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Nutritional status affects Igf1 regulation of skeletal muscle myogenesis, myostatin, and myofibrillar protein degradation pathways in gopher rockfish (*Sebastes carnatus*)

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ABSTRACT

Insulin-like growth factor-1 (Igf1) regulates skeletal muscle growth in fishes by increasing protein synthesis and promoting muscle hypertrophy. When fish experience periods of insufficient food intake, they undergo slower muscle growth or even muscle wasting, and those changes emerge in part from nutritional modulation of Igf1 signaling. Here, we examined how food deprivation (fasting) affects Igf1 regulation of liver and skeletal muscle gene expression in gopher rockfish (*Sebastes carnatus*), a nearshore rockfish of importance for commercial and recreational fisheries in the northeastern Pacific Ocean, to understand how food limitation impacts Igf regulation of muscle growth pathways. Rockfish were either fed or fasted for 14 d, after which a subset of fish from each group was treated with recombinant Igf1 from sea bream (*Sparus aurata*). Fish that were fasted lost body mass and had lower body condition, reduced hepatosomatic index, and lower plasma Igf1 concentrations, as well as a decreased abundance of *igf1* gene transcripts in the liver, increased hepatic mRNAs for Igf binding proteins *igfbp1a*, *igfbp1b*, and *igfbp3a*, and decreased mRNA abundances for *igfbp2b* and a putative Igf acid labile subunit (*igfals*) gene. In skeletal muscle, fasted fish showed a reduced abundance of intramuscular *igf1* mRNAs but elevated gene transcripts encoding Igf1 receptors A (*igf1ra*) and B (*igf1rb*), which also showed downregulation by Igf1. Fasting increased skeletal muscle mRNAs for *myogenin* and *myostatin1*, as well as ubiquitin ligase F-box only protein 32 (*fbxo32*) and muscle RING-finger protein-1 (*murf1*) genes involved in muscle atrophy, while concurrently downregulating mRNAs for myoblast determination protein 2 (*myod2*), *myostatin2*, and myogenic factors 5 (*myf5*) and 6 (*myf6* encoding Mrf4). Treatment with Igf1 downregulated muscle *myostatin1* and *fbxo32* under both feeding conditions, but showed feeding-dependent effects on *murf1, myf5*, and *myf6*/Mrf4 gene expression indicating that Igf1 effects on muscle growth and atrophy pathways is contingent on recent food consumption experience.

1. Introduction

The growth hormone (GH)/insulin-like growth factor-1 (Igf1) somatotropic endocrine axis regulates growth, development, and aging in teleost fishes (Duan, 1997; Power et al., 2001; Bergan-Roller and Sheridan, 2018). In fish as in other vertebrates, the synthesis and release of Igf1 from the liver is stimulated by GH from the pituitary gland (Leung et al., 2008). Changes in food consumption including the quantity and nutritional composition of diet affect GH/Igf1 axis activation and alter liver Igf1 synthesis (Duan, 1997; Picha et al., 2008; Beckman,

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2011; Reindl and Sheridan, 2012; Bergan-Roller and Sheridan, 2018). Ultimately, those shifts in liver Igf1 synthesis cause changes in circulating Igf1, which then modulates somatic growth via actions on tissues including skeletal muscle (Duan et al., 1993; Mommsen and Moon, 2001; Velloso, 2008; Cleveland and Weber, 2010; Bergan-Roller and Sheridan, 2018). Such nutritional regulation of Igf1 production and, ultimately, growth has been recorded in a wide taxonomic variety of fish species experiencing experimentally-generated variation in food ration amounts or feeding/fasting conditions (Pérez-Sánchez et al., 1995; Beckman et al., 2004a; Pierce et al., 2005; Bower et al., 2008; Breves et al., 2014, 2016; Hack et al., 2018, 2019; Strobel et al., 2020; Bersin et al., 2023).

The picture emerging from studies of growth regulation in fish indicates that the effects of Igf1 on peripheral tissues relies on several other components in the GH/Igf1 signaling pathway including local Igf1 production, Igf binding proteins (Igfbps) that modulate Igf1 availability in blood circulation or target tissues, and multiple Igf1 receptors (Greene and Chen, 1999; Bach, 2018; Shimizu and Dickhoff, 2017). Igfbp genes were duplicated one or more times across the evolution of teleost fishes depending on the taxonomic lineage (Shimizu and Dickhoff, 2017; Allard and Duan, 2018), and the relative expression of the different Igfbps modulates the availability of Igf1 for action on target tissues (Allard and Duan, 2018; Eppler et al., 2010; Franz et al., 2016). Some Igfbps have even been shown to have cellular actions independent of Igf1 (Firth and Baxter, 2002). While liver synthesis of Igfbps is thought to be the primary source for these binding proteins in blood circulation, evidence for peripheral tissue expression of *igfbp* genes – and also the *igf1* gene – implies a role for tissue-specific autocrine or paracrine regulation of Igf1 action. Similar to other endocrine regulatory pathways (e.g., Lema and Kitano, 2013), the complex structure and multi-tissue expression of hormones, binding proteins, and receptors in GH/Igf1 endocrine signaling provides multiple steps for regulatory control under changing developmental stages or environmental conditions.

Characterizing how Igf1 actions on skeletal muscle growth are regulated by Igfbps and other factors under differing conditions of food intake is critically important for understanding modulation of somatic growth (Fuentes et al., 2013), as skeletal muscle comprises approximately 40–60% of overall body mass in most teleost fish (Weatherley and Gill, 1985). Changes in muscle growth rate due to food restriction or deprivation involve regulatory changes in myogenesis or muscle wasting. Unlike mammals, many teleost fishes evolved indeterminant growth and will continue to recruit and activate muscle stem cells to increase muscle mass via both hyperplasia and hypertrophy throughout life (Weatherley and Gill, 1987; Fauconneau and Padboef., 2000; Froehlich et al., 2013). Similar to mammals, however, skeletal muscle growth in fish is mediated by several myogenic factors that directly bind DNA to upregulate muscle- and sarcomeric-specific genes involved in muscle differentiation and growth (Buckingham, 1994; Watabe, 2001; Hernández-Hernández et al., 2017; Zammit, 2017). Those myogenic transcription factors include myoblast determination protein (*myod*), which promotes histone acetylation to turn on genes involved in myoblast and myotube formation (Cao et al., 2010), and *myogenin*, which is critical in myogenic differentiation and myocyte fusion during muscle development (Sassoon et al., 1989; Wright et al., 1989; Nabeshima et al., 1993; Ganassi et al., 2018). In addition, muscle growth also is regulated by myogenic factors 5 (*myf5*) and 6 (*myf6*, which encodes the muscle-specific regulatory factor 4 [Mrf4] protein) that regulate myoblast differentiation via actions on myokine expression and muscle progenitor cell proliferation and differentiation (Buckingham, 1994; Kassar-Duchossoy et al., 2004; Lazure et al., 2020). However, under conditions of insufficient energy intake, fish will lose muscle mass due to muscle protein breakdown and atrophy. These effects occur via an alternative set of regulatory factors such as myostatins that inhibit growth-promoting pathways (Garikipati and Rodgers, 2012a), as well as by the action of several atrophy-related genes including the ubiquitin ligases F-box only protein 32 (Fbxo32) – also called atrogin-1 and muscle atrophy F-box (MAFbx) – which increases in muscle under conditions of fasting (e.g., Gomes et al., 2001), and muscle RING-finger protein-1 (MuRF1), which mediates degradation of sarcomeric muscle proteins during atrophy (Bodine et al., 2001; Kedar et al., 2004; Glass and Roubenoff, 2010).

While Igf1 clearly regulates muscle growth in fishes (Castillo et al., 2004; Montserrat et al., 2007; Fuentes et al., 2013), it is not fully clear how the sensitivity and regulatory responses of muscle growth and atrophy pathways to Igf1 shift to generate somatic growth modulation when fish experience changing food conditions. In the present study, we examined how recent nutritional experience modulates liver Igf1 and Igfbp expression, skeletal muscle Igf signaling pathways, and muscle growth- and atrophy-associated gene pathways in juvenile gopher rockfish (*Sebastes carnatus*). Gopher rockfish are a nearshore rockfish that is one among dozens of *Sebastes* rockfish species in the North Pacific Ocean targeted by commercial or recreational fisheries. Several *Sebastes* rockfishes have been studied extensively in the context of fishery management (e.g., Chin et al., 2013; Loury et al., 2015; Bellquist et al., 2019) and for their potential for aquaculture (e.g., Nakagawa et al., 2007; Guo et al., 2017; Zhang et al., 2020; Lee et al., 2022), and also have been studied recently for extensive species variation in longevity (Kolora et al., 2021). Here, we tested how variation in recent feeding status modulated Igf1 regulation of Igf-related gene expression pathways (*igf1,* Igfbps, etc.) in the liver and Igf-signaling, myogenesis, and muscle atrophy in skeletal muscle to understand how the effects of Igf1 on these pathways are differentially shaped by recent feeding experience. Specifically, we maintained juvenile gopher rockfish under conditions of either continuous feeding or fasting for 14 d, after which a subset of fish from both the 'feeding' and 'fasted' groups was given a single injection of Igf1. We then examined how those differences in recent feeding status influenced Igf1 regulation of *igf1* and Igfbp gene expression in the liver, as well as skeletal muscle expression of Igf1 receptors A (*igf1ra*) and B (*igf1rb*), Igfbps, and myogenesis or muscle atrophy genes including MyoD-encoding paralogs *myod1* and *myod2* (Macqueen and Johnston, 2008), *myogenin*, *myf5, myf6, myostatin1* and *myostatin2*, *fbxo32*, and *murf1*.

2. Materials and methods

2.1. Animals

Young-of-the-year juvenile gopher rockfish (*S. carnatus*) were collected between May and September 2016 using a Standard Monitoring Unit for the Recruitment of Fishes (SMURF) (e.g., Ammann, 2004; Wilson et al., 2008) that was positioned in the water column at a depth of approximately 1–2 m under California Polytechnic 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). Juvenile fish collected from the SMURF were held at the CCMS pier in 340 L, flow-through tanks with sand-filtered seawater under ambient photoperiod, salinity (~33‰) and temperature (~11–15 °C) conditions. Fish were fed *ad libitum* daily with commercial fish pellet feed (BioPro 2 pellets, BioOregon, Longview, WA, USA) for ~1.5–2 years prior to starting the experimental ration treatments. BioPro 2 feed composition is minimum 50% protein and 22% lipid, and maximum 1.0% fiber, 13% ash, and 8.5% moisture. All experimental procedures were approved by the Animal Use and Care Committee of California Polytechnic State University, San Luis Obispo (Protocol # 1504).

