

## ABSTRACT

TURANO, MARC JOHN. Effect of cyclic feeding on compensatory growth and water quality in hybrid striped bass, *Morone chrysops* X *M. saxatilis* culture. (Under the direction of Harry V. Daniels and Russell J. Borski).

A series of pond and tank studies were conducted to determine if compensatory growth (CG) could be elicited in hybrid striped bass (HSB; *Morone chrysops* x *M. saxatilis*) through manipulations in feeding that involve sequential cycles of feed deprivation followed by daily satiation feeding. Growth, feed efficiency (FE), hepatosomatic index (HSI), intraperitoneal fat ratio (IPF), and condition factor (CF) were assessed at intervals throughout the growth trials to characterize the nutritional status (catabolic state) and CG response of HSB. Measurements of pituitary growth hormone (GH) cell activity (gene expression, protein stores), and plasma GH were assessed in tank studies to identify the potential catabolic and anabolic role of this growth promoting hormone during feed deprivation and CG. Lastly, the effects of the cyclic feeding regimes on water quality were assessed in pond trials. In each of the three studies, growth compensation, defined by growth rates that exceed that of control fish fed daily, was observed when treatment fish were realimentated to a control daily satiation feeding following a period of feed deprivation. Cyclic feeding increased overall FE of treatment fish over the controls by 10.8-40.0 % in the first pond study (HSB fingerlings), and 7.0-8.5 % in the second pond study (HSB food fish) but not in the tank study. Hepatosomatic index and CF varied significantly with feed deprivation and refeeding, and were useful predictors of the CG response following feed deprivation. There was an inverse relationship between all GH measurements and nutritional status, with no reported compensatory changes in GH. Changes in water quality parameters due to cyclic

feeding were only observed in the pond study with HSB food fish. Ponds subjected to the cyclic feeding regimes had 25-38 % lower levels of total phosphorus, 23.9-41.3 % less soluble reactive phosphorus, and 11.6-27.8 % less chlorophyll-a. Based on these results, CG can be repeatedly induced in HSB in both ponds and tanks, albeit full growth compensation was not achieved under the cyclic feeding conditions used in these studies. Regardless, these studies suggest that cyclic feeding can improve overall FE and water quality in pond-raised HSB. Further studies are required to ascertain the optimal catabolic conditions needed to induce full growth compensation during HSB production.

EFFECT OF CYCLIC FEEDING ON COMPENSATORY GROWTH AND WATER  
QUALITY IN HYBRID STRIPED BASS, *Morone chrysops* X *M. saxatilis* CULTURE

by

MARC JOHN TURANO

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Approved by:

Dr. Harry V. Daniels

Dr. Russell J. Borski

Dr. Ronald G. Hodson

Dr. Thomas M. Losordo

Dr. T. Gibson Gaylord

## DEDICATION

To my mom, Mary Ann, and dad, Barry, thank you for your love, support, and the opportunity of higher education. To my wife, thank you for your unconditional love, support, and sacrifice throughout the past few years. And yes, no more school.

## BIOGRAPHY

Marc Turano is a native New Yorker who grew up on Long Island's coast. After High School graduation, he left NY to attend the University of North Carolina at Wilmington where he completed a B.S. degree in Marine Biology. Following graduation from UNCW, Marc entered the graduate program at Texas A&M University- Corpus Christi and fulfilled the requirements for a M.S. degree in Mariculture. During his Masters program, he worked as a research technician at the University of Texas- Marine Science Institute. Following completion of a Masters degree, Marc returned to North Carolina and was hired in a joint position between the North Carolina Cooperative Extension Service (NCCES) and Brunswick Community College. This joint position was eventually shifted to include NCCES and NC Sea Grant. In addition to his extension program activities, he was then tasked to administer the Blue Crab Research Program, a \$500,000/year granting program for research concerning blue crabs. After working for 3 years in this position, he entered North Carolina State University as a Ph.D. student in the Department of Zoology. He continued his role as administrator of the Blue Crab Research Program while also conducting research on compensatory growth in hybrid striped bass. Marc's research approach combines the disciplines of physiology, nutrition, and water quality to address issues of basic science as well as their commercial application. When not working with blue crabs or hybrid striped bass, he enjoys spending time with his wife and daughter. In addition, Marc's hobbies include fishing, scuba diving, and, in trying to hold on to his youth, rugby.

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## Chapter 1

### Critical Considerations for the Comparison of Hybrid Striped Bass (*Morone chrysops* x *M. saxatilis*) Compensatory Growth Trials in Tanks to Ponds: A Review

#### Abstract

Compensatory growth (CG) is a period of accelerated growth that follows periods of limited growth due, for example, to feed deprivation or low temperatures. Interest in CG has developed for its potential use in aquaculture, as the CG response is often accompanied by enhanced growth rates and feed efficiency (FE), as observed in tank trials. Few studies have evaluated CG of pond-reared fish. Considering that most fish are raised in earthen ponds, the development of techniques to enhance growth rates and FE in these systems could prove useful in reducing feed costs and improving water quality management. Hybrid striped bass, *Morone chrysops* x *M. saxatilis*, (HSB) farming is one industry that could benefit from the development of methods to induce and control CG. Improvements in the FE of farm-reared HSB reduces feed costs and assists in improving water quality. This review attempts to identify important considerations in conducting experiments on CG in ponds, with reference to how different designs may affect water quality.

Tanks studies have utilized periodic feed restriction/deprivation followed by refeeding to induce CG. Increases in growth rate and FE, have been elicited through short term (< 1-week) and prolonged (1-week or greater) feed deprivation. Because of inherent differences between ponds and tanks, such as presence of natural prey, lower stocking density, and daily and seasonal variations in temperature, photoperiod, and water quality, it seems likely that prolonged feed deprivation periods would prove more suitable to pond

studies. Additionally, prolonged cyclic feeding regimes would likely have a greater affect on water quality as phytoplankton, the major producers of dissolved oxygen, relies on nutrients, particularly phosphorus, originating from feed. Depriving fish of feed, therefore, may be a means of dampening excessive fluctuations in phytoplankton abundance, and dissolved oxygen levels caused by high feeding rates. Due to the ability of phytoplankton to store phosphorus, feed deprivation periods need to be sufficient to allow phosphorus to be depleted and phytoplankton abundance to decrease. However, feed restriction/deprivation periods to minimize phytoplankton population flux should be balanced against excessive weight loss, and subsequent decreases in production.

Hybrid striped bass are an important cultured fish. Estimates of FE average 50 % (Feed conversion ratio; FCR 2.0) during final growout (Hodson, 1995). During this period, feed input is at a maximum, and increases in FE have a greater impact on feed costs (more feed is utilized) and on reducing nutrient inputs to the pond. Therefore, the benefits of controlling CG in HSB pond production can be shown in costs savings, and decreased risks with respect to management of water quality. Because little is known about whether the CG response of fish observed in tanks will be similar to fish grown in ponds, further research on pond production of fish is warranted.

## Introduction

Compensatory growth (CG), or “catch-up” growth, has been defined as a physiological process whereby an organism accelerates its growth after a period of restriction, to attain the weight of cohorts whose growth was never hampered (Hornick et al., 2000). Compensatory growth has been reported in several terrestrial vertebrates (Wilson and Osborne, 1960) and has drawn interest for its potential to improve growth rate and feed efficiency [FE= (weight gain/ food fed) x 100]. Many aquatic species have also been shown to exhibit CG (reviewed by Ali et al., 2003) with similar improvements. However these studies were conducted in tanks or aquaria. To date, there are only two published studies of CG that have been conducted in ponds (Kim and Lovell, 1995; Li et al., 2005). Both studies evaluated CG in channel catfish, and only one (Li et al., 2005) assessed changes in water quality variables. Hence, it little is known about how planned periods of feed deprivation affect water quality in ponds.

Experiments to elicit a CG response in fish have applied varying lengths of feed deprivation followed by periods of refeeding, termed cyclic feeding. Some studies reported short-term improvements in growth and FE for an initial period following realimentation, then returning to normal levels thereafter (Kim and Lovell, 1995; Johansen et al., 2001; Wang et al., 2000; Xie et al., 2001). Other studies reported increases in overall FE (Chatakondi et al., 2001; Quinton and Blake, 1990). Improved growth may decrease production time while increased FE could reduce costs by enhancing nutrient utilization and assisting in water quality management, particularly in pond production. Wasted nutrients from feed are direct losses, in terms of costs, and can later impact water quality, causing

mortalities, reduced growth, or added labor and water usage to manage the water quality decline.

One established aquaculture species with the potential to benefit from the development of methods to control CG is the hybrid striped bass (HSB), *Morone chrysops* x *M. saxatilis*, (Skalski et al., 2005). A major portion of farmed HSB are produced in ponds (57 %; Carlberg et al, 2005). Practical methods to decrease wasted feed or increase FE without adverse effects on growth would be useful to improve the economics and waste management of HSB production.

### **Sources of Variation Inherent to Pond Studies**

Ali et al. (2003) reviewed dozens of experiments on CG with fish. Of the 77 experiments reviewed, 76 were conducted in tanks under controlled conditions. Further. Only one additional study (Li et al., 2005) was conducted in ponds. It is likely that the CG response reported in the tank experiments would be difficult to reproduce in ponds, since the presence of natural prey, lower stocking density, and daily and seasonal variations in temperature, photoperiod, and water quality can affect fish growth during a long-term field study. There is a relationship between severity of feed deprivation (ration size and length of deprivation) and the extent of the CG response (Quinton and Blake, 1990; Hayward et al., 1997). Therefore, experimental treatments that deprive fish of feed may not be entirely effective in a pond study because of the inability to completely eliminate natural productivity. The presence of natural prey in ponds could alter the CG response when it is dependent on periodic feed deprivation, particularly if the species of interest is an opportunistic feeder such as the HSB. This problem would likely be compounded in studies

using smaller fish because although fish readily consume both natural prey and artificial feed (Kelly and Kohler, 1996), smaller fish may benefit more from natural prey, and utilize this source during deprivation periods.

Stocking density has been shown to affect feeding behavior (Kemeh and Brown, 2001). Oftentimes, increased densities, such as those found in tank studies, lead to more vigorous feeding (Pitcher and Parrish, 1993). Ponds are stocked at lower densities than tanks (ponds, 0.4-0.7 fish/ m<sup>3</sup>; tanks, 60-88 fish/ m<sup>3</sup>) so reduced feeding activity relative to tanks may affect the CG response. Furthermore, many species, including HSB, exhibit schooling behavior, a pattern that may be important to increased feeding activity. Kemeh and Brown (2001) reported significant increases in standing crop biomass with higher stocking density (from 2.5-15.0 kg/m<sup>3</sup>) without sacrificing weight gain in tank production of HSB. Further, no schooling behavior was observed with stocking densities less than 5.0 kg/m<sup>3</sup>. Hence, decreased densities in ponds may limit the strength of the refeeding response by decreasing hyperphagia and altering fish growth during this critical period.

Pond studies of CG must also consider environmental factors such as temperature and photoperiod. Ponds are subjected to diurnal and seasonal temperature fluctuations whereas tank studies are more controlled. Temperature may alter the CG response, as temperatures outside the optimal growth range may negatively affect growth and/or FE. It has also been suggested that a decrease in metabolic rate caused by feed deprivation may be one of the key factors in the CG response, as allocation of nutrients following realimentation can be directed toward tissue deposition rather than maintenance (Broekhuizen et al., 1994). If this hypothesis is correct, increased temperatures may prevent the reduction of metabolic rate to a sufficient level such that significant gains in FE are not achieved. In addition to temperature,

it is well known that natural photoperiod is a primary trigger for sexual maturation (Bromage et al., 2001). Alterations in nutrient partitioning (from growth to maturation) as a result of changes in day length depress growth and would likely affect the CG response. Therefore, experiments with spring-spawners such as the HSB should be conducted prior to sexual maturation or after the end of the spawning season. While tank studies provide valuable insight into the factors that control CG, the lack of environmental control in pond studies make the direct application of results from tank trials difficult without an understanding of how variations in the pond environment may affect fish growth.

### **Why Conduct Pond Studies?**

Although tank studies generate data from a controlled environment, replicated pond studies offer the ability to evaluate biological processes under conditions not accounted for in indoor tanks. Because the majority of US aquaculture production (63 %) is carried out in ponds (ERS-USDA, 2004), methods to improve pond production efficiencies could impact a significant portion of the industry. Specifically, of the 11.5 million pounds of HSB production in 2003, approximately 57 % was from ponds (Carlberg et al, 2005). Feed is the second highest cost in HSB production (D'Abramo et al., 2004) and has direct impacts on growth, fish health, and water quality. Increased nutrient utilization results in reduced feed cost per unit production. Development of techniques to control CG in ponds could reduce costs and help manage water quality in HSB culture through overall improvements in FE. As more dietary ingredients are utilized for growth, less are available to ponds in the form of waste, thereby mitigating the negative effects of poor water quality. Also, application of

cyclic feeding regimes may reduce production costs associated with labor, as there would be periods where feeding is absent or its frequency reduced.

A portion of feed added to ponds for fish production is ultimately released to the water increasing levels of nitrogen, phosphorous, and organic matter which can lead to water quality problems or be released as pollutants into the environment. Nitrogenous waste in the form of ammonia results from the direct release from the gills of fish, and the microbial breakdown of uneaten feed, urea, and fecal nitrogen (Tucker and Boyd, 1985). Excessive ammonia and nitrite can lead to mortalities due to toxicity (Oppenborn and Goudie, 1993), or impaired growth rates at sub-acute levels. Phosphorous is a major nutrient in feed, and is a limiting factor in freshwater phytoplankton production. It can indirectly lead to low dissolved oxygen levels, increasing the risk of mortalities when phytoplankton communities consume oxygen during night-time hours. Phosphorus is readily absorbed by phytoplankton promoting growth of these communities. Although phytoplankton are the primary producers of oxygen in the fish ponds, they can become the main consumers of dissolved oxygen, when photosynthesis ceases. Excessive phytoplankton populations can reduce oxygen to lethal or stressful levels during the night-time. Hence, management of phytoplankton blooms through feed management is a necessary component of fish culture in ponds. Dissolved oxygen levels are also affected by the accumulation of organic matter. Increased loading of organic matter results in increased biological oxygen demand by stimulating microbial breakdown (Daniels and Boyd, 1989).

In addition to the direct impacts of wasted feed to the pond environment, accumulation of nutrients may be a concern when ponds are drained or flushed. The potential for regulation of pond discharge by the United States Environmental Protection Agency has

increased the need to identify methods to reduce nutrients in pond effluents. With the growing trend of environmental awareness by aquaculture associations that promote environmentally responsible production methods, there is also widespread interest in the economic advantages of products certified to be, “environmentally friendly,” (Boyd, 2003). Hence, the development of methods to control CG in fish under pond production conditions could result in benefits beyond savings in feed costs.

### **Comparison of Feeding Regimes: Restricted Feeding vs. Prolonged Feed Deprivation**

It is unknown whether results from studies of CG in tanks can be directly applied to ponds, however, feeding methods employed in tank studies provide a starting-point for the design of pond studies. Experiments on CG in different species can be categorized as using either restrictive feeding or prolonged feed deprivation. For purposes of this review, restrictive feeding, is complete feed deprivation for less than one week, or feeding a decreased percentage of body weight (maintenance ration) as compared to control fish. Prolonged feed deprivation is considered complete feed deprivation for one week or greater.

In the first of two published replicated pond trials of CG, Kim and Lovell (1995) subjected advanced catfish fingerlings, *Ictalurus punctatus*, to restricted feeding regimes of zero, three, six, and nine weeks, during which time fish were fed every three days. Following feed restriction, fish were fed daily to satiation. At the conclusion of the 18-week experiment, fish subjected to the 3-week feed restriction regime were equal in weight to the control groups fed daily throughout the experiment. Complete growth compensation was achieved after three weeks of refeeding. All other treatment groups were significantly smaller than the control and three-week treatment fish. Morphometric indices, total fat, protein, and

moisture as well as dressout were not different among groups at the completion of the trial. Interestingly, fish in the three-week treatment were fed only seven of 21 days during the restriction period, yet were ultimately similar in final weight to control fish. These findings have significant implications for commercial production in that both labor costs and nutrient loading could be reduced during the restriction period. Unfortunately, only dissolved oxygen levels were monitored, with no effect from feed restriction observed. Hence it is unknown whether the feed restriction periods had an affect on water quality.

Similarly, in another published pond study of CG, Li et al. (2005) reported no difference in net production between channel catfish offered cyclic feeding regimes of 1:6, 1:4, and 2:5 (days not fed: days fed) and normally fed control fish. No significant differences in any of the water quality parameters were observed between treatment and control ponds. The experimental treatments used in this study included only feed restriction rather than complete feed deprivation. Hence, the decrease in feed rate may not have been sufficient in length to observe differences in water quality. Nevertheless, production practices could be adopted in which a feeding regime similar to the three-week restriction (Kim and Lovell, 1995) or the more short-term restriction (Li et al., 2005) is applied to increase FE and decrease costs associated with labor.

In a study of restricted feeding in tanks (Chatakondi et al., 2001), catfish fingerlings were fed daily to satiation (controls) and growth was compared with those deprived of feed for one, two, or three days and then refed for as long as feed consumption was statistically higher than that of control fish. Following the 10-week trial, average growth rates of treatment fish were 40 %, 180 %, and 191 % greater than the control fish for those deprived for one, two, and three days, respectively. In addition, final weights of fish subjected to the

three day feed deprivation cycles were significantly greater than all other treatments including controls, while all treatment fish had significantly higher FE's than control fish. The results of this study confirm the findings reported by Kim and Lovell (1995) and Li et al. (2005), and further demonstrate the potential for catfish to overcome periods of feed restriction by regaining lost weight. However, treatment fish in the study by Chatakondi et al. (2001) exhibited increased FE at the termination of the trial, whereas Kim and Lovell (1995) did not report improvements in FE and Li et al. (2005) showed modest improvements, albeit not statistically different from control fish. If the feeding regimes in pond studies could yield increases in FE and full growth compensation, farmers would benefit from both reductions in feed cost and water quality issues without sacrificing production.

In another study on restrictive feeding in tanks, two groups of Atlantic salmon, *Salmo salar*, were fed either at 50 % of a pre-determined optimal feeding level for eight weeks, followed by an eight week period of feeding to satiation, or to satiation for 16 weeks (Johansen et al. 2001). At the end of the trial, weight gain and feed intake between control and treatment fish were statistically similar, but FE was not elevated in fish offered the restricted feeding regime. These results suggest that Atlantic salmon have the capacity to fully compensate for growth through increased feed consumption. Additionally, increased growth rates in this study were initiated through feeding a maintenance ration. This method, although slightly different from the studies with catfish, yielded similar results in terms of overall growth, suggesting that complete feed deprivation may not be necessary to initiate a CG response.

Although the studies on restricted feeding differ in the duration and extent of feed restriction, the response, complete compensation for lost growth and/or increased FE, was

similar. However, when attempting to quantify an effect on water quality, application of a restricted feeding regime to pond trials would likely have little detectable effect because of the limited periods without feed inputs. Daily feeding provides a constant supply of nutrients to both fish and phytoplankton. Despite fish production being of primary concern, management of phytoplankton has direct effects on oxygen production, hence, timing and magnitude of nutrient delivery is key for phytoplankton management. However, because phytoplankton have the capacity to store and use phosphorus, a process termed luxury consumption (Boyd, 1990), the short-term duration of restricted feeding regimes would likely have little effect on their abundance. In pond studies, CG experiments using prolonged feed deprivation may prove more suitable to trigger both a CG response and an effect on water quality as longer periods of nutrient deprivation would likely be required to allow phosphorus stores to be depleted and phytoplankton abundance to decline.

Several studies have reported growth compensation of fish when subjected to prolonged feed deprivation followed by refeeding. Quinton and Blake (1990) evaluated different feeding regimes for rainbow trout, *Oncorhynchus mykiss*. Fish were exposed to either daily feeding at 5 % body weight (control), or one of five other regimes which cycled between alternating weeks of feed deprivation followed by refeeding at 3, 5, or 7 % body weight. Fish subjected to 3-weeks feed deprivation/ 3-weeks refeeding at 3 % body weight grew more than fish exposed to other regimes. In addition, when fish fed daily were compared with fish cycled on the 3-week regime, there was no significant difference in weight of either group after 12 weeks, with control fish ingesting 264 % more feed. Hence, temporary improvements in growth rates were sufficient to compensate for lost weight, and overall FE was increased.

Similarly, in another study using extended feed deprivation, full growth compensation was observed with hybrid tilapia, *Oreochromis mossambicus* x *O. niloticus* (Wang et al., 2000). When fish deprived of feed for one, two, or four weeks followed by four weeks of refeeding were compared to control fish fed twice daily to satiation, those deprived of feed for one week were not statistically different in weight following the completion of the 8-week trial. No difference was observed in FE, or protein and energy retention efficiency between control and treatment fish during refeeding. Similar results were also observed by Xie et al. (2001) with gibel carp (*Carassius auratus gibelio*). It appears then that increases in feed consumption would account for the catch-up growth observed in cyclic-fed fish in this study. Because CG has been shown to occur in response to prolonged feed deprivation, and it is likely long periods of nutrient deprivation are needed to affect water quality, the feeding regimes used in the studies by Wang et al. (2000) and Xie et al. (2001) could prove more effective for use in pond trials.

In some studies, only partial growth compensation and/or intermediate increases in FE were achieved. Although these studies may not be as effective in enhancing water quality, they nevertheless display the potential for a CG response, which, upon further experimentation, could enhance water quality. For example, in an experiment with catfish fingerlings, fish were fed either twice daily to satiation for 14 weeks or deprived of feed for four weeks followed by twice daily feeding to satiation for 10 weeks (Gaylord and Gatlin, 2000). After eight weeks, treatment fish gained 179 % of their initial weight, while control fish gained 231 % of their initial weight. Full growth compensation was not achieved. However, a temporary increase in both growth and FE was observed, compared with control fish. The authors concluded that a feed deprivation period of four weeks may be excessive

and not allow full catch-up, however, the decreased overall weight gain may be outweighed by improvements in water quality in pond application. Hence, timing of feed deprivation may be as important as the duration. If a feeding regime similar to the one utilized in the Gaylord and Gatlin (2000) experiment was applied during periods of high temperatures and poor water quality, risk of fish mortalities due to poor water quality may be decreased enough to justify the lack of complete growth compensation. Additionally, during these periods of poor water quality, growth may be hampered in control ponds (satiation feeding), hence there may be no apparent difference in weight between fish in control ponds and those undergoing partial compensation.

### **Nutrient Availability and Feed Timing**

The studies mentioned above have shown that feed deprivation of greater than one week, followed by feeding to satiation can trigger a CG response in some fish. Applying these designs to pond studies must account for timing of nutrient delivery for phytoplankton bloom management. Because no previous studies have evaluated pulsatile additions of feed to ponds, relevant data are lacking, although pond fertilization regimes can provide nutrients in a similar manner. Typical fertilization regimes include adding fertilizers more frequently at the start of the production season to stimulate primary production. The algal bloom is then managed by maintain plankton abundance, as fertilizers are then added weekly or bi-weekly. The maintenance addition of fertilizers is similar to the feeding regimes in CG studies when prolonged periods (>1-week) of feed deprivation or lack of nutrient input are initiated. Comparisons can be made between nutrient additions (particularly phosphorus) from fertilizer and those from feed inputs. Studies are available on the effects of additions of

fertilizer to production ponds. Since variations in phosphorus availability, as measured by soluble reactive phosphorus, can alter the growth of phytoplankton, understanding the flow of phosphorus following its addition to the pond is necessary.

Although there is a lack of data on additions of phosphorus to ponds via feed inputs, a number of studies have been conducted to determine the effects of phosphorus from fertilizers on pond production. In a study to determine the availability of phosphorus from three forms of phosphorus fertilizers, Boyd et al. (1981) found that total phosphorus (TP) and soluble reactive phosphorus (SRP) peaked one day following fertilization, and decreased to stable levels within 10-15 days. Chlorophyll-a concentrations, a measure of phytoplankton abundance, started to increase within 1-day and peaked 5-8 days following fertilization, hence, there is an immediate but slow response in phytoplankton production following increased SRP levels. Rushton and Boyd (2000) further examined phosphorus availability by measuring TP and SRP beginning seven hours after fertilization of 12 earthen ponds. SRP levels peaked at seven hours post-fertilization, while TP concentrations peaked beginning at 32 hours depending on type of fertilizer used. The rapid decrease in SRP only three hours after its peak was caused by phytoplankton uptake which suggests that phosphorus in liquid fertilizers is readily available to phytoplankton, and can rapidly alter productivity. Since pond studies on post-feeding phosphorus release have not been reported, it is unknown how the rate and availability of phosphorus released from feed will compare to that of fertilizers.

The fate of nutrients in pond water can be as important as determining their availability as it assists in locating the target of their greatest impact. Phosphorus is removed from pond water primarily by phytoplankton, bacteria, and sediment (Boyd, 1990). Hence, to follow the flow of phosphorus added to ponds, one must consider these three avenues of

removal. In an experiment to identify the uptake of phosphorus by phytoplankton and sediment, Boyd and Musig (1981) measured SRP levels at various intervals from water samples containing phytoplankton only, and samples containing sediment without phytoplankton. Depending on concentration of chlorophyll-a, mean percent uptake of phosphorus at 2, 4, 8, and 24 hours post-fertilization was 24.1, 30.1, 33.0, and 41.0 %, respectively, although, in one sample, 97 % of the phosphorus was removed as early as two hours following fertilization. In experiments with sediment only, phosphorus uptake was inversely proportional to the amount of inorganic phosphorus present in the sediment at the start of the trial. Greater than 80 % of the SRP was removed within 15 days of fertilization by adsorption to the sediment. The authors concluded that phosphorus is taken up very rapidly by phytoplankton, whereas sediments, a major sink for nutrients, vary in the degree of phosphorus adsorption. These studies demonstrate the rapid uptake of phosphorus from pond waters and the primary role of phytoplankton in this process. Additions of phosphorus from feed inputs would likely be absorbed similarly, however, the timing of its availability (release to the water) is unknown.

