ABSTRACT


Compensatory growth (CG) is a period of growth acceleration that exceeds normal rates after animals are alleviated of certain growth-stunting conditions. While the endocrine control of growth in fishes and other vertebrates is regulated primarily through the growth hormone (GH) / insulin-like growth factor (IGF) axis, the hormonal dynamics regulating accelerated (compensatory) phases of growth are poorly understood. We assessed whether CG responses could be induced in hybrid striped bass (HSB; *Morone chrysops* X *Morone saxatilis*) through feeding and/or temperature manipulation, and then examined whether components of the GH/IGF endocrine growth axis could be contributing to the response.

We found that CG responses can be elicited in HSB following periods of 1) partial feed restriction, 2) complete feed restriction and 3) a combination of low water temperatures and complete feed restriction, with full catch-up growth being achieved in the latter. The most dramatic periods of CG were preceded by a catabolic state marked by body weight loss, depressed hepatosomatic index values and an endocrine state of GH resistance. Upon alleviation of the growth-stunting condition, HSB displayed significant elevations in growth rate that exceeded controls by as much as 4 times. This CG response was accompanied by increases in feed intake (hyperphagia), improved feed conversion and a reversal of GH resistance.
The onset of CG in feed manipulation studies was marked by significant elevations in total hepatic IGF-I mRNA and plasma IGF-I, indicating that elevated synthesis and secretion of the growth factor may mediate rapid growth responses. Strong correlations between systemic IGF-I and specific growth rate (SGR) suggest that circulating levels of IGF-I may serve as a biomarker of growth in HSB and perhaps other fishes. The 40kDa IGF binding protein (IGFBP) may also contribute to CG, since plasma levels increased in concordance with IGF-I and growth. Although little is known about the mitogenic effects of IGF-II in adult vertebrates, changes in hepatic IGF-II mRNA paralleled changes in body weight prior to and during CG, strengthening the hypothesis that it is an important regulator of variable growth rates in fishes. Associated with declines in growth and IGF-I, we found that feed deprivation caused reductions in Type I GH receptor (GHR1) gene expression which were subsequently restored during realimentation. Hence, the GHR may be a critical mediator of the changes in IGF-I and growth rates observed prior to and during CG.

Aside from endocrine (circulating) contributions, insulin-like growth factors may also act locally to regulate tissue growth. A ten-fold increase in skeletal muscle IGF-I mRNA from previously depressed levels was seen during realimentation, suggesting the growth factor may act in a paracrine/autocrine fashion to stimulate cell proliferation and facilitate rapid growth that is characteristic of CG.

Taken together, these data show that HSB undergo considerable phases of accelerated growth when preceded by sufficient catabolism, and that an up-regulation of endocrine
IGF-I, -II, 40kDa IGFBP) and paracrine/autocrine (IGF-I) components of the GH/IGF axis likely facilitate the response. Compensatory growth protocols in HSB provide a good model system to elucidate the underlying metabolic and endocrine mechanisms of poor, normal and accelerated (compensatory) growth in fishes. Our results with cold-banking and feed deprivation show that CG protocols can improve overall feed conversion by as much as 30% with no loss in biomass, providing a practical method for reducing production costs of HSB, an important aquacultured species.
Characterization of Compensatory Growth in Hybrid Striped Bass (*Morone chrysops* X *Morone saxatilis*): Hormones and Mechanisms

by

Matthew Eugene Picha

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

Physiology Program

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2007

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Dr. Craig Sullivan            Dr. Russell Borski
Chair of Advisory Committee
DEDICATION

To old and new friends and especially to my family, who have offered continued support, perspective and humor throughout this endeavor.
BIOGRAPHY

Matthew Eugene Picha was born in 1977 in Waimea, Kauaii. After moving to Burlington, Washington at an early age, he developed an unknowing interest for biology by wading down the Samish River for cutthroat trout and by salmon fishing in Puget Sound with his Grandpa. After graduating from Burlington-Edison High School in 1995, he attended college at Washington State University where he graduated in 2000 with an Animal Sciences degree, knowing towards the end that his specific interests lie in fish biology. He confirmed this suspicion through a husbandry internship at the Monterey Bay Aquarium and then took the next year off to work, snowboard and apply to graduate programs which could facilitate this now career ambition. He found a good fit at North Carolina State University with Dr. Russell Borski, who for the past six years has helped turn this life-long interest into a career.
ACKNOWLEDGEMENTS

I would first and foremost like to acknowledge my advisor and friend Dr. Russell Borski for taking a chance on an unknown student with virtually no formal lab experience. I would also like to thank him for his continual encouragement and guidance with research and career development, along with his generous support for professional meetings. My committee, whose wide range of expertise in disease, nutrition, metabolism and reproduction no doubt made me a better scientist, also deserves special recognition. My sincere thanks go especially to Craig Sullivan, who gave me unlimited access to any and all of his research equipment and facilities.

The work contained herein would not have been possible without assistance from a number of current and former undergraduate and graduate students in the Borski lab. Their contributions are very appreciated and have not gone unnoticed. Fish technicians at NCSU, PAFL and the Tidewater Research Station also need special recognition for their diligence in fish husbandry and accommodations for experiments.

Finally, I would like to thank my friends and family for their unconditional support and for their ability to make me laugh and maintain perspective on a regular basis.

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF TABLES</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
</tbody>
</table>

## CHAPTER I. Discordant Regulation of Hepatic IGF-I mRNA and Circulating IGF-I During Compensatory Growth in a Teleost, the Hybrid Striped Bass (*Morone chrysops X Morone saxatilis*)

- Abstract: 2
- Introduction: 4
- Materials and Methods: 6
- Results: 12
- Discussion: 18
- Acknowledgements: 26
- References: 27

## CHAPTER II. Endocrine and Paracrine Dynamics of Insulin-like Growth Factors During Compensatory Growth in a Teleost Fish

- Abstract: 40
- Introduction: 42
- Materials and Methods: 44
CHAPTER III. Complete Compensatory Growth In Hybrid Striped Bass (Morone chrysops X Morone saxatilis) Through Combined Cold-Banking and Feeding Manipulations

Abstract

Introduction

Materials and Methods

Results

Discussion

Acknowledgements

References

CHAPTER IV. Endocrine Biomarkers of Growth and Applications to Aquaculture: A Mini-Review of Growth Hormone, Insulin-like Growth Factor-I and IGF Binding Proteins as Potential Growth Indicators in Fishes

Abstract
LIST OF TABLES

CHAPTER I.
Table 1  Correlation ($r^2$) between hepatosomatic index (HSI), plasma IGF-I (IGF-I), hepatic IGF-I mRNA copy # (IGF-I copy #) and specific growth rate (SGR) during the catabolic state (days 54-84) and the subsequent CG response (days 85-114) of the second feeding cycle in treatment fish. Values were also determined for control fish over the same time period (days 54-114)..............................................................38

CHAPTER II
Table 1  Primer and Taq Man probe sequences for various hybrid striped bass genes measured in liver and muscle tissues by quantitative RTPCR............................................................72
Table 2  Feed conversion ratios and feed consumption of control fish fed to satiation 2X daily and treatment fish subjected to 3 weeks feed restriction (days 0-21) followed by 6 weeks of refeeding 2X daily to satiation (days 22-42, 43-63).......................................................73
Table 3  Skeletal muscle mRNA levels (copy number / ng RNA) for IGF-II, IGFR and GHR1 in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction (days 0-21) followed by 6 weeks of refeeding (days 22-63).................................................................81
CHAPTER III

Table 1  Total lengths and condition factors in groups of HSB that were either fed (F) or starved (S) during the initial temperature phase at 24°C (days 0-23) and/or during the 14°C cold-banked (i.e. overwintering) period (days 24-114).................................................................108

Table 2  Specific growth rates (SGR; %/day), feed consumption (%BW/day) and feed conversion ratios (FCR) for all groups during the first 3 weeks (days 115-134) and the following 2 weeks (days 135-148) of refeed at 24°C. Overall FCR (day 0-148) is also indicated for all groups.................................................109

Table 3  Fluctuations in hepatosomatic index and adiposomatic index in groups of HSB that were either fed (F) or starved (S) during the initial temperature phase at 24°C (days 0-23) and/or during the 14°C cold-banked (i.e. overwintering) period (days 24-114). All fish were subsequently fed during the second 24°C temperature phase (days 115-148). Control HSB (F-F) were fed throughout the entire experiment (days 0-148).........................................................110

CHAPTER IV

Table 1  Correlations ($r^2$) between plasma IGF-I and specific growth rate (weight) (SGR: ln W2 – ln W1)/(t2 – t1) X 100) in fishes induced through specific manipulations.........................................................155
Table 2  Dynamics in the GH-IGF system relative to altered states of growth elicited by various manipulations……………………………………..156
## LIST OF FIGURES

### CHAPTER I.

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.</td>
<td>Response of mean body weight of HSB fed on a normal (control) and cycled (treatment) regimen</td>
<td>32</td>
</tr>
<tr>
<td>Figure 2.</td>
<td>Specific growth rates (SGR) for individual HSB plotted against their corresponding mean weight for a given time interval</td>
<td>33</td>
</tr>
<tr>
<td>Figure 3.</td>
<td>Hepatosomatic index (HSI) of HSB fed on a normal (control) and cycled (treatment) regimen</td>
<td>34</td>
</tr>
<tr>
<td>Figure 4.</td>
<td>Responses of (A) feed consumption (% body weight consumed / day) and (B) feed conversion ratio (FCR) for fish fed on a normal (control) and cycled (treatment) regimen</td>
<td>35</td>
</tr>
<tr>
<td>Figure 5.</td>
<td>Responses of (A) plasma IGF-I and (B) hepatic IGF-I mRNA in HSB fed on a normal (control) and cycled (treatment) regimen</td>
<td>36</td>
</tr>
<tr>
<td>Figure 6.</td>
<td>Changes ($\Delta x / \Delta t$) in (A) growth (grams), (B) hepatic IGF-I mRNA (copy # / ng total RNA) and (C) plasma IGF-I (ng/ml) during a catabolic state (days 54-84) and the subsequent CG response (days 85-114) in treatment HSB. Parameters were also analyzed for controls, fed a daily ad libitum diet, during these same time periods</td>
<td>37</td>
</tr>
</tbody>
</table>
CHAPTER II.

Figure 1. Mean body weight (A) and specific growth rates (B) (SGR; %/day) of control HSB fed to satiation 2X daily and treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding…….74

Figure 2. Hepatosomatic index of control HSB fed to satiation 2X daily and treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding 2X daily to satiation……………………………….75

Figure 3. Hepatic mRNA levels of IGF-I in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding 2X daily to satiation…………………76

Figure 4. Total hepatic mRNA levels for (A) IGF-II and (B) GHR1 in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding 2X daily to satiation………………………………………………………………….77

Figure 5. Plasma (A) IGF-I (ng/ml) and (B) 40kDa IGF binding protein (arbitrary density units, ADU) in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding 2X daily to satiation……………………………………78

Figure 6. Correlation between plasma IGF-I (ng/ml) and specific growth rate (SGR; %/day) in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction followed by 6 weeks of refeeding 2X daily to satiation………………………………………………………………….79
Figure 7. Insulin-like growth factor-I (IGF-I) mRNA expression in muscle tissue in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding 2X daily to satiation.................................................................80

CHAPTER III.

Figure 1. Growth of hybrid striped bass (HSB) raised in tanks under feeding and temperature manipulation (N = 2 tanks/group with 60+ fish/tank).....106
Figure 2. Specific growth rates (SGR) for individual HSB plotted against their mean body weights during the refeeding period at 24°C (days 115-134).....107
Figure 3. Transmission electron microscopy from HSB liver sections following feeding and temperature manipulation.................................111

CHAPTER IV.

Figure 1. The endocrine control of growth in teleost fishes..............................157
CHAPTER I

Discordant Regulation of Hepatic IGF-I mRNA and Circulating IGF-I During Compensatory Growth in a Teleost, the Hybrid Striped Bass
(Morone chrysops X Morone saxatilis)\(^1\)

\(^1\)Published in General and Comparative Endocrinology. 2006. 147, 196-205 by Matthew E. Picha, Jeffrey T. Silverstein and Russell J. Borski.
ABSTRACT

Compensatory growth (CG) is a period of growth that exceeds normal rates after animals are alleviated of certain growth-stunting conditions. Little is known, however, about the endocrine control of CG in teleosts. So, our aim was to induce CG in juvenile hybrid striped bass (HSB, *Morone chrysops x Morone saxatilis*) through manipulations in feeding regimen, and then determine whether changes in circulating insulin-like growth factor-I (IGF-I) and hepatic IGF-I gene expression accompany the CG response. A considerable catabolic state was induced in HSB fed a total of two times over 4 weeks (once each in the 2nd and 3rd week). Negative energy balance was evidenced through weight loss (-3.4 % BW) and a significant drop in hepatosomatic index (HSI) from a value of 3.71 to 1.46. Upon realimentation, in which HSB were fed *ad libitum* 2X/day, a significant CG response was observed over a 4-week period. The CG response was characterized by an elevated specific growth rate, hyperphagia, restoration of the HSI and an improvement in feed conversion, all relative to controls that were fed *ad libitum* 2X/day throughout the experiment. Moreover, the CG response and catabolic state preceding it were marked by a discordant regulation in the expression of hepatic IGF-I mRNA and plasma IGF-I levels, the latter parameter paralleling changes in growth (*r^2 = 0.56, P < 001*). The catabolic state was accompanied by an 82% increase in hepatic IGF-I mRNA while levels of plasma IGF-I were significantly depressed relative to controls. During the subsequent CG response, however, hepatic IGF-I mRNA decreased by 61% while plasma IGF-I increased by 86%. The underlying mechanisms for this inverse regulation of hepatic IGF-I mRNA and circulating IGF-I is uncertain, but may reflect alterations in hepatic IGF-I mRNA production, stability, and translation such that hepatic
IGF-I mRNA is accumulated during periods of catabolism and then rapidly translated and released into circulation when conditions improve. These results suggest that CG can be induced in HSB following a sufficient catabolic state and that systemic IGF-I may be an important mediator of the accelerated growth rate characteristic of CG.

Key Words: Compensatory growth; IGF-I; Hybrid striped bass; Teleost
INTRODUCTION

The natural history of numerous teleost fishes involves periods in which biotic and abiotic variables can severely limit growth. In particular, fishes can experience episodes of reduced feed availability and/or low temperatures, which result in varying degrees of growth depression. When conditions improve, a period of accelerated weight gain known as compensatory growth (CG) is often observed (Wilson and Osbourne, 1960; Morgan and Metcalfe, 2001; Ali et al., 2003). Compensatory growth has been demonstrated in many, but not all, teleosts and appears to be dependent on a variety of factors including the degree of growth suppression and catabolism prior to the response (for review see Ali et al. 2003). Nevertheless, the hormonal control of the rapid weight gain that characterizes CG is poorly understood. Furthermore, while much attention has focused on the CG response in fishes generally, little is known about potential CG responses in hybrid striped bass (HSB; *Morone chrysops* x *Morone saxatilis*) specifically. In particular, while we have recently demonstrated that individual-reared HSB can exhibit significant CG responses (Skalski et al., 2005), it is uncertain whether the response might occur in HSB under group-housed, intensive culture conditions. Since CG is characterized by accelerated growth and improved feed conversion, the response has the potential to improve cultivation of this economically valuable finfish.

The growth hormone (GH) - insulin-like growth factor (IGF) axis is central to the control of growth in teleost fishes, as well as in other vertebrates (Jones and Clemmons, 1995; Oksbjerg et al., 2004; Wood et al., 2005). Insulin-like growth factor-I (IGF-I), a 70 AA
polypeptide produced primarily in the liver, is involved in cell differentiation and proliferation and ultimately body growth (Moriyama et al., 2000). Although endocrine IGF-I of hepatic origin is thought to account for the majority of somatic growth, autocrine and paracrine effects may also play a significant role (Chauvigne et al., 2003).

Correlation between plasma IGF-I and growth rates have been demonstrated in some fish species (Pierce et al., 2001; Uchida et al., 2003). Additionally, catabolic states induced through complete feed restriction significantly reduced hepatic IGF-I mRNA levels in coho salmon and tilapia (Duan and Plisetskaya, 1993; Uchida et al., 2003). In salmonids, a subsequent refeed period restored values to control levels (Duan and Plisetskaya, 1993). In many instances, periods of feed deprivation have also induced states of GH resistance in which circulating levels of GH rise (Duan and Plisetskaya, 1993; Perez-Sanchez and LeBail, 1999) but the number of hepatic GH receptors (GHR) decline (Gray et al., 1992; Mori et al., 1992). The latter response reduces the capacity of GH to stimulate hepatic-derived IGF-I production while elevated levels of circulating GH may still promote lipolysis and skeletal growth (Storebakken et al., 1991; Harmon and Sheridan, 1992; MacKenzie et al., 1998).

While the endocrine response to feed deprivation has been well examined, the subsequent CG response remains virtually undefined in teleosts and other ectotherms. The purpose of this study was to examine the potential for CG in group-housed HSB subjected to manipulations in feeding regimen and to investigate whether changes in hepatic IGF-I mRNA and the circulating peptide might accompany the CG response. We also examined
the temporal and metabolic dynamics of CG through measurements of feed conversion ratio (FCR), hyperphagia and hepatosomatic index (HSI). We were also interested in determining how a limited diet, as opposed to complete feed restriction used in most CG protocols, might affect the CG response. This is because reduced feeding is likely more reflective of the seasonal declines in prey availability seen by many species.

MATERIALS AND METHODS

Experimental Design

Freshwater, juvenile hybrid striped bass (HSB) (*Morone chrysops* x *Morone saxatilis*) (120-135 g) were transported from Pamlico Aquaculture Field Laboratory (Aurora, NC) and transferred to tanks at North Carolina State University (Raleigh, NC). Fish were allowed to acclimate to water quality conditions (hardness: 125 mg/L; alkalinity: 200 mg/L) for a period of 14 days before individually tagging them with FD-94 Anchor Tags (Floy Tag and Manufacturing Inc., Seattle, WA). HSB representing control and treatment groups were stocked in duplicate in 1350-L circular tanks (80 fish/tank) within recirculating systems. Systems were equipped with 80-watt UV sterilizers and biofiltration. Two additional weeks were allowed for post-tagging recovery before initiation of the experiment (Time 0). Control HSB were fed *ad libitum* twice daily throughout the entire 114 day experiment. Treatment HSB were placed on sequential, 4-week cycles of reduced and then normal (*ad libitum* twice daily) feeding levels. During the first 4-week cycle of reduced feeding, HSB were fed a total of five days over four weeks (*ad libitum* twice daily ~1 time/week). During the second cycle of reduced feeding, HSB were fed two days over four weeks (once each in the 2nd and 3rd week).
The deviation in the second reduced feeding cycle was intended to induce a greater catabolic state. Both groups were fed a Zeigler 5.0 mm floating pellet (Zeigler Bros Inc., Gardners, PA). Photoperiod was kept on a 12:12 LD cycle. Water temperature was maintained at 23.03°C +/- 0.06 SEM and the experiments were conducted from July through October.

Sample Collection

Body weights (g), standard lengths (mm) and tissue samples (liver, blood) were taken at initiation of the experiment as well as at the end of each feeding cycle. In addition, tissue samples were collected 4 and 11 days into the second refeed period (days 88 and 95). 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 tricane methanesulfonate (MS 222; Argent Chemical Laboratories, Redmond, WA). Blood was collected using heparinized 1cc syringes with 22-gauge needles. Blood was then dispensed into heparinized 1.5 ml tubes containing aprotinin. Plasma was extracted by centrifugation at 8000 x g for 15 min at 4º C. Liver samples were removed, snap frozen in liquid nitrogen and then stored, along with plasma, at -70º C. HSB were deprived of feed 18 h prior to sampling or group weight and length determinations.

To assess variation in feed consumption among individual fish, group-housed HSB were fed a 42% protein, 16% crude fat commercial trout-type floating diet (prepared at the National Fish Technology Center in Bozeman, MT, U.S.A.) labeled with leaded glass
ballotini beads of 0.4 to 0.6 mm diameter (type H beads, Sigmund Lindner GmBH, Germany) (Silverstein et al. 1999). The beads were mixed into the feed at the rate of 1% (w/w). The labeled diet was fed for one meal, six days into the first refeed period (day 34). Beginning one hour after feeding the labeled diet, radiographs of each HSB were taken with a Soeye SY-31-90P X-ray unit (Soeye, South Korea). Radiographs were taken at a distance of 60 cm and energy of 70kVP/20mA for 0.1 sec using Image-Tek-G (American X-ray, www.amxs.com) 400-speed high contrast film with a detail screen. A standard curve for the number of radio-opaque particles was produced from radiographs of duplicate samples of 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 g of labeled feed. Radio-opaque beads in the stomach were counted for each fish using the Metamorph © image analysis software (Universal Imaging Corp., Downingtown, PA) and compared with the standard curve to estimate the weight of feed consumed. Two days prior to dispensing the labeled feed, HSB were fed an unlabeled feed, in which wheat flour replaced the glass beads, to acclimate fish to the new feed. Immediately following day 34, HSB were returned to their normal feed (Zeigler 5.0 mm floating pellet).

Growth rate and metabolic parameters

Hepatosomatic index (HSI) was calculated as (liver wt (g) / body wt (g)) X 100 and feed conversion ratio (FCR) as (feed consumed (g))/(weight gain (g)). Percent body weight consumed per day was calculated as (total feed consumption per cycle / # of days in cycle)/(total fish weight estimated at midcycle) X 100. Specific growth rate (SGR) was calculated as (ln W2 – ln W1)/(t2 – t1) X 100, where W2 is the weight at the end of the growth interval and W1 is the weight at the beginning of the growth interval.
Since growth rates in fish often have an allometric relationship to body mass and because fish tend to weigh less after periods of feed deprivation relative to controls, it is necessary to compare SGRs of treatment HSB to that of similarly-sized controls. This is to ensure that elevated SGRs in cyclic-fed animals are due to a CG response and not because smaller fish are growing at faster rates. However, when SGRs of individual controls were plotted against their mean weights during all respective time intervals, we found no allometric relationship (Fig. 2; $r^2 = 0.011$). Further, an ANCOVA showed no significant effect of mean weight on SGR. Taken together, growth rates for controls in our experiment were not a function of size. This allowed for direct comparisons between treatment SGRs at various time intervals and control SGRs for all time intervals without allometric growth rates confounding the analysis.