2.2. Experimental feeding and Igf1 treatments

Juvenile gopher rockfish were weighed, measured, and tagged intraperitoneally using passive integrated transponder (PIT) tags (7 mm, Loligo Systems, Inc., Viborg, Denmark). Tagged fish were assigned to experimental 340 L tanks (0.97 m diameter x 0.48 m depth) in May 2018

with ten fish per tank and two replicate tanks per treatment combination for a total $n = 20$ fish per treatment. Fish were assigned systematically to tanks based on body size parameters so that body mass and length distributions were statistically similar among the tanks (no differences between treatment tanks: body mass, $p = 0.8729$; standard length [SL], $p = 0.9325$). Fish were maintained under ambient photoperiod on flow-through filtered seawater (~33 ppt), and water temperatures were recorded every 10 min throughout the experimental period (HOBO Pendant Temperature/Light Data Logger, Onset Computer Corp., Bourne, MA, USA). Water temperature averaged 12.79 ± 0.97 °C (mean ± SD; range: 11.04–15.00 °C) over the duration of the experiment.

During an initial 14 d acclimation period, all fish were fed twice each day for a total daily ration of 9% mass of feed per fish wet mass. That feeding amount was selected to exceed a satiation-level ration. After that acclimation period, rockfish were again measured and weighed (day 0), and then fish in half the tanks were changed to a treatment of complete food deprivation (fasting) for 14 d, while fish in the other tanks continued to be maintained on the 9% ration amount. After 14 d of continued feeding or fasting, fish were anesthetized with tricaine methanesulfonate (MS222), weighed and measured again (day 14), and then injected intraperitoneally with saline vehicle (0.9% saline solution with 0.1% bovine serum albumin; 1 μl per g body mass) or recombinant gilthead sea bream Igf1 (1 μg per 1 g body mass; >98% purity, ProSpec TechnoGene Ltd., Ness-Ziona, Israel). All fish in both feeding treatments were fasted 24 h prior to injection and were not fed again post-injection. After injection, fish were maintained in their same experimental growth tanks for 16–24 h, and then euthanized using MS222 and again weighed and measured (day 15). For all $n = 20$ fish from each treatment group, blood and tissues were collected for hormone and gene expression analyses. Blood was collected in heparinized microhematocrit capillary tubes following caudal ablation. Blood was centrifuged at 3000×*g* for 10 min at 4 °C, and the resulting plasma was stored at −80 °C. The liver and a sample of fast-twitch (white) skeletal muscle from the left side of the body posterior to the dorsal fin were dissected, frozen in liquid N_2, and stored at −80 °C.

2.3. Plasma Igf1 quantification

Plasma concentrations of Igf1 were quantified using a time-resolved fluoroimmunoassay (TR-FIA) method (Small and Peterson, 2005), which is described in detail elsewhere (Ferriss et al., 2014) and had been previously validated for use in *Sebastes* rockfishes (Hack et al., 2018, 2019) and other fishes of order Scorpaeniformes (Strobel et al., 2020). This TR-FIA method was developed from the RIA method described by Shimizu et al. (1999). For individual fish with plasma volumes less than 20 μl, the plasma was pooled with that from another fish of the same treatment condition prior to hormone extraction, ultimately resulting in final sample sizes of $n = 10$–15 per treatment. Igf1 was extracted from gopher rockfish plasma using a 4x volume solution of 87.5% ethanol and 12.5% 2M HCl acid, centrifuged, and then resuspended in ice cold 0.855 M Tris Base. Extract (20–35 μl) was then assayed using antiserum to recombinant barramundi (*Lates calcarifer*) Igf1 (GroPep BioReagents, Ltd., Thebarton, SA, Australia) (Degger et al., 2000). Assays were run using dissociation enhanced lanthanide fluorescence immunoassay (DELFIA®, PerkinElmer) anti-rabbit IgG-coated 96-well plates and custom Eu-labeled recombinant salmon Igf1 (GroPep BioReagents, Ltd.). The standard curve was made using recombinant salmon Igf1. Eight samples from the 'fasted/control injection' treatment group and one sample from the 'fed/control injection' treatment were above the 85% binding threshold of the assay (<8.0 ng/ml) but still generated plasma Igf1 values above the lowest Igf1 concentration standard (2.0 ng/ml) used for the standard curve. Since duplicate Igf1 values generated by those samples were >0 ng/ml, it was clear that those samples displaced from the antibody; however, we lacked the ability to precisely quantify how much label was displaced as the values were below the 8.0 ng/ml sensitivity of the assay based on % binding. For those samples, we conservatively assigned a randomly generated value between 0 ng/ml and the sensitivity of the assay (8.0 ng/ml).

2.4. Measurement of RNA:DNA ratio

RNA:DNA in liver and skeletal muscle tissues was measured using a spectrofluorimetric method from Grémare and Vétion (1994) that was subsequently modified by Kawaguchi et al. (2013). Frozen tissue was digested at 4 °C using Proteinase K (20 mg/ml; Invitrogen) in a 0.5 ml solution of 20 mM phosphate buffered saline with 0.15 M NaCl (pH 7.5). After digestion, 0.1% sodium dodecyl sulfate (56 μl) was added. Samples were then incubated for 15 min on ice with mixing every 3 min, and finally centrifuged for 15 min at 4500×*g* and 4 °C. The supernatant was then used for quantification of RNA:DNA.

Total nucleic acid concentrations were measured spectrophotometrically using Thiazole orange (Sigma-Aldrich) while DNA concentrations were quantified using Hoechst 33258 (Sigma-Aldrich). To quantify DNA, a 100 μl volume of supernatant was combined with 50 μl of PBS buffer and 5 μl of 0.02 mg/ml Hoechst 33258 solution that was then incubated for 30 min at 37 °C. 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, 50 μl of PBS buffer and 5 μl of Thiazole orange (4 μg/ml) were added to a 100 μl volume of supernatant, which was then assayed at 490 nm excitation and 545 nm emission. Standard curves for both assays were comprised of purified DNA from chum salmon, *Oncorhynchus keta* (Sigma-Aldrich). All standards were assayed in triplicate, and samples assayed in duplicate. The intra-assay % CV was 8.8% for the Hoechst 33258 assay, and 5.1% for the Thiazole orange assay.

2.5. Reverse transcription real-time quantitative PCR

Total RNA was extracted from tissues using TriReagent (Molecular Research Center, Inc.) with bromochloropropane as the phase separation reagent. Total RNA was DNase I treated (TURBO DNA-free Kit, Ambion) and quantified by spectrophotometry (P300 NanoPhotometer, Implen; 260:280 ratios ≥1.96). Total RNA was then reverse transcribed using 5.6 μl of 5x 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 Units/μl; Promega Corp.), 0.25 μl nuclease-free H_2O, 1.05 μl GoScript™ reverse transcriptase (Promega Corp.), and 14 μl of RNA template (30.0 ng/μl for liver RNA, 32.5 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 used to measure relative mRNA levels of genes in liver and skeletal muscle. All quantitative PCR reactions were run as 10 μl volumes containing 5 μl of iTaq™ Universal SYBR Green Supermix (BioRad Laboratories, Inc.), 1.5 μl of nuclease-free H_2O, 1.5 μl of cDNA, and 1 μl each of forward primer (10 μM) and reverse primer (10 μM). Gene specific SYBR Green primers were designed to partial cDNAs sequenced from gopher rockfish (GenBank accession nos. MW940098 to MW940107) using primers previously designed for use in *Sebastes serranoides* and *S. caurinus* (Hack et al., 2018, 2019), or to consensus regions of target genes identified in the assembled genomes of *Sebastes aleutianus* (GenBank assembly no. GCA_001910805), *S. umbrosus* (GCA_015220095), *S. rubrivinctus* (GCA_000475215), and *S. minor* (GCA_001910765). Primers were subsequently checked against a genome for gopher rockfish (GCA_916700855) that became available after the start of this study. Primers were also designed to cDNAs from gopher rockfish for elongation factor-1α (*ef1α*, MW940108) and 60S ribosomal protein L17 (*rpl17*, MW940109), as well as to the tubulin α-1a (*tuba*) gene identified in *Sebastes* genomes. Those *ef1α*, *rpl17*, and *tuba* genes were used as endogenous references in qPCR analyses. When possible, SYBR Green primer sets 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 S1. The specificity of each primer set was confirmed by Sanger sequencing select PCR products (Molecular Cloning Laboratories, South San Francisco, USA).

Because one of the genes targeted by this study – Igf binding protein acid labile subunit (Igfals) – had not been previously studied in detail in teleost fishes, a larger partial cDNA encoding 1496 bp of this putative *igfals* cDNA (GenBank accession no. OK350156) was amplified by PCR from RNA extracted from the liver of a gopher rockfish and Sanger sequenced (Molecular Cloning Labs, South San Francisco, CA, USA). Nucleotide sequences for the nested sets of gene-specific oligo primers used to amplify that gopher rockfish *igfals* cDNA are provided in Supplemental Materials, Table S2. The identify of gopher rockfish *igfals* was confirmed by phylogenetic reconstruction using deduced amino acid sequences of that cDNA and previously described cDNAs for *igfals* from other vertebrates (Supplemental Materials, Fig. S1).