The dynamics of nutrient flow following additions of fertilizer are rapid, as phosphorus ( $P_2O_5$ ) is added in a form readily available by phytoplankton. It would be appropriate to assume that the resulting reaction from additions of phosphorus in feed would be less pronounced. There is less phosphorus in feed as a percent in comparison to fertilizers. Pond fertilizers most often used by fish farmers contain 34 % phosphorus as  $P_2O_5$  (Boyd, 1990), added at a rate of 4kg/ha, depending on alkalinity and pond productivity. These additions result in rapid increases of phosphorus ranging from 0.20-0.30 ppm assuming a 4-acre-ft pond. Dietary phosphorus concentrations typically range from 0.5-2.0 % and feeding

levels in production ponds depend on stocking density, hence, phosphorus inputs will vary. In order for final phosphorus levels to be equivalent to recommended fertilizer applications, 300 lbs of feed containing 1.0 % phosphorus would need to be added per acre, assuming 100 % of the phosphorus in the feed was available to the phytoplankton. In foodfish production, these feeding levels are two to three times higher than normal. The response of phytoplankton to phosphorus from feed inputs would also be affected by phosphorus availability. Because phosphorus from feed is ultimately available in fish excretory products, the time necessary for digestion and excretion would likely result in a delayed availability of phosphorus and the subsequent response by phytoplankton.

In studies of fish growth in ponds, it is assumed that all feed is consumed by fish, although excess undigested feed may remain in the pond. Hence the availability of phosphorus is generally thought to come from excretory products after digestion and absorption. Attempts to quantify phosphorus excretion in fish have had varied results because of differences in dietary phosphorus inclusion, availability, and species requirements. In studies with rainbow trout, total phosphorus retention averaged 60 % (Rodehutsord et al., 2000; Coloso et al., 2001, 2003), while, Ballestrazzi et al. (1998) observed phosphorus retention amounts of 17.9-19.1 % in European Seabass, *Dicentrarchus labrax*. The drastic differences could be the result of higher availability of phosphorus supplementation in the trout diets, whereas seabass diets might contain phosphorus from ingredients other than phosphorus supplementation. Additionally, overall dietary levels varied between the studies, with 0.10-1.20 % for the trout and 1.78-2.05 % dry matter for the seabass study. The differences in overall phosphorus levels in the studies is of interest because it has been found that non-fecal phosphorus excretion, the major excretion route for phosphorus in fish,

increases exponentially after basal phosphorus intake levels are met (Rodehutsord et al., 2000). Hence, diets used in the seabass study may be over-formulated in terms of phosphorus and/or provide phosphorus in forms not readily available to fish. Estimates of phosphorus retention can be used to help predict the effect of feed additions to pond water. Using the prior example of feeding 45 kg/day (100lbs./day) of a diet containing 1.0 % phosphorus, if only 18 % were retained in the fish (Ballestrazzi et al. 1998), the feed would contribute 0.075 ppm phosphorus to the pond water. The availability of phosphorus would therefore be greatly reduced relative to typical fertilizer additions. Hence, direct affects of phosphorus from feed on phytoplankton production would likely be reduced at these levels. However, phosphorus excretion levels do not always reflect actual amounts of available phosphorus in the environment. As shown in ponds, phosphorus availability after fertilization may vary, but is rapidly taken up by phytoplankton and adsorbed to sediment. Studies to determine the amounts of phosphorus entering the culture environment have measured SRP at varying intervals after feeding in tanks. Soluble reactive phosphorus levels have been observed to peak between 0.5-1 hours post-feeding (Coloso et al., 2003, McIntoch et al., 2004) and 1.5-3 hours post-feeding (Ballestrazzi et al, 1998). Levels of SRP in the tank trial by Coloso et al (2003) reached 0.11 mg/L during the morning feeding, and 0.24 mg/L following the evening feeding. Ballestrazzi et al. (1998) observed average SRP levels of 0.35-0.39 mg/L following feeding. Interestingly, peak values for SRP in these tanks studies exceeded those in the pond fertilization trials. The reasons for the initial spike in SRP following feeding are not known since all feed is consumed rapidly and digestion is not complete within three hours. However, Coloso et al. (2003) suggested that the release in SRP immediately following feeding resulted

from feeding-induced excretion of phosphorus. Regardless of the source, the release of phosphorus in any form provides nutrients for phytoplankton production.

The availability of phosphorus in water following feeding is rapid, usually within 1-3 hours, and is similar to the response observed in fertilizer studies. The response in ponds could be similar, but requires further attention due to the presence of phytoplankton, sediment, and increased levels of bacteria and organic matter. Although there are inherent amounts of organic matter and bacteria in tank systems, uptake or adsorption of phosphorus by these sources is greatly reduced in comparison to phytoplankton and sediment. Nevertheless, the relationship between feeding and nutrient availability could be altered by cyclic feeding regimes.

### **Potential for Compensatory Growth in HSB**

One species with the potential for exhibiting CG is HSB. Based on the current knowledge of the mechanisms of CG, use of feed deprivation in an attempt to mimic periodic feeding, is one method of stimulating a CG response. Gurney et al., (2003) notes, “In the natural environment, [HSB, similar to other species], exploit food supplies whose spatial, temporal, and seasonal variability implies periods of near or actual starvation.” These instances can be observed during juvenile stages where planktonic prey distribution can be patchy, as well as adult stages during over-wintering and seasonal migrations (Shultz et al, 2002). Once prey abundance increases, HSB may compensate for lost growth. The HSB aquaculture industry could benefit from reduced production costs due to improved FE. Furthermore, a significant portion of the HSB industry consists of pond production (approximately 57 % in 2004), emphasizing the wide application to the industry.

Compensatory growth has been reported in HSB. Picha et al. (in press) conducted a 120-day growth trial in which phase II HSB (120-130g) were subjected to one of two feeding regimes; twice daily to satiation or cyclic feeding consisting of four weeks of restrictive feeding (one feeding to satiation per week), followed by four weeks of feeding to satiation. Intermediate sampling revealed a rapid increase in growth and FE compared to controls. This response then returned to normal levels, and no statistical difference in overall FE was observed. Hence, HSB show a similar CG response to feed deprivation as other fish in tank studies.

### **Summary**

Compensatory growth in fish is a promising means of improving FE and water quality management in ponds. There is a considerable body of literature documenting CG of fish in tanks, but fundamental differences between tank and pond environments may limit the direct application of tank protocols to pond culture. Some of these differences in ponds relative to tanks that should be considered in the development of CG protocols are the presence of natural foods, decreased fish densities, and environmental variables such as photoperiod and temperature. The mechanisms that trigger growth compensation have yet to be clearly defined, hence methods to reliably elicit the response have not been developed. Based on the current understanding of the mechanisms of CG reported in the literature, and of pond nutrient cycling, it is likely that only the protocols involving prolonged feed deprivation (>1 week) will cause a detectable CG response in ponds.

Hybrid striped bass are an important cultured fish with potential for increasing FE in pond production. The benefits of controlling CG to increase FE in HSB pond production can

be shown in costs savings, and decreased risks with respect to management of water quality. Further, because little is known about whether the CG response of fish observed in tanks will be similar to fish grown in ponds, further pond research is clearly warranted.

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## Chapter 2

### Compensatory Growth in Pond-Reared Hybrid Striped Bass

(*Morone chrysops* x *M. saxatilis*) Fingerlings

#### Abstract

Compensatory growth (CG) or “catch-up growth” is a period of super-accelerated growth following a period of sub-optimal conditions (i.e. lack of prey availability or over-wintering). To evaluate the potential for CG in HSB culture, a 16-week growth trial in 12, 0.1 ha earthen ponds was conducted. Approximately 2850 fish (mean weight  $\pm$  std.;  $3.2 \pm 1.10$ ) were stocked into ponds and subjected to one of four cyclic feeding regimens of feed deprivation/refeeding. Treatment regimens included a 0-week control (fed twice daily to apparent satiation) and cycles of either one (1-week), two (2-week), or four (4-week) weeks of feed deprivation followed by one, two, or four weeks of feeding to apparent satiation. Fish subjected to the 4-week feeding regimen were also offered feed once every other week during the deprivation period. Growth, specific growth rate (SGR), hepatosomatic index (HSI), intraperitoneal fat ratio (IPF), and condition factor (CF) were measured every other week, while overall growth, feed efficiency [FE: (weight gain/feed fed) x 100], and survival was calculated at the trial termination. The effect of these feeding regimens on water quality was examined by monitoring pH, turbidity, total ammonia nitrogen, nitrite-nitrogen, nitrate-nitrogen, soluble reactive phosphorus, and chlorophyll-a weekly, total nitrogen and phosphorous biweekly, and dissolved oxygen and temperature twice daily. Cyclic feeding elicited partial CG, with fish subjected to the 2-week regimen having significantly higher

SGR than 0-week controls during all but the final refeeding period. FE was higher for all fish in the cyclic regimens, although only FE for fish in the 2-week regimen was statistically greater than the controls. HSI was the most responsive measure and significantly decreased in the 2 and 4-week treatments during feed deprivation and overcompensated during the refeeding period. Condition factor also varied with feeding cycle and proved to be an effective non-lethal measure of predicting a CG response. No statistical differences in water quality parameters were observed. The increase in FE warrants further research for practical application. Future pond studies with fingerling HSB fish should be conducted with emphasis on feed deprivation periods of two weeks and refeeding periods of at least twice that of the feed deprivation.

## Introduction

Hybrid striped bass (*Morone chrysops* x *M. saxatilis*; HSB) culture is a significant part of the US aquaculture industry with total production for 2004 at 12 million pounds (Carlberg 2005). High costs of production and low fillet yield have slowed expansion (Carlberg, 2005). Regulations on water usage and discharge could further negatively impact efforts to expand production and affect economic viability. Economic sustainability will therefore rely on the development of methods to reduce production costs and minimize environmental impacts. One means of improving production and management of water quality is to develop methods that take advantage of compensatory growth (CG). This response also known as “catch-up” growth is a physiological process whereby an organism exhibits accelerated growth after a period of restricted development, usually due to reduced feed intake, in order to reach the weight of animals whose growth was never restricted (Hornick et al., 2000).

Compensatory growth has been reported in terrestrial (reviewed by Wilson and Osbourn, 1960) and aquatic (reviewed by Ali et al., 2003) animals. Quinton and Blake (1990) observed complete growth compensation in rainbow trout, *Oncorhynchus mykiss* fed a cyclic regimen of three weeks feed restriction followed by three weeks refeeding compared with fish fed daily throughout the 12-week study. Full growth compensation was also observed in Atlantic salmon, *Salmo salar*, subjected to an eight week restricted feeding regimen (50 % of a pre-determined optimal feeding level) followed by an eight week period of refeeding in excess (Johansen et al. 2001). Compensatory growth has also been elicited in non-salmonid species. Hybrid sunfish (*Lepomis cyanellus* x *L. macrochirus*) doubled their growth rate and surpassed control fish (overcompensated) when subjected to two days of feed deprivation

followed by refeeding periods persisting until the hyperphagic response ceased (Haywood et al., 1997). This was the first and only study to report over-compensation. However overcompensation was not reported when the study was repeated with group-housed as opposed to individually-housed fish (Hayward et al., 2000). Channel catfish have also been shown to exhibit CG, both temporary and complete CG (Kim and Lovell, 1995; Gaylord and Gatlin, 2000; Chatakondi and Yant, 2001; Li et al., 2005). Thus, the duration and extent of the CG response appears to be species-specific and dependent on the specific feed deprivation/refeeding cycle utilized.

The above studies have all been conducted in tanks, and to date, only two studies have been carried out in ponds (Kim and Lovell, 1995; Li et al., 2005). Advanced catfish fingerlings, *Ictalurus punctatus*, subjected to a restricted feeding regimen (fed every three days) for three weeks were not significantly different from control fish fed daily at the end of an 18-week growth trial (Kim and Lovell, 1995). In addition, whole body fat, protein, and moisture, as well as dress-out percentage were not different between groups at the completion of the trial. Similarly, Li et al. (2005) reported no difference in net production between channel catfish offered cyclic feeding regimes of 1:6, 1:4, and 2:5 (days not fed: days fed) and normally fed control fish. Production practices could be adopted in which a feeding regime similar to the three-week restriction (Kim and Lovell, 1995) or the more short-term restriction (Li et al., 2005) is applied to increase FE and decrease costs associated with labor. Further, increases in FE may prove beneficial to water quality providing additional potential for using cyclic feeding in production practices.

Although tank studies generate data from a controlled environment, replicated pond studies offer the ability to evaluate biological processes under pond conditions that cannot be

fully controlled. In addition, the majority of HSB culture is conducted in ponds (57 %). Therefore, affects of cyclic feeding regimens on cost savings and/or water quality could have practical application to HSB producers. To this end, a 16-week pond study was conducted to evaluate the effectiveness of cyclic feeding regimens to elicit a CG response in fingerling hybrid striped bass grown in ponds and to mediate water quality problems.

## **Materials and Methods**

### *Growth and Body Indices*

A 16-week growth trial was conducted at the Tidewater Research Station in Plymouth, North Carolina beginning in June 2003. Twelve, 0.1-ha ponds were stocked with approximately 2,850 fish/pond (28,500 fish/ ha) and allowed to acclimate for 5 d. During acclimation, fish were fed twice daily at 20 % body weight. Following acclimation 30 fish, randomly chosen from three ponds, were measured for initial length (mean  $\pm$  std.; 67.2 mm  $\pm$  7.05) and weight (mean  $\pm$  std.; 3.2 g  $\pm$  1.10). Additionally, liver and intraperitoneal fat weights were obtained to determine hepatosomatic index (HSI= wet liver weight x 100/body weight) and intraperitoneal fat ratio (IPF= wet weight of fat x 100/body weight).

Four feeding regimens consisting of alternating cycles of feed deprivation and refeeding were randomly assigned in triplicate to the 12 ponds. During all feeding periods fish were fed to apparent satiation twice daily with a commercially available HSB diet (45 % protein and 12 % lipid; Melick Aquafeeds, Catawissa, PA. USA). During the first four weeks, fish were offered a set ration of 15 % body weight due to the difficulty in determining apparent satiation. The four treatments were as follows; a 0-week treatment (control) consisted of daily feeding, 1-week and 2-week treatments consisted of alternating equivalent

periods of feed deprivation then refeeding for one and two weeks, respectively, and a 4-week treatment subjected fish to feed deprivation for four weeks followed by refeeding for four weeks. During the feed deprivation period for the 4-week treatment, fish were fed one day every other week (twice during the feed deprivation period).

Growth was measured at week four, and then every two weeks by seine sampling a section of each pond (bisecting the long axis) and obtaining a group wet weight (approximately 50 fish/ pond). Specific growth rate  $\{SGR = [100 \times (\ln \text{Weight}_f - \ln \text{Weight}_i)] / (\text{Time}_f - \text{Time}_i)\}$  was calculated based on average group weights of fish from each pond. At each sampling period, 10 fish were sacrificed to obtain individual weight, length, HSI, IPF and condition factor  $[CF = (\text{weight in grams}/\text{length}^3 \text{ in mm}) \times 10^5]$ . Following the termination of the growth trial, each pond was drained approximately 45 cm then harvested by seining. Total harvest weight was recorded, and a subsample from each pond (range: 143-200 fish/pond) was taken for individual weight and length measurements and to calculate coefficient of variation (CV). Additionally, 10 fish/pond were frozen at -20 C for proximate analysis (AOAC, 1995).

### *Water Quality*

Water quality parameters were measured throughout the study to determine the effects of cyclic feeding on water quality. Temperature and dissolved oxygen (DO) were recorded twice daily (0800 and 1600h) with a YSI 550 (Yellow Springs Instrument Company, Yellow Springs, Ohio, USA). Water samples were taken weekly using a 90 cm water column sampler (Boyd and Tucker 1992) and analyzed for pH (Orion 720A pH meter; Thermoelectron Corp., Waltham, MA, USA), turbidity (DRT 100B turbidimeter, HF

Scientific Inc., Fort Myers, FL, USA), total ammonia-nitrogen (TAN), nitrite-nitrogen (NO<sub>2</sub>-N), nitrate-nitrogen (NO<sub>3</sub>-N), and soluble reactive phosphorous (SRP; APHA et al., 1995). Weekly chlorophyll-a measurements were also taken using methods described by Pechar (1987). Total nitrogen (TN) and total phosphorous (TP) were measured every two weeks (APHA et al., 1995). Nightly aeration (2400h-0830h) was applied to each pond via 0.75-hp (6.6 hp/ha) paddlewheel aerators (Southern Machine Welding Inc., Quinton, AL, USA). Emergency aeration was provided if morning DO levels were below 5 ppm until this level was reached and/or if afternoon DO levels were below 7 ppm. Emergency hours of aeration were recorded.

#### *Statistical Analysis*

Growth, HSI, IPF ratio, and CF were analyzed at two week intervals while FE was compared at eight and 16 weeks when all fish had been subjected to at least one or two complete feed deprivation/refeeding cycles, respectively. Overall averages for water quality measurements were compared across treatments. All measurements were analyzed using a one-way analysis of variance (ANOVA) to determine significant differences ( $P < 0.05$ ) among treatment means. Tukey's least significant difference test was used to separate significant differences between treatment means (Steel et al., 1997). All statistical analyses were conducted using the Statistical Analysis System (SAS; software version 8.2, Cary, NC, USA).

## Results

Results of production variables (growth and FE) were analyzed at eight and 16-weeks to coincide with the time when all treatments had completed at least one or two production cycles respectively.

### *Weeks 0-8*

Fish readily adapted to the feeding regimens with no effect on overall survival. Mean total production for ponds assigned the 0-week regimen (control) was 62.3 % higher than all other treatments (Table 1). Following the first eight weeks of the study, a time at which all fish had been subjected to at least one complete feeding cycle, no significant differences were observed in weight gain (Figure 1). Specific growth rates were calculated following the first four weeks of the trial since a set ration was offered during feeding periods for this duration (Table 2). Fish in the 2-week treatment had a significantly higher SGR than control fish and 1-week cycled fish, but SGR was not different from 4-week treatment fish at week 8. Specific growth rate of fish in the 4-week regimen was significantly higher than all other treatments at 6 wk.

Feed efficiency during the first eight weeks was notably improved for all cyclic fed fish ranging from 101.5-117.0 % (FCR= 0.99-0.85) compared to 88 % (FCR=1.14) for the 0-week fish (Table 2), however, only fish in the 4-week treatment had significantly higher FE than control fish.

### *Weeks 8-16*

During the final eight weeks of the trial, fish subjected to the 0-week feeding regimen were significantly larger (Figure 1). Fish cycled in the 2-week treatment had a significantly higher SGR than the controls at week 12 (2-weeks after refeeding) (Table 2). An increase in SGR was observed for fish in the 4-week regimen during the second complete cycle (weeks 14 and 16), however, the effect was not statistically different from controls during these periods. Similar to the first eight weeks, FE of all treatment fish was improved, however only fish in the 2-week regimen displayed statistically higher FE (98.3 %) (FCR=1.02) than that of 0-week fish (70.2 %) (FCR=1.42)

In order to monitor fish health and energy partitioning, HSI and IPF were measured throughout the study. Based on the rapid fluctuations in HSI levels following feed deprivation and refeeding, the liver appeared to be a highly responsive organ to variations in feed consumption (Figure 2). Liver weights of fish subjected to cyclic feeding regimens decreased and reached their lowest mass within 2 weeks of feed deprivation, and, upon refeeding, exceeded that of 0-week fish. As shown by fish offered the 4-week regimen, liver weights reach control levels by the fourth week of refeeding. The rapid liver response in both treatments was similar during all complete feeding cycles. Intraperitoneal fat was not as responsive to the feeding regimens as HSI levels. Figure three shows variations in IPF ratios for all treatments during the trial. Intraperitoneal fat varied early for the 2 and 4-week treatments, however, as fish grew larger, fluctuations in fat deposition stabilized.

Condition factor was calculated during the study as a non-lethal means of evaluating the effect of cyclic feeding on fish. Condition factor of fish in the 2-week treatment decreased significantly with feed deprivation and recovered during refeeding (Figure 4). A

maximum of two weeks of refeeding was necessary for the CF to return to levels similar to 0-week fish, as indicated by fish in the 2 and 4-week regimens. In addition to CF, cyclic feeding did not seem to affect overall body composition. No significant differences were observed in fish for whole body percent dry matter (range: 31.7-33.3 %), protein (range: 47.0-49.2 %), and fat (range: 35.1-37.3 %) (Table 3). Further, the different feeding strategies did not influence size variation in fish since there were no differences in CV among fish in each treatment (Table 1).

### **Discussion**

Fish respond to periods of feed deprivation by increasing SGR and/or FE during the refeeding period (reviewed by Ali et al., 2003). These responses in various fish species resulted in partial or complete growth compensation, with one study reporting overcompensation (Hayward et al, 1997). Following the first eight weeks of the current trial, no significant differences in growth were observed between cycled fish despite being fed only 50 % of the total feed offered to the 0-week treatment. However, the mean FE for the 1, 2, and 4-week feeding regimens was 24.5 % higher than that of the 0-week treatment. Both growth and FE results following the first eight weeks appear to have resulted from both a CG response as shown by the increase in SGR, as well as through an increase in consumption of natural productivity. Gut content analyses indicated the presence of both plant matter and zooplankton in addition to feed pellets. Similar to the first 8-weeks, FE for fish in the 1, 2, and 4-week feeding regimens was higher than that of the 0-week regimen at the completion of the trial. Feed efficiency of fish offered the 2-week feeding regimen was 40 % higher ( $P<0.05$ ) than the 0-week regimen. The elevated FE has important cost-saving implications,

however, savings as a result of increased FE came at the expense of lost growth, as final weights of fish in the 0-week regimen were significantly higher than all cyclic fed fish. The lack of complete growth compensation indicates that the CG response, as indicated by increases in SGR was temporary and not long enough to overcome lost weight. Temporary increases in growth rates have also been observed in channel catfish (Gaylord and Gatlin 2000) where it was suggested that the period of feed deprivation may have been excessive, not allowing complete catch-up. Similarly, in this study, the extent and duration of super-accelerated growth (CG response) was not sufficient to overcome lost growth.

Shorter feed deprivation periods and longer refeeding periods have been shown to result in full growth compensation (Kim and Lovell, 1995; Chatakondi and Yant, 2001; Hayward and Wang 2001; Johansen et al. 2001; Miglavs and Jobling, 1989). In this study, the shortest period of feed deprivation (1-week) did not result in a significant increase in SGR upon refeeding when compared to normally fed fish (0-week treatment). However, fish offered the 2-week cyclic regimen displayed both increased SGR during the refeed and significantly higher FE at the end of the trial. Hence, two weeks of feed deprivation is sufficient to trigger a measurable CG response that leads to improved FE in fingerling HSB. Since the final weight of fish in the 2-week feeding regimen did not reach those in the 0-week treatment, it would seem that although a CG response was elicited by 2-weeks of feed deprivation, the increase in growth rate was not of sufficient magnitude or duration to compensate for lost growth. It is well known that growth, measured as a percentage of body weight, is more rapid in smaller fish than larger ones (Mommsen, 2001). Therefore, it is possible that the rapid growth rates of smaller fish used in this study, may have precluded the ability to elicit even greater growth rates through manipulation of feeding practices. Perhaps

a better CG response and hence full catch-up growth can be achieved in larger, slower-growing fish, or in production of market-sized animals. Nevertheless, strategies to increase the length of the CG response in fast growing fingerlings, such as regimens which include longer refeeding periods may result in a more complete CG response.

Variations in the magnitude and duration of CG seen in studies of various fish suggests the response is species-specific (Hayward and Wang, 2001) and highly dependent on the life history of the species being examined. The exact mechanisms underlying the CG response have yet to be elucidated (Ali et al., 2003). Broekhuizen et al., (1994) suggested a two-part model which describes an attainment of an optimal ratio between reserve and structural tissue. During feed deprivation, the ratio falls below this “ideal” level, at which time appetite increases, but maintenance is steady. If feed deprivation persists, fish will minimize maintenance costs to increase chances of survival. Upon locating food, consumption is increased beyond the normal maintenance level in order to replenish the ratio, however, the maintenance cost stays at a minimum. The resulting increase in nutrient intake and decreased maintenance costs allows for more rapid growth, particularly in the form of muscle tissue. In this study, HSI levels were monitored in an attempt to estimate metabolic condition and provide an indication of sufficient feed deprivation (reserve tissue). Hepatic tissue responded rapidly to feed deprivation and reached a minimum level after two weeks. Further reductions in HSI were not observed even after four weeks of feed deprivation. The reduction in liver size was likely due to glycogen depletion and may represent a maintenance state of metabolism. Gaylord and Gatlin (2000) observed a decrease in channel catfish liver glycogen levels after 14 days of feed deprivation. In this study, HSI levels rebounded above those in the 0-week treatment during the refeeding period. The rapid overcompensation of the

liver could be a response mechanism where fish store excess glycogen in anticipation of future periods without feed. In this study, overcompensation of HSI (increase in liver mass) coincided with rapid increases in SGR during the refeeding period, and partly contributed to the rapid weight gain. Reduction of the HSI to 1.5 in fish in the 2-week treatment, and 2.2 in fish in the 4-week treatment led to significant increases in SGR during refeeding. Hence, a reduction in liver size may be an important indicator for a potential CG response. However, the transient nature of the CG response observed in this study seemingly corresponds to the restocking of nutrients in the liver and return to normal growth. Therefore, if HSI is to be used as an indicator of metabolic function, identifying feeding regimens that reduce HSI levels and do not result in rapid overcompensation in the liver upon refeeding could result in a longer CG response and lead to complete compensation. A similar pattern of rapid decline during feed deprivation and overcompensation after refeeding was observed in IPF ratios of Arctic Charr (Miglav and Jobling 1989). Arctic Charr fed a restricted diet for eight weeks followed by satiation feeding for 8 weeks did not completely compensate for lost growth when final weights were compared to control fish. Whole body lipid to lean body mass ratios were similar for both groups at the trial completion, leading the authors to suggest that the repletion of lipid stores led to a halt in the CG response and the failure to fully compensate for lost growth.

