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Overcompensation of circulating and local insulin-like growth factor-1 during catch-up growth in hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) following temperature and feeding manipulations

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ABSTRACT

Teleosts and other aquatic ectotherms have the ability to withstand prolonged periods of low water temperatures (cold-acclimation) and fasting, and can often respond with phases of accelerated (compensatory) growth when favorable conditions are restored. We assessed whether complete feed restriction prior to (24 °C, days 0–23) and/or during (14 °C, days 24–114) a simulated period of cold-acclimation could elicit episodes of compensatory growth (CG) and catch-up growth upon warm-up to 24 °C and satiation feeding (days 115–148). Control hybrid striped bass (HSB: *Morone chrysops* × *Morone saxatilis*) were fed to satiation throughout the entire experiment under these temperature fluctuations. Compensatory growth and ultimately catch-up growth were achieved in groups of HSB that were deprived of feed during either the initial period at 24 °C (days 0–23), during the cold-acclimation period (14 °C, days 24–114), or during both of these periods (days 0–114). Further, it appears that HSB are better able to compensate for weight loss when skeletal length is not significantly compromised during the treatment period, which occurred in HSB feed restricted during cold-acclimation only. The most dramatic CG responses were defined by specific growth rates (SGRs) up to 4.2 times that of controls and were accompanied by hyperphagia and improvements in temporal and overall feed conversion. Levels of plasma insulin-like growth factor (IGF)-1 and muscle IGF-1 mRNA were significantly correlated to growth rate for all groups throughout the experiment ($R^2 = 0.40, 0.23$, respectively), with an overcompensation of both observed in HSB with the most elevated SGRs during the CG response. Interestingly, opposing trends were observed between muscle mRNA expression of growth hormone receptor (GHR)-1 and -2, with fasting at 24 °C and 14 °C resulting in depressed levels of GHR-1 and elevated levels of GHR-2 relative to controls. Levels of muscle myostatin (MSTN)-1 mRNA were significantly depressed in HSB fasted at 24 °C and/or 14 °C while MSTN-2 mRNA was lower following initial feed restriction at 24 °C. Likewise, levels of unprocessed pro-MSTN (precursor) and mature MSTN protein were both depressed in fasted fish at 24 °C. This study demonstrates that a previous period of feed restriction and cold-acclimation followed by realimentation at more favorable water temperatures produces a strong CG response and catch-up growth in fish. These studies also suggest that an overcompensation of circulating and local IGF-1 along with changes in MSTN mRNA and protein expression may contribute to accelerated growth rates characteristic of CG. Furthermore, our studies indicate that overall feed conversion can improve by as much as 30% with CG induced through temperature and feeding manipulations with no adverse effects on growth of HSB. This raises the possibility that CG protocols can improve production efficiency of HSB and other temperate teleosts in pond or tank culture.

1. Introduction

In teleost fishes and other aquatic ectotherms, periods of depressed water temperature can result in reduced growth rates through decreases in both metabolism and prey availability (Clarke and Johnston, 1999). The onset of better rearing conditions, however, can result in episodes of compensatory growth (CG) whereby growth is accelerated

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relative to size-matched controls (Morgan and Metcalfe, 2001). Catch-up growth, where body mass or skeletal length of stunted fish completely converges with that of controls, can occur independently or in conjunction with CG and therefore the terms are not synonyms (Jobling, 2010). The CG response has been documented in numerous teleosts along with mammals and birds and is typically accompanied by hyperphagia and improved feed conversion (Largo, 1993; Yu et al., 1990). Although fish are the most studied of the vertebrate taxa, most studies have focused on feeding manipulation alone to induce CG responses (Ali et al., 2003). By contrast, surprisingly little is known about the effects of a combination of water temperature and feeding manipulations, despite the biological and aquaculture implications for temperate-zone fish.

The endocrine control of growth in fishes and other vertebrates is regulated primarily through the growth hormone (GH)/insulin-like growth factor (IGF) axis and is influenced by variables such as nutrition, temperature and stress (Beckman, 2011; Reindl and Sheridan, 2012; Reinecke, 2010). Under anabolic conditions, pituitary-derived GH binds to one or both of its hepatic receptors (GHR-1, -2) to stimulate the production and subsequent release of insulin-like growth factor-I (IGF-1) into the circulation. Systemic IGF-1 can then act on target tissues to promote cell proliferation, differentiation and ultimately body growth (Wood et al., 2005). GH may also act directly on target tissues such as skeletal muscle to stimulate IGF-1 production, which in turn can act in a paracrine/autocrine fashion to promote tissue growth (Duan et al., 2010). Accordingly, levels of circulating and locally-produced IGF-1 have been correlated to specific growth rate in numerous teleosts and may provide a useful biomarker of growth in fishes (Beckman, 2011; De-Santis and Jerry, 2007; Picha et al., 2008a). Myostatin (MSTN), a negative regulator of skeletal muscle growth in mammals, may also influence growth in HSB, although no definitive evidence exists regarding its function in fishes (Rodgers and Garikipati, 2008). Indeed, variables affecting growth such as nutrition, temperature, growth hormone and cortisol have all been shown to regulate transcription of the duplicated MSTN genes (MSTN-1, -2) in fishes, while regulation of the protein remains much less studied. Despite evidence supporting the role of the GH/IGF axis and MSTN in regulating growth in fishes, few studies have addressed how most components correspond to variable growth phases, especially during the accelerated growth state seen with CG.

Hybrid striped bass (HSB: *Morone chrysops* × *Morone saxatilis*) and its related species are valuable recreational and aquacultured fishes that are exposed to natural periods of over-wintering in lakes/reservoirs and aquaculture ponds located within temperate environments. While CG responses have been elicited in HSB through partial and full feed restriction (Picha et al., 2006, 2008b; Skalski et al., 2005; Turano et al., 2007, 2008), no studies have assessed whether CG can be induced through both temperature and feed manipulations. In fact, the potential to induce CG responses through manipulation of these specific variables has been assessed in a limited number of fishes in general. The purpose of this study, therefore, was to determine if HSB undergo CG and catch-up growth following feed restriction prior to and/or during prolonged overwintering conditions and to assess potential involvement of insulin-like growth factor-1, myostatins and growth hormone receptors.

2. Materials and methods

2.1. Experimental design

Phase II juvenile hybrid striped bass (180–220 g) were transported from ponds at the Tidewater Research Station (Plymouth, NC) to indoor circular tanks at the Pamlico Aquaculture Field Laboratory (Aurora, NC). Opercular tags (Newport Band and Tag Co.; Newport, KY) were applied to all HSB, which were then evenly distributed between eight 1100 l circular tanks (70 HSB/tank) divided between 2 freshwater flow-through systems. Salinity was kept below 1 ppt for the majority of the experiment, but was increased to ~10 ppt on two occasions to assist with handling stress after sampling (days 114, 134). In both cases the salinity gradually returned to <1 ppt within 7 days. Sampling did not occur during each salinity drop. Each of the 4 treatment groups (see below) was evenly and randomly assigned between the 2 systems (2 tanks total/treatment). Each system was equipped with biofiltration, UV sterilization, and supplied with freshwater derived from the Castle Haynes Aquifer, the same water source used by HSB farmers in eastern North Carolina. All HSB were feeding (Melick Aquafeed 4.0 mm floating; Catawissa, PA) 6 days prior to initiation of the study. Photoperiod was kept on a 12L:12D schedule throughout the experiment.

All fish were subjected to the following water temperature fluctuations: days 0–23 at 24 °C, days 24–114 at 14 °C and days 115–148 at 24 °C. Unless specifically noted, days 24–114 will be referred to as the cold-acclimation period, even though this includes water temperature transition periods from 24 to 14 °C (days 24–34) and from 14 to 24 °C (days 107–114). Optimal growth for HSB occurs at 25–27 °C, and therefore rearing at 14 °C is considered a period of cold-acclimation (Hodson, 1995). Temperature data was recorded with Hobo pendant data loggers (Bourne, MA). The experiment ran from September 14, 2006 (Time 0) until February 10, 2007 (day 148). Data collected from 2002 to 2007 indicates that average pond temperatures in Aurora, NC drop from 21 °C to 14.5 °C between October and November. Cold-acclimation in this experiment began on October 18, 2006.

