

**Lack of growth enhancement by exogenous growth hormone treatment in yellow perch
(*Perca flavescens*) in four separate experiments**

**Sissel Jentoft¹, Nicole Topp¹, Matthew Seeliger¹, Jeffrey A. Malison¹, Terence P. Barry¹,
James A. Held¹, Steven Roberts² and Frederick Goetz³**

¹Aquaculture Program, University of Wisconsin-Madison, Department of Animal Sciences,
1675 Observatory Dr., Madison, WI 53706, USA

²Marine Resources Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA
02543, USA

³Great Lakes WATER Institute, University of Wisconsin-Milwaukee, 600E. Greenfield Ave.,
Milwaukee, WI 53204, USA

Correspondence to

Sissel Jentoft

Agricultural University of Norway

Department of Mathematical Sciences and Technology

P.O. Box 5003

N-1432 Ås

Norway

Fax: +47 64 94 88 10

Tel: +47 64 94 86 98

e-mail:sissel.jentoft@imt.nlh.no

Abstract

The effect of exogenous growth hormone (GH) treatment on the growth of juvenile yellow perch (*Perca flavescens*) was investigated in four experiments. In the first two experiments juvenile yellow perch were reared at either 13°C or 21°C, and injected weekly with bovine GH (bGH) at 0.1, 1.0 or 10.0 µg/g body weight for 84 days. No significant growth enhancement in GH-treated fish was measured in fish in either of the experiments. In the third experiment, juvenile yellow perch were treated with estradiol-17β (E₂, 15 µg/g of diet), bGH (1.0 µg/g body weight) injected weekly, or both hormones for 70 days at 21°C. E₂ alone stimulated growth, but no further growth stimulation occurred in the E₂ + bGH treated fish. In addition, no growth enhancement was found in fish treated with bGH alone. We measured no difference in serum insulin-like growth factor-I (IGF-I) levels between the treatment groups at 12 and 24 h after the final injection of GH, however, a drop in IGF-I levels after 24 h was revealed. In a fourth study, the effect of recombinant yellow perch GH (rypGH, 0.2 or 1.0 µg/g body weight) injected weekly was evaluated in yellow perch juveniles. The fish were reared for 42 days at 18°C. Neither dose of growth hormone improved growth compared to control-injected and non-injected fish. Taken together, the lack of effect of mammalian GH or rypGH in our experiments suggest (1) low-affinity between these hormones and the GH receptor in yellow perch, (2) that the endogenous GH levels were already at biologically maximal levels, or (3) that other endocrine factors are needed in order for GH to promote yellow perch growth. The reduction in IGF-I levels 24 h after handling suggests a negative effect of handling stress on the GH-IGF-I axis in yellow perch.

Key words: Yellow perch, GH, growth, IGF-I, E₂

1. Introduction

The yellow perch (*Perca flavescens*) is a popular food fish in the mid-western United States, and an important aquaculture industry has emerged to supply the high market demand for this species. A major constraint to the expansion of this industry, however, is the slow growth rate of yellow perch (Malison et al., 2003). To date, research directed at improving the growth of cultured yellow perch has focused on producing all-female stocks, as female yellow perch grow significantly faster than males (Schott et al., 1978; Malison et al., 1988). Sex steroids regulate this sexually dimorphic growth pattern. Estradiol-17 β (E₂) and androgens have positive and negative effects on growth, respectively (Malison et al., 1985; Malison et al., 1987; Malison et al., 1988). E₂ appears to promote growth in yellow perch by stimulating appetite (Malison et al., 1988), and enhancing the production of growth hormone (GH) (Roberts et al., 2004). Roberts et al. (2004) recently cloned and sequenced the yellow perch GH gene, and showed that the pituitary GH content increases markedly in the early spring, coincident with a dramatic increase in the growth rate of the fish. Other than these data, very little is known about the role of GH in mediating growth in yellow perch.

