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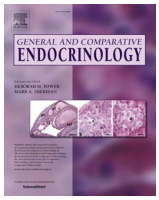

# Cortisol reduces insulin-like growth factor-1 (Igf1) and alters liver Igf binding protein (Igfbp) and muscle myogenic gene expression in blue rockfish (*Sebastes mystinus*)

Hayley M. Mapes [a], Janae E. Shew [a], Henry M. Marden [a], Meredith L. Journey [b,c,1], Brian R. Beckman [c], Sean C. Lema [a,*]

[a] Biological Sciences Department, Center for Coastal Marine Sciences, California Polytechnic State University, San Luis Obispo, CA, 93407, USA
[b] Lynker Technology, 202 Church St SE #536, Leesburg, VA 20175, USA
[c] Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, Washington, 98112, USA

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ABSTRACT

Fish experiencing stressful conditions often show elevated circulating concentrations of the glucocorticoid hormone cortisol, which regulates physiological processes including intermediary metabolism and somatic growth. Prior studies point to cortisol inhibiting growth via changes to growth hormone (Gh)/insulin-like growth factor-1 (Igf1) signaling. However, the mechanisms by which cortisol alters Gh/Igf1 pathways in fishes are not well understood. Here, we explored how cortisol influences growth-related pathways in adult blue rockfish (*Sebastes mystinus*) by administering a single intraperitoneal dose of exogenous cortisol and examining effects on plasma Igf1 and liver mRNA levels for *igf1* and Igf binding proteins (Igfbps), as well as expression patterns of select myogenesis or muscle atrophy-regulating genes in skeletal muscle. Cortisol-treated rockfish had elevated plasma cortisol and glucose concentrations 5 h and 24 h after cortisol administration. Rockfish also showed lower plasma Igf1 concentrations 24 h after cortisol administration, even though relative expression of *igf1* mRNAs in the liver was unaffected. Rockfish given cortisol had higher liver mRNA levels for the Igfbp genes *igfbp1a* and *igfbp1b*, which encode type 1 Igfbps with proposed inhibitory influences on Igf1 stimulation of somatic growth. Cortisol-treated fish also expressed elevated liver mRNA levels of *igfbp2a*, *−5b* and *−6*, but lower liver *igfbp3a* gene transcript abundance, as well as reduced mRNAs in skeletal muscle for myoblast determination protein 2 (*myod2*), a transcriptional activator of myocyte differentiation. These findings show that sustained (24 h) elevated cortisol can lower circulating Igf1 and alter Igfbp expression, which may bring about reduced growth for fish experiencing prolonged stressors.

## 1. Introduction

Cortisol is the primary glucocorticoid secreted in response to external stressors in actinopterygian teleost fishes (Barton, 2002; Mommsen et al., 1999). When an external stressor is perceived, corticotrophin releasing factor (Crf) released by axonal connections from hypothalamic neurons stimulates pituitary gland release of adrenocorticotropic hormone (Acth) and the subsequent secretion of cortisol as part of the hypothalamic-pituitary-interrenal (HPI) endocrine axis response to stressors (Barton, 2002; Mommsen et al., 1999; Gorissen and Fick, 2016). Elevated cortisol in the blood then regulates a suite of secondary physiological responses including shifts in intermediary metabolism and energy availability (Alfonso et al., 2021; Sadoul and Vijayan, 2016; Islam et al., 2022; Canosa and Bertucci, 2023).

*Abbreviations:* Acth, adrenocorticotropic hormone; Crf, corticotrophin-releasing factor; Gh, growth hormone; Igf1, insulin-like growth factor 1; Igf2, insulin-like growth factor 2; Igfbp, insulin-like growth factor binding protein; Igfals, igfbp acid labile subunit; Myod1, myoblast determination protein 1; Myod2, myoblast determination protein 2; Myog, myogenin; Fbxo32, ubiquitin ligases F-box only protein 32; Murf1, muscle RING-finger protein-1; HPI, hypothalamic-pituitary-interrenal axis; Mafbx, muscle atrophy F-box gene.

* Corresponding author.
*E-mail address:* slema@calpoly.edu (S.C. Lema).
[1] Present address: Wyoming Cooperative Fish and Wildlife Research Unit, Department of Zoology and Physiology, University of Wyoming, Laramie, WY, USA.

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Depending on the degree and duration of cortisol elevation, effects of elevated cortisol may include increases in blood glucose (Ashaf-Ud-Doulah et al., 2019; Islam et al., 2020; Pérez-Casanova et al., 2008; Phuc et al., 2017; Samaras et al., 2018), shifts in appetite and food intake (Bernier et al., 2004; Bernier and Peter, 2001; Madison et al., 2015; McCormick et al., 1998), and changes in somatic growth (Sadoul and Vijayan, 2016; Valenzuela et al., 2018).

Previous research in teleost fishes has indicated that cortisol inhibits anabolic growth processes in part via effects on endocrine growth hormone (Gh)/insulin-like growth factor-1 (Igf1) signaling (Davis and Peterson, 2006; Small et al., 2006; Madison et al., 2015; Sadoul and Vijayan, 2016). The Gh/Igf1 system is a key regulator of somatic growth in fishes and has been shown to be an important mechanism for shifting somatic growth rates in response to changes in nutritional status (Gabillard et al., 2006; Beckman, 2011; Fuentes et al., 2013; Picha et al., 2008; Reindl and Sheridan, 2012). Gh secreted into blood circulation by the pituitary stimulates liver Igf1 production, which regulates somatic growth via actions on tissues including skeletal muscle (Duan et al., 2010; Fuentes et al., 2013; Le Roith et al., 2001; Wood et al., 2005). Those effects of Igf1 on skeletal muscle growth occur via upregulation of myogenic transcription factor genes (i.e., myoblast determination protein [*myoD*], myogenin [*myog*], and myogenic factors 5 [*myf5*] and 6 [*myf6*]) and inhibition of atrophy-related genes including F-box only protein 32 (*fbxo32*; also called atrogin-1) and muscle RING-finger protein-1 (*murf1*) (e.g., Bersin et al., 2023a). In mammals, cortisol has been shown to regulate the expression of several of those genes (Pandurangan et al., 2014). However, it remains unclear if cortisol regulates these pathways in teleost fishes (Sadoul and Vijayan, 2016).

Despite the importance of Gh/Igf1 signaling for somatic growth, it is not fully understood how cortisol modulates Gh/Igf1 signaling. Fish exposed to acute stressors or treated with supplemental cortisol can show altered plasma Gh (Davis and Peterson, 2006; Deane and Woo, 2006, 2008; Rotllant et al., 2001) as well as reduced circulating Igf1 concentrations (Kajimura et al., 2003; Davis and Peterson, 2006; Small et al., 2006), although declines in Igf1 have not been detected in all fishes examined (Breves et al., 2020; Yamaguchi et al., 2021). Some of the effects on Igf1 appear due to cortisol-induced downregulation of liver Gh receptor expression (Small et al., 2006; Saera-Vila et al., 2009; Nakano et al., 2013). For instance, using isolated hepatocytes from Mozambique tilapia (*Oreochromis mossambicus*), cortisol was observed to inhibit the induction of *igf1* mRNAs by Gh, providing evidence that cortisol can modulate fish liver hepatocyte Igf1 synthesis sensitivity to Gh (Pierce et al., 2011).

Several recent studies have also indicated that cortisol can regulate Igf binding protein (Igfbp) expression (Breves et al., 2020; Yamaguchi et al., 2021). As another component of Igf signaling, Igfbps modulate Igf1 actions by either facilitating Igf1 transport and Igf1 receptor activation, or sequestering Igf1 to reduce receptor binding (Shimizu and Dickhoff, 2017). In some cases, Igfbps have their own biological activity independent of Igf hormone binding by activating Igf1 receptors or other receptors (e.g., transforming growth factor-β5 receptor, peroxisome proliferator-activated receptor γ) (Baxter, 2015; Chan et al., 2009; Clemmons, 2007, 2016; Duan and Xu, 2005; Huang et al., 2003). In mammals, six types of Igfbps are present in blood. In humans, those Igfbps bind > 99 % of Igf1 in circulation (Frystyk et al., 1994). Teleost fish genomes contain orthologs of those same six types of *igfbp* genes, although several *igfbp* genes duplicated into multiple paralogs in fishes (Daza et al., 2011). Some Igfbps in teleost fishes appear to perform similar functions as in mammals. Teleost type 1 Igfbps such as Igfbp1a and Igfbp1b have inhibitory actions on Igf1 stimulation of somatic growth, similar to that in mammals (Allard and Duan, 2018; Shimizu and Dickhoff, 2017). Other teleost Igfbps, however, appear to have diverged in function from their mammalian counterparts. IGFBP3/5 binds ~ 75–80 % of IGF1 in circulation in humans (in a ternary complex with an IGF acid-labile subunit [IGFALS]) (Baxter and Martin, 1989); however, Igfbp2b appears to be the primary Igfbp facilitating Igf1 transport for growth-promoting action in salmonid fishes (Shimizu et al., 2003, 2011; reviewed by: Shimizu and Dickhoff, 2017).