*Real-Time Quantitative PCR for Hepatic IGF-I mRNA*

Total RNA was isolated from liver using the guanidium thiocyanate procedure (Chomczynski and Sacchi, 1987) and further extracted with lithium chloride to remove excess glycogen (Puissant and Houdebine, 1990). Total RNA was then resuspended in RNAse-free water and double DNAse treated with DNA-free (Ambion, Austin, TX) to eliminate any potential genomic DNA and RNA/DNA heteroduplexes. The integrity of RNA preparations from randomly selected samples was confirmed by visual inspection of ethidium bromide-stained 18S and 28S ribosomal RNA bands after electrophoresis through 1% agarose.
Total RNA from each sample was then quantified with Ribogreen RNA according to the manufacturer’s procedure (Ribogreen RNA Quantitation; Molecular Probes, Eugene, OR) and diluted to a standard concentration of 20ng/ul. Hepatic IGF-I mRNA was quantified in triplicate by a SYBR Green Real Time PCR (BioRad iCycler iQ) assay (Qiagen Inc., Valencia, CA) using specific primers (Forward: ACTACTGCTGTGCGTCCTC; Reverse: GCAGCACTCGTCCACAATG) corresponding to the signal peptide and D domains of the HSB IGF-I precursor mRNA (Gen Bank Accession No. AF402669.1). This resulted in the production of a 184 base pair amplicon. Sample RNA was first reverse transcribed at 50°C for 30 min. This was followed by reverse transcriptase inactivation and Hot Star Taq activation at 95°C for 15 min. Forty-five cycles of PCR were subsequently performed, each consisting of denaturation at 94°C for 15 s, annealing at 53°C for 30 s and extension at 83°C for 30 s. Real-time measurements were made during the extension step. Melting curve analysis denoted the formation of a single gene product which, upon sequence analysis (University of Chicago Cancer Research Center DNA Sequence Facility) was confirmed to be *Morone chrysops x Morone saxatilis* IGF-I precursor mRNA. Absolute quantification of unknown samples was accomplished by converting cycle threshold (Ct) values to IGF-I copy number (Bustin 2000). To do this, IGF-I cDNA was synthesized from a plasmid vector, run on a 1.2% agarose gel and subsequently purified (QIAquick® gel extraction kit; Qiagen Inc., Valencia, CA). Following purification, IGF-I cDNA was quantified via Nanodrop (ND-1000 Spectrophotometer) and converted to copy number/ul via the following formula: (ng DNA/ul) / (transcript length x 660) x 6.023 x 10^{23}. Subsequently, the stock of IGF-I cDNA (quantified as previously mentioned) was serially diluted (1.0 x 10^{5}, 10^{6}, 10^{4} and
and each dilution run on the SYBR Green Real Time PCR assay to generate a Ct value vs. log quantity of IGF-I copy number standard curve ($r = 0.916; y = -3.4x + 40.7$). IGF-I copy number per ng of total RNA for unknown samples was then calculated by entering unknown Ct values into the standard curve equation, taking the anti-log and then dividing by total RNA loaded per sample. Thus, hepatic IGF-I mRNA is expressed as copy number / ng total RNA. Similar to that previously reported and reviewed by Bustin (2000), our initial validation studies show that normalization of target RNA to total RNA (measured by the Ribogreen method) yields similar results as that for normalization to 18S RNA.

Radioimmunoassay for Circulating IGF-I

Circulating levels of total IGF-I were measured from acid/ethanol extracted HSB plasma by a commercially-available ‘Universal’ fish IGF-I radioimmunoassay (RIA) (GroPep, Adelaide, Australia). Serially diluted HSB plasma was shown to produce a displacement curve that parallels that of the standard, verifying its validity for measures of circulating IGF-I in HSB (Davis and Peterson, 2003). All samples were run in triplicate.

Statistical Analysis

Body weight was analyzed by Repeated Measures analysis followed by an LS Means test. All other data, unless otherwise noted in the figure legend, was analyzed with a two-way ANOVA (treatment X time) followed by Fisher’s LSD test. Correlations between specific growth rate, HSI, circulating IGF-I and hepatic IGF-I mRNA copy number were
analyzed by regression analysis using Statistica 7.0 (Stat Soft, Tulsa, OK). Statistical significance was set at a level of $P \leq 0.05$. All data is presented as mean ± SEM.

**RESULTS**

*Effects of feeding regimen on growth*

At the end of the first restricted feeding cycle (days 0-28), in which HSB were fed a total of 5 times over 28 days (~1 time/week), fish gained an average of 5.4 g in body weight (Fig. 1). During the subsequent refeed period (days 29-53), then, these same HSB had a SGR 30% higher than control fish that were fed continuously over the entire experiment (1.14 ± 0.06 %/day for treatments vs. 0.88 ± 0.03 %/day for controls), although the effect was not statistically different (Fig. 2). Hence, CG was not observed during this refeed period. Treatment HSB were only fed twice over the next 4 weeks (days 54-84; fed once each in the second and third weeks) and lost an average of 3.4 % BW. The ensuing refeed period (days 85-114), then, resulted in SGRs 64% higher than that of control fish (1.44 ± 0.05 %/day for treatments vs. 0.88 ± 0.03 %/day for controls; P < 0.001). Because of this elevated SGR relative to controls, we refer to this interval as a period of CG.

Furthermore, HSB were sampled from both controls and treatments at days 88 and 95, 4 and 11 days into the realimentation period where the CG response was observed in treatment fish. The highest SGRs for the entire experiment were for treatment HSB during days 85-88 (2.86 ± 0.17 %/day), followed by those for days 85-95 (1.98 ± 0.10 %/day) and 85-114 (1.44 ± 0.05 %/day). Based on fish sampled throughout the experiment, control and treatment groups were composed of the same ratios of female: male fish (43% female:57% male). Therefore, potential sex differences in growth
of HSB, whereby females grow faster than males beginning around 500 g BW (Davis and Ludwig, 2004), cannot account for the observed differences in growth rates seen among control and treatment fish in these studies.

*Hepatosomatic Index*

HSI for control and treatment HSB were similar at Time 0 (3.90 ± 0.18 for treatments vs. 3.71 ± 0.18 for controls) (Fig. 3). Control HSB showed a gradual yet significant decrease ($P < 0.001$) in HSI during the first 53 days of normal feeding (day 53 = 2.89 ± 0.15) at which point it plateaued for the remainder of the experiment. HSI for treatment animals significantly declined ($P < 0.001$) during the first restricted feeding period (day 28; HSI = 2.49 ± 0.09) even though a slight increase in body weight was observed. During the subsequent refeed period, days 29-53, the HSI was restored to values similar to those at Time 0.

During restricted feeding days 54-84, in which treatment HSB lost body weight, HSI precipitously dropped to levels below that of the first restricted feeding period (1.46 ± 0.07). During the final refeed period (days 85-114), overcompensation of HSI relative to controls was observed by day 95 (4.00 ±0.10 for treatments vs. 3.08 ±0.06 for controls) and remained elevated until the end of the experiment. This overcompensation was also evident when comparing HSI to similar-sized control fish, not just to controls evaluated at the same sample points. For instance, treatment HSB at day 114 are the same weight as controls at day 84 (271.8 g for treatments vs. 270.1 g for controls), yet HSI is approximately 25% higher in treatment fish ($P <0.001$). This overcompensation in HSI
for treatment animals at days 95 and 114 is significantly elevated relative to control fish at all time points beyond day 53 of the experiment. During the day 54-114 time interval in which CG was achieved following a catabolic state, the HSI was positively correlated to SGR ($r^2=0.88$, $P < 0.001$; Table 1) in treatment groups. No correlations were observed between these two parameters in control fish over the same time period.

**Feed Consumption and Conversion**

Feed consumption was determined by calculating the percentage body weight consumed per day (% bw/day) for each tank of fish. During the first refeed period (days 29-53), in which SGRs for treatments did not exceed that of controls, treatment HSB consumed a greater (1.60 ± 0.04 % bw/day), yet statistically similar percentage of feed as controls (1.26 ± 0.19 % bw/day) (Fig. 4A). They also had a slightly lower FCR, but values relative to controls were not significantly different (1.34 ± 0.04 for treatments vs. 1.42 ± 0.11 for controls) (Fig. 4B). On day 34, six days into this initial refeed period, HSB were fed a labeled feed to allow individual consumption rates to be monitored (N = 142 fish/group). Feed consumption was statistically similar between treatments (1.99 ± 0.06 % bw/day) and controls (1.94 ± 0.05 % bw/day). This similarity was maintained even when consumption data was separated by small- and large-sized fish. This indicates that the selected feeding regimen did not create a feeding hierarchy, at least during the first refeed period.

The second refeed period (days 85-114) rendered treatment HSB with a significantly higher SGR than controls, a canonical characteristic of CG. During this time, feed
consumption was significantly higher \((P < 0.01)\) in treatments \((1.84 \pm 0.09 \% \text{ bw/day})\) relative to controls \((1.11 \pm 0.09 \% \text{ bw/day})\). Feed conversion was also significantly improved \((\text{FCR: } 1.17 \pm 0.02 \text{ for treatments vs. } 1.47 \pm 0.01 \text{ for controls}; P < 0.05)\) in fish that exhibited the CG response (Figs. 4A + B).

**Endocrine Indices**

Circulating levels of IGF-I (ng/ml) were similar between control \((41.6 \pm 3.6)\) and treatment \((38.3 \pm 3.3)\) HSB at the initiation of the experiment (Fig. 5A). Control fish, which were fed an *ad libitum* diet throughout the entire experiment, experienced a gradual and significant increase \((P < 0.001)\) in plasma IGF-I through day 53 \((65.7 \pm 7.1)\). From this point values plateaued for the remainder of the experiment, although a slight spike was observed at day 95. Similar responses in hepatic IGF-I mRNA (copy # / ng total RNA) were also observed in control fish (Fig. 5B). After beginning at statistically similar levels as treatment fish \((88.8 \pm 11.7 \text{ for controls vs. } 118.23 \pm 17.5 \text{ for treatments})\), a gradual and significant increase \((P < 0.01)\) in hepatic IGF-I mRNA was observed by day 53. This was followed by a plateau, with the exception of day 95, for the remainder of the experiment.

During the initial restricted feeding period (days 0-28), treatment HSB gained 5.4 g of body weight. During this time, circulating levels of plasma IGF-I were similar to control fish, and then declined slightly following the refeed period to levels below control animals. There was no statistical difference in plasma IGF-I between treatment fish at time 0 and the end of the first refeed period. Hepatic IGF-I mRNA levels significantly
increased (P < 0.01) in treatment fish during the first restricted feeding period. This was
followed by a slight, albeit insignificant, increase in IGF mRNA upon refeeding.

In the second restricted feeding cycle (days 54-84) treatment animals experienced a 6.2 g
average loss in body weight. During this time, plasma IGF-I in treatment fish decreased
by 14% relative to levels observed at the end of the previous refeed period. Plasma IGF-I
was also 43% lower than control fish at the end of this second restricted feed (38.9 ± 3.3
for treatments vs. 69.1 ± 5.6 for controls; P < 0.001). In contrast to the depressed plasma
IGF-I levels, hepatic IGF-I mRNA increased by 82% to levels over 2-fold higher than
controls (P < 0.001). Paradoxically, plasma IGF-I concentration was near its lowest
levels while hepatic IGF-I mRNA was at its highest levels at the end of this restricted
feeding period. Upon refeeding (days 85-114), where a considerable CG response (as
measured by an elevated SGR) was observed, there was a rapid and dramatic increase in
plasma IGF-I along with a decline in hepatic IGF-I mRNA in treatment HSB. By day 88,
4 days into the CG response, circulating IGF-I increased by 47% while mRNA levels
decreased by 45%. These trends continued through day 95 and by day 114, plasma IGF-I
had increased by 86% while hepatic IGF-I mRNA had decreased by 61%. Interestingly,
the most dramatic increase in plasma IGF-I occurred during the first 4 days of the CG
response, which also corresponds to the largest decrease in hepatic IGF-I mRNA and the
highest SGR. During the day 54-114 time interval in which CG was achieved following a
catabolic state, circulating IGF-I positively correlated to SGR (r² = 0.56; P < 0.001) and
HSI (r² = 0.58; P < 0.001), while hepatic IGF-I mRNA copy number was negatively
correlated to SGR (r²= 0.42; P < 0.001) and HSI (r²= 0.34; P < 0.001), in individuals
from the treatment group (Table 1). There was also a weak, albeit significant, negative correlation between circulating IGF-I and hepatic IGF-I mRNA copy number in treatment fish ($r^2=0.17$, $P < 0.05$; Table 1). No significant correlations were observed between these different parameters in control fish over the same time period (Table 1).

To further characterize the catabolic state (weight loss during days 54-84) and CG response (elevated SGR during days 85-114) evident in the second restricted and refeed cycle, we assessed changes ($\Delta x / \Delta$ time) in growth, hepatic IGF-I mRNA and plasma IGF-I between these two time periods in treatment fish (Fig. 6). The restricted feeding period, days 54-84, resulted in a negative change in growth (g) and plasma IGF-I (ng/ml). Hepatic IGF-I mRNA (copy # / ng total RNA), however, underwent a positive change during this period. During the CG response, significant inversions were observed for each of these variables. The CG response resulted in a positive change in growth and plasma IGF-I while hepatic IGF-I mRNA levels experienced a negative inversion. During the 54-114 day time interval changes in circulating IGF-I ($\Delta$ IGF-I/$\Delta$ time) correlated positively to SGR ($r^2 = 0.63$, $P < 0.001$) while changes in IGF-I mRNA ($\Delta$ IGF-I mRNA copy number/$\Delta$ time) correlated negatively to SGR ($r^2 = 0.66$, $P < 0.001$) in treatment animals (data not shown). For these correlations, changes in IGF-I and IGF-I mRNA copy number were derived by subtracting individual values at days 84 and 114 from group averages at the beginning of the respective time intervals, (days 54 and 85 respectively)(i.e. $\Delta$ IGF-I/$\Delta$ time for the 54-84 time interval = [individual plasma IGF-I value at day 84 – group mean plasma IGF-I value at day 54] / [84 - 54 days]).
Changes in these same parameters were also analyzed for control fish for days 54-84 and 85-114 (Fig. 6). Controls were fed a normal, *ad libitum* diet (2X / d) throughout the entire experiment, including during these two time periods. Not surprisingly, no significant changes were observed for growth, hepatic IGF-I mRNA or plasma IGF-I between these intervals.

**DISCUSSION**

In this study we demonstrate that compensatory growth (CG), characterized by an elevated SGR, can be induced in HSB following a sufficient catabolic state. The response is accompanied by hyperphagia, an improvement in feed efficiency and an overcompensation of a previously depressed HSI. An inverse relationship between hepatic IGF-I mRNA and circulating levels of IGF-I both preceding and during the CG response was also observed. In particular, the catabolic state rendered significant increases in hepatic IGF-I mRNA along with depressed levels of plasma IGF-I, followed by significant inversions of both of these endocrine measures during CG.

Compensatory growth is a period of growth acceleration that exceeds normal rates after animals or plants are alleviated of certain growth-stunting conditions. While fish are the most studied of the vertebrate taxa, the phenomenon has also been documented in mammals, birds and even some invertebrates (Wilson and Osbourne, 1960; Sibly and Calow, 1986). Within the life history of many animals, a fluctuation in food availability is a common growth-stunting condition that can render a CG response (Bilton and Robins, 1973; Broekhuizen et al., 1994). Several teleosts, including Atlantic salmon
(Nicieza and Metcalfe, 1997), rainbow trout (Quinton and Blake, 1990; Nikki et al., 2004), channel catfish (Kim and Lovell, 1995; Gaylord and Gatlin, 2001) and European minnow (Russell and Wootton, 1992) show either partial or full growth compensation, while others lack a CG response altogether (for review see Ali et al. 2003) following feed manipulations. We demonstrate that group-housed HSB show a strong CG response following sufficient periods of feed restriction. These results are similar to those we recently reported with individual-reared HSB (Skalski et al., 2005). However, compared with the individual-reared animals that showed complete catch-up growth following adequate periods of catabolism, full growth compensation was not observed in the present study with group-reared HSB (Fig. 1). We postulate that had the initial restricted feeding period rendered a CG response similar to that observed in individual fish (Skalski et al. 2005) or that of the second refeed period in the present study, full growth compensation might have been achieved in group-reared HSB by the end of the experiment.

Consistent with the results reported here, the degree of catabolism may be critical to eliciting a CG response (Weatherly and Gill, 1981; Russell and Wootton, 1992; Jobling and Koskela, 1996; Boersma and Wit, 1997). In particular, CG was not observed after the first restricted feeding period (days 0-28) where HSB gained a slight amount of weight (5.4 g or 4.0% BW) and SGR was not different from controls (see Figs. 1 and 2, days 29-53). In addition, other characteristics that often accompany CG in fishes, including hyperphagia and improved feed conversion, were not observed as well (see Fig. 4). By contrast, HSB demonstrated CG after a considerable catabolic state was induced during
the second restricted feeding cycle (days 54-84). This preceding state was marked by a slight decrease in body mass (-6.2 g or -3.4% BW) along with a dramatic decrease in HSI from 3.70 to 1.46, a final value significantly below that of the first restricted feeding cycle (HSI = 2.49). As a result, CG was observed upon refeeding (days 85-114, Fig. 2). The most exaggerated period of CG, as defined by elevated SGRs, occurred during the first 4 followed by 11 and 30 days of the response (Fig. 2). Thus, it would seem that the endocrine, metabolic and behavioral factors that facilitate CG may be contributing most significantly during the initial stages of the response. Significant improvements in metabolic efficiency (Fig. 4B) along with considerable hyperphagic responses (Fig. 4A) were also observed during this 30-day period of accelerated growth (days 85-114) and represent critical factors by which CG might be achieved. Full CG after a slight weight gain has been documented, however, in juvenile coho salmon (Damsgard and Dill, 1998), suggesting that the response may be species specific or dependent on rearing temperature.

Although hepatic energy stores constitute a small proportion of the overall reserves available to fish, they represent important reservoirs during the initial period of food deprivation (Collins and Anderson, 1995). Based on our studies, they may also be used as indicators for catabolism and the potential to undergo CG. As mentioned previously, the first cycle of restricted feeding caused a significant drop in HSI to 2.49, while the second restricted feeding period produced a decline in HSI to 1.46, the latter resulting in a considerable CG response upon refeeding. Thus, there may be a threshold level of catabolism that must be reached in feed manipulation studies in order to induce CG.
Follow-up studies in similar-sized HSB affirmed that when values for HSI fall below 1.55, CG is induced upon realimentation (unpublished data).

The role of the somatotrophic axis on growth in teleost fishes has been the focus of many recent studies and reviews (Perez-Sanchez and Le Bail, 1999; Bjornsson et al., 2002; Chauvigne et al., 2003; Beckman et al., 2004; Biga et al., 2004). Within this axis, circulating levels of IGF-I, a mitogenic peptide produced primarily in the liver, have been correlated to both positive and negative SGRs (Pierce et al., 2001; Uchida et al., 2003). However, the temporal dynamics in circulating IGF-I and hepatic IGF-I gene expression during episodes of CG have yet to be assessed. During the considerable catabolic state of the second restricted feed period, circulating levels of IGF-I declined but then rebounded dramatically during the subsequent CG response. The most dramatic increase in plasma IGF-I occurred during the first 4 days of the response, which corresponds to the highest SGR during the entire CG period (days 85-88; see Figure 2). Overall, there was a strong positive correlation between SGR and circulating IGF-I among treatment fish that showed a significant CG response following an adequate period of catabolism (days 54-114; see Table 1). Interestingly, while the CG response was characterized by elevated SGRs relative to controls, it was not accompanied by an overcompensation of plasma IGF-I. Thus, while these results suggest that circulating IGF-I may be crucial to regulating variable growth rates, the accelerated growth response observed during CG may be facilitated in part by the relative increase or change in plasma IGF-I (see Fig 6) rather than by absolute concentrations alone. Indeed, we found an even stronger correlation between SGR and changes in plasma IGF-I levels ($\Delta$ plasma IGF-I / $\Delta$ time)
than that between SGR and absolute IGF-I concentrations. This raises the possibility that target tissues are more sensitive to or respond more efficiently to changes in circulating IGF-I than absolute hormone concentrations per se. This is further verified by data that demonstrate the highest SGR corresponds to the largest incremental increase in IGF-I four days after the onset of CG in HSB. Regardless, the results suggest that circulating IGF-I is an important mediator of the accelerated growth observed during CG in HSB, and perhaps in other teleosts as well.

The same catabolic state that led to depressed plasma IGF-I levels also produced a significant increase in hepatic IGF-I gene expression that was 2-fold higher than control levels (day 84). This finding is surprising considering that hepatic sources of IGF-I are the primary contributor to circulating hormone levels (Sjogren et al., 1999; Shamblott et al., 1995). Our findings also contrast those from two previous studies. After two weeks of complete feed restriction in tilapia, *Oreochromis mossambicus*, hepatic IGF-I mRNA was significantly lower than levels in control fish fed a normal diet (Uchida et al., 2003). In coho salmon, *Oncorhynchus kisutch*, it took 28 days of complete feed restriction before hepatic IGF-I mRNA experienced a significant decrease. Upon 14 days of refeeding in the salmon, however, IGF-I mRNA returned to control levels (Duan and Plisetskaya, 1993). It is worth noting that these studies employed complete feed restriction, while our regimen was that of partial feed restriction. Interestingly, while the 30-day period of CG in our experiment produced an 86% net increase in plasma IGF-I, this same interval rendered a 61% net decrease in hepatic IGF-I mRNA from its previous elevated state. Furthermore, the first 4 days of this response were not only associated with
the largest increase in plasma IGF-I and highest SGR but also saw the largest decrease in hepatic IGF-I mRNA. Based on the trends during this entire CG period and the catabolic state preceding it, this study represents the first to our knowledge of an inverse regulation of plasma IGF-I and hepatic IGF-I mRNA levels in non-mammalian vertebrates.