As a non-lethal measure of the effect of feeding regimen on the fish, CF was monitored through the study. Condition factor values coincided with the feeding cycle for fish subjected to the 2 and 4-week treatments. During feed deprivation, CF decreased significantly from 0-week controls in both 2 and 4-week treatment fish. Within two weeks of refeeding, fish offered the 2 and 4-week cyclic regimens returned to 0-week control levels.

Data for the 1-week treatment could not be analyzed because sampling periods were always following a complete feed deprivation/refeeding cycle. Since CF measurements show a pattern that follows feed deprivation and refeeding, it may be used as an indicator of a potential CG response.

Cyclical feeding of fish could promote hierarchal feeding and hence variation in fish size, if the largest more dominant fish consume a greater amount of feed during the refeeding period. This does not appear to be the case in this study, as there were no significant variations in final weights of fish among treatment and control fish. This suggests that feed offered during the refeeding periods was enough to allow for equal access to the entire fish population. Additionally, results of proximate composition indicated that all fish were similar in whole body protein, lipid, and moisture at the end of the study further indicating no negative effects of the feeding cycles. Similar results were obtained with rainbow trout (Quinton and Blake 1990; Miglavs and Jobling, 1989; Kim and Lovell, 1995; Wang et al., 2000) These results have further implications for commercial production in that implementation of cyclic feeding strategies do not have negative effects on overall body composition of the fish.

The increases in FE observed in cyclic fed fish in the current study would not only reduce feed costs, but may have positive impacts on water quality. Only 25 % to 30 % of the nitrogen and phosphorus applied to ponds in feeds is recovered during the harvest (Boyd and Tucker, 1998). Hence, a large amount of potential waste remains in the pond and must be assimilated. Despite differences in FE between treatments, significant differences in water quality variables were not observed in this study. Although stocking was similar to that of commercial fingerling production (Hodson, 1995), average total feed input did not reach

problematic levels. Cole and Boyd (1986) reported feeding rates up to 56 kg/ha required relatively little aeration, whereas ponds with feeding rates of 112 kg/ha required almost constant nightly aeration. In this study the highest maximum daily feeding rate reached 63.87 kg/ha, below the level requiring constant nightly aeration.

### **Summary and Conclusions**

Cyclic feeding was successful in eliciting a CG response in fingerling HSB grown in ponds. Fish offered the 2-week cyclic feeding regimen had a significantly higher SGR during refeeding periods and higher overall FE compared to other treatment groups and control fish. Based on the feeding regimens in this study, complete growth compensation was not observed. When compared to other studies, it seems that although 2-weeks of feed deprivation was sufficient to cause a CG response, the subsequent 2-weeks of refeeding was insufficient to allow complete catch-up. A longer refeeding period is likely needed to allow fish to regain lost growth. The HSI was a highly responsive measure of feed deprivation, and feeding strategies to gradually replenish energy stores during refeeding could result in a longer period of growth compensation. Practical application of these regimens is possible if farmers are willing to sacrifice weight gain for increased FE. Further, improvements in water quality were not observed in this study, likely due to reduced stocking density and feed inputs. Future pond studies with fingerling HSB should be conducted with emphasis on feed deprivation periods of two weeks and refeeding periods of at least twice that of the feed deprivation. Additional studies should also be conducted with larger fish to observe whether the CG response in slower growing individuals is enhanced and if pond water quality is impacted to a greater extent. Further, the use of HSI as an indicator of metabolic condition

seems promising, and future studies should be directed toward hepatic function and the mechanisms that control nutrient partitioning.

Table 1. Mean production variables ( $\pm$  SEM) of fingerling hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed daily to satiation (control) or equal periods of feed deprivation/refeeding during a 16-week study in ponds.

Treatment	Production (kg/ha)	Survival ( %)	Coefficient of Variation for fish weight (%)
0-week (control)	2149.6 $\pm$ 479.2 <sup>a</sup>	81.7 $\pm$ 12.0 <sup>a</sup>	31.0 $\pm$ 4.2 <sup>a</sup>
1-week (1/1)	1174.1 $\pm$ 146.5 <sup>a</sup>	67.3 $\pm$ 1.6 <sup>a</sup>	28.0 $\pm$ 0.8 <sup>a</sup>
2-week (2/2)	1322.4 $\pm$ 43.9 <sup>a</sup>	79.7 $\pm$ 5.5 <sup>a</sup>	32.0 $\pm$ 8.0 <sup>a</sup>
4-week (4/4)	1450.1 $\pm$ 100.3 <sup>a</sup>	85.2 $\pm$ 9.1 <sup>a</sup>	26.0 $\pm$ 1.7 <sup>a</sup>

\*Means with different letters represent significant differences ( $P < 0.05$ ).

Table 2. Mean growth parameters of fingerling hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed daily to satiation (control) or equal periods of feed deprivation/refeeding during a 16-week study in ponds

Parameter	Treatment	Week						
		4	6	8	10	12	14	16
SGR	0-week (Control)	5.4	3.2b	3.6b	2.1a	0.7b	2.8a	0.0
	1-week (1/1)	5.2	3.0b	3.6b	0.5ab	0.6b	1.6ab	1.9
	2-week (2/2)	4.8	0.8b	5.7a	0.1ab	3.0a	0.2b	1.9
	4-week (4/4)	3.5	6.1a	4.4ab	-0.6b	0.0b	3.0a	1.6
	<i>P</i> -value	0.0593	0.0014	0.0203	0.0357	0.0072	0.0183	0.0764
FE (%) (FCR) <sup>3</sup>	0-week (Control)			88.8a (1.13)				70.2b (1.42)
	1-week (1/1)			101.5ab (0.99)				77.8ab (1.29)
	2-week (2/2)			115.8ab (0.86)				98.3a (1.02)
	4-week (4/4)			117.0b (0.90)				90.4ab (1.1)
	<i>P</i> -value			0.0442				0.0414

\*Means with different letters represent significant differences at  $P=0.05$  level.

<sup>1</sup>Specific growth rate {SGR= [100 x ln Wt.<sub>f</sub> - ln Wt.<sub>i</sub>]/(T<sub>f</sub>-T<sub>i</sub>)},

<sup>2</sup>Feed efficiency [FE= 100 x (weight gain / feed fed)]

<sup>3</sup>Feed conversion ratio (FCR= feed fed / weight gain)

Table 3. Final proximate composition (mean  $\pm$  SEM) of fingerling hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed daily to satiation (control) or equal periods of feed deprivation/refeeding during a 16-week study in ponds.

Treatment	Protein ( %)	Lipid ( %)	Dry matter ( %)
0-week (control)	47.50 $\pm$ 0.4	37.27 $\pm$ 0.6	33.27 $\pm$ 0.3
1-week (1/1)	49.22 $\pm$ 1.3	35.14 $\pm$ 1.2	35.14 $\pm$ 0.9
2-week (2/2)	47.02 $\pm$ 0.9	35.45 $\pm$ 0.8	35.45 $\pm$ 0.7
4-week (4/4)	47.96 $\pm$ 0.8	36.54 $\pm$ 0.6	36.54 $\pm$ 0.4

Figure 1. Weight (g) of fingerling hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (0-week control) or consecutive cycles of equal periods of feed deprivation followed by twice daily feeding to satiation (1-week, 2-week, and 4-week). Arrows indicate start of refeeding period. (\* represents significant difference from 0-week controls  $P < 0.05$ )

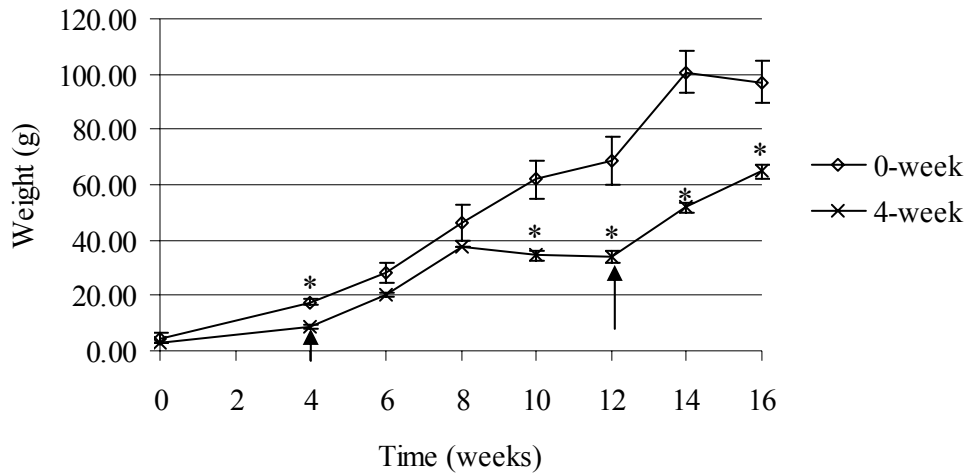
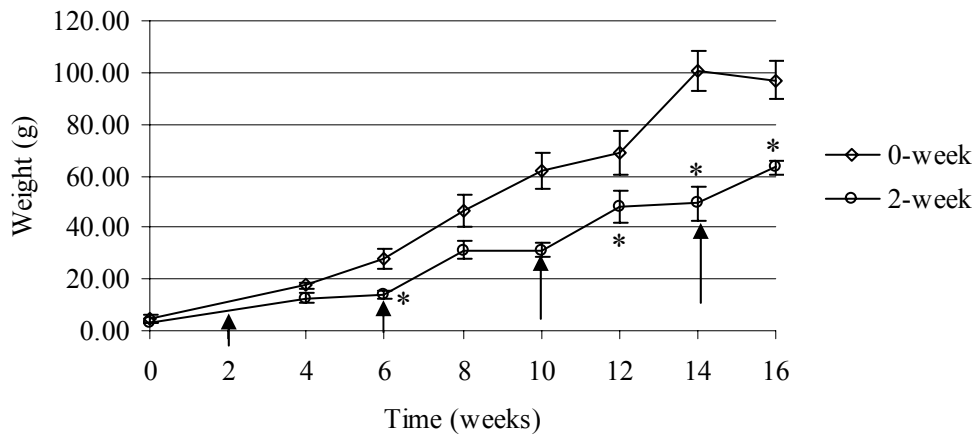
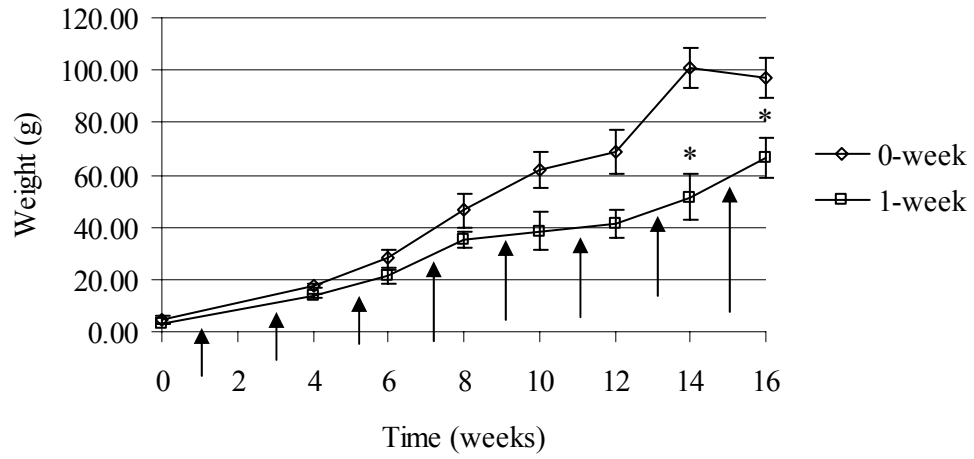


Figure 2. Hepatosomatic index (HSI= liver weight x 100/fish weight) of fingerling hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (0-week control) or consecutive cycles of equal periods of feed deprivation followed by twice daily feeding to satiation (1-week, 2-week, and 4-week). Arrows indicate start of refeeding period. (\* represents significant difference from 0-week controls  $P<0.05$ )

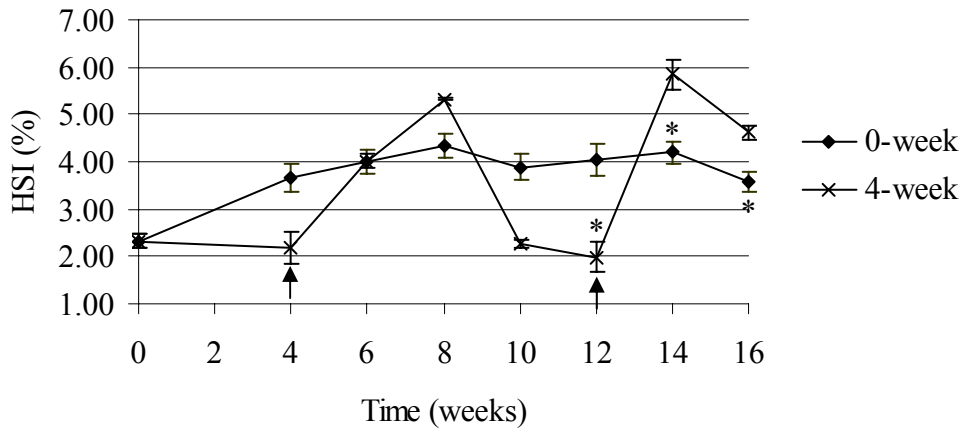
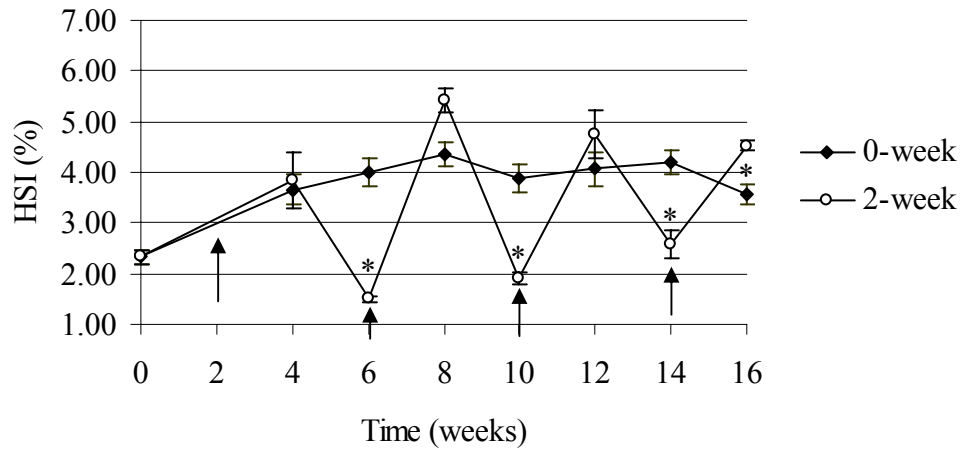
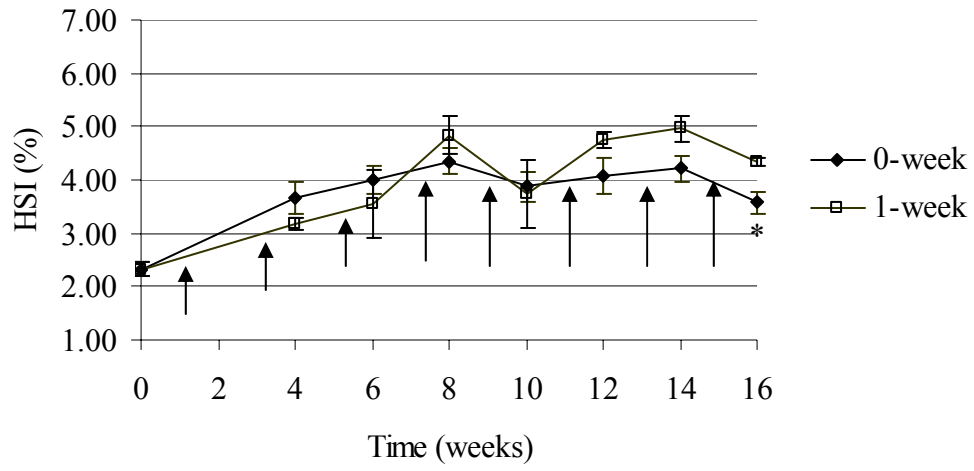


Figure 3. Intraperitoneal fat (IPF) ratio (IPF= fat weight x 100/fish weight) of fingerling hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (0-week control) or consecutive cycles of equal periods of feed deprivation followed by twice daily feeding to satiation (1-week, 2-week, and 4-week). Arrows indicate start of refeeding period. (\* represents significant difference from 0-week controls  $P<0.05$ )

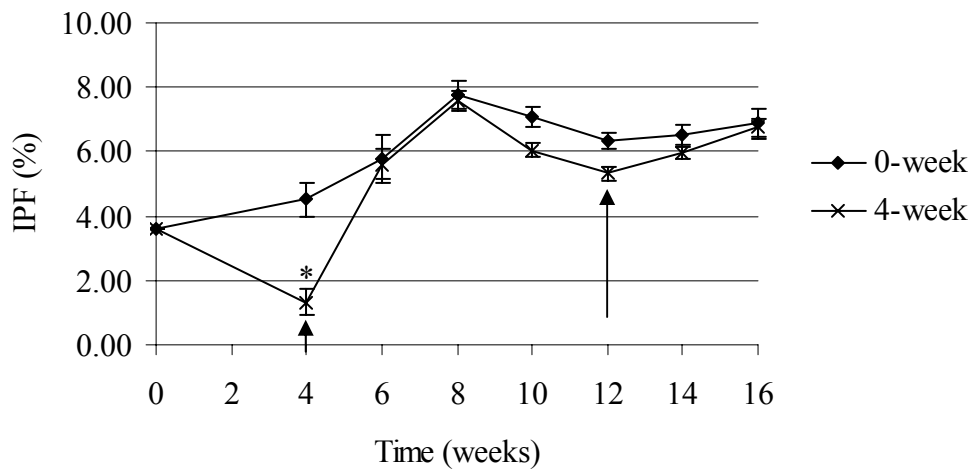
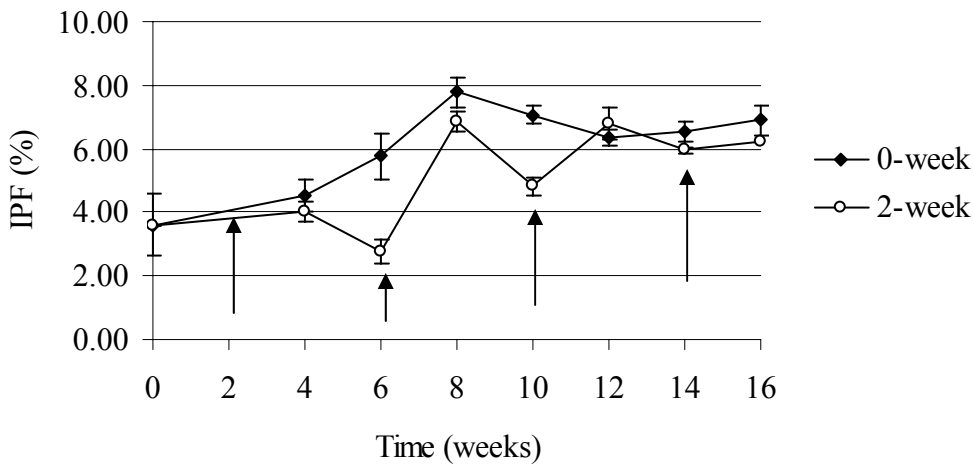
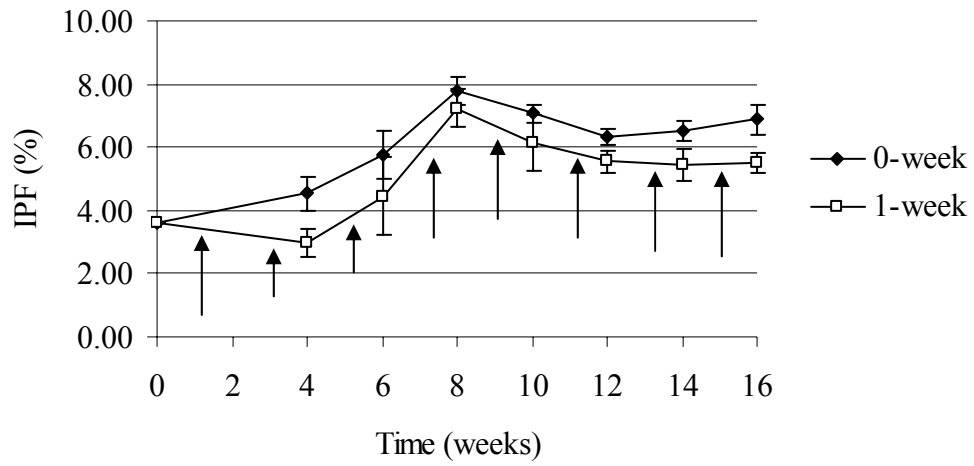
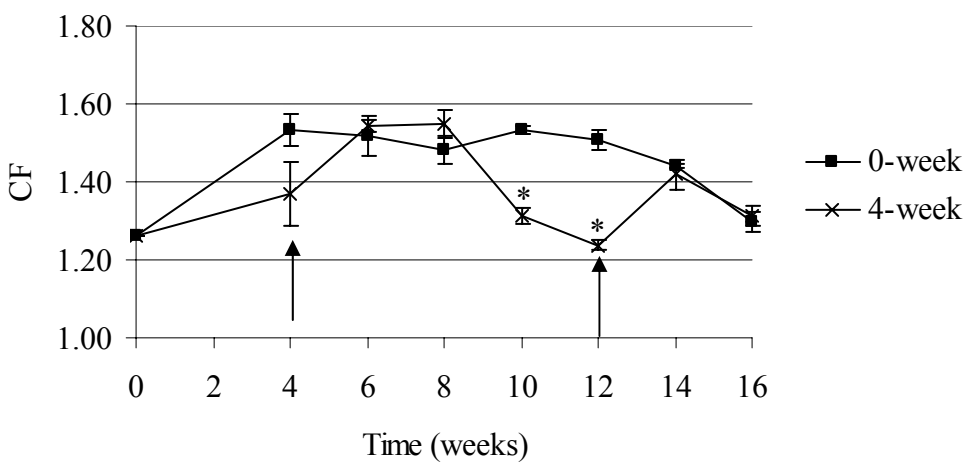
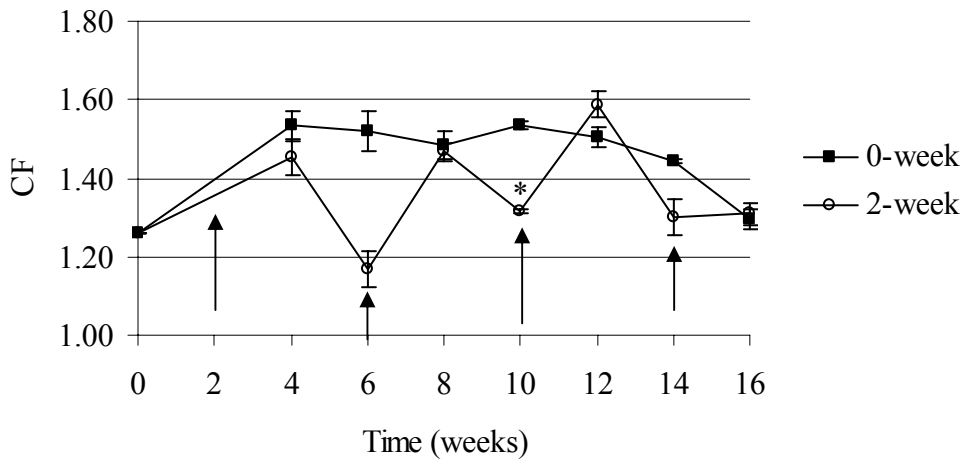
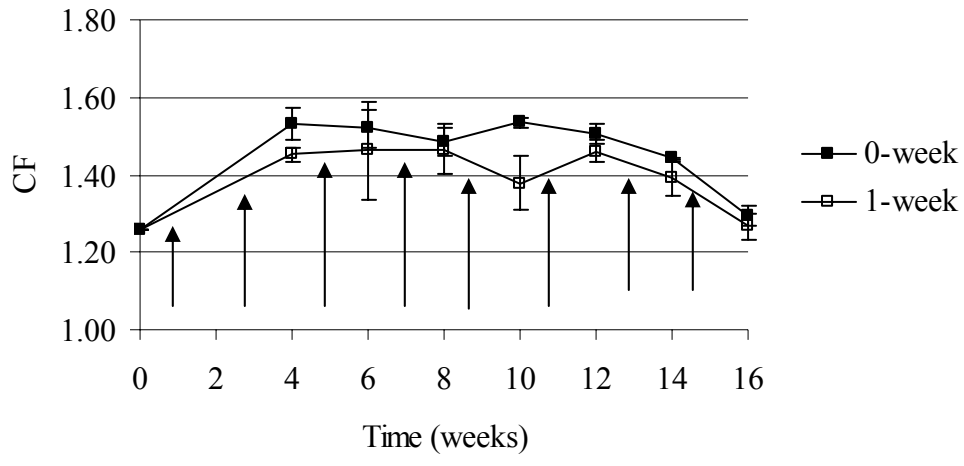


Figure 4. Condition factor [CF= (fish wt./length<sup>3</sup>) x 10<sup>4</sup>] of fingerling hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (0-week control) or consecutive cycles of equal periods of feed deprivation followed by twice daily feeding to satiation (1-week, 2-week, and 4-week). Arrows indicate start of refeeding period. (\* represents significant difference from 0-week controls  $P<0.05$ )



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## Chapter 3

### Effects of Cyclic Feeding Regimes on Compensatory Growth and Water Quality in Hybrid Striped Bass (*Morone chrysops* x *M. saxatilis*) Foodfish in Ponds

#### Abstract

An 18-week study was conducted in 12, 0.1 ha ponds to evaluate the impacts of cyclic feeding regimes on production and water quality in hybrid striped bass foodfish. Approximately 840 hybrid striped bass [HSB; mean weight (std.); 91.08 g (8.18)] were stocked into each pond (8400 fish/ha; 3360 fish/ acre) and fed according to one of three feeding regimes. The three feeding regimes included a control (fed twice daily to apparent satiation), and cycles of three weeks feed deprivation followed by three or six weeks of feeding to apparent satiation (3/3 and 3/6, respectively).

