Shimizu, M., 2013. Responses of insulin-like growth factor (IGF)-I and two IGF-binding protein-1 subtypes to fasting and re-feeding, and their relationships with individual growth rates in yearling masu salmon (*Oncorhynchus masou*). Comp. Biochem. Physiol. A 165, 191–198.
Kawanago, M., Takemura, S., Ishizuka, R., Shioya, I., 2014. Plasma components and hepatic insulin-like growth factor expression indicate nutritional status in yellowtail *Seriola quinqueradiata*. N. Amer. J. Aquacult. 76, 143–152.

Kelley, K.M., Siharath, K., Bern, H.A., 1992. Identification of insulin-like growth factor-binding proteins in the circulation of four teleost fish species. J. Exp. Zool. 263, 220–224.
Kelley, K.M., Haigwood, J.T., Perez, M., Galima, M.M., 2001. Serum insulin-like growth factor binding proteins (IGFBPs) as markers for anabolic/catabolic condition in fishes. Comp. Biochem. Physiol. B 129, 229–236.
Krause, E.T., Honarmand, M., Wetzel, J., Naguib, M., 2009. Early fasting is long lasting: differences in early nutritional conditions reappear under stressful conditions in adult female Zebra finches. PLoS ONE 4, e5015.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 33, 1870–1874.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.
Larsen, D., Beckman, B., Walton, D., 2001. The effect of low temperature and fasting during the winter on metabolic stores and endocrine physiology (insulin, insulin-like growth factor-I, and thyroxine) of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 123, 308–323.
Laviola, L., Natalicchio, A., Giorgino, F., 2007. The IGF-I signaling pathway. Curr. Pharm. Des. 13, 663–669.
Lefevre, S., McKenzie, D.J., Nilsson, G.E., 2017. Models projecting the fate of fish populations under climate change need to be based on valid physiological mechanisms. Glob. Change Biol. 23, 3449–3459.
Lema, S.C., Kitano, J., 2013. Hormones and phenotypic plasticity: implications for the evolution of integrated adaptive phenotypes. Curr. Zool. 59, 506–525.
Li, Y., Xiang, J., Duan, C., 2005. Insulin-like growth factor-binding protein-3 plays an important role in regulating pharyngeal skeleton and inner ear formation and differentiation. J. Biol. Chem. 280, 3613–3620.
Logerwell, E.A., Mantua, N., Lawson, P.W., Francis, R.C., Agostini, V.N., 2003. Tracking environmental processes in the coastal zone for understanding and predicting Oregon coho (*Oncorhynchus kisutch*) marine survival. Fish. Oceanogr. 12, 554–568.
Martin, J.L., Baxter, R.C., 1992. Insulin-like growth factor binding protein-3: biochemistry and physiology. Growth Regul. 2, 88–99.
Maures, T., Chan, S.J., Xu, B., Sun, H., Ding, J., Duan, C., 2002. Structural, biochemical and expression analysis of two distinct insulin-like growth factor I receptors and their ligands in zebrafish. Endocrinology 143, 1858–1871.
Metzger, D.C., Luckenbach, J.A., Shimizu, M., Beckman, B.A., 2012. Normalizing for biology: accounting for technical and biological variation in levels of reference gene and insulin-like growth factor 1 (*igf1*) transcripts in fish livers. Comp. Biochem. Physiol. A 163, 7–14.
Mingarro, M., Vega-Rubín De Celis, S., Astola, A., Pendón, C., Valdivia, M.M., Pérez-Sánchez, J., 2002. Endocrine mediators of seasonal growth in gilthead sea bream (*Sparus aurata*): the growth hormone and somatolactin paradigm. Gen. Comp. Endocrinol. 128, 102–111.
Montserrat, N., Gabillard, J.C., Capilla, E., Navarro, M.I., Gutiérrez, J., 2007. Role of insulin, insulin-like growth factors, and muscle regulatory factors in the compensatory growth of the trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 150, 462–472.
Most, J., Tosti, V., Redman, L.M., Fontana, L., 2017. Calorie restriction in humans: an update. Ageing Res. Rev. 39, 36–45.
Navarro, I., Gutiérrez, J., 1995. Fasting and starvation. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and Molecular Biology of Fishes. Elsevier, New York, pp. 394–434.
Navarro, I., Gutiérrez, J., Planas, J., 1993. Changes in plasma glucagon, insulin and tissue metabolites associated with prolonged fasting in brown trout (*Salmo trutta fario*) during two different seasons of the year. Comp. Biochem. Physiol. A 102, 401–407.