As nearly all Igf1 in blood circulation appears bound to Igfbps (Frystyk et al., 1994), the dynamics of Igfbp expression are crucial to Igf1 regulation of growth. And yet, only a few studies have examined how elevated cortisol affects Igfbp expression in fish. In one study, channel catfish (*Ictalurus punctatus*) fed supplemental cortisol for 4 weeks had elevated plasma levels of a ~ 20 kDa Igfbp protein, ostensibly a type 1 Igfbp in that species (Peterson and Small, 2005). Rainbow trout (*Oncorhynchus mykiss*) infused with supplemental cortisol for up to 42 d showed elevated liver gene transcript abundances for *igfbp1* and *igfbp2* (Madison et al., 2015). In Atlantic salmon (*Salmo salar*), cortisol upregulated liver 1b-type *igfbp* mRNA expression (Breves et al., 2020). To date, however, no study on cortisol regulation of teleost Igfbp expression has assessed effects on all six types of Igfbps, and more work needs to be done to understand how the full complement of duplicated Igfbps of teleost fishes may be regulated by cortisol.

Here, we examined how a short term (24 h) sustained elevation of cortisol influences plasma Igf1 and liver *igfbp* gene expression in adult blue rockfish (*Sebastes mystinus*). Blue rockfish belong to a larger clade of Pacific Ocean rockfishes (*Sebastes* spp.) that are important for commercial and recreational fisheries in the Northeast Pacific Ocean (Hyde and Vetter, 2007). Many of these species have been monitored by fisheries managers along the West Coast of North America for the past thirty years following a near population crash (Love et al., 2002), and several *Sebastes* rockfish species are being developed for aquaculture (e.g., Zhang et al., 2020, 2021; Lee et al., 2022). In the current study, adult blue rockfish were administered a single dose of exogenous cortisol and then examined for effects on plasma Igf1 and liver mRNA levels of several Igf-pathways genes including *igf1* and all identified rockfish *igfbps*. At the same time, cortisol-treated fish were also assessed for effects on skeletal muscle expression of the Myod genes *myod1* and *myod2*, *myogenin*, *myostatin1*, and the ubiquitin ligase-encoding genes *fbxo32* and *muRF1* to evaluate how a sustained, 24 h elevation of cortisol influences gene expression related to myogenesis and muscle atrophy.

## 2. Materials and methods

### 2.1. Animal collection

Adult male and female blue rockfish (*S. mystinus*) (total length > 19 cm) were collected between March and September 2022 by hook-and-line fishing using baited shrimp flies from nearshore regions (< 30 m depth) of the ocean near Morro Bay, California, USA. Immediately after collection, fish were placed into an aerated holding tank receiving ambient flow-through seawater. Fish were transferred to California Polytechnic State University's Center for Coastal Marine Sciences pier facility in Avila Beach, California, USA, where they were maintained in circular 340 l tanks (0.97 m diameter, 0.48 m depth). Tanks were located outdoors under ambient photoperiod and were supplied with flow-through, filtered seawater under conditions of the local ocean. Fish were fed frozen market squid *ad libitum* daily. Three weeks after collection, fish were tagged with passive integrative transponders (PIT) tags (7 mm, Loligo Systems, Inc., Viborg, Denmark) to allow for individual identification during the experiment. All experimental procedures were approved by the Institutional Animal Use and Care Committee of California Polytechnic State University, San Luis Obispo (Protocol # 2108).

### 2.2. Cortisol treatment

After at least 3 months of acclimation to captivity conditions, rockfish were reapportioned to one of six 340 l experimental tanks with flow-through, filtered seawater. Fish were assigned to experimental tanks systematically with 3 or 4 fish per tank to balance body size variation across tanks (no differences in total length [TL, $F_{1,18} = 0.0411$, p =

0.999] or in body mass [$F_{1,18} = 0.008$, $p = 1.00$]). Mean fish body size at the time of tank assignment was 22.4 ± 2.8 cm total length (TL; mean ± SD) and 208.25 ± 75.79 g body mass. The average body mass ($p = 0.613$) and length ($p = 0.949$) of cortisol- and vehicle-injected fish were also similar between the hormone injection treatments as described further below (Appendix A, Supplementary Fig. S1a,b). The ratio of females:males, as determined by visual assessment of gonadal sex at the time of tissue sampling, was also similar across experimental cortisol- and vehicle-injection treatment groups ($p = 0.795$), as described below, with $n = 4$ or 5 males and $n = 7$ females in each injection treatment group (Appendix A, Supplementary Fig. S1c). Throughout the experimental period, fish were maintained under ambient photoperiod and local ocean conditions of temperature (mean: 14.98 ± 0.97 °C, mean ± SD) and salinity (~33–34 ppt). Fish were fed frozen market squid *ad libitum* once daily.

Tanks were randomly assigned to either a cortisol-administered or vehicle-administered (*control*) treatment, with three replicate tanks per each treatment (n = 11 fish for cortisol injection, $n = 12$ for vehicle control). All fish were fasted for 24 h and then lightly anesthetized (buffered tricaine methanesulfonate, MS222, Syndel Aquaculture, Ferndale, WA, USA) prior to injection. Anesthetized fish were weighed, and ~ 0.2 ml of blood was collected from the caudal vasculature (e.g., Lawrence et al., 2020) via syringe for a baseline (*pre-injection*) sample. Each fish was then injected intraperitoneally with either cortisol (Sigma, St. Louis, MO, USA) at 40 μg·g$^{-1}$ body mass, or vehicle solution (0.9 % NaCl, pH 7.0 with 16.6 % ethanol) at a volume of 2.5 μl per g body mass. Fish were then recovered from anesthesia in an aerated 22 l bucket and returned to their respective experimental 340 l tank. At time + 5 h after injection, fish were again netted, lightly anesthetized, and subject to a 2nd blood collection (+ 5 h sampling time), after which fish were again returned to their experimental tanks.

Then, at + 24 h after injection, fish were euthanized using MS222 and again measured for total length (TL) and body mass. Blood was collected from the caudal vasculature using heparinized capillary tubes. Following blood collection, gonads were removed and examined visually for sex identification. The liver and a sample of skeletal muscle (fast twitch 'white' muscle) from the left side of each fish posterior to the dorsal fin were dissected and flash frozen in liquid $N_2$. Liver and skeletal muscle tissues were subsequently stored at −70 °C for later RNA extraction. All blood samples throughout the experiment were collected within 6 min of netting a fish from its experimental tank. Blood samples were centrifuged at 3,000 g for 10 min to collect plasma. Plasma collected at each of the sampling time points (0 h, + 5 h and + 24 h) was stored at −70 °C.

### 2.3. Cortisol quantification

Plasma from the pre-injection (0 h), + 5 h post-injection, and + 24 h post-injection sampling times was used to quantify total cortisol concentrations. Cortisol was measured by enzyme immunoassay (EIA) according to methods described in Carey and McCormick (1998). Microtiter plates (Corning/Costar Easy Wash Microtiter plates) were coated with 150 μl of rabbit anti-cortisol (polyclonal, Fitzgerald Industries International) at a final concentration of 1:10,000 in coating buffer (0.05 M, carbonate-bicarbonate, pH 9.6). Plates were incubated at 37 °C for 3 h and then washed 5x with a solution of 0.15 M NaCl and 0.05 % Tween 20. The wash solution was removed by inverting the plate and tapping dry. Plates were then blocked with 250 μl of EIA buffer (0.1 M phosphate, 0.15 M NaCl, with 0.1 % bovine serum albumin) for 30 min, after which this solution was removed, and 150 μl of EIA buffer was added back, along with 2.5 μl of standard or plasma sample and 100 μl of cortisol-horseradish peroxidase (cortisol-HRP) conjugate (1:500,000; Fitzgerald Industries International). Plates were tightly sealed and incubated overnight at 25 °C in the dark. Standards were run in triplicate, while plasma samples, blanks, and non-specific binding (NSB) wells were run in duplicate.

After overnight incubation, plates were dumped of their contents and washed 5X with 250 μl·well$^{-1}$ of wash buffer. Then, 200 μl of TMB (3,3′,5,5′-tetramethylbenzidine containing 0.01 % hydrogen peroxide) (1-Step™ Turbo TMB-ELISA, ThermoScientific) was added to each well. Plates were read (650 nm) at room temperature using a Victor X4 microplate reader (Perkin Elmer) with shaking until the desired optical density was reached. Then 100 μl of 1.0 M $H_2SO_4$ was added to each well to stop the color reaction, and an endpoint reading was taken (450 nm). The standard curve ($r^2 > 0.99$) for the assay ranged from 2.5 to 500 ng·ml$^{-1}$, and the intra-assay coefficient of variation was 8.7 %.

### 2.4. Quantification of Igf1

Plasma Igf1 concentrations were quantified using a time-resolved fluoroimmunoassay (Small and Peterson, 2005). This TR-FIA was originally developed from an RIA described by Shimizu and colleagues (2000) and had been validated and used previously for *Sebastes* rockfishes (e.g., Hack et al., 2018, 2019; Bersin et al., 2023a, 2023b) as well as the related cabezon (*Scorpaenichthys marmoratus*) (Strobel et al., 2020). Methods for this Igf1 time-resolved fluoroimmunoassay are described in detail elsewhere (Ferriss et al., 2014; Hack et al., 2018). In brief, 25 μl of plasma was extracted using a 4x volume solution of 87.5 % ethanol and 12.5 % 2 M HCl acid and then resuspended in 0.855 M Tris Base. Extracts were assayed using recombinant barramundi (*Lates calcarifer*) anti-Igf1 antiserum (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 Igf1 (GroPep BioReagents, Ltd.). Igf1 was only quantified in plasma from the pre-injection and + 24 h post-injection sampling times, due to limited plasma volumes from the + 5 h sampling time.

