

# The effect of temperature, stress, and cortisol on plasma IGF-I and IGFbps in sunshine bass

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## Abstract

The mechanisms through which stress and cortisol regulate insulin like growth factor-I (IGF-I) and insulin like growth factor binding proteins (IGFBPs) were studied in sunshine bass, by measuring plasma IGF-I and IGFBPs in fish maintained at 5, 10, 15, 20, 25, or 30 °C, fish subjected to an acute 15 min confinement stress at 25 and 30 °C, and fish fed 100 mg cortisol/kg feed. Plasma IGF-I concentrations were higher at 25 and 30 °C than at 20 °C and below. A 15 min confinement stress resulted in a decrease in IGF-I 2 h post-confinement. Plasma concentrations of IGFBP with molecular weights of 24, 28, and 33 kDa were similar for fish acclimated to different temperatures, except for 5 °C where a 33-kDa IGFBP was significantly reduced. After a 15 min low-water stress at 25 °C, a 33-kDa IGFBP was reduced and IGFBPs with molecular weights of 24 and 28 kDa were increased at 2 and 6 h, respectively. A 15 min low-water stress at 30 °C, resulted in no change in levels of a 33-kDa IGFBP over the 6-h recovery period. However, levels of a 24- and 28-kDa IGFBP were significantly increased at 2 and 6 h, respectively. A single feeding with 100 mg cortisol/kg feed increased plasma cortisol but did affect plasma concentrations of IGF-I or any of the three IGFBPs. Acute stress appears to result in a decrease in IGF-I, but the mechanism of the decrease does not appear to be caused by cortisol released during the stress.

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## 1. Introduction

Sunshine bass, a hybrid produced by crossing a female white bass with a male striped bass (Harrell and Webster, 1997) has become the preferred hybrid for use in aquaculture and it has become important to identify optimal conditions for growth. The primary environmental determinates of fish growth are nutrition and temperature. Other secondary influences include photoperiod and stress the fish are exposed to during aquaculture operations. Growth of fish is regulated by components of the pituitary/liver/somatic tissue axis. Experiments with hypophysectomy, growth hormone (GH) replacement therapy and GH transgenics have demonstrated the importance of GH in stimulating fish

growth (Donaldson, 1981; McLean and Donaldson, 1993). GH from the pituitary stimulates the liver to synthesize and release insulin-like growth factor-I (IGF-I). Evidence in fish also suggests that IGF-II may also be regulated by GH (Shablott et al., 1995; Peterson et al., 2005). IGF-I is now thought to have direct effects on somatic tissues that result in growth. Experiments in knock-out mice estimate that IGF-I alone accounts for 35% of growth (Lupu et al., 2001). Growth of fish is also thought to be regulated by IGF-I. Plasma IGF-I concentrations decrease in unfed fish while GH does not change (Uchida et al., 2003), or even increases (Small et al., 2002). Plasma IGF-I concentrations and growth rate in a number of fish have been shown to be correlated and has been suggested as a tool for assessing the potential of new diets (Dyer et al., 2004). Plasma IGFs are bound in the plasma to IGF binding proteins (IGFBPs) and the role of the IGFBPs as possible growth regulators is now being studied (Kelley et al., 2001). IGFBPs are thought to

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increase the half-life of IGFs and may transport them to target tissues. There are multiple forms of IGFs and some are upregulated in anabolic states (Kelley et al., 1992; Kelley et al., 2002; Siharath et al., 1995a; Shimizu et al., 1999) while some are upregulated during catabolic states (Shimizu et al., 1999; Siharath et al., 1996; Duan et al., 1999; Perez et al., 2000; Peterson and Small, 2004). For example, The 35- to 50-kDa fish IGFs are upregulated by GH and are correlated with somatic growth in the striped bass (Siharath et al., 1995b), coho salmon (*Oncorhynchus kisutch*; Kelley et al., 1992; Shimizu et al., 1999), and tilapia (*Oreochromis mossambicus*; Park et al., 2000). The  $\leq 31$ -kDa fish IGFs are upregulated in catabolic states and inversely correlated with somatic growth (Siharath et al., 1996; Duan et al., 1999; Perez et al., 2000; Kelley et al., 2001; Peterson and Small, 2004).