Partial compensatory growth (CG) was observed in both cyclic feeding treatments; however, the response was insufficient for the fish to completely regain lost weight. Final mean weight of fish in the control treatment (477.9g) was significantly larger ( $P<0.05$ ) than fish from both cyclic treatments, followed by fish in the 3/6 (404.7g) and 3/3 (353.8g) treatments, respectively. Mean feed efficiency (FE) for the 3/3 and 3/6 treatments was 7.8 % greater than the controls. Specific growth rate (SGR) of fish in the 3/3 treatment increased during all three refeeding periods, and was significantly greater than controls during weeks nine through 12 (392 %) and weeks 15-18 (347 %) while SGR for fish in the 3/6 treatment was significantly higher than controls (90 %) only during the first three weeks of the first

feeding cycle. Hepatosomatic index and CF were highly responsive measures that closely followed the feeding cycle. Of the water quality variables measured, total phosphorus (TP) was 32 % lower in the cyclic fed treatments than control ponds. Soluble reactive phosphorus was 41 % and 24 % lower in ponds offered the 3/3 and 3/6 cyclic feeding treatments respectively, however, statistical differences were only observed between control and 3/3 treatment ponds. Overall, partial CG was observed in HSB foodfish grown in ponds, although three weeks of feed deprivation was excessive and did not allow for complete growth compensation. Further studies are needed to identify the best feeding regimes which allow for complete growth compensation and a reduction of phosphorus in effluents.

## Introduction

Seasonal variations of food supply in the aquatic environment cause many species of fish to endure periods of starvation (van Dijk et al., 2005). Once food becomes available, some species exhibit compensatory growth (CG), or periods of accelerated weight gain exceeding that of fish not exposed to food shortages (Hornick et al., 2000). In some studies, increased growth rates have been reported to result in complete compensation; while in others the response only results in partial compensation (see review by Ali et al., 2003). Interest in CG has increased because of its potential use in aquaculture, specifically to enhance feed efficiency (FE). Improvements in FE in farm-raised fish can reduce production costs and potentially assist in water quality management.

The use of cyclic feeding regimes in fish production attempts to mimic the natural fluctuation in prey availability and trigger CG, however, because the exact mechanisms of CG are poorly understood (Hornick et al., 2000), the ideal feeding cycles have not been identified. Quinton and Blake (1990) suggest that factors such as sex, state of maturity, diet composition, and severity of feed restriction may influence the strength and extent of the compensatory response. Further, prior studies on CG suggest the response is species-specific (Hayward and Wang, 2001). In pond culture of hybrid striped bass foodfish, fish are typically harvested prior to sexual maturation (1.5 lbs.). Although diet composition can contribute to the CG response, understanding the physiological response to feed deprivation and initial growth following refeeding could provide baseline data which can be enhanced through future diet manipulation.

Three hypotheses have been suggested to explain the mechanisms behind CG, 1) hyperphagia or food consumption above normal levels, 2) maintenance of an ideal ratio of

structural to reserve tissue, 3) increases in FE. Some studies have postulated that hyperphagia is responsible for the increased growth rates during the CG response (Bull and Metcalf, 1997; Rueda et al., 1998; Wang et al. 2000; Johansen et al., 2001; Gurney et al., 2003; Tian and Qin, 2004; Ali et al., 2003). In each case, hyperphagia was observed following each fasting period. Hence, identifying the optimum duration of feed deprivation that causes the greatest hyperphagic response may result in a complete CG response.

In another proposed explanation for the CG response, Broekhuizen et al. (1994) suggested that there is an ideal ratio of structural to reserve tissue, which is upset during feed deprivation. Extended periods without feed result in decreased metabolic function and decreased energy requirements. Immediately following refeeding, metabolism remains at a basal level, while feed intake is rapidly increased, creating a condition in which a larger percent of the nutrients consumed are directed toward protein accretion. Once metabolic function returns to normal levels, the CG response ceases. Similar suggestions have been made by Gaylord and Gatlin (2001) in which catfish increased their rate of energy deposition without an increase in dietary energy requirement. Further, examples of reducing metabolic rate and maintaining it at a basal level during realimination has been shown in beef cattle (Yambayamba et al., 1996, Carstens et al., 1991).

Lastly, Boujard et al. (2000) suggested that increases in FE during refeeding are responsible for the CG response. Fish may utilize feed more efficiently following periods of feed deprivation and growth rates increase without hyperphagia. Regardless of the exact mechanism, feed deprivation followed by refeeding triggers a measurable CG response, and evaluations of these different cyclic feeding regimes could help to identify the cycle that

results in the best improvements in FE and lead to a better understanding of the mechanisms involved.

Few studies have evaluated cyclic feeding regimes in ponds. Kim and Lovell (1995) reported that feeding channel catfish every third day for three weeks did not significantly alter final weight gain, providing evidence of CG. Only dissolved oxygen was measured in the study, hence the effect of feed restriction on nutrient levels could not be evaluated. Li et al. (2005) reported no difference in net production between channel catfish offered cyclic feeding regimes of 1:6, 1:4, and 2:5 (days not fed: days fed) and normally fed control fish. Since pond culture is conducted in a dynamic and biologically diverse environment, as opposed to more controlled tank experiments, additional factors must be taken into consideration in the design of the study. Specifically, because of the availability of natural prey, extended periods of feed deprivation may be necessary to obtain a similar metabolic state as fish exposed to shorter feed deprivation periods in tanks. Additionally, seasonal fluctuations in temperature can influence the amount of time necessary to reduce metabolism, and may also affect growth during the refeed. Hence, there is a need to conduct studies on the CG response in ponds to determine if results similar to the tank studies can be obtained. In addition, use of cyclic feeding regimes may also have an overall effect on pond water quality, particularly if FE is increased. Enhancements in FE may help to improve overall nutrient utilization while periods of feed deprivation could be used during periods of maximal feeding to assist in management of phytoplankton, thereby reducing the risk of dissolved oxygen (DO) depletion. The purpose of this study was to identify to what extent cyclic feeding could elicit a CG response in pond reared HSB foodfish and to determine the effects of cyclic feeding on pond water quality.

## Methods

An 18-week growth trial was conducted at the Tidewater Research Station in Plymouth, NC. Twelve 0.1 ha ponds were stocked in May, 2004 with approximately 840 fish/pond (8400 fish/ha), mean weight 78.0 g. Fish were allowed to acclimate for two weeks during which time they were fed twice daily to satiation. Following the acclimation period, each pond was assigned one of three feeding regimes; a control, fed twice daily to apparent satiation, or three weeks of feed deprivation followed by 3 or 6 weeks of twice daily feeding to apparent satiation (3/3 and 3/6, respectively). The 3/3 treatment went through three complete cycles whereas the 3/6 treatment complete two cycles. Each treatment had four replicates. After the two-week acclimation period, fish were sampled from each pond to obtain an initial weight. Ten fish from each pond were sacrificed to obtain individual weights, lengths, and liver and intraperitoneal fat weights. Hepatosomatic index (HSI= wet liver weight x 100/body weight) and intraperitoneal fat ratio (IPF= wet weight of fat x 100/body weight) and condition factor [CF; (weight in grams/length<sup>3</sup> in mm) x 10<sup>5</sup>] were calculated. Growth and body indices were measured every three weeks for 18 weeks. Specific growth rate {SGR; [100 x (ln weight<sub>f</sub> - ln weight<sub>i</sub>)] / (Time<sub>f</sub> - Time<sub>i</sub>)} was calculated based on average group weights of fish from each pond beginning at 3 wk, while feed efficiency [FE; (weight in grams/weight of feed) x 100] was calculated at the end of the trial (18 wk). Following the trial termination, each pond was drained approximately 45 cm, and harvested twice by seining. Growth and body indices were sampled, and total harvest weight (kg/ha) recorded.

### *Water Quality*

Water quality was measured during the trial to determine the effects of cyclic feeding on various water quality parameters. Water samples were taken weekly using a 90-cm water column sampler (Boyd and Tucker 1992) and analyzed for pH (Orion 720A pH meter; Thermoelectron Corp., Waltham, MA, USA), turbidity (DRT 100B turbidimeter, HF Scientific Inc., Fort Myers, FL, USA), total ammonia-nitrogen (TAN), nitrite-nitrogen (NO<sub>2</sub>-N), nitrate-nitrogen (NO<sub>3</sub>-N), and soluble reactive phosphorous (SRP) (APHA et al., 1995), and chlorophyll-a (Pechar, 1987). Total nitrogen (TN) and total phosphorous (TP) were measured every two weeks (APHA et al., 1995). Total ammonia nitrogen and SRP were also measured one, three, and five days following each refeeding period in treatment and control ponds.

Temperature and dissolved oxygen (DO) were recorded twice daily (0800 and 1600h) with a YSI 550 (Yellow Springs Instrument Company, Yellow Springs Ohio, USA). Nightly (2400h-0830h) aeration was applied to each pond via 0.75-hp paddlewheel aerators (Southern Machine Welding Inc., Quinton, AL, USA). Additional hours of aeration were provided if morning DO levels were below 5 ppm until this level was reached and/or if afternoon DO levels were below 7 ppm. Additional hours of aeration were recorded.

### *Statistical Analysis*

Production, final weights, and overall water quality measurements were analyzed using a one-way analysis of variance (ANOVA) followed by a Tukey's least significant difference test to separate the means. Additionally, pre-planned contrasts were conducted to

compare each treatment separately against the control for overall TP, SRP, and chlorophyll-a. Intermediate sampling for growth, body indices, and water quality were analyzed between control fish and each treatment individually using a t-test (Steel et al., 1997). Survival data were arcsin transformed before analysis; however, treatment means are presented for simplicity. Significant differences were determined at the  $P < 0.05$  level for all growth and body indices data, while water quality parameters were considered significant at the  $P < 0.10$  level. All statistical analyses were conducted using the Statistical Analysis System (SAS; v8.2, Cary, NC, USA).

Upon initial data analyses, it was found that one pond assigned the control treatment was incorrectly stocked. Hence, all data analyses were conducted based on three replicates for the control treatment, and four replicates for cyclic treatments.

## **Results**

### *Growth and Body Indices*

All experimental fish adapted well to the feeding regimes. No significant differences were found for survival (%), overall production (kg/ha), and FE (%). Production was the highest for the control treatment (2528.2 kg/ha) followed by the 3/6 (2485.9 kg/ha) and 3/3 (1703.9 kg/ha) treatments, respectively (Table 4). Feed efficiency was 7.0 % and 8.5 % higher than controls in fish subjected to the 3/3 and 3/6 feeding regimes, respectively. Fish in the 3/3 treatment were exposed to three cycles of feed deprivation/refeeding, while fish in the 3/6 treatment were subjected to only two complete cycles. Fish in the control treatment were significantly larger (477.9g) than both cyclic feeding treatments, followed by fish in the 3/6 (404.7g) and 3/3 (353.8) treatments, respectively (Figure 5). During the first three weeks, fish

in both cyclic feeding treatments gained an average of 5g, despite the lack of feed. During subsequent feed deprivation periods, 3/3 treatment fish lost 0.5 % and 13.3 % body weight, and 3/6 treatment fish lost 12.2 % body weight. Fish in the 3/3 treatment significantly increased SGR above fish in control ponds during all refeeding periods, while the SGRs of fish in the 3/6 treatment were only statistically higher than controls during the first 3-weeks of the first refeeding periods (Figure 6).

Liver weight closely followed changes in the feeding cycle (Figure 7). Hepatosomatic indices for all cyclic fed fish decreased significantly compared to control fish after each three-week feed deprivation period. During refeeding, overcompensation of the liver was observed for fish in both cyclic treatments. Hepatosomatic index returned to control levels following six weeks of refeeding as observed in 3/6 treatment fish.

Intraperitoneal fat levels varied early in the trial, however this variation diminished after week nine (Figure 8). Following the first three weeks of feed deprivation, the IPF ratio of fish in both cyclic treatments significantly decreased, and then rebounded to control levels after three weeks of refeeding. Intraperitoneal fat levels of fish in the 3/3 treatment followed a similar pattern to that of the first during the second feeding cycle. There were no differences in IPF ratio following week nine. Condition factor varied with feeding cycle for both treatments (Figure 9). Fish subjected to the cyclic feeding regimes showed a significant decrease in CF during each feed deprivation period, which returned to levels similar to that of control fish during the three weeks of refeeding. The additional three weeks of refeeding offered to fish in the 3/6 treatment resulted in a statistically higher CF, but only during the first refeeding period. All CF measurements were statistically similar at the end of the trial.

### *Water quality*

Significant differences ( $P < 0.10$ ) were found in overall TP, SRP, and chlorophyll-a (Table 5). Total phosphorus in both cyclic fed treatments was significantly lower than control ponds. Ponds in the 3/3 and 3/6 treatments had 38 % and 25 % less TP than the control treatment. Chlorophyll-a was 28 % and 12 % lower in the 3/3 and 3/6 treatments respectively than in control ponds, and SRP was 41 % and 24 % lower than controls in the 3/3 and 3/6 treatments respectively. Chlorophyll-a and SRP levels of ponds subjected to the 3/3 treatment were statistically lower than control ponds. Total phosphorus was significantly lower in the 3/3 treatment than in control ponds at all sampling periods except 0 and 14 wks (Figure 10). The 3/6 treatment had lower TP levels than control ponds throughout the study, however, statistical differences were only observed at 3 and 5 wks. Soluble reactive phosphorus was sensitive to feeding cycle for the first nine weeks of the study, decreasing with feed deprivation and increasing with refeeding for ponds in both cyclic regimes (Figure 11). Statistical differences were only observed however, between control and 3/3 treatment ponds at 12 and 15 wks. Soluble reactive phosphorus was significantly lower in the 3/6 treatment ponds than control ponds at 18 wk. Following week nine, SRP in cyclic fed ponds remained below that of control ponds for the remainder of the study, although not statistically. Finally, chlorophyll-a measurements closely followed the feeding cycles, decreasing during feed deprivation and increasing during refeeding (Figure 12). However, this pattern was observed only after week three as all ponds had an increase in chlorophyll-a during the first three weeks of the study, regardless of feed offered. Statistical differences in chlorophyll-a were only observed between the control and 3/3 treatment ponds at 15 and 18 wks, and between between 3/6 treatment ponds and the controls at week three. There were no other statistical

differences in other water quality parameters observed, and no patterns could be determined from samples taken 1, 3 and 5 days during the refeeding periods.

## **Discussion**

A partial CG response was observed for all fish offered the cyclic feeding regimes. The greatest changes in SGR were observed for fish in the 3/3 treatment, followed by those in the 3/6 and control regimes, respectively. Overall FE increased by 7.0 % and 8.5 % in 3/3 and 3/6 treatment fish, respectively over control fish. However, the increases in SGR and FE did not lead to complete compensation. The response was either not long enough or of sufficient strength to overcome the reduction in weight during feed deprivation periods. A number of studies on CG in fish have reported partial growth compensation (Gaylord and Gatlin, 2000; Thorpe et al. 1990; Reimers et al., 1993; Morgan and Metcalfe, 2001; Johansen et al., 2001; Quinton and Blake, 1990; Jobling et al., 1993; Hayward and Wang, 2001; Hayward et al., 2000). Overly long or short periods of feed deprivation are possible causes for partial compensation. Gaylord and Gatlin (2000) suggested that four weeks of feed deprivation for channel catfish was excessive and prevented complete growth compensation. Likewise, in this study, three weeks of feed deprivation was lost growth opportunity as well as, in some cases, weight loss. Interestingly, cyclic fed fish had an average of 7 % weight gain during the first feed deprivation period but lost weight during subsequent feed deprivation periods. Fish in the 3/3 treatment lost 0.5 % and 13.3 % body weight during the second and third feed deprivation periods, while fish in the 3/6 treatment lost 12.2 % body weight during the second feed deprivation period. Failure to fully compensate for lost growth was either a result of excessive weight loss and/or excessively long deprivation periods. For

each feed deprivation period, there is a period of lost growth opportunity not presented to normally fed fish. However, if these periods also cause weight loss, the increase in SGR upon refeeding may not be sufficient to overcome both. In this experiment, fish in the 3/6 treatment weighed approximately 73 g less than controls at the end of the trial. There was a loss of approximately 32 g during the second feeding cycle, with the remaining 41g of weight incurred as lost growth opportunity. Feeding regimes that do not cause weight loss, but rather maintain weight during feed deprivation periods, and still elicit a CG response could lead to complete compensation. For example, Tain and Qin (2004) demonstrated that Barramundi (*Lates calcarifer*) subjected to feed rations of 50 % and 75 % of satiation for two weeks, followed by satiation for five weeks fully compensated for the decreased growth during the deprivation period. Barramundi did not lose weight during the feed deprivation period, however growth was less than that of control fish. Instead of complete feed deprivation, Kim and Lovell (1995) showed that channel catfish subjected to a feeding regime of feeding every third day to satiation for three weeks could fully compensate for the decrease in weight gain after six weeks of feeding to satiation. Again, no weight loss was observed during feed deprivation. Hence, it may be possible to stimulate a CG response (through partial feeding) to overcome decreased growth but not weight loss.

Of interest between the two cyclic feeding regimes in this study is the similarity in timing and severity of weight loss. Fish in both treatments showed modest weight gains during the first feed deprivation period, and those in the 3/3 treatment only lost 0.5 % body weight during the second feed deprivation period. The majority of weight loss occurred at weeks 12 and 15, during which fish in treatments 3/6 and 3/3 lost 32 and 36g, respectively. The weight gain observed during the first three weeks of the trial was likely the result of fish

consuming natural prey in the ponds, particularly juvenile crawfish, although both plant and insect matter were observed during gut content analyses. Further, the water temperature and season could have played a role during this period. The experiment was started during the first week in May, when mean temperatures were approximately 23 C. Although growth is not optimal at this temperature, FE is improved over higher temperatures (Ronald Hodson, affiliation NC Sea Grant, pers. comm.). Further, this time frame coincides with the spring season, a period in which striped bass would normally undergo increased feeding and growth, following the winter period. Experiments which subject fish to periods of low temperatures, mimicking the overwintering season, have been shown to stimulate a CG response when temperatures were increased (Mortensson and Damsgard, 1993; Chmylevskii, 1998; Maclean and Metcalfe, 2001; Purchase and Brown, 2001). Hence, the combination of improved FE, the ability to feed on natural productivity, and seasonality may reduce the negative effects of feed deprivation during the spring season.

The largest weight loss (12-13 % body weight) experienced by fish in this study occurred during feed deprivation periods initiated in the warmest months (July and August). It is hypothesized that cyclic feeding regimes will need to be shortened at higher temperatures, as excessive periods of feed deprivation during warmer temperatures could lead to a loss of weight that cannot be regained with the CG response.

In addition to measuring body mass, liver weight was monitored throughout the study to determine if HSI could be a measure of metabolic state and an indicator of a CG response. For all cycles, feed deprivation resulted in a sharp decrease in HSI levels for all treatment fish. Following refeeding, these levels increased beyond that of control fish. The reduction in liver mass was likely due to the rapid mobilization of glycogen, as reported in channel catfish

(Gaylord and Gatlin, 2000). Once these stores are utilized, energy must be obtained from other stores, during which time a shift in metabolism occurs. Mehner and Weiser (1994) reported that reduction in muscle and liver glycogen corresponded with a decrease in dissolved oxygen consumption and change in swimming activity of juvenile yellow perch *Perca flavescens*. If the model proposed by Broekhuizen et al. (1994) is correct, this period of basal metabolism could increase the scope for growth if, during the initial refeeding period, metabolic levels are maintained at a basal level. Further, if reduction in liver mass is a true indicator of metabolic shift, the HSI could provide a simple measure of an appropriate length of feed deprivation. In this study, a reduction of HSI levels to at least 1.9 resulted in an increased SGR in cyclic fed fish. Feed deprivation during periods of increased temperatures resulted in further declines in HSI, however the subsequent CG response did not increase beyond the initial response during the cooler months. Because fish were sampled every three weeks, it could not be determined if HSI was reduced to a minimal level earlier than the three week period of feed deprivation. If HSI is a predictor of a resulting CG response, halting the feed deprivation period based on the period at which HSI is reduced to a minimum could prevent excessive weight loss during any season. For example, since metabolic rate increases with temperature, HSI levels will likely decrease more rapidly during periods of higher temperatures. During the summer months, a shorter feed deprivation period may result in a similar weight loss and CG response pattern to that of a longer feed deprivation period during the cooler season.

Although HSI was responsive to feeding cycle, fish must be sacrificed in order to determine the measurement. If cyclic feeding regimes were to be used in commercial production, a simple, non-lethal measurement would be useful. Length measurements were

taken throughout the study to calculate CF, and to determine if CF could be used as a non-lethal measure of feeding cycle. Since length is not lost during feed deprivation, changes in weight can be observed in comparison to linear growth. Condition factor decreased significantly with three weeks of feed deprivation and rebounded to control levels after three weeks of refeeding. For fish in the 3/6 treatment, CF remained slightly elevated after six weeks of refeeding. Hence, the use of CF as a measure of sufficient feed deprivation is possible in HSB foodfish. If a cycle is identified which results in complete compensation, simple weight and length measurements can be used in field experiments and on farms without sacrificing fish.

In addition to affecting fish growth patterns, the use of cyclic feeding regimes may also affect pond water quality. Feed deprivation periods used in cyclic feeding effectively reduce nutrient inputs that lead to phytoplankton abundance. Since phosphorus is a limiting nutrient to phytoplankton growth, the fate of phosphorus from feed is important in the management of water quality. Further, because phytoplankton have the capacity to store and use phosphorus, a process termed luxury consumption (Boyd, 1990), the use of cyclic feeding to mediate water quality must include feed deprivation periods sufficient in length to allow phosphorus to be depleted and phytoplankton abundance to decrease. In this study, of the water quality parameters measured, TP, SRP, and chlorophyll-a were lower in the 3/3 and 3/6 treatments versus the controls. Mean overall TP was 38 % and 25 % lower in 3/3 and 3/6 ponds, respectively, than in control ponds. The reduction in TP was caused, in part, by the decreased levels of feed offered to the treatment ponds during the 18-week study. However, ponds subjected to the 3/6 feeding regime had significantly less TP, and did not differ in the total amount of feed offered. Hence the reduction of phosphorus must have been caused by

the slight increase in FE by 3/6 treatment fish. These findings could have significant implications in water quality and effluent management. If phytoplankton do not store as much phosphorus because of the pulsatile nature of cyclic feeding regimes, excessive phytoplankton growth could be reduced in addition to the reductions in overall phosphorus levels, an important consideration when discharging water.

Although reductions in TP can have significant impacts on water quality and effluent management, the amount of phosphorus as SRP provides an indicator of the percent of phosphorus available to phytoplankton and other plant sources in the pond. Since, higher SRP levels could lead to rapid increases in phytoplankton blooms, reductions in SRP could further assist in bloom management. Mean soluble reactive phosphorus for the entire study was lower in ponds subjected to the 3/3 treatment when compared to control ponds. Soluble reactive phosphorus in ponds subjected to the cyclic regimes seemed more sensitive to feed during the first nine weeks, during which SRP levels reached their highest level for ponds in both treatments. Following this period, SRP levels stabilized to the end of the study for the 3/6 ponds, and increased only at week 18 for the 3/3 ponds. A similar reduction in chlorophyll-a was also observed for ponds subjected to the 3/3 treatment. Additionally, chlorophyll-a measurements varied with feeding cycle. During the first 3 weeks, chlorophyll-a increased in all ponds, despite the lack of feed input in treatment ponds. Previous experiments conducted in these ponds have shown similar phytoplankton blooms following initial filling of the ponds, and could be a result of naturally occurring phosphorus in the well water. Following week nine, chlorophyll-a levels in all treatment ponds were lower than control ponds, although only statistically lower in 3/3 ponds during the last 6-weeks of the study. This pattern of sensitivity to feeding cycle could have important implications if

repetitive feeding cycles are applied to pond production. Apparently, variations in feed inputs during the early part of the production season (May-June) influence SRP and chlorophyll-a levels to a greater extent than during the latter part of the season. Hence reductions in feed inputs during the first 2 months of production appear to affect TP, SRP, and chlorophyll-a levels for the remainder of the growing season. The use of three weeks of feed deprivation followed by six weeks of refeeding could be utilized in commercial HSB production if farmers are willing to sacrifice lost growth for increased FE and improvements in water quality. Benefits would best be observed during the spring and early summer months of the production season. Future studies to determine if cyclic feeding regimes implemented solely during the early production season will result in complete compensation and an overall benefit to water quality. Other studies should continue to identify optimal feed deprivation: refeeding periods to cause the most complete CG response.

## Summary

Three weeks of feed deprivation was sufficient to induce a partial CG response in pond reared HSB foodfish. Specific growth rate of treatment fish was higher than controls during the refeeding periods. However, the higher SGR during refeeding was insufficient to regain the weight lost during feed deprivation. Mean FE was 7.8 % greater in treatment fish but was not statistically different from control fish. Hepatosomatic index and CF were useful measures in predicting a CG response and varied significantly with feed deprivation and refeeding. Further benefits from the use of the cyclic feeding regimes could be observed by the significant reductions in overall TP, SRP, and chlorophyll-a which could have implications in water quality management.

Table 4. Mean production variables ( $\pm$  SEM) for hybrid striped bass (*Morone chrysops* x *M. saxatilis*) foodfish subjected to twice daily feeding to satiation (control) or cycles of three weeks feed deprivation followed by three or six weeks twice daily feeding to satiation during an 18-week compensatory growth study in ponds.