### 2.5. Glucose quantification

Plasma glucose concentrations were quantified using the Invitrogen™ Amplex® Red Glucose/Glucose Oxidase Assay Kit (ThermoFisher Scientific). Plasma (25 μl) was diluted 1:4 in 1x reaction buffer and assayed in duplicate. Glucose standards from 0.406 to 100 μM were run in triplicate. A 50 μl volume of each sample or standard was incubated with a solution containing 0.5 μl Amplex® Red reagent stock solution (10 mM), 1 μl horseradish peroxidase solution (10 U/ml), and 1 μl of glucose oxidase solution (100 U/ml) in 47.5 μl 1x reaction buffer for 30 min at room temperature, before being read at 550 nm on a Victor X4 microplate reader (Perkin Elmer). The intra-assay % CV for the assay was 2.9 %, and inter-assay % CV was 3.5 %.

### 2.6. Real-time quantitative PCR

Total RNA from liver and skeletal muscle tissues was extracted using TriReagent® (Molecular Research Center, Inc.), followed by DNase I treatment (TURBO DNA-free kit, Life Technologies). DNase treated RNA was quantified with a spectrophotometer (P300 NanoPhotometer, Implen, Inc.). To standardized RNA concentrations ahead of reverse transcription, liver RNA samples were diluted to 200 ng·μl$^{-1}$, and muscle RNA was diluted to 47 ng·μl$^{-1}$ before being reverse transcribed using the Invitrogen High Capacity cDNA Reverse Transcription Kit (Invitrogen). Each 26 μl reverse transcription reaction contained MultiScribe™ reverse transcriptase enzyme (50 U/μL), 25x dNTP mix (100 mM), 10X random primers, 10X reverse transcription buffer, and nuclease-free water, as well as Recombinant RNasin™ Ribonuclease Inhibitor (Promega) and a 1:2 vol of total RNA. Reverse transcription was conducted under the following thermal parameters: 25 °C for 10 min and 37 °C for 120 min, followed by 85 °C for 5 min to inactivate the reverse transcriptase enzyme (T100 Thermal Cycler; BioRad Laboratories, Inc., Hercules, California, USA).

Real-time quantitative PCR was performed using SYBR™ Green intercalating dye to measure relative gene transcript abundance for several genes involved in Igf hormone signaling and myogenesis. Quantitative PCR was performed according to the guidelines presented by Bustin and coworkers (2009). All quantitative PCR reactions were run as 10 µl volumes containing 5 µl PowerUp™ SYBR™ Green Master Mix, 0.65 µl each of forward and reverse oligo primer (10 mM), 2.2 µl nuclease-free water, and 1.5 µl cDNA. Liver cDNA samples were analyzed using a CFX96™ Real-Time PCR Detection System (BioRad Laboratories, Inc.) under a thermal profile of 50 °C for 2 min followed by 95 °C for 2 min and then 42 cycles of 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 1 min, subsequently followed by a melt curve analysis. Skeletal muscle samples were analyzed on a QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific). Standard curves for qPCR were made using RNA pooled from fish representing both treatment groups. Standards for each tissue were then serially diluted and assayed in triplicate. Correlation coefficients ($r^2$) were > 0.95 for the standard curves for each gene. All experimental samples were assayed in duplicate. Each qPCR run included samples without cDNA as 'no template controls' (NTCs), and DNA contamination was evaluated by assaying RNA samples that were not reverse-transcribed. Resulting PCR efficiencies were calculated for each gene transcript measured using the following formula: % efficiency = [10(1/slope) − 1] · 100. Mean PCR efficiencies for each gene transcript quantified are provided in Appendix A, Supplementary Table S1.

Gene specific primers described previously were designed to consensus regions of partial cDNAs encoding Igf-related or myogenesis-related gene transcripts from several *Sebastes* rockfishes including gopher rockfish (*S. carnatus*) (Bersin et al., 2024; Bersin et al., 2023a, 2023b), copper rockfish (*S. caurinus*) (Hack et al., 2019) and olive rockfish (*S. serranoides*) (Hack et al., 2018). These primer sequences were examined against the blue rockfish genome (GenBank assembly accession no. GCA_916701245) to confirm primer nucleotide identity. Primer sets for use in SYBR™ Green qPCR were designed to target cDNAs for *igf1* and *igf2,* Gh receptors 1 (*ghr1*) and 2 (*ghr2*), ten *igfbp* genes, and an Igf acid labile subunit gene (*igfals*) recently identified in *Sebastes* rockfishes (Bersin et al., 2024; Bersin et al., 2023a). In addition, relative mRNA levels for *igf1* and *igf2* as well as several genes involved in myogenesis or muscle degradation/atrophy were examined in skeletal muscle, including myogenic Myod genes *myod1* and *myod2*, myogenin (*myog*), *myostatin1*, and muscle atrophy-associated ubiquitin ligases *fbxo32* and *muRF1* (see also, Bersin et al., 2023a).

Nucleotide sequences for each primer set used for SYBR™ Green qPCR are provided in Appendix A, Supplementary Table S1. When possible, primers were designed to span an intron boundary. All primers were synthesized by Eurofins MWG Operon (Huntsville, AL, USA). While melt curve analyses were conducted for each qPCR run, the specificity of each primer set was also confirmed by Sanger sequencing select PCR products (Molecular Cloning Laboratories, South San Francisco, USA). Gene transcripts encoding elongation factor-1$\alpha$ (*ef1$\alpha$*) and tubulin α-1a (*tuba*) were used as references for normalization of liver gene expression analyses, while *ef1$\alpha$* alone was used as the reference gene for skeletal muscle analyses. For the liver, relative mRNA abundance was normalized to the geometric mean value of *ef1$\alpha$* and *tuba*, which was unaffected by treatment conditions. For skeletal muscle, *ef1$\alpha$* alone was used as relative mRNA levels of this gene were not affected by cortisol treatment, while other genes examined as possible references showed near significant expressional changes due to cortisol. Relative mRNA values for each gene transcript are plotted as normalized to the mean expression value for the vehicle-injected (*control*) group.

### 2.7. Statistical analyses

One factor ANOVA models were used to test for differences in body length (TL) or mass among tanks at the time of initial assignment to experimental tanks, as well as at the time of tissue sampling (+24 h) after injections. Contingency analysis was used to test for differences in sex distribution between the injection treatment groups. As initial statistical comparisons showed no effects of 'sex' on any of the physiological variables measured, 'sex' was removed as a factor in all further statistical models.

Repeated-measures ANOVA models with experimental injection treatment and time as factors were used to test for effects of cortisol treatment on plasma cortisol, glucose, and Igf1. When significant main or interaction effects of treatment were observed, targeted pairwise comparisons were conducted using Bonferroni-corrected Student's t tests to identify significant differences between treatment groups (cortisol or vehicle injection) at a sampling time point: pre-injection baseline (*pre*), + 5 h post injection, or + 24 h post-injection.

For relative mRNA abundances in liver and skeletal muscle tissues, data were $\log_{10}(x + 1)$ transformed prior to further analysis if variances were identified as unequal (Bartlett tests). For each gene transcript measured, Student's t tests were used to identify any significant differences in relative mRNA levels between injection treatments. All tests were two-tailed and performed according to an overall $\alpha = 0.05$, and all tests were calculated using JMP Pro 16.0 software (SAS Institute Inc., Cary, NC, USA).

## 3. Results

### 3.1. Plasma cortisol and glucose concentrations

Plasma cortisol concentrations were similar between the experimental groups at the baseline sampling time (pre-injection 0 h) prior to administration of cortisol or vehicle (*control*) solutions (df = 20.568, t = 0.006, p = 0.996). Cortisol injection, however, significantly elevated plasma cortisol concentrations beginning at the + 5 h sampling time compared to vehicle-injected rockfish, and cortisol remained elevated in cortisol-treated rockfish + 24 h after hormone administration (Fig. 1) (injection*time interaction, $F_{2,20} = 36.395$, $p < 0.0001$). Pairwise comparisons revealed cortisol to be elevated in cortisol-treated rockfish at both the + 5 h (t = -4.128, p = 0.0005) and + 24 h (t = -8.077, p < 0.0001) sampling times compared to fish administered vehicle solution only.

Plasma glucose concentrations were similar at the 0 h baseline sampling time but subsequently became elevated following injection treatments in both cortisol- and vehicle-injected rockfish (Fig. 2)

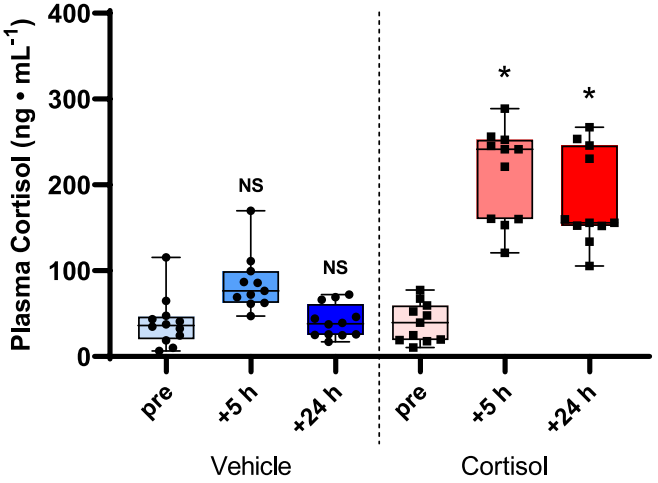


**Fig. 1.** Cortisol-injected blue rockfish showed increased plasma cortisol + 5 h and + 24 h after hormone administration. Box plots denote median (center line), 25th –75th percentile interval, and whiskers indicate min to max values. Data points indicate individual fish values. Asterisks indicate significant pairwise comparisons: * p < 0.05. 'NS' denotes for non-significant relative to 0 h baseline of the same treatment.