Glucocorticoids are known to decrease growth in teleosts (Mommensen et al., 1999). The mechanisms through which glucocorticoids inhibit growth may involve the GH/IGF-I/IGFBP network. In a recent study with tilapia, *Oreochromis mossambicus*, cortisol injection increased IGFs of four different sizes (24, 28, 30, and 32 kDa) in the plasma by 2 h without affecting plasma IGF-I levels (Kajimura et al., 2003). A reduction of plasma IGF-I and liver IGF-I mRNA was observed 24 and 48 h after cortisol administration (Kajimura et al., 2003). In addition, no change was observed in plasma or pituitary GH at any time point examined (Kajimura et al., 2003). In a study with channel catfish, an increase in a  $\sim 20$ -kDa IGF was observed in plasma of catfish fed cortisol (Peterson and Small, 2005). A high dose cortisol treatment (400 mg/kg diet) increased pituitary GH mRNA expression approximately 10-fold while liver IGF-I mRNA expression was not different between cortisol treated fish and controls (Peterson and Small, 2005). Cortisol treatment also decreased plasma levels of IGF-I (Peterson and Small, 2005). The results of the tilapia and catfish studies suggest a mechanism whereby cortisol reduces IGF-I sensitivity to GH and increases lower molecular weight IGFs. As in mammals, this may be one mechanism through which cortisol inhibits growth.

Exposure to a variety of stressful conditions often results in decreased growth of fish. Such conditions result in an increase of plasma cortisol, which is suspected to have a role in growth reduction. Administration of cortisol in the diet of channel catfish resulted in a dose dependent reduction in growth, which was shown to be related to increased gluconeogenesis (Davis et al., 1985). Cortisol may also have additional metabolic effects on growth by affecting components of the somatotrophic axis.

The mechanisms controlling these physiological changes in IGF-I and IGFs appear to be conserved across species, but there is no information on anabolic and catabolic roles of IGF-I or IGFs as they relate to stress in sunshine bass. The present study was done to examine the effects of acute stress and dietary cortisol on plasma levels of IGF-I and IGFs in sunshine bass, which represent an important aquaculture species in the United States.

## 2. Materials and methods

Sunshine bass were produced at Keo Fish Farm (Keo, AR, USA) and were trained to pelleted feed in ponds for about 2 months. The fish were then transferred to 23 °C well water and raised until they were about 18 months old. A random sample of the fish weighed  $191.7 \pm 57.8$  g (mean  $\pm$  SD,  $n = 24$ ) at the beginning of the experiment and was in the range of fish referred to as phase II fingerlings (Hodson and Hayes, 1989). All studies were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee at the Harry K. Dupree Stuttgart National Aquaculture Research Unit, USDA/ARS. For each temperature tested, six fish were stocked into each of seven, and 60-l aquaria provided with aeration and flowing well water at 23 °C, the same temperature they had been raised for over eight months. All tanks were held at the same temperature and each experimental temperature was achieved at a rate of change of less than 3 °C per day by passing the water through a gas heater or a cooling coil. When the desired temperature was reached (5, 10, 15, 20, 25, or 30 °C) fish were held for 5 days before an experiment was conducted. Food was offered at up to 2% of the body weight per day, although fish held at the lower temperatures ate very little. This feeding rate is considered to be a maintenance ration. After the completion of each experiment, the water temperature was adjusted to the next experimental temperature.

Fish acclimated to 25 and 30 °C were subjected to a low-water stress. These temperatures were used because they bracket the optimal temperature range for growth of this fish (Woiwode and Adelman, 1984, 1991). At the beginning of each experiment, a group of six fish from a single tank was sampled and the remaining tanks were exposed to a low-water stress by turning off the water and replacing the standpipe with a shorter one, thereby lowering the water level from 60 l to 5 l within 5 min. This water level was selected so that the fish were submerged but were unable to maintain their posture in the tank. The second group of six fish in a tank was sampled 15 min after removal of the tall standpipe. Water flow was restored, the tall standpipe replaced, and the third sample taken 1 h after replacing the standpipe. Complete restoration of the 60-l volume required 45 min. Subsequent samples of six fish each were taken at 2, 6, 24, and 48 h after replacing the standpipe. No fish survived after 6 h in fish stressed at 30 °C. Plasma cortisol was measured in these fish that were subjected to a low-water stress and published previously (Davis, 2004).