Quantitative real-time RT-PCR (qRT-PCR) was performed in accordance with the guidelines presented by Bustin et al. (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 as 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 = $[10(^{1/slope}) - 1]\bullet 100$, and are provided for each set of primers in Supplemental Materials, Table S1. Correlation coefficients (r^2) were >0.96 for the standard curve for each gene. For each tissue, the geometric mean value of *ef1α*, *rpl17*, and *tuba* 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, but was affected in the liver by feeding treatment ($p = 0.0002$). The geometric reference gene value in liver was therefore also normalized to RNA:DNA ratio, as suggested by Metzger et al. (2012) when applying qRT-PCR methods for quantifying liver mRNA levels in fasted teleost fishes. The RNA:DNA ratio in the liver varied among treatment groups (one-factor ANOVA, ration*injection interaction: $F_{1,76} = 4.513$, $p = 0.0369$), with fasted, saline 'control' injected rockfish exhibiting lower RNA:DNA ratios (Supplemental Materials, Fig. S2). In muscle, RNA:DNA ratio did not vary significantly among treatments. All relative mRNA abundance data were then plotted as a relative level normalized to the mean value of that gene observed in rockfish from the fed, 'control' injection treatment.

2.6. Statistical analyses

Growth rates of fish were compared by calculating mass-specific growth rate (mass SGR) values. First, a *t*-test was used to compare mass SGRs for rockfish in the fed and fasted feeding treatments from day 0 to day 14, prior to hormone administration. To examine in more detail how fish changed body mass over that 14 d period as well after hormone administration, a repeated-measured ANOVA model was used to test for feeding and hormone treatment effects on body mass from day 0 to day 15, the day of tissue sampling after hormone administration. Mass SGR calculated from day 14–15 was further compared using a two-factor ANOVA model to test for effects of 'feeding' or 'fasting' food ration treatment, hormone injection with recombinant 'Igf1' or saline 'control,' and the interaction between these feeding and hormone effects on the loss of body mass in that final day of the experiment where all fish were fasted following hormone administration. Body condition factor (k) was calculated for each fish both prior to (day 0) and after exposure to the experimental treatments (day 14 and 15) using the following formula: (mass/standard length^3) · 100. Body condition values were initially examined between fish assigned to the feeding and fasting treatments using a one-factor ANOVA as part of the systematic assignment of fish to treatment tanks. Body condition was again examined for effects of feeding and Igf1 exposure treatments using a repeated-measures ANOVA model, followed by ANOVA models on day 14 and 15 data values separately. Hepatosomatic index (HSI, calculated as: [liver mass/body mass] · 100) values were similarly assessed using two-factor ANOVA models.

Relative mRNA levels in liver and skeletal muscle tissues were examined for normality and equivalence of variances using both a Bartlett test and a Levene's test. When variances were found to be unequal, mRNA data for that gene were $\log_{10}(x+1)$ transformed. Gene transcript abundance data were subsequently analyzed using two-factor ANOVA models with fed/fasting, control/Igf1 injection, and the interaction between those factors. For gene transcripts with significant feeding and hormone treatment main effects or interactions, *post hoc* pairwise comparisons were conducted using Tukey HSD multiple comparisons. When only a single main effect was identified, only the ANOVA main effect statistic is reported. All tests performed were two-tailed using an $\alpha = 0.05$, and were calculated using JMP® 16.0 software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Variation in growth rate, body condition, and HSI

Juvenile gopher rockfish experiencing food deprivation for 2 weeks declined by an average of 8.8% in body mass, while rockfish continuously fed over the 14 d period increased an average of 3.3% in mass. That decline was also reflected as a lower mass-specific growth rate (SGR) for fasted than fed rockfish from day 0 to the day of measurement (day 14) prior to hormone administration ($t = 12.763$, $p < 0.0001$) (Fig. 1a). Examination of body mass changes over that 14 d time period and also during the ~1 d period after hormone administration revealed that while fed rockfish gained body mass over the 14 d period of feeding, fasted rockfish declined in mass during that period (repeated-measures ANOVA, feed*time interaction: $F_{2,75} = 253.247$, $p < 0.0001$) (Fig. 1b). Fish in all treatment groups declined in body mass from day 14 to the time of blood and tissue collection (day 15) as a result of all fish being deprived for food for that period after hormone administration (Fig. 1b); those body mass declines after hormone administration were larger for fish from the fed treatment than from the fasted treatment, likely due to mass loss associated with gastrointestinal emptying in rockfish from the fed treatment (feed effect: $F_{1,76} = 4.451$, $p = 0.0382$) (Fig. 1c). While body condition factor (k) was similar initially (day 0) between fish assigned to the fed and fasted ration treatments (feed effect: $t = -0.901$, $p = 0.371$), k values were affected by both feed treatment and hormone injection over the 15 experimental period (repeated-measures ANOVA, time*feed effect*hormone effect interaction: $F_{2,75} = 4.419$, $p = 0.0153$) (Fig. 1d). On day 14 – after 14 d of feeding/fasting but before hormone administration – rockfish in the fed treatment showed a higher body condition than fish in the fasted treatments (feed effect: $F_{1,76} = 36.698$, $p < 0.0001$). That difference in body condition between feeding treatments was again observed at the 15 d time point, following hormone injection (feed effect: $F_{1,76} = 32.565$, $p < 0.0001$). Hormone treatment had no effect on body condition factor values in either fed or fasted rockfish. Liver HSI was also significantly lower in food-deprived rockfish than in fed fish (feed effect: $F_{1,76} = 173.929$, $p < 0.0001$) (Fig. 1e).

3.2. Circulating Igf1 and hepatic igf1 gene transcript abundance

Plasma Igf1 concentrations were higher in fed rockfish than in fasted rockfish (feed effect: $F_{1,45} = 34.812$, $p < 0.0001$), and were significantly elevated in Igf1-treated fish from both feeding conditions (hormone effect: $F_{1,45} = 7.224$, $p < 0.0001$) (Fig. 2). The food treatment differences in plasma Igf1 seem to reflect variation in endogenous Igf1

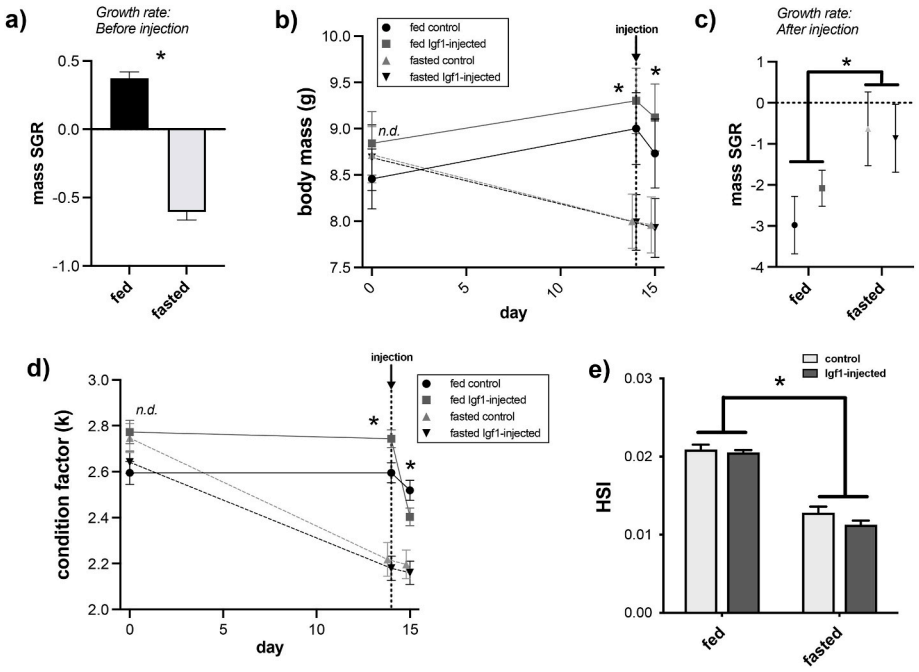

Fig. 1. (a) Fasted rockfish had a negative mass-specific growth rate (mass SGR) that differed from fed rockfish after 14 d of fasting (before hormone administration). (**b**) Corresponding with that lower mass SGR in fasted rockfish, fasting resulted in a decline in body mass over the 14 d period of food deprivation, while fed fish increased in mass over that same period (feed effect: day 14, $p = 0.0010$; day 15, $p = 0.0051$). (**c**) Over the ~1 d after hormone injection, fed fish exhibited a negative mass SGR, likely due to GI tract evacuation (feed effect: $p = 0.0382$). Treatment symbols are the same as in (**c**). (**d**) Rockfish in all treatment groups had similar body condition (k) at the start of the experiment (day 0). Body condition declined in fasted rockfish to be lower than fed fish at the time of hormone injection (day 14) as well as 16–24 h after injection (day 15). (**e**) Hepatosomatic index (HSI) values were higher in fed rockfish than fasted fish at the time of tissue sampling (day 15) (feed effect: $p < 0.0001$). Data are plotted as mean ± SEM values, and *n. d.* notation denotes 'no differences' on that sampling day. Sample sizes are $n = 20$ fish per treatment.

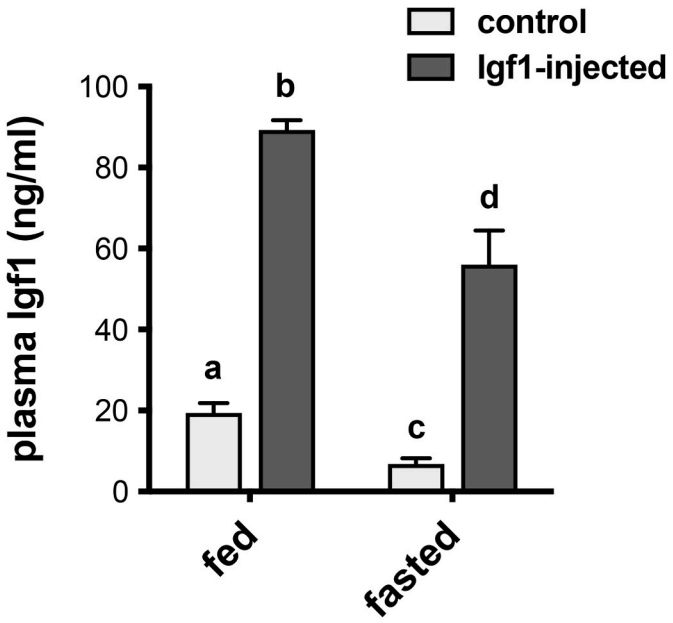

Fig. 2. Plasma Igf1 concentrations were elevated in fed rockfish compared to fish that were fasted and were also increased in both feeding groups following Igf1 injection. Data are plotted as mean ± SEM values. Letters indicate *post hoc* pairwise difference among treatments (Tukey HSD test).