Since all HSB experienced the same temperature regimen, the 4 treatment groups were based on feeding manipulations during the initial period at 24 °C (days 0–23) and the cold-acclimation period at 14 °C (days 24–114). Treatments were as follows: S–S fish were starved (S) at both 24 °C and 14 °C; F–S fish were fed (F) at 24 °C and starved at 14 °C; S–F fish were starved at 24 °C and fed at 14 °C; and F–F or control fish were fed during both of these intervals. All treatment groups were fed during the final rearing period at 24 °C (days 115–148) to assess potential CG responses. All feeding was to apparent satiation 1×/day (5×/week) between 7 and 9 am. Mortality because of treatment was only observed in the S–S group, and amounted to 1.4%. However, one S–S tank was lost because of a plumbing malfunction at the end of the cold-acclimation period, which left one remaining tank of tagged fish for the refeed period at 24 °C (see Section 2.8, Statistical analysis methods).

2.2. Sample collection

Body weights (g) and standard lengths (mm) were taken for all fish at initiation of the experiment (Time 0), at the end of the initial 24 °C rearing period (day 23), at the end of 14 °C cold-acclimation following the transition to 24 °C (day 114), and 19 and 34 days into the final 24 °C rearing period (days 134 and 148). Tissues (liver, muscle, mesenteric fat, blood) were taken at these same time points as well as following the transition from 24 to 14 °C (day 34), during and at the end of the cold-acclimation period (days 57 and 106) and 13 days into the final 24 °C rearing period (day 127). When group weights and lengths were taken, HSB were anesthetized using buffered quinaldine sulfate (B.L. Mitchell Inc., Leland, MS). When terminal sampling was necessary (to collect tissue samples), HSB were anesthetized using buffered tricaine methanesulfonate (MS 222; Argent Chemical Laboratories, Redmond, WA). HSB were deprived of feed 22–24 h prior to sampling when water temperatures were at 24 °C, and 44–48 h prior when water temperatures were at 14 °C. Sampling on each date began between 8 and 10 am. All experimental procedures were approved by the North Carolina State University Animal Care and Use Committee.

2.3. Growth rate and metabolic/energy indices

Specific growth rate (SGR) was calculated as $[(\ln W_2 - \ln W_1) / (T_2 - T_1) \times 100]$, where W_2 is the weight at the end of the growth interval and W_1 is the weight at the beginning of the growth interval, while $T_2 - T_1$ represents the duration (days) of the growth interval. Feed conversion ratio (FCR) was calculated as [feed consumed /

weight gain]. Percent body weight consumed per day (% BW/day) was calculated as [(total feed consumption per cycle / # of days in cycle) / (total fish weight estimated at midcycle) × 100]. All calculations were measured in grams.

2.4. Radioimmunoassays

Circulating levels of total IGF-1 were measured from acid/ethanol extracted plasma by radioimmunoassay (RIA) using recombinant barramundi IGF-1 as tracer and standard, rabbit anti-barramundi IGF-1 primary antibody (Novozymes GroPep; Adelaide, Australia) and goat anti-rabbit secondary antibody (Sigma; St. Louis, Missouri) according to previously described methods (Shimizu et al., 2000; Picha et al., 2006, 2008a). All samples were run in duplicate.

2.5. Gene cloning

Hybrid striped bass MSTN-2 was partially cloned in order to design effective primers and probes for measures of mRNA by quantitative real time PCR (qRTPCR). Total RNA was extracted from HSB muscle samples using a TRI Reagent isolation solution (Molecular Research Center; Cincinnati, OH), DNase-treated (Ambion; Austin. TX) and then quantified via NanoDrop spectrophotometry (NanoDrop Technologies; Wilmington, DE). One μg of total RNA was reverse transcribed using oligo d(t) primers and then 10% of the RT reaction used for PCR (Qiagen; Valencia, CA). Degenerate MSTN-2 primers (forward: 5′-CAGCAAGCAG ATGAGGYTSCACAG-3′; reverse: 5′-AGCCTGCAGCAGAGACTTGATG-3′) were designed using known *Sparus aurata* (GenBank accession number: AY046314) and *Umbrina cirrosa* (AY059386) growth differentiation factor 8b (now considered MSTN-2; Rodgers and Garikipati, 2008) sequences. PCR cycling conditions were as follows: 1 cycle at 95 °C for 2 min; 40 cycles at 95 °C for 30 s, 48–56 °C for 30 s and 72 °C for 1 min; and 1 cycle at 72 °C for 5 min. The PCR product was identified on a 1.5% agarose gel and then purified and concentrated with a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). The PCR amplicon was sequenced at the University of Chicago DNA sequencing facility and verified by BLAST search (NCBI: Bethesda, MD).

2.6. Quantitative real-time PCR

Quantitative real time PCR (qRTPCR) primers and Taq Man probes for IGF-1, GHR-1 and GHR-2 were previously designed and validated for hybrid striped bass (Picha et al., 2008a). Those for MSTN-1 (for 5′-AGATGACAACAGGGATGTGGTTATG-3′; rev 5′-AAGAGAAAAAGCAGCA CCTTGGT-3′; probe: 5′-CGACGAGCACGCCATCA-3′) were designed from striped bass MSTN-1 (AF290910) while those for MSTN-2 (for 5′-ATGAGGTTGCACAGCATCAAGT-3′; rev 5′-CAGCAGCTGGCGGATCAT-3′; probe 5′-CCTCAGCATCCTGCGGCTCGAC-3′) were designed from the HSB MSTN-2 amplicon (GU230828) generated in this study. No cross-reactivity was observed using MSTN-1 primers and probe with MSTN-2 cDNA template or when using MSTN-2 primers and probe with MSTN-1 cDNA. Quantitative real time PCRs were expressed as copy #/ng RNA. according to previously published methods (Picha et al., 2008b).

2.7. Myostatin western analysis

Whole skeletal muscle samples (4 mg) were homogenized in lysis buffer (0.3 M Tris–HCl, 5% SDS, 50% glycerol, 100 mM dithiothreitol) and allowed to sit at room temperature for 1 h to isolate total proteins. The supernatant was collected and total protein concentrations were quantified using BCA Protein Assay (bicinchoninic acid, Pierce Biotechnology). Muscle proteins (10 μg) were separated on polyacrylamide gels (4–20% gradient, Pierce) in Tris–HEPES–SDS running buffer and subsequently transferred to nitrocellulose membranes (4 μm) for blotting. Blots were blocked with non-fat dry milk and myostatin immnoreactive peptides (MIPs) detected with anti-brook trout myostatin antibody (BtAb6) (Biga et al., 2004). With BtAb6, two primary immunoreactive peptides at ~23 and 50 kDa were detected, consistent with processed mature MSTN (homo-dimer) and unprocessed pro-MSTN (precursor MSTN), respectively. These immunoreactive peptides detected correspond to the predicted sizes of striped bass MSTN based upon the respective cDNA sequences alone (www.bioinformatics.org/sms/prot_mw.html). In addition, these MIPs correspond to previous reports in brook and rainbow trout (Biga et al., 2004; Roberts and Goetz, 2003) and in zebrafish and giant danio (Biga and Meyer, 2009). This antibody was previously validated by pre-absorption with the immunogen used to make the antibody (Biga et al., 2004). A decrease in immunoreactive band intensity was detected in pooled muscle extracts that was positively correlated to the amount of immunogen used in the pre-absorption. Primary antibody binding was detected with an anti-rabbit IgG–horseradish peroxidase conjugate (Pierce) and West Dura Luminescence Reagent detection kit (Pierce). A FluorChem FC2 Chemiluminescence Imager (Alpha Innotech) was utilized to capture luminescence and AlphaEase FC Analysis software was utilized to quantify MIP intensities between treatment groups using arbitrary densitometry units for comparison.