The effect of plasma cortisol on IGF-I in the absence of confinement stress was done by feeding fish one time with cortisol mixed in the feed at 100 mg/kg feed. The cortisol feed was prepared by dissolving the appropriate amount of cortisol in ethyl alcohol and spraying the feed with an atomizer. The feed was then mixed and spread out to dry before use. Six fish were stocked into each of seven, 60-l aquaria with flowing 23 °C well water. Prior to feeding cortisol fish were fed control feed at about 2% of the body weight per day for 7 days. The day before the experiment began, the fish were not fed to insure a robust feeding episode. At the beginning of the experiment one group was sampled as before and the remaining fish fed 2% of the body weight per day of control or cortisol containing feed, and groups of six fish from each feeding group were sampled at 0.25, 1, 2, 6, 24, and 48 h after feeding.

Sampling for all experiments was done rapidly, without anesthesia, utilizing three individuals to minimize the effect of sampling on hormonal changes. Sampling was usually completed in less than 3 min. Blood was collected from the caudal vessels in the hemal arch with heparinized syringes, centrifuged and the plasma frozen for later analysis.

Plasma IGF-I and cortisol concentrations were measured by radioimmunoassay with commercial kits from GroPep Ltd., Adelaide, S.A., Australia, and Diagnostics Corporation, Los Angeles, CA, respectively, and both have been previously validated for sunshine bass (Davis and Peterson, in press). Plasma IGFs were quantified using Western ligand blotting (Peterson et al., 2004). Quantity One software (Bio-Rad, Hercules) was used to calculate peak intensities of IGFs. Peak intensity values are reported as arbitrary densitometric units (ADU) and their means. Plasma glucose was determined by the glucose oxidase procedure (Sigma Diagnostics, No. 510A, St. Louis, MO, USA).

For the temperature experiments IGF-I concentrations were compared by analysis of variance followed by Tukey's multiple range test when significance was indicated. For the fish stressed at 25 and 30 °C, values were compared with the initial concentrations and for the cortisol feeding experiments the values for the experimental fish were compared with their respective control by Student's *t* test. All fish in a tank were sampled at the same time. Removing one fish from a tank would stress the remaining fish and change the density of fish in the tank. Individual fish served as the experimental unit for each variable measured. In all cases a probability level of  $p < 0.05$  was considered significant.

**3. Results**

Fish acclimated to 25 and 30 °C had similar and statistically higher plasma IGF-I concentrations than fish acclimated to 5, 10, 15, or 20 °C (Fig. 1). Three IGFBPs of 24, 28, and 33 kDa were detected in the fish plasma (Fig. 8). Fish acclimated to 5, 10, 15, 20, 25 or 30 °C had similar 28- and 24-kDa plasma IGFBP concentrations. Fish acclimated to 5 °C had a slightly but significantly reduced 33-kDa IGFBP compared to the other temperatures ( $p < 0.05$ ) (Fig. 2).

IGF-I concentrations were significantly lower for fish exposed to a 15 min low-water stress compared to the unstressed initial group after 2 h at 25 °C (Fig. 3A), and for 2 and 6 h in fish tested at 30 °C (Fig. 4A). IGF-I concentrations were higher at the end of the 15 min stress exposure in fish acclimated to 30 °C than in the initial group. In the same experimental fish, there were alterations in plasma levels of IGFBPs. In the 25 °C-held fish, the 15-min low-water confinement resulted in slightly reduced levels of the 33-kDa IGFBP and increased levels of the 24- and 28-kDa IGFBPs by 2 and 6 h after treatment, while all proteins returned to normal by 24 h posttreatment (Fig. 3B). In the 30 °C-held fish, the 24- and 28-kDa IGFBPs showed a more dramatic 3-fold increase at 2 and 6 h posttreatment but the fish did not survive to the 24 h or later time points (Fig. 4B). Levels of a 33-kDa IGFBP were similar over the 6-h recovery period (Fig. 4B).