Growth in fish is primarily regulated by the GH-insulin-like growth factor-I (IGF-I) axis (Björnsson, 1997; Duan, 1998; Moriyama et al., 2000). Hypothalamic hormones control the pituitary production of GH (Donaldson et al., 1979; Harvey, 1993; Peter and Marchant, 1995), which, in turn, stimulates hepatic production of IGF-I, the principal mediator of somatic growth (Duan, 1998; Moriyama et al., 2000). The GH-IGF-I axis is well conserved in vertebrate evolution, and the structure and the biological potency of piscine IGF-I are similar to their mammalian homologs (Duan, 1998). The exogenous administration of various mammalian and piscine GHs, both native and recombinant, have been shown repeatedly to increase the growth rates of teleosts (see reviews Donaldson et al., 1979; McLean and

Donaldson, 1993). To better understand the function of the GH-IGF-I axis in yellow perch, the effects of exogenous bovine GH (bGH) and recombinant yellow perch GH (rypGH) treatment on growth and serum levels of IGF-I were investigated in juvenile yellow perch.

2. Materials and methods

2.1 Experiments 1 and 2

Juvenile yellow perch obtained from Willow Creek Aquaculture, LLC, Berlin, WI, USA were acclimated to either 13°C (Experiment 1) or 21°C (Experiment 2) at the University of Wisconsin-Madison, Madison, WI, USA, over a 4-week period. For each temperature, 64 fish were randomly distributed into eight 110-L flow-through tanks. The eight fish in each tank were individually fin clipped. At the start of experiments 1 and 2, the mean fish weights were $11.4 \pm 0.5\text{g}$ and $13.8 \pm 0.4\text{g}$, respectively. The fish were divided into four treatments groups; two tanks per treatment, injected weekly with either purified bGH (National Hormone and Peptide Program, USA) at 0.1, 1.0, or 10.0 $\mu\text{g/g}$ body weight or saline water as control, and raised for 84 days. The bGH was dissolved in 0.85% NaCl (pH = 9.5), and the injection volume was 100 $\mu\text{l}/10\text{ g}$ body weight for all treatment groups. The bGH shared 35% sequence identity to the native yellow perch GH (Roberts et al., 2004). The light intensity was the same in all treatment groups and the photoperiod was constant L:D 16:8. The fish were hand-fed twice a day approximately 4% of body weight per day (Silver Cup trout food, Murray, Utah). Body weight and length of individual fish were recorded every third week. In experiment 2 the sex of each fish was determined at the end of the study.

2.2 Experiment 3

Age 1+ yellow perch (Willow Creek Aquaculture, LLC, Berlin, WI, USA) were acclimated to 21°C at the University of Wisconsin-Madison, Madison, WI, USA for 4 weeks. The fish ($n =$

124) were individually PIT-tagged, and randomly distributed into twelve 110-L flow through tanks at 10 or 11 fish in each tank. In addition, 5 or 6 untagged fish were distributed to achieve a rearing density of 16 fish per tank. At the start of the experiment the average fish weight was 33.9 ± 0.7 g. There were four treatment groups replicated three times: injected weekly with saline, E_2 (15 μ g/g of diet) in combination with weekly injections of saline, purified bGH (National Hormone and Peptide Program, USA) (1.0 μ g/g body weight) injected weekly, or both hormones. The experiment was conducted for 70 days. E_2 was administered via the diet according to the method of Malison et al. (1985). The bGH was dissolved in 0.85% NaCl (pH = 9.5), and the injection volume was 100 μ l/30 g body weight for all treatment groups. The light intensity was the same in all treatment groups and the photoperiod was constant L:D 16:8. The fish were hand-fed twice a day approximately 3% of body weight per day (Silver Cup trout food, Murray, Utah). Body weight and length of the tagged fish were recorded every second week. The sex of each fish was determined at the end of the study.

After the final weight and length measurements, an additional injection of bGH (1.0 μ g/g body weight) was administered to the bGH treated fish, and blood samples were collected from all treatment groups 12 (n = 6) and 24 (n = 3) h post-injection. The blood samples were allowed to clot at 4°C overnight, centrifuged at $3,000 \times g$ for 20 min, and the serum samples were collected and stored at -40°C until assayed for IGF-I. The levels of IGF-I were measured in triplicate by using a commercial fish IGF-I radioimmunoassay (RIA) kit according to the manufacturer's instructions (GroPep, Australia). The RIA kit uses recombinant tuna IGF-I as radiolabeled tracer (125 I) and standard. The assay kit was validated for measuring yellow perch IGF-I by verifying that serial dilutions of yellow perch serum inhibited the binding of

radiolabeled IGF-I in parallel with the IGF-I standards. Serum samples were extracted with acid ethanol to remove IGF-I binding proteins.