2.8. Statistical analysis

Body weight was analyzed by Repeated Measures analysis followed by Fisher's LSD test for predetermined comparisons between groups. Specific growth rate plotted against mean body weight was assessed with an ANCOVA (covariates: SGR and mean weight) followed by Fisher's LSD test. All other data was analyzed with separate two-way ANOVA's (treatment × time) for days 0–23, 24–114 and 115–148 followed by Fisher's LSD tests. This included statistical analysis both 1) between treatments within each time point and 2) within treatments across all time points. For days 24–114, where sampling occurred at different water temperatures due to temperature transition periods, data were assessed with an ANCOVA (covariates: variable and temperature). Where necessary, data were log-transformed to pass homogeneity of variance tests. Statistical analyses were performed with Statistica 7.0 software (StatSoft, Tulsa, OK). The N value for all growth and body index data (weight, length, SGR) was represented by individually-tagged fish, while feed consumption and conversion (% BW/day, FCR) were represented by the number of tanks (N = 2). Because a tank in the S–S group was lost due to a plumbing malfunction just prior to reefed (day 114) (N = 1), statistics could not be run with feed consumption and conversion data past day 114 for this group. However, statistics were run on all remaining parameters that used individual fish as the N value (N = 36). Statistical significance was set at a level of $P \leq 0.05$.

3. Results

3.1. Effects of temperature and feeding on growth indices

During the initial period at 24 °C (days 0–23), F–F controls and F–S treatment fish were fed to apparent satiation and gained 11 and 13% of their body weights (BW), respectively (Fig. 1A; $P < 0.05$). Meanwhile, S–F and S–S treatment groups were starved during this time and lost 7% of their BW ($P < 0.05$). Control fish continued to be fed during the subsequent period of cold-acclimation at 14 °C (days 24–114) and therefore continued to grow, albeit at a slower rate than at 24 °C ($P < 0.001$). Fed–starved HSB were completely feed restricted at 14 °C and lost 14% of their body weight during this time ($P < 0.05$), while S–S HSB starved at the initial 24 °C phase and during cold-acclimation lost an additional 10% of body weight at 14 °C ($P < 0.05$). From Time 0 to the end of the cold-acclimation period (days 0–114), F–F and S–F groups had undergone net growth while F–S and S–S treatments had undergone net weight loss (% BW gain: F–F = 21.0%; S–F = 3.2%; F–S = −3.2%; S–S = −17.0%).

Interestingly, while the F–S treatment group lost a considerable amount of weight following feed restriction at 14 °C (day 114) and

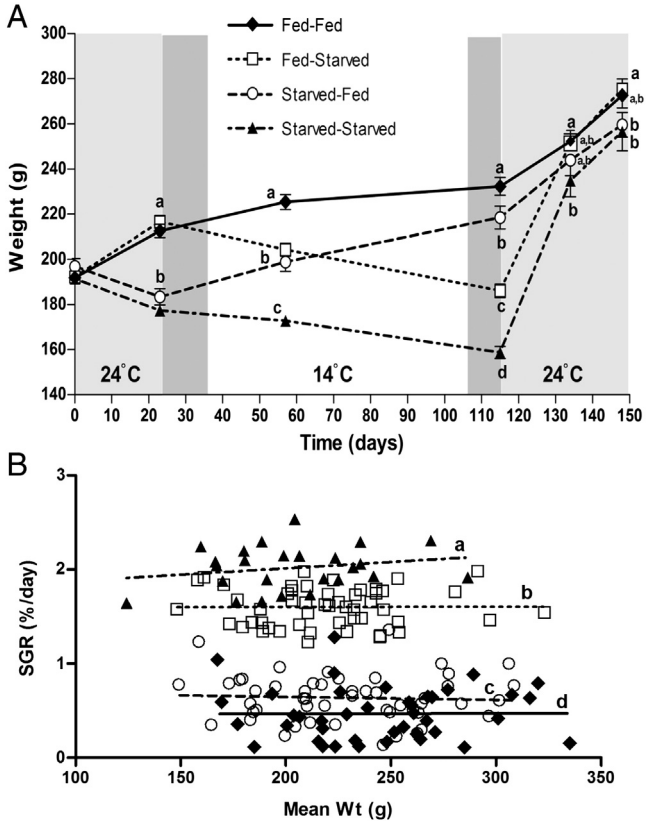

Fig. 1. (A) Mean body weights (g) of hybrid striped bass (HSB) in response to feeding and temperature manipulations. All groups of HSB were exposed to the following temperature fluctuations: rearing at near-optimal, 24 °C water temperatures (days 0–23; light gray area), transition to 14 °C (days 24–34; dark gray area), prolonged cold-acclimation at 14 °C (days 35–106), transition to 24 °C (days 107–114; dark gray area) and 24 °C (days 115–148). Control fish (Fed–Fed) were fed to satiation throughout the entire experiment. Treatment HSB were either fed (F) or starved (S) during the initial period at 24 °C and during prolonged cold-acclimation, the latter including both temperature transition periods. All groups were placed on the control feeding regimen upon return to 24 °C. (B) Specific growth rates (SGRs) as a function of body weight for tagged HSB during the re-feed period at 24 °C (days 115–134). Compensatory growth responses were observed in all treatment groups, as indicated by elevated SGRs relative to controls (Fed–Fed). Different lower case letters represent significant differences between groups within each time point ($P < 0.05$). The absence of letters at any given time point indicates no significant differences between groups.

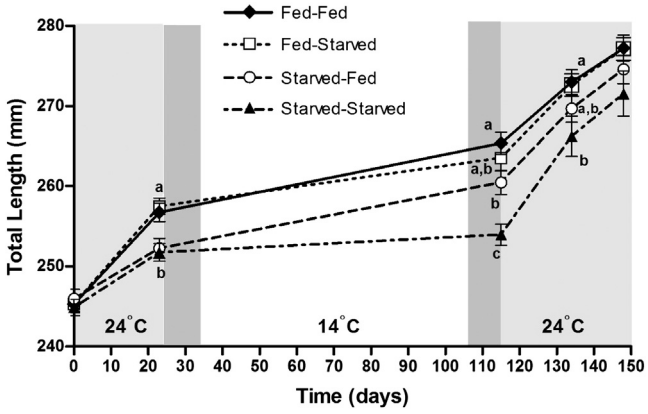

Fig. 2. Total lengths (mm) following initial rearing at 24 °C (day 23), after the prolonged cold-acclimation period at 14 °C and transition to 24 °C (day 114), and following 3 and 5 weeks (days 134 and 148, respectively) at 24 °C. All data are presented ± SEM. Different lower case letters represent significant differences ($P < 0.05$) between groups within each time point.

weighed significantly less than F–F controls (Fig. 1A; $P < 0.001$), total lengths between these groups were nearly identical (Fig. 2). This suggests that although feed restriction during cold-acclimation leads to weight loss, total length gain is minimally affected. However, when feed restriction at 24 °C precedes feed restriction at 14 °C (S–S treatment), length gain during cold-acclimation appears to be compromised, as S–S treatments only gained 0.01% of length during cold-acclimation (days 24–114) while F–S treatments gained 2.3% (Fig. 2; $P < 0.05$).