Treatment	Final weight (g)	Production <sup>1</sup> (kg/ ha)	Survival (%)	Feed efficiency (%)	Total feed (kg)
Control (n=3)	477.9 $\pm$ 10.3 <sup>a</sup>	2528.2 $\pm$ 99.7	78.1 $\pm$ 1.8	71.9 $\pm$ 1.3 FCR <sup>2</sup> = 1.39	3511.9 $\pm$ 79.7 <sup>a</sup>
3/3 (n=4)	353.8 $\pm$ 7.8 <sup>c</sup>	1703.9 $\pm$ 76.1	81.3 $\pm$ 3.4	76.9 $\pm$ 2.7 FCR <sup>2</sup> = 1.30	2218.8 $\pm$ 80.5 <sup>b</sup>
3/6 (n=4)	404.7 $\pm$ 7.7 <sup>b</sup>	2485.9 $\pm$ 407.3	88.8 $\pm$ 4.5	78.0 $\pm$ 2.6 FCR <sup>2</sup> = 1.28	3148.5 $\pm$ 403.8 <sup>a</sup>
Pr>F	<i>P</i> <0.0001	<i>P</i> =0.1651	<i>P</i> =0.2068	<i>P</i> =0.2742	<i>P</i> =0.0243

\*Means followed by different letters indicates a significant difference (*P*<0.05)

<sup>1</sup>Production= (final fish weight x final number of fish) / (840 fish x initial stocking weight)

<sup>2</sup> Food conversion ratio (FCR = food fed/ weight gain)

Table 5. Mean ( $\pm$ SEM) overall pond water quality variables during an 18-week growth trial in which fish were fed twice daily to satiation (control), or cycles of three weeks of feed deprivation followed by twice daily feeding to satiation for three or six weeks, 3/3 and 3/6 respectively.

Treatment	pH	Turbidity	TAN <sup>1</sup>	NO <sub>2</sub> -N <sup>2</sup>	NO <sub>3</sub> -N <sup>3</sup>	SRP <sup>4</sup>	Chlorophyll-a	TN <sup>5</sup>	TP <sup>6</sup>
Control (n=3)	8.78 $\pm$ 0.03	50.3 $\pm$ 3.30	0.08 $\pm$ 0.02	0.01 $\pm$ 0.00	0.02 $\pm$ 0.01	0.46 $\pm$ 0.08	138.21 $\pm$ 17.60	4.84 $\pm$ 0.23	0.72 $\pm$ 0.07 <sup>a</sup>
3/3 (n=4)	8.77 $\pm$ 0.05	50.7 $\pm$ 5.51	0.16 $\pm$ 0.06	0.03 $\pm$ 0.01	0.09 $\pm$ 0.06	0.27 $\pm$ 0.04*	99.79 $\pm$ 16.49*	4.59 $\pm$ 0.16	0.44 $\pm$ 0.01 <sup>b</sup>
3/6 (n=4)	8.82 $\pm$ 0.07	55.7 $\pm$ 8.60	0.13 $\pm$ 0.03	0.03 $\pm$ 0.01	0.05 $\pm$ 0.02	0.35 $\pm$ 0.06	122.22 $\pm$ 5.29	4.46 $\pm$ 0.19	0.54 $\pm$ 0.05 <sup>b</sup>
Pr>F	P=0.8166	P=0.4766	P=0.4972	P=0.4316	P=0.1563	P=0.1563	P=0.2083	P=0.4457	P=0.0079

Means followed by different letters indicates a significant difference ( $P<0.10$ ).

\* Significantly different from controls as indicated by pre-planned contrast ( $P<0.10$ )

<sup>1</sup> Total ammonia nitrogen

<sup>2</sup> Nitrite-nitrogen

<sup>3</sup> Nitrate-nitrogen

<sup>4</sup> Soluble reactive phosphorus

<sup>5</sup> Total nitrogen

<sup>6</sup> Total phosphorus

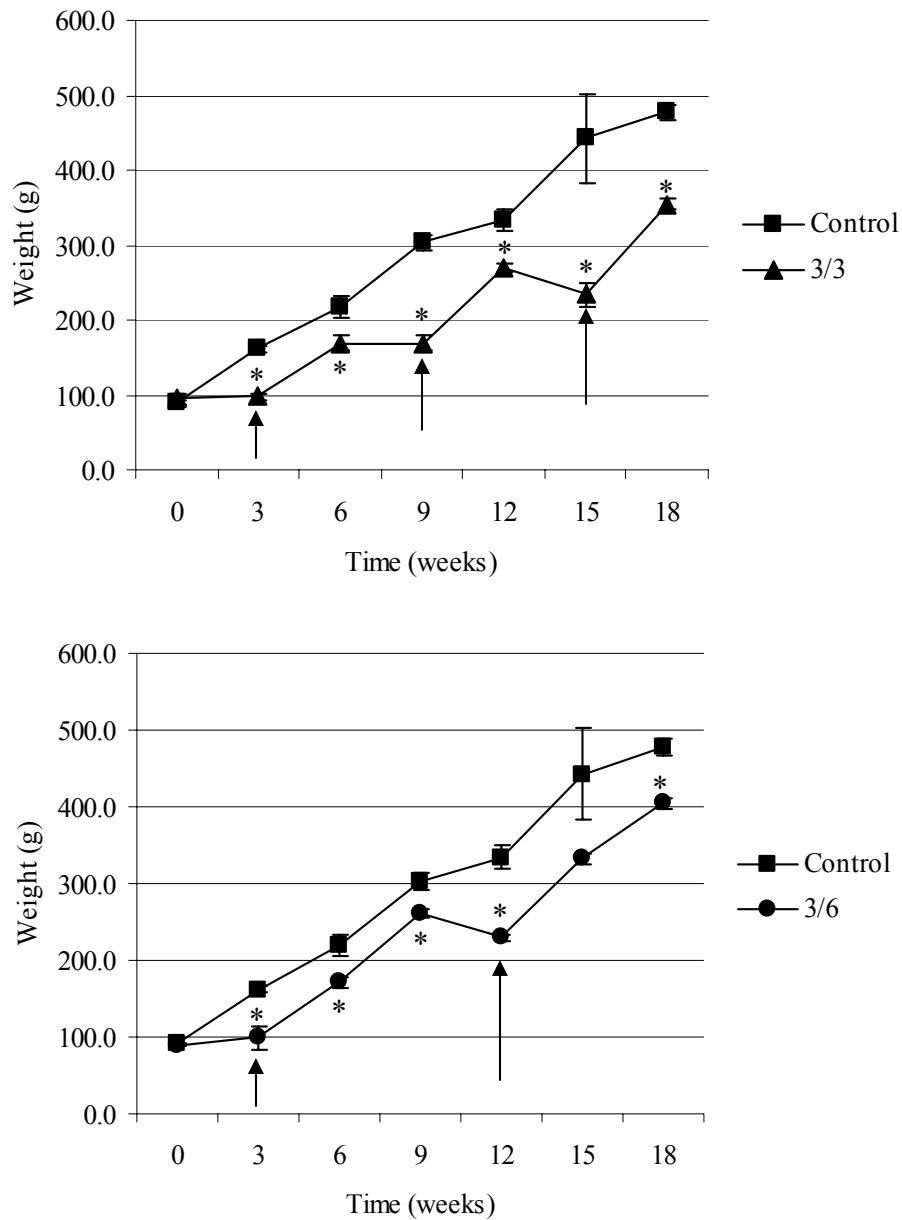


Figure 5. Weight (g) of foodfish hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or consecutive cycles of three weeks feed deprivation followed by three or six weeks of twice daily feeding to satiation, 3/3 and 3/6 respectively. Arrows indicate start of refeeding. (\*represents significant difference from controls,  $P < 0.05$ )

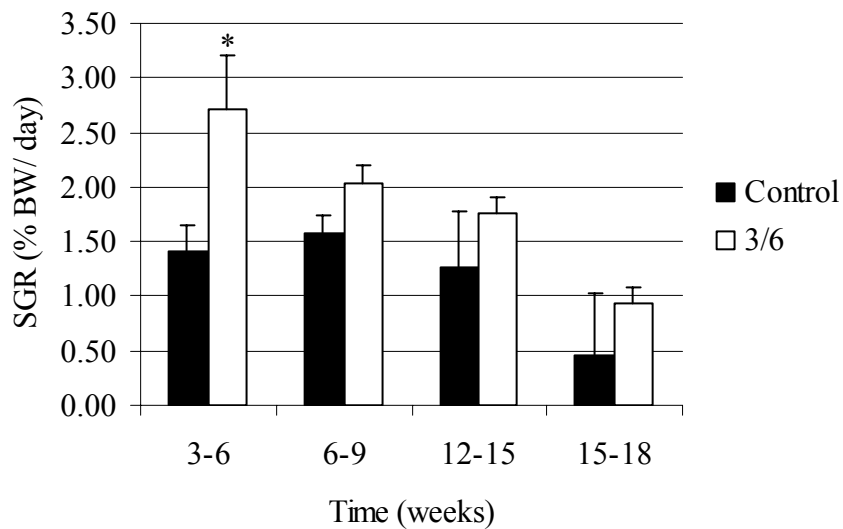
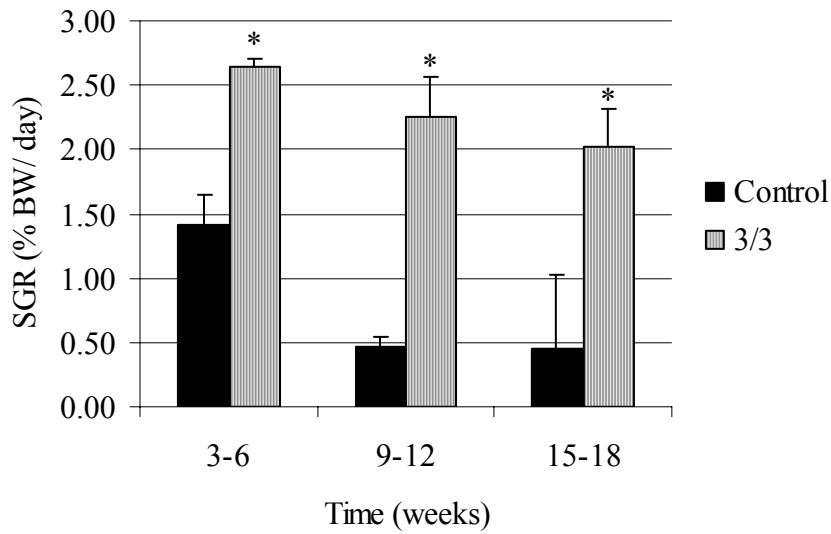


Figure 6. Specific growth rate  $\{SGR = [100 \times (\ln \text{Weight}_f - \ln \text{Weight}_i)] / (\text{Time}_f - \text{Time}_i)\}$  of foodfish hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or consecutive cycles of three weeks feed deprivation followed by three or six weeks of twice daily feeding to satiation, 3/3 and 3/6 respectively. Arrows indicate start of refeeding. (\*represents significant difference from controls,  $P < 0.05$ )

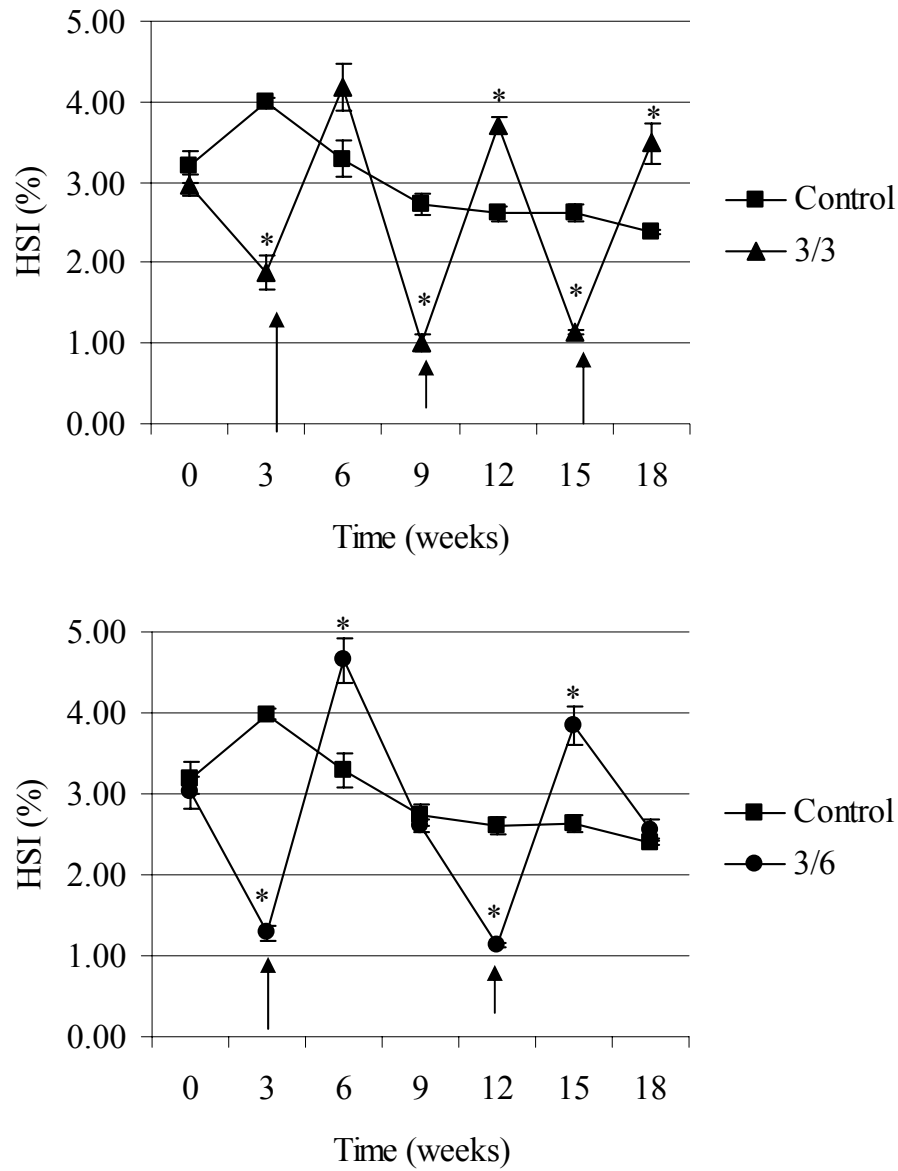


Figure 7. Hepatosomatic index (HSI= liver weight x 100/fish weight) of foodfish hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or consecutive cycles of three weeks feed deprivation followed by three or six weeks of twice daily feeding to satiation, 3/3 and 3/6 respectively. Arrows indicate start of refeeding. (\*represents significant difference from controls,  $P < 0.05$ )

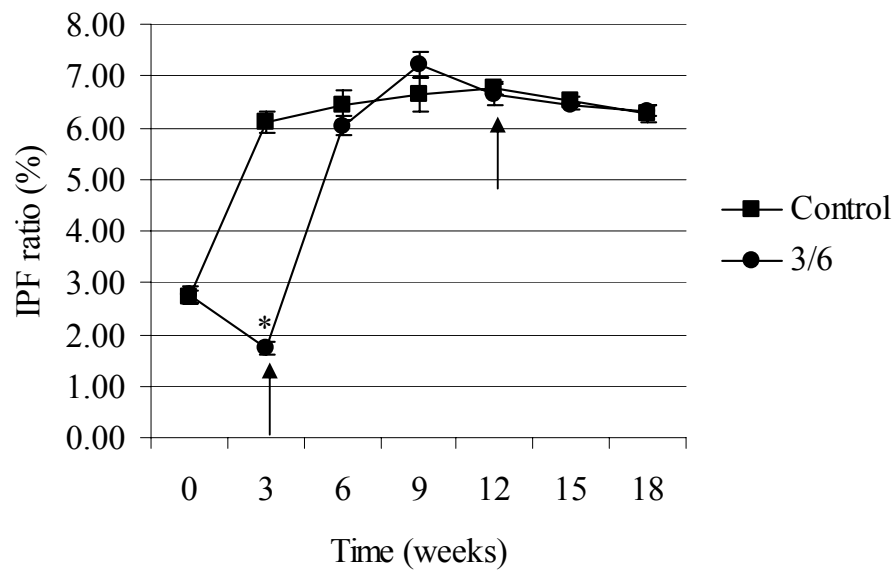
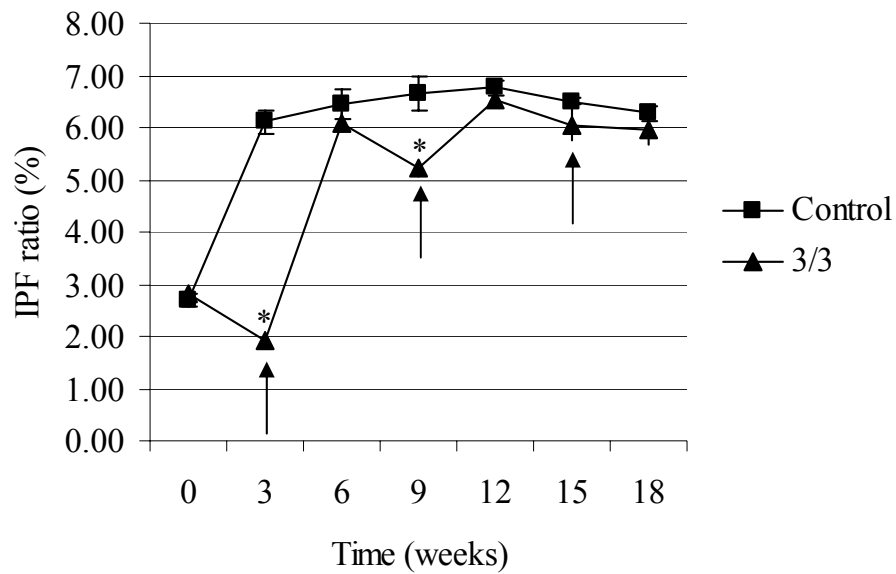


Figure 8. Intraperitoneal fat (IPF) ratio (IPF= fat weight x 100/fish weight) of foodfish hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or consecutive cycles of three weeks feed deprivation followed by three or six weeks of twice daily feeding to satiation, 3/3 and 3/6 respectively. Arrows indicate start of refeeding. (\*represents significant difference from controls,  $P < 0.05$ )

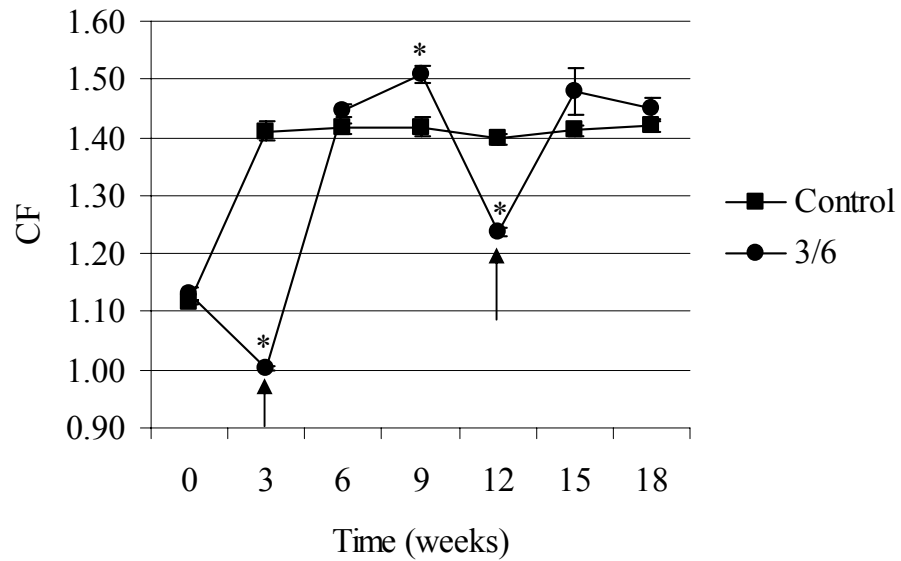
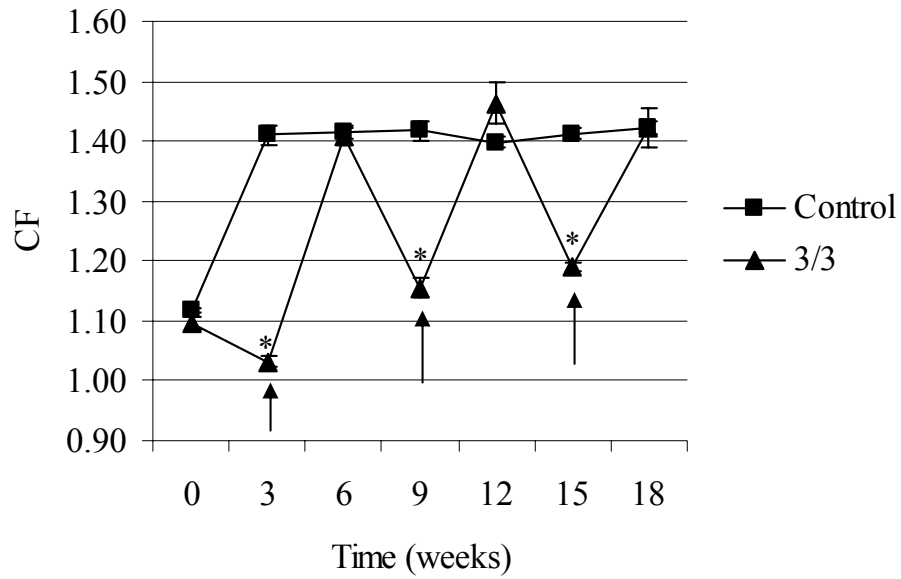


Figure 9. Condition factor [ $CF = (\text{fish wt.}/\text{length}^3) \times 10^4$ ] of foodfish hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or consecutive cycles of three weeks feed deprivation followed by three or six weeks of twice daily feeding to satiation, 3/3 and 3/6 respectively. Arrows indicate start of refeeding. (\*represents significant difference from controls,  $P < 0.05$ )

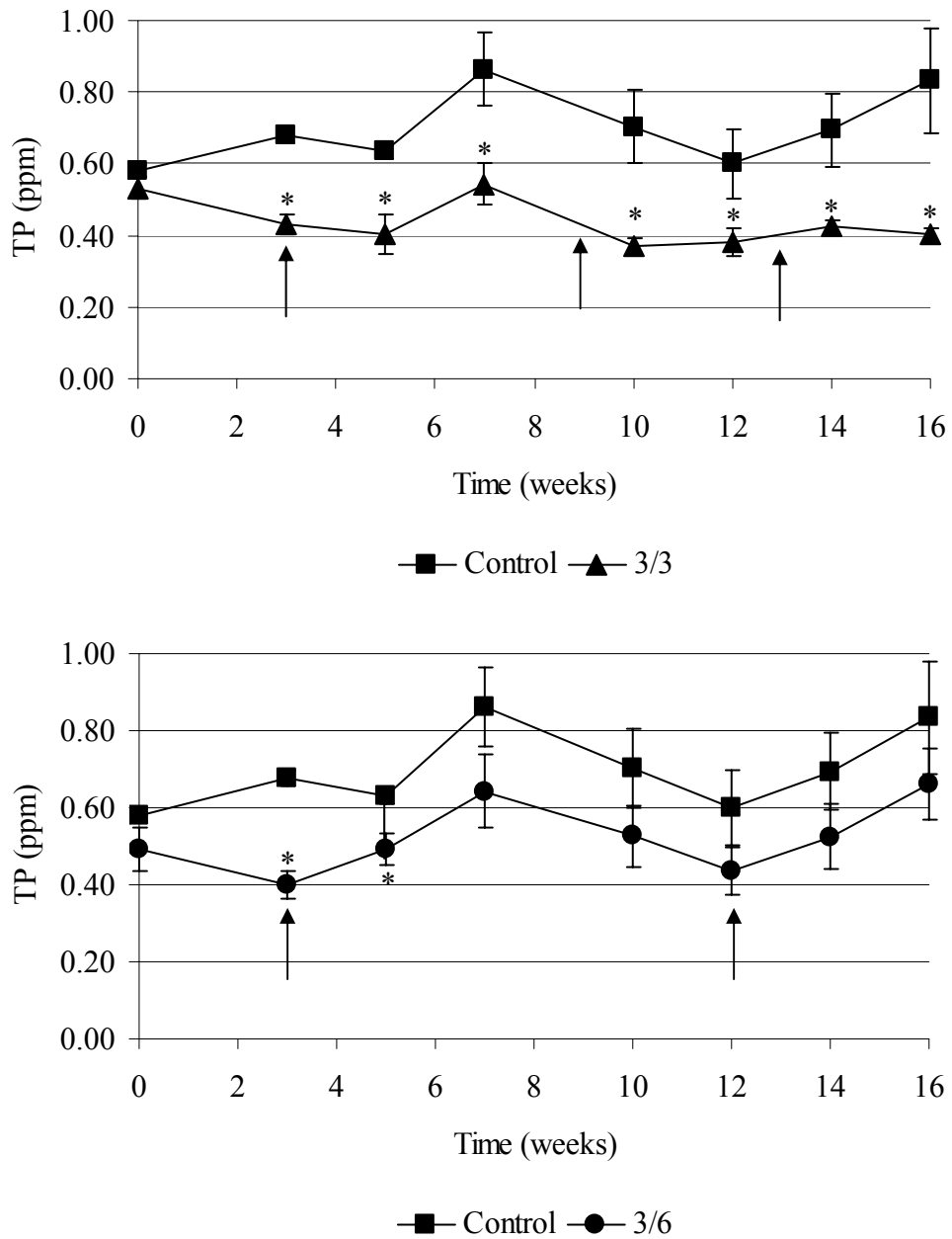


Figure 10. Mean total phosphorus (TP) levels of ponds during an 18-week hybrid striped bass (*Morone chrysops* x *M. saxatilis*) growth trial in which fish were fed twice daily to satiation (control) or consecutive cycles of three weeks feed deprivation followed by three or six weeks of twice daily feeding to satiation, 3/3 and 3/6 respectively. Arrows indicate start of refeeding. (\*represents significant difference from controls,  $P < 0.10$ )