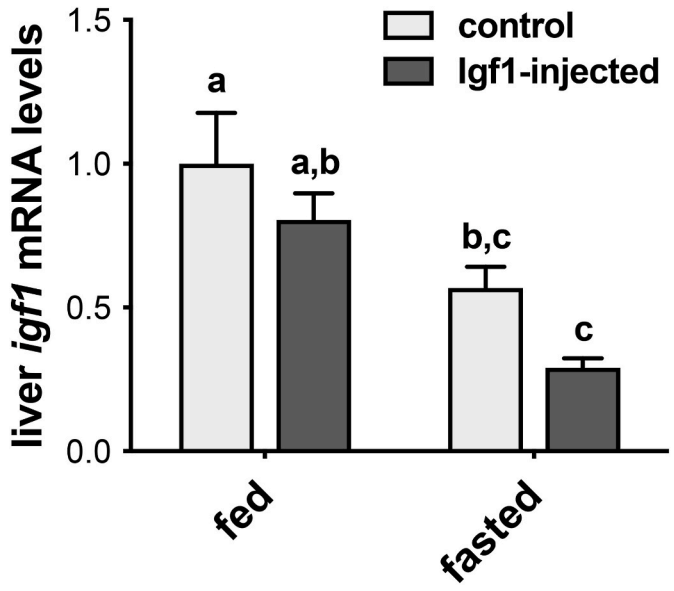

Fig. 3. Differences in liver *igf1* mRNA levels associated with feeding treatment (fed or fasted) and Igf1 injection. Letters indicate *post hoc* pairwise difference among treatments (Tukey HSD test).

synthesis, as liver *igf1* mRNA abundance was >40% higher in fed fish than in fasted fish (feed effect: $F_{1,73} = 25.1022$, $p < 0.0001$) (Fig. 3). In both the fed and fasted groups, rockfish injected with recombinant sea bream Igf1 had lower liver *igf1* gene transcript levels relative to fish in the same food treatment not dosed with exogenous Igf1 (hormone effect: $F_{1,73} = 5.242$, $p = 0.0249$), implying a downregulation of hepatic Igf1 production in rockfish administered exogenous Igf1.

3.3. Liver Igf binding protein and Igf acid labile subunit mRNA levels

Relative levels of mRNAs encoding *igfbp1a* were more abundant in the liver of fasted rockfish than fed fish, and were elevated in both treatments by Igf1 with a significantly larger increase response to Igf1 in fasted fish (feed*hormone interaction: $F_{1,72} = 12.8712$, $p = 0.0006$) (Fig. 4a). Similarly, liver *igfbp1b* mRNAs were at higher abundance in fasted fish than fed fish (feed effect: $F_{1,73} = 11.0142$, $p = 0.0014$), and increased 4- to 8-fold in fish injected with Igf1 in both feeding treatments (hormone effect: $F_{1,73} = 43.1920$, $p < 0.0001$) (Fig. 4b). While liver *igfbp2a* gene transcript abundance did not vary with either feed or Igf1 treatment (Fig. 4c), hepatic *igfbp2b* mRNA levels were higher in fed than in fasted fish (feed effect: $F_{1,73} = 35.2173$, $p < 0.0001$), and were also at reduced relative abundance in Igf1-treated rockfish from both food ration treatments (hormone effect: $F_{1,73} = 7.6524$, $p = 0.0072$) (Fig. 4d). Liver *igfbp3a* mRNAs were at higher abundance in fasted fish

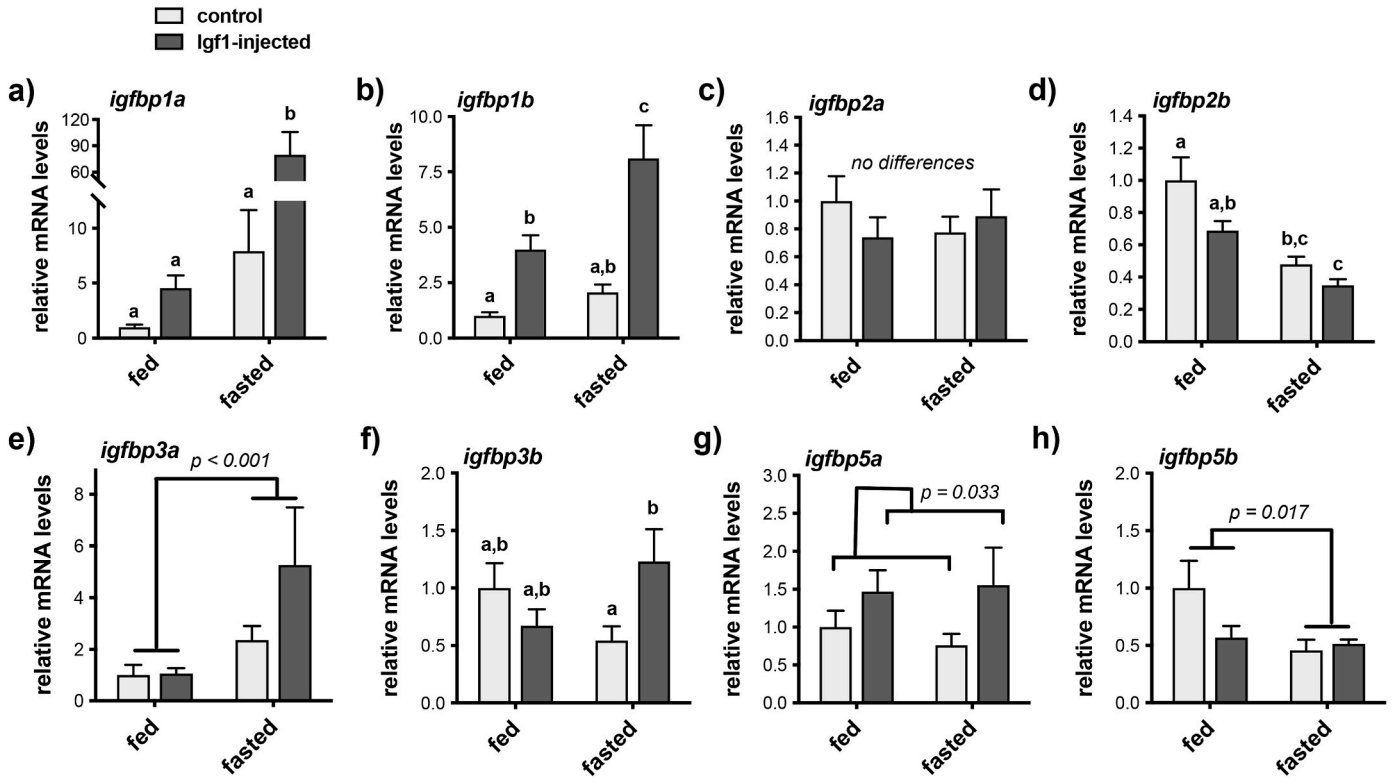

Fig. 4. Relative mRNA levels for IGF binding protein (Igfbp) types 1, 2, 3 and 5 in the liver. Letters indicate pairwise differences among treatments (Tukey HSD tests) for transcripts with significant effects of both feeding and hormone treatment. Lines denote main effect differences when only a single main effect was significant. Data are plotted as mean ± SEM. Y-axis scales differ among gene transcripts.

than fed fish (feed effect: $F_{1,73} = 20.0786$, $p < 0.0001$), but were not significantly affected by Igf1, despite the appearance of an increase in liver *igfbp3a* mRNA levels in fasted fish following recombinant Igf1 treatment (Fig. 4e). In contrast, liver *igfbp3b* mRNAs showed an increase in relative abundance in response to exogenous Igf1 only in fasted fish (feed*hormone interaction: $F_{1,73} = 6.6781$, $p = 0.0118$) (Fig. 4f). Gene transcripts encoding *igfbp5a* in the liver were at similar abundances in fed and fasted fish, but increased in both food treatments after injection with Igf1 (hormone effect: $F_{1,73} = 4.7052$, $p = 0.0333$) (Fig. 4g), although the extent of this Igf1-induced variation was insufficient to be significant in *post hoc* pairwise comparisons. Liver *igfbp5b* mRNA abundance, however, was affected by fasting (feed effect: $F_{1,73} = 5.9556$, $p = 0.0171$) with fasted rockfish exhibiting lower relative *igfbp5b* mRNA levels than fed fish (Fig. 4h). Transcript abundance for *igfbp5b* in the liver was not altered by exogenous Igf1.

Transcript levels of the *igfals* gene were at higher relative abundance in the liver of fish continually fed compared to those deprived of food (feed effect: $F_{1,73} = 23.093$, $p < 0.0001$) (Fig. 5). No effects of Igf1 hormone treatment were observed on liver *igfals* mRNA levels in either fed or fasted rockfish.

3.4. Interactions between fasting and Igf1 on skeletal muscle gene expression

Gene transcripts encoding myogenic regulatory factor proteins showed changes in relative abundance in skeletal muscle due to food deprivation and Igf1. While muscle mRNA abundance for *myod1* was unaltered by either food deprivation or Igf1 (Fig. 6a), *myod2* mRNA levels were higher in fed fish (Fig. 6b) (feed effect: $F_{1,76} = 26.4381$, $p < 0.0001$). Transcripts encoding *myf5* were reduced in the skeletal muscle of fasted fish, and showed induced expression by Igf1, but only under conditions of food deprivation (Fig. 6c) (feed*hormone interaction: $F_{1,76} = 7.0692$, $p = 0.0096$). Transcripts encoding *myf6* were likewise lower in the skeletal muscle of rockfish experiencing fasting (Fig. 6d) and exhibited feeding-contingent regulation by Igf1 (feed*hormone interaction: $F_{1,69} = 6.4281$, $p = 0.0135$), with Igf1 increasing the relative abundance of *myf6* mRNAs in the muscle of fasted fish only. Relative levels of *myogenin* mRNAs were elevated in fish fasted (Fig. 6e) (feed effect: $F_{1,76} = 15.755$, $p = 0.0002$). Igf1 did not have any statistically significant effect on the relative abundance of *myogenin* mRNAs in either fed or fasted rockfish.