The mechanisms underlying the discordance in hepatic IGF-I mRNA and circulating IGF-I levels during catabolism and the CG response observed in these studies is uncertain. It is well established that IGF-I is a potent inhibitor of pituitary GH synthesis and secretion in vertebrates, including Morone species as well as other teleosts (Fruchtman et al. 2000, 2001). Preliminary evidence shows that circulating GH levels rise in HSB following complete feed restriction and are then restored to levels of control fish following realimentation to satiation feeding (data not shown). In light of these data and the negative feedback control of GH secretion by IGF-I, it is possible that a surge in circulating IGF-I during the CG response seen in this study following limited feeding might suppress GH secretion, which would lead to GH-dependent reductions in IGF-I mRNA levels. Likewise, an opposite regulation might be seen during the catabolic state or periodic feeding period that precedes the CG response, where low circulating IGF-I cause elevations in GH secretion and hence increased IGF-I transcription and higher IGF-I mRNA levels. However, this explanation does not take into account the possibility that a state of GH-resistance is induced in HSB during the catabolic state preceding CG, whereby a downregulation of hepatic GHR suppresses the stimulating effects of GH. This would, in turn, lead to reductions in both hepatic IGF-I gene expression and
circulating IGF-I levels as has been observed in salmonids and mammals (Pierce et al., 2005; Beauloye et al., 2002). Moreover, considering that the liver is thought to be the primary source of circulating IGF-I and that the hormone is secreted as its produced and not stored in vesicles (Daughaday and Rotwein, 1989; Plisetskaya, 1998), we would expect changes in circulating IGF-I to correspond with changes in hepatic IGF-I mRNA levels regardless of whether or not a GH-resistant state is observed. Consistent with this, we found that the dynamics in plasma IGF-I were similar to that of hepatic IGF-I mRNA in control fish (Fig. 5). Thus it would appear that additional mechanisms, possibly those involving the differential regulation of IGF-I mRNA transcripts, mRNA stability and translation, along with the involvement of endocrine factors other than GH, might account for the discordance in hepatic IGF-I mRNA and circulating IGF-I levels observed in treatment fish in this study.

In fetal rat hepatocytes, it has been shown that stability of IGF-I mRNAs is higher in the presence of insulin than in its absence (Goya et al. 2001), perhaps through the down-regulation of RNase activity or the presence of alternative 3’ untranslated terminal repeats within IGF mRNA transcripts (Nielsen et al. 1999). In primary cultures of tilapia hepatocytes, treatment with 3,5,3’-triiodothyronine (T3) results in the doubling of IGF-I mRNA expression after 6 hours with similar trends seen with in vivo experiments (Schmid et al., 2003). Thus, if periodic feeding during the catabolic state rendered temporal bursts of insulin and T3 and perhaps an increase in hepatic sensitivity to these hormones, then IGF-I mRNA transcription and increased stability could potentially result in its tissue accumulation, or at least in elevated levels relative to control fish. During the
subsequent CG response, the significant drop in previously elevated hepatic IGF-I mRNA levels and the corresponding increase in depressed plasma IGF-I could be explained in a similar fashion. That is, when feed resources become consistency available it is possible that newly synthesized and existing hepatic IGF-I mRNA transcripts become more prone to translation. This would not only account for their precipitous decline during the initial stages of the CG response but for the rapid increase in circulating IGF-I and could, in part, contribute to the elevated specific growth rates in HSB. Interestingly, in vitro assays with Hep3B cells have demonstrated that, relative to rapidly growing cells, quiescent cells have higher levels of IGF-II mRNA (Scheper et al. 1996). Then, when a mitogenic signal is received, large amounts of mRNA may be directed to the polysomes for translation while subsequent down-regulation of IGF-II mRNA transcripts prevents overproduction of the peptide (Scheper et al. 1996).

In conclusion, we have found that HSB can undergo periods of CG when the response is preceded by a sufficient catabolic state evident by slight weight loss along with a drop in HSI to values < 1.5. The CG response following this negative energy balance is characterized by an elevated SGR, hyperphagia, improved feed conversion and increases in plasma IGF-I along with declines in hepatic IGF-I mRNA expression. Although further studies are required to address the underlying mechanisms for the discordant regulation of hepatic IGF-I gene expression and circulating hormone levels, it would appear the latter is an important mediator of CG in HSB. Studies such as this one are critical to understanding the endocrine control of CG in the natural environment since partial feed restriction, rather than complete fasting used in most studies, is likely more
reflective of seasonal fluctuations in prey availability where resources may become more sporadic but are not entirely absent for sustained periods.

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Figure 1. Response of mean body weight of HSB fed on a normal (control) and cycled (treatment) regimen. Grey bars denote periods of restricted feeding in the treatment group. Asterisks represent significant differences between groups (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). (N = 67-158 animals/group).
Figure 2. Specific growth rates (SGR) for individual HSB plotted against their corresponding mean weight for a given time interval. Each line represents the linear best fit for the group of fish. The length of each line represents the weight range of the fish for each time interval. SGRs for controls are plotted for the entire experiment. SGRs for treatments are plotted during refeed periods only. Significant differences between lines (groups) are indicated by different letters (P < 0.05). Data were analyzed with an ANCOVA followed by Tukey’s HSD.
Figure 3. Hepatosomatic index (HSI) of HSB fed on a normal (control) and cycled (treatment) regimen. Grey bars denote periods of restricted feeding in the treatment group. Asterisks represent significant differences between groups (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) (N=12 animals/group).
Figure 4. Responses of (A) feed consumption (% body weight consumed / day) and (B) feed conversion ratio (FCR) for fish fed on a normal (control) and cycled (treatment) regimen. Data are shown for time periods that reflect the treatment refeed periods only. Asterisks represent significant differences between groups (*$P < 0.05$; **$P < 0.01$) ($N = 2$ tanks/group).
Figure 5. Responses of (A) plasma IGF-I and (B) hepatic IGF-I mRNA in HSB fed on a normal (control) and cycled (treatment) regimen. Grey bars denote periods of restricted feeding in the treatment group. Asterisks represent significant differences between groups (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) (N=12).
Figure 6. Changes ($\Delta x / \Delta t$) in (A) growth (grams), (B) hepatic IGF-I mRNA (copy #/ng total RNA) and (C) plasma IGF-I (ng/ml) during a catabolic state (days 54-84) and the subsequent CG response (days 85-114) in treatment HSB. Parameters were also analyzed for controls, fed a daily ad libitum diet, during these same time periods. Each parameter was analyzed with a two-sample t-test. Asterisks represent significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) (N=2).
Table 1. Correlation ($r^2$) between hepatosomatic index (HSI), plasma IGF-I (IGF-I), hepatic IGF-I mRNA copy # (IGF-I copy #) and specific growth rate (SGR) during the catabolic state (days 54-84) and the subsequent CG response (days 85-114) of the second feeding cycle in treatment fish. Values were also determined for control fish over the same time period (days 54-114). Data for HSI, IGF-I and IGF-I copy # are derived from individual fish at days 84 (endpoint of catabolic state) and 114 (endpoint of CG response). Data for SGR are calculated from individually tagged fish during the 54-84 and 85-114 day growth periods. $r^2$ values with a (-) represent correlations with negative slopes.

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N = 22-24 animals/parameter for both control and treatment groups.

* $P < 0.05$

*** $P < 0.001$. 

38
CHAPTER II

Endocrine and Paracrine Dynamics of Insulin-like Growth Factors
During Compensatory Growth in a Teleost Fish
ABSTRACT

Compensatory growth (CG) is a period of growth acceleration that exceeds normal rates after animals are alleviated of certain growth-stunting conditions. In hybrid striped bass (HSB, *Morone chrysops* X *M. saxatilis*), 3 weeks of complete feed restriction resulted in a growth-stunting / catabolic condition that, when relieved, was sufficient to render a subsequent phase of CG. Specifically, the catabolic state was characterized by a 7.7% loss in body weight, a drop in hepatosomatic index (HSI) values below a previously established threshold, and an endocrine state of growth hormone (GH) resistance marked by depressed levels of hepatic Type I GH receptor (GHR1) and insulin-like growth factor-I (IGF-I) mRNA, along with considerable decreases in plasma IGF-I. The state of catabolism also resulted in significant declines in hepatic IGF-II gene expression and in circulating 40kDa IGF binding protein (IGFBP). Upon realimentation, specific growth rates (SGR) were significantly higher than similar-sized controls for 3 weeks, and thus constituted a period of CG. The response was driven both behaviorally and metabolically by hyperphagia and improvements in feed conversion. Hepatic GHR1, IGF-I, and IGF-II mRNA levels increased rapidly during realimentation, exceeding that of control fish by 3 weeks of refeeding, and then returning to basal levels after the CG response subsided. Plasma IGF-I and 40kDa IGFBP also increased during the accelerated growth phase of the CG response. Plasma IGF-I was significantly correlated to SGR throughout the study ($R^2 = 0.54; P < 0.001$). Local paracrine/autocrine actions of IGF-I may have also contributed to the response, as levels of skeletal muscle IGF-I mRNA increased 10 fold during CG. There were no significant changes in muscle GHR1, IGF-II, and IGF receptor mRNA between control and treatment animals during feed restriction or
realimentation. Taken together, these data suggest that both endocrine and paracrine/autocrine components of the GH-IGF axis are responsible for periods of accelerated (compensatory) growth in HSB.
INTRODUCTION

Growth in teleost fishes involves a complex array of neuroendocrine processes under the direct influence of variables such as temperature, nutrition and stress. Fish are an excellent model to study the physiological control of variable and often dramatic differences in growth rate since they exhibit indeterminate and relatively plastic rates of growth. Compensatory growth is a period of growth acceleration that exceeds normal rates after animals are alleviated of certain growth-stunting conditions (Wilson and Osbourn 1960; see Ali et al. 2003 for review). Experimental regimes that elicit states of compensatory growth (CG) provide the opportunity to elucidate the underlying mechanisms of stunted (negative), normal and accelerated (compensatory) growth. While numerous studies in fishes have examined the endocrine growth axis during periods of weight loss, typically induced through feed restriction (see Picha et al. 2008 for review), few have examined the response of the axis during the subsequent period of CG when the stunting condition is removed.

The endocrine control of growth in fishes and other vertebrates is regulated primarily through the growth hormone (GH)/insulin-like growth factor (IGF) axis (Luckenbach et al. 2007; reviewed by Reinecke 2006). Under anabolic conditions, GH released from the pituitary acts on its hepatic receptors to stimulate the production and release of insulin-like growth factor-I (IGF-I) into the circulation, where it then acts on target tissues to promote cell proliferation, differentiation and ultimately body growth (reviewed by Wood et al. 2005). Accordingly, its levels have been correlated to specific growth rate in numerous teleosts (Uchida et al. 2003; Beckman et al. 2004; Picha et al. 2006, 2008;
Vera Cruz et al. 2006) and it has recently been shown that the presence or absence of a single IGF-I nucleotide polymorphism is a major determinant of body size in dogs (Sutter et al. 2007). In addition to hepatic binding, GH can also act directly on target tissues such as skeletal muscle to stimulate the production of IGF-I, which in turn may act in a paracrine/autocrine fashion to stimulate tissue growth (Chauvigne et al. 2003). The potential contribution of locally produced IGF-I to anabolism is further underscored by studies in GH transgenic tilapia (*Oreochromis niloticus*), which showed elevated growth and skeletal IGF-I mRNA but reduced plasma IGF-I relative to wild type controls (Epler et al. 2007). Unlike postnatal mammals, circulating as well as locally-produced insulin-like growth factor-II (IGF-II) may also have mitogenic effects in adult fishes (Peterson et al. 2004; Gabillard et al. 2006; Terova et al. 2007). The anabolic effects of both IGF-I and –II are mediated by IGF binding proteins (IGFBPs), four of which have been identified in fishes at the protein level (IGFBP-1, -2, -3, -5) (Shimizu et al. 2003; Kamangar et al. 2006). Insulin-like growth factor binding protein-3 (40-50 kDa), most commonly associated with anabolic states in fishes, may facilitate IGF actions by increasing IGF half-life in the blood and by mediating their transport from the vascular space to target tissues (reviewed by Kelley et al. 2006).

The purpose of this experiment was to examine the growth, metabolic and endocrine characteristics of hybrid striped bass (*Morone chrysops X M. saxatilis*) during stunted, normal and accelerated (compensatory) growth. Specifically, we were interested in the IGF dynamics at the gene expression and protein level during catabolism (weight loss).
and more particularly during the period of rapid (compensatory) growth that follows the growth stunting condition.

**MATERIALS AND METHODS**

*Animals and Experimental Design*

Freshwater, juvenile (71.4 g ± 0.8 g; mean ± SEM) hybrid striped bass (HSB; *Morone chrysops X Morone saxatilis*) were transported from ponds at the Tidewater Research Station (Plymouth, NC) to indoor tanks at North Carolina State University (Raleigh, NC). One week following transport, fish were evenly distributed to eight 650-L tanks within two identical freshwater recirculating systems (4 tanks/system) equipped with biofiltration and UV sterilization. Fish were allowed to acclimate to photoperiod (12L:12D) and water quality parameters (hardness = 170mg/L; alkalinity = 250mg/L) for one month prior to the start of the experiment. All fish were maintained on a daily feeding regimen prior to initiation of the experiment. Two tanks from each of the recirculating systems were randomly assigned to control and treatment groups (N = 4 tanks/group at 50 fish/tank). Control fish were fed 2X daily to apparent satiation throughout the 63-day experiment. Treatment fish were fasted for the initial 3 weeks (days 0-21) of the experiment, after which time they were placed on the control, twice daily satiation diet for 6 weeks (days 22-63). Water temperature averaged 24.4°C ± 0.04°C (mean ± SEM) throughout the experiment.
Sample Procedures

Body weights (g) and total lengths (mm) were taken from both control and treatment groups at the following points: Time 0, after 3 weeks of feed restriction in treatments (day 21), and after 3 and 6 weeks of refeed (days 42 and 63). Blood and tissue (liver, muscle) samples were taken from a subsample of fish at these same time points, as well as 8 days into the refeed period for treatments (day 29) to better track the time-course response of variables during CG. Group weights and lengths were taken in fish anesthetized with quinaldine sulfate (B.L. Mitchell Inc., Leland, MS) and terminal sampling for blood and tissue collection was conducted on fish anesthetized with buffered tricane methanesulfonate (MS 222; Argent Chemical Laboratories, Redmond, WA). Blood was collected using heparinized 1cc syringes with 22-gauge needles, dispensed into heparinized 1.5 ml tubes containing aprotinin and kept on ice. Plasma was separated by centrifugation at 4º C and stored at -70º C until analyses. Liver and muscle samples were rapidly removed, snap frozen in liquid nitrogen and then stored at -70º C. Fish were deprived of feed 20 h prior to sampling or group weight and length determinations.

Growth rate and metabolic calculations

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 growing interval. Hepatosomatic index (HSI) was calculated as \[
(liver wt / body wt) \times 100
\] and feed conversion ratio (FCR) as \[
(feed consumed / weight gain)\]. Percent body weight
consumed per day (% BW/d) at specific intervals throughout the experiment was calculated as \([(\text{total feed consumption per time interval}) / (\text{total fish weight estimated at midpoint of the interval}) \times 100]\).

**Gene cloning**

Striped bass (*Morone saxatilis*) IGF-II (180 bp) and Type I GHR (480 bp) gene were 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 striped bass liver samples using 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 ug 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 IGF-II primers (Forward: 5’-TGTGGRGGAGARCTGGTGGA-3’; Reverse: 5’-ACTTGGCRGGTTTGGCACAG-3’) were designed within the B and A/D domains of the IGF-II gene using known *Cottus scorpus* (Genbank Accession Number: Y16643), *Oncorhynchus mykiss* (M95184), *Paralichthys olivaceus* (AF091454) and *Oreochromis mossambicus* (Y18691) IGF-II sequences. Degenerate GHR primers (Forward: 5’-TCCTGCACCYAAAATYAAAGG-3’; Reverse: 5’-CTGGGSCCCYCCAGTGTGG-3’) were designed within the Box 1 region and downstream of the Box 2 region of the cytoplasmic domain using Type 1 GHR sequences from *Scophthalmus maximus* (Genbank Accession Number: AF352396), *Acanthopagrus schlegelii* (AF502071), *Sparus aurata* (AF438176), *Oreochromis mossambicus* (AB115179) and *Dicentrarchus labrax* (AF438177). PCR cycling
conditions were as follows for both genes: 1 cycle at 95°C for 15 min; 30 cycles of 94°C for 30 sec, 50-65°C for 30 sec and 72°C for 1 min; and 1 cycle at 72°C for 10 min. PCR products were ligated into pCR 2.1 vectors and transformed using INVαF' chemically competent E. coli cells (Invitrogen; Carlsbad, CA). Plasmids were sequenced at the University of Chicago DNA sequencing facility and verified by BLAST search (NCBI: Bethesda, MD).

**RNA isolation for quantitative real-time PCR**

Total RNA was extracted from liver samples using TRI Reagent isolation solution with sequential high salt (Molecular Research Center, Cincinnati, OH) and LiCl precipitation steps intended to remove glycogen contamination (Barlow et al. 1963). The mean 260:230 nm ratio for these RNA samples ranged from 1.35 to 2.28 as determined by Nanodrop spectrophotometry. This wide range and low values in 260:230 ratios suggested residual polysaccharide contamination (Nanodrop Technical Support Bulletin T009), which can artificially alter RNA quantification since glycogen can absorb at 260 nm (unpublished data). Therefore, samples were further purified with Plant RNA Isolation Aid (Ambion; Austin, TX) to completely remove glycogen, as was indicated by 260:230 values between 2.0-2.2 measured for all samples. This, combined with sample 260:280 ratios of 1.9-2.0, indicates high purity of nucleic acids and minimal contamination by proteins and polysaccharides. Muscle samples were extracted using the standard Tri Reagent RNA isolation protocol (Molecular Research Center; Cincinnati, OH).
Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) primers and Taq Man probes for hybrid striped bass (Order Perciformes; Family Moronidae) were designed for IGF-I, IGF-II, IGFR and the GHR1 in regions with low identity to related genes (Table 1). IGF-I primers and probe showed no cross-reactivity when run with IGF-II cDNA template. Likewise, no cross-reactivity was observed using IGF-II primers and probe with IGF-I cDNA template. HSB IGFR primers and probe were designed within the tyrosine kinase domain (AF402674) that only has 63% nucleic acid sequence similarity to insulin receptor of another perciform, the tilapia (*Oreochromis mossambicus*, AF493794). The GHR1 primers and probe were designed in the region that exhibits only 28% similarity to the European seabass GHR2 (*Dicentrarchus labrax*, Family Moronidae, AF438177).

Following total RNA extraction, muscle and liver RNA from experimental samples was double DNase-treated, diluted to the same concentration and then subjected to reverse transcription (RT) using random hexamer primers (Applied Biosystems; Foster City, CA). Enzymatic reactions for all samples for each tissue type were performed at the same time with identical reagents, as to reduce the variability of DNase and RT efficiency between samples. Ten (liver) or 100 ng (muscle) of cDNA from the RT reaction was then loaded with 900 nM primers and 250 nM probes into 20 ul qRT-PCR reaction volumes containing Brilliant QPCR master mix (1X) (Stratagene; La Jolla, CA). Quantitative RTPCR assays for each gene were run on an ABI 7900 Thermal Cycler (Applied Biosystems; Foster City, CA) under the following conditions: 1 cycle at 50°C for 2 minutes, 1 cycle at 95°C for 10 minutes and then 40 cycles of 95°C for 15 seconds
and 60°C for 1 minute. No amplification (DNase treated-RNA instead of cDNA) as well as no template (no cDNA) controls were run in parallel with experimental samples to confirm the absence of genomic DNA and qRTPCR reagent contamination, respectively. The absolute quantity of specific muscle mRNAs was calculated using cDNA copy number standard curves (10⁰ to 10⁻⁹ copy number / ng total RNA) generated through gene specific PCR amplification and isolation according to previously established procedures (Bustin 2000; Picha et al. 2006). Copy number data for all experimental samples was then normalized to total RNA loaded into each qRTPCR assay. Muscle qRTPCR data are therefore expressed as copy number per ng RNA. Our initial studies found that changes in mRNA are similar whether normalized to total RNA or to the β-actin housekeeping gene.

Liver IGF-I mRNA levels are expressed as both copy number as a fraction of total RNA (copy number per ng RNA), as previously described for muscle, and as total copy number for the entire liver relative to body weight [(ng total liver RNA X copy number of gene per ng RNA) / (g body weight)]. We argue that the latter is more physiologically relevant for quantifying liver gene expression levels when hepatosomatic indices fluctuate dramatically due to nutritional state. Therefore, this method was used to normalize hepatic GHR1 and IGF-II mRNA data. We found here and previously that mRNA was similar whether expressed as copy number per ng total RNA or when normalized to the housekeeping gene 18S (Picha et al. 2006). Our studies found that hepatic β-actin was regulated in the liver by nutritional state.
Radioimmunoassay

Circulating levels of total IGF-I were measured from acid/ethanol extracted plasma by radioimmunoassay (RIA) using recombinant barramundi IGF-I as tracer and standard, rabbit anti-barramundi IGF-I 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). Barramundi IGF-I was iodinated using the chloroamine-T method and purified by column chromatography. Tracer ($^{125}$I-barramundi IGF-I) was diluted to 20,000 cpm for each assay tube. Our previous validation shows that serially diluted HSB plasma produces a displacement curve that parallels that of the barramundi standard (Picha et al. 2006). All samples were run in triplicate.