2.3 Experiment 4

Age 1+ yellow perch (n = 96) hatched and reared at the University of Wisconsin-Madison, Lake Mills, WI, USA, were randomly distributed into twelve 110-L flow-through tanks for a five-week acclimatization period. The eight fish in each tank were individually fin clipped. The fish (initial mean weight = 11.4 ± 0.5 g) were reared for 42 days at 18°C. There were four treatment groups replicated three times: rypGH injected weekly at 0.2 µg/g body weight, rypGH injected weekly at 1.0 µg/g body weight, injected control or un-injected control. The rypGH was dissolved in D1-Dithiothreitol (DTT); which was also used as the control injection. The injection volume was 100 µl/10 g body weight. The rypGH shared 100% sequence identity to the native yellow perch GH (Roberts et al., 2004). The light intensity was the same in all treatment groups and the photoperiod was constant L:D 16:8. The fish were hand-fed twice a day approximately 4% of body weight per day (Silver Cup trout food, Murray, Utah). Body weight and length of individual fish were recorded every third week. The sex of each fish was determined at the end of the study.

2.4 Statistics

Weight and length gain were analyzed by a one-way (treatment, Experiment 1) or two-way (treatment and sex, Experiments 2, 3 and 4) ANOVA. Differences in growth rates between the treatment groups were further examined by LSD (least significant difference). Serum IGF-I levels were analyzed by a two-way ANOVA (treatment and sex), and IGF-I levels were correlated (linear regression) with weight gain. The level of significance used for all tests was $P < 0.05$.

3. Results

3.1 Experiment 1

No differences in weight or length gain were found between the control fish and the bGH-treated fish (Fig. 1a and b), regardless of treatment dose.

3.2 Experiment 2

No differences in weight or length gain were found between any of the GH treatment groups (Fig. 2a and b). For all treatments females grew faster than males, and there was no treatment \times sex interaction (data not shown).

3.3 Experiment 3

E₂ had a positive effect on growth in both females and males, while no effect of bGH was found (Fig. 3a and b). Fish treated with a combination of E₂ and bGH showed no further growth enhancement compared to the fish treated with E₂ alone (Fig. 3a and b). In all treatments females grew faster than males (data not shown), and no treatment \times sex interaction was found. There was no effect of hormone treatment on serum IGF-I levels (Table 1) at either 12 or 24 h post-injection, although IGF-I levels were lower at 24 h post-injection than at 12 h post-injection (Table 1). Furthermore, there were no differences in IGF-I levels between females and males in any of the treatment groups. No correlation between IGF-I levels and weight gain was measured (data not shown).

3.4 Experiment 4

Yellow perch treated with rypGH at 1.0 μ g/g body weight gained less weight and length than fish in the other treatment groups, while no growth differences were found between un-

injected control fish, injected control fish and fish treated with rypGH at 0.1 $\mu\text{g/g}$ body weight (Fig. 4a and b). There was no main effect of sex, nor was there a treatment \times sex interaction.

4. Discussion

The failure of bGH or homologous rypGH treatment to promote growth in yellow perch is in contrast to other fish species in which exogenous GH treatment markedly stimulates growth under both optimal (Weatherley and Gill, 1987; Skyrud et al., 1989; Silverstein et al., 2000; Hunt et al., 2000) and sub-optimal (Adelman, 1977; Kelly et al., 1996; Leedom et al., 2002) rearing conditions. Further, the GH treatments induced no increase in IGF-I levels in our studies, as has been shown in several other fish species (Cao et al., 1989; Funkenstein et al., 1989; Moriyama, 1995; Moriyama et al., 1995; Shamblott et al., 1995). A positive correlation between IGF-I levels and growth has also been demonstrated in teleosts (Duan et al., 1995; Silverstein et al., 2000; Pierce et al., 2001; Kajimura et al., 2001; Uchida et al., 2003). However, the lack of correlation between IGF-I levels and weight gain found in yellow perch has also been demonstrated in the closely related species gilthead seabream (*Sparus aurata*) (Metón et al., 2000).