Muscle myostatin mRNA levels were also altered by both food deprivation and Igf1. Skeletal muscle *myostatin1* mRNAs increased under fasting conditions (Fig. 6f) (feed effect: $F_{1,76} = 4.019$, $p = 0.0486$) and were downregulated by Igf1 in fish from both feeding treatments (hormone effect: $F_{1,76} = 6.300$, $p = 0.0142$; no significant interaction). Transcripts encoding *myostatin2* exhibited a pattern of regulation distinct from *myostatin1*. Fasting decreased muscle *myostatin2* mRNA abundance (Fig. 6g). The effects of Igf1 on *myostatin2*, however, varied with feeding experience such that Igf1 treatment reduced *myostatin2* mRNA levels in fed fish but increased mRNAs for this gene in fasted rockfish (feed*hormone interaction: $F_{1,76} = 6.067$, $p = 0.0160$). Muscle degradation pathway genes *fbxo32* and *murf1* were likewise regulated by both fasting and Igf1. Relative expression of *fbxo32* mRNAs in skeletal muscle was higher in fasted fish (Fig. 6h) (feed effect: $F_{1,76} = 36.215$, $p < 0.0001$) and exhibited lower relative levels in both fed and fasted fish following Igf1 treatment (hormone effect: $F_{1,76} = 9.906$, $p = 0.0024$; no significant interaction). Muscle *murf1* transcript abundance was elevated due to fasting and showed opposing patterns of regulation by Igf1 depending on recent feeding experience (Fig. 6i), with Igf1 increasing muscle *murf1* mRNA abundance in fed rockfish but reducing abundance in fasted fish (feed*hormone interaction: $F_{1,76} = 15.397$, $p = 0.0002$).

The relative abundance of *igf1* mRNAs was also found to be lower in the skeletal muscle of fasted rockfish compared to fed rockfish (feed effect: $F_{1,76} = 10.718$, $p = 0.0016$) (Fig. 7a). Muscle *igf1* mRNA levels

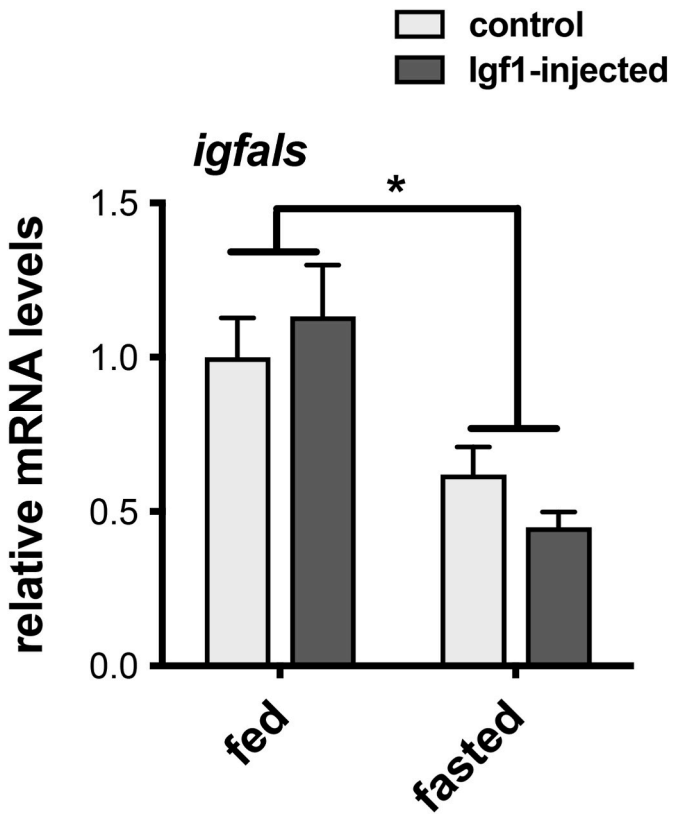

Fig. 5. Transcripts encoding a putative Igf acid labile subunit (*igfals*) gene were lower in rockfish experiencing fasting. Data are plotted as mean ± SEM values. Lines indicate the significant difference in *igfals* mRNA levels between feeding treatments ($p < 0.0001$).

were not, however, altered following treatment with Igf1, implying that this fasting-mediated decline in muscle *igf1* transcript abundance was not a result of the decline in circulating Igf1 concentrations caused by nutritional stress (see Fig. 2). Skeletal muscle of fasted rockfish exhibited higher relative levels of gene transcripts encoding Igf1 receptor A (*igf1ra*) (feed effect: $F_{1,76} = 6.081$, $p = 0.0159$) (Fig. 7b). Muscle *igf1ra* mRNA levels were also, however, affected by Igf1 treatment, with Igf1-injected rockfish showing a reduction in muscle *igf1ra* mRNA abundance (hormone effect: $F_{1,76} = 6.708$, $p = 0.0115$). Similar opposing food and Igf1-influenced patterns of variation were observed for Igf1 receptor B transcript abundance in skeletal muscle (Fig. 7c), with fasted rockfish exhibiting higher muscle *igf1rb* mRNA levels (feed effect: $F_{1,75} = 5.695$, $p = 0.0195$) and rockfish treated with Igf1 showing lower muscle *igf1rb* mRNA abundance (hormone effect: $F_{1,75} = 9.865$, $p = 0.0024$).

Transcripts encoding Igfbp genes *igfbp1a*, *igfbp1b*, and *igfbp2a* were each at lower relative levels in the skeletal muscle (fast-twitch) of fasted rockfish than fed fish, but increased back to fed fish levels in fasted fish given Igf1, despite these genes being unaffected by Igf1 in fed fish (*igfbp1a*: feed*hormone interaction: $F_{1,76} = 10.036$, $p = 0.0022$; *igfbp1b*: feed*hormone interaction: $F_{1,74} = 12.216$, $p = 0.0008$; *igfbp2a*: feed*-hormone interaction: $F_{1,76} = 8.556$, $p = 0.0045$) (Fig. 8a,b,c). No effect of fasting or Igf1 was observed on muscle *igfbp2b* mRNA levels (Fig. 8d), and qPCR reaction Ct values for *igfbp2b* suggested that this Igfbp gene shows low expression in skeletal muscle. Muscle *igfbp3a* mRNAs were elevated in fasted fish compared to fed fish (feed effect: $F_{1,76} = 16.002$, $p = 0.0001$) (Fig. 8e); Igf treatment did not affect *igfbp3a* expression in either feeding group. No effects of feeding treatment or Igf1 were observed for muscle *igfbp3b* or *igfbp5b* mRNAs (Fig. 8f,h). However, muscle *igfbp5a* mRNAs were lower in relative abundance in fasted fish compared to fed fish, with the effects of Igf1 appearing to up- or down-regulate *igfbp5a* mRNA abundance depending on whether fish were fed or fasted, respectively (feed*hormone interaction: $F_{1,75} = 5.147$, $p = 0.0262$) (Fig. 8g).

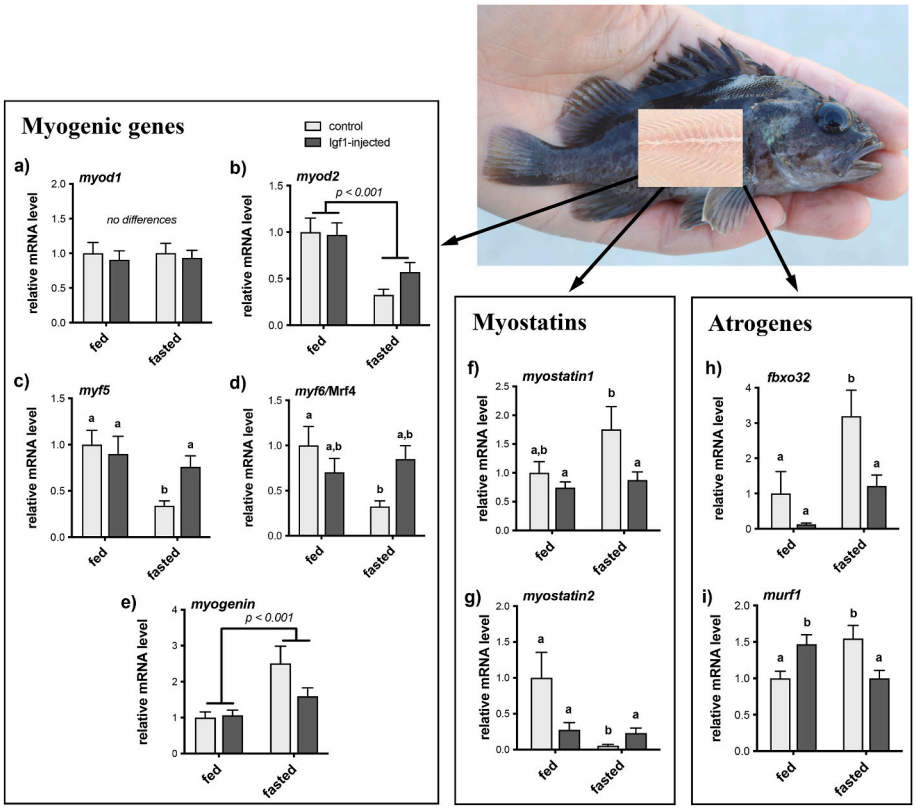

Fig. 6. Effects of fasting and Igf1 on relative expression levels of myogenesis genes encoding myoblast determination proteins 1 (*myod1*)(**a**) and 2 (*myod2*) (**b**), *myogenic factor 5* (*myf5*)(**c**), myogenic factor 6 (*myf6*)(**d**), and *myogenin* (**e**) in the skeletal muscle of juvenile gopher rockfish. Also shown are mRNA levels of skeletal muscle *myostatin1* (**f**) and *myostatin2* (**g**), and the muscle atrophy genes (atrogenes) ubiquitin ligase F-box only protein 32 (*fbxo32*) (**h**) and muscle RING-finger protein-1 (*murf1*) (**i**). Data are plotted as mean ± SEM. Letters indicate pairwise differences among treatments (Tukey HSD tests) for transcripts with significant effects of both feeding and hormone treatment. Lines denote main effect differences when only a single main effect was significant. Y-axis scales differ among gene transcripts.