Plasma cortisol changes due to the 15 min confinement stress were published separately and were found to induce a typical stress response characterized by an increase in cortisol and glucose which was temperature dependent (Davis, 2004). Plasma cortisol reached about 200 ng/ml

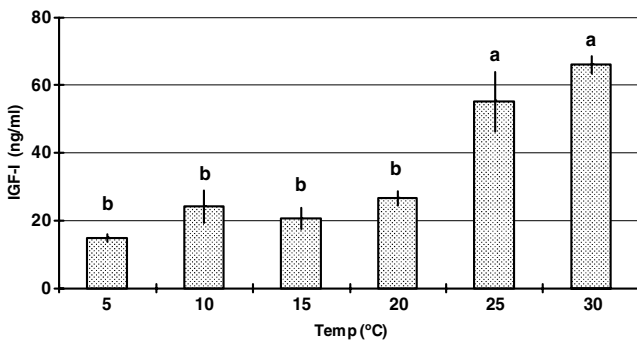


Fig. 1. Plasma IGF-I concentrations in sunshine bass acclimated to different temperatures for 5 days. Values represent the mean of 6 fish ± SE. Similar letters above the means represent statistically similar subsets by Tukey's multiple range test at  $p < 0.05$ .

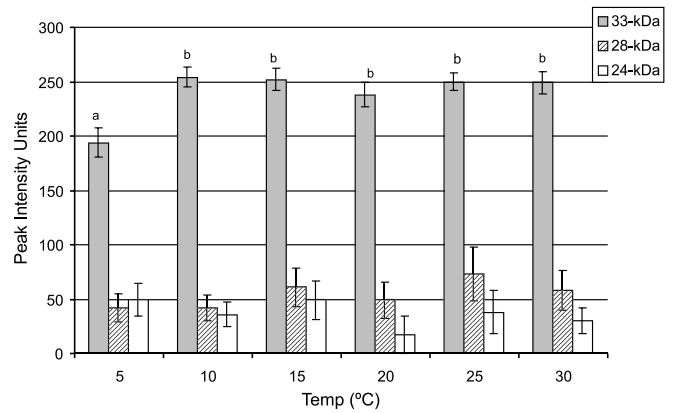


Fig. 2. Plasma IGFBP concentrations in sunshine bass acclimated to different temperatures for 5 days. Values represent the mean of 6 fish ± SE. Similar letters above the means represent statistically similar subsets by Tukey's multiple range test at  $p < 0.05$ .