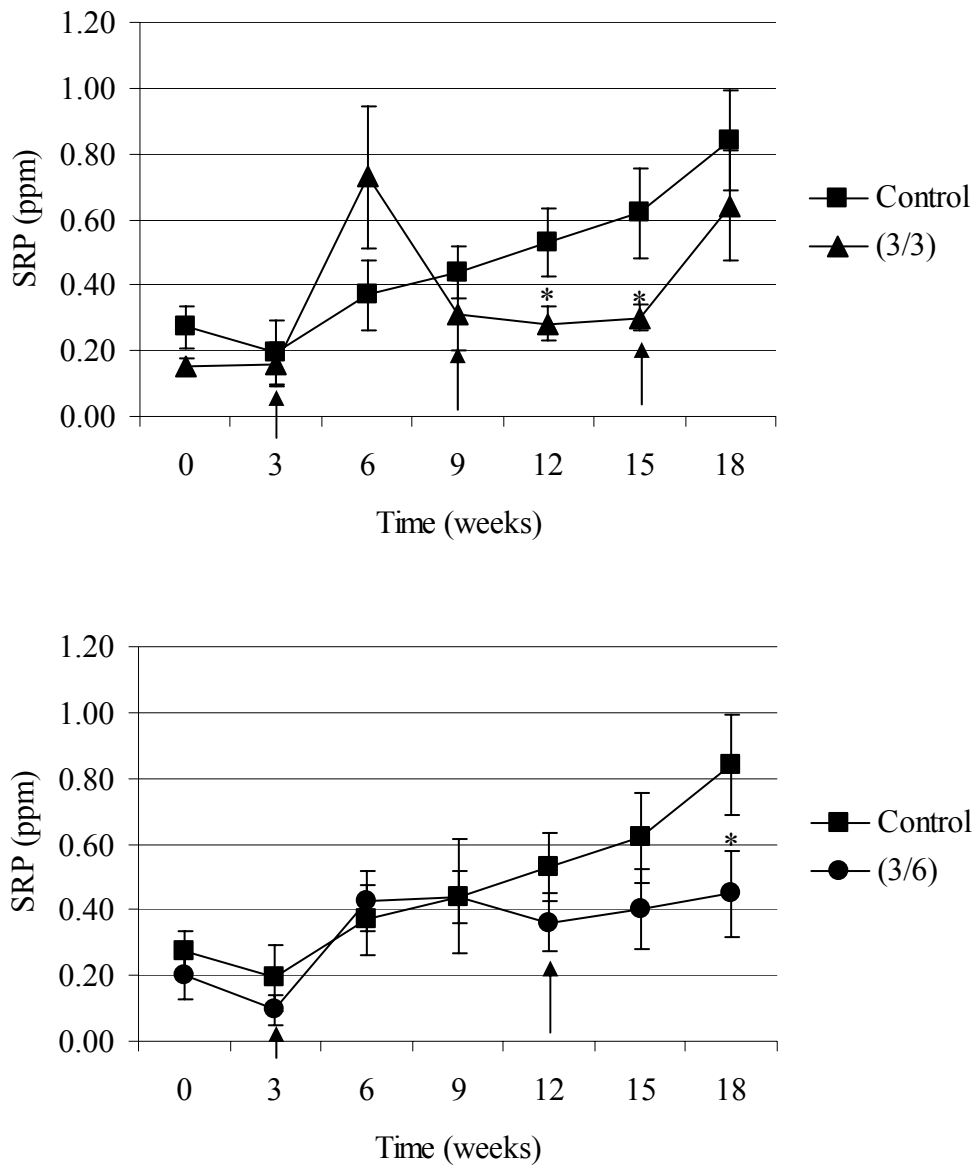


Figure 11. Mean soluble reactive phosphorus (SRP) levels in ponds during an 18-week hybrid striped bass (*Morone chrysops* x *M. saxatilis*) growth trial in which fish were fed twice daily to satiation (control) or consecutive cycles of three weeks feed deprivation followed by three or six weeks of twice daily feeding to satiation, 3/3 and 3/6 respectively. Arrows indicate start of refeeding. (\*represents significant difference from controls,  $P < 0.10$ )

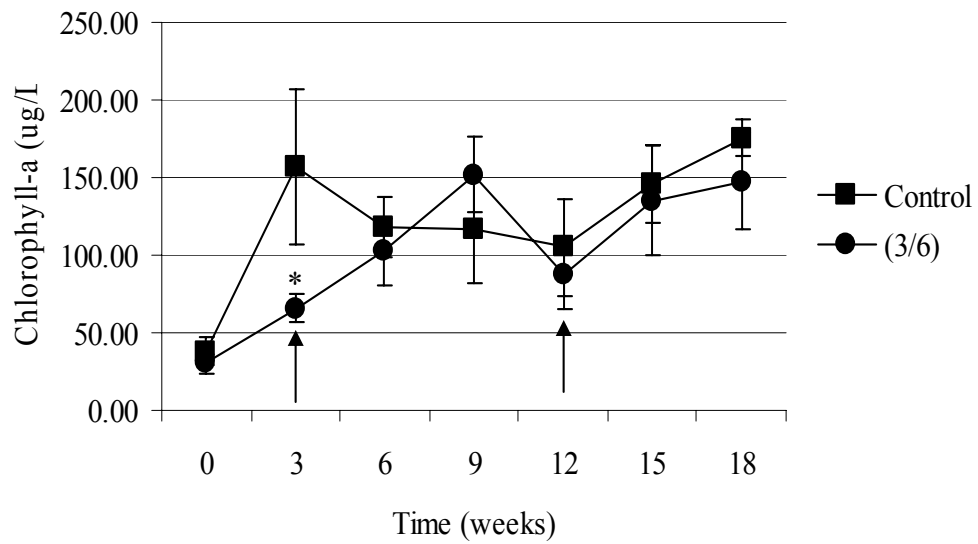
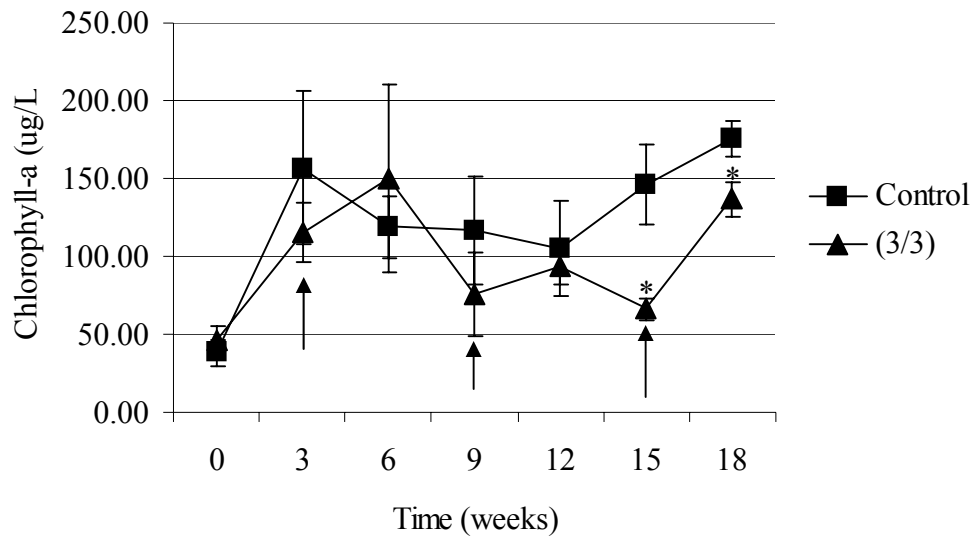


Figure 12. Mean chlorophyll-a levels in ponds during an 18-week hybrid striped bass (*Morone chrysops* x *M. saxatilis*) growth trial in which fish were fed twice daily to satiation (control) or consecutive cycles of three weeks feed deprivation followed by three or six weeks of twice daily feeding to satiation, 3/3 and 3/6 respectively. Arrows indicate start of refeeding. (\*represents significant difference from controls,  $P < 0.10$ )

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## Chapter 4

Regulation of plasma growth hormone and pituitary growth hormone gene expression and synthesis during compensatory growth in a teleost fish, the hybrid striped bass, *Morone chrysops* x *M. saxatilis*

### Abstract

An 18-week growth study was conducted to evaluate the compensatory growth (CG) response and changes in energy reserves, pituitary growth hormone (GH) gene expression and content, and circulating GH levels in hybrid striped bass (HSB), *Morone chrysops* x *M. saxatilis* induced to grow at different rates through alterations in feeding levels. HSB were subjected to either a treatment feeding regimen consisting of three weeks of complete feed deprivation followed by six weeks of twice daily satiation feeding or a control regimen where fish were fed twice daily to satiation throughout the course of the experiment. The design of the study allowed for assessment of growth and endocrine parameters over two consecutive feeding cycles. During the first feeding cycle, three weeks of feed deprivation caused a slight decline in body weight and significant decreases in hepatosomatic index (HSI) condition factor (CF), and intraperitoneal fat ratio (IPF). In contrast to that observed with growth, pituitary GH mRNA, pituitary GH content, and plasma GH increased by two-fold following three weeks of feed deprivation. Upon refeeding, treatment fish showed a significant CG response. This was characterized by elevations in specific growth rate (SGR), feed consumption, and feed efficiency (FE). The increases in these variables eventually subsided to levels observed in control fish during the last 3 weeks of the refeed. IPF and CF were fully

restored, while an over-compensation in the HSI relative to control animals was observed in treatment fish during the refeed period. In contrast to that observed during feed deprivation, pituitary GH mRNA and protein levels as well as plasma GH rapidly declined to levels observed in control fish within one week of refeeding. Similar changes in growth, energy reserve indices, feed consumption, FE, and pituitary GH mRNA and protein stores were generally observed in treatment fish during the second feed deprivation and refeed cycle. However, we did not see any significant elevations in plasma GH during the second restricted feed period in treatment fish, despite seeing increases in pituitary GH mRNA and GH stores over the same period. Moreover, elevations in feed consumption and SGR observed in treatment fish upon realimentation was sustained for the full six weeks of the refeed period of the second feeding cycle. There were significant negative correlations between depletions in energy stores and pituitary GH mRNA and protein levels over both feeding cycles. Overall, these results suggest that repeated induction of a catabolic state, reflective of reduced energy reserves is sufficient to trigger states of CG. The CG response is accompanied by elevations in SGR, feed consumption and FE. It appears that the changes in SGR characteristic of the catabolic state and CG response that follows, is accompanied by paradoxical increases and declines in pituitary GH synthesis and secretion, respectively. This discordance in pituitary GH activity with SGR, may be a consequence of the prevailing levels of insulin-like growth factor-I (IGF-I) seen during catabolic and anabolic states. IGF-I is a powerful negative feedback inhibitor of GH secretion and synthesis and its levels often correlate to growth status in HSB. Therefore, when levels of systemic IGF-I are low as observed during periods of poor growth, pituitary GH production and secretion may increase. The elevation in circulating GH levels seen here during feed deprivation may serve an

adaptive purpose, to minimize protein degradation. When conditions improve, such as that observed during CG, a rise in circulating IGF-I would exert a more intense negative feedback inhibition on the pituitary to restore GH synthesis and circulating GH levels to that observed in animals fed on a normal nutritional plane.

## Introduction

Fish growth has been described as flexible (Mommsen 2001), and is affected by a number of factors including season, temperature, prey availability, stage of sexual maturity, size of fish, and presence of other conspecifics. Due to seasonal variations in food supply, many fish species must endure periods of starvation (van Dijk et al., 2002). Once food becomes available, some species exhibit compensatory growth (CG), a phenomenon whereby animals exhibit rapid growth that exceeds that of normal individuals never previously exposed to growth-stunting conditions. The underlying mechanisms of CG are poorly understood, although three explanations have been proposed. These include hyperphagia (Bull and Metcalf, 1997; Rueda et al., 1998; Jobling and Johansen, 1999; Wang et al. 2000; Johansen et al., 2001; Ali et al., 2003; Gurney et al., 2003; Tian and Qin, 2004;), maintenance of an ideal structural:reserve tissue ratio (Broekhuizen et al., 1994 ), and increases in feed efficiency [ $FE = (\text{weight gain} / \text{feed fed}) \times 100$ ] (Boujard et al., 2000) during the realimentation period encompassing CG. Regardless of which of these variables contribute to a CG response, little is known of the endocrine mediators of CG in vertebrates generally, and teleost specifically. This is despite the fact that endocrine factors are the central physiological (e.g. growth) integrators of environmental and genetic cues.

The growth hormone (GH)/ insulin-like growth factor I (IGF-I) system is the main growth axis in fish and its role in mediating the CG response remains poorly understood. Growth hormone is well known for its role in growth regulation in fish (Mommsen, 2001). It has been shown in mammals to work both directly at the tissue level as well as indirectly through stimulation of IGF-I (Cao et al., 1989; Duan and Inui, 1990; Peter and Marchant, 1995). Exogenous GH treatment stimulates food intake, amino acid uptake and nitrogen

retention and improves food conversion (Markert et al., 1977; Gill et al., 1985, Johnsson and Bjornsson, 1994; Farmanfarmaian and Sun, 1999).

Studies to elicit a CG response in fish have utilized cyclic feeding regimes, which include periods of feed deprivation followed by periods of refeeding (see review by Ali et al., 2003). These studies varied between complete feed deprivation and levels of feed restriction or maintenance rationing, and depending on the nutritional plane, either a partial compensation, full compensation, or over-compensation in growth has been achieved following realimentation.

Although a number of energy reserve parameters, including hepatosomatic index (HSI), intraperitoneal fat ratio (IPF), condition factor (CF), and proximate body composition have been measured in CG experiments, relatively few studies have addressed regulation of pituitary GH cell activity and secretion with respect to cyclic feeding and the CG response. In rainbow trout (*Oncorhynchus mykiss*) plasma GH levels are reduced in animals fed once per week over six weeks relative to those fed more frequently every 3 or 5 days over the same time period (Farbridge et al., 1992). Plasma GH levels recovered following daily ad libitum feeding. In contrast, Duan and Plisetskaya (1993) reported significant increases in plasma GH, during four weeks of fasting in coho salmon (*Oncorhynchus kisutch*), which upon refeeding recovered to levels of control fish fed throughout the study. Fasting had no effect on pituitary GH content.

A number of other studies have evaluated dynamics in plasma GH or pituitary GH stores during feed deprivation only. In most cases, increases in plasma GH were observed with starvation (Wagner and McKeown, 1986; Barrett and McKeown, 1989; Sumpter et al., 1991; Perez-Sanchez et al., 1994; Kanisawa et al., 1995; Rand-Weaver et al., 1995). In

tilapia, pituitary GH content increases in 21-day fasted female and male tilapia (Weber and Grau, 1999). In contrast to that observed with coho salmon (Duan and Plisetskaya, 1993), both plasma GH and pituitary GH content increase in European eel (*Anguilla anguilla*) following 3-months fasting (Marchelidon et al., 1996). In the only study to evaluate pituitary GH mRNA stores in fish during fasting, Small (2002) found that 2-weeks of feed deprivation increases both pituitary GH mRNA levels and plasma GH in striped bass (*Morone saxatilis*). Collectively, these results suggest that the responsiveness of pituitary somatotrophs may vary depending on species and degree of catabolism. To date, no studies have concurrently measured GH mRNA, pituitary GH stores, and plasma GH during periods of feed deprivation or during the realimentation period in teleosts.

The aim of this study was to evaluate dynamics in pituitary GH mRNA, pituitary GH stores and plasma GH during repeated cycles of feed deprivation followed by satiation feeding in the context of the CG response in a commercially important finfish, the hybrid striped bass (HSB; *Morone chrysops* x *M. saxatilis*). Specific growth rates, HSI, IPF ratio, and CF measurements were also evaluated to determine the relationship between energy reserves, growth, plasma GH, pituitary GH content, and mRNA levels.

## **Methods**

### *Experimental system*

Two identical recirculating aquaculture systems located at North Carolina State University were utilized during the 18-week study. Each system consisted of four, 640L semi-square polyethylene tanks equipped with UV sterilization (80 W, Emperor Aquatics, Pottstown, PA, USA) and biological filtration. Animals were maintained in freshwater

(hardness 125ppm, alkalinity 200ppm) at 25 C and under a constant 12h:12h, light:dark photoperiod. Water quality parameters, dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite-nitrogen (NO<sub>2</sub>), alkalinity, hardness, and pH were all maintained at constant levels well within the tolerance limits for HSB.

Eight days prior to the start of the study, approximately 560 fish (mean weight  $\pm$  SEM;  $62.8 \pm 1.58$ g) obtained from the Tidewater Research Station, Plymouth, NC, were stocked into eight experimental tanks (70 fish/tank). Each of the four tanks in both systems was randomly assigned one of two treatment feeding regimes to result in two replicates of each treatment per system (4 replicates/ group). The treatment feeding regimes consisted of twice daily feeding to satiation (control), and three weeks of complete feed deprivation followed by twice daily feeding to satiation for six weeks (treatment). During the acclimation period, all fish were fed to satiation using a commercial HSB diet of 40 % protein/ 10 % lipid (Zeigler Bros. Inc., Gardners, PA, USA). Food was offered at 0900 and 1700. Following a two-week acclimation period, fish were graded to obtain 60 fish per tank of a similar size.

### *Sampling*

Sampling was conducted on two consecutive days for each time period with measurements taken at week zero, three, four, six, nine, 12, 15, and 18. During the first day of each sampling period, four fish from each tank were removed and anesthetized by immersion in 0.2mg/L MS-222. Individual fish were measured for total length, and weight. Blood was then taken from the caudal vasculature via a heparinized syringe and 21-gauge needle. Blood was transferred to heparinized 1.5 ml eppendorf tubes containing 24 $\mu$ l of

aprotinin (3 mg/ml), centrifuged at 8000 x g, at 4 C for 15 min. Plasma was removed and stored at -20 C.

Following decapitation, the pituitary gland was removed, placed in 1.5 ml eppendorf tubes and then flash frozen in liquid nitrogen and stored at -80 C. The liver and intraperitoneal fat was removed and weighed to calculate HSI (wet liver weight x 100 / body weight), and IPF ratio (wet weight of fat x 100 / body weight). For the second day of each sampling period (not including week 4), remaining fish in each tank were removed and anesthetized with buffered quinaldine sulfate (B.L. Mitchell Inc. Leland, MS, USA). Individual length and weight measurements were taken for mean growth analyses, and to calculate specific growth rate (SGR;  $[100 \times \ln(\text{Weight}_f) - \ln(\text{Weight}_i)] / (\text{Time}_f - \text{Time}_i)$ ) and condition factor (CF;  $[(\text{weight in grams} / \text{length}^3 \text{ in mm}) \times 10^5]$ ). Mean feed consumption was measured throughout the experiment and analyzed at each sampling period. Feed consumption was calculated as a percent body weight of fish between two sample periods  $[(\text{feed consumed} / \text{number of fish}) / \text{mean fish weight}_f - \text{mean fish weight}_i]$ . Feed efficiency [FE;  $(\text{weight gain} / \text{feed consumed}) \times 100$ ] was also calculated after each sampling period.

#### *Pituitary growth hormone mRNA and protein extraction*

Due to the relatively small size of fish at the initiation of the experiment, pituitary glands from two animals were pooled for each pituitary GH and GH mRNA determination. A total of 16 animals were sampled/group/time point for a total of eight separate measurements per group. Pituitary cytoplasmic RNA was isolated according to previously described methods (Borski et al. 1996). In brief, pituitaries were thawed on ice, and homogenized in 10mM Tris HCL, 1 mM Disodium EDTA, and 0.5 % Tween-20 (pH of 7.5). The

homogenate was centrifuged for 7 min (18000 x g), and a 5 $\mu$ l aliquot of cytosol was placed in 45 $\mu$ l of radioimmunoassay (RIA) buffer (0.05 % Tween-20, 0.8 % NaCl, 0.05M Disodium EDTA, and 0.01M phosphate; pH 7.5) and stored at -20 C until pituitary GH determinations via RIA. Total RNA was extracted with a phenol-chloroform-isoamyl alcohol mixture (100:100:1; pH 5.2) and precipitated overnight in a solution of 40  $\mu$ l 20 % KOAc (pH 5.1) and a 1ml of 0.2M NaCl : 100 % ETOH (1:2 v/v). Extracted RNA for all samples was treated with DNA-free™ (Ambion Inc.) to remove any potential genomic DNA contamination following the manufacturer's protocol, except that the volume of DNase-I enzyme was increased to 2  $\mu$ l (4 units) per reaction and the incubation time was extended to 1 hr. Total RNA concentration was determined in triplicate for each sample using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Purity of RNA preparation (A260/A280) ranged from 1.6 to 2.1.

#### *Quantitative Real Time RT-PCR*

Gene-specific primers for qRT-PCR were designed from known cDNA sequences for striped bass GH (Cheng et al., 1995) using Primer Express 2.0 software (Applied Biosystems, Foster City, CA) and recommendations from Bustin (2000). Primer sequences were as follows: (GH forward primer) 5'-ACTGCTGGCCTGCTTCAAA-3', (GH reverse primer) 5'-TTTGCCACCGTCAGGTAGGT-3'. All reactions were performed in duplicate. The total volume of each reaction was 25  $\mu$ l and final concentrations were as follows: 0.5x Quantitect SYBR Green RT-PCR master mix (Quiagen), 0.5 $\mu$ M forward and reverse primers, 0.5  $\mu$ l Quantitect RT mix, and 1.0 ng of total RNA template. Quantitative PCRs were performed for each sample in duplicate with a GeneAmp 5700 Sequence Detection System

(Applied Biosystems) using the following thermal cycling conditions: 50 C for 30 min (RT reaction), 95 C for 15 min (PCR activation), followed by 40 cycles at 95 C for 15 sec, 55 C for 30sec, and 72 C for 30 sec. A “no amplification” control (NAC) was run in duplicate on each 96-well plate. For these reactions, the 0.5 $\mu$ l Quantitect RT mix was replaced with 0.5  $\mu$ l of RNase-free water (Sigma-Aldrich), but was otherwise handled identically. These reactions should reveal genomic DNA contamination in HSB GH mRNA sample preparations. A no template control (NTC) was also run in duplicate for each 96-well plate and contained RNase-free water rather than RNA template. This negative control should reveal PCR carryover contamination in reagents, if present. No detectable amplification over 40 cycles of qRT-PCR was observed in the NTCs and NACs included in each reaction. Standard curves for total RNA were generated by serially diluting a pool of pituitary RNA (concentrations ranged from 0.01 to 100 ng). There was a strong correlation ( $R^2 = 0.99$ ) between cycle threshold values to amount of template delivered to the reaction. Relative GH mRNA abundance was normalized to total RNA for all experimental samples as suggested by Bustin (2000) (for more details see Relative Standard Curve Method; ABI Prism 7700 Sequence Detection System, User Bulletin 2, P/N 4303859 Rev. A, Stock No. 777802-001 available from Applied Biosystems).

### *GH Iodination*

Purified striped bass GH was iodinated using the chloramine-T method (Ayson et al. 1993; Jackson, 1998). Homologous hormone was solubilized in 0.01N acetic acid and 0.2% Triton X-100 (1  $\mu$ g striped bass GH/  $\mu$ l reaction volume). The iodination reaction was initiated by the addition of 1.0 mCi (10 $\mu$ l) of NaI<sup>125</sup> and 20  $\mu$ l chloramine-T. The mixture was

incubated for 2.5 min at room temperature and the reaction was terminated by the addition of 20  $\mu$ l of sodium metabisulfite solution (2.5 mg/ ml elution buffer [0.1M Tris HCL, 0.1% Triton X-100, pH 7.2]). Free iodine was separated from labeled  $^{125}\text{I}$ -GH by gel filtration through an econocolumn (1 X 20cm) containing Sephadex G75 previously saturated with one column volume of saturation buffer (0.1M Tris-HCL, 1.0% Triton X-100 pH 7.2). The iodinated GH mixture was then applied to the column and eluted in three column volumes of elution buffer. Fractions containing  $^{125}\text{I}$ -GH were pooled for use as radioligand in the RIA.

#### *GH Radioimmunoassay*

Pituitary GH content and plasma GH were measured by a previously validated homologous RIA (Jackson, 1998). The RIA was performed using a double-antibody method under disequilibrium conditions. Assay buffer was 0.01M phosphate buffer (pH 7.3), 0.14M NaCl, 0.1% Triton X-100, and 1% BSA. In preliminary studies, we found that striped bass GH (sbGH) antiserum, diluted 1:6,000 in EDTA-PBS (0.01M PBS, pH 7.3, 50mM EDTA) and 1% normal rabbit serum (NRS-EDTA-PBS), binds approximately 22-30% of iodinated sbGH. Plasma(50  $\mu$ l) was measured in duplicate. For measures of pituitary GH content samples were first diluted 1:8000 in assay buffer, and then measured in triplicate by RIA using 50  $\mu$ l of diluted sample. For plasma and pituitary extract, 50  $\mu$ l of sample and standards were diluted to 150  $\mu$ l with 50  $\mu$ l of sbGH antiserum in NRS-EDTA-PBS (1:6000 dilution) and 50 $\mu$ l of  $^{125}\text{I}$  sbGH (approximately 10,000 cpm). All tubes were vortexed and incubated overnight at 4 C. Following incubation, 100 $\mu$ l of EDTA-PBS buffer containing goat anti-rabbit  $\gamma$ -globulin (diluted 1:80) and 10% polyethylene glycol (PEG) was added to each tube and incubated for 2 hr at room temperature. Following incubation, 100  $\mu$ l of RIA buffer was

added to all standard and sample tubes, vortexed, and centrifuged at 1300 x *g* at 4 C for 60 min. The supernatant was aspirated and bound fractions were counted on a Packard Multi-Prius 2 gamma counter. Nonspecific binding represented 5% of total binding. Values for pituitary GH content were normalized to mean body weight and are reported as (ng/ml)/(g BW).

### *Statistical Analyses*

Initial statistical comparisons indicated no differences within treatment groups between separate rearing systems. Therefore, data from all tanks within a treatment group were pooled and, unless otherwise noted, analyzed with a t-test for each sampling point (Steel et al., 1997). Linear regression analysis was also conducted for all GH measurements and body indices. Statistical significance was set at a level of  $P \leq 0.05$ . All data is presented as mean  $\pm$  SEM.

## **Results**

### *Growth and Body Indices*

Control fish initially grew at around 1.5 % BW/day through the first six weeks, at which point the rate of growth gradually declined to around 1.0 % BW/day by the end of the experiment (Figs. 14 and 16). During initial feed deprivation, treatment fish showed a slight decline in body weight (Fig. 14), accompanied by similar reductions in all energy reserve indices, including HSI and IPF, as well as CF (Figs. 18-20). Upon refeeding through 3 weeks, SGR increased by 99 %, feed consumption by 66 %, and FE by 30 %, relative to controls over the same time period (Figs. 15-17). These parameters subsided to control levels

by the sixth week of realimentation (weeks 15-17). Similar patterns in SGR, feed consumption and FE were observed during the second restricted and realimentation period. Food consumption, SGR, and FE increased by 58 %, 90 %, and 17 %, respectively, compared to controls following 3-weeks of refeeding (week 15). The response decreased slightly by the end of 6-weeks of refeeding (week 18), although both food consumption and SGR remained statistically greater than control fish. Compared with the first feeding cycle, the CG response, characterized by elevated SGR and food consumption, appears to have persisted throughout the entire 6-week realimentation period of the second feeding cycle. The overall FE (mean  $\pm$  SEM) measured over the course of the experiment was not significantly different in treatment ( $85.6 \pm 1.06$ ) versus control ( $87.4 \pm 1.01$ ) fish.