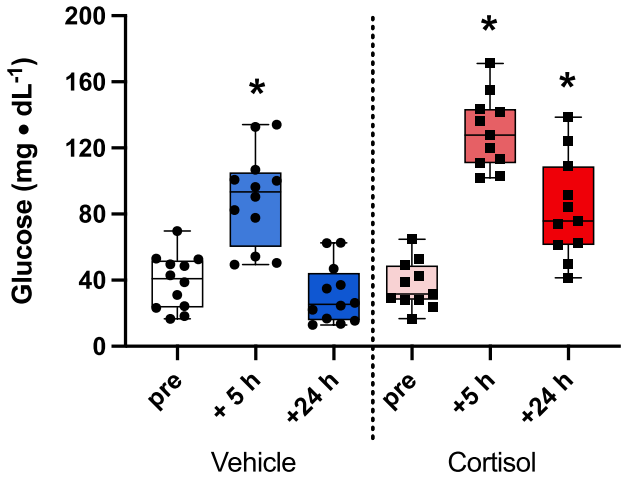


**Fig. 2.** Plasma glucose concentrations in cortisol- and vehicle-injected rockfish. Box plots denote median (center line), 25th −75th percentile interval, and whiskers indicate min to max values. Data points indicate individual fish values. Asterisks indicate significant pairwise comparisons relative to pre-treatment (0 h) values: * $p < 0.05$. 'NS' denotes for non-significant relative to 0 h baseline of the same treatment.

(injection*time interaction, $F_{2,20} = 10.502$, $p = 0.0008$). At + 5 h after hormone or vehicle injection, glucose concentrations were ~ 2-fold elevated above baseline (0 h) levels in vehicle-injected rockfish, and more than 3-fold increase in cortisol-treated fish. At + 24 h, however, plasma glucose remained ~ 2-fold elevated in cortisol-injected fish but had returned to baseline (0 h) levels in rockfish injected with vehicle solution only.

### 3.2. Cortisol effects on plasma Igf1 and liver igf, ghr, and igfbp gene expression

Plasma Igf1 concentrations were altered by the administration of exogenous cortisol (Fig. 3) (injection*time interaction, $F_{1,21} = 5.583$, $p = 0.0279$). While plasma Igf1 was similar in fish from the treatment groups at the baseline (0 h) sampling time prior to hormone injection ($p = 0.290$), Igf1 was significantly lower in cortisol-treated fish than in vehicle-injected fish at + 24 h following cortisol administration ($t = 3.986$, $df = 17.908$, $p = 0.0009$).

Cortisol administration did not have any effects on the relative abundances of mRNAs encoding *igf1* ($p = 0.8589$) or *igf2* ($p = 0.7087$) in the liver (Fig. 4). Similarly, exogenous cortisol did not alter liver mRNA abundances of GH receptor 1 (*ghr1*) (Fig. 5a; $p = 0.1251$) or for *ghr2* (Fig. 5b; $p = 0.9462$). However, cortisol-injected fish on average showed more than ~ 3-fold higher relative mRNA levels for *igfbp1a* ($t = -4.747$, $p = 0.0004$) and ~ 2-fold higher levels of *igfbp1b* mRNAs ($t = -2.756$, $p = 0.0131$) in the liver (Fig. 6a,b). Gene transcripts encoding *igfbp2a* ($t = -3.205$, $p = 0.0045$) (Fig. 6c), *igfbp5b* ($t = -3.122$, $p = 0.0054$) (Fig. 6i), and *igfbp6* ($t = -2.859$, $p = 0.0098$) (Fig. 6j) were also at higher relative levels in cortisol-treated rockfish. Rockfish treated with exogenous cortisol also had significantly lower liver mRNA abundance for *igfbp3a* ($t = 2.494$, $p = 0.0226$) (Fig. 6e). There were no significant differences between injection treatments in liver relative mRNA expression levels for Igfbp genes *igfbp2b* ($p = 0.2404$), *igfbp3b* ($p = 0.9297$), *igfbp4* ($p = 0.7467$), or *igfbp5a* ($p = 0.6826$) (Fig. 6d,f-h). Liver mRNA levels encoding a putative *igfals* gene also were unaffected by cortisol treatment ($p = 0.1479$) (Fig. 7).

### 3.3. Effects of exogenous cortisol on skeletal muscle gene expression

Rockfish given supplemental cortisol did not show any differences in skeletal muscle *igf1* mRNA levels (Fig. 8a)($p = 0.4759$) or in *igf2* mRNA

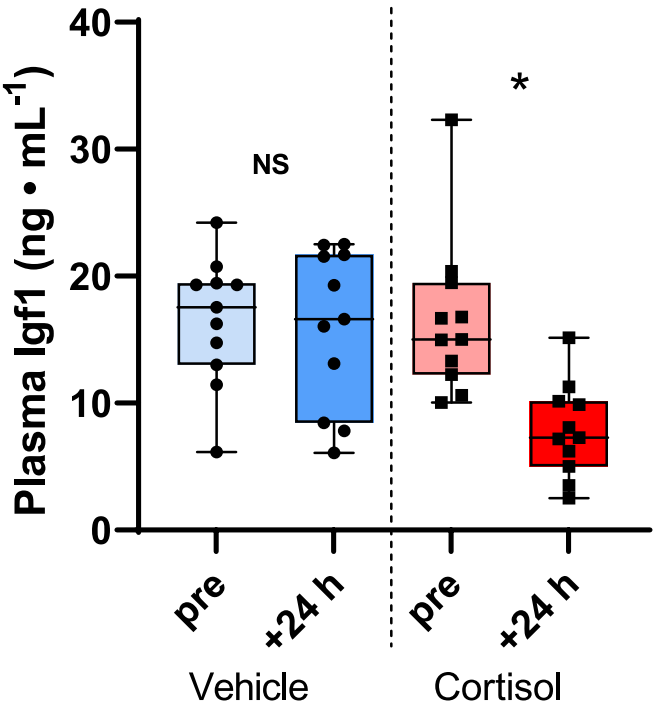


**Fig. 3.** Cortisol effects on plasma Igf1 levels. Box plots denote median (center line), 25th-75th percentile interval, and whiskers indicate min to max values. Data points indicate individual fish values. Asterisk indicates significant pairwise comparisons: * $p < 0.05$. 'NS' denotes for non-significant relative to 0 h baseline of the same treatment.

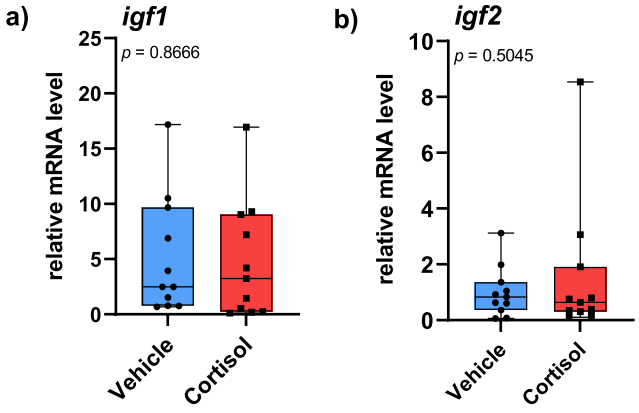


**Fig. 4.** Relative mRNA levels for **(a)** *igf1* and **(b)** *igf2* in the liver + 24 h after receiving respective injection treatments. Box plots denote median (center line), 25th-75th percentile interval, and whiskers indicate min to max values. Data points indicate individual fish values. Asterisks denote significant difference with respective p-values indicated on each graph.

levels (Fig. 8b)($p = 0.8093$). Cortisol-injected rockfish, however, had a significantly lower relative expression level of *myod2* mRNAs ($t = 3.425$, $p = 0.0026$) in skeletal muscle + 24 h after cortisol treatment compared to fish given vehicle solution only (Fig. 9b). None of the other transcripts examined encoding myogenic or muscle degradation genes differed in relative expression in skeletal muscle after cortisol treatment; relative mRNA levels for *myod1* ($p = 0.8246$), *myogenin* ($p = 0.1905$), *myostatin1* ($p = 0.3631$), *muRF1* ($p = 0.1913$), and *fbxo32* ($p = 0.9535$) were similar in relative expression between cortisol- and vehicle-injected rockfish + 24 h after administering the injection treatments (Fig. 9a,c-f).