Western Ligand Blot

Hybrid striped bass 40 kDa IGFBP was measured in plasma by Western blot according to previously published procedures used for striped bass (Siharath et al. 1995). HSB plasma samples (4 ul) along with rat serum (+ control) were run on discontinuous 4% stacking, 12% separating SDS-PAGE gels under non-reducing conditions. Following electrophoresis, proteins were electro-transferred overnight onto nitrocellulose membranes, stained with Coomassie blue and then blocked with TBS + 1% BSA for 4 hours. Hybridization took place overnight at room temperature using $^{125}$I-barramundi IGF-I at 200,000 cpm/ml incubating buffer. Membranes were exposed to film in cassettes using intensifying screens at -70C. All data was analyzed using Image Quant 5.2 software (GE Healthcare, USA) and is expressed as arbitrary density units (ADU).
**Statistical Analyses**

Body weight was analyzed by Repeated Measures ANOVA followed by Fisher’s LSD test for predetermined comparisons (Steele and Torrie 1980). Correlations were analyzed by regression analysis. All other data was analyzed with a two-way ANOVA (treatment X time) followed by Fisher’s LSD test. Statistical analyses were performed with Statistica 7.0 software (Stat Soft, Tulsa, OK). The N value for all growth (weight, SGR) and feed (%BW/d, FCR) data was represented by mean values of tanks, while individual fish served as the N value for all endocrine parameters. Statistical significance was set at a level of $P \leq 0.05$. All data is presented as mean ± SEM.

**RESULTS**

*Effects of cyclic feeding on growth and metabolic indices*

Three weeks of complete feed restriction (days 0-21) in treatment fish resulted in a 7.7% decrease in body weight (Figure 1A), indicated by a negative SGR (-0.38 ± 0.05) (Figure 1B). During the subsequent 3 weeks of refeed (days 22-42), however, SGRs for treatment fish were significantly higher ($P < 0.05$) than those for controls at all growth intervals throughout the experiment (Figure 1B). Since growth rates are dependent on size and show an allometric relationship to body mass, it is also important to compare growth of similar sized fish. We found that elevations in SGR in treatment fish during the realimentation period was still higher compared with control fish of a similar initial size (Time 0 controls = 71.4 ± 1.3 g; day 21 treatments = 65.8 ± 0.7 g). The elevated SGR that characterizes CG subsequently declined back to basal levels during weeks 4-6 of
realimentation (days 43-63), where there was no significant difference in SGR in treatment fish relative to size-matched controls (control SGR days 22-42 = 1.3 ± 0.1 %/day; treatment SGR days 43-63 = 1.1 ± 0.2 %/day; Figure 1).

In conjunction with the weight loss experienced during feed restriction (days 0-21), HSI values for treatment fish dropped 3.5-fold (3.85 ± 0.11 to 1.11 ± 0.11; P < 0.001) and were significantly lower than controls (Figure 2; P < 0.001). Eight days into realimenation and the initial CG response (day 29), treatment HSI values increased by 168%, and reached higher levels than controls by 3 weeks (day 42: Controls = 3.53 ± 0.09; Treatments = 4.60 ± 0.16; P < 0.001). This overcompensation persisted through the end of the experiment (day 63: Controls = 3.27 ± 0.07; Treatments = 4.85 ± 0.14; P < 0.001).

**Feed Consumption and Feed Conversion**

Control fish, which were fed 2X daily to apparent satiation, had a gradual yet significant increase in feed conversion ratio throughout the experiment (Table 2) (P < 0.01), coupled with a gradual and significant decrease in feed consumption (% BW/d) (Table 2) (P < 0.001). While no data was available for either of these parameters during feed restriction for treatment HSB, the subsequent CG response (days 22-42) was marked by both hyperphagia (2.46 ± 0.05 % BW/d for treatments; 1.48 ± 0.05 % BW/d for controls; P < 0.01) and improved feed conversion (0.87 ± 0.03 for treatments; 1.14 ± 0.07 for controls; P < 0.01; Table 2). Both feed consumption and feed conversion ratios returned to control values 4-6 weeks into the refeeding period in treatment fish, coinciding with the decline
in CG response. Overall FCR (days 0-63) for treatment fish was statistically similar to that of controls. Interestingly, a highly significant and positive correlation was obtained between feed consumption (% BW/d) and HSI values ($R^2 = 0.64; P < 0.001$), suggesting that HSI may be used as a biomarker for varying degrees of feeding intensity, including periods of feed deprivation.

**Gene Cloning**

Sequencing results indicated that the striped bass amplicon generated with degenerate IGF-II primers shared 98% nucleotide identity with the closely related European sea bass (*Dicentrarchus labrax*) IGF-II (Terova et al. 2007), confirming we cloned a partial sequence of IGF-II in striped bass. The striped bass amplicon generated with degenerate GHR1 primers shared 84 and 94% nucleotide identity with gilthead sea bream (*Sparus aurata*) (Saera-Vila et al. 2005; Jiao et al., 2006) and European sea bass GHR1 sequences, respectively, and only 58 and 59% identity with GHR2 sequences from these same species. Based on these identities with closely-related species, we consider the striped bass amplicon a partial clone of the putative type I GHR and hence refer to it here as GHR1. It should be noted, however, that it is not certain whether the GHR1 in non-salmonid fishes is a true GHR (Jiao et al. 2006; Li et al. 2007) or instead the somatolactin receptor (SLR) (Fukada et al. 2005; Fukamachi et al. 2005; Pierce et al. 2007). While SL has been shown to bind more strongly to the GHR1 than GH in salmonids (Fukada et al. 2005; Fukamachi et al. 2005), only GH, and not SL, could activate the GHR1 in gilthead seabream (Jiao et al. 2006), a more closely related species to the striped bass (Order Perciformes). Furthermore, injection of SL resulted in the elevation of hepatic IGF-I
mRNA in coho salmon (Duan et al. 1993), indicating that there may be consequences for growth regardless of the classification.

**Hepatic Gene Expression**

When hepatic IGF-I qRTPCR data was normalized to copy number / ng total RNA (Figure 3A), 3 weeks of feed restriction in treatment fish (days 0-21) resulted in a 27% increase in IGF-I copy number (P < 0.05), followed by a subsequent decline 8 days into realimentation and the CG response (day 29; P < 0.001). These same trends were observed when data was normalized to the housekeeping gene 18S, and suggests that IGF-I mRNA may be accumulated during feed restriction and rapidly translated during CG (Picha et al. 2006). While data normalized in either of these manners is an accurate reflection of gene expression trends relative to a fraction of total liver RNA, it does not take into account the total amount of mRNA produced by the liver and presumably released into the circulation. Indeed, during nutritional manipulations the liver shows dramatic fluctuations in size relative to body weight, as indicated by HSI values in this study (Figure 2). Similarly, total hepatic RNA content also fluctuates with HSI (R² = 0.41; P < 0.001), whereby smaller HSI values in feed deprived fish have the least amount of total liver RNA, and vice versa for fish in an anabolic state (Figure 2). Therefore, in order to determine the actual content of a specific mRNA produced in response to nutritional status, it is important to account for gene expression output for the entire liver. For this reason, results for IGF-I and all other hepatic mRNA species are expressed as total liver mRNA as a function of body weight (total liver copy number / BW). This
normalization, like that for many endocrine organs whose size changes with physiological state, better reflects the biological significance of gene expression patterns.

When hepatic IGF-I gene expression was expressed as total amount of hepatic mRNA as a function of body weight (liver IGF-I copy number / body weight), control fish experienced no significant variations from time 0 values throughout the experiment (Figure 3B). The pattern of gene expression in control fish using this more appropriate method of normalization is virtually identical to that seen when hepatic gene expression is normalized as a fraction of total RNA (Figure 3A). This would be expected in livers where little change occurs in HSI. Following feed restriction, treatment HSB experienced a 3.5-fold decline in total hepatic IGF-I mRNA levels (P < 0.001; Figure 3B) that was 64% lower than control fish at day 21 (P < 0.01). Upon refeeding, levels rose 8 days into the CG response, the shortest time point measured (day 29; P < 0.05). By three weeks of realimentation, a 530% increase in total IGF-I mRNA was observed that exceeded control values (day 42; P < 0.001). This dramatic rise in hepatic IGF-I mRNA, which coincided with the duration of the CG response, was subsequently restored to control levels by the end of the experiment.

With regard to hepatic IGF-II gene expression, no significant changes in total mRNA levels were observed for control fish during the initial 3-weeks, after which time levels slowly declined and plateaued by the end of the experiment (Figure 4A). Treatment HSB, on the other hand, experienced an over 6-fold decline in total liver IGF-II mRNA following 3 weeks of complete feed restriction (P < 0.001). Despite this precipitous
decline, levels rose by 377% (P < 0.001) and had completely caught up to controls 8 days into refeeding and the CG response. After this initial increase, values continued to rise for the remainder of the CG response (day 42), exceeding that of control fish by 2.7-fold (P < 0.001; Figure 4A). However, this elevation at day 42 relative to controls may be diminished, at least somewhat, when considering mRNA levels as an allometric function of body weight. Similar to trends for hepatic IGF-I gene expression, IGF-II mRNA declined back to control levels over the last 3 weeks of realimentation (days 43-63), the period over which CG was not observed.

Alterations in GHR1 mRNA was similar to that observed for IGF-II mRNA. In controls, no significant changes were observed during the first 29 days of the experiment, after which time they underwent a slight decrease and then remained level for the remainder of the trial (Figure 4B). Treatment fish experienced a 5-fold decline in total hepatic GHR1 mRNA levels during 3 weeks of feed deprivation (P < 0.001), followed by a rapid, 3.2-fold increase upon 8 days of refeeding (P < 0.001). By 3-weeks of realimentation, GHR1 levels surpassed that of control fish (P < 0.05) and reached similar levels by the end of the experiment.

Plasma IGF-I

Plasma IGF-I rose slightly in control fish over the course of the experiment (Figure 5A). By contrast, treatment HSB experienced a 61% decline in plasma IGF-I (P < 0.001) following feed restriction (days 0-21) to a value that was significantly lower than control levels at any time during the experiment (P < 0.001) (Figure 5A). Upon refeeding, plasma
IGF-I increased by 8 days and reached control levels by 3 weeks of realimentation, after which point levels remained similar to controls. A significant correlation was seen with plasma IGF-I and SGR ($R^2 = 0.54$, $P < 0.001$; Figure 6). Responses in plasma IGF-I in treatment fish subjected to the CG feeding protocol were also similar to that observed for hepatic IGF-I mRNA ($R^2 = 0.50$, $P < 0.001$; data not shown).

**Plasma 40kDa IGFBP**

The 40 kDa IGFBP, thought to be a putative mammalian IGFBP-3 (Siharath et al. 1995; Shimizu et al. 2003), displays distinct expression patterns in relation to feed restriction and CG (Figure 5B). Specifically, levels of the 40kDa IGFBP decreased by 74% ($P < 0.001$) following 3 weeks of feed restriction (days 0-21) to values well below those of controls ($P < 0.001$). Similar to that observed with circulating IGF-I, the 40kDa IGFBP was completely restored to control values 3 weeks into the CG response when fish were refed.

**Skeletal Muscle Gene Expression**

The catabolic state induced through complete feed restriction in treatment fish resulted in an 89% decrease in skeletal muscle IGF-I mRNA ($P < 0.001$), which was also significantly lower than controls at this same time ($P < 0.05$; Figure 7). IGF-I mRNA increased 10 fold ($P < 0.001$) from their previously depressed levels and were completely restored to control values by 8 days of realimentation. Gene expression levels were similar between control and treatment groups for the remainder of the experiment. There
were no significant differences in skeletal muscle IGF-II, IGFR or GHR mRNA levels either within treatment across time or between treatments within time (Table 3).

**DISCUSSION**

This study demonstrates that a sufficient period of feed restriction and catabolism can induce subsequent periods of CG upon refeeding in hybrid striped bass (HSB). The response is characterized by elevated specific growth rates (SGR), hyperphagia and improved feed conversion. Furthermore, total hepatic IGF-I, IGF-II and GHR1 mRNA increases during CG from previously depressed levels observed with catabolism. The CG response was also accompanied by considerable increases in plasma IGF-I, a 40kDa IGFBP and skeletal gene expression of IGF-I. No change in skeletal muscle gene expression was detected for IGFR, IGF-II or the GHR1 during the different metabolic states associated with CG feeding protocols.

Compensatory growth is a period of growth acceleration that exceeds normal rates after animals are alleviated of certain growth-stunting conditions. In fishes, feeding manipulation remains the most common means of inducing the response, with periods of feed restriction and typically some degree of weight loss being followed by satiation feeding (see Ali et al. 2003 for review). In those species where CG has been demonstrated, either partial or full catch-up growth was induced in Atlantic halibut (Heide et al. 2006), channel catfish (Gaylord et al. 2000), European sea bass (Terova et al. 2006), hybrid sunfish (Hayward et al. 1997) and rainbow trout (Montserrat et al. 2007) through variations of this protocol. In HSB, partial feed restriction has lead to full
compensation in individually-housed fish (Skalski et al. 2005), along with partial CG in group-housed HSB in both ponds (Turano et al. 2007) and tanks (Picha et al. 2006).

In this study we demonstrate that tank-raised, group-housed HSB exhibit strong CG responses following complete feed restriction, and that the response is characterized by an almost 2-fold increase in SGR relative to control fish over the same time interval. An additional consideration that has received little attention in most CG studies (see Ali et al. 2003), including those evaluating the endocrine basis of the response, is the allometric relationship between growth rates and body mass (Jobling 1994). Indeed, smaller fish tend to grow faster than larger fish, raising the question as to whether elevations in SGR of growth-stunted fish are the result of their smaller body size or a ‘true’ CG response. We found that SGR was 50% higher than control fish during the CG response when initial body size was accounted for (control fish day 0-21, 1.70 %BW/day versus treatment fish day 22-42, 2.52 %BW/day). This is in agreement with our earlier reports in HSB where CG was induced following a period of partial feed restriction (Skalski et al. 2005; Picha et al. 2006).

Hyperphagia and improved feed conversion were observed alongside the elevated SGRs during the CG response (day22-42; Table 2). Hence, these behavioral and metabolic responses likely contribute to phases of accelerated growth associated with CG. Based on previous reports and results herein, it would appear that a certain catabolic threshold is necessary to drive these higher growth rates, feed consumption and improved feed conversion. In previous studies we found that a combination of low HSI (< 1.5) and
body weight loss are necessary to induce subsequent CG responses (Picha et al. 2006; Turano et al. 2007). In the current study both of these conditions were met following feed restriction (7.7% BW decrease, Figure 1A; HSI = 1.11, Figure 2).

Feeding manipulation protocols that induce periods of stunted (negative), normal and accelerated (compensatory) growth can provide methods for elucidating the endocrine control of variable growth rates and its control by the GH-IGF axis. We found that circulating IGF-I paralleled altered growth states during CG feeding protocols, declining during growth depression and increasing during CG. Likewise, total hepatic IGF-I mRNA levels also changed in a fashion similar to both circulating hormone and growth, suggesting that the synthesis and secretion of hepatic IGF-I are important in mediating growth responses observed with stunted and rapid growth states. Interestingly, we found that IGF-I mRNA levels exceeded control fish during the CG response, raising the likely possibility that elevated transcript levels may contribute to the rapid rise in circulating IGF-I during CG. The significant correlation between total hepatic IGF-I mRNA and circulating IGF-I supports this notion, and that suggesting the liver is the primary source of circulating hormone, which has been firmly established in mammalian models using selective knockouts of the hepatic IGF-I gene (Yakar et al. 1999; Sjogren et al. 1999).

The majority of circulating IGF-I in fish is bound to IGFBPs, making them prime mediators of the mitogenic effects of IGF-I (Shimizu et al. 1999; Duan et al. 2005). Based on MW and physiological response to feeding manipulation, the 40kDa IGFBP detected in this study is likely the mammalian equivalent of IGFBP-3, which is reported
to increase IGF-I half-life and regulate its availability to target tissues (Kelley et al. 2001; Shimizu et al. 2003; Rodgers et al. 2007). Accordingly, levels of the 40kDa IGFBP paralleled those of circulating IGF-I, declining during catabolism and increasing to levels of control fish by termination of the CG response. These studies are the first to show circulating 40kDa IGFBP trends during CG responses, with the strong up-regulation during realimentation possibly contributing to elevated plasma IGF-I by increasing its half-life. This also corroborates earlier studies in which similar trends in 40kDa IGFBP were observed with fluctuations in nutritional state of the more primitive salmonid teleosts (Beckman et al. 2004; Pierce et al. 2005). Evidence in mammals also suggests that some IGFBPs may have ligand-independent mitogenic effects (see Duan 2005), underscoring the need to further investigate their role in fishes during CG.

During catabolic states preceding CG responses or following most periods of feed restriction in fishes, a state of GH resistance is induced in which elevated plasma GH is generally countered by depressed hepatic GHR binding and gene expression, which ultimately results in low levels of both hepatic IGF-I mRNA and plasma IGF-I (Pierce et al. 2005; Norbeck et al. 2007). In an alternative form of GH resistance seen during feeding manipulation, plasma GH increases and hepatic IGF-I gene expression declines with no change in hepatic GHR mRNA (Fox et al. 2006; Gabillard et al. 2006), raising the possibility that GHR signaling may be impaired (Beauloye et al. 2002). In this investigation, GHR1 mRNA levels declined during feed deprivation along with hepatic IGF-I mRNA and circulating IGF-I peptide. This, along with evidence that pituitary GH mRNA, GH content and plasma GH increase in these fish (Turano et al. 2006;
unpublished results) suggests a GH resistance in HSB, although further research is required to confirm that GHR binding declines in conjunction with mRNA levels, as has been recently shown for rainbow trout (Norbeck et al. 2007). Previous work in HSB has shown that IGF-I binds with high affinity to the pituitary and acts as a powerful negative feedback inhibitor of GH synthesis and secretion (Fruchtman et al. 2000; Fruchtman et al. 2002). It is likely, therefore, that elevations in pituitary GH activity and plasma GH levels are a reflection of the prevailing low circulating IGF-I seen during states of catabolism and vice versa during anabolism.

As previously indicated, we found that total hepatic IGF-I mRNA levels decline during catabolism and increase upon realimentation. However, when hepatic IGF-I gene expression is normalized according to standard procedures, i.e. to copy number / ng RNA or to a housekeeping gene, an elevation in IGF-I mRNA is observed during the catabolic state of feed deprivation in HSB (Figure 3A). To this end, other studies in fishes using similar normalizations have reported either increases or no change in hepatic IGF-I mRNA following feed restriction (Picha et al. 2006; Ayson et al. 2007; Pierce et al. 2007). The lack of concordance in hepatic IGF-I mRNA and circulating hormone may be resolved by the alternative method of mRNA normalization presented here. When measuring mRNA levels in an organ whose size relative to body weight changes with nutritional state, we argue that it is more appropriate to consider hepatic gene expression with respect to total organ output rather than as gene expression per unit of total RNA. Since HSI fluctuates dramatically between feed restriction and CG (Figure 2) and its values correlate to total liver RNA, expressing hepatic gene expression data as a function
of the total amount in the organ relative to body size seems most appropriate (total liver copy number / BW). Indeed, the amount of transcript produced by the entire liver relative to the size of the fish provides a more accurate indication of the IGF-I levels present in the circulation. A similar qRT-PCR normalization scheme was utilized in gene expression analyses of spermatogenesis in rainbow trout where testes size varies considerably with reproductive state (Kusakabe et al. 2006).

Relative to IGF-I, little is known about the role of IGF-II in postnatal growth despite its high expression levels in the liver of fish, ability to bind with equal affinity as IGF-I to the IGF receptor, and potential for up-regulation by GH (Shamblott et al. 1995; Vong et al. 2003; Fruchtman et al. 2002; Peterson et al. 2005). In HSB, total hepatic IGF-II mRNA levels fell dramatically during feed deprivation and rose very rapidly upon realimentation, eventually exceeding those of control fish by day 21 of the experiment. We found no difference in muscle IGF-II mRNA expression between control and treatment fish. Studies in European seabass showed both muscle and liver IGF-II changed with growth state and feeding (Terova et al. 2007). Hepatic and muscle IGF-II mRNA is also higher in fast versus slow-growing families of catfish (Peterson et al. 2004). In rainbow trout, both hepatic IGF-II mRNA and plasma IGF-II were depressed during fasting and were subsequently up-regulated during refeeding (Gabillard et al. 2006). Our results suggest that IGF-II may contribute to the rapid growth characteristic of CG and the negative growth seen with catabolism. This, along with the research of others, strengthens the hypothesis that IGF-II might serve as an important regulator of
postnatal growth in fish, although evidence for direct mitogenic effects of IGF-II is still lacking in teleosts.

Aside from the endocrine effects of IGF-I and possibly IGF-II, local paracrine/autocrine dynamics of IGF-I may also contribute to the phase of accelerated growth. The importance of locally-produced IGF-I to growth has gained considerably more attention due to studies in mice that show hepatic IGF-I gene deletion results in depressed levels of plasma IGF-I but little change in body weight (Sjogren et al. 1999; Yakar et al. 1999). Similarly, transgenic tilapia over-expressing GH were 2-fold heavier than wild types yet had depressed levels of plasma IGF-I and elevated skeletal muscle IGF-I gene expression (Eppler et al. 2007). In studies here, we found that skeletal muscle IGF-I mRNA increased 10 fold from previously depressed catabolic levels and was completely restored to control values by 8 days into the CG response (Figure 7). These findings and those in rainbow trout and European sea bass (Montserrat et al. 2007; Terova et al. 2007) show that paracrine IGF-I may facilitate the rapid growth characteristic of CG responses in fish. We speculated that the IGF receptor might increase to enhance tissue sensitivity to IGFs during CG. However, we found that gene expression of the receptor in skeletal muscle was not altered with either feed deprivation or the rapid growth phase characteristic of CG. This does not preclude the possibility, however, that the receptor itself might be up-regulated or that it might show greater responsiveness to rapid fluctuations in systemic or local (muscle) IGF-I compared with relatively constant levels of ligand with fish on continuous daily feeding (Borski et al. 2000).
In conclusion, we have found that complete feed restriction can prime HSB for CG responses and that this catabolic state is characterized by weight loss, a drop in HSI below threshold levels and an endocrine state of GH resistance. During the subsequent phase of refeeding and the ensuing CG response, elevations in growth rates are likely driven by a combination of hyperphagia, improved feed conversion and heightened levels of IGF-I, 40kDa IGFBPs and possibly IGF-II. Our results also suggest that paracrine/autocrine IGF-I may be contributing to enhanced growth during CG. In addition, we propose that hepatic mRNA levels of specific genes be expressed as the total amount produced by the organ. This method provides a more physiologically relevant idea of the amount of gene produced by a tissue whose size changes dramatically with nutritional state.