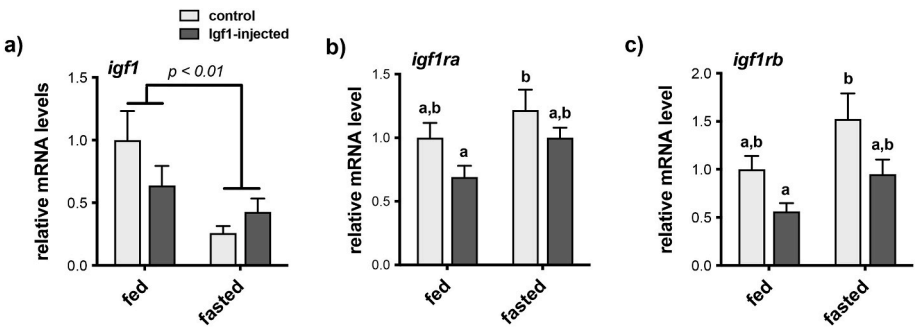

Fig. 7. Skeletal muscle mRNA levels for *igf1* (**a**) and the Igf1 receptors *igf1ra* (**b**) and *igf1rb* (**c**). Data are plotted as mean ± SEM values. Letters indicate pairwise differences among treatments (Tukey HSD tests) for transcripts with significant effects of both feeding and hormone treatment. Lines denote main effect differences when only a single main effect was significant.

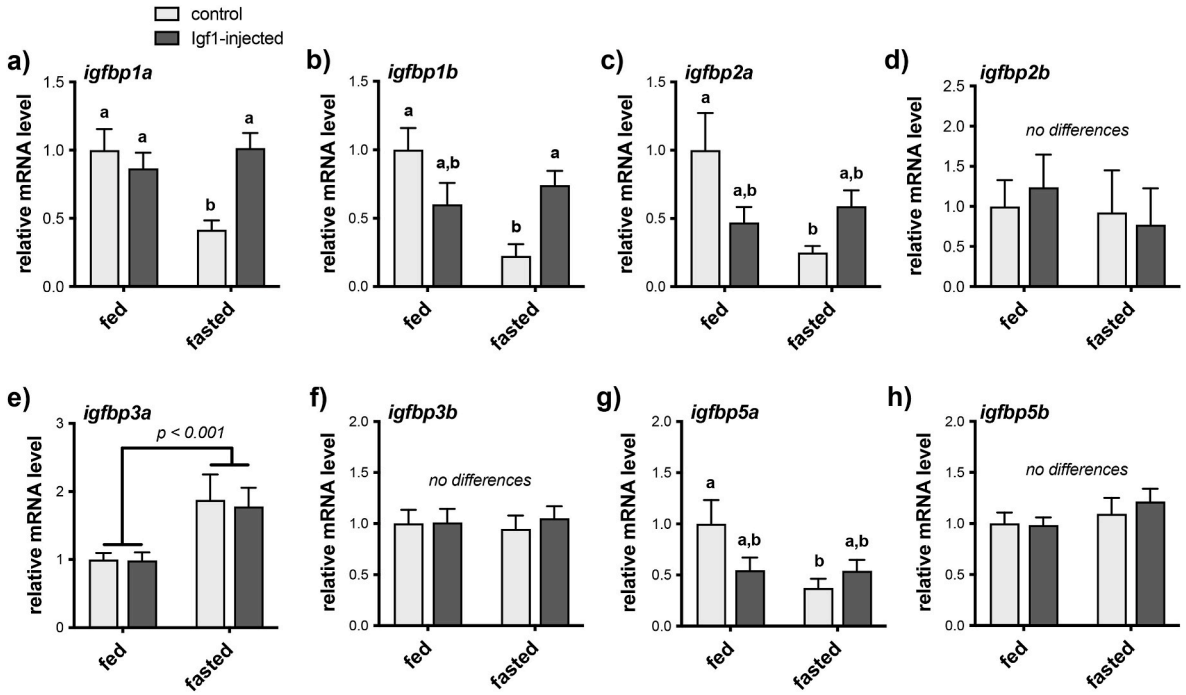

Fig. 8. Relative mRNA levels for IGF binding protein (Igfbp) types 1, 2, 3 and 5 in the skeletal muscle of juvenile gopher rockfish fed or fasted for 14 d, and administered exogenous Igf1 or vehicle control. Data are plotted as mean ± SEM values, and letters indicate pairwise differences among treatments (Tukey HSD tests). Lines denote main effect differences when only a single main effect was significant. Y-axis scales differ among gene transcripts.

4. Discussion

As in other fishes, somatic growth in *Sebastes* rockfishes is affected by the quantity and quality of food resources available (Miller et al., 1967; Miller and Geibel, 1973; Oh et al., 2008; Hack et al., 2018, 2019). In the present study, juvenile gopher rockfish were placed under conditions of either continued feeding or food deprivation to examine how nutritional variation would impact Igf1 action in liver and skeletal muscle. As expected, gopher rockfish experiencing fasting for 14 d lost body mass and showed a lower body condition factor (k). Those changes in growth and condition were paralleled by lower circulating Igf1 and alterations to Igf signaling pathways in both the liver and skeletal muscle. What is more, the nutritional stress of fasting affected how exogenous Igf1 regulated several important components of Igf signaling (i.e., *igf1* and *igfbp* gene expression) in those tissues, and also altered Igf1 regulation of genes controlling myogenesis and myofibrillar protein degradation in skeletal muscle indicating that these pathways can shift responses to Igf1 depending on recent nutritional and growth experience.

4.1. Fasting and Igf1 regulation of liver Igf components

The lower plasma Igf1 observed here in fasted juvenile gopher rockfish is consistent with previous observations in juvenile copper rockfish, where food deprivation for 12 d also reduced circulating Igf1 (Hack et al., 2019). Igf1 was also found to be lower when juvenile copper rockfish and olive rockfish were maintained on a reduced ration amount (Hack et al., 2018, 2019). Igf1 in blood circulation is derived largely from liver Igf1 synthesis, and studies in a wide variety of fishes have observed food deprivation to lower circulating Igf1; that lower Igf1 is often accompanied by reduced *igf1* mRNA levels in the liver (e.g., Duan, 1997, 1998; Pierce et al., 2005; Small et al., 2006; Vera Cruz et al., 2006; Ayson et al., 2007; Montserrat et al., 2007; Kawanago et al., 2014; Hack et al., 2019). As expected from those studies in other teleost species, food-deprived gopher rockfish in the current study exhibited reduced liver *igf1* mRNA abundance. A similar decline in liver *igf1* mRNA was observed in copper rockfish fasted for 12 d (Hack et al., 2019), implying that changes in somatic growth in fasted fish are in part caused by a fasting-induced reduction in hepatic Igf1 production.

Rockfish treated with Igf1 also had lower hepatic *igf1* transcript abundance. Igf1 exerts negative feedback effects on pituitary GH in teleost fishes (Pérez-Sánchez et al., 1992; Blaise et al., 1995; Huang et al., 1998; Moriyama et al., 2000), as it does in mammals (e.g., Romero et al., 2012). We were unable to quantify GH in the present study and therefore do not have a picture of how plasma GH concentrations responded to the experimentally elevated Igf1 concentrations. While GH can become elevated in fish experiencing fasting (e.g., Gómez-Requeni et al., 2005; Gabillard et al., 2006; Norbeck et al., 2007), it is expected that the excess recombinant Igf1 provided in this study would have eventually downregulated pituitary GH production in both fed and fasted gopher rockfish. Lower GH production might subsequently reduce hepatic production of Igf1. Alternatively, Igf1 interacts with thyroid hormones (Giavoli et al., 2017; Kineman et al., 2018) and insulin (Kineman et al., 2018), which also can influence hepatocyte Igf1 synthesis. In mammals, insulin regulates hepatocyte GH receptor expression (Phillips et al., 1998; Dong et al., 2008), and changes in insulin or other hormone regulators of hepatocyte function – or even excess Igf1 binding insulin receptors – could have contributed to lower liver *igf1* mRNAs in Igf1-injected rockfish.

In addition to changes in liver *igf1* expression, fasting and Igf1 administration each altered liver gene expression of several Igfbps. Several of the six Igfbps present in mammals have been duplicated in teleost fishes (Daza et al., 2011), and prior studies of Pacific rockfish and other scorpaeniform fishes indicated these taxa have duplications in the type 1, 2, 3 and 5 *igfbp* genes (Hack et al., 2019; Strobel et al., 2020). Here, we observed that fasting and Igf1 each induced significant elevations in liver mRNAs for *igfbp1a*, *-1b,* and *-3a,* but downregulated *igfbp2b* mRNAs, with that overall pattern suggesting a general stimulation of liver catabolic pathways. Fasting-induced increases in liver transcript abundances for *igfbp1a* and *igfbp1b* were previously observed in copper rockfish deprived of food for 12 d (Hack et al., 2019), as well as in cabezon – another scorpaeniform fish – fasted for 14 d (Strobel et al., 2020). Hack et al. (2018) also detected greater liver *igfbp1a* and *-1b* mRNA abundances in olive rockfish maintained on reduced feed for 98 d. Those findings in rockfishes and other scorpaeniform fishes generally agree with what has been recorded in teleost fishes more broadly, where elevated liver *igfbp1a* and *-1b* mRNAs or protein concentrations result from reduced food intake or fasting (e.g., Shimizu et al., 1999, 2006; Peterson and Small, 2004; Kawaguchi et al., 2013; Breves et al., 2014, 2016; Bersin et al., 2023; for review, see Shimizu and Dickhoff, 2017). Given how consistent that pattern of liver *igfbp1a* and *-1b* upregulation has been across studies in fish, it has been hypothesized that teleost Igfbp1a and -1b act as negative regulators of Igf1 action; under conditions of food limitation, hepatic type 1 Igfbp expression may be upregulated to increase the proportion of Igf1 bound to Igfbp1, thereby reducing Igf1 availability to act on target tissues and promote growth (Shimizu and Dickhoff, 2017; Garcia de la serrana and Macqueen, 2018).