Hepatosomatic index was measured as an indicator of metabolic state to determine its suitability in predicting the catabolic state possibly needed to elicit a CG response. The HSI of control fish remained relatively constant initially, and then decreased slightly over time (Figure 18). Not surprisingly, HSI proved to be a highly responsive measurement in fish on the cyclic feed regime. Following three weeks of feed deprivation where a slight loss in body weight was observed, the HSI of treatment fish decreased dramatically to 1.11, relative to that seen in control fish (HSI = 4.00). There was an over-compensation in HSI in treatment fish during realimentation. Upon refeeding the HSI increased within one week by 200 %, and rebounded further to levels that exceeded controls by the sixth week of refeeding (week 9). A similar pattern in treatment fish was observed during the second feeding cycle, although the HSI following feed deprivation was higher (HSI = 1.54) than that observed after the first starvation period (HSI = 1.11). Intraperitoneal fat ratio was also measured to assess potential changes in fat deposition. Intraperitoneal fat ratio of treatment fish decreased significantly

after the first three weeks of feed deprivation, and subsequently returned to control levels by the end of the first three weeks of refeeding (Figure 19). Intra-peritoneal fat levels subsequently remained at levels of control fish thereafter.

As an additional measure of energy content and as a potential non-lethal parameter for predicting a CG response, CF was monitored throughout the study. Lost weight significantly affected CF, as feed deprivation periods in both cycles resulted in a rapid decrease in CF, which returned to control levels after 3-weeks of refeeding (Figure 20).

### *Endocrine Indices*

Pituitary GH mRNA and GH content, and plasma GH levels were similar between control and treatment fish at the initiation of the experiment. In control fish, steady-state GH mRNA levels slowly declined over the course of the experiment, although a slight increase was observed at week 9 of the experiment (Figure 21). Both pituitary GH content (Figure 22) and plasma GH (Figure 23) levels in control fish remained relatively stable throughout the study, with final levels slightly reduced from that at time zero.

Significant differences were observed in cyclic fed fish for all three endocrine parameters during the initial feeding cycle. For the first feeding cycle, feed deprivation resulted in significant elevations in GH mRNA (214 %), pituitary GH content (99 %), and plasma GH (204 %) compared to control fish (Figs. 21-23). The elevation in GH mRNA rapidly declined to levels observed in control fish within one week of refeeding (week 4) with levels remaining near that of control fish thereafter (Fig. 21). Similarly, pituitary GH content of treatment fish also declined to levels in control fish within 1-week of refeeding (Fig. 22). Further reductions to levels below control fish was seen for the remainder of the

realimentation phase of the first feeding cycle. Plasma GH levels in cyclic fed fish similarly declined to control levels one-week into the realimentation periods (Fig. 23). The rapid return of these plasma and pituitary GH parameters to control levels within the first three weeks of refeeding were concurrent with significant increases in SGR as well as the replenishment of energy stores as observed with HSI, IPF ratio, and CF. Linear regression analysis of treatment fish during the first refeeding period, revealed significant negative correlations between pituitary GH mRNA and HSI, IPF ratio, and CF (Table 6). Likewise, significant negative correlations were found for pituitary GH content and HSI, IPF ratio, and CF during the same period. For plasma GH, significant negative correlations were only observed with HSI and CF.

During the second feeding cycle, significant increases were observed in GH mRNA (143 %) and GH pituitary content (116 %) after three weeks of feed deprivation. Both parameters fell to control levels within 3 weeks (week 15) of the refeed period, the earliest time point measured following the onset of refeeding. Levels remained similar to control fish over the remainder of the study. Although, no significant increases in plasma GH were observed during the feed deprivation phase of the second cycle, levels did drop by almost 60 % during the first 3-weeks of refeeding. Significant negative correlations in pituitary GH mRNA and GH stores were seen with HSI and CF in treatment fish during the refeeding period of the second feed cycle (Table 1).

## **Discussion**

Repeated cycles of feed deprivation followed by satiation feeding was sufficient to elicit strong CG responses in HSB, although the two sequential phases of CG elicited in this

study did not result in complete “catch-up” growth or full growth compensation. A number of other studies have reported partial growth compensation in fish (Gaylord and Gatlin, 2000; Thorpe et al. 1990; Reimers et al., 1993; Morgan and Metcalfe, 2001; Johansen et al., 2001; Quinton and Blake, 1990; Jobling et al., 1993; Hayward and Wang, 2001; Hayward et al., 2000), owing the lack of complete compensation to excessive feed deprivation cycles, insufficient refeeding periods, or an abbreviated response overall. In this study, control fish were 48.2 g larger than treatment fish at the end of the study, with 19.8 g of lost weight occurring as a result of feed deprivation, and the remaining 28.4 g difference from lost growth opportunity during the realimentation periods. Hence, it appears that the combination of both lost growth opportunity in addition to weight loss results in a growth debt, which cannot be overcome during six-week refeeding periods. Interestingly, the majority of weight loss occurred during the second cycle (10.2 %) versus the first cycle (7.7 %) despite equivalent feed deprivation periods. The basis for this discrepancy in weight loss is unclear, but may reflect the presence of an internal biological clock. Evidence for an internal clock was reported by Van Dijk et al. (2005). Despite holding temperature and photoperiod constant, juvenile roach, *Rutilus rutilus*, exposed to a 21 d feed deprivation period during the winter season had a significantly larger liver mass, and higher liver lipid content, white muscle protein and glycogen content than fish used in the same study conducted during the summer. Similarly, HSB in this study may be better prepared for feed deprivation periods during the initial feeding cycles, because the experiment was started in spring, whereas increased weight loss occurred during the summer. Interestingly, there was a temporal difference between the first and second feed cycle in the growth response during realimentation. During the first cycle, refeeding led to significant increases in food

consumption, FE, and SGR, which persisted for 3-weeks of feeding, while the SGR and food consumption response continued for the full 6-weeks of the second refeeding period. It is possible that if the last refeed period of the second cycle had been extended when the CG response persisted, treatment animals may have achieved a final body weight closer to that of control fish. The prolonged CG response observed during the second refeeding period may have resulted from the increased amount of weight loss experienced during the feed deprivation period, as it has been reported that the CG response depends on the length and severity of feed deprivation (Quinton and Blake, 1990). The extended CG response could also be a learned response to previous feed deprivation periods. Similar responses have been reported in experiments using repetitive feeding cycles (up to four), in which increases in SGR (Wu et al., 2003, Zhu et al., 2004), food consumption (Ali and Wooten, 2001; Wu et al., 2002; Wu et al., 2003, Zhu et al., 2004), and FE (Wu et al., 2003) were improved with each cycle.

In order to better understand the physiological basis of CG, measures of energy (HSI, IPF ratio, and CF) were monitored throughout the experiment. Complete feed deprivation during both cycles resulted in precipitous declines in HSI and CF, while a decline in IPF ratio was only observed during the first feeding cycle. Similar changes in HSI and CF have been observed in channel catfish (Gaylord and Gatlin, 2000), although IPF ratio declined more slowly than that observed here. The reduction in energy stores likely represents a shift from energy storage toward maintenance metabolism (MacKenzie et al., 1998). The shift in metabolism, as proposed by Broekhuizen et al. (1994) could contribute to the CG response. If low metabolic rates are maintained from the period of starvation into the initial realimentation period, more energy could then be directed toward growth. Hybrid striped

bass required three weeks of refeeding to replenish energy stores to control levels, with HSI levels overcompensating at three and six weeks into the refeeding period. Although a similar pattern was observed during the second feeding period, no change was observed in IPF ratio over this period. It seems then that larger-sized HSB may preferentially mobilize energy stores from the liver, rather than fat stores following periods of feed deprivation.

Replenishment of energy stores coincided with the CG response of both feed cycles.

However, during the first cycle, this replenishment seemed to result in a halt to the CG response within 3 weeks of refeeding, while restoration of energy parameters during the second cycle resulted in a CG response lasting the full 6-weeks of refeeding. Hence, although a CG response can be predicted by a sufficient reduction in HSI to a value of at least 1.5 (Fig. 18; Picha et al., in press), the extent of the response cannot.

Measurements of energy reserves such as HSI, IPF and CF may offer some understanding of metabolic state and may prove useful in predicting a CG response.

However, the underlying physiological mechanisms through which nutrient intake activates an increase in somatic growth in fish are not fully understood (MacKenzie et al., 1998).

Although endocrine mediators such as GH and IGF-I have been shown to play key roles in fish growth (Mommsen, 2001), their function in the CG response is poorly understood.

Growth hormone mRNA, pituitary content, and plasma levels were measured in this study to characterize the possible function of pituitary somatotrophs in the CG response. This study reports for the first time that feed deprivation causes a concomitant increase in pituitary GH mRNA levels, pituitary GH content and plasma GH in teleosts. Levels of pituitary GH mRNA, pituitary GH content, and plasma GH increased by 125 %, 97 %, and 71 %, respectively during the first 3-weeks of starvation. Similar increases in these parameters were

seen in the second deprivation period, although no significant increase in plasma GH was observed. The magnitude of change in GH mRNA and pituitary GH increased during the second feeding cycle (weeks 9-12) compared to that observed during the first feeding cycle (weeks 0-3). The concomitant increase in both GH mRNA and GH stores along with elevations in plasma GH suggests higher synthesis and secretion of GH from the pituitary during periods of starvation. However, we cannot rule out that reduced clearance rates may also contribute to elevations in plasma GH seen during the feed deprivation periods. Small et al. (2002) reported a 33 % increase in GH mRNA and a significant increase in plasma GH in striped bass, similar to that observed here. The mechanism(s) that underlie the increases in GH synthesis and secretion observed in HSB during feed deprivation is uncertain. Studies have shown that food deprivation may reduce secretion of hypothalamic somatostatin, a peptide known to inhibit GH secretion in teleosts (Thomas et al., 1990). Starvation has also been shown to inhibit hepatic GH receptor expression, GH binding, IGF-I expression, and/or circulating IGF-I levels (Maes et al., 1983, Deng et al., 2004; Fukada et al., 2004; Gray et al., 1992; Perez-Sanchez et al., 1994; Perez-Sanchez et al., 1995). Since previous studies, including those in HSB (Fruchtman et al. 2000, 2002), have shown IGF-I to be a potent negative feedback inhibitor of GH secretion and synthesis, reductions in circulating IGF-I would lead to increased GH secretion during states of GH resistance when hepatic GH receptors are downregulated. Our recent studies show that restricted or maintenance feeding reduces circulating IGF-I in HSB (Picha et al., in press) and preliminary results from these studies (data not shown) indicate complete feed deprivation may also reduce circulating IGF-I. Future studies are required to assess if a state of GH-resistance or downregulation of GH receptors induced by a low nutritional plane might contribute to reductions in hepatic IGF-I

synthesis and circulating hormone, and hence, the elevations in pituitary GH activity observed in the present investigations.

The rapid increase in GH in feed deprived fish seems contradictory to its known role in growth promotion (Donaldson et al., 1979), although studies have suggested a catabolic role for GH in addition to its well-established anabolic properties (Leatherland and Nuti, 1982; Sumpter et al., 1991). Growth hormone has lipolytic activity and promotes protein synthesis in vertebrates, including teleosts (Sumpter et al. 1991). The increase in GH during feed deprivation could be a means of conserving muscle protein while ensuring a ready supply of free fatty acids and glycerol during states of catabolism. Interestingly, during the first feeding cycle, increases in pituitary GH synthesis and plasma GH (all measures) were accompanied by a reduction in energy stores, specifically liver and intraperitoneal fat.

Although the reduction in HSI is likely due to glycogen mobilization (Gaylord et al., 2000), the significant reduction in IPF ratio could be evidence of the lipolytic effects of GH.

Interestingly, there was a less dramatic elevation in pituitary GH mRNA and GH stores, and no significant increase in plasma GH during the second feeding cycle relative to the first starvation cycle. During the second period, IPF ratio did not decline below levels in control fish during feed deprivation, suggesting that an elevation in levels of circulating GH, may be important in mobilizing intraperitoneal fat during catabolism.

Realimentation following the first feed deprivation period caused a rapid decline and full recovery in GH mRNA, pituitary GH content, and plasma GH levels to control levels within one week, similar to results on plasma GH reported in juvenile coho salmon (Duan and Plisetskaya, 1993). Growth hormone mRNA and pituitary GH further declined after three weeks of refeeding, and could be the result of increased IGF-I production and more

pronounced negative feed-back regulation of GH synthesis during the CG response (Duan and Plisetskaya, 1993; Picha et al., in press). The concomitant decreases in the GH measurements during both refeeding periods also correlated to increases in every energy reserve parameter measured. Hence, the return of energy content to components of the viscera provides some mechanism for GH reduction, likely the result of improved nutritive state and increased production of IGF-I.

### **Summary and Conclusions**

A cyclic feeding regime of three weeks feed deprivation followed by six weeks of refeeding was sufficient to elicit a CG response in HSB. The CG response, however, was not sufficient to result in complete compensation. It is evident that the loss of weight during feed deprivation periods leads to excessive growth debts, which cannot be overcome despite the significant, albeit temporary, increases in FE and SGR. Hence, experiments that reduce the level of weight loss during the feed deprivation period should be evaluated for the scope of the CG response and the potential for complete compensation.

During the first feeding cycle, the rapid reduction and restoration in GH mRNA and pituitary GH to levels of control animals fed throughout the experiment may be part of the CG response, insofar as it may reflect elevations in systemic IGF-I and/or restoration of hepatic GH receptors. Despite weight loss during feed deprivation, GH levels increased suggesting a possible alternative role for GH during periods of poor nutritional status. These results suggest that all measures of GH are highly dependent on nutritional state and that pituitary somatotrophs may play a role in conserving enough energy reserves during periods

of catabolism, so when conditions improve fish may exhibit supra-accelerated growth, characteristic of CG.

Table 6. Correlation between body indices (HSI, IPF, and CF) and GH mRNA and pituitary GH content during the six-week refeed period of both treatment cycles.

Factors	Feed cycle	Regression Equation	R <sup>2</sup>	N	P-value
GH mRNA and HSI	1 <sup>st</sup>	y= -0.501x + 2.892	0.5905	29	P<0.0001
GH mRNA and IPF	1 <sup>st</sup>	y= -0.978x + 4.449	0.5010	29	P<0.0001
GH mRNA and CF	1 <sup>st</sup>	y= -5.528x + 7.442	0.5779	29	P<0.0001
Pituitary GH and HSI	1 <sup>st</sup>	y= -0.319x + 1.996	0.4567	32	P<0.0001
Pituitary GH and IPF	1 <sup>st</sup>	y= -0.631x + 3.030	0.4331	32	P<0.0001
Pituitary GH and CF	1 <sup>st</sup>	y= -3.509x + 4.886	0.4560	32	P<0.0001
GH mRNA and HSI	2 <sup>nd</sup>	y= -0.426x + 2.341	0.5678	24	P<0.0001
GH mRNA and CF	2 <sup>nd</sup>	y= -3.477x + 5.277	0.4815	24	P=0.0002
Pituitary GH and HSI	2 <sup>nd</sup>	y= -0.220x + 1.489	0.4995	24	P=0.0001
Pituitary GH and CF	2 <sup>nd</sup>	y= -2.005x + 3.267	0.5300	24	P<0.0001

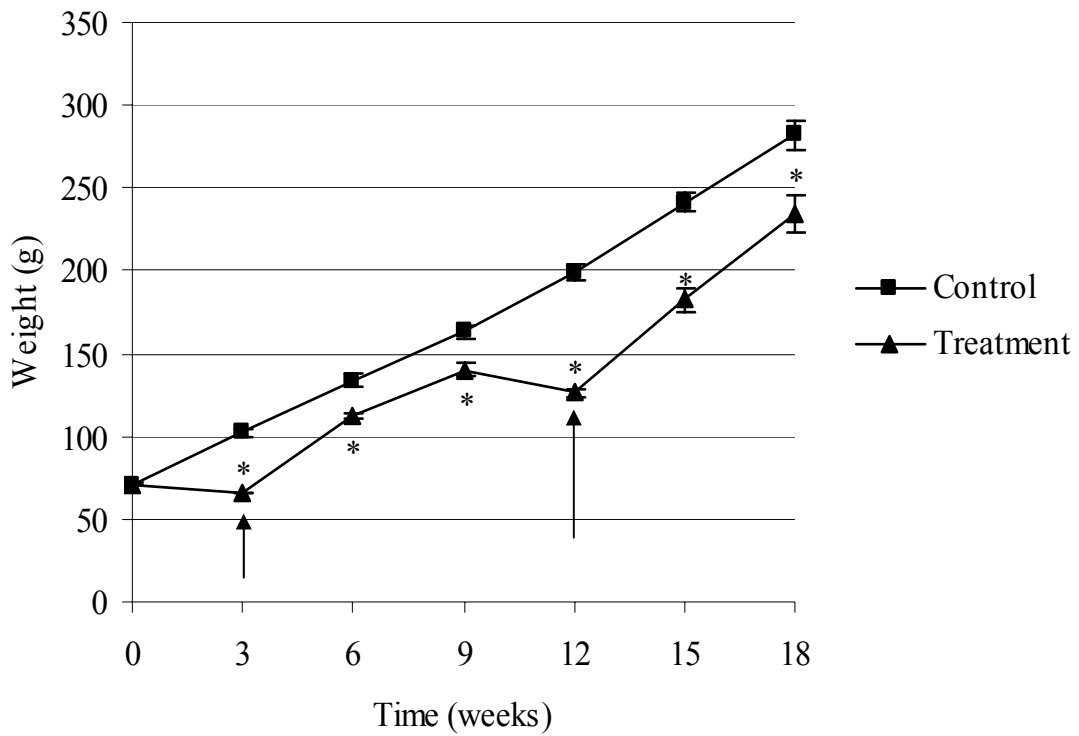


Figure 13. Growth (weight) of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

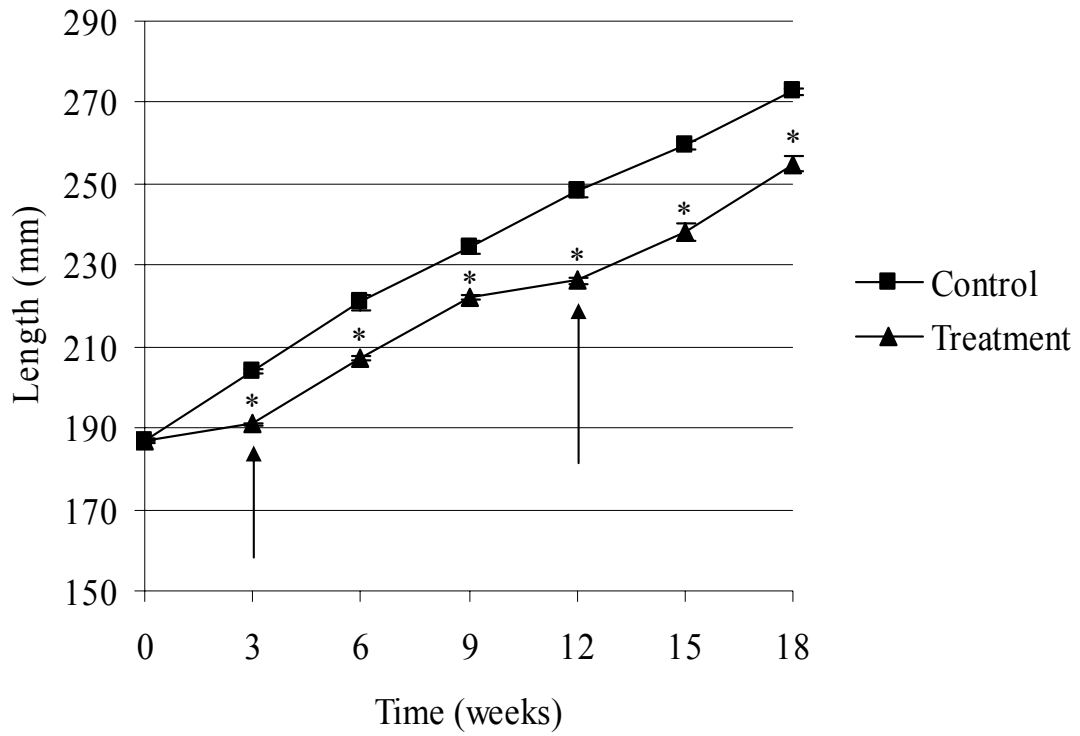


Figure 14. Growth (length) of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

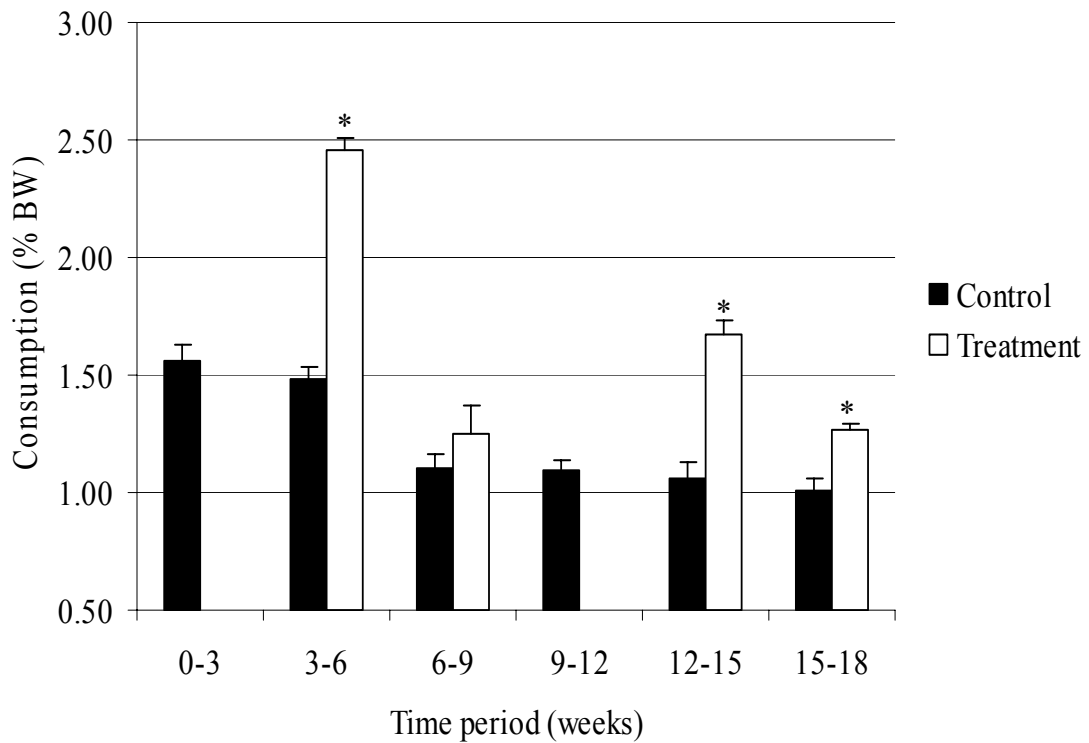


Figure 15. Food consumption (% BW) of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

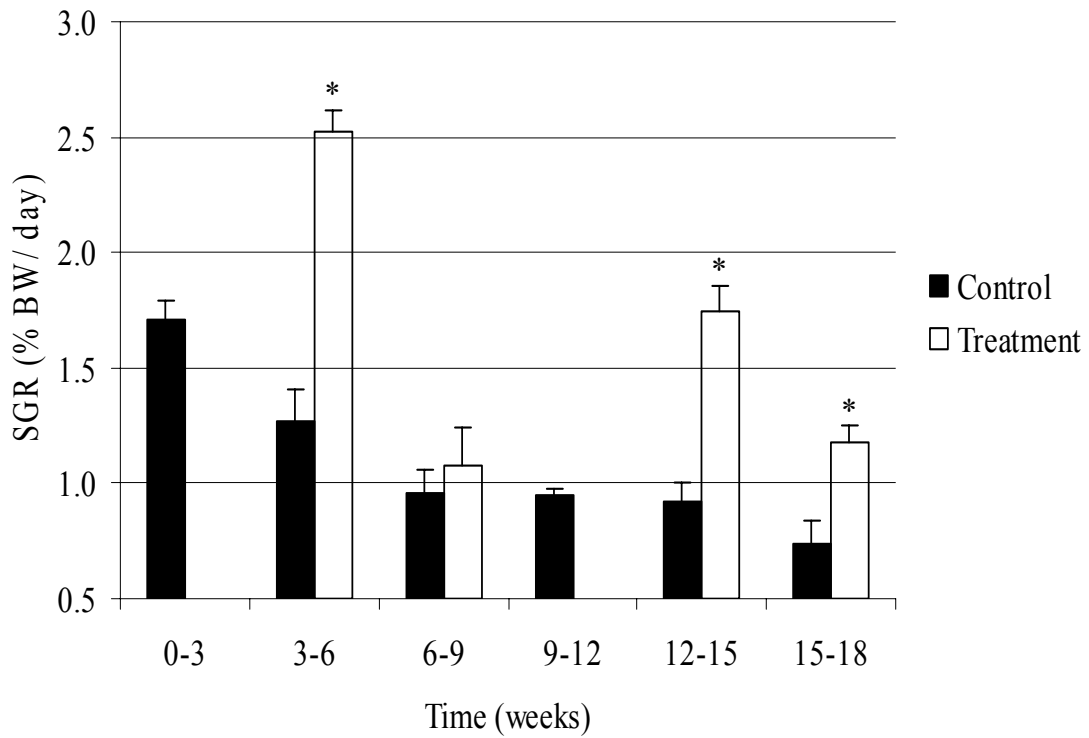


Figure 16. Specific growth rate (SGR;  $[100 \times \ln(\text{Weight}_f) - \ln(\text{Weight}_i)] / (\text{Time}_f - \text{Time}_i)$ ) of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