**ACKNOWLEDGEMENTS**

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Table 1
Primer and Taq Man probe sequences for various hybrid striped bass genes measured in liver and muscle tissues by quantitative RTPCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>For primer</th>
<th>TaqMan probe</th>
<th>Rev primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I</td>
<td>5’-TTGTGTGTGGAG AGAGAGGCTTT-3’</td>
<td>5’-TTTCAGTAAACCT ACAGGCTATGGCC-3’</td>
<td>5’-TGACCGCC GTGCATTG-3’</td>
</tr>
<tr>
<td>IGFR</td>
<td>5’-CGCTCTCTCCG ACCGAAAGAG-3’</td>
<td>5’-TGGTCAGCCTGT CACTCCCTCTCT-3’</td>
<td>5’-GACCGGCCATCT GAAGCATCTTC-3’</td>
</tr>
<tr>
<td>IGF-II</td>
<td>5’-AAACAACAGAC GGCCCCAGAA-3’</td>
<td>5’-CGTGGGATCGTGAAGGAGTGGTTTCTCC-3’</td>
<td>5’-AGCAAGTGGAG GCCACAGCTA-3’</td>
</tr>
<tr>
<td>GHR I</td>
<td>5’-TCAGCAACCACA TGAACATAGGA-3’</td>
<td>5’-TGCCAATGTCAT CAGCTTCCCCTC-3’</td>
<td>5’-GGCAGGGCC TGAGTCATC-3’</td>
</tr>
<tr>
<td>18S</td>
<td>5’-TGAAAAACATTC TTGGCAATAGGC-3’</td>
<td>5’-TTCGCTTTTCTGC CGTCTTCCCGC-3’</td>
<td>5’-GCCGCTAGAGGT GAATTTCTTG-3’</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5’-GCCTTCTCTC CCTCAGTATGG-3’</td>
<td>5’-CCTGCAGGAATC CAGAGACCACC-3’</td>
<td>5’-CGCAGCTTCAT GATGCTTTGT-3’</td>
</tr>
</tbody>
</table>
Table 2
Feed conversion ratios and feed consumption of control fish fed to satiation 2X daily and treatment fish subjected to 3 weeks feed restriction (days 0-21) followed by 6 weeks of refeeding 2X daily to satiation (days 22-42, 43-63). No data was available for treatments during days 0-21 because fish were not fed during this time. Treatment HSB had improved feed conversion and increased feed consumption during the CG response (days 22-42).

<table>
<thead>
<tr>
<th>Days</th>
<th>Control a</th>
<th>Treatment b</th>
<th>Control a</th>
<th>Treatment b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-21</td>
<td>0.97 ± 0.01</td>
<td>N/A</td>
<td>1.56 ± 0.07</td>
<td>N/A</td>
</tr>
<tr>
<td>22-42</td>
<td>1.14 ± 0.07</td>
<td>0.87 ± 0.03 **</td>
<td>1.48 ± 0.05</td>
<td>2.46 ± 0.05 **</td>
</tr>
<tr>
<td>43-63</td>
<td>1.20 ± 0.07</td>
<td>1.23 ± 0.08</td>
<td>1.11 ± 0.06</td>
<td>1.25 ± 0.12</td>
</tr>
</tbody>
</table>

**P < 0.01 represents significant differences from controls at the same time point.
\[ aP < 0.001 \] represents significant differences across time within the control group.
\[ bP < 0.001 \] represents significant differences across time within the treatment group.
N = 4 tanks /group with 30-50 fish/group
Figure 1. (A) Mean body weight (g) and (B) specific growth rates (SGR; %/day) of control HSB fed to satiation 2X daily and treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding. CG indicates a period of compensatory growth for treatment fish as defined by elevated SGRs relative to similar-sized controls (time 0 controls = 71.4 ± 1.3g; day 21 treatments = 65.8 ± 0.7g). Asterisks represent significant differences between groups at each time point (**P < 0.01; ***P < 0.001). (N = average values of individual tanks with 4 replicate tanks/group and 30-50 fish/tank). Letters represent significant differences within and between groups across time (P < 0.05).
Figure 2. Hepatosomatic index of control HSB fed to satiation 2X daily and treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding 2X daily to satiation. Asterisks represent significant differences between groups at each time point (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) (N = 16 fish/group).
Figure 3. Hepatic mRNA levels of IGF-I in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding 2X daily to satiation. Hepatic mRNA levels were expressed as (A) IGF-I copy number / ng total RNA and (B) total hepatic IGF-I copy number / body weight. Asterisks represent significant differences between groups at each time point (***(P < 0.001) (N = 6-8 fish/group).
Figure 4. Total hepatic mRNA levels for (A) IGF-II and (B) GHR1 in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding 2X daily to satiation. Asterisks represent significant differences between groups at each time point (*$P < 0.05$; ***$P < 0.001$) (N = 6-8 fish/group).
Figure 5. Plasma (A) IGF-I (ng/ml) and (B) 40kDa IGF binding protein (arbitrary density units, ADU) in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding 2X daily to satiation. Asterisks represent significant differences between groups at each time point (**$P < 0.01$; ***$P < 0.001$) (N = 6-8 fish/group).
Figure 6. Correlation between plasma IGF-I (ng/ml) and specific growth rate (SGR; %/day) in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction followed by 6 weeks of refeeding 2X daily to satiation. Specific growth rates (days 0-21, 22-42, 43-63) reflect the pairing of mean weights of fish with mean plasma IGF-I values from 8 tanks (4 tanks/group). Fish were sampled at day 21, 42 and 63.
Figure 7. Insulin-like growth factor-I (IGF-I) mRNA expression in muscle tissue in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction (grey bar) followed by 6 weeks of refeeding 2X daily to satiation. Data is expressed as IGF-I copy number per ng total RNA. Grey bar denotes a period of feed restriction for treatment fish. Asterisks represent significant differences between groups at each time point (*P < 0.05) (N = 8-16 fish/group).
Table 3
Skeletal muscle mRNA levels (copy number / ng RNA) for IGF-II, IGFR and GHR1 in control HSB fed to satiation 2X daily and in treatment fish subjected to 3 weeks feed restriction (days 0-21) followed by 6 weeks of refeeding (days 22-63).

<table>
<thead>
<tr>
<th>Day</th>
<th>IGF-II Control</th>
<th>IGF-II Treatment</th>
<th>IGFR Control</th>
<th>IGFR Treatment</th>
<th>GHR1 Control</th>
<th>GHR1 Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>131.5 ± 10.8</td>
<td>131.5 ± 10.8</td>
<td>655.3 ± 91.7</td>
<td>655.3 ± 91.7</td>
<td>7703.4 ± 361.2</td>
<td>7703.4 ± 361.2</td>
</tr>
<tr>
<td>21</td>
<td>173.1 ± 16.4</td>
<td>169.6 ± 16.4</td>
<td>439.7 ± 87.6</td>
<td>499.4 ± 106.9</td>
<td>9751.4 ± 864.0</td>
<td>8381.7 ± 744.9</td>
</tr>
<tr>
<td>29</td>
<td>135.6 ± 18.1</td>
<td>98.8 ± 5.1</td>
<td>368.6 ± 31.5</td>
<td>333.2 ± 43.7</td>
<td>7883.7 ± 1026.6</td>
<td>8259.5 ± 609.7</td>
</tr>
<tr>
<td>42</td>
<td>186.2 ± 18.6</td>
<td>164.1 ± 18.6</td>
<td>827.8 ± 121.6</td>
<td>736.5 ± 93.1</td>
<td>8447.9 ± 1567.0</td>
<td>8740.8 ± 905.3</td>
</tr>
<tr>
<td>63</td>
<td>185.3 ± 18.0</td>
<td>167.6 ± 31.9</td>
<td>165.3 ± 51.6</td>
<td>313.2 ± 89.8</td>
<td>10,174.2 ± 735.2</td>
<td>9847.3 ± 998.4</td>
</tr>
</tbody>
</table>

Time 0 samples were combined between groups for each gene
*values reflect (copy number/ng RNA) X 1000
No significant differences were reported between groups for each gene (P < 0.05).
N = 8 fish/group
CHAPTER III

Complete Compensatory Growth In Hybrid Striped Bass (*Morone chrysops* X *Morone saxatilis*) Through Combined Cold-Banking and Feeding Manipulations
ABSTRACT

Teleosts and other aquatic ectotherms have the ability to withstand prolonged periods of low water temperatures and starvation, and can often respond with phases of accelerated (compensatory) growth when favorable conditions are restored. For fishes located within temperate zones, including pond-reared aquacultured species, seasonal fluctuations in water temperature can result in natural periods of over-wintering (cold-banking). We assessed whether or not feed restriction prior to (24°C, days 0-23) and/or during (14°C, days 24-114) a simulated period of cold-banking could elicit episodes of compensatory growth upon warm-up to 24°C and satiation feeding. Control hybrid striped bass (HSB: *Morone chrysops* X *Morone saxatilis*) were fed to satiation throughout the experiment under these identical temperature fluctuations. Complete catch-up growth was achieved in groups of HSB that were deprived of feed during either the initial period at 24°C (days 0-23) or the cold-banked period (14°C, days 24-114), or during both of these periods (days 0-114). The response was defined by specific growth rates up to 4.2 times that of controls and was accompanied by hyperphagia and improvement in feed conversion. Furthermore, the catabolic state preceding compensatory growth (CG) in feed restricted HSB was accompanied by a diminution of hepatic cell and organ size (hepatic atrophy) and an apparent accumulation of lipids in the liver that were alleviated upon refeed. This is the first study, to our knowledge to achieve full catch-up growth through feed restriction and temperature manipulation in any non-salmonid teleost. Furthermore, our studies suggest that a previous period of cold-banking and feed restriction followed by a return to more favorable rearing conditions can improve overall feed conversion by as much as 30% with no adverse effects on growth of HSB. This raises the possibility that
CG protocols have a strong potential to improve production efficiency of HSB in pond or tank culture.
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 (Fry 1971; Kaushik 1986; Clarke 1999). The onset of better rearing conditions, however, can result in episodes of growth acceleration known as compensatory growth (CG) (Wilson and Osbourne, 1960; Morgan and Metcalfe, 2001). The CG response has been documented in numerous teleosts along with mammals and birds, and is typically, but not always, accompanied by elevated growth rates, hyperphagia and improved feed conversion (Yu et al. 1990; Largo 1993; Hornick et al. 2000; Ali et al. 2003). Although fish are the most studied of the vertebrate taxa, most studies have focused on feeding manipulation alone to induce CG responses (see Ali 2003 for review). By contrast, surprisingly little is known about the effects of a combination of water temperature and feeding manipulation on CG responses in fish. A recent study in brown trout suggests that full catch-up growth occurs in the wild, albeit at the cost of some mortality (Johnsson and Bohlin 2006). Other studies, limited to salmonids, also suggest that temperature alone may be effective in inducing CG (Mortensen and Damsgard 1993; MacLean and Metcalfe 2001).

Hybrid striped bass (HSB: Morone chrysops X Morone saxatilis) and its related species are a valuable recreational and aquacultured species, including in the United States where fish are exposed to natural periods of over-wintering in lakes/reservoirs and in aquaculture ponds in temperate environments. While CG responses have been elicited in HSB through partial and full feed restriction, (Skalski et al. 2005; Picha et al. 2006;
Turano et al. 2007), no studies within the family Moronidae or of nonsalmonids generally have assessed whether CG can be induced through both temperature and feed manipulation, despite the basic biological and practical implications it may have on all temperate-zone fish. The purpose of this study, therefore, was to determine if a CG response occurs in HSB following feed restriction prior to and during prolonged overwintering conditions. We also assessed various morphological and somatic indices to evaluate the metabolic conditions that preceded, accompanied and followed the CG response.

**MATERIALS and METHODS**

*Experimental Design*

Phase II juvenile hybrid striped bass (180-220g) 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 1100L circular tanks (70 HSB / tank) divided between 2 partial freshwater flow-through systems. Each of the 4 treatment groups (see below) were 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: 1) days 0-23 at 24°C, 2) days 24-114 at 14°C and 3) days 115-148 at 24°C. Throughout this manuscript, days 24-114 will be referred to as the cold-banked period, even though this includes one week water temperature transition periods each for 24-14°C and 14-24°C. Optimal growth for HSB occurs at 25-27°C, and therefore rearing at 14°C is considered a period of cold-banking (Hodson 1995). Temperature data was recorded with Hobo pendant data loggers (Bourne, MA).

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-banked period at 14°C (days 24-114). Treatments are as follows: S-S fish were starved (S) at 24°C and starved at 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; 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 compensatory growth (CG) responses. All feeding was to apparent satiation 1X / day (5X / week) between 7 and 9am. Mortality because of treatment was only observed in the S-S group, and only amounted to 1.4%. However, one S-S tank was lost because of a plumbing malfunction at the end of the cold-banked period, which left one remaining tank for the refeed period at 24°C (see Statistical Analysis methods).
Sample collection

Body weights (g), standard lengths (mm) and tissue indices (liver, mesenteric fat) were taken at initiation of the experiment (Time 0), at the end of the initial 24ºC rearing period (day 23), at the end of cold-banking (day 114), and 19 and 34 days into the final 24ºC rearing period (days 134 and 148). Additional liver samples for transmission electron microscopy were taken after cold-banking just prior to when temperature was transitioned from 14 to 24ºC (day 106). 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 tricane methanesulfonate (MS 222; Argent Chemical Laboratories, Redmond, WA). HSB were deprived of feed 22-24 h prior to sampling when water temperatures were at 24ºC, and 44-48 h prior when water temperatures were at 14ºC. Sampling on each date began between 8-10 am.

For transmission electron microscopy (TEM), liver samples were excised, cut to 1 mm thickness, placed in McDowell’s and Trump’s 4F:1G fixative and then kept at 4ºC until processing. For processing, samples were rinsed twice with 0.1 M sodium phosphate buffer (pH 7.2) and then placed in 1% osmium tetroxide in the same buffer for 1 hr at room temperature. Samples were rinsed 2 times in distilled water and dehydrated in an ethanolic series culminating in two changes of 100% acetone. Tissues were then placed in a mixture of Spurr (2) resin and acetone (1:1) for 30 min, followed by 2 hr in 100% resin with 2 changes. Finally, samples were placed in fresh 100% resin in molds and polymerized at 70ºC for 8 hrs to 3 days. Semi-thin (0.25-0.5 um) sections were cut with
glass knives and stained with 1% toluidine blue-O in 1% sodium borate. Ultrathin (70-90 nm) sections were cut with a diamond knife, stained with methanolic uranyl acetate followed by lead citrate and examined with a transmission electron microscope. Samples were processed at the Laboratory for Advanced Electron and Light Optical Methods at the North Carolina State University College of Veterinary Medicine (Raleigh, NC).

Growth rate and metabolic/energy indices

Specific growth rate (SGR) was calculated as \[\frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 100\], 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. Condition factor (K) was calculated as \[\frac{wt}{length^3 (cm)} \times 100\], hepatosomatic index (HSI) as \[\frac{(liver \, wt \, / \, body \, wt)}{X \, 100}\], adiposomatic index as \[\frac{(adipose \, wt \, / \, body \, wt)}{X \, 100}\], and feed conversion ratio (FCR) as \[\frac{[feed \, consumed \, / \, weight \, gain]}{X \, 100}\]. Percent body weight consumed per day (% BW/d) was calculated as \[\frac{[total \, feed \, consumption \, per \, cycle \, / \, \# \, of \, days \, in \, cycle]}{[total \, fish \, weight \, estimated \, at \, midcycle]} \times 100\]. All body, liver and feed weights for these calculations were measured in grams.

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 a two-way ANOVA (treatment X time) followed by Fisher’s LSD test. Statistical analyses were performed
with Statistica 7.0 software (Stat Soft, Tulsa, OK). The N value for all growth and body indice data (weight, length, SGR, K, HSI, ASI) was represented by individually-tagged fish, while feed consumption and conversion (%BW/d, FCR) was 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 refeed (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. Statistical significance was set at a level of $P \leq 0.05$.

RESULTS

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 (Figure 1). Meanwhile, S-F and S-S treatment groups were starved during this time and lost 7% of their BW. Control fish continued to be fed during the subsequent period of cold-banking at 14°C (days 24-114) and therefore continued to grow, albeit at a slower rate than at 24°C ($P < 0.001$). After being starved initially at 24°C, S-F treatments were fed during the cold-banked period and grew at nearly a 2-fold faster rate than the F-F controls at this same time ($P < 0.001$), indicating that feed deprivation at 24°C can lead to a compensatory growth (CG) response upon refeed at 14°C. Fed-starved HSB were completely feed restricted at 14°C and lost 14% of their body weight during this time, while S-S HSB starved at the initial 24°C phase and during cold-banking lost an additional 10% of body weight (Figure 1). From Time 0 to the end of the cold-banked
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%). The degree of weight gain/loss was also reflected in statistically different condition factor (K) indices, with net weight gain being associated with higher K values and net weight loss being associated with lower K values (Table 1).

Interestingly, while the F-S treatment group lost a considerable amount of weight following feed restriction at 14ºC (day 114) and weighed significantly less than F-F controls (Figure 1; P < 0.001), total lengths between these groups were nearly identical (Table 1). This suggests that although feed restriction during cold-banking leads to severe weight loss, total length gain is minimally affected. However, when feed restriction at 24ºC precedes feed restriction at 14ºC (S-S treatment), length gain during cold-banking appears to be compromised, as S-S treatments only gained 0.01% of length during cold-banking (days 24-114) while F-S treatments gained 2.3% (Table 1).

Regardless of the feeding manipulations that occurred at 24ºC (days 0-23) and/or 14ºC (days 24-114), all groups of HSB were placed on the control (F-F) ration (fed 1X daily to apparent satiation) after water temperatures had transitioned back to 24ºC (day 115). HSB were weighed after 19 days of refeeding (days 115-134) to assess potential CG responses, and then weighed after an additional 2 weeks of refeed (days 135-148) to determine the duration of the response. As indicated by elevated specific growth rates relative to F-F controls, all groups of fish underwent CG responses during the initial 19 days after warm-up (Figure 2; 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/lowest condition factors following cold-banking displaying the highest growth rates following refeeding at 24°C. Specifically, SGRs for S-S and F-S treatment HSB were 4.3 and 3.4 fold higher than controls, respectively, during the initial 19 days of refeeding (Figure 2; Table 2; P < 0.001). Complete body weight catch-up was achieved in S-F and F-S groups by day 134 (Control = 252.3 ± 4.8; 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) (Figure 1). 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 (Table 1). By the end of the experiment (day 148), however, S-S fish weights and lengths were statistically similar to controls (Figure 1; Table 1), while F-S weights were greater (P < 0.05) than both S-F and S-S treatments (Figure 1). 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; Table 2).

Hyperphagia, Feed Conversion and the Compensatory Growth Response

The compensatory growth 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. The most dramatic CG response was observed in S-S fish, which had SGRs 4.2 times greater than F-F controls (Figure 2). They also fed at a 2.8-fold higher rate (%BW/d) and converted feed 100% more efficiently during this time (Table 2). Overall feed conversion (days 0-148) was 30% more efficient relative to controls (Table 2). Although a full statistical analyses could not be done on S-S fish after day 114 due to the
loss of one tank replicate, 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, 2007). 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 within each treatment were consistent throughout the experiment, we expect that the lost replicate would have behaved similar to its counterpart.

Full body weight catch-up was achieved in the F-S group after 19 days of refeed. Not surprisingly, this group fed at a 2.2-fold greater rate and converted feed 100% more efficiently than F-F controls during this time (P < 0.01; Table 2). Overall feed conversion (days 0-148) was also more efficient, albeit not quite statistically significant (P < 0.09; Table 2).

Despite being starved during the initial 24°C period, feeding during the cold-banked period rendered a net weight gain for S-F fish (Figure 1). Regardless of this anabolic state, the S-F group had elevated SGRs relative to controls during the initial 19 days of the second 24°C feeding period (days 115-134; Figure 2). Hence, these fish underwent a CG response, albeit a mild one relative to F-S and S-S groups. Accordingly, feed consumption and conversion in S-F fish was intermediate to control and F-S and S-S fish,
with feed conversion being significantly better than control fish during the CG phase of
the second 24°C feeding period (P < 0.05; Table 2). S-F fish exhibited poorer overall
feed conversion than F-S and S-S fish and no significant difference from control animals
(Table 2).

Energy Indices/Reserves and Liver Histology

Hepatosomatic index in F-F controls rose from time 0 values through feeding at both
24°C (days 0-23) and 14°C (days 24-114) (Table 3) and gradually dropped during 19 and
34 days into the 24°C warm-up period. In S-F fish, a significant drop in HSI was
observed at 24°C with starvation. Values were restored to control levels after refeed at
14°C. Similar to the trend with control fish, a gradual fall in HSI was observed after water
temperatures returned to 24°C, but levels remained well above that seen at the initiation
of the experiment. In F-S treatment fish, feed restriction during the cold-banked period
led to a 78% drop in HSI (P < 0.001; Table 3). Upon refeed at 24°C, which induced
hyperphagia and a CG response, HSI increased by 515% to values well above controls (P
< 0.001). HSI remained well above controls by day 148 even after the CG response and
hyperphagia had subsided. S-S treatment fish had HSI values that were 60% lower than
controls after starvation at 24°C (P < 0.001). Interestingly, values did not significantly
decrease any further during the subsequent 90-day period of feed restriction and cold-
banking. Following 19 days of refeeding where the most pronounced hyperphagic
response was induced, HSI increased by 370% to values above all other groups, including
F-S HSB which were also undergoing hyperphagia and CG (see day 134, Table 3).
Values remained elevated until the end of the experiment when CG and hyperphagia were no longer apparent.