Regardless of the feeding manipulations that occurred at 24 °C (days 0–23) and/or 14 °C (days 24–114), all groups of HSB were placed on the control (F–F) ration (fed to satiation 5 days/week) after water temperatures had transitioned back to 24 °C (day 115). All groups of fish underwent CG during the initial 19 days after warm-up, as indicated by elevated specific growth rates relative to F–F controls (Fig. 1B; $P < 0.05$). Furthermore, the response was proportional to the prior degree of catabolism, with S–S and F–S groups with the greatest weight loss and lowest condition factors and hepatosomatic index (HSI; data not shown) following cold-acclimation displaying the highest growth rates following refeeding at 24 °C. Specifically, SGRs for S–S and F–S treatment HSB were 4.2 and 3.4 fold higher than controls, respectively, during the initial 19 days of refeeding (Fig. 1B; $P < 0.001$). Complete body weight catch-up (catch-up growth) was achieved in S–F and F–S groups by day 134 (control = 252.3 ± 4.8 g; S–F = 243.8 ± 6.8; F–S = 251.2 ± 4.3), while S–S weights remained statistically lower than controls (S–S = 234.8 ± 7.11 g) (Fig. 1A). Similar lengths (mm) were also observed at day 134 between F–F (controls), S–F and F–S groups, with the S–S group remaining lower than control HSB (Fig. 2). By the end of the experiment (day 148), S–S fish weights and lengths were statistically similar to controls (Fig. 1A; Fig. 2), while F–S weights were greater ($P < 0.05$) than both S–F and S–S treatments. Compensatory growth responses subsided following the initial 19 days of refeed, as SGRs became statistically similar between all groups during the final 2 weeks of the study (days 135–148; data not shown).

3.2. Hyperphagia, feed conversion and the compensatory growth response

The CG response that occurred for 19 days upon refeeding at 24 °C (day 115–134) was accompanied by hyperphagia and/or improved feed conversion in all groups. In S–S fish, which exhibited the most dramatic CG response, feed consumption (% BW/day) was 2.8-fold higher (Fig. 3) and feed conversion 51% improved (Fig. 4A) relative to controls. Overall feed conversion (days 0–148) was improved by 30% relative to controls (Fig. 4B). Although a full statistical analyses could not be done on S–S fish after day 114 due to the loss of one tank replicate, quantitative evidence indicates that individual fish in the remaining replicate showed similar changes in HSI and growth rate during CG, suggesting individual fish ate and converted feed at similar levels. This would be consistent with previous CG studies showing that HSI can be used as a biomarker for feed consumption, and that CG feeding protocols result in similar feeding rates between individual fish within a tank (Picha et al., 2006). Thus, our results on feed consumption and FCR in S–S fish calculated from one replicate tank are likely highly meaningful and reflective of that from numerous individuals or N values. Furthermore, since feed consumption and FCR values between tanks within each treatment were consistent throughout the experiment, we expect that the lost replicate would have behaved similar to its counterpart.

Compensatory growth and catch-up growth achieved in the F–S group after 19 days of refeeding (days 115–134) were accompanied by a 2.2-fold greater feeding rate and a 50% improvement in feed conversion during this time ($P < 0.01$; Figs. 3, 4A). Overall feed conversion (days 0–148) was also more efficient, albeit not quite statistically significant ($P < 0.09$; Fig. 4B).

3.3. Plasma IGF-1

In control (F–F) fish fed throughout the study, plasma IGF-1 remained steady during the initial 24 °C rearing period (days 0–23;

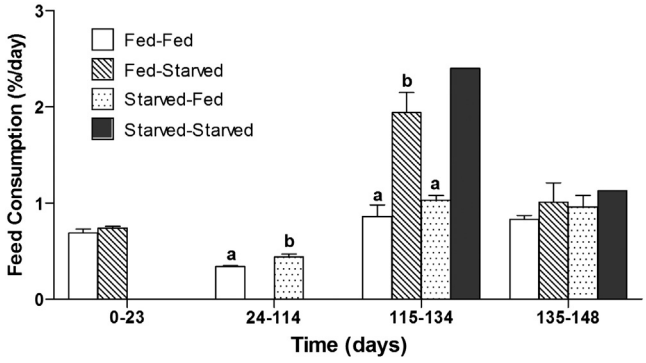

Fig. 3. Feed consumption (% BW/day) for all groups of HSB throughout the experiment. Days 0–23 at 24 °C and days 24–114 at 14 °C represent feeding regimen treatment periods. S–F treatments were starved from days 0 to 23 and then fed from days 24 to 114. F–S treatments were fed from days 0 to 23 and starved from days 24 to 114, while S–S treatments were starved during both of these periods (days 0–114). All groups were placed on the control feeding regimen from days 115 to 148 at 24 °C. Control fish (F–F) were fed throughout the entire experiment. Because feed consumption and FCR data are calculated with tank as the n value, and because an S–S tank was lost at day 115, statistical analysis could not be performed on this group for these parameters. Data for the remaining S–S replicate (n = 1) is shown. All data are presented ± SD. Different lower case letters represent significant differences ($P < 0.05$) between groups within each time point.

Fig. 5A). Plasma IGF-1 values declined as growth rates dropped during exposure to cooler (14 °C) water temperature ($P < 0.05$; day 56). Concentrations remained lower at the end of the 14 °C period (day 106), but rose steadily during the 7 day transition from 14 to 24 °C (days 107–114) to pre cold-bank levels. During the final feeding period at 24 °C (days 115–148), a continued rise in plasma IGF-1 ($P < 0.05$) corresponded with increased growth rates in control fish. While F–S HSB fed initially at 24 °C maintained steady levels of plasma IGF-1 similar to controls, concentrations subsequently dropped by 49%

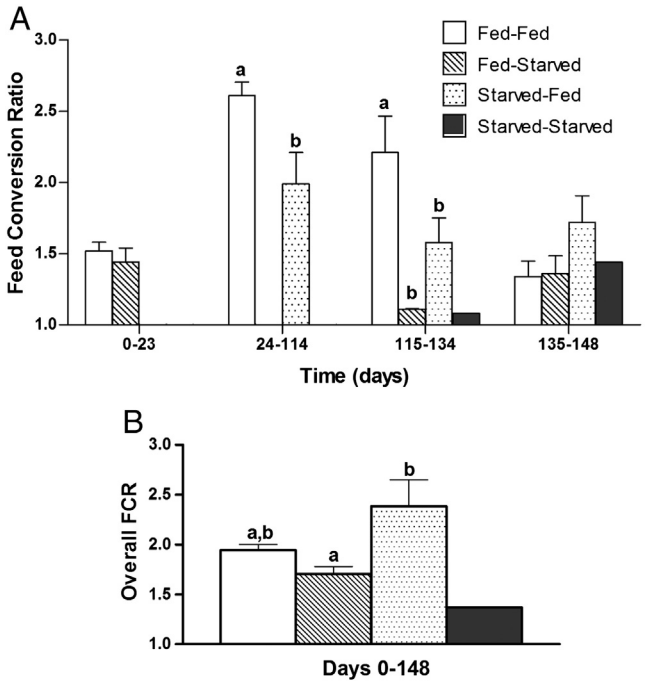

Fig. 4. (A) Temporal and (B) overall feed conversion ratios (FCRs). Days 0–23 at 24 °C and days 24–114 at 14 °C represent feeding regimen treatment periods. All groups were placed on the control feeding regimen from days 115 to 148 at 24 °C. Control fish (F–F) were fed throughout the entire experiment. Because FCR data is calculated with tank as the n value, and because an S–S tank was lost at day 115, statistical analysis could not be performed on this group for these parameters. Data for the remaining S–S replicate (n = 1) is shown. All data are presented ± SD. Different lower case letters represent significant differences ($P < 0.05$) between groups within each time point.