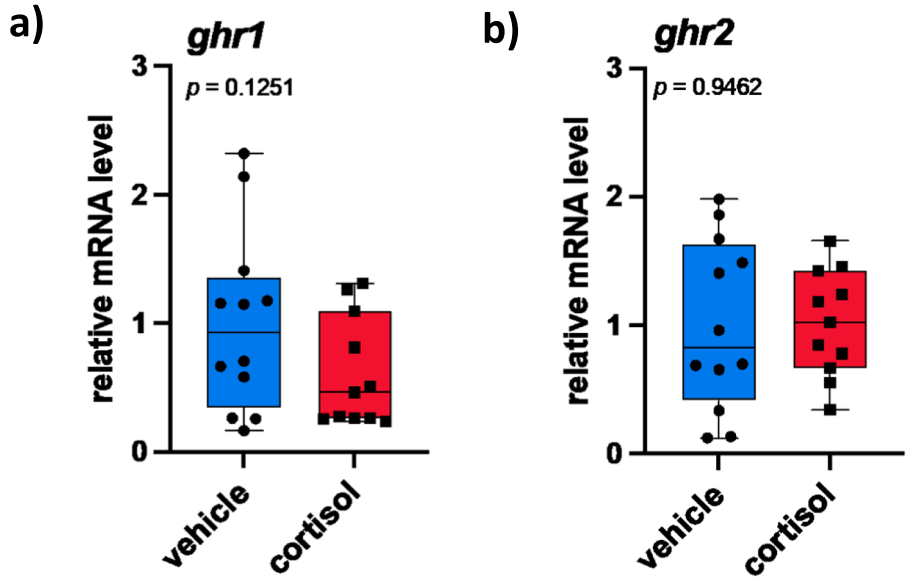


**Fig. 5.** Relative mRNA levels for Gh receptors (**a**) *ghr1* and (**b**) *ghr2* in the liver were unaffected by exogenous cortisol treatment. Box plots denote median (center line), 25th-75th percentile interval, and whiskers indicate min to max values. Data points indicate individual fish values.

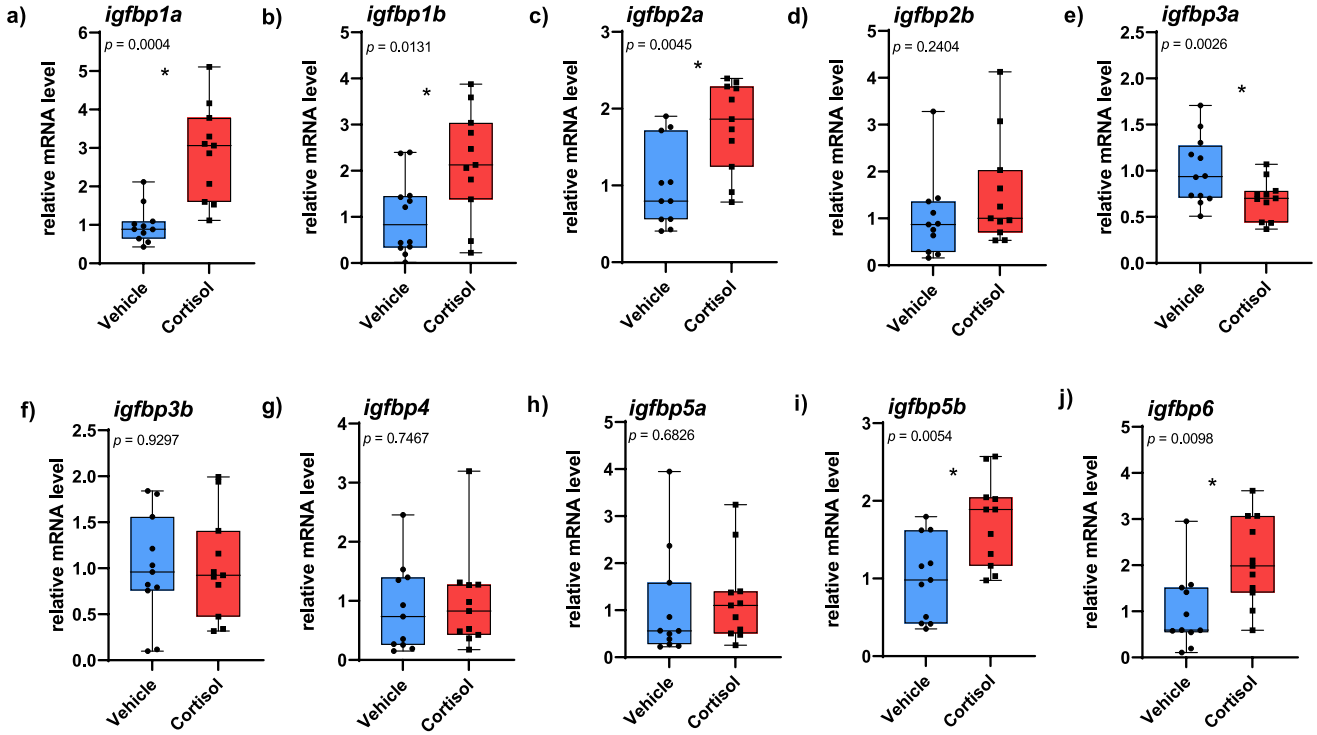


**Fig. 6.** Relative mRNA levels for Igf binding protein (*igfbp*) genes in the liver 24 h after receiving respective injection treatments. Box plots denote median (center line), 25th-75th percentile interval, and whiskers indicate min to max values. Data points indicate individual fish values. Asterisks indicate significant difference with respective p-values indicated on each graph.

## 4. Discussion

Blue rockfish given a single intraperitoneal injection of cortisol showed elevated plasma cortisol compared to vehicle-injected control fish both + 5 h and + 24 h following hormone injection. That single cortisol dose of cortisol increased plasma cortisol from a baseline average of 39.7 ng·ml$^{-1}$ to 212.9 ng·ml$^{-1}$ by + 5 h after injection, with cortisol concentrations remaining elevated (182.9 ng·ml$^{-1}$, avg.) + 24 h after injection. While little information is available regarding circulating cortisol concentrations in *Sebastes* rockfishes, the baseline cortisol values measured here are lower than those in a previous study with gopher rockfish (*S. carnatus*), which recorded baseline plasma cortisol in a range of 130 to 154 ng·ml$^{-1}$ (Duryea, 2014).

Supplemental cortisol induced elevated plasma glucose in cortisol-injected rockfish at both the + 5 and + 24 h time points. Although + 5 h cortisol values in vehicle-administered rockfish were not statistically higher than baseline pre-injection values, vehicle-injected fish had higher plasma glucose concentrations at that + 5 h sampling time

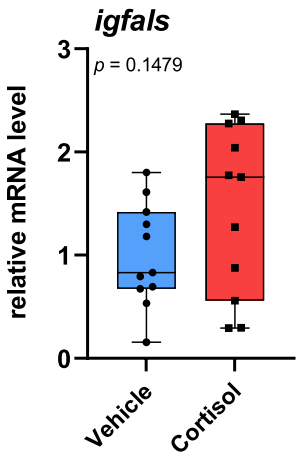


**Fig. 7.** Liver mRNA levels for an Igfbp acid labile subunit-like gene (*igfals)* did not differ between vehicle- and cortisol-treated rockfish. Box plots denote median (center line), 25th-75th percentile interval, and whiskers indicate min to max values. Data points indicate individual fish values.

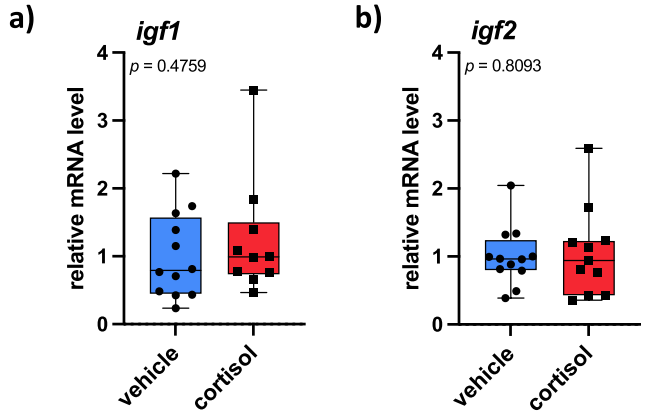


**Fig. 8.** Relative mRNA levels for (**a**) *igf1* and (**b**) *igf2* in skeletal muscle were unaffected 24 h after treatment with exogenous cortisol. Box plots denote median (center line), 25th-75th percentile interval, and whiskers indicate min to max values. Data points indicate individual fish values.

relative to baseline. That change in glucose suggests that vehicle-administered rockfish experienced short-term HPI axis activation from the handling and injection procedure. Even so, rockfish given exogenous cortisol had statistically higher plasma cortisol and glucose concentrations than vehicle-injected fish at + 5 h and continued to experience elevated cortisol and glucose at the + 24 h sampling time, when liver and skeletal muscle tissues were collected for gene expression analysis. As such, differences between cortisol- and vehicle-injected fish in this study might be best interpreted as a comparison between fish experiencing a sustained, 24 h elevation in cortisol (*cortisol-injected treatment*) versus those that experienced an acute HPI axis activation sufficient to elevate plasma glucose but not enough to sustain elevated glucocorticoids + 5 h after the stress of handling and injection (*vehicle-injected treatment*).

### *4.1. Cortisol decreased plasma Igf1 without altering liver igf1 mRNA levels*

Blue rockfish had lower plasma Igf1 + 24 h after cortisol injection. Only a few studies have examined cortisol effects on plasma Igf1 in teleost fishes (Table 1). While several studies have observed cortisol-mediated declines in circulating Igf1, other studies have not detected any effects. Male tilapia (*Oreochromis mossambicus*) given an intraperitoneal injection of cortisol (10 or 50 $\mu g \cdot g^{-1}$ body mass), for instance, exhibited lower plasma Igf1 + 24 and + 48 h after hormone administration without any change in plasma Gh (Kajimura et al., 2003). Channel catfish fed dietary cortisol for 4 weeks also had lower plasma Igf1 concentrations (Peterson and Small, 2005). However, Atlantic salmon parr given slow-release cortisol implants showed no changes in plasma Igf1 (Breves et al., 2020). Similarly, cortisol injection (40 $\mu g \cdot g^{-1}$ body mass) had no effect on circulating Igf1 in masu salmon (*Oncorhynchus masou masou*) or amago salmon (*O. masou ishikawae*) parr (Yamaguchi et al., 2021).