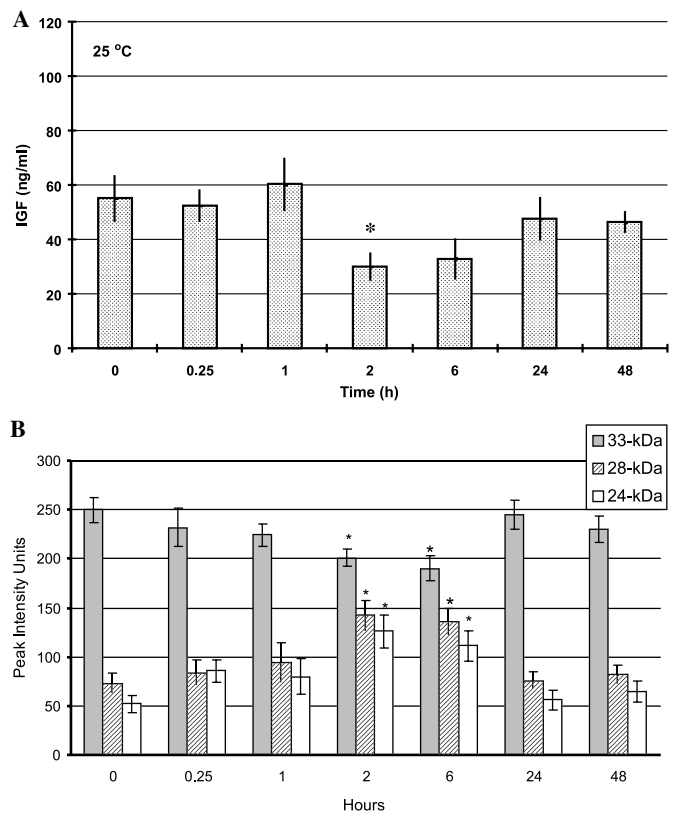


Fig. 3. (A) Plasma IGF-I concentrations in sunshine bass exposed to a 15 min (0.25 h) low-water confinement stress at 25 °C and recovery after 1, 2, 6, 24, and 48 h. (B) Plasma IGFBP concentrations in sunshine bass exposed to a 15 min (0.25 h) low-water confinement stress at 25 °C and recovery after 1, 2, 6, 24, and 48 h. Values represent the mean of 6 fish ± SE. \*values significantly different from the initial values by Student's *t* test at the  $p < 0.05$ .

and glucose concentrations were about 100 mg/dl after 15 min in fish stressed at temperatures of 20 °C and were higher than at the lower temperatures. Plasma cortisol recovered to pre-stressed concentrations by 2 h after removal of the stress, however, glucose concentrations recovered more slowly.

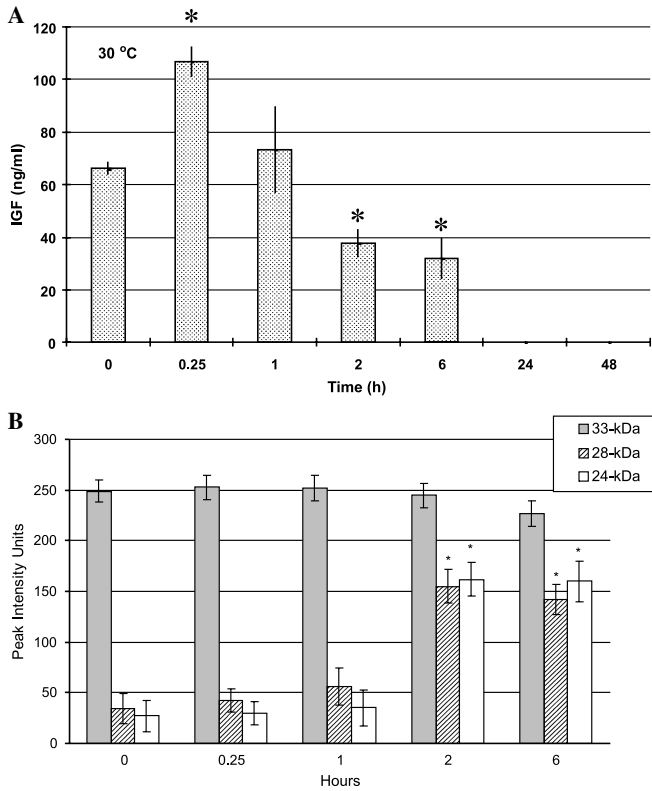


Fig. 4. (A) Plasma IGF-I concentrations in sunshine bass exposed to a 15 min (0.25) low-water confinement stress at 30°C and recovery after 1, 2, and 6 h. No fish survived after 6 h. (B) Plasma IGF-BP concentrations in sunshine bass exposed to a 15 min (0.25 h) low-water confinement stress at 30°C and recovery after 1, 2, and 6 h. Values represent the mean of 6 fish + SE. \* values significantly different from the initial values by Student's *t* test at the *p* < 0.05.