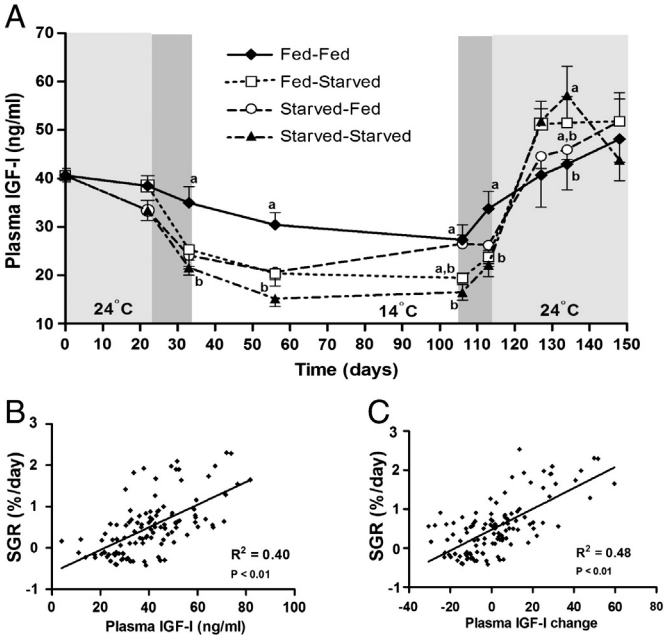

Fig. 5. (A) Plasma IGF-1 (ng/ml) dynamics in hybrid striped bass (HSB) undergoing various feeding and temperature manipulations. Different lower case letters represent significant differences between groups within each time point ($P < 0.05$). The absence of letters at any given time point indicates no significant differences between groups. Correlations between specific growth rates (SGRs) versus (B) plasma IGF-1 (ng/ml) and (C) changes in plasma IGF-1 were calculated from sampled HSB in all control and treatment groups throughout the experiment. Specifically, SGRs from days 1–23, 24–114, 115–134 and 135–148 were plotted against (B) plasma IGF-1 values at the end of that growth interval or against (C) changes in plasma IGF-1 values during that growth interval.

($P < 0.001$) during fasting at 14 °C (days 24–106). Upon refeeding at 24 °C, which corresponded with a considerable CG response (days 115–134), plasma IGF-1 increased by 117% ($P < 0.001$) and remained elevated relative to control even as the CG response subsided (days 135–148). HSB that were initially starved at 24 °C (days 0–23; S–F, S–S treatments) experienced weight loss and a significant drop ($P < 0.01$) in plasma IGF-1. In S–S fish that continued to be starved at 14 °C (days 24–106), plasma IGF-1 dropped by an additional 51% to levels significantly ($P < 0.01$) below controls. Upon refeeding at 24 °C (days 115–134), plasma IGF-1 increased by 98% to levels 1.3× above controls ($P < 0.05$). This significant increase and overcompensation in IGF-1 also corresponded to the most dramatic CG response. Circulating IGF-1 subsequently dropped to control values after the CG response had subsided (day 148).

Taken together, plasma IGF-1 values for all groups of HSB generally followed trends for growth rate, which fluctuated due to both feeding and temperature manipulations. This is reflected in a significant correlation between plasma IGF-1 and SGR ($R^2 = 0.40$; $P < 0.001$; Fig. 5B), which was calculated from time intervals encompassing the entire experiment. More so, an even stronger correlation ($R^2 = 0.48$; $P < 0.001$; Fig. 5C) was derived when changes in plasma IGF-1 were plotted against SGR.

3.4. Muscle IGF-1 mRNA

After initial rearing at 24 °C (days 0–23), HSB that had been fasted (S–F, S–S) had significantly ($P < 0.01$) lower levels of muscle IGF-1 mRNA than fed fish (F–F, F–S groups; Table 1). Levels generally stabilized within groups following prolonged 14 °C cold-acclimation (day 106) as indicated by statistically similar IGF-1 levels. Following the temperature transition from 14 to 24 °C muscle IGF-1 mRNA was similar in all groups except S–S HSB, whose levels were 4.8× lower than controls ($P < 0.001$). In F–S HSB, a considerable CG response was accompanied by a 217% increase in muscle IGF-1 (days 115–127; $P < 0.001$) to levels

Table 1
Muscle IGF-1 mRNA expression (copy #/ng total RNA) in response to feeding and temperature manipulations. All groups of HSB were exposed to the following temperature fluctuations: rearing at near-optimal, 24 °C water temperatures (days 0–23), transition to 14 °C (days 24–34), prolonged cold-banking at 14 °C (days 35–106), transition to 24 °C (days 107–114) and 24 °C (days 115–148). Groups of hybrid striped bass underwent various feeding manipulations prior to day 114, at which time all treatments were fed to apparent satiation until the end of the experiment. S–F treatments were starved from days 0 to 23 and then fed from days 24 to 114. F–S treatments were fed from days 0 to 23 and starved from days 24 to 114, while S–S treatments were starved during both of these periods (days 0–114). Control fish (F–F) were fed throughout the entire experiment.

	Time 0	d 23	d 34	d 57	d 106	d 114	d 127	d 134	d 148
Muscle IGF-1 mRNA									
F–F (control)	56.5 ± 8.2	88.7^{a} ± 10.4	56.1^{a} ± 10.8	32.3^{a} ± 7.1	33.4 ± 14.1	55.1^{a} ± 9.4	42.8^{c} ± 4.4	39.2^{c} ± 8.9	30.0 ± 7.2
S–F	56.5 ± 8.2	37.5^{b} ± 4.7	52.9^{a} ± 11.3	13.1^{a,b} ± 3.2	31.4 ± 5.7	51.7^{a} ± 6.7	83.2^{b} ± 15.5	72.8^{b} ± 19.4	36.2 ± 6.7
F–S	56.5 ± 8.2	88.7^{a} ± 10.4	24.0^{b} ± 2.6	11.0^{a,b} ± 3.0	22.2 ± 5.2	35.1^{a} ± 9.2	111.7^{a,b} ± 24.6	47.7^{b,c} ± 4.8	26.1 ± 6.4
S–S	56.5 ± 8.2	37.5^{b} ± 4.7	10.7^{b} ± 1.6	4.6^{b} ± 1.2	11.6 ± 3.3	11.4^{b} ± 2.6	117.7^{a} ± 16.4	137.7^{a} ± 17.5	59.0 ± 10.5

All data are presented ± SEM.
Different lower case letters represent significant differences ($P < 0.05$) between groups within each time point.

2.6× above controls ($P < 0.001$). Similar to F–S HSB, the S–S treatment group underwent a dramatic CG response upon realimentation and experienced an overcompensation in muscle IGF-1 mRNA. In fact, levels increased by 930% 12 days into the response when animals were refed and warmed up to 24 °C (day 127; $P < 0.001$). Overall, muscle IGF-1 mRNA was significantly correlated to SGR ($R^2 = 0.23$; $P < 0.001$), with samples taken from all groups of fish throughout the experiment.

3.5. Muscle GHR-1, -2 mRNA

Following the initial 23 day rearing period at 24 °C, starved fish (S–F, S–S) had significantly lower muscle GHR-1 mRNA levels than fed HSB (F–F, F–S) ($P < 0.05$; Table 2). These differences became exaggerated during cold-banking (days 24–106) and resulted in GHR-1 levels that were nearly 3× higher in fed (F–F) relative to continually starved (S–S) fish at the end of this period. Interestingly, decreases in fed controls during warm-up (14–24 °C; days 107–114) rendered values statistically similar to starved HSB. These levels remained statistically similar for the remainder of the experiment (24 °C; days 114–148), despite refeeding and a significant CG response in the starved HSB.

Contrary to that for GHR-1, GHR-2 values were significantly higher in starved (S–F, S–S) relative to fed (F–F, F–S) HSB following the initial 23 day rearing period at 24 °C ($P < 0.001$; Table 2). Additional increases during cold-banking (days 24–106) in continually starved (S–S) fish resulted in GHR-2 values which were nearly 9× higher than controls. Values in F–S fish which had been starved during cold-banking only were also 5× higher than controls. Despite a significant decrease during warm-up (14–24 °C; days 107–114) in the S–S group, GHR-2 was still over 6× higher than fed controls. A considerable CG response in S–S fish upon refeeding at more optimal water temperatures (24 °C; days 114–148) was accompanied by a precipitous decline in GHR-2 mRNA to control levels (day 134), at which point values remained statistically similar for all groups through the remainder of the experiment.