Igf1 signaling has been extensively studied in a number of teleost fish species, and increases in feeding rate and food intake have been linked repeatedly to increased plasma Igf1, which ultimately enhances somatic growth (Pérez-Sánchez et al., 1995; Beckman et al., 2004a,b; Pierce et al., 2005; Wilkinson et al., 2006; Cameron et al., 2007; Bower et al., 2008; Breves et al., 2014, 2016; Hack et al., 2019). Recent studies in Pacific rockfishes and other related scorpaeniform fishes have likewise shown positive associations between circulating Igf1, growth rate, and the expression of myogenesis-related genes in skeletal muscle (Bersin et al., 2023a, 2023b; Hack et al., 2018, 2019; Strobel et al., 2020). Given that higher Igf1 in blood circulation is linked to faster somatic growth, our observation here of lower Igf1 in blue rockfish with sustained (24 h) elevated cortisol suggests those fish may also experience a reduction in somatic growth rate, if that reduced Igf1 is sustained over days.

For a full picture of how cortisol affects Igf1 and growth, one must consider how acute and chronic cortisol treatments induce different physiological states. Based on studies to date, short periods ($\leq$0.25 to 6 h) of elevated cortisol – time durations approximating an acute stress response – do not appear to affect plasma Igf1 (Table 1). Acutely elevated cortisol thus probably does not change growth in any measurable way, but may temporarily reallocate energy (i.e., glucose) to restore reserves. In contrast, cortisol-induced declines in Igf1 appear generally consistent across fishes given continuous/chronic administration of cortisol for periods of 24 h or greater (Table 1; but see Yamaguchi et al., 2021). Reductions in circulating Igf1 thus might be a mechanism to lessen somatic growth under chronic stressor exposures. Such a downregulation of growth could be an adaptive response to shift energy allocation toward essential needs during chronic stress. Even so, experimental paradigms that chronically elevate cortisol could generate range of growth-related outcomes from slightly reducing growth to severe muscle wasting, depending on the duration, dose, and method of experimental cortisol supplementation. To better interpret results from future studies looking at cortisol effects on Igf1 signaling pathways, it will be important for studies to measure physiological parameters indicative of anabolic/catabolic status to place any observed Igf1 responses within the context of metabolic state.

Given that the liver is the primary tissue for Igf1 synthesis and release into blood circulation, we also examined whether hepatic Gh receptor and *igf1* gene expression was altered by the experimentally elevated cortisol concentrations. Even though plasma Igf1 levels were reduced + 24 h after cortisol administration, the relative abundance of *igf1* mRNAs in the liver did not differ between cortisol- and vehicle-injected blue rockfish at that + 24 h time point. While relative mRNA levels do not consistently reflect abundances of proteins generated, the absence of any difference in liver *igf1* mRNA levels between cortisol- and vehicle-treated rockfish may indicate that the decline in plasma Igf1 was not a result of reduced liver Igf1 production. Similarly, mRNA levels for Gh receptors *ghr1* and *ghr2* in the liver did not differ between fish treated with cortisol or vehicle control. Gh binding to liver Gh receptors is a key first step toward increasing the hepatic synthesis and secretion of Igf1. Treating gopher rockfish with supplemental Gh has been previously shown to upregulate liver *ghr1* and *ghr2* mRNA abundance, with those increases relating to a rise in plasma Igf1 (Bersin et al., 2024). Hepatic *ghr1* mRNAs – but not *ghr2* mRNAs – were also found to be upregulated in gopher rockfish concomitant with an increase in plasma Igf1 resulting from refeeding after an extended period of food deprivation (Bersin

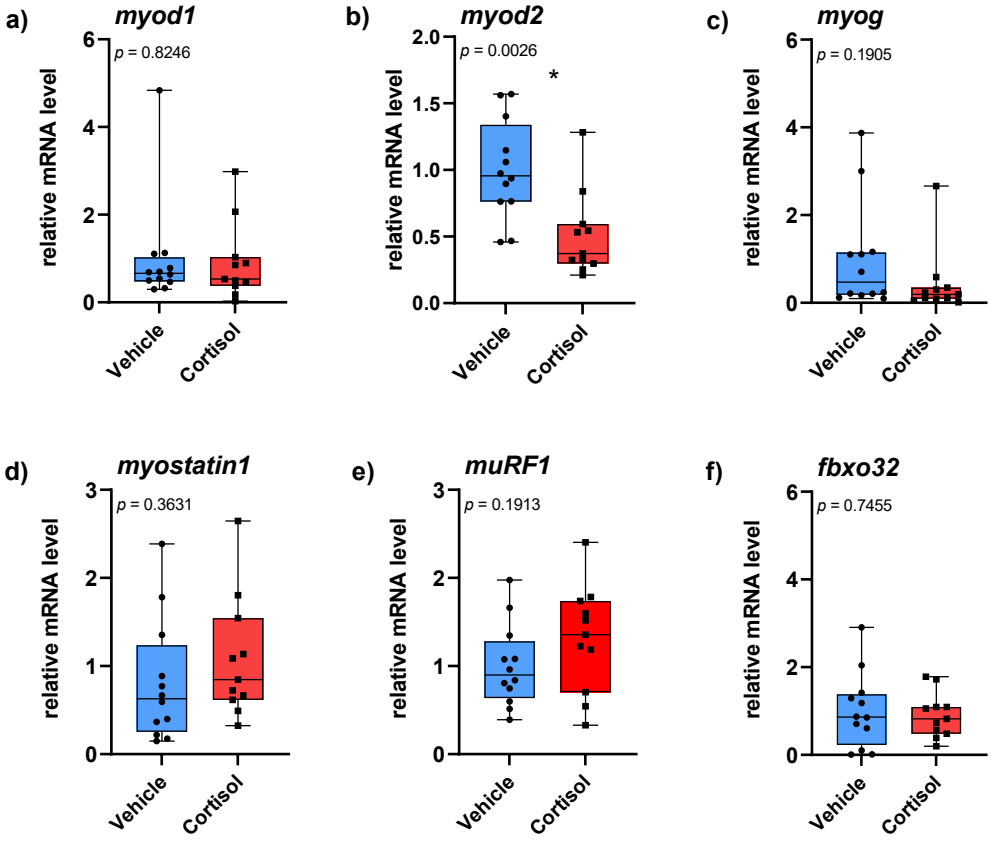


**Fig. 9.** Skeletal muscle relative mRNA levels for **(a)** myoblast determination protein-1 (*myod1*), **(b)** myoblast determination protein-2 (*myod2*), **(c)** myogenin (*myog*), **(d)** *myostatin1*, **(e)** muscle RING-finger protein-1 (*muRF1*), and **(f)** ubiquitin ligase F-box only protein 32 (*fbxo32*) in skeletal muscle + 24 h after receiving respective injection treatments. Box plots denote median (center line), 25th-75th percentile interval, and whiskers indicate min to max values. Data points indicate individual fish values. Asterisks denote significant difference with respective p-values indicated on each graph.

**Table 1**
Summary of studies testing plasma Igf1 responses to exogenous cortisol administered as either a single injection (single / acute administration) or continuously via food or cannulation (continuous / chronic administration).

| Type of cortisol administration | Species | Method of hormone administration | Cortisol dose(s) | Post-administration testing time(s) | Plasma Igf1 responses | Reference |
|---|---|---|---|---|---|---|
| **Single / acute administration** | | | | | | |
| | Mozambique tilapia (*Oreochromis mossambicus*) | Single intraperitoneal injection (in soybean oil) | 2 or 10 $\mu g \cdot g^{-1}$ body mass | 2, 4, 8 and 24 h | No effect at 2 ,4 and 8 h; Decreased at 24 h (10 $\mu g \cdot g^{-1}$ dose only) | Kajimura et al., 2003 |
| | | Single intraperitoneal injection (in soybean oil) | 2, 10 or 50 $\mu g \cdot g^{-1}$ body mass | 24 or 48 h | Decreased at 24 and 48 h (10 and 50 $\mu g \cdot g^{-1}$ doses only) | Kajimura et al., 2003 |
| | Atlantic salmon (*Salmo salar*) | Single intraperitoneal injection (in 1:1 vegetable oil:shortening) | 10 or 40 $\mu g \cdot g^{-1}$ body mass | 3 or 14 d | No effect at 3 or 14 d | Breves et al., 2020 |
| | masu salmon (*Oncorhynchus masou*) | Single intraperitoneal injection (in buffer with 15.9 % ethanol) | 40 $\mu g \cdot g^{-1}$ body mass | 24 or 48 h | No effect at 24 or 48 h | Yamaguchi et al., 2021 |
| | blue rockfish (*Sebastes mystinus*) | Single intraperitoneal injection (0.9 % NaCl with 16.6 % ethanol) | 40 $\mu g \cdot g^{-1}$ body mass | 24 h | Decreased at 24 h | Mapes et al. (this study) |
| **Continuous / chronic administration** | | | | | | |
| | channel catfish (*Ictalurus punctatus*) | Dietary (fed once per d) | 200 or 400 $mg \cdot kg^{-1}$ feed per d | 4 weeks | Decreased at 4 weeks (both 200 and 400 $mg \cdot kg^{-1}$ feed doses) | Peterson and Small, 2005 |
| | channel catfish (*Ictalurus punctatus*) | Dietary (fed once per d) | 200 $mg \cdot kg^{-1}$ feed per d | 4 weeks | Decreased at 4 weeks | Small et al., 2006 |
| | hybrid striped bass (*Morone saxatilis*) x white bass (*M. chrysops* | Dietary (one feeding event) | 100 $mg \cdot kg^{-1}$ feed | 0.25, 1, 2, 6, 24 and 48 h | No effect at 0.25, 1, 2, or 6 h; Decreased at 48 h | Davis and Peterson, 2006 |
| | rainbow trout (*Oncorhynchus mykiss*) | Continuous dosing via vascular cannulation | 20 or 40 $\mu g \cdot g^{-1}$ body mass | 14, 28 and 42 d | No effect at 14 or 28 d; Decreased at 42 d (40 $\mu g \cdot g^{-1}$ dose only) | Madison et al., 2015 |

et al., 2023b). And yet, the absence of any changes in liver Gh receptor mRNAs or *igf1* mRNA expression in cortisol-treated blue rockfish in the current study suggests that the observed decline in circulating Igf1 after cortisol administration may not have been due to a change in liver Igf1 synthesis. While mechanism(s) leading to the lower Igf1 in cortisol-treated blue rockfish remain unclear at this time, a change in Igf1 degradation rates – possibly via altered protease activity (e.g., Tejada et al., 2016) – is one possible explanation that should be explored in future studies, as little work has been done to date on the dynamics of Igf1 degradation and clearance from blood.