When fish were fed 100 mg cortisol/kg feed, plasma cortisol was significantly higher than controls after 15 min and reached a peak 2 h after feeding and remained significantly higher than controls for 48 h after feeding (Fig. 5). Plasma glucose was significantly higher from cortisol fed fish than

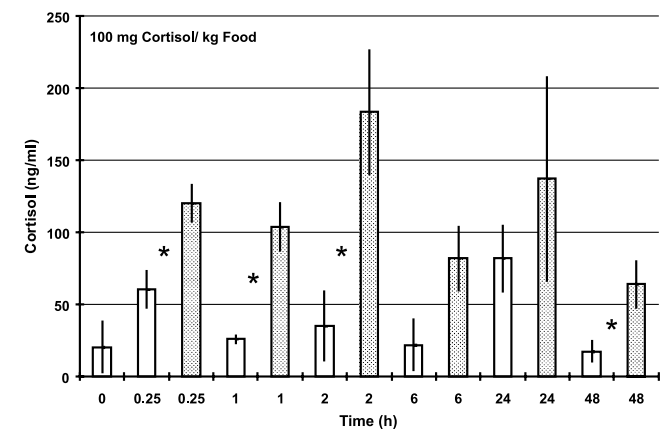


Fig. 5. Plasma cortisol concentrations in sunshine bass fed a control diet (open bars) or 100 mg/kg cortisol (dark bars) mixed in the feed at 0 time. Blood samples were taken after 0.25, 1, 2, 6, 24, and 48 h and fish were held at 23°C. Values are the mean of 6 fish + SE. \* statistical difference between controls and cortisol fed fish.

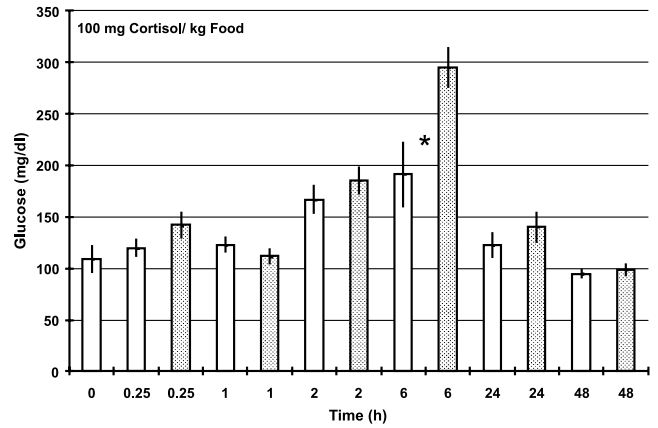


Fig. 6. Plasma glucose concentrations in sunshine bass treated as in Fig. 5.

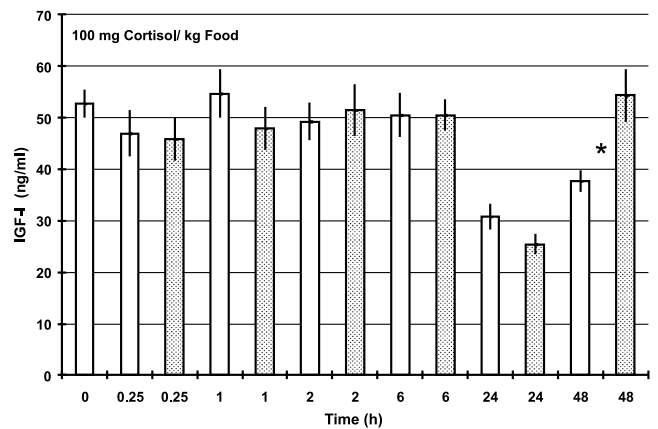


Fig. 7. Plasma IGF-I concentrations in sunshine bass treated as in Fig. 5.

for controls 6 h after feeding (Fig. 6). Plasma IGF-I concentrations were significantly different from controls only 48 h after feeding. IGF-I levels were significantly lower 24 h after feeding in both the cortisol and control fed fish, which were similar to each other (Fig. 7). Dietary cortisol had no effect on plasma concentrations of IGF-BPs (data not shown).