Liver transcript abundance for *igfbp3a* was elevated by fasting and also exhibited a ~2.5-fold increase after Igf1 treatment in rockfish experiencing fasting, but not in fish continuously fed. Prior studies in tilapia (*Oreochromis mossambicus*) and yellowtail (*Seriola quinqueradiata*) previously showed liver gene transcript abundance for *igfbp3* to be elevated following GH administration (Cheng et al., 2002; Pedroso et al., 2009). A previous study using copper rockfish also observed higher relative levels of liver *igfbp3a* mRNAs – but not *igfbp3b* mRNAs – in fasted fish (Hack et al., 2019). Notably, however, Igf1 only increased liver *igfbp3a* mRNA relative abundance in fasted rockfish – and not in fed fish. That pattern of Igf1 effects on *igfbp3a* differs from the consistently higher abundance of *igfbp1a* and *-1b* mRNAs observed after Igf1 treatment of rockfish under both fed and fasted conditions, suggesting distinct mechanisms of regulatory control for type 1 and type 3a Igfbps in rockfish liver.

Unlike *igfbp1a*, *-1b*, and *-3a*, transcripts encoding *igfbp2b, -3b,* and *-5b* each were at lower relative abundance in the liver of fasted gopher rockfish, which is consistent with findings in copper rockfish fasted for 12 d (Hack et al., 2019). Shimizu and Dickhoff (2017) proposed that Igfbp2b may be the major carrier of Igf1 in blood circulation in fish. If that is indeed the case, fasting-induced downregulation of *igfbp2b* mRNAs in the liver might be linked to reduced Igf1 transport to peripheral target tissues and diminished Igf1 action under conditions of energy limitation. Studies in salmonid fishes have shown that circulating Igf1 and Igfbp2b (salmon 41-kDa Igfbp) decline with food deprivation (Beckman et al., 2004a, 2004b; Pierce et al., 2005; Gabillard et al., 2006; Shimizu et al., 2009; Cleveland and Weber, 2013). Rainbow trout with mutated *igfbp2b1* and *-2b2* genes showed reduced plasma Igfbp2b and Igf1 with the greatest declines of Igfbp2b occurring in fish smallest in size (Cleveland et al., 2018, 2020). While those findings support the idea that Igfbp2b may be the major carrier protein for Igf1 in fish (Shimizu and Dickhoff, 2017), much less is known about the functions of Igfbps 3–6 in fish (e.g., Garcia de la serrana and Macqueen, 2018). Hack et al. (2019) observed reduced hepatic *igfbp5a* and *-5b* transcript abundance in fasted copper rockfish, but other studies in tilapia and trout found Igfbp5 gene expression to be unaffected by fasting (Gabillard et al., 2006; Breves et al., 2014). Broadly, Igfbp5 has been linked to skeletal muscle development and bone growth in vertebrates (Duan and Xu, 2005; Ren et al., 2008; Safian et al., 2012). Even still, the functions of Igfbp5 in fish remains largely unknown and should be the focus of future investigations.

Notably, the relative abundance of an *igfals* gene in the liver of juvenile gopher rockfish also changed with food deprivation. In mammals, IGFALS binds to binary complexes comprised of IGF1 and either IGFBP3 or IGFBP5; that interaction extends the half-life of the IGF1-IGFBP complex to, ultimately, enhance IGF1 transport to target tissues (Baxter, 1990; Boisclair et al., 2001; Domené and Domené, 2020). In humans, ~80–85% of IGF1-IGFBP3/5 in circulation is bound to IGFALS (Boisclair et al., 2001), and deficiencies in IGFALS lead to impaired growth and short stature, as well as reduced blood IGF1 and IGFBP3 concentrations (Ueki et al., 2000; Domené et al., 2004, 2011; reviewed by Domené and Domené, 2020). Recently, other studies independently identified *igfals* genes in zebrafish (*Danio rerio*) (Landi et al., 2020) and walking catfish (*Clarias magur*) (Haldar et al., 2022). At this time, however, we are unaware of any study having yet isolated an Igfals protein from fish, and it remains unknown whether this putative teleost Igfals functions similarly to mammalian IGFALS.

4.2. Food deprivation influences Igf1 regulation of myogenic and Igf-related pathways

As an anabolic hormone, Igf1 binds skeletal muscle cells (Castillo et al., 2002) and can increase the rate of skeletal muscle growth (Mommsen and Moon, 2001; Fuentes et al., 2013). Evidence for the growth-promoting effects of Igf1 in teleost fishes comes from a wide variety of studies. For instance, weekly injections of juvenile tilapia with recombinant tilapia Igf1 resulted in increased growth (Chen et al., 2000), and exogenous Igf1 has been shown to increase skeletal muscle amino acid uptake, protein synthesis rate, myoblast proliferation, and myotube differentiation in rainbow trout myocytes (Castillo et al., 2004; Cleveland and Weber, 2010). As in mammals, skeletal muscle growth in teleost fishes occurs via changes in a complex suite of genes, many of which are regulated by myogenic regulatory factor genes encoding transcription factors (Buckingham, 1994; Watabe, 2001; Hernández-Hernández et al., 2017; Koganti et al., 2021). As fish increase or decrease growth rates under varying environmental conditions, those myogenesis-promoting pathways are counterbalanced by atrophy-related gene pathways that mediate protein breakdown and muscle atrophy under stressful conditions such as prolonged energy limitation. In fish, both myogenic and muscle atrophy gene pathways are regulated by Igf1 (Fuentes et al., 2013). Effects of Igf1 on muscle differentiation and growth likely occur from both Igf1 in blood circulation and from muscle-specific production of Igf1. Igf1 derived from

both sources has the potential to act within skeletal muscle cells to regulate protein synthesis via changes in myogenic regulatory factors, myostatin expression, and several Igf signaling pathway components (i.e., receptors, Igfbps), all of which influence muscle growth (Cleveland and Weber, 2010; Fuentes et al., 2013).

In the current study, fasting decreased skeletal muscle expression of myogenic regulatory factors *myod2*, *myf5*, and *myf6*/Mrf4 in juvenile gopher rockfish and led to increased expression of muscle atrophy genes *fbxo32* and *murf1*. Fasted rockfish also showed corresponding negative growth measured as a ~9% decline in body mass and negative mass-specific growth rate (Fig. 1). Taken together, those findings imply a downregulation of myogenesis pathways and enhancement of muscle atrophy gene pathways in rockfish experiencing nutritional stress from food deprivation. Transcript abundance of the myogenic factor *myogenin* was also altered by fasting, but *myogenin* showed a disparate pattern of change compared to the other myogenic genes with higher relative mRNA levels in the skeletal muscle of food deprived rockfish.

The transcriptional regulation of myogenic regulatory factors is a critical component of muscle growth, and Igf1 regulation of those factors plays a key role in satellite cell proliferation and myoblast differentiation (reviewed by: Mommsen and Moon, 2001; Fuentes et al., 2013). Although the temporal sequence of expression of myogenic regulatory factors can vary between fish species (Watabe, 2001), myogenic gene expression generally shows a sequential pattern with each transcription factor contributing to broader changes that promote muscle growth (Bower et al., 2009; Fuentes et al., 2013; Nebo et al., 2013; García de la serrana et al., 2014). Using myogenic cells from gilthead sea bream (*Sparus aurata*), García de la serrana et al. (2014) found that *myf5* and *myod2* mRNAs were elevated during the first few days (day 2–3) of culture, but then declined as *myf6* and *myogenin* mRNAs increased at days 8–12, corresponding to the timing of differentiation into myocytes. In that same study, García de la serrana et al. (2014) observed that sea bream experiencing food deprivation had lower mRNA levels of *myf5* and *myod2* in skeletal muscle (fast twitch). Based on those findings, the lower muscle *myf5* and *myod2* mRNA levels observed here in food deprived gopher rockfish suggests a decline in muscle satellite cell recruitment and proliferation. Myf5 and MyoD function in the determination of myoblast cells (Chen and Tsai, 2008), which develop into myotubes under the influences of other genes including *myogenin*. That functional role is supported by knockdown studies: double *myf5* and *myoD* mutant mice fail to develop precursor myoblasts (Rudnicki et al., 1993; Kablar et al., 2003). Similarly, morpholino knockdown of *myf5* or *myod* in zebrafish alters cranial muscle development (Lin et al., 2006), while simultaneous knockdown of both genes slowed muscle differentiation and blocked *myf6*/Mrf4 and *myogenin* expression (Hinits et al., 2009). Importantly, Mrf4 does not appear to function in myogenic precursor cell proliferation, but instead regulates later stages of myogenesis including muscle fiber alignment (Chen and Tsai, 2008), although those Mrf4 expressional dynamics vary among muscle groups (Hinits et al., 2007). On the whole, our findings here that mRNAs encoding *myod2*, *myf5*, and *myf6*/Mrf4 were all reduced in abundance in the muscle of rockfish experiencing fasting suggests a general downregulation of myogenic progenitor cell proliferation and early differentiation by conditions of nutritional stress.

The relative expression of myogenic genes in muscle in fasted – but not fed – rockfish was also altered by Igf1 in patterns suggestive of Igf1 normalizing or even rescuing muscle tissue growth-promoting pathways (Fig. 6c, d, and 8a-c: e.g., expression of *myf5* and *myf6*/Mrf4 myogenic genes, expression of muscle *igfbp1a*, *-1b*, and *-2a* genes) in fish experiencing nutritional stress. While it is well established that Igf1 regulates myogenesis in fish and other vertebrates (Mommsen and Moon, 2001; Velloso, 2008; Fuentes et al., 2013), there is also evidence for those Igf1 effects being context dependent. Garikipati and Rodgers (2012a) observed that Igf1 both increased proliferation of cultured myosatellite cells from rainbow trout and induced elevated expression of *myf5* and *myod1* – as well as *myogenin* – in those cells during myosatellite-to-myotube differentiation, but not consistently or at other timings of myogenesis (Garikipati and Rodgers, 2012b). Jiménez-Amilburu et al. (2013) found Igf1 to function synergistically with GH to upregulate *myogenin* and *myf6*/Mrf4 transcript abundance in cultured muscle satellite cells of gilthead sea bream, but only during the period of myotube differentiation. In this study, we observed upregulation of *myod2*, *myf5*, and *myf6*/Mrf4 mRNAs in the skeletal muscle of fasted rockfish only, suggesting Igf1 plays a key role in regulating the expression of myogenic regulatory factors underlying myogenesis (Fuentes et al., 2013), but also that the actions of Igf1 may vary with prior feeding experience and possibly the stage of cellular myogenesis.