Even though liver *igf1* mRNA abundance was unaffected by elevated cortisol in blue rockfish, studies in other teleost fishes have observed changes in liver *igf1* gene expression associated with elevated cortisol. Cortisol thus may have impacts on *igf1* expression in some fishes, or at some time scales or cortisol doses different than those used in the current experiment. For instance, Atlantic salmon parr given slow-release cortisol implants showed lower liver *igf1* mRNA levels – and elevated liver *igf2* mRNAs – 3 d after commencing cortisol treatment (Breves et al., 2020). Immature rainbow trout infused chronically with cortisol showed higher *igf1* mRNAs in the liver after 14 and 28 d (Madison et al., 2015). Similarly, channel catfish fed dietary cortisol for 28 d showed elevated liver *igf1* mRNAs, even though plasma Igf1 declined (Peterson and Small, 2005). Those other studies treated fish with exogenous cortisol continuously over several days, and it is possible that a longer duration of cortisol elevation was necessary to detect alterations in liver *igf1* mRNA abundance in blue rockfish. Since that liver *igf1* gene regulation can be modulated by several factors, it is also possible that cortisol effects on liver *igf1* gene expression vary with context of the endocrine stress response (Pierce et al., 2011), such as the type of stressor or duration of elevated cortisol (e.g., Kajimura et al., 2003; Madison et al., 2015; Breves et al., 2020).

### 4.2. Cortisol-induced changes in liver Igfbp expression

In addition to lower circulating Igf1, cortisol-treated rockfish had altered liver mRNA expression for several Igfbps. Igfbps modulate the availability of Igf1 in blood circulation and within tissues or cells, with some Igfbps (e.g., type 1) acting as negative regulators of Igf1 availability and action, while other Igfbps facilitate Igf1 transport and the hormone's growth-promoting effects (Macqueen et al., 2013; Shimizu & Dickhoff, 2017; Allard & Duan, 2018; Garcia de la Serrana and Macqueen, 2018). Here in adult blue rockfish, Igfbp-encoding genes *igfbp1a*, *−1b*, *−2a, −5b*, and *−6* were all expressed at higher mRNA levels in the liver of cortisol-treated fish after 24 h of sustained cortisol elevation. Gene transcripts encoding *igfbp3a*, in contrast, were at lower abundance in the liver of blue rockfish treated with cortisol. Several other Igfbp transcripts (*igfbp2b, −3b, −4,* and *−5a*) were unaltered in relative hepatic expression following cortisol elevation. Studies in *Sebastes* rockfishes have observed type-specific liver *igfbp* gene regulation with variation in food intake (Hack et al., 2018; Bersin et al., 2024; Bersin et al., 2023a, 2023b; Hack et al., 2019) or experimental administration of Igf1 (Bersin et al., 2023a) or Gh (Bersin et al., 2024). The type-specific *igfbp* expressional responses to cortisol in the current study thus likely reflect the disparate functions of different teleost Igfbps (Allard and Duan, 2018; Garcia de la Serrana and Macqueen, 2018; Macqueen et al., 2013; Safian et al., 2012; Shimizu and Dickhoff, 2017).

Blue rockfish treated with cortisol had 2–3 fold higher liver mRNA levels for *igfbp1a* and *igfbp1b*. Teleost type 1 Igfbps are generally thought to have inhibitory influences on Igf1 action (Kajimura and Duan, 2007; Shimizu & Dickhoff, 2017; Garcia de la Serrana and Macqueen, 2018). Hepatic *igfbp1a* and *igfbp1b* mRNA levels increase in *Sebastes* rockfishes experiencing reduced food intake or complete food deprivation (Hack et al., 2018, 2019; Bersin et al., 2024; Bersin et al., 2023a, 2023b). Studies with other teleosts have likewise found liver type 1 Igfbp gene expression to increase in fish subjected to catabolic conditions (Pierce et al., 2006; Breves et al., 2016), and recent evidence has even tied elevated type 1 Igfbp production in fish under food deprivation to reproductive inhibition (Li et al., 2024).

The primary mode of action for type 1 Igfbps appears to be to encumber Igfs in blood circulation or within tissues to suppress Igf1 availability (Kajimura and Duan, 2007). Our observation here that liver *igfbp1a* and *igfbp1b* mRNAs were more abundant in cortisol-treated rockfish thus suggests a cortisol-induced intensification of Igfbp1 inhibition of Igf1 action. Cortisol has been shown to increase the production of IGFBP1 in mammals (Unterman et al., 1993; Levitt Katz et al., 1998; Shimizu & Dickhoff, 2017), and the upregulation of Igfbp type 1 protein in blood circulation has previously been shown in fishes both after stressful experiences including confinement or handling (Kelley et al., 2001; Davis and Peterson, 2005, 2006; Peterson and Small, 2005) as well as after treatment with cortisol or dexamethasone (Pierce et al., 2006; Yamaguchi et al., 2021). For instance, tilapia given cortisol showed elevated plasma Igfbp proteins of 24, 28, 30, and 32 kDa sizes, as well as reduced plasma Igf1 and decreased liver *igf1* mRNA levels (Kajimura et al., 2003). Channel catfish treated with dietary cortisol likewise had higher plasma levels of a 20-kDa Igfbp protein – likely a type 1 Igfbp (Peterson and Small, 2005). Along those same lines, Atlantic salmon given cortisol showed increased liver mRNA levels for two salmonid type 1 Igfbp genes: *igfbp1b1* and *igfbp1b2* (Breves et al., 2020). Our observation here in blue rockfish that liver *igfbp1a* and *igfbp1b* gene transcript abundances were higher in cortisol-treated fish provides further evidence for cortisol upregulating teleost type 1 Igfbp expression. Notably, while teleost Igfbp1 is primarily thought to modulate Igf1 action by encumbering Igf1 (Shimizu & Dickhoff, 2017; Garcia de la Serrana and Macqueen, 2018), there is evidence from mammals that IGFBP1 may also directly modulate pituitary GH production (Cingel-Ristić et al., 2004). While we did not quantify plasma Gh here in blue rockfish, altered negative feedback of Igfbp1 on Gh could be an alternative avenue for modulating Igf1 production during physiological stress, and future studies should examine possible functional role for Igfbp1 directly modulating Gh in teleost fishes.

Supplemental cortisol also altered liver type 2 and type 3 *igfbp* gene expression in blue rockfish. *Sebastes* rockfishes have two Igfbp2 genes: *igfbp2a* and *igfbp2b* (Bersin et al., 2023a, 2023b; Hack et al., 2019). Here, cortisol-injected blue rockfish showed higher hepatic mRNA levels for *igfbp2a* after cortisol administration, but no changes were seen in liver *igfbp2b* mRNAs. In a separate study with Atlantic salmon, fish given slow-release cortisol implants did not show any changes in liver *igfbp2a, −2b1,* or *−2b2* mRNA levels 3 or 14 d after commencing cortisol dosing; however, plasma Igf1 also did not change in that study (Breves et al., 2020). In a study with rainbow trout, liver *igfbp2* mRNA levels increased ~ 2-fold after 14 d of cortisol infusion (Madison et al., 2015). Along those same lines, cortisol increased plasma Igfbp2b protein in masu salmon 2 d after injection (Yamaguchi et al., 2021).

Shimizu and Dickhoff (2017) proposed Igfbp2b to be the main carrier of Igf1 in fish blood, a role analogous to that of Igfbp3 in humans. However, the role of Igfbp2a in fishes is less well understood and is likely different from Igfbp2b, given that these type 2 Igfbps show dissimilar patterns of gene regulation (Shimizu and Dickhoff, 2017; Garcia de la Serrana and Macqueen, 2018). Previous work in *Sebastes* rockfishes found that fasting reduced liver *igfbp2b* mRNA levels without affecting *igfbp2a* mRNAs (Bersin et al., 2024; Bersin et al., 2023a; Hack et al., 2019), suggesting divergent roles during nutritional stress. Studies in salmon and trout support that idea, as several experiments with those taxa observed declines in both Igfbp2b protein (salmon 41 kDa Igbfp) and Igf1 in blood circulation during fasting (Beckman et al., 2004b; Pierce et al., 2005; Shimizu et al., 2009; Cleveland and Weber, 2013). Experimentally mutating *igfbp2b1* and *igfbp2b2* genes in rainbow trout using CRISPR/Cas9 reduced plasma Igfbp2b and Igf1, and impacted somatic growth (Cleveland et al., 2018). While such evidence points to a role for Igfbp2b in somatic growth, comparable data are unavailable for Igfbp2a. Our observation here that liver mRNA levels of *igfbp2a* – but not *igfbp2b* mRNAs – were altered by cortisol may point to a role for Igfbp2a

in modulating Igf signaling during physiological stress.