Norbeck, L.A., Kittilson, J.D., Sheridan, M.A., 2007. Resolving the growth-promoting and metabolic effects of growth hormone: differential regulation of GH–IGF-I system components. Gen. Comp. Endocrinol. 151, 332–341.
Paloheim, J.E., Dickie, L.M., 1966. Food and growth of fishes: III. Relations among food body size and growth efficiency. J. Fish. Res. Board Canada 23, 1209–1248.
Pedroso, F.L., de Jesus-Ayson, E.G.T., Cortado, H.H., Hyodo, S., Ayson, F.G., 2006. Changes in mRNA expression of grouper (*Epinephelus coioides*) growth hormone and insulin-like growth factor I in response to nutritional status. Gen. Comp. Endocrinol. 145, 237–246.
Pedroso, F.L., Fukada, H., Masumoto, T., 2009. In vivo and in vitro effect of recombinant salmon growth hormone treatment on IGF-I and IGFBPs in yellowtail *Seriola quinqueradiata*. Fish. Sci. 75, 887–894.
Pérez-Sánchez, J., Martí-Palanca, H., Kaushik, S.J., 1995. Ration size and protein intake affect circulating growth hormone concentrations, hepatic growth hormone binding and plasma insulin-like growth factor-I immunoreactivity in a marine teleost, the gilthead sea bream (*Sparus aurata*). J. Nutrition 125, 546–552.
Peterson, B.C., Small, B.C., 2004. Effects of fasting on circulating IGF-binding proteins, glucose, and cortisol in channel catfish (*Ictalurus punctatus*). Domest. Anim. Endocrinol. 26, 231–240.
Peterson, B.C., Bilodeau-Bourgeois, A.L., Small, B.C., 2009. Response of the somatotropic axis to alterations in feed intake of channel catfish (*Ictalurus punctatus*). Comp. Biochem. Physiol. A 153, 457–463.
Peterson, B.C., Waldbieser, G.C., 2009. Effects of fasting on IGF-I, IGF-II, and IGF-binding protein mRNA concentrations in channel catfish (*Ictalurus punctatus*). Domest. Anim. Endocrinol. 37, 74–83.
Picha, M.E., Silverstein, J.T., Borski, R.J., 2006. Discordant regulation of hepatic IGF-I mRNA and circulating IGF-I during compensatory growth in a teleost, the hybrid striped bass (*Morone chrysops* X *Morone saxatilis*). Gen. Comp. Endocrinol. 147, 196–205.
Picha, M.E., Turano, M.J., Beckman, B.R., Borski, R.J., 2008a. Endocrine biomarkers of growth and applications to aquaculture: a minireview of growth hormone, insulin-like growth factor (IGF)-I, and IGF-binding proteins as potential growth indicators in fish. N. Am. J. Aquacult. 70, 196–211.
Picha, M.E., Turano, M.J., Tipsmark, C.K., Borski, R.J., 2008b. Regulation of endocrine and paracrine sources of Igfs and Gh receptor during compensatory growth in hybrid striped bass (*Morone chrysops* X *Morone saxatilis*). J. Endocrinol. 199, 81–94.
Pierce, A.L., Beckman, B.R., Shearer, K.D., Larsen, D.A., Dickhoff, W.W., 2001. Effects of ration on somatotropic hormones and growth in coho salmon. Comp. Biochem. Physiol. B 128, 255–264.
Pierce, A.L., Shimizu, M., Beckman, B.R., Baker, D.M., Dickhoff, W.W., 2005. Time course of the GH/IGF axis response to fasting and increased ration in chinook salmon (*Oncorhynchus tshawytscha*). Gen. Comp. Endocrinol. 140, 192–202.
Pierce, A.L., Fox, B.K., Davis, L.K., Visitacion, N., Kitahashi, T., Hirano, T., Grau, E.G., 2007. Prolactin receptor, growth hormone receptor, and putative somatolactin receptor in Mozambique tilapia: tissue specific expression and differential regulation by salinity and fasting. Gen. Comp. Endocrinol. 154, 31–40.
Polakof, S., Mommsen, T.P., Soengas, J.L., 2011. Glucosensing and glucose homeostasis: from fish to mammals. Comp. Biochem. Physiol. B 160, 123–149.
Power, D.M., Llewellyn, L., Faustino, M., Nowell, M.A., Björnsson, B.Th., Einarsdottir, I.E., Canario, A.V.M., Sweeney, G.E., 2001. Thyroid hormones in growth and development of fish. Comp. Biochem. Physiol. C 130, 447–459.
Ranke, M.B., 2015. Insulin-like growth factor binding-protein-3 (IGFBP-3). Best Prac. Res. Clin. Endocrinol. Met. 29, 701–711.
Rajaram, S., Baylink, D.J., Mohan, S., 1997. Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr. Rev. 18, 801–831.
Reindl, K.M., Sheridan, M.A., 2012. Peripheral regulation of the growth hormone - insulin-like growth factor system in fish and other vertebrates. Comp. Biochem. Physiol. A 163, 231–245.