**4. Discussion**

Juvenile hybrid bass demonstrate maximum growth at 27–30°C (Kellogg and Gift, 1983; Woiwode and Adelman, 1991), although some of these studies were done with palmetto bass, a hybrid produced by a cross of female striped bass and a male white bass. Data from the present study suggest that plasma IGF-I concentrations are also highest over the temperatures at which growth is optimal and that sunshine bass likely have a similar optimal growth temperature as the palmetto bass. Studies that directly compare the growth of the two hybrids have found similar growth between the hybrids (Harrell, 1997). Chinook salmon separated by size and grown at low and higher temperatures had little effect of IGF-I due to size; however, fish raised in warm water had higher growth and plasma IGF-I concentrations (Beckman et al., 1998), suggesting that

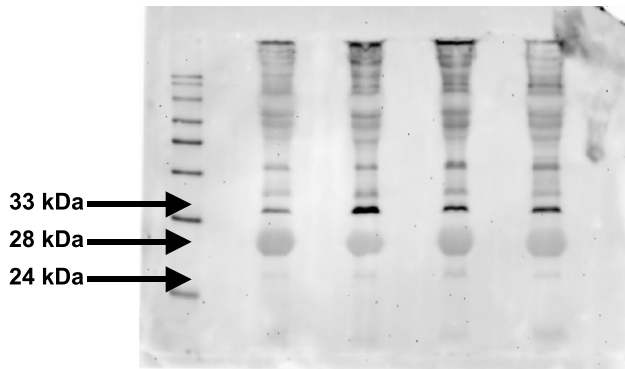


Fig. 8. Representative Western ligand blot. Samples were taken from four sunshine bass exposed to a 15 min (0.25 h) low-water confinement stress at 30 °C after 1 h. Plasma IGFbps with molecular weights of 24, 28, and 33 kDa are shown.

environmental factors such as photoperiod or temperature may modulate growth and development and thus levels of IGF-I. Plasma IGF-I levels of coho salmon showed a decline with temperature decrease (Larsen et al., 2001). Strain and temperature effects on growth and feeding consumption in channel catfish were also associated with higher IGF-I concentrations in fish injected with recombinant bovine growth hormone (Silverstein et al., 2000).

Plasma cortisol changes due to the 15 min confinement stress were published separately and were found to induce a typical stress response characterized by an increase in cortisol and glucose which was temperature dependent (Davis, 2004). Plasma cortisol reached about 200 ng/ml and glucose concentrations were about 100 mg/dl after 15 min in fish stressed at temperatures of 20 °C and were higher than at the lower temperatures. Plasma cortisol recovered to prestressed concentrations by 2 h after removal of the stress, however, glucose concentrations recovered more slowly. Although the mechanism of action is not clear, a decrease of IGF-I concentration occurred 2 h after the confinement stress in fish tested at 25 and 30 °C. Recovery to pre-stress conditions had begun 6 h after the confinement in fish tested at 25 °C but remained low in fish tested at 30 °C. The increase in IGF-I after 15 min of confinement in fish tested at 30 °C may have been due to a massive release due to the physical activity observed during confinement. Cortisol mixed in the diet has also been shown to decrease the growth rate of channel catfish (Davis et al., 1985; Peterson and Small, 2005).

Fish plasma typically reveals three IGFBP bands at 20–25, 28–30, and 40–50 kDa (Shimizu et al., 2005). Similarly, we detected IGFbps at 24, 28, and 33 kDa in sunshine bass. These IGFbps are similar in molecular weight to IGFbps reported in striped bass (Siharath et al., 1995a,b, 1996; Fukazawa et al., 1995). However, in fish fed cortisol, only the 33-kDa IGFBP was consistently detected. This is in agreement with our previous work with sunshine and palmetto bass in which we could only consistently detect a 33-kDa IGFBP (Davis and Peterson, 2005). It is not clear why we were unable to detect the three IGFbps in all

samples. However, in the samples that we did detect the two lower molecular weight IGFbps, levels were similar between cortisol and control fed fish. The 33-kDa IGFBP was detected in all of the sampled fish.