Messenger RNAs for *igfbp3a* were also at lower abundance in the liver of blue rockfish treated with cortisol. We are unaware of any other study in fishes having examined type 3 Igfbp gene expression responses to cortisol. However, studies in *Sebastes* rockfishes have repeatedly found liver *igfbp3a* mRNAs to become elevated from food deprivation, while *igfbp3b* mRNAs were unaffected (Bersin et al., 2023a; Bersin et al., 2024; Hack et al., 2019). Copper rockfish given reduced rations for 152 d had ~ 3.5-fold elevated liver *igfbp3a* mRNA levels, and subsequently depriving those fish of food entirely for 12 d raised liver *igfbp3a* mRNAs ~ 10-fold (Hack et al., 2019). In a separate study, refeeding gopher rockfish a single satiation meal after 30 d of fasting reduced liver *igfbp3a* mRNA levels without effects on *igfbp3b* mRNAs (Bersin et al., 2023b). However, given that *igfbp3a* mRNAs declined here in blue rockfish following cortisol treatment, it seems unlikely for those observed increases in hepatic *igfbp3a* transcript expression (e.g., Bersin et al., 2023a, 2023b; Hack et al., 2019, 2024) to be related to nutrition-related changes in cortisol.

Much less is known about the functions of Igfbp types 4, 5, and 6 in teleost fishes (Garcia de la Serrana and Macqueen, 2018). In mammals, IGFBPs 4–6 are in present in blood, albeit type 5 and 6 IGFBPs are low in concentration (Rajaram et al., 1997; see also, Shimizu and Dickhoff, 2017). In teleost fishes, however, Igfbp 4 and 6 do not appear to be in blood circulation and instead may be acting within cells or extracellular fluids of tissues (Shimizu and Dickhoff, 2017). Mammalian IGFBP4 has been suggested to inhibit growth (Zhou et al., 2003), while mammalian IGFBP5 may serve to both stimulate and inhibit growth depending on the type of cell or tissue (Garcia de la Serrana and Macqueen, 2018). Mammalian IGFBP5 is strongly regulated by IGF1 (Mohan et al. 1996; Ye and D'Ercole, 1998) and appears to influence bone growth and muscle development (Schneider et al., 2002; Duan and Xu, 2005; Ren et al., 2008). However, the roles of type 5 Igfbps in teleost fishes are unresolved (Garcia de la Serrana and Macqueen, 2018), and different teleost *igfbp5* paralogs might have different functions (e.g., Zeng et al., 2016; see also Bersin et al., 2023a).

Blue rockfish also had higher liver *igfbp6* mRNA levels following treatment with cortisol. Mammalian IGFBP6 has stronger binding affinity for IGF2 than for IGF1 (Bach, 2015, 2018). Igfbp6 function in teleost fishes, however, is almost completely unstudied (Garcia de la Serrana and Macqueen, 2018). Despite structural similarity between teleost and mammalian type 6 Igfbps (Macqueen et al., 2013), it is not clear whether teleost Igfbp6 binds Igf1 or Igf2 with greater affinity. One study in the Indian catfish *Clarias magur* found faster growing genetic family lines to have lower *igfbp6* transcript abundances in the liver (Ram et al., 2023). A separate study in zebrafish (*Danio rerio*) revealed that overexpressing either *igfbp6a* or *igfbp6b* genes during embryonic development led to reduced development rate and growth (Wang et al., 2009). Liver expression of Igfbp6, however, appears to be generally low in teleost fishes (Garcia de la Serrana and Macqueen, 2018), and whether variation in hepatic Igfbp6 production plays any role in regulating somatic growth remains to be determined.

### 4.3. Acutely elevated cortisol influences skeletal muscle myod2 expression

Here we also found that relative mRNA levels for *myod2* – but not *myod1* – were significantly reduced in rockfish skeletal muscle after treatment with cortisol. The sustained 24 h of elevated cortisol did not, however, affect relative mRNA levels for any of the other myogenesis-regulating genes quantified besides *myod2*, despite some of these genes having previously been shown to be regulated by glucocorticoids in fish (e.g., *myostatin,* Weber et al., 2005; Galt et al., 2014, 2016). Similarly, cortisol treatment did not alter skeletal muscle mRNA levels of either *igf1* or *igf2*. While food deprivation has been shown to diminish muscle *igf1* expression in rockfishes (Bersin et al., 2024; Bersin et al., 2023a; Hack et al., 2019), the absence of cortisol effects on muscle *igf1* here in blue rockfish suggests that those nutritional effects are likely independent of any fasting-related changes in circulating cortisol.

MyoD promotes histone acetylation of DNA to upregulate other genes promoting myoblast and myotube formation (Cao et al., 2010; Hernández-Hernández et al., 2017). Cortisol has been shown to reduce muscle expression of *MYOD* in mammalian models (Pandurangan et al., 2014), suggesting a role for stress-induced glucocorticoid elevations in downregulating myogenesis. In teleost fishes including *Sebastes* rockfishes, MyoD-encoding genes have evolved into at least two paralogs: *myod1* and *myod2* (Macqueen and Johnston, 2008). Notably, differences in skeletal muscle regulation of *myod1* and *myod2* were recently observed in gopher rockfish experiencing fasting, where only *myod2* mRNAs were downregulated (Bersin et al., 2023a). In those same gopher rockfish, exogenous Igf1 had no effect on muscle *myod1* or *myod2* expression (Bersin et al., 2023a). A similar lack of Igf1 effects on skeletal muscle *MYOD* expression has been observed in mammals (e.g., Miyake et al., 2007). Given that, the reduced relative *myod2* mRNA levels observed here in cortisol-treated blue rockfish appears more likely to be a direct result of cortisol action, rather than a secondary effect of the reduced plasma Igf1 in cortisol-treated fish.

In mammals, increased cortisol concentrations in blood under chronically stressful conditions leads to increased skeletal muscle protein breakdown, decreased protein synthesis, and atrophy of skeletal muscle. That promotion of muscle wasting in mammals occurs via both cortisol-mediated upregulation of atrophy gene pathways (i.e., MAFbx, MuRF1) and the inhibition of anabolic pathways for protein synthesis (Braun and Marks, 2015). In teleost fishes, cortisol may have similar effects both promoting catabolic breakdown of skeletal muscle and inhibition the anabolic pathways underling myogenesis via activation of glucocorticoid receptors, which have been previously shown to regulate myogenesis gene expression patterns in fishes (e.g., Nesan et al., 2012; Faught and Vijayan, 2019). Even though we did not observe changes in muscle *fbxo32* or *muRF1* gene expression here in cortisol-treated rockfish, other studies provide evidence for cortisol upregulation of muscle atrophy pathways. RNA-seq analyses of skeletal muscle myotubes from red cusk-eel (*Genypterus chilensis*) treated with exogenous cortisol showed elevated expression of atrogenic genes *fbxo32* and *foxo1*, a regulator of *fbxo32* (Aedo et al., 2015). In that same study, red cusk-eel exposed to repeated handling stress for 5 d had highly upregulated *fbxo32* expression and downregulated *myod1* and *myod2* transcript expression in skeletal muscle (Aedo et al., 2015). Here, our observation that 24 h of elevated cortisol downregulated muscle *myod2* expression in blue rockfish without altering *fbxo32* or *muRF1* gene expression suggests that glucocorticoids first downregulate anabolic pathways promoting myogenesis in adult *Sebastes* rockfish, and that longer durations of elevated cortisol might be necessary before fish begin cortisol-associated muscle wasting and loss.

## 5. Conclusions

Sustained experimental elevation of plasma cortisol for 24 h altered Igf1 signaling in adult blue rockfish by reducing plasma Igf1 and upregulating liver mRNA levels for type 1 Igfbp genes *igfbp1a* and *igfbp1b*, both of which encode Igfbps purported to inhibit the growth-promoting actions of Igf1. Those observations suggest persistent cortisol elevation for 24 h modulates Igf1 signaling in several ways that together are likely to reduce somatic growth. Cortisol-treated rockfish also showed diminished skeletal muscle mRNA expression of *myod2*, a transcriptional activator of other genes promoting myocyte differentiation and, ultimately, skeletal muscle growth. Taken together, these findings provide evidence that fish experiencing sustained (24 h) elevations in cortisol such as those associated with persistent, chronic stresses are likely to also experience reduce somatic growth rate via changes in Igf1 signaling.

## CRediT authorship contribution statement

**Hayley M. Mapes:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Janae E. Shew:** Methodology, Funding acquisition. **Henry M. Marden:** Methodology, Funding acquisition. **Meredith L. Journey:** Methodology. **Brian R. Beckman:** Writing – review & editing, Supervision, Resources, Methodology, Formal analysis. **Sean C. Lema:** Conceptualization, Methodology, Resources, Writing – review & editing, Formal analysis, Visualization, Supervision, Funding acquisition.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2024.114659.

## Data availability

Data will be made available on request.

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