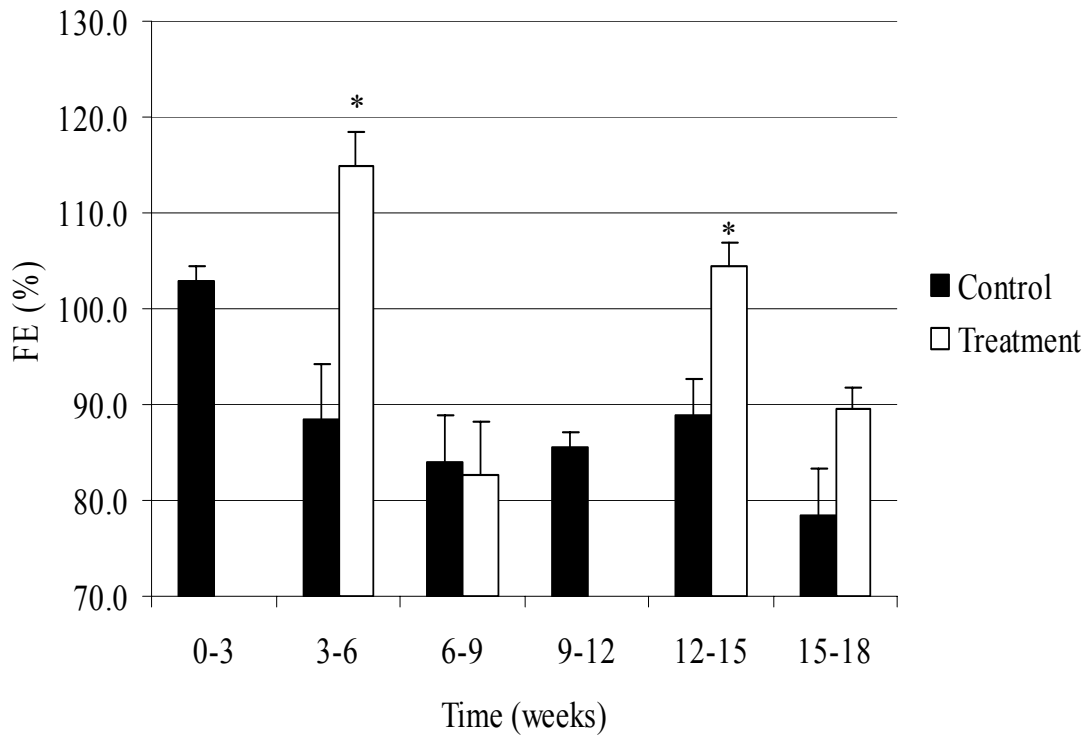


Figure 17. Feed efficiency (weight gain/feed fed x 100) of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

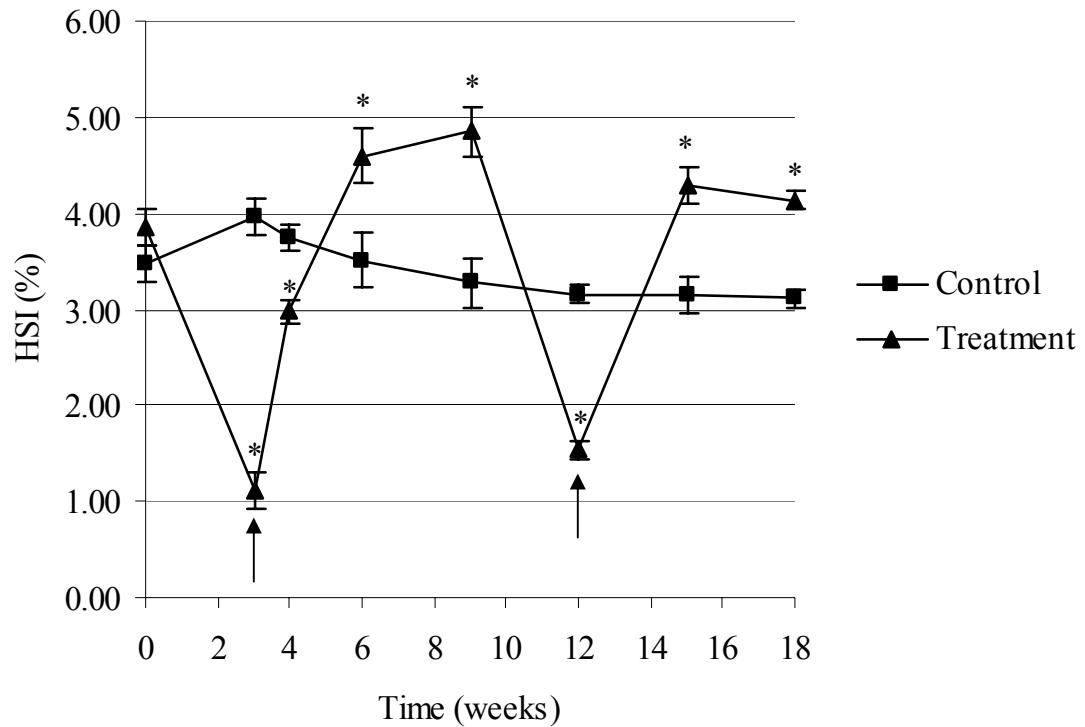


Figure 18. Hepatosomatic index (liver weight/body weight x 100) of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

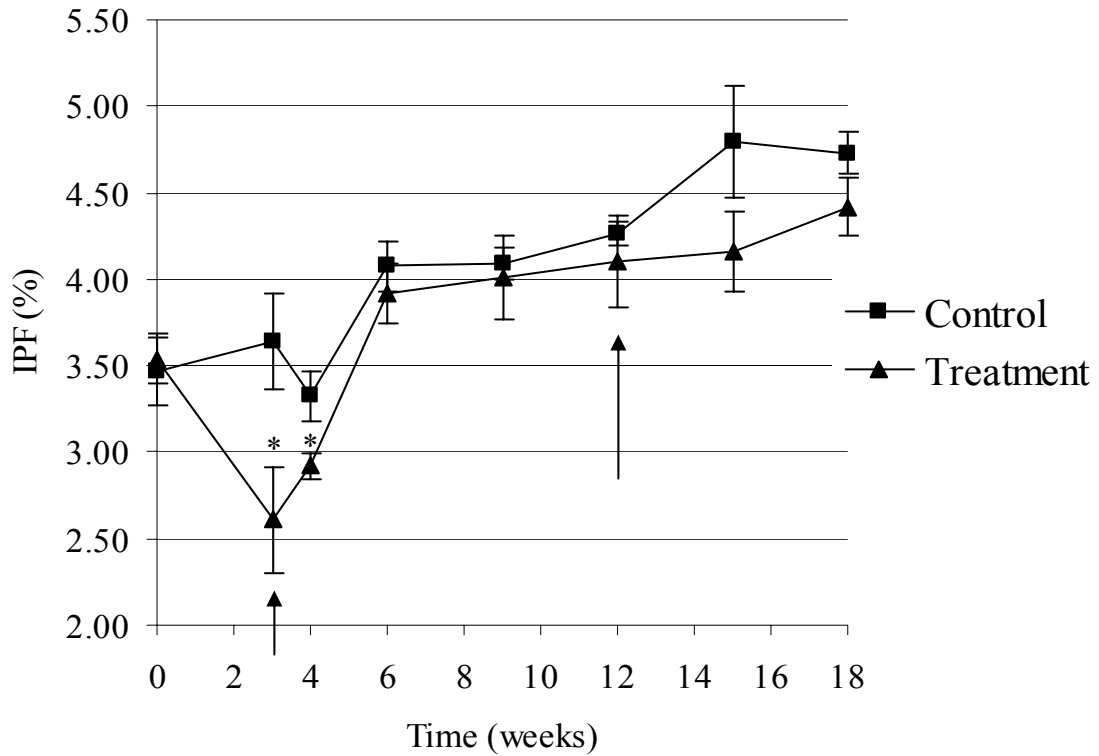


Figure 19. Intrapерitoneal fat ratio (fat weight/body weight x 100) of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

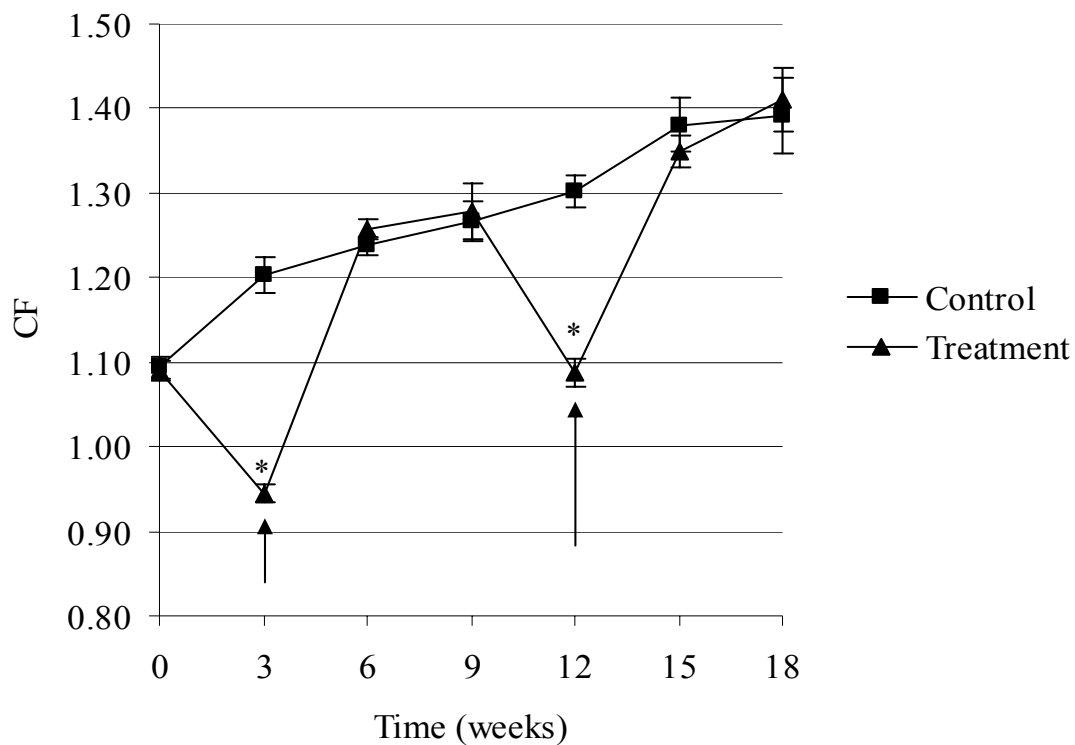


Figure 20. Condition factor  $[(\text{weight in grams} / \text{length}^3 \text{ in mm}) \times 10^5]$  of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

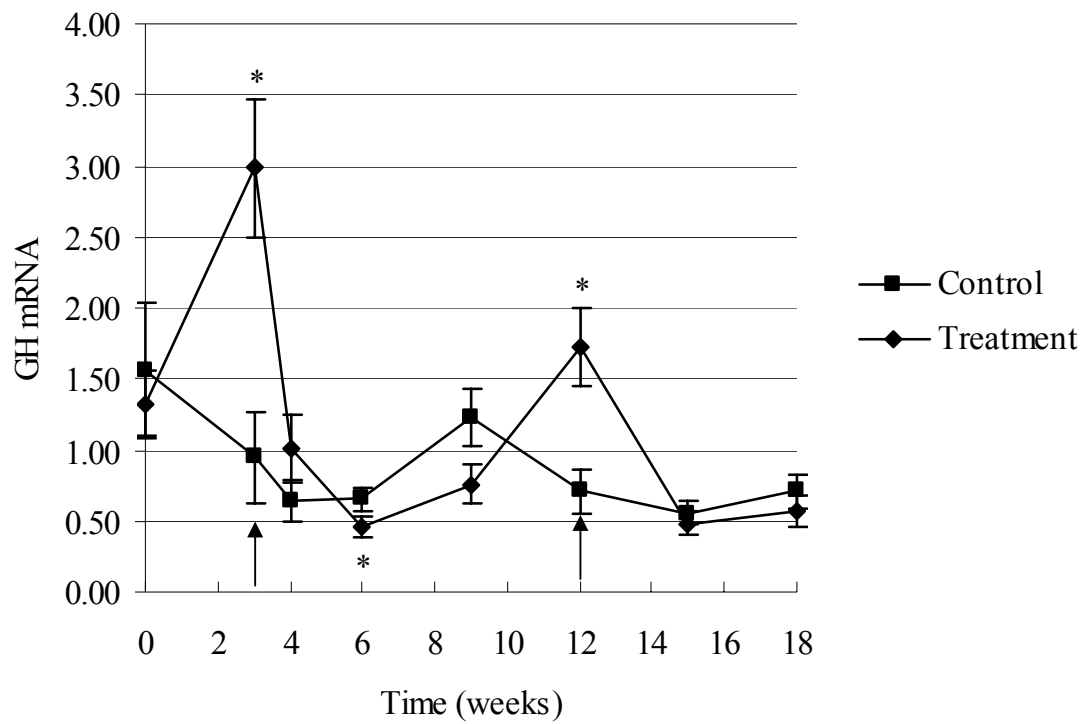


Figure 21. Growth hormone mRNA normalized to total RNA in the pituitary of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

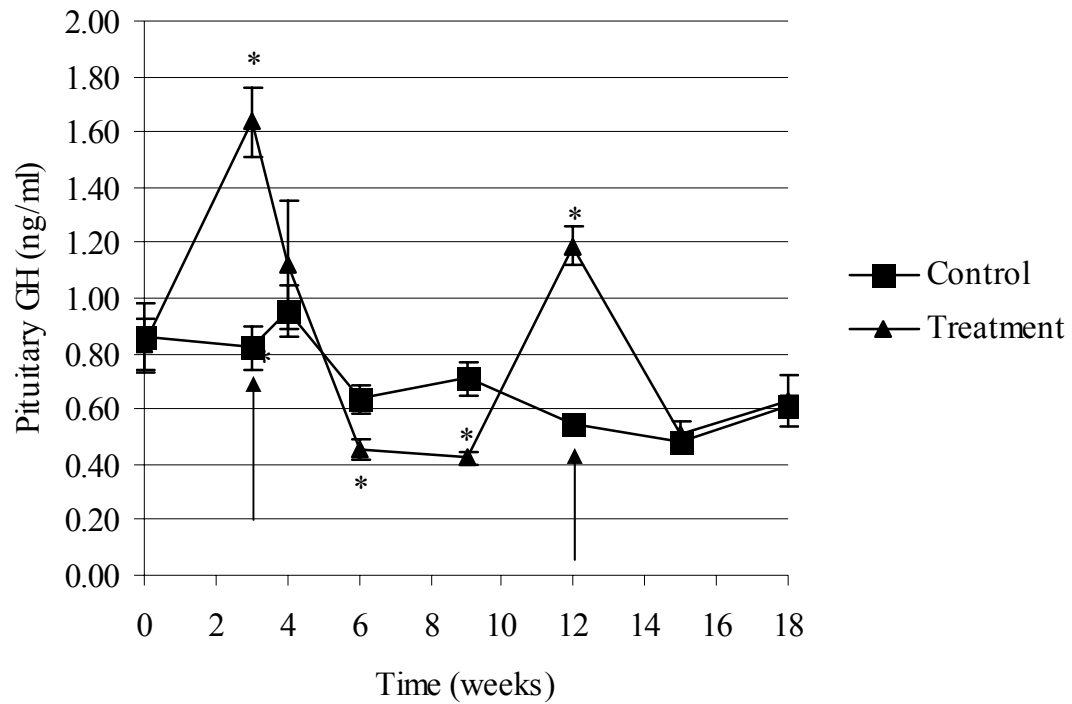


Figure 22. Pituitary GH content in hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

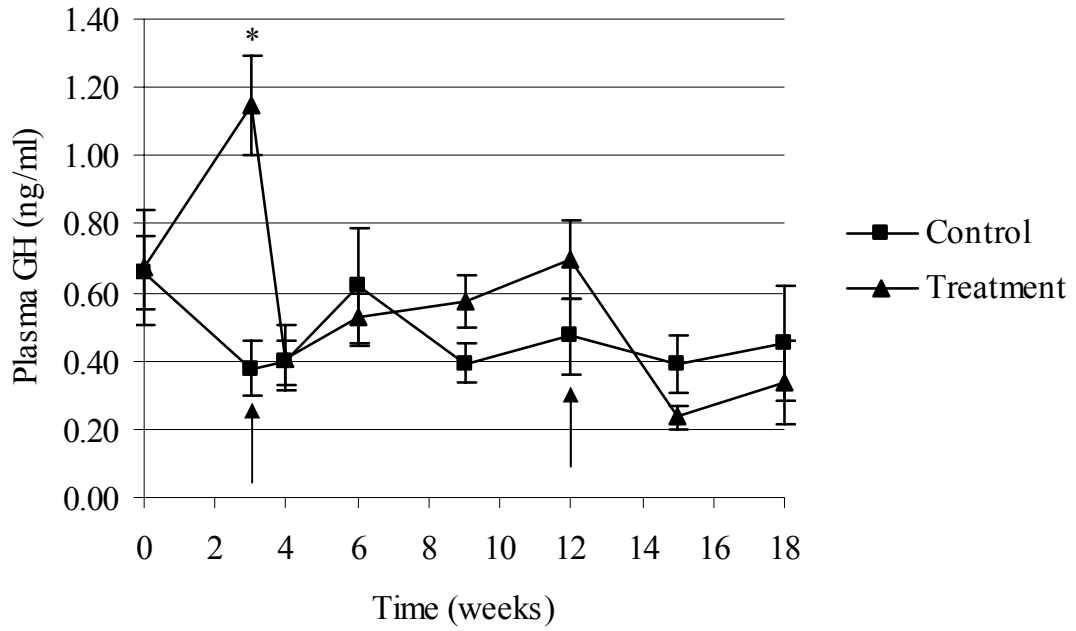


Figure 23. Plasma GH levels in hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two consecutive cycles of three weeks feed deprivation followed by six weeks of refeeding to satiation twice daily (treatment). Arrows indicate start of refeeding period. (\* represents significant difference between groups at each respective time point,  $P < 0.05$ )

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## Chapter 5- Summary

The cyclic feeding regimes used in the two pond studies and one tank study elicited partial compensatory growth (CG) in hybrid striped bass (HSB). The response was demonstrated by dramatic, albeit temporary, increases in SGR and FE. The combination of lost weight, and lost growth opportunity during the period of feed deprivation resulted in a growth debt, which could not be overcome by the partial CG response. In each of the three experiments conducted, repeated cycles of feed deprivation followed by refeeding were used to stimulate a CG response. A consistent difference in weight loss was observed between early and late feeding cycles in all three studies and suggests that seasonal differences in the fish's response to feed deprivation may exist. Woiwode and Adelmade (1991) reported that weight loss significantly increased and FE decreased with increasing temperature in hybrid striped bass (*Morone saxatilis* x *M. chrysops*), while photoperiod had no effect on weight loss during starvation. Temperature could account for some of the weight loss discrepancy in pond-reared fish, as temperatures increased with the progression of the study, however, changing temperature was not a factor in the tank study. It is possible that an internal biological clock is responsible for the discrepancy in weight loss between feed deprivation periods in the two feeding cycles. Evidence for an internal clock was reported by Van Dijk et al. (2005). Despite holding temperature and photoperiod constant, juvenile roach, *Rutilus rutilus*, exposed to a 21 day feed deprivation period during the winter season had a significantly larger liver mass, and higher liver lipid content, white muscle protein and glycogen content than fish used in the same study conducted during the summer. Similarly, HSB in these studies may be better prepared for feed deprivation periods during the initial

feeding cycles, because all three experiments were initiated during the spring, whereas increased weight loss occurred during the summer.

Estimates of energy storage as measured by HSI and CF proved to be reliable predictors of a robust CG response, and taken together with measures of GH provide good indicators of growth and nutritional status of the fish.

Increases in FE were observed as a part of the CG response. In both pond studies, overall FE of treatment fish exceeded that of control fish, a result not observed in the tank study. Fish subjected to the cyclic feeding regime in the tank study substantially increased FE during the first three weeks of each refeeding period, however the response was temporary. Of all five cyclic feeding regimes evaluated in the three studies, three weeks of feed deprivation followed by six weeks of refeeding (3/6) resulted a final weight nearest to control fish, in addition to significantly increasing FE (in the pond study) when compared to control fish. Further, the 3/6 feeding regime in the pond study also led to reductions in TP, SRP, and chlorophyll-a. Hence, farmers could adopt the 3/6 cyclic feeding regime to improve water quality if some growth could be sacrificed.

### **Comparison of study results: tanks versus ponds**

It was hypothesized that inherent differences between tank systems and the pond environment would likely lead to differences in the CG response. Further, in order to benefit from both the FE aspects of the CG response, as well as those attributed to water quality improvements, adjustment of the optimum cyclic feeding regimes from tank studies would be necessary to have similar results in ponds. The differences in the feeding regimes used in tanks and ponds can be illustrated by the feeding regime of three weeks of feed deprivation

followed by six weeks of refeeding (3/6). The 3/6 treatment was evaluated in both a tank and pond study. Although, these studies were not a designed comparison, some general observations between the results can be made.

Overall, pond-reared fish reached a larger size, 408 and 405 g for control and treatment fish, respectively, while final weights for tank-reared fish were 282 and 234 g for control and treatment fish, respectively. Feed efficiency was lower in pond reared fish, 71.9 (FCR= 1.4) and 78.0 % (FCR= 1.3) for control and treatment fish, than in tank-reared fish 87.4 (FCR= 1.1) and 85.6 % (FCR= 1.2) for control and treatment fish although treatment fish in the pond study increased their FE by 8.5 % over control fish. Differences between the tank and pond studies were also observed during each feeding cycle.

During the first feed deprivation cycle, tank-reared fish lost 7.7 % body weight, while those in the pond gained 11 % of their body weight (Figure 24). Natural prey sources in the pond study likely contributed to the weight gain, mediating the overall weight loss of fish during the feed deprivation periods. Despite the differences in changes in body weight, the SGR of fish in both studies increased by 99 % (tank) and 91 % (pond) over control fish during the first refeeding period. The increased SGR in both studies subsided to control levels after three weeks.

In contrast to the first feeding cycle, the CG response during the second feeding cycle was similar between the tank and pond studies. Treatment fish in both studies lost weight, 10.2 % and 12.2 % body weight for the tank and pond fish, respectively. During the first three weeks of refeeding, SGR of fish in the tanks and ponds increased 89.7 % and 39.4 % above control levels, however, SGR of fish in the pond study was not significantly different from control fish. The increases in SGR remained elevated in the tank reared fish (60.0 %)

and pond-reared fish (108.6 %), however, due to the variation in pond reared fish, this increase was not statistically significant. Of interest is the duration of elevated SGR during the second feeding cycle, lasting six weeks in the tank study, compared to only three weeks during the first feeding cycle, despite a similar feed deprivation period. The increase in SGR in pond-reared fish during this time period was also elevated, however not significantly. The increased loss of weight in both tank and pond-reared fish during the second feeding cycle could have triggered the longer CG response. However, the net effect of the weight loss combined with temporary increases in SGR was partial CG and lower final weight of cyclic fed fish.

Taken together, these results suggest that future research on cyclic feeding should concentrate on the use of a single feeding cycle, conducted in the early part of the production season. A single feeding cycle minimizes weight loss, and may lead to a more complete CG response. Further, since the effects of the feeding cycles on water quality seemed more beneficial during the first two months of production, cessation of feeding during this period may assist in water quality management for the entire season.

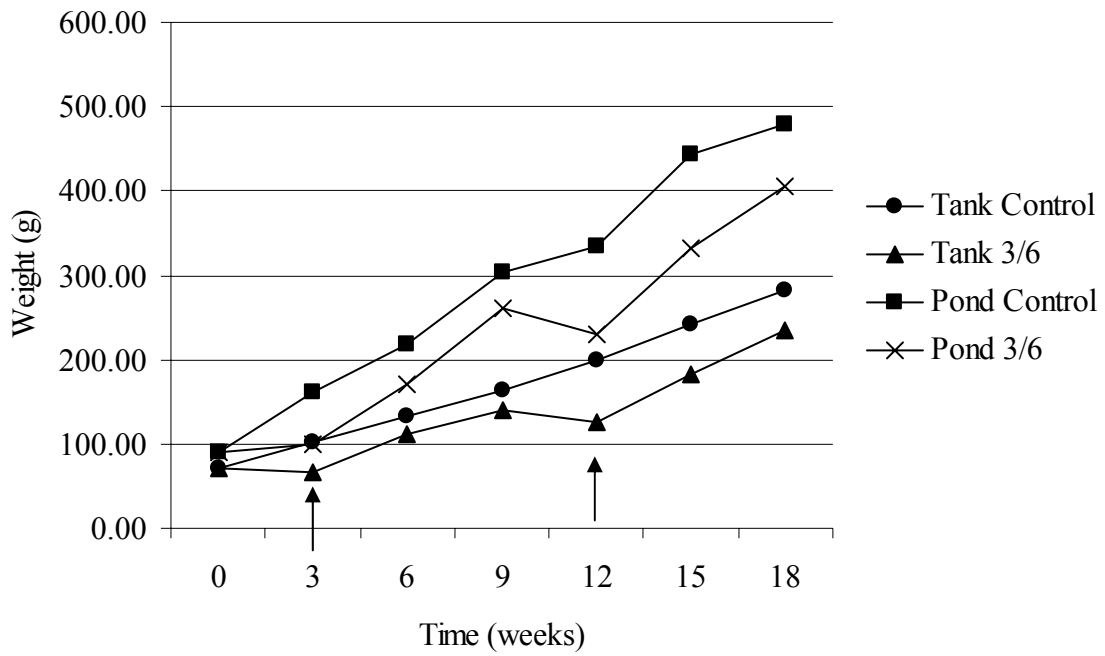


Figure 24. Growth (weight) of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) fed twice daily to satiation (control) or two cycles of three weeks feed deprivation followed by six weeks of refeeding (3/6) in a tank and pond study. Arrows indicate the start of refeeding.