When fish were acclimated to 5, 10, 15, or 20 °C, plasma IGFBP concentrations were similar except at the 5 °C temperature. At 5 °C, a 33-kDa IGFBP was reduced compared to the other temperatures. This is the first report of the effect of temperature on levels of IGFbps in any species. It is not clear why the reduction in concentrations of a 33-kDa IGFBP was only observed at 5 °C when the optimum temperature range for maximum growth is at 27–30 °C (Kellogg and Gift, 1983; Woiwode and Adelman, 1991). It has been suggested that the 33-kDa IGFBP is associated with growth and may be similar to the mammalian IGFBP-3 (Siharath et al., 1995a).

When fish were exposed to a 15 min low-water stress at 25 °C, a 33-kDa IGFBP was reduced at 2 and 6 h post-stress. In addition, two IGFbps with molecular weights of 24 and 28 kDa were increased at 2 and 6 h post-stress at 25 °C. When fish were exposed to a 15 min low-water stress at 30 °C, levels of a 33-kDa IGFBP were similar over the 6-h recovery period. However, levels of a 24 and 28 kDa were increased at 2 and 6 h, respectively.

Withholding food can affect IGF-I, binding protein and cortisol concentrations, however, these changes usually do not occur until a week or more of fasting (unpublished data). Since fish held at the lower temperatures used here do not feed well, any effect of the low temperatures and the lack of feeding cannot be determined.

Plasma cortisol concentration 2 h after feeding the cortisol diet was about 175 ng/ml and similar to concentrations induced by a 15 min confinement exposure, about 200 ng/ml (Davis, 2004). Highest cortisol concentrations due to confinement are reached immediately after a 15 min confinement and have returned to initial levels after 2 h at 23 °C. The increase in cortisol in the control fish at 15 min and 24 h must have been due to activity around the tank when bleeding the other groups. Glucose increases in both control and cortisol fed groups after feeding was likely caused by absorption of the meal and was augmented by the action of cortisol 6 h after the meal. A similar temporal pattern of cortisol and glucose change was observed in fish receiving 100 or 200 mg (data not shown for 200 mg) cortisol/kg feed. Fish fed the lower amount more closely quantitatively reflected the amount of endogenous cortisol release induced by a 15 min confinement stressor (Davis, 2004) although the temporal component for feeding cortisol was slower. However, in spite of the very large increase in plasma cortisol induced by feeding cortisol there was no decrease in plasma IGF-I in contrast to results reported for tilapia (Kajimura et al., 2003). They found a decrease in IGF-I, and IGF-I mRNA 24–48 h after injection of cortisol. Judging from their previous work from which the doses were selected (Eckert et al., 2001), the amount of cortisol injected likely resulted in pharmacological concentrations of cortisol and therefore may not represent an acute physiological

response. A role for increased glucose in the stress induced decrease of IGF-I cannot be ruled out.

Neither a 24- nor 28-kDa IGFBP were upregulated due to feeding cortisol. This is in contrast to what has been observed in other fish studies. Kelley et al. (2001) compared three catabolic states in fish: fasting, untreated insulin-dependent diabetes mellitus, and stress. Under all conditions, cortisol concentrations were increased and an increase in one or both low molecular weight IGFbps (24 and 30kDa) (depending on species) was observed. In a study with tilapia, *O. mossambicus*, Kajimura et al. (2003) demonstrated a relationship between cortisol and four low molecular weight IGFbps (24, 28, 30, and 32kDa). Similarly, a ~20-kDa IGFBP was observed in the plasma of catfish fed dietary cortisol (Peterson and Small, 2005). In both the tilapia and catfish studies, cortisol treatment also decreased plasma levels of IGF-I. The results of these studies suggest a mechanism whereby cortisol reduces IGF-I sensitivity to GH and increases lower molecular weight IGFbps. As in mammals, this may be one mechanism through which cortisol inhibits growth. What role (if any) cortisol has on regulating IGF-I and IGFbps in sunshine bass is unclear.

Our data suggest plasma IGF-I concentrations are highest at temperatures that support growth and that an acute confinement results in a decrease of IGF-I. IGF-I levels recover fairly rapidly at 25 °C but are more persistent in fish confined at 30 °C. The secretion of cortisol or glucose did not cause the confinement induced decrease of IGF-I at 2 h. The decrease may have been caused by a general activation of the sympathetic nervous system that also occurs during acute stress.

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