3.6. Gene cloning, muscle MSTN-1 and MSTN-2 mRNA

Sequencing results indicate that the HSB (*M. chrysops* × *M. saxatilis*) 471 base pair amplicon generated with degenerate MSTN-2 primers shares 92 and 93% nucleotide identity with shi drum (*U. cirrosa*) and gilthead seabream (*S. aurata*) MSTN-2 and only 65–67% nucleotide identity with shi drum, gilthead seabream and striped bass (*M. saxatilis*) MSTN-1. This indicates that the generated amplicon represents a partial sequence of MSTN-2 in HSB (GU230828).

MSTN-1 mRNA was significantly higher in fed (F–F, F–S) relative to starved (S–F, S–S) HSB following the initial 24 °C rearing period ($P < 0.05$; Table 3). Regardless of feeding history at 24 °C, groups that were fed during cold-acclimation (F–F, S–F) experienced increases in MSTN-1 expression while levels declined in HSB starved during this time (F–S, S–S), resulting in significantly higher levels in fed groups at the end of a cold-bank period (day 106). During the final rearing period at 24 °C, MSTN-1 levels were similar between all groups by day 127 and throughout the remainder of the experiment.

Trends for muscle MSTN-2 gene expression initially followed those of MSTN-1. Specifically, MSTN-2 mRNA was elevated in fed (F–F, F–S) relative to starved (S–S, S–F) HSB following initial rearing at 24 °C ($P < 0.05$; Table 3) and through 33 days of cold-acclimation (day 57). However, levels converged and became variable by the end of cold-acclimation, generally resulting in non-significant differences between groups for the remainder of the experiment.

3.7. Muscle MSTN protein

Skeletal muscle levels of precursor MSTN (~50 kDa) declined significantly ($P < 0.01$; Fig. 6A) in S–S fish fasted during the initial 24 °C

Table 2
Muscle GHR1 and GHR2 mRNA expression (copy #/ng total RNA) in response to feeding and temperature manipulations. All groups of HSB were exposed to the following temperature fluctuations: rearing at near-optimal, 24 °C water temperatures (days 0–23), transition to 14 °C (days 24–34), prolonged cold-banking at 14 °C (days 35–106), transition to 24 °C (days 107–114) and 24 °C (days 115–148). Groups of hybrid striped bass underwent various feeding manipulations prior to day 114, at which time all treatments were fed to apparent satiation until the end of the experiment. S–F treatments were starved from days 0 to 23 and then fed from days 24 to 114. F–S treatments were fed from days 0 to 23 and starved from days 24 to 114, while S–S treatments were starved during both of these periods (days 0–114). Control fish (F–F) were fed throughout the entire experiment.

Treatment	Muscle GHR1 mRNA				Muscle GHR2 mRNA			
	F–F (control)	S–F	F–S	S–S	F–F (control)	S–F	F–S	S–S
Time 0	151,163 ± 9495	151,163 ± 9495	151,163 ± 9495	151,163 ± 9495	2270 ± 212	2270 ± 212	2270 ± 212	2270 ± 212
d 23	157,953^{a} ± 10,755	121,415^{b} ± 11,760	157,953^{a} ± 10,755	121,415^{b} ± 11,760	7908^{a} ± 1658	16,450^{b} ± 2523	7908^{a} ± 1658	16,450^{b} ± 2523
d 34	179,376^{a,b} ± 20,110	248,567^{b} ± 11,072	144,114^{a,c} ± 13,385	92,132^{c} ± 15,768	2435^{a} ± 457	2585^{a} ± 482	17,053^{b} ± 1416	28,064^{c} ± 4225
d 57	300,620^{a} ± 62,483	183,945^{b} ± 35,469	103,159^{c} ± 26,751	73,120^{c} ± 11,996	4710^{a} ± 1752	2794^{a} ± 688	12,515^{a,b} ± 3744	16,958^{b} ± 3006
d 106	284,286^{a} ± 79,520	171,565^{b,c} ± 34,732	185,927^{b} ± 36,777	98,523^{c} ± 18,344	7704^{a} ± 2508	7921^{a} ± 2692	38,843^{b} ± 9046	68,134^{c} ± 9295
d 114	118,268 ± 10,080	121,713 ± 26,692	85,078 ± 6284	64,475 ± 15183	4951^{a} ± 1354	4832^{a} ± 827	22,740^{b} ± 3142	30,678^{b} ± 6594
d 127	63,668 ± 13,402	87,290 ± 16,453	69,262 ± 19,003	98,497 ± 14,382	4307 ± 751	5369 ± 1498	5216 ± 2219	2847 ± 514
d 134	63,876 ± 21,240	78,088 ± 10,769	43,024 ± 12,707	49,919 ± 12,125	3416^{a,b} ± 933	5032^{b} ± 972	1271^{a} ± 435	1205^{a} ± 239
d 148	44,656^{a,b} ± 14,139	38,652^{a,b} ± 8172	33,292^{a} ± 12,181	76,413^{b} ± 10,615	3475 ± 1052	2973 ± 667	1346 ± 411	2485 ± 492

All data are presented ± SEM.
Different lower case letters represent significant differences ($P < 0.05$) between groups within each time point.

Table 3
Muscle MSTN-1 and MSTN-2 mRNA (copy #/ng total RNA) dynamics in response to feeding and temperature manipulations. All groups of HSB were exposed to the following temperature fluctuations: rearing at near-optimal, 24 °C water temperatures (days 0–23), transition to 14 °C (days 24–34), prolonged cold-banking at 14 °C (days 35–106), transition to 24 °C (days 107–114) and 24 °C (days 115–148). Groups of hybrid striped bass underwent various feeding manipulations prior to day 114, at which time all treatments were fed to apparent satiation until the end of the experiment. S–F treatments were starved from days 0 to 23 and then fed from days 24 to 114. F–S treatments were fed from days 0 to 23 and starved from days 24 to 114, while S–S treatments were starved during both of these periods (days 0–114). Control fish (F–F) were fed throughout the entire experiment.

Treatment	Muscle MSTN-1 mRNA				Muscle MSTN-2 mRNA			
	F–F (control)	S–F	F–S	S–S	F–F (control)	S–F	F–S	S–S
Time 0	11,168 ± 1349	11,168 ± 1349	11,168 ± 1349	11,168 ± 1349	150.5 ± 11.2	150.5 ± 11.2	150.5 ± 11.2	150.5 ± 11.2
d 23	15,419^{a} ± 2232	9579^{b} ± 1173	15,419^{a} ± 2232	9579^{b} ± 1173	164.8^{a} ± 16.9	107.2^{b} ± 13.9	164.8^{a} ± 16.9	107.2^{b} ± 13.9
d 34	12,737 ± 2799	14,207 ± 2182	10,725 ± 1538	7577 ± 2560	91.6 ± 9.8	92.9 ± 14.1	111.7 ± 12.1	70.8 ± 18.6
d 57	14,686^{a} ± 3484	11,318^{a,b} ± 2132	5334^{b} ± 1764	4082^{b} ± 1053	215.5^{a} ± 44.2	129.0^{b} ± 20.7	112.9^{b,c} ± 33.2	52.8^{c} ± 10.7
d 106	21,022^{a} ± 7293	19,990^{a,b} ± 5271	11,697^{b,c} ± 1860	7355^{c} ± 1122	191.3 ± 61.8	142.4 ± 27.5	196.0 ± 18.6	137.5 ± 9.0
d 114	16,254^{a} ± 2729	17,181^{a} ± 2316	6284^{b} ± 1003	10,003^{b} ± 3146	178.6 ± 16.1	177.6 ± 38.9	126.4 ± 24.4	124.2 ± 19.7
d 127	6614 ± 1491	11,688 ± 2408	10,443 ± 3070	11,010 ± 1058	98.8 ± 20.2	113.0 ± 20.4	155.8 ± 23.8	149.9 ± 28.8
d 134	4980 ± 2044	6958 ± 1389	1999 ± 413	4033 ± 891	65.2 ± 16.5	93.7 ± 13.5	43.1 ± 9.8	92.4 ± 13.1
d 148	3498 ± 1219	3932 ± 1145	3935 ± 1642	7578 ± 2086	61.9 ± 15.4	65.5 ± 14.0	60.3 ± 16.9	95.4 ± 24.0

All data are presented ± SEM.
Different lower case letters represent significant differences ($P < 0.05$) between groups within each time point.

rearing period (days 0–23) with levels 35% less than fed controls ($P < 0.05$). Levels of the 50 kDa precursor MSTN in S–S fish subsequently rebounded to control values despite continued fasting through the cold-acclimation period (days 24–114) and remained statistically similar to control values during the CG response that occurs with refeeding at 24 °C (days 115–127).