Reinecke, M., Björnsson, B.Th., Dickhoff, W.W., McCormick, S.D., Navarro, I., Power, D.M., Gutiérrez, J., 2005. Growth hormone and insulin-like growth factors in fish: where we are and where to go. Gen. Comp. Endocrinol. 142, 20–24.
Ren, H., Yin, P., Duan, C., 2008. IGFBP-5 regulates muscle cell differentiation by binding to IGF-II and switching on the IGF-II auto-regulation loop. J. Cell Biol. 182, 979–991.
Sadoul, B., Vijayan, M.M., 2016. Stress and growth. In: In: Schreck, C.B., Tort, L., Farrell, A.P. (Eds.), Biology of Stress in Fish: Fish Physiology, vol. 35. Elsevier, Inc., London, UK, pp. 167–205.
Saera-Vila, A., Calduch-Giner, J.-A., Pérez-Sánchez, J., 2005. Duplication of growth hormone receptor (GHR) in fish genome: gene organization and transcriptional regulation of GHR type I and II in gilthead sea bream (*Sparus aurata*). Gen. Comp. Endocrinol. 142, 193–203.
Safian, D., Fuentes, E.N., Valdés, J.A., Molina, A., 2012. Dynamic transcriptional regulation of autocrine/paracrine igfbp1, 2, 3, 4, 5, and 6 in the skeletal muscle of the fine flounder during different nutritional statuses. J. Endocrinol. 214, 95–108.
Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Salem, M., Kenney, P.B., Rexroad III, C.E., Yao, J., 2010. Proteomic signature of muscle atrophy in rainbow trout. J. Proteomics 73, 778–789.
Salgin, B., Marcovecchio, M.L., Hill, N., Dunger, D.B., Frystyk, J., 2012. The effect of prolonged fasting on levels of growth hormone-binding protein and free growth hormone. Growth Horm. IGF Res. 22 (2), 76–81.
Salih, D.A.M., Tripathi, G., Holding, C., Szestak, T.A.M., Gonzalez, M.I., Carter, E.J., Cobb, L.J., Eisemann, J.E., Pell, J.M., 2004. Insulin-like growth factor-binding protein 5 (Igfbp5) compromises survival, growth, muscle development, and fertility in mice. Proc. Natl. Acad. Sci. U.S.A. 101, 4314–4319.
Shanley, D.P., Kirkwood, T.B.L., 2006. Caloric restriction does not enhance longevity in all species and is unlikely to do so in humans. Biogerontology 7, 165–168.
Shimizu, M., Swanson, P., Dickhoff, W.W., 1999. Free and protein-bound insulin-like growth factor-I (IGF-I) and IGF-binding proteins in plasma of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 115, 398–405.
Shimizu, M., Swanson, P., Fukada, H., Hara, A., Dickhoff, W.W., 2000. Comparison of extraction methods and assay validation for salmon insulin-like growth factor-1 using commercially available components. Gen. Comp. Endocrinol. 119, 26–36.
Shimizu, M., Hara, A., Dickhoff, W.W., 2003. Development of an RIA for salmon 41 kDa IGF-binding protein. J. Endocrinol. 178, 275–283.
Shimizu, M., Beckman, B.R., Hara, A., Dickhoff, W.W., 2006. Measurement of circulating salmon IGF binding protein-1: assay development, response to feeding ration and temperature, and relation to growth parameters. J. Endocrinol. 188, 101–110.
Shimizu, M., Cooper, K.A., Dickhoff, W.W., Beckman, B.R., 2009. Postprandial changes in plasma growth hormone, insulin, insulin-like growth factor (IGF)-I, and IGF-binding proteins in coho salmon fasted for varying periods. Am. J. Physiol. 297, R352–R361.
Shimizu, M., Dickhoff, W.W., 2017. Circulating insulin-like growth factor binding proteins in fish: their identities and physiological regulation. Gen. Comp. Endocrinol. 252, 150–161.
Small, B.C., Peterson, B.C., 2005. Establishment of a time-resolved fluoroimmunoassay for measuring plasma insulin-like growth factor I (IGF-I) in fish: effect of fasting on plasma concentrations and tissue mRNA expression of IGF-I and growth hormone (GH) in channel catfish (*Ictalurus punctatus*). Domest. Anim. Endocrinol. 28, 202–215.
Small, B.C., Murdock, C.A., Waldbieser, G.C., Peterson, B.C., 2006. Reduction in channel catfish hepatic growth hormone receptor expression in response to food deprivation and exogenous cortisol. Domest. Anim. Endocrinol. 31, 340–356.
Sonntag, W.E., Lynch, C.D., Cefalu, W.T., Ingram, R.L., Bennett, S.A., Thornton, P.L., Khan, A.S., 1999.. Pleiotropic effects of growth hormone and insulin-like growth factor (IGF)-1 on biological aging: inferences from moderate caloric-restricted animals. J. Gerontol. A 54, B521–B538.