Some of that variation in Igf1 effects on myogenic regulatory genes may emerge in part from differences in Igf receptor expression within skeletal muscle (Castillo et al., 2002; Fuentes et al., 2013). In the gopher rockfish studied here, fasting increased Igf1 receptor A and B expression in skeletal muscle while treatment with Igf1 had the opposing effect of causing lower relative mRNA levels for both Igf1 receptors. Juvenile copper rockfish fasted for 12 d similarly had elevated *igf1ra* and *igf1rb* mRNAs in skeletal muscle (Hack et al., 2019). Chauvigné et al. (2003) similarly observed that food deprivation increased the abundance of mRNAs encoding *igf1ra*, but not *igf1rb*, in the skeletal muscle of rainbow trout, although it should be noted that another recent study in trout found increased ration amount to be associated with increased skeletal muscle *igf1rb* mRNA levels (Weber et al., 2022). Changes in muscle Igf1 receptor expression may alter tissue sensitivity to Igf1, which could help counter the decline in circulating Igf1 observed in fish experiencing nutritional stress (e.g., Gabillard et al., 2006; Breves et al., 2014; Hack et al., 2019; Izutsu et al., 2022).

Several studies in teleost fishes have also documented changes in *igf1* and Igfbp transcript abundances in skeletal muscle caused by food reduction or deprivation (Gabillard et al., 2006; Bower et al., 2008; Amaral and Johnston, 2011; Macqueen et al., 2011; Safian et al., 2012). As seen in copper rockfish experiencing either lower food rations or fasting (Hack et al., 2019), fasted gopher rockfish exhibited lower levels of *igf1* mRNAs in skeletal muscle, as well as lower abundances of transcripts encoding Igfbp types -2a and -5a, but elevated mRNAs for *igfbp3a*. While it is hypothesized that synthesis of these proteins within skeletal muscle is related to auto- or paracrine regulation of growth, studies directly assessing the functions of Igfbps in fish muscle are limited, and the large – and varying – number of duplications of *igfbp* genes in teleost taxa complicates direct comparisons across species. Illustrating these challenges, Macqueen et al. (2011) explored whether differences in muscle Igfbp gene expression might underlie growth variation in Arctic charr (*Salvelinus alpinus*) populations with either 'dwarfed' or 'non-dwarf' growth phenotypes. While these populations consistently differed in muscle *igfbp4* expression, other Igfbp genes including *igfbp2.1* varied a lot among populations and even showed dissimilar responses to fasting/refeeding in populations expressing similar growth phenotypes (Macqueen et al., 2011). Even so, muscle-derived Igfbps appear to regulate Igf1 action and muscle growth, at least in some fish species and under some contexts (e.g., Duan and Xu, 2005; Fuentes et al., 2013; Shimizu and Dickhoff, 2017; Allard and Duan, 2018; Garcia de la serrana and Macqueen, 2018). In fine flounder (*Paralichthys adspersus*), for instance, muscle *igfbp2* and *igfbp5* mRNAs were downregulated, and *igfbp3* mRNA upregulated, during food deprivation, with transcript levels of each gene displaying the expected opposing patterns of change when the fasted fish were again fed (Safian et al., 2012). Using cultured myotube cells from Atlantic salmon, Bower and Johnston (2010) found several Igfbps changed gene expression during myotube maturation: mRNA levels for *igfbp2.2, -5.1* and *-5.2* were downregulated as myotubes differentiated, while *igfbp4* transcripts increased during that same period. Such observations point to a complex pattern of Igfbp gene expression in fish skeletal muscle during myogenesis. While our current study in gopher rockfish was not designed to uncover details of that temporal Igfbp expression, the data presented here reinforce the proposition that Igfbps play a role in shifting the dynamics of muscle growth,

and provide evidence that circulating Igf1 status and recent feeding experience interact to affect muscle Igfbp gene expression in binding protein type-specific patterns in teleost fishes.

4.3. Food deprivation and Igf1 effects on muscle atrophy-related genes and myostatins

In mammals, muscle wasting caused by starvation is regulated in part via expression of the genes *Fbxo32* (atrogin-1) and *Murf1* (Bodine et al., 2001; Lecker et al., 2004), which encode ubiquitin-proteasome ligases involved in proteolysis (Glass, 2003; Koyama et al., 2008; Deshaies and Joazeiro, 2009). In teleost fish, increased expression of these atrophy-related genes occurs during body mass loss from food deprivation. Withholding food for 1–3 weeks increased skeletal muscle mRNA abundances of *fbxo32* and *murf1* in juvenile tilapia (Nebo et al., 2017), and immature fine flounder fasted for as little as 1 week showed significant increases in muscle *fbxo32* and *murf1*, with mRNAs for those genes returning to pre-fasting levels after refeeding (Fuentes et al., 2012). And, in a series of studies with rainbow trout, fish fasted for 4 weeks showed elevated *fbxo32* expression in both fast- and slow-twitch skeletal muscle (Cleveland and Evenhuis, 2010) while also exhibiting increased expression in fast-twitch muscle under conditions of reduced feeding (Cleveland and Burr, 2011). In the present study, we found relative *fbxo32* and *murf1* mRNAs increased in the fast-twitch skeletal muscle of gopher rockfish fasted for 14 d, a pattern generally consistent with what has been observed previously in other fishes experiencing food restriction.

We also observed that treatment of gopher rockfish with Igf1 altered skeletal muscle mRNA levels of both *fbxo32* and *murf1*, although the pattern of regulation differed between these genes. Muscle *fbxo32* mRNA levels were lower in both fasted and fed rockfish given supplemental Igf1. However, transcripts encoding *murf1* were less abundant in the skeletal muscle of fasted rockfish treated with Igf1, but increased following Igf1 treatment in rockfish experiencing continuous feeding. In prior work, Igf1 induced gene transcription of both *fbxo32* and *murf1* in cultured myosatellite cells from juvenile rainbow trout (Cleveland and Weber, 2010), and treatment of rainbow trout with Igf1 stopped *fbxo32* mRNAs from increasing *in vivo* after 2 weeks of fasting (Cleveland et al., 2009). It is not clear why gene transcript abundance for *murf1* – but not *fbxo32* – showed a feeding condition-dependent response to Igf1 in the muscle of juvenile rockfish. In human myocytes, Igf1 reduced relative mRNA levels for both *MURF1* and *ATROGIN-1* (*FBXO32*) with those effects of Igf1 occurring on differing time scales for the two genes (Sacheck et al., 2004). It is thus possible that the single sampling time point in our study with Igf1-injected gopher rockfish was insufficient to detect regulatory changes in both *fbxo32* and *murf1*.

Myostatin is a negative regulator of muscle mass in mammals (e.g., Amthor et al., 2007; Shelton and Engvall, 2007), and these proteins likewise regulate cell proliferation and tissue growth in fish (Gabillard et al., 2013). Co-treatment of Igf1-stimulated cultured myoblasts from rainbow trout with human myostatin inhibited cell proliferation, but not myoblast differentiation (Seiliez et al., 2012). However, gene duplications in teleost fishes have also led to multiple myostatin genes (Maccatrozzo et al., 2001; Gabillard et al., 2013). In cultured, proliferating myosatellite cells from rainbow trout, Igf1 downregulated both *myostatin1a* and *-1b* gene expression, but upregulated *myostatin2* expression (Garikipati and Rodgers, 2012a, 2012b), a pattern similar to that observed here in the skeletal muscle of gopher rockfish. Although fasted gopher rockfish expressed lower *myostatin2* mRNAs in skeletal muscle, *myostatin1* mRNAs were elevated in those same fish. That pattern is inconsistent with other studies in fishes where fasting did not affect skeletal muscle *myostatin1* gene expression (Chauvigné et al., 2003; Weber and Bosworth, 2005). However, given that myostatin expression has been shown to be modulated by cortisol in some fish species, but not others (e.g., Galt et al., 2016), it is also possible that the dissimilar feeding/fasting-dependent responses of *myostatin1* and *-2* observed here in gopher rockfish could be related to fasting-related changes in glucocorticoid status. Whatever the mechanism, our findings here provide more evidence that poor nutrition alters muscle myostatin expression, which in turn may contribute to the downregulation of myogenesis (e.g., Amali et al., 2004) and an altered ability for a subsequent increase in liver Igf1 secretion – perhaps related to the consumption of more nutritious food or successful foraging – to stimulate muscle growth.

5. Conclusions

Taken as a whole, findings presented here indicate that changes in plasma Igf1 related to recent feeding experience regulate skeletal muscle gene expression associated with myogeneis and muscle growth in gopher rockfish. Fasted rockfish showed lower plasma Igf1 and reduced liver *igf1* mRNA levels, as well as elevated *igfbp1a, -1b*, and *-3a* mRNAs, consistent with fasted fish being in a catabolic state. Fasted rockfish also exhibited increased skeletal muscle transcript abundances for *myostatin1* and protein ligase genes *fbxo32* and *murf1*, as well as reduced gene expression for myogenic factors *myod2*, *myf5* and *myf6*/Mrf4, implying a downregulation of myogenesis pathways concurrent with an upregulation of muscle atrophy pathways when rockfish experience nutritional stress. While Igf1 modulated relative mRNA levels of some of those genes in the skeletal muscle of rockfish experiencing both feeding and fasting conditions, the effects of Igf1 on several key myogenesis-related genes including *myf5, myf6*, and *murf1* was observed to be dependent on feeding experience. That finding suggests recent feeding success by rockfish influences the sensitivity of skeletal muscle myogenesis pathways to Igf1, which has implications for how rapidly rockfish might be able to shift excess energy to growth under changing conditions of food abundance or quality in their ocean environments.

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CRediT authorship contribution statement

Theresa V. Bersin: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Formal analysis, Visualization. **Kasey L. Cordova:** Conceptualization, Methodology, Writing – review & editing, Formal analysis, Funding acquisition. **E. Kate Saenger:** Methodology, Writing – review & editing, Funding acquisition. **Meredith L. Journey:** Methodology, Formal analysis. **Brian R. Beckman:** Methodology, Formal analysis, Resources, Writing – review & editing, Supervision, Funding acquisition. **Sean C. Lema:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing, Formal analysis, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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