Similar to trends described above, mature MSTN (~23 kDa) levels significantly decreased ($P < 0.01$; Fig. 6B) in S–S fish fasted during the initial 24 °C rearing period and were 49% lower than fed controls at this time ($P < 0.05$). Levels in S–S fish returned to control values upon continued fasting and cold-acclimation and remained statistically similar during subsequent refeeding at 24 °C.

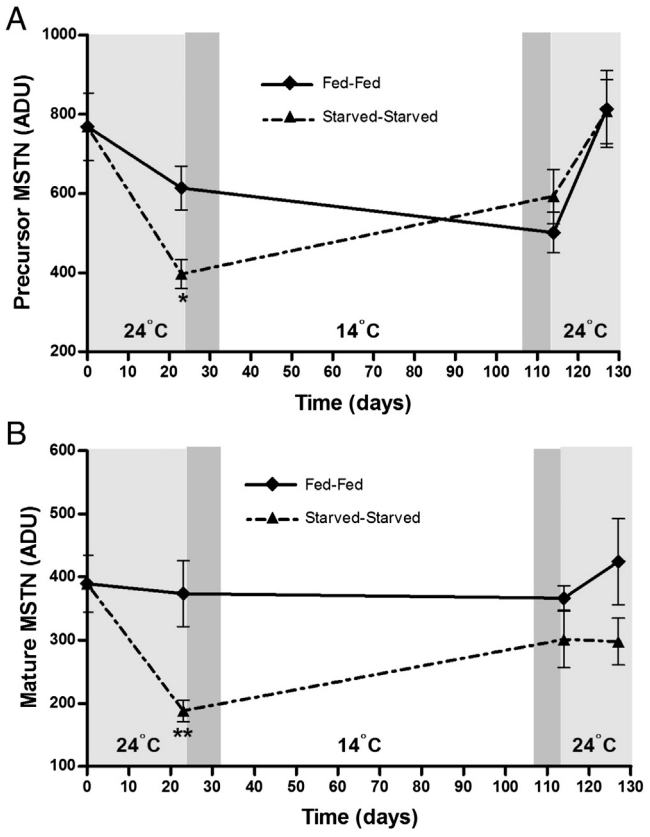

Fig. 6. (A) Precursor (~50 kDa) and (B) mature (~23 kDa) MSTN proteins (ADU) in skeletal muscle of HSB in response to feeding and temperature manipulations. Control (F–F) fish were fed to apparent satiation throughout the entire period. All data are presented ± SEM. Asterisks represent significant differences ($P < 0.05$) between groups within each time point.

4. Discussion

This study demonstrates that both compensatory and catch-up growth can be achieved in HSB when feed restriction prior to and/or during cold-acclimation is followed by a return to satiation feeding and more optimal water temperatures. The response is characterized by significant elevations in growth rate and feed consumption, along with considerable improvements in temporal and overall feed conversion. The most dramatic CG was preceded by extreme catabolism, characterized by weight loss. Furthermore, it appears that HSB are better able to compensate for significant degrees of weight loss when skeletal length is not significantly compromised during the treatment period. The growth response for all treatment groups was accompanied by parallel changes in plasma IGF-1 and muscle IGF-1 mRNA, with significant overcompensations of both occurring in HSB exhibiting the most dramatic CG response. Interestingly, catabolism resulted in depressed levels of skeletal muscle MSTN-1, -2 mRNA along with MSTN precursor and mature peptides. Opposing trends were observed between muscle GHR-1 and -2 mRNA during catabolism, with depressed levels of GHR-1 and elevated levels of GHR-2. To our knowledge, this is the first study to achieve compensatory and catch-up growth through feed restriction and temperature manipulation in any fish species, and has tremendous potential to improve production efficiency of temperate aquacultured teleosts. Our studies also suggest that an overcompensation of endocrine and paracrine IGF-1 may be a driver for achieving catch-up growth in fishes, and perhaps other vertebrates.

Because of seasonal fluctuations in both water temperature and feed availability, fish have adapted the ability to withstand prolonged periods of low water temperatures and starvation. The ability to rapidly recover from a period of growth depression may be a trait that is consistently selected for in the natural environment to reduce potential size-dependent mortality (Holtby et al., 1990; Nicieza and Metcalfe, 1997). The period of accelerated/compensatory growth following an episode of growth cessation has been demonstrated in a number of animal species, especially fishes. Compensatory and/or catch-up growth following feed restriction alone has been achieved in hybrid sunfish, Atlantic halibut, European sea bass and HSB (Foss et al., 2009; Hayward et al., 1997; Skalski et al., 2005; Terova et al., 2006), as well as following exposure to low temperature in Atlantic salmon, Arctic charr and brown flounder (Huang et al., 2008; Maclean and Metcalfe, 2001; Mortensen and Damsgard, 1993). However, growth responses following exposure to both of these variables have been much less studied, especially under captive conditions where all experimental variables can be controlled.

Since both parental lines (white bass *M. chrysops*; striped bass *M. saxatilis*) experience periods of overwintering and low prey availability in their natural environments (Hurst et al., 2000; Kohler, 1997), it is not surprising that HSB can undergo CG responses following similar

conditions. Indeed, fish that were starved prior to and during cold-acclimation or just over the cold-acclimation period showed dramatic CG responses upon refeeding at more optimal temperatures relative to size-matched controls. Comparing growth rates of similar-sized fish is critical to assessing CG responses, since SGR has an allometric relationship to body mass whereby smaller fish tend to grow at faster rates than larger individuals (Jobling, 2010). Due to this allometric relationship it is also possible to achieve catch-up growth without undergoing CG. In this study, compensatory and catch-up growth occurred in all treatment groups during the realimentation period of refeeding and warmup. In European sea bass, a closely-related species to HSB, elevated growth rates but not catch-up growth was reported following complete feed restriction for 35 days at suboptimal temperatures (Pastoureaud, 1991). Interestingly, delayed catch-up growth was observed in captive-reared coho salmon following exposure to ambient water temperatures and feed manipulation (Triebenbach et al., 2009). Specifically, 10 weeks of complete feed restriction at sub-optimal winter temperatures (Nov 19–Jan 28) did not result in elevated growth rates upon 3.5 months of subsequent satiation feeding (Jan 29–May 12). However, catch-up growth was achieved upon transfer to seawater which was 4 °C warmer, corroborating results of delayed catch-up growth also observed in Atlantic salmon (Dickhoff et al., 1989). Since SGRs during the catch-up period were not compared to those of similarly-sized fish in both of these salmonid studies, it is possible that catch-up growth was achieved simply because smaller fish generally grow faster than larger ones, and not because of CG per se. Regardless, when taken together with our results in HSB it appears that temperate-zone fish tend to have plastic and resilient rates of growth, particularly with regard to environmental manipulations involving feeding and temperature. This not only provides rearing protocol flexibility for aquaculture producers but can also improve production efficiency through gains in overall feed conversion.