Uchida, K., Kajimura, S., Riley, L.G., Hirano, T., Aida, K., Grau, E.G., 2003. Effects of fasting on growth hormone/ insulin-like growth factor I axis in the tilapia, *Oreochromis mossambicus*. Comp. Biochem. Physiol. A. 134, 429–439.

Van Leeuwen, T.E., Rosenfeld, J.S., Richards, J.G., 2012. Effects of food ration on SMR: influence of food consumption on individual variation in metabolic rate in juvenile coho salmon (*Oncorhynchus kisutch*). J. Anim. Ecol. 81, 395–402.

VenTresca, D.A., Houk, J.L., Paddack, M.J., Gingras, M.L., 1996. Early life-history studies of nearshore rockfishes and lingcod off Central California, 1987-92. Mar. Res. Div. Admin. Rep. 96-4, California Dept. Fish Game, Monterey, CA.

Vera Cruz, E.M., Brown, C.L., Luckenbach, J.A., Picha, M.E., Bolivar, R.B., Borski, R.J., 2006. Insulin-like growth factor-I cDNA cloning, gene expression and potential use as a growth rate indicator in Nile tilapia, *Oreochromis niloticus*. Aquaculture 251, 585–595.

von Biela, V.R., Kruse, G.H., Mueter, F.J., Black, B.A., Douglas, D.C., Helser, T.E., Zimmerman, C.E., 2015. Evidence of bottom-up limitations in nearshore marine systems based on otolith proxies of fish growth. Mar. Biol. 162, 1019–1031.

Waples, R.S., Audzijonyte, A., 2016. Fishery-induced evolution provides insights into adaptive responses of marine species to climate change. Front. Ecol. Environ. 14, 217–224.

Wilkinson, R.J., Porter, M., Woolcott, H., Longland, R., Carragher, J.F., 2006. Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic salmon and rainbow trout. Comp. Biochem. Physiol. A 145, 214–224.

Wilson, J.R., Broitman, B.R., Caselle, J.E., Wendt, D.E., 2008. Recruitment of coastal fishes and oceanographic variability in central California. Estuar. Coastal Shelf Sci. 79, 483–490.

Wood, A.W., Duan, C.M., Bern, H.A., 2005. Insulin-like growth factor signaling in fish. Int. Rev. Cytol. 243, 215–285.

Won, E.T., Borski, R.J., 2013. Endocrine regulation of compensatory growth in fish. Front. Endocrinol. 4, 74.

Wurtsbaugh, W.A., Davis, G.E., 1977. Effects of fish size and ration level on the growth and food conversion efficiency of rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 11, 99–104.

Zheng, D.-G., Zhou, C.-X., Lin, S.-T., Chen, J., Jiang, X.-Y., Zou, S.-M., 2017. Two grass carp (*Ctenopharyngodon idella*) insulin-like growth factor-binding protein 5 genes exhibit different yet conserved functions in development and growth. Comp. Biochem. Physiol. B 204, 69–76.

Zhong, Y., Lu, L., Zhou, J., Li, Y., Liu, Y., Clemmons, D.R., Duan, C., 2011. IGF binding protein 3 exerts its ligand-independent action by antagonizing BMP in zebrafish embryos. J. Cell Sci. 124, 1925–1935.