Because of the wide range of doses and conditions tested, we do not believe that the failure of GH to promote growth in our experiments was due to methodological problems, such as improper dose or rearing conditions. Rather, one explanation for our results is that neither bGH nor rypGH bind and/or activate the GH receptor in yellow perch. In tilapia (*Oreochromis mossambicus*) recombinant bGH showed low-binding affinity to the GH receptor relatively to the native tilapia GH (Ng et al., 1992; Leedom et al., 2002). With regard to our results with rypGH, we suggest that the protein may have been improperly folded to interact with the GH receptor. The poor growth achieved when treated with high dosages of

rypGH could indicate that the protein was blocking the GH receptors, and competing with native yellow perch GH.

A second explanation of our results is that the 16:8 L:D photoperiod used in all four of our experiments may have promoted maximal endogenous GH synthesis and release in yellow perch, resulting in insensitivity to additional exogenous GH treatment. Extrinsic factors such as photoperiod and temperature were shown by Roberts et al. (2004) to influence the GH content in the pituitary of yellow perch, reaching a peak level in May. In Eurasian perch (*Perca fluviatilis*) the GH pituitary content was also shown to reach maximum levels in May/June at the time of longest photoperiod (Swift and Pickford, 1965). Additionally, long photoperiod (18:6 or 24:0 L:D) has been documented to strongly enhance the growth performance in juvenile Eurasian perch (Jourdan et al., 2000).

A third possible explanation for our results is that other endocrine factors are needed in order for GH to promote yellow perch growth. Because of its growth-promoting ability in yellow perch, we postulated that exogenous E₂ might work in concert with GH to stimulate growth. We found, however, that bGH induced no further growth enhancement in E₂-treated yellow perch. Additionally, the serum IGF-I levels were no higher in fast growing E₂-treated fish than in bGH treated fish or control fish, nor in fast growing females than in males. This finding supports the hypothesis that the growth-promoting effects of E₂ in yellow perch are probably not mediated via IGF-I. Roberts et al. (2004), however, reported that E₂ treatment increased pituitary levels of GH. In other fish species sex steroids have been documented to mediate the GH-IGF-I axis (Holloway and Leatherland, 1997a; Holloway and Leatherland, 1997b; Riley et al., 2002), and estrogens are documented to affect the levels of IGF binding

proteins (IGFBPs) (Fukazawa et al., 1995). Thus, the positive effect of estrogens on growth in yellow perch could be mediated by the GH-IGF-I axis through actions of IGFBPs.

A fourth reason for the lack of an effect of GH on growth in our studies could be related to negative effect of handling stress. In Experiment 4, we found no negative effects of injections per se on growth. All potential negative effects of handling cannot be ruled out, however, since all groups of fish including the un-injected control fish were handled once a week at the time of the injection. Yellow perch are known to have a powerful cortisol response to handling stress (Head and Malison, 2000), and cortisol has been documented to reduce the levels of IGF-I in tilapia (Kajimura et al., 2003). Serum IGF-I levels decrease one to two days after handling in several fish species, including Atlantic salmon (*Salmo salar*), silver perch (*Bidyanus bidyanus*), and black bream (*Acanthopagrus butcheri*) (Dyer et al., 2004). Our findings that IGF-I levels declined from 12 to 24 h after the final injection suggests that IGF-I levels may be impacted by handling stress in yellow perch.

Acknowledgements

This work was supported by the University of Wisconsin-Madison College of Agricultural and Life Sciences and School of Natural Resources; the Wisconsin Department of Natural Resources; the University of Wisconsin Sea Grant College Program, National Oceanic and Atmospheric Administration, US Department of Commerce; the State of Wisconsin (Federal Grant NA46RG0481, Project No. R/AQ-38); and the USDA NOAA Project R/A-05-99, grant #NA86RG0048 to FG and SR. This study was also funded by the Norwegian Research Council (NFR). We thank Roar Elvegård for good assistance at the fish lab and Jennifer Bunner for helping with the IGF-I measurements. We also acknowledge NIDDK's National Hormone & Peptide Program and Dr. A.F. Parlow for the shipment of purified bovine GH.