Despite a continuous feeding regimen, adiposomatic index (ASI) underwent a gradual yet significant decrease in control fish during initial feeding at 24°C as well as during cold-banking, after which time it plateaued for the remainder of the experiment at 24°C (Table 3). Interestingly, the lone significant differences between groups came at day 114, when feed restriction during cold-banking resulted in depressed ASI values in F-S and S-S HSB. Values were restored to control levels after refeeding at 24°C.

Lipids were ultrastructurally identified from HSB liver sections through transmission electron microscopy (Figure 3). At the end of the initial period at 24°C, feed restricted S-S HSB appeared to have a greater accumulation of lipids relative to control fish fed daily (Figure 3B versus 3A). This accumulation appeared to be exacerbated following an additional period of feed restriction combined with cold-banking (Figure 3D). After refeeding at 24°C, hepatic lipid levels appeared significantly reduced in S-S HSB from their previous starvation/overwintering state and closely resembled that of control fish fed throughout the experiment (Figure 3F versus 3E). Quantitative assessments of total liver lipid levels will be required to definitively determine whether more lipid is present in the livers of feed-restricted versus fed fish.
DISCUSSION

This study demonstrates that CG can be achieved in HSB following a combination of temperature and feeding manipulation. The response is characterized by considerable elevations in growth rate and feed consumption, along with significant improvements in feed conversion. While the CG response was preceded in one instance by a general anabolic state, the most dramatic states of CG were preceded by extreme states of catabolism characterized by weight loss and depressed HSI and ASI values, and possibly hepatic atrophy. 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. To our knowledge, this is the first study in nonsalmonid fishes to demonstrate that a return to more favorable conditions following a previous period of cold-banking and feed restriction can lead to full catch-up growth.

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 (Broekhuizen et al., 1994; Van Dijk et al., 2005). 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; Lundqvist et al., 1994; Nicieza and Metcalfe 1997). The period of accelerated growth following an episode of growth cessation known as CG has been demonstrated in a number of animal species (Wilson and Osbourne, 1960; Morgan and Metcalfe, 2001). Since both of the parental lines used to create hybrid striped bass (white bass *Morone chrysops*; striped bass *M. saxatilis*) experience periods of overwintering and low prey
availability in their natural environments (Karas 1993; Kohler 1997), the potential for HSB to undergo CG responses following these same conditions seems apparent. Indeed, relative to controls (F-F) fed throughout the experiment, fish that were starved prior to and during cold-banking (S-S treatments) or just over the 14°C cold-banking period (F-S treatments) showed dramatic CG responses upon refeeding at 24°C. These fish grew at rates that well exceeded those of control fish over the realimentation period and, more appropriately, relative to control fish of a similar initial body size. Complete catch-up growth occurred after 19 days (days 115-134) in the F-S group (Figure 1), while the S-S group had statistically similar weights as controls by 34 days of refeed (day 148). Full catch-up following feeding and temperature manipulation was also achieved in brown trout *Salmo trutta* (Johnsson and Bohlin 2006). Previous studies in other salmonids, the Arctic charr *Salvelinus alpinus* and Atlantic salmon *Salmo salar* also show complete catch-up growth following temperature manipulations alone (Mortensen and Damsgard 1993; MacLean and Metcalfe 2001).

While increasing water temperatures (14 to 24°C) and the associated Q10 effects (Angiletta et al. 2004) are likely responsible for elevated growth rates in all groups of HSB following cold-banking (Figure 2; Table 2), the mechanisms responsible for growth rates that exceed those of controls (CG responses) are less clear. Hyperphagic responses are a commonly-observed behavior driving CG, and several lines of evidence suggest that a humoral signal released in proportion to adipose tissue sends negative feedback to appetite centers in the brain (Bull and Metcalf 1997; Johansen et al. 2001, 2002). That is, low adipose stores (i.e. during starvation) result in reduced negative feedback and
increased appetite. Indeed, hyperphagic responses during CG in S-S and F-S treatment
groups were preceded by significant depressions in ASI, with feed consumption returning
to normal rates after ASI reached control levels (Table 3). Associated with these
elevations in feed consumption are significant improvements in feed conversion, which
were observed in all 3 groups undergoing CG in this study (Table 2). Other contributions
to accelerated growth might include lower cumulative maintenance costs of treatment
fish that are smaller for longer periods of time than control animals (Skalski et al. 2005),
heightened responses in endocrine growth factors and their receptors (Wood et al. 2005;
Picha et al. 2006, 2008) and an enhanced ability to synthesize muscle proteins. The latter
occurs in Atlantic cod *Gadus morhua* during temperature-induced CG (Treberg et al.
2005).

The growth and energetic indices of our study also correspond with the two-compartment
model of CG initially proposed by Broekhuizen et al. (1994), which assumes that fish
have an ‘ideal’ reserve to structure ratio, and that their behavior and physiology will be
modified to maintain it. In particular, this model suggests that the regulation of growth
depends on the balance between tissue types which are broadly divided into reserve and
structural components; reserves referring to fat and glycogen deposits along with
mobilizable parts of the musculature (protein), and structural components referring to the
skeletal, circulatory and nervous tissue. The combination of increasing skeletal length
along with decreasing protein (body weight), glycogen (HSI) and lipid (ASI) stores that
occurred in this study during feed deprivation and cold-banking (S-S, F-S treatments) led
to dramatic reductions in reserves relative to body length (Tables 1 and 3). This change
in reserve:structure ratio is exemplified by the reduced condition factors (Table 1) seen in S-S and F-S fish, and was subsequently restored during CG through modifications in behavior (hyperphagia) and physiology (feed conversion, endocrine, metabolic). A similar restoration of reserve:structure ratio was observed during CG in European sea bass *Dicentrarchus labrax* exposed to low temperature and feed restriction. Full catch-up growth, however, was not achieved in this study (Pastouread 1991). With regards to structure, it is interesting to note that the most successful body weight catch-up occurred in HSB whose skeletal gain was not compromised despite having lost significant amounts of body weight during the catabolic state. This was observed in F-S treatments, who were fed initially at 24°C but then starved at 14°C, suggesting that lowered metabolic maintenance costs due to reduced temperatures 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 S-S treatments starved prior to and during cold-banking had significantly lower lengths relative to controls and did not catch-up in body weight after the initial 19 days of refeeding, as was observed with F-S treatments.

While the mobilization of energy reserves (protein, glycogen, lipids) allow fish to withstand prolonged periods of feed deprivation, pathological conditions may arise from these catabolic processes. In HSB deprived of feed at 24°C, transmission electron microscopy revealed an apparent hepatic accumulation of lipids relative to controls, a condition known as hepatic steatosis. This condition, which appears to be exacerbated by additional feed restriction at 14°C (Figure 3), has been documented in mammals.
(Kalderon et al. 2000; Jensen et al. 2001) as well as in fish (Cabellero et al. 2004; Deng et al. 2004; Ibarz et al. 2007) and may result from a metabolic defect in the processing of free fatty acids during catabolism (Kumar et al. 2004). Specifically, while the majority of free fatty acids (FFA) from adipose tissue lipolysis serve as metabolic fuel, excess lipolysis can lead to the reesterification of FFAs back into triglycerides in the liver (triglyceride recycling). The triglycerides then associate with very low density lipoproteins before they are sent into the circulation and taken up by adipose tissue (Jensen et al. 2001; Donnelly et al. 2005; Tocher et al. 2005). However, any metabolic defect with the triglyceride recycling process, such as a decline in enzymes critical to the cycle which is not uncommon during catabolism, could lead to hepatic lipid accumulation and steatosis (Kumar et al. 2004). Following refeeding and the CG response, lipid levels appeared to return to that of controls, indicating that the condition is reversible. Quantitative data, however, will be required to validate these assessments and definitively determine whether more lipid is present in the livers of feed-restricted versus fed fish.

In summary, we have found that HSB can undergo complete 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 feed conversion by as much as 30%. Based on the broad array of energetic fluctuations that occurred throughout the experiment, this species and rearing protocol could provide a valuable model system to study the metabolic and endocrine mechanisms underpinnings stunted, normal and accelerated (compensatory)
growth states. Since this protocol reflects the periods prior to, during and following the natural over-wintering period for HSB and other temperate-zone teleosts, results also have both biological and practical (aquaculture-related) applications. Specifically, the capacity to reduce feed costs through a 30% improvement in feed efficiency with no loss in biomass has profound implications in reducing production costs of HSB culture in tanks and possibly ponds.

ACKNOWLEDGEMENTS

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REFERENCES


Figure 1. Growth of hybrid striped bass (HSB) raised in tanks under feeding and temperature manipulation (N = 2 tanks/group with 60+ fish/tank). All groups of HSB were fed (F) or starved (S) during the initial temperature phase at 24°C (dark grey bar) and/or during the 14°C cold-banked (i.e. overwintering) period. All fish were subsequently fed during the second 24°C temperature phase (dark grey bar). Control fish (F-F) were fed throughout the entire experiment (days 0-148). “T” within the light grey bars refers to transition periods where temperatures were adjusted from 24-14°C and 14-24°C. Compensatory growth (CG) responses were observed in all treatment groups upon refeeding at 24°C relative to controls. Different lower case letters represent significant weight differences between groups at each time point (P < 0.05).
Figure 2. Specific growth rates (SGR) for individual HSB plotted against their mean body weights during the refeeding period at 24°C (days 115-134). Compensatory growth responses were observed in all treatment groups, as indicated by elevated SGRs relative to control fish (F-F) fed throughout the entire experiment (days 0-148). The most dramatic CG response was observed in S-S HSB previously starved at 24°C (days 0-23) and 14°C (days 24-114), followed by F-S HSB fed at 24°C and starved at 14°C, and then by S-F HSB starved at 24°C and fed at 14°C. Different lower case letters indicate significant differences between groups (P < 0.05).
Table 1
Total lengths and condition factors in groups of HSB that were either fed (F) or starved (S) during the initial temperature phase at 24ºC (days 0-23) and/or during the 14ºC cold-banked (i.e. overwintering) period (days 24-114). All fish were subsequently fed during the second 24ºC temperature phase (days 115-148). Control HSB (F-F) were fed throughout the entire experiment (days 0-148).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Length (mm)</th>
<th>Condition Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-F</td>
<td>S-F</td>
</tr>
<tr>
<td>Time 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>245.3 ± 1.1</td>
<td>246.0 ± 1.1</td>
</tr>
<tr>
<td>d 23</td>
<td>256.7 ± 1.14</td>
<td>252.3 ± 1.14</td>
</tr>
<tr>
<td>d 114</td>
<td>265.3 ± 1.4</td>
<td>260.5 ± 1.5</td>
</tr>
<tr>
<td>d 134</td>
<td>273.0 ± 1.6</td>
<td>270.0 ± 1.7</td>
</tr>
<tr>
<td>d 148</td>
<td>277.2 ± 1.7</td>
<td>274.6 ± 1.8</td>
</tr>
</tbody>
</table>

All data are presented ± SEM.
Different lower case letters represent significant differences (P < 0.05) between groups within each time point.
Table 2
Specific growth rates (SGR; %/day), feed consumption (%BW/day) and feed conversion ratios (FCR) for all groups during the first 3 weeks (days 115-134) and the following 2 weeks (days 135-148) of refeed at 24°C. Overall FCR (day 0-148) is also indicated for all groups. Statistical analysis could not be performed on the S-S group for feed consumption and FCR because one tank was lost due to a plumbing malfunction (see Statistical Analyses methods). Data for the remaining S-S replicate (n=1) is shown.

<table>
<thead>
<tr>
<th></th>
<th>SGR</th>
<th>Feed Consumption</th>
<th>FCR</th>
<th>Overall FCR</th>
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<td>Days</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>SGR</td>
<td>Feed Consumption</td>
<td>FCR</td>
<td></td>
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<tr>
<td>Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>115-134</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>135-148</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>F-F</td>
<td>0.47 ± 0.04</td>
<td>0.86 ± 0.12</td>
<td>2.21 ± 0.26</td>
<td>1.95 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>± 0.05</td>
<td>± 0.04</td>
<td>± 0.11</td>
<td>± 0.05</td>
</tr>
<tr>
<td>S-F</td>
<td>0.64 ± 0.03</td>
<td>1.03 ± 0.05</td>
<td>1.58 ± 0.17</td>
<td>2.39 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>± 0.05</td>
<td>± 0.12</td>
<td>± 0.19</td>
<td>± 0.26</td>
</tr>
<tr>
<td>F-S</td>
<td>1.60 ± 0.03</td>
<td>1.93 ± 0.21</td>
<td>1.11 ± 0.00</td>
<td>1.71 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>± 0.5</td>
<td>± 0.20</td>
<td>± 0.13</td>
<td>± 0.08</td>
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<tr>
<td>S-S</td>
<td>2.02 ± 0.05</td>
<td>2.40 ± 1.13</td>
<td>1.08 ± 1.44</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>± 0.10</td>
<td>± 0.10</td>
<td>± 0.10</td>
<td>± 0.10</td>
</tr>
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</table>

All data are presented ± SEM. Different lower case letters represent significant differences (P < 0.05) between groups within each time point.
Table 3
Fluctuations in hepatosomatic index and adiposomatic index in groups of HSB that were either fed (F) or starved (S) during the initial temperature phase at 24°C (days 0-23) and/or during the 14°C cold-banked (i.e. overwintering) period (days 24-114). All fish were subsequently fed during the second 24°C temperature phase (days 115-148). Control HSB (F-F) were fed throughout the entire experiment (days 0-148).

<table>
<thead>
<tr>
<th>Time day</th>
<th>Hepatosomatic Index</th>
<th>Adiposomatic Index</th>
</tr>
</thead>
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<tr>
<td></td>
<td>F-F</td>
<td>S-F</td>
</tr>
<tr>
<td>0</td>
<td>2.87 ± 0.13</td>
<td>2.64 ± 0.12</td>
</tr>
<tr>
<td>23</td>
<td>3.83a ± 0.23</td>
<td>1.52b ± 0.10</td>
</tr>
<tr>
<td>114</td>
<td>4.36a ± 0.37</td>
<td>4.37a ± 0.32</td>
</tr>
<tr>
<td>134</td>
<td>3.48a ± 0.21</td>
<td>3.62a ± 0.13</td>
</tr>
<tr>
<td>148</td>
<td>3.27a ± 0.24</td>
<td>3.57a ± 0.14</td>
</tr>
</tbody>
</table>

All data are presented ± SEM.
Different lower case letters represent significant differences (P < 0.05) between groups within each time point.
Figure 3. Transmission electron microscopy from HSB liver sections following feeding and temperature manipulation. Panels A, C and E represent hepatic sections from F-F control fish following (A) the initial period at 24ºC, (C) after subsequent cold-banking at 14ºC and (E) after 19 days of the final 24ºC period. Control F-F fish were fed throughout the entire experiment. Panels B, D and F represent hepatic sections from S-S treatment fish following (B) starvation at 24ºC, (D) starvation during cold-banking and (F) after 19 days of refeeding at 24ºC. An intracellular lipid droplet is indicated by an arrow in panels A and B.
CHAPTER IV

Endocrine Biomarkers of Growth and Applications to Aquaculture: A Mini-Review of Growth Hormone, Insulin-like Growth Factor-I and IGF Binding Proteins as Potential Growth Indicators in Fishes

\[^1\]Published in North American Journal of Aquaculture, by Matthew E. Picha, Marc J. Turano, Brian R. Beckman and Russell J. Borski.
ABSTRACT

Growth in fishes and other vertebrates is under endocrine control, particularly through the growth hormone (GH)-insulin-like growth factor (IGF) axis. For this reason, it has been of interest to aquaculture researchers and the industry to establish endocrine biomarkers which can both reflect and potentially predict growth rates in fishes subject to various biotic and abiotic manipulations. Ultimately, by understanding the hormones which control growth and utilizing them as biomarkers, it is hoped that achieving optimal growth conditions in the aquaculture environment can be expedited through reducing the need for lengthy and costly grow-out trials. While the most appropriate endocrine biomarkers for growth can be both species and situation specific, IGF-I may be the most promising candidate for measures of instantaneous growth in fish. This is based on the direct contributions of IGF-I in regulating cell proliferation and ultimately somatic growth, along with its previously established correlations with specific growth rate (SGR) in fishes under various conditions that alter growth. However, other endocrine indices such as growth hormone (GH) and IGF binding proteins (IGFBPs) are also important contributors and may in some instances prove a strong corollary to growth rate. This review discusses the potential utility of GH, IGF-I and IGFBPs as growth biomarkers for those manipulations most relevant to the aquaculture industry, namely feeding regimen, diet composition, temperature, photoperiod and stress.
INTRODUCTION

For aquaculture producers, the ability to minimize production costs is critical to competing in a global market place. Since endocrine responses to both environmental and genetic variables ultimately control growth in fishes and other vertebrates, an evaluation of these hormonal indices could prove useful in establishing biomarkers for growth. Therefore, we will build the case that endocrine indices might not only provide useful biomarkers for growth, but also an endocrine foundation for understanding growth variation that will help design efficient rearing regimens for cultured fish. To do this we will review the utility of certain endocrine factors as potential growth biomarkers in warm- and cool-water aquacultured fishes and analyze their practicality for use in the industry.

In fishes, as in mammals, the endocrine control of growth works through the growth hormone (GH) - insulin-like growth factor (IGF) axis (Figure 1) (reviewed by Oksbjerg et al. 2004; Wood et al. 2005; Reinecke 2006). Endogenous (i.e. nutritional state, humoral factors) and exogenous (i.e. temperature, photoperiod) cues integrated by the hypothalamus lead to the subsequent release of either stimulatory (GH-releasing hormone) or inhibitory (somatostatins) signals onto somatotrophs (GH-producing cells) in the anterior pituitary (see Bjornsson et al. 2002; Canosa et al. 2007 for reviews). Under anabolic conditions, GH released from the pituitary enters the circulation and binds to hepatic GH receptors (GHR), stimulating the synthesis and release of IGF-I into the bloodstream. Insulin-like growth factor-I, a 7.5kDa single chain polypeptide, is responsible for cell differentiation and proliferation, the stimulation of processes related
to skeletal elongation and ultimately for body growth (see Duan 1997; LeBail et al. 1998 for reviews). Plasma IGF-I, in turn, acts in a negative feedback fashion to inhibit GH secretion from the pituitary (Fruchtman et al. 2000). Hence, GH and IGF levels are tightly controlled to maintain the appropriate growth homeostasis. While hepatically-derived IGF-I accounts for the majority of the circulating peptide (Shamblott et al. 1995), GH can also stimulate the synthesis of IGF-I in non-hepatic tissues such as skeletal muscle (Kajimura et al. 2001). If translated, this locally-produced IGF-I can then act in a paracrine-autocrine manner to initiate cell proliferation (Chauvigne et al. 2003; Pedroso et al. 2005). Additional evidence also suggests that GH may have anabolic effects on these tissues independent of IGF-I, at least in mammals (reviewed by Ohlsson et al. 1998). Since the majority of IGF-I in the extracellular space and plasma is bound to IGF binding proteins (IGFBPs), they are central modulators of IGF actions. These carrier proteins not only increase IGF half-life, but can mediate their transport from the vascular space to target tissues, determine tissue distribution and inhibit or potentiate IGF activities. They also bind IGF-I with similar affinities as the type-1 IGF receptor (IGFR) (reviewed by Kelley et al. 2002; Duan and Xu 2005; Kelley et al. 2006). Approximately four IGFBP homologs of mammalian IGFBPs have been identified in fishes at the protein level (IGFBP-1, -2, -3, -5), while five full IGFBP coding sequences (IGFBP-1, -2, -3, -5 and -6) and a partial sequence for a sixth (IGFBP-4) have been reported at the molecular level (Kamangar et al. 2006). In general, in accordance with their functions in mammals, IGFBP-1 (20-29 kDa) and IGFBP-2 (31-39 kDa) are typically up-regulated in catabolic states, potentially sequestering plasma IGF-I and thus preventing any anabolic effects (Duan et al. 1999; Bauchat et al. 2001; Maures and Duan 2002; Shimizu et al. 2005).
Insulin-like growth factor binding protein-3 (40-50 kDa), on the other hand, is associated with anabolic states and may facilitate IGF actions (Shimizu et al. 2003a), while the actions of IGFBP-5 (76-90 kDa) are less clear (Johnson et al. 2003). It should be noted that while the 40-50 kDa IGFBP is most similar to mammalian IGFBP-3 based on physiological responses, molecular weight and the type of glycosylation, (Shimizu et al. 2003a), its amino acid sequence is most homologous with human IGFBP-2 (Kamangar et al. 2006). For the purpose of this review, the 40-50kDa IGFBP will be referred to as IGFBP-3.

The endocrine control of growth is complex, and for this reason this manuscript is not intended to be an exhaustive review of the GH-IGF axis or its control of growth in fishes. Rather, it will focus on some of the major factors which are most applicable to the aquaculture industry with respect to growth improvement. Since IGF-I is ultimately responsible for body growth (McCormick et al. 1992; Negatu and Meier 1995), this growth factor would seem a most appropriate candidate as a growth biomarker, and evidence discussed herein would suggest that this might be the case. However, the efficacy of specific endocrine factors as growth indicators are likely both species and situation specific and dependent on various components within the GH-IGF axis. In this review we will focus on the major circulating proteins within the growth-endocrine system, specifically GH, IGF-I and IGFBPs; their utility in assessing disparities in growth rate due to feeding regimen, diet composition, temperature, photoperiod and stress will be discussed (Table 2). Since protein levels determine phenotype, we will focus primarily on plasma hormone measurements rather than tissue mRNA levels.
While the ability to use these endocrine indices as growth biomarkers hinges on deducing correlations with specific growth rate (SGR: \( \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \times 100 \)) under the conditions being tested, surprisingly few correlations have been made between plasma IGF-I and SGR (Table 1). Even fewer have been made between SGR and either IGFBPs or GH (see discussion below for examples). For this reason, well planned, quantitative studies will have to be performed to determine whether the lack of correlations are simply due to a deficiency of studies designed specifically for this purpose or rather due to the absence of a physiological relationship. With this in mind, it is hoped that researchers will fill these voids to expedite the use of endocrine biomarkers for facilitating growth improvement in aquacultured fishes.