While increasing the water temperature to a more favorable level (14 to 24 °C) and the associated Q10 effects (Angilletta et al., 2004) are likely responsible for elevated growth rates in all groups of HSB following cold-acclimation, including controls, the mechanisms responsible for CG responses are less clear. Hyperphagia is a commonly-observed behavior during CG and is typically accompanied by significant improvements in temporal feed conversion, both of which tend to subside during cessation of the response (Ali et al., 2003). Indeed, both of these behavioral and metabolic modifications were observed during accelerated growth in this study. Specifically, S–S and F–S HSB feed consumption rates during CG were 2.8 and 2.2 times higher, respectively, than controls with feed conversion ratios improving by 51 and 50%. In addition, overall feed conversion was improved by 30% in the S–S group.

It is interesting to note that the most successful body weight catch-up occurred in F–S HSB whose skeletal gain was not compromised during the treatment period, despite experiencing considerable reductions in body weight, energy reserves and condition factor. Lowered metabolic maintenance costs associated with reduced temperature may allow more energy reserves to be utilized for skeletal growth (Van Ham et al., 2003). Indeed, compensating for lost skeletal growth may be a limiting factor in achieving full body weight catch-up, as was also observed in gilthead seabream (Bavcevic et al., 2010).

The endocrine control of growth during feed manipulation has been relatively well-studied in fishes, although the characterization of compensatory growth remains far from resolved. In this study we show that levels of circulating IGF-1 and, less robustly, muscle IGF-1 mRNA have significant, positive correlations with growth rate despite dramatic fluctuations in feeding (fasting, normal, hyperphagia), growth rate (catabolic, normal anabolic, supra-anabolic) and water temperature (14, 24 °C). More so, changes in plasma IGF-1 (Δplasma IGF-1/Δtime) resulted in an even stronger correlation with SGR, raising the possibility that CG may be facilitated in part by the relative increase in plasma IGF-1 rather than by absolute concentrations alone. That is, target tissues may be more sensitive to plasma IGF-1 following periods of starvation through enhanced receptor binding or sensitivity. Therefore, lesser absolute amounts but greater changes in plasma IGF-1 may lead to disproportionate hormonal responses and ultimately enhanced hyperplasia and perhaps hypertrophy (Montserrat et al., 2007; Picha et al., 2006).

While this scenario is plausible, we also show that the greatest CG response (S–S treatment) was accompanied by a significant overcompensation of both circulating IGF-1 protein and locally-produced (muscle) IGF-1 mRNA, with respective levels 1.3 and 3.5 times higher than control values after 19 days of CG. Likewise, in the F–S treatment muscle IGF-1 was 2.6 times higher than controls 12 days into CG. In several fish studies, depressed plasma IGF-1 during starvation leads to a full recovery during refeeding (Fox et al., 2010; Gabillard et al., 2006; Montserrat et al., 2007; Norbeck et al., 2007), or in some cases a mild overcompensation (Imsland et al., 2008; Shimizu et al., 2009). Similarly muscle IGF-1 mRNA may show either an increase, complete restoration or overcompensation in refed fish relative to controls (Bower et al., 2008; Fox et al., 2010; Hagen et al., 2009; Peterson and Waldbieser, 2009; Terova et al., 2007). In each of these cases, however, SGR and changes in endocrine variables were not assessed relative to fish mass (e.g. in similar-sized fish) and therefore CG was not truly assessed, despite the possibility that it may have occurred in some of the investigations. In the present studies we show that SGR, circulating IGF-1 and muscle IGF-1 mRNA expression are expressed at levels substantially higher during CG relative to similar-sized cohorts never subjected to growth-stunting conditions. The results suggest that rises in locally and systemic sources of IGF-1 may contribute to CG with overcompensation in these variables supporting full growth compensation. Our findings also support the notion that plasma IGF-1 and perhaps muscle IGF-1 mRNA may serve as indicators of growth whether rates are stunted, normal or accelerated (compensatory).

Interestingly, opposing trends were observed between muscle mRNA expression of GHR-1 and -2 during catabolism, resulting in depressed levels of GHR-1 and elevated levels of GHR-2. This indicates that the duplicated GHRs may have evolved alternate functions (Fukada et al., 2005; Fukamachi et al., 2005; Jiao et al., 2006; Li et al., 2007; Pierce et al., 2007; Reindl et al., 2009). Elevations in plasma GH (Picha et al., 2009) and depressed muscle GHR-1 and IGF-1 mRNA during catabolism in this study suggest that GH may work through GHR-1 to stimulate IGF-1 transcription. Conversely, elevated plasma GH and muscle GHR-2 mRNA during catabolism in HSB and other teleosts (Gabillard et al., 2006; Picha et al., 2008b; Pierce et al., 2007; Saera-Vila et al., 2005) suggests that GH may work through the GHR-2 isoform to facilitate mobilization of muscle lipids (Inui et al., 1985) and/or to limit proteolysis (i.e. protein sparing; Gamrin et al., 2000) as adaptive responses to catabolism, or may even inhibit MSTN expression to facilitate subsequent CG responses (see below).

The role of MSTN as a negative regulator of skeletal muscle growth in mammals has been well defined, as evident by the “double muscling” phenotype in MSTN-null mice and cattle (Rodgers and Garikipati, 2008). While MSTN may function similarly in fishes (Acosta et al., 2005; Xu et al., 2003), its role has not been clearly defined. We show that sustained fasting at 24 °C and then at 14 °C results in depressed levels of skeletal muscle MSTN-1 mRNA relative to fed controls, followed by a convergence with controls during the subsequent CG response. This contrasts with the increase in skeletal muscle MSTN-1 mRNA seen during starvation and recovery with refeeding in European sea bass (Terova et al., 2006). We also show depressed levels of MSTN-2 mRNA during starvation at 24 °C and partially through starvation at 14 °C. Skeletal muscle levels of both precursor and mature MSTN proteins were also depressed during initial starvation at 24 °C, corroborating trends with mRNA. Depressed levels of both MSTN mRNA and protein during starvation in HSB seem counter to its proposed role in inhibiting skeletal muscle growth and differentiation. That is, it seems logical that MSTN would increase and skeletal muscle proliferation decrease during catabolism. However, these depressed MSTN levels could be facilitating subsequent CG responses by allowing for skeletal muscle hyperplasia during

catabolism. If so, the skeletal muscle cell framework would be in place when feed becomes available and hyperphagia ensues, resulting in rapid hypertrophy and CG without cell proliferation being a limiting factor. Interestingly, our endocrine measures support this possibility. Growth hormone has been shown to suppress MSTN expression in GH-deficient adults and in muscle cell cultures (Liu et al., 2003), while a decrease in skeletal muscle MSTN mRNA and protein levels was observed with GH-treated giant danio (Biga and Meyer, 2009). Indeed, elevated plasma GH (Picha et al., 2009) and GHR-2 mRNA during catabolism could be a direct mechanism by which MSTN is depressed and subsequent CG responses achieved. This is the first study to document trends in MSTN mRNA and protein during CG in any species, and represents an intriguing mechanism by which animals may recover from periods of stunted growth.

In summary, we found that HSB undergo both compensatory and catch-up growth when various combinations of feed restriction and low water temperatures are followed by favorable rearing conditions. The response is accompanied by elevated growth rates, hyperphagia and improvements in overall feed conversion by as much as 30%, along with overcompensations of both circulating and locally-produced IGF-1. A discordance in regulation of skeletal muscle GHR isoforms along with a decline of MSTN during growth stunted states may help facilitate appropriate energy mobilization while also supporting enhanced muscle growth when conditions improve and CG ensues. Since the temperature protocol used here reflects the periods prior to, during and following the natural over-wintering period for HSB and other temperate-zone teleosts, these results also have direct aquaculture applications. Specifically, the capacity to reduce feed costs through improvement in feed efficiency with no loss in biomass has profound implications in reducing production costs of HSB culture in tanks and possibly ponds. Based on the broad array of energetic fluctuations that occurred throughout the experiment, this species and rearing protocol could also provide a valuable model system to study the metabolic and endocrine mechanisms underpinning stunted, normal and accelerated (compensatory) growth.

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