**BIOMARKER UTILITIES**

**Feeding Regimen**

Optimizing feeding regimen with respect to growth and metabolic efficiency is a goal of any aquaculture producer. Feeding rate and frequency, for instance, not only vary with species but with water temperature and body size as well. However, feeding tables which consider these variables have not been developed or optimized for many aquacultured fishes. Furthermore, alternative feeding regimens which deviate from typical daily feeding recommendations, such as those that might elicit compensatory growth responses or maximize feed utilization, are in some instances proving more appropriate. Based on measures of plasma IGF-I, IGFBPs and perhaps GH, it may be possible to develop these optimal feeding regimens on a species-specific basis.
Feed Restriction
A common initial means of assessing the relations of a hormone to nutritional status and thus growth is to measure their concentrations in fed versus feed deprived fish. In adult channel catfish *Ictalurus punctatus*, 2 weeks of fasting rendered significant depressions in plasma IGF-I relative to fed fish, while plasma GH showed no change. It was not until 4 weeks of fasting that plasma GH displayed the canonical increases associated with catabolism in fishes (Table 2; Small and Peterson 2005). This initially paradoxical relationship of elevated circulating GH during episodes of poor or negative growth (Duan and Plisetskaya 1993; Perez-Sanchez and LeBail 1999) is likely due, in part, to a state of GH resistance. That is, elevated plasma GH is accompanied by a reduction in the number of hepatic GHRs (Gray et. al. 1992; Mori et. al. 1992), preventing GH from stimulating the production of hepatic-derived IGF-I and ultimately depressing somatic growth (reviewed by MacKenzie et. al. 1998; Thissen et al. 1999). Depressed plasma IGF-I during catabolism may also facilitate the elevations in plasma GH through a reduction in negative feedback by IGF-I on GH synthesis and secretion in the pituitary (Fruchtman et al. 2000; Figure 1). Meanwhile, the elevated levels of circulating GH may still promote lipolysis for energy utilization and protein-sparing purposes (MacKenzie et. al. 1998). These results in adult catfish are consistent with a similar study in fingerling channel catfish where 3 weeks of fasting resulted in significant reductions in plasma IGF-I and no change in circulating GH (Small 2005). This early response of plasma IGF-I relative to GH was also observed in freshwater-acclimated Mozambique tilapia *Oreochromis mossambicus*, where 2 weeks of fasting resulted in significant reductions of IGF-I and growth but no change in GH (Uchida et al. 2003). Strong correlations between IGF-I and
SGR were demonstrated in this as well as other studies (Table 1). In seawater-acclimated tilapia *O. mossambicus*, decreases in plasma IGF-I coincided with increases in plasma GH after 4 weeks of fasting (Fox et al. 2006), although specific correlations between growth and endocrine factors were not determined in this study. Thus, circulating levels of IGF-I appears to be a more acute biomarker for disparities in growth due to feed restriction than plasma GH in both channel catfish and tilapia species. In hybrid striped bass *Morone chrysops x Morone saxatilis*, a considerable catabolic state induced through 4 weeks of partial feed restriction (fed twice over 4 weeks) rendered depressed concentrations of circulating IGF-I along with a significant IGF-I and SGR correlation (Table 1; Picha et al. 2006). Three weeks of complete feed restriction in this same species resulted in depressed plasma IGF-I and elevated plasma GH relative to fed controls (M. Picha unpublished results; Turano 2006).

While the trends discussed above suggest roles for both plasma IGF-I and GH during catabolic and anabolic states, the earliest samples in all cases were taken after 2 weeks of feed restriction. Much information would be gained if, instead, the temporal dynamics of IGF-I and GH were established through more frequent sampling. This would allow for the determination of the earliest point at which changes in feeding frequency coincided with changes in endocrine indices and the point at which these biomarkers may become viable. In Chinook salmon *Oncorhynchus tshawytscha*, for instance, increases in plasma GH were detected by day 3 of fasting while IGF-I and IGFBP-3 decreases were detected after 4 days (Pierce et al. 2005). Following 4 weeks of fasting in rainbow trout, immediate changes in plasma IGF-I and GH were also observed upon refeeding.
Specifically, significant increases in previously depressed plasma IGF-I values were recorded after 4 days of realimentation while only 1 day of re-feeding was required for previously elevated plasma GH levels to be completely reduced down to control values (Gabillard et al. 2006). Therefore, unlike in catfish and tilapia, either plasma GH or IGF-I in salmonids may be an effective indicator of differences in growth due to feeding regimen (Table 2). Indeed, based on these previous studies, it appears that a combination of elevated GH and depressed plasma IGF-I may serve as biomarkers for the catabolic state (negative energy balance) induced through fasting. It would be both interesting and informative, however, to assess whether graded degrees (lengths) of fasting in fact resulted in graded changes in either of these hormones or instead, if a threshold level of catabolism resulted in dramatic, all-or-nothing endocrine changes. It should also be noted that while this approach for hormone assessment may be appropriate for feeding regimens which utilize “on and off” feeding or for evaluating catabolic versus anabolic states, comparing hormone levels of fed and fasted fish may not be an appropriate general approach to assessing a growth biomarker. Rather, assessing endocrine correlates of growth in animals showing graded degrees of weight gain should be distinguished from those looking at positive growth (weight gain) versus negative growth (weight loss).

*Ration Size*

Positive correlations between SGR and plasma IGF-I have also been made by feeding variable ration sizes to achieve a broad range of positive growth rates, at least in coho salmon *Oncorhynchus kisutch* (Table 1; Pierce et al. 2001; Beckman et al. 2004a, 2004b, 2004c) and hybrid striped bass ($r^2= 0.86$, M. Picha unpublished data). Collectively, the strongest correlations from these studies were derived from growth rates that reflected the
most recent growth stanzas (2 to 6 weeks), establishing IGF-I as a biomarker of recent growth. In one instance it was even found that plasma IGF-I was more strongly related to growth rate than either size or condition factor (Beckman et al. 2004b). Within these studies, moderate changes in nutritional plane (feeding level) resulted in parallel changes in plasma IGF-I and IGFBP-3, with additional positive correlations being made between plasma IGF-I and IGFBP-3 (Beckman et al. 2004a, 2004c). In Chinook salmon, mild reductions in nutritional plane produced increases in IGFBP-1. Specifically, increases in IGFBP-1 were seen 2 weeks following a ration reduction of 2% to 0.5% body weight per day (BW/d), and 4 weeks following a ration reduction of 1% to 0.5% BW/d (Shimizu et al. 2006). Interestingly, no changes in plasma IGF-I were detected following the 1% to 0.5% BW/d reduction (Shimizu et al. 2006). Variable ration sizes given to Nile tilapia Oreochromis niloticus also yielded a gradient of SGR values that were positively correlated to hepatic IGF-I mRNA expression (Vera Cruz et al. 2006). Collectively, these results among the different fish species indicate that plasma IGF-I, IGFBP-3 and possibly hepatic IGF-I mRNA may prove useful as positive and rapid (within two weeks) indicators of growth (Table 2), while IGFBP-1 may serve as a negative indicator. In fact, IGFBP-1 may be the most sensitive of these factors, at least based on studies in salmonids.

Growth hormone either changed little or showed an inverse relationship to feed consumption and growth in response to different ration sizes in salmonids (Table 2; Pierce et al. 2001; Beckman et al. 2004a). Similarly, gilthead sea bream Sparus aurata fed graded ration sizes showed an inverse relationship between positive SGR and plasma
GH (Company et al. 1999a). These results are consistent with other studies that assessed suboptimal, yet positive growth in sea bream (Perez-Sanchez et al. 1995, Marti-Palanca et al. 1996; see also Diet Composition section). The disparity between GH and growth rate may very well be mediated by the prevailing levels of IGF-I and its negative feedback effects on the pituitary. That is, as plasma IGF-I increases with higher growth rates, GH levels decline due to an amplified negative feedback, with the opposite trends occurring during lower growth states observed with reduced ration size.

**Compensatory Growth**

An important aspect to the validity of growth biomarkers is the capacity to assess differences between poor, normal and rapid growth states. Compensatory growth, an example of the latter, is the phenomenon by which animals exhibit accelerated growth that exceeds normal rates following growth-stunting conditions. Compensatory growth (CG) has been well studied in fishes (see Ali et al. 2003 for review) and carries potential benefits to aquaculturists in the form of improved overall growth rates and feed efficiencies, along with reduced labor costs and improved water quality. The CG model also allows one to assess the utility of potential bioindicators and the underlying endocrine mechanisms mediating poor, normal and rapid growth. Additionally, since CG is typically preceded by a period of negative growth, biomarkers can be evaluated in animals exposed to sequential shifts in metabolic state, *e.g.* catabolism to extreme anabolism, in comparison to those reared under continuous conditions. These types of measurements may better reflect the naturally fluctuating conditions fish often experience as part of their life history, or in those cultured fishes raised under ambient conditions in seasonal environments.
While some CG studies have focused on manipulations such as temperature, salinity, density and oxygen levels as a means of inducing the response, the vast majority involve some form of feeding manipulation, typically a period of feed restriction followed by re-feeding (reviewed by Ali et al. 2003). The overwhelming majority of studies, however, have not addressed physiological indicators of CG, particularly the endocrine mediators of the accelerated growth response in fishes. Studies in hybrid striped bass show that animals subject to an initial period of feed restriction exhibit a robust CG response upon re-feeding (Skalski et al. 2005; Picha et al. 2006; Turano et al. 2007). During the catabolic state induced through restricted feeding, circulating levels of IGF-I declined, but then rebounded dramatically during the subsequent CG response. The most dramatic increase in plasma IGF-I occurred during the initial 4 days of the 28-day response (Table 2), which also corresponded to the highest SGR observed among either controls (fish fed normally throughout) or animals on the CG regimen. Overall, there was a strong positive correlation between SGR and circulating IGF-I with compensatory growth (Table 1; Picha et al. 2006). Interestingly, while the CG response was characterized by elevated SGRs, IGF-I levels did not exceed that of control fish. Thus, while circulating IGF-I may be crucial to regulating variable growth rates, the relative increase or change in plasma IGF-I rather than absolute concentrations alone may be a more critical regulator of the accelerated growth response observed during CG. Indeed, we found an even stronger correlation between SGR and changes in plasma IGF-I levels (Δ plasma IGF-I / Δ time) than that between SGR and absolute IGF-I concentrations (Picha et al. 2006). It is well established that secretory pulses of hormones, including those within the endocrine-
growth axis, may be more effective regulators of target organs than absolute hormone levels (Borski et al. 2000). This sensitivity of target tissues to bolus hormone release may well be mediated in part by changes in the hormone receptor(s) or amplification of the cellular transduction cascade. Supportive of this hypothesis are data from rainbow trout and gilthead seabream in which 4 weeks of fasting in both species increases specific binding for IGF-I in muscle tissue (Montserrat et al. 2007a, 2007b). Hence, heightened IGFR numbers or sensitivity may lead to disproportionate increases in mitogenic activity, particularly when IGF-I increases from depressed or baseline levels. Therefore, despite being a good indicator of growth, it may be more critical to determine the relative changes in IGF-I to distinguish relative differences in growth rates under anabolic conditions (normal versus accelerated growth).

Collectively, changes in systemic IGF-I appear to be a good and reasonably rapid (≤ 4 days) indicator of growth under feed manipulation protocols where extreme shifts in metabolism occur. In addition to systemic IGF-I, it is also important to consider locally produced IGF-I. Muscle IGF-I mRNA is up-regulated in rainbow trout Oncorhynchus mykiss (Chauvigne et al. 2003; Montserrat et al. 2007a) and hybrid striped bass (M. Picha unpublished data) following periods of fasting. Presumably, this IGF-I is being translated to act in a paracrine-autocrine manner, and hence this pool of IGF-I may also contribute to greater growth during CG, especially given the up-regulation of IGF-I binding in muscle tissue following catabolism (Montserrat et al. 2007a, 2007b).
Diet Composition

Feed formulation is critical to fish health and growth and to aquaculture economics. Carnivorous fish, for instance, generally require formulated feeds that contain 40-50% protein. Because of these high protein requirements, much of which is derived from costly fishmeal, 40-70% of variable (operational) costs of most cultured species can be attributed to feeds. Therefore, ongoing research is aimed at supplementing diets with lipid and carbohydrates to support maintenance metabolism to allow the maximal amount of protein for growth (protein-sparing) (Stickney 1994). Additionally, the incorporation of plant protein and oil in lieu of fishmeal and fish oil is also being extensively investigated. It would be highly useful to circumvent lengthy and costly grow-out trials by using endocrine biomarkers such as plasma IGF-I and GH for testing various feed formulations on growth performance during brief exposure to test diets.

Protein Levels

In barramundi *Lates calcarifer* fed diets with variable amounts of crude protein for 6 weeks, strong correlations were found between plasma IGF-I and SGR with a single end point measure (Table 1; Dyer et al. 2004a). Consequently, it is possible that grow-out trials such as these could be reduced in length to the point where discernable and significant differences in plasma IGF-I are first detected, and that these subtle endocrine changes may reflect current as well as future body weight gain. In order to design efficient (short) feed trials, however, it will be necessary initially to employ a serial sampling design in order to determine the temporal sequence of hormone changes and how this relates to differences in growth and size. In particular, do changes in IGF-I precede, coincide with or follow divergences in size or growth rate? This barramundi
study also showed that plasma IGF-I was positively correlated to dietary protein levels, indicating the growth factor may prove a reliable indicator of optimal protein levels required for growth. Initial trials in this same species suggest the effects of dietary energy levels on growth might also be assessed by IGF-I measurements (Nankervis et al. 2000).

In contrast to observations of circulating IGF-I, GH is generally negatively correlated to growth and dietary protein levels, as was observed in fingerling gilthead seabream (Perez-Sanchez et al. 1995). Similarly, sea bream fingerlings fed high protein – low lipid diets exhibited greater growth rates, better feed conversion and lower plasma GH than those on a low protein – high lipid diet (Marti Palanca et al. 1996). An additional study with this same species was less clear as there was little effect of variable protein to lipid ratios (55P:9L vs. 46P:17L) on SGR and inconsistent changes in GH levels (Company et al. 1999a). Sea bream fed isoproteic and isoenergetic diets with variable ratios of indispensable and dispensable amino acids (IAA:DAA) displayed no differences in SGR (Gomez-Requeni et al. 2003). Despite this lack of effect on SGR, fish fed lower proportions of IAA displayed hormone levels consistent with lower growth (low plasma IGF-I and elevated plasma GH) and also had nutritional characteristics consistent with poorer growth efficiency (lower feed conversion and nitrogen retention). Thus, the endocrine characters of these fish reflected some disruption in the relations between nutrition and growth even though the growth rates, as measured, did not differ. This suggests that IGF-I and GH measures may be useful for making inferences about feeds and nutritional conditions of fishes beyond simply growth rate, including the efficiency
of lipid utilization, protein sparing effects and nutrient retention, among others (MacKenzie et al. 1998; Company et al. 1999b; Perez-Sanchez and LeBail 1999).

Plant Proteins and Oils

Because of the high cost and finite availability of fishmeal for aquaculture feeds, research regarding its substitution with plant protein sources is increasing (Hardy 1996), as are the corresponding investigations on the effects of protein replacement on the GH-IGF-I system. Given the limitations of plant protein substitution in certain fish species, namely in carnivores (i.e. palatability, digestion, disease) (reviewed by Gatlin III et al. 2007), it will be important to assess their suitability on a species-by-species basis based on their effects on growth, for which endocrine biomarkers may serve as a proxy and potential predictor. In juvenile channel catfish, isoproteic diets (28%) containing various percentages of fish meal (0, 4 and 8%) supplemented accordingly with plant protein were evaluated for their effects on commercially important traits. In two of the three genetic strains tested (Mississippi “normal” and USDA 303), diets with higher levels of fish meal resulted in elevated SGRs and plasma IGF-I, along with generally improved feed conversion (Table 2; Li et al. 2006). In the third genetic strain (NWAC 103), however, fish meal percentage had no significant effect on SGR, coincident with steady levels of IGF-I. When all three strains were evaluated together, a significant and positive correlation was achieved between SGR and plasma IGF-I (Table 1). Thus, circulating IGF-I appears to be an appropriate biomarker for growth in all three genetic strains of this omnivorous species. In rainbow trout, a carnivore, diets with increasing levels of plant protein replacement (0, 50, 75 and 100%) resulted in graded declines in IGF-I and
increasing levels of GH, along with significant decreases in growth rates and feed efficiency (Gomez-Requeni et al. 2005). In nearly identical experimental manipulations, gilthead sea bream showed strikingly similar growth and endocrine trends. That is, 50, 75 and 100% plant protein replacement resulted in significant decreases in growth rate and feed efficiency which paralleled increases in plasma GH (Gomez-Requeni et al. 2004). These decreases in growth rate also resulted in significant declines in plasma IGF-I, supporting the notion that IGF-I may also prove useful as an indicator of growth in these types of studies. Similarly, when gilthead sea bream diets were formulated with graded levels of vegetable oil mixture that replaced fish oil, differences in growth corresponded to significant changes in plasma IGF-I. Specifically, diets containing 100% fish oil along with 33 and 66% vegetable oil replacement all resulted in similar weights and plasma IGF-I levels at the end of the 11 week study, while fish fed diets with 100% vegetable oil replacement had significantly reduced growth and depressed circulating IGF-I concentrations (Benedito-Palos et al. 2007). Taken together, both GH and IGF-I appear to provide relevant but discordant signals with regard to growth performance and diet formulations in these studies; that is, increases in plant protein and vegetable oil that resulted in decreased growth also showed reduced IGF-I and increased GH.

While the trends seem clear for these studies on catfish, rainbow trout and sea bream, caution must be taken when drawing direct conclusions between plant protein percentages and regulation of the endocrine growth axis. Specifically, the elevated plasma GH or depressed plasma IGF-I may not be due to the effects of plant protein replacement on the somatotrophic axis directly, but rather due to the palatability of plant-
based feeds. Indeed, in the catfish (Li et al. 2006) and gilthead sea bream (Gomez-Requeni et al. 2004) studies, increasing the percentage of plant protein replacement resulted in significant decreases in feed intake, which in and of itself may be directing the endocrine and growth changes. Thus, it appears that measures of IGF-I and GH do not provide answers as to why a feed performed poorly (palatability, protein quality); instead, they reliably indicate differences in growth regardless of differences in diet composition. This is an essential characteristic for a growth biomarker to be useful for comparative feed trials.

**Temperature**

With unlimited feed availability, increases in temperature often enhance growth up to a species-specific physiological limit. However, the mechanisms mediating temperature effects on growth and regulation of the GH-IGF axis are not well understood (Mommsen 2001). Isolating the effects of temperature alone on the growth axis has proven difficult as studies that employ satiation feeding result in variable feed intake and growth rates based on water temperature. However, a good growth biomarker should detect changes in growth regardless of whether it is mediated by the environmental factor (e.g. temperature) alone, feed intake or both. To this end, we review literature on temperature effects on growth where it may be mediated by alterations in feed intake or not.

*Temperature and GH*

Growth hormone regulation by rearing temperature has not been clearly established in fishes or any other vertebrate. Indeed, as most work on endocrine growth physiology has
occurred in homeothermic mammals and birds, investigations into relations between temperature, growth, and the GH-IGF-I system are almost unique to fishes. In an effort to determine the role of temperature in hormone regulation in rainbow trout, plasma GH levels were compared in fish maintained at similar growth rates through feed manipulations and exposed to rearing temperatures of 8, 12, and 16ºC (Gabillard et al. 2003a). It was found that low temperature reduces plasma GH, indicating that temperature affects circulating GH levels independent of any effects on growth. In gilthead seabream and smolting Atlantic salmon, increasing water temperatures were associated with increases in feed intake, growth and plasma GH (McCormick et al., 2000; Mingarro et al. 2002), with significant positive correlations between plasma GH and feed intake in the seabream study (Mingarro et al. 2002). It should be noted that while seabream were on a natural photoperiod and increases in GH coincided with increases in day length, smolting Atlantic salmon were held on a constant photoperiod. Taken together, it appears that GH may, in part, drive alterations in growth under different water temperatures. This positive relationship between circulating GH, feed intake and growth rate contrasts with the discordance between GH and growth discussed earlier with catabolic versus anabolic states (see Feeding Regimen and Diet Composition), but is similar to that associated with photoperiod discussed in a later section (see Photoperiod).

Temperature and the IGF System
Increasing environmental temperatures resulted in increased growth and plasma IGF-I levels in Chinook salmon (Beckman et al, 1998), Atlantic salmon Salmo salar (McCormick et al. 2000), rainbow trout (Gabillard et al. 2003b; Taylor et al. 2005), coho salmon (Larsen et al. 2001) and gilthead sea bream (Mingarro et al. 2002). Within these
studies, significant correlations between plasma IGF-I and SGR were established in gilthead sea bream, although natural photoperiod may have contributed to the temperature effect on growth seen in this species (Table 1; Mingarro et al. 2002). Hepatic IGF-I mRNA was positively correlated to growth rates of Nile tilapia reared at temperatures ranging from approximately 18°C to 32°C (Vera Cruz et al. 2006). Furthermore, correlated increases in growth rates and plasma IGF-I levels were also reported in southern flounder *Paralichthys lethostigma* grown at 23°C versus 28°C (Luckenbach et al. 2007). In a 3-year study in Atlantic cod *Gadus morhua*, mean plasma IGF-I levels showed a circannual pattern that positively correlated with ambient water temperature under ambient photoperiod conditions. Correlations of IGF-I with individual growth rates were relatively weak, but this may have been due to the longer-term measure of growth activity and IGF-I levels (around every 3 months) relative to the shorter-term fluctuations in ambient temperature to which fish were exposed between sampling intervals (Davie et al. 2007). In rainbow trout, positive correlations in plasma IGF-I, growth rate and water temperature were found when fish were raised for 6 months under ambient water temperatures that gradually fluctuated between 2 and 16°C and were sampled at monthly intervals (Taylor et al. 2005). It is possible that the elevations in growth and IGF-I seen in these studies are mediated by differences in feed consumption, as feed intake was shown to increase with temperature. Consistent with this, little change was observed in plasma IGF-I in rainbow trout reared at different temperatures (8, 12 and 16°C) but given feeding rations that resulted in similar growth rates (Gabillard et al. 2003b).
Direct tests of whether IGF-I or 41 kDa IGFBP (IGFBP-3) can be used as growth biomarkers for fish reared at different temperatures have been conducted with juvenile coho salmon (Beckman et al. 2004c). Growth of individually tagged fish was monitored at 2 – 3 week intervals over a nine week period as one group of fish experienced a temperature decrease from 11°C to 7°C while control fish remained at 11°C. Positive and significant correlations were found between growth and both IGF-I and 41 kDa IGFBP on all four sampling dates for control fish (Table 1). While correlations between growth and either IGF-I or 41 kDa IGFBP were disrupted on the first two sampling dates for fish subjected to the temperature decrease, positive and significant correlations between both IGF-I and 41 kDa IGFBP and growth were subsequently re-established for the fish held at cooler temperatures. This indicates that IGF-I or 41 kDa IGFBP may be used as biomarkers for growth in fish regardless of differences in rearing temperature, although a sufficient temporal period of acclimation may be required prior to comparisons being made. However, since this is only one study with one species, further work is needed to assess the efficacy of IGF-I and 41 kDa IGFBP as growth biomarkers across temperatures.

Collectively, studies in various salmonid and nonsalmonid species point to IGF-I as a potentially effective biomarker for growth rates under varying rearing temperatures. It would appear that its usefulness extends to gradual or seasonal changes in temperature, although a brief period of acclimation may be required prior to analysis when abrupt temperature shifts are made. It would also appear that much of the changes in plasma IGF-I and growth that occur with temperature are mediated through alterations in
temperature-dependent feed consumption. Interestingly, unlike that observed with the state of “GH resistance” associated with episodes of feed restriction, GH and IGF-I both show positive correlations to growth with seasonal temperature variation. From this perspective, both circulating GH and IGF-I together might prove useful in assessing and predicting growth in fish under variable temperature regimes where feed availability is unlimited (Table 2).

**Photoperiod**

Photoperiod or changes in photoperiod clearly have strong affects on growth in some fishes (Boeuf and Le Bail 1999). Consequently, light cycle manipulations are becoming common in many commercial aquaculture programs to directly increase growth, change the seasonal timing of smolting (introduction into seawater) and to inhibit maturation which maintains investment in somatic rather than gonadal tissue. Early work suggests that at least some photoperiod driven changes in growth can be traced to changes in the GH-IGF axis (Kourmandjian et al. 1976; Marchant and Peter 1986). Thus, a key test for a putative growth index is how photoperiod manipulation affects the status of GH and IGF-I as biomarkers for growth.

*Spring equinox*

Some of the earliest work on photoperiod, growth and GH took place in salmonids and was directly concerned with mechanisms controlling smolting. However, identifying the effects of photoperiod on relations between GH levels and growth in salmonids during smoltification is difficult because of GH’s overlapping osmoregulatory, behavioral, metabolic and growth-stimulating actions (Stefansson et al. 1991; McCormick et al. 1993).
1995). This may be further compounded by interactions with other seasonal cues such as varying water temperature and feeding rates (Bjornsson et al. 1989; McCormick et al. 2000). Nevertheless, it is now well established that plasma GH levels increase during the spring parr-smolt transformation and that the increasing photoperiod-day length is of overriding importance as a “zeitgeber” for these increases in GH levels (for review see Bjornsson 1997). The increase in plasma GH during smolting results in a strong, positive, nonlinear correlation between plasma GH and SGRs (Bjornsson et al. 1995). Thus, GH relations to growth are opposite those described previously (see Feeding Regimen and Diet Composition). However, a negative correlation was found between GH and growth rate in adult salmon over the course of the summer-fall (both natural and manipulated photoperiods) (Nordgarden et al. 2005), demonstrating that a positive correlation between GH and growth is not a common feature in adult salmon. Finally, it has been found that a late spring – summer increase in GH in gilthead seabream appears to correlate with seasonal increases in growth rate (Perez-Sanchez et al. 1995; Mingarro et al. 2002). This work, together with that in goldfish (Marchant and Peter 1986), suggests that spring increases in GH and growth are not limited to salmon smolts and indicates that a disruption of “normal” relations between GH and growth (see Feeding Regimen and Diet Composition) may be found during the spring equinox, at least in some fishes.

In contrast to GH, the relations between IGF-I and growth rate remain positive during spring photoperiod increases and smolting in salmon (Beckman et al. 1998; Larsen et al. 2001). Similarly, IGF-I and growth rate are positively correlated (see Table 1) through
the spring-summer period in gilthead seabream (Perez-Sanchez et al. 1995; Mingarro et al. 2002).

**Autumn equinox**

Autumnal changes in IGF-I and GH have been described in Chinook salmon (Pierce et al. 2001) and gilthead sea bream (Mingarro et al. 2002) which appear to be dissociated from feeding rates and temperature along with developmental events such as smolting and reproduction. Both studies suggest that seasonally changing photoperiod might provide the cue for these altered hormone levels. Autumnal peaks in IGF-I were found in rainbow trout, but it was suggested that the peaks were not photoperiod induced as fish held under constant photoperiod also displayed an IGF-I peak in September (Taylor et al. 2005). Instead, it was suggested that the IGF-I peak might have been related to the changes in water temperature and feeding rate found in their experiment. In post-smolt coho salmon, the relations between individual growth rate and individual plasma IGF-I levels were assessed on four occasions ranging from July through September (Beckman et al. 2004). On each date, a positive and significant relation between IGF-I and growth was found. However, plasma IGF-I levels increased from July to September even though temperature and ration were held constant, resulting in a shift in the IGF-I and growth relation as fish approached the autumn equinox. Together, these studies suggest that while IGF-I and growth are generally positively related, the exact nature of this relation may change seasonally. Thus, caution may be appropriate for using IGF-I as a growth index to compare samples taken during different seasons.
Multiple photoperiods
In addition to naturally changing photoperiods, fish in aquaculture are subjected to a number of different yet constant photoperiod regimes, ranging from constant short day to constant long day to continuous (24 hour) light. In hybrid striped bass, 3 weeks of long day (16h light : 8h dark) versus short day (8h light : 16h dark) light cycles resulted in insignificant differences in both growth and plasma IGF-I (Davis and McEntire 2006). Indeed, it appears that in many cases a treatment period of months rather than weeks is required before variable photoperiod regimens result in appreciable changes in body weight (Boeuf and LeBail 1999). This may be an opportunity, however, to gauge whether noticeable changes in plasma IGF-I precede significant changes in growth, which ultimately could allow for shortened photoperiod experiments. In rainbow trout, IGF-I and growth rates were compared in two experiments (Taylor et al. 2005). In the first, fish were subjected to either natural photoperiod, constant short day (8:16) or constant long day (18:6) from June through December, with naturally fluctuating temperatures and the resulting variations in feed intake. A positive and significant correlation between mean plasma IGF-I values and growth rates was found for samples taken at approximately monthly intervals and compared among all treatments (see Table 1). A second experiment employing natural photoperiod, short day (8:16) and continuous light treatments from April to October found similar results: mean IGF-I was positively correlated to mean growth rate for all samples combined (although p = 0.07). To examine the effects of photoperiod on growth in Atlantic Cod, eight different photoperiod treatments were used which utilized different combinations of long day constant and natural photoperiods with a seasonally varying temperature (Davie et al. 2007). When growth and plasma IGF-I relations were compared over two periods (January to April and
April to July), they found positive (both trials) and significant (1st trial) relations between mean IGF-I (measured at the end of the growth period) and mean growth over each three month period (see Table 1) (Davie et al. 2007).

These studies demonstrate a common positive relation between IGF-I and growth for fish held under different photoperiods, suggesting IGF-I may act as a growth index regardless of photoperiod. A cautionary note is necessary as these studies were conducted with seasonally varying temperatures and concomitantly varying feeding rates. It is therefore difficult to isolate the sole effect of photoperiod in these studies (Taylor et al. 2005). Studies specifically designed to test whether growth and IGF-I relations are consistent regardless of photoperiod will have to be conducted to clearly demonstrate whether IGF-I provides a stable growth index.

**Stress**

Due to the intimate association with their aquatic medium, fishes are inherently susceptible and sensitive to a wide range of stressors that disrupt homeostasis and ultimately lead to growth suppression (for review see Conte 2004). In aquaculture settings, stressors may include high density, poor water quality, handling, transport and acclimation, among others. The stress response in fishes involves the release of catecholamines (norepinephrine and epinephrine) and glucocorticoids (cortisol) (reviewed by Perry and Bernier 1999; Barton 2002), with cortisol being the most frequently utilized indicator of stress. This is despite the resistance in cortisol responses that often occur with acute or chronic stress. Crosstalk likely exists between the stress
and growth axes; in particular, cortisol is linked to inhibition of various components of
the IGF axis and thereby the attenuation of growth (Pankhurst and Van Der Kraak 1997;
Mommsen 1999; Davis 2006). Likewise, in salmonids, glucocorticoids have been shown
to stimulate the synthesis and production of IGFBP-1 from hepatocytes (Pierce et al.
2006), a protein whose expression is up-regulated during poor growth states such as those
associated with stress (Kelley et al. 2001). Therefore, while cortisol may be an
appropriate biomarker for the portion of the stress response concerning energy
metabolism and hydromineral balance (Vijayan et al. 1993; Reid et al. 1998), plasma
IGF-I, IGFBPs and perhaps GH may represent a direct indicator of the effects of acute
and chronic stressors on growth. If so, then protocols for procedures on aquaculture
operations which result in stress responses such as handling or transport could be
optimized as to minimize the stress-induced effects on growth. Interestingly, in rainbow
tROUT, these relationships between growth and plasma GH, IGF-I and cortisol are also
being used for genetic selection of stress responses associated with better growth
performance (Lankford and Weber 2006; Weber and Silverstein 2007).

In Mozambique tilapia, intraperitoneal cortisol injections caused an initially rapid (< 2 hr)
increase in low MW IGFBPs (potential IGFBP-1 and -2) and decreases of higher MW
IGFBPs (potential IGFBP-3), followed by recovery at 24 hr (Kajimura et al. 2003). The
putative roles of these variably sized IGFBPs in fish are generally consistent with
mammalian counterparts, whereby low MW IGFBPs increase under catabolic conditions,
while higher MW IGFBPs increase with anabolic states (Kajimura et al. 2003; review
Kelley et al. 2006). Cortisol was also effective in reducing circulating IGF-I by 24 hr,
albeit five-fold higher concentrations of cortisol were used relative to that in regulating IGFBPs (Kajimura et al. 2003). In channel catfish, dietary cortisol treatment for four weeks suppresses growth by 50%, feed intake by 30%, reduces circulating IGF-I levels and up-regulates a low MW (~20kDa) IGFBP (Peterson and Small 2005; Small et al. 2006). Thus, it would appear that exogenous treatment with the stress hormone cortisol leads to a consistent inhibition of IGF-I and a high MW IGFBP while stimulating low MW IGFBP(s). Surprisingly, few studies outside those on catfish have assessed growth alongside endocrine-growth responses to exogenous cortisol.

In Atlantic salmon parr subjected to once daily handling stress for 40 days (e.g. long-term chronic stress), plasma GH was significantly higher than unstressed controls both prior to and 7 hours following an acute handling stress on day 40 (McCormick et al. 1998). Since chronic stress was associated with lower growth rates, these elevated GH concentrations may be involved in energy mobilization to accommodate the increased energetic demands of stress. At the same time, however, plasma IGF-I was higher than controls at 3 and 7 hours following the stressor on day 40 (McCormick et al. 1998). Similarly, dramatic elevations in cortisol but no changes in plasma IGF-I were observed immediately following a 3 hour confinement stress in yearling steelhead trout *Oncorhynchus mykiss* (Liebert and Schreck 2006). While it may be that an up-regulation of low MW IGFBPs is suppressing the growth-promoting effects of elevated or sustained levels of circulating IGF-I, it indicates that measures of a single hormone during stressful situations may not always be representative of short and long-term growth status, and that more comprehensive measures (i.e. cortisol, IGF-I and IGFBPs) will provide a clearer
picture. In contrast to that observed in salmonids, a 15-minute low water stress in hybrid striped bass rendered significant depressions in both plasma IGF-I and a 33 kDa IGFBP (potential IGFBP-3) along with considerable increases in low MW IGFBPs (putative IGFBP-1 and -2) by 2-6 hours post-stress (Davis and Peterson 2006). Interestingly, plasma cortisol was only elevated for 1 hour following the stressor, suggesting that changes in plasma IGF-I and IGFBPs may be a more appropriate biomarker for gauging the temporal effects that acute stressors may exert on somatic growth, if any. Additional studies involving handling or confinement stress in southern Bluefin tuna *Thunnus maccoyii*, silver perch *Bidyanus bidyanus* and black bream *Acanthopagrus butcherii* also led to depressions in plasma IGF-I, some within 12 hours of the stressor (Dyer et al. 2004b). Furthermore, jack mackerel *Trachurus symmetricus* subjected to a confinement then handling stress showed strong increases in low MW IGFBPs 60 seconds following the handling stress, similar to that observed for plasma cortisol (Kelley et al. 2001).

Results to date suggest that IGFBPs may be a sensitive indicator of stress (increase in low MW IGFBPs, reduction in a high MW IGFBP) and, along with IGF-I, could provide an accurate indication of the long-term effects of stress on growth. Detailed studies analyzing the temporal patterns of plasma cortisol, IGF-I and IGFBPs in direct relation to growth will be required before growth biomarkers reflecting the effects of specific stressors are established.

**CONCLUSION**

Establishing endocrine biomarkers either responsible for or reflective of growth in fishes has broad applications to aquaculture researchers and the industry. In particular, by
establishing correlations between hormonal indices and specific growth rates under various biological conditions, lengthy grow-out trials intended to establish optimal diet formulations, feeding regimens, handling protocols or rearing conditions (i.e. temperature, photoperiod, salinity) may be circumvented. It also provides valuable endocrine data regarding the underlying control of growth in fishes, which ultimately may be required for growth optimization.

It would appear among all the hormones controlling somatic growth, that IGF-I is the most promising candidate for measures of instantaneous growth in fish. This is due to the causal relationship the growth factor plays in directly regulating cellular proliferation and ultimately somatic growth in vertebrates. A recent study in dogs shows that IGF-I is crucial to regulating body size (Sutter et al. 2007) and previous investigations using IGF-I knockout mice established that IGF-I is responsible for much of postnatal growth (Lupu et al. 2001). With respect to the numerous studies on growth regulation in fishes, relatively few have established correlations between plasma IGF-I and SGR (Table 1), a criterion for biomarker efficacy. However, it will have to be determined whether the lack of correlations are simply due to a deficiency of studies designed specifically for this purpose or rather due to the absence of a physiological relationship. The development and validation of a commercial IGF-I RIA (GroPep, Adelaide, Australia; Shimizu et al. 1999; Shimizu et al. 2000; Dyer et al. 2004b) in the past decade with the highly conserved IGF-I ligand and a “universal” antibody for fish (GroPep, Adelaide, Australia) should prove highly useful for IGF-I biomarker testing in various fish species. Furthermore, the recent development of homologous radioimmunoassays (RIA) for salmon IGFBP-1 and -3
(Shimizu et al. 1999; Shimizu et al. 2003a, 2003b; Shimizu et al. 2006) should also enable decisive testing of these carrier proteins for biomarker potential, especially since RIAs have greater precision and the ability to process a large numbers of samples relative to traditional Western IGFBP ligand blot analyses. Based on current literature it appears that IGF-I, and possibly GH or IGFBPs where appropriate (see Table 2), might best serve as putative biomarkers for growth when the variables of interest (ration size, diet composition, temperature, etc.) are held relatively constant, and particularly when they are not confounded by multiple environmental factors that serve as covariates (simultaneous temperature and photoperiod manipulations).

Clearly, the utility of growth biomarkers should be based not only on their relation to current growth status but ideally on the ability to predict future growth rates, assuming the variable being tested is held relatively constant. The predictive value of early endocrine measures on subsequent growth performance has yet to be established for any of the components of the GH-IGF growth axis. Additionally, if one is to predict or assess the relative quality of a test variable it is important to know how rapidly the endocrine biomarker might change. Based on this review, it would appear that changes in IGF-I may occur within days and that correlations reflect growth rates within the last two weeks. With more frequent sampling rather than end-point analysis, however, a better resolution of the temporal dynamics between endocrine parameters and growth rate can be established. An additional point to consider is whether subtle changes in growth are reflected by distinguishable changes in the biomarker. This feature in particular could support the practicality of endocrine biomarker use over that of direct weight measures,
although this may require using larger sample sizes to potentially reduce standard errors and establish statistical significance,

While this review has been intentionally limited in scope, it is important to note that hormonal factors that were not discussed herein (i.e. IGF-II, neuropeptide Y) might also reflect growth status, whether through their direct regulation on tissue proliferation or indirectly through control of appetite, respectively. Indeed, the heritability of appetite seems to be a driving force in divergent growth rates between different strains of the same fish species (Silverstein 2002). Likewise, as pointed out in this review, many of the variables regulating IGF-I, GH or IGFBPs and growth might also be doing so through changes in feed intake.

ACKNOWLEDGEMENTS

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REFERENCES


**Table 1.** Correlations ($r^2$) between plasma IGF-I and specific growth rate (weight) (SGR: ln W2 – ln W1)/(t2 – t1) X 100) in fishes induced through specific manipulations. All correlations are statistically significant. Letters following each correlation represent references to their original manuscripts: a, Uchida et al. 2003; b, Picha et al. 2006; c, Pierce et al. 2001; d, Beckman et al. 2004a; e, Beckman et al. 2004b; f, Beckman et al. 2004c; g, Dyer et al. 2004a; h, Taylor et al. 2005; i, Mingarro et al. 2002; j, Davie et al. 2007; k, Li et al. 2006. Other studies discussed in the text but not included in the table because of lack of correlative analysis also showed similar patterns regarding fluctuations between IGF-I and growth.

<table>
<thead>
<tr>
<th>Feeding regimen</th>
<th>Nutrient Composition</th>
<th>Temperature x Photoperiod</th>
<th>Crude Protein %</th>
<th>Genetic Strain x Fish Meal %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilapia</td>
<td>0.55, a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid striped bass</td>
<td>0.56, b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coho salmon</td>
<td>0.72, c</td>
<td>0.68, d</td>
<td>0.72, e</td>
<td>0.47, f</td>
</tr>
<tr>
<td>Atlantic salmon</td>
<td>0.67, g</td>
<td>0.78, h</td>
<td>0.67, i</td>
<td></td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>0.73, j</td>
<td>0.65, g</td>
<td></td>
<td>0.70, k</td>
</tr>
<tr>
<td>Gilthead sea bream</td>
<td></td>
<td></td>
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<tr>
<td>Atlantic cod</td>
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<td></td>
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<tr>
<td>Barramundi</td>
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<tr>
<td>Channel Catfish</td>
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</tbody>
</table>
Table 2. Dynamics in the GH-IGF system relative to altered states of growth elicited by various manipulations. Upward arrows reflect positive relations between the hormone and the variable; Downward arrows indicate negative relations; Horizontal arrows indicate no change. Multiple indicator arrows represent differing results between studies. — indicates that data is not available for the particular variable. Abbreviations: SGR = specific growth rate; IGF-I = insulin-like growth factor-I; GH = growth hormone; IGFBPs = insulin-like growth factor binding proteins; BP = binding protein. Binding protein numbers are based on their putative mammalian homologs. BP-1 and -2 are typically associated with catabolic states while BP-3 is typically associated with anabolic states.

<table>
<thead>
<tr>
<th>Feed</th>
<th>SGR</th>
<th>Plasma IGF-I</th>
<th>Plasma GH</th>
<th>IGFBPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restriction-Deprivation</td>
<td>↓</td>
<td>↓</td>
<td>↑ ↔</td>
<td>(BP-3) ↓</td>
</tr>
<tr>
<td>Increased Ration Size</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>(BP-1) ↓; (BP-3) ↑</td>
</tr>
<tr>
<td>Compensatory Growth</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Increased Crude Protein %</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>—</td>
</tr>
<tr>
<td>Plant Protein Supplementation</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimal Rearing Temperature</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Photoperiod</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Increasing Daylength</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol Injections</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
<td>(BP-1) ↑</td>
</tr>
<tr>
<td>Stress</td>
<td>↓</td>
<td>↑ ↔</td>
<td>↑</td>
<td>(BP-1, -2) ↑; (BP-3) ↓</td>
</tr>
</tbody>
</table>

\(^a\) the effects of temperature on growth may be confounded by photoperiod

\(^b\) the effects of photoperiod on growth may be confounded by temperature
Figure 1. The endocrine control of growth in teleost fishes. Growth hormone, in response to various exogenous and endogenous cues, stimulates the synthesis and secretion of IGF-I peptide from the liver, which causes proliferation of target tissues. Rises in IGF-I, in turn, lead to an inhibition of GH synthesis and secretion through a classic negative feedback loop. IGF-I produced locally may also contribute to cell proliferation. IGFBPs from hepatic and non-hepatic sources can inhibit or potentiate the biological actions of IGF-I. Abbreviations: GH = growth hormone; IGF-I = insulin-like growth factor-I; IGFBPs = insulin-like growth factor binding proteins.