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Figure legends

Fig. 1. Mean (a) weights (g) and (b) lengths (mm) of yellow perch reared at 13°C and injected weekly with bGH (Experiment 1). Data represent $\bar{u} \pm \text{SEM}$, $n = 2$. There were no differences between treatments.

Fig. 2. Mean (a) weights (g) and (b) lengths (mm) of yellow perch reared at 21°C and injected weekly with bGH (Experiment 2). Data represent $\bar{u} \pm \text{SEM}$, $n = 2$. There were no differences between treatments.

Fig. 3. Mean (a) weights (g) and (b) lengths (mm) of yellow perch treated with E_2 at 15 $\mu\text{g/g}$ diet and/or injected weekly with bGH at 1.0 $\mu\text{g/g}$ body weight (Experiment 3). Data represent $\bar{u} \pm \text{SEM}$, $n = 3$. Fish treated with E_2 grew faster than the other groups, and there were no other differences between treatments.

Fig. 4. Mean (a) weights (g) and (b) lengths (mm) of yellow perch injected weekly with rypGH (Experiment 4). Data represent $\bar{u} \pm \text{SEM}$, $n = 3$. Fish treated with rypGH at 1.0 $\mu\text{g/g}$ body weight grew slower in weight and length than the other groups.

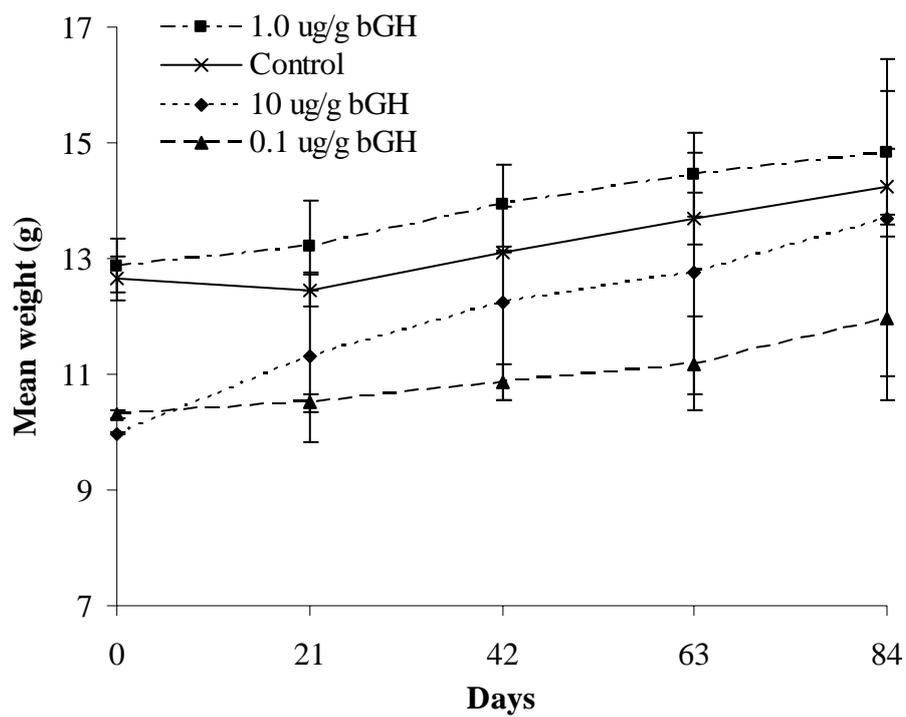


Fig 1a

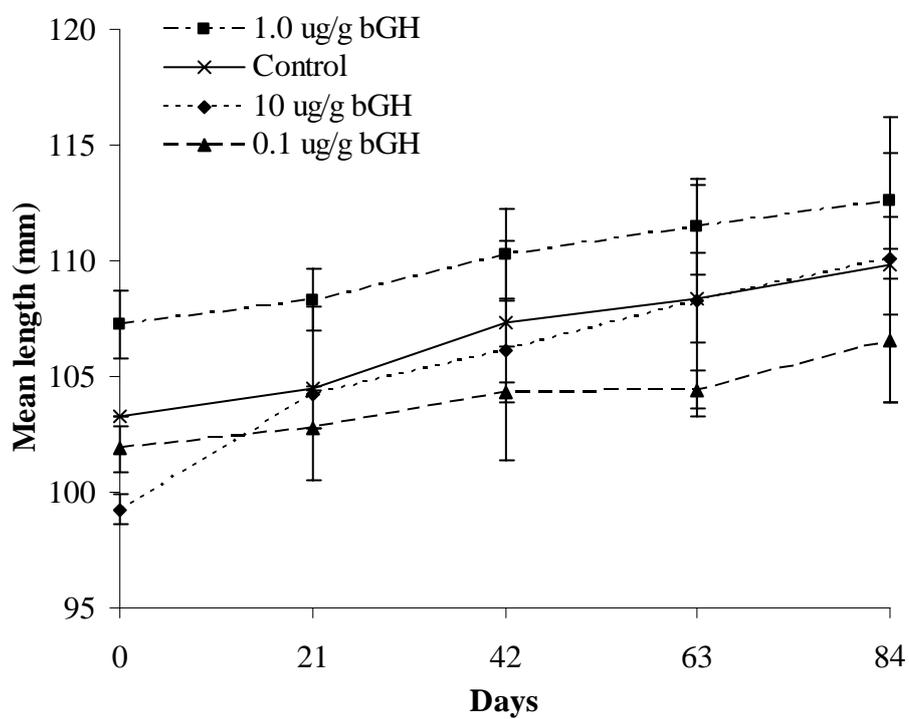


Fig 1b

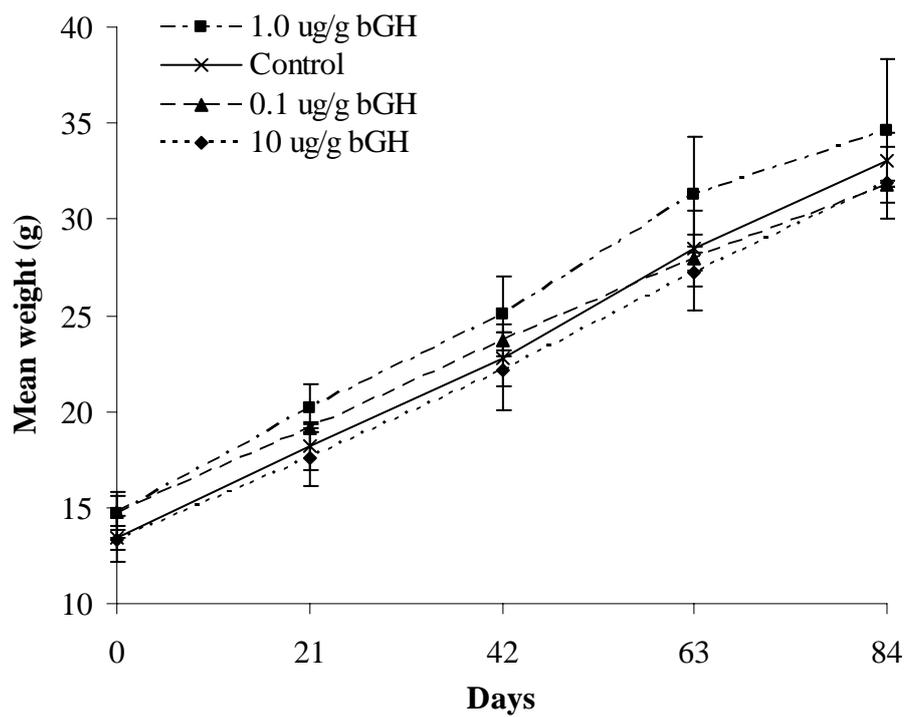


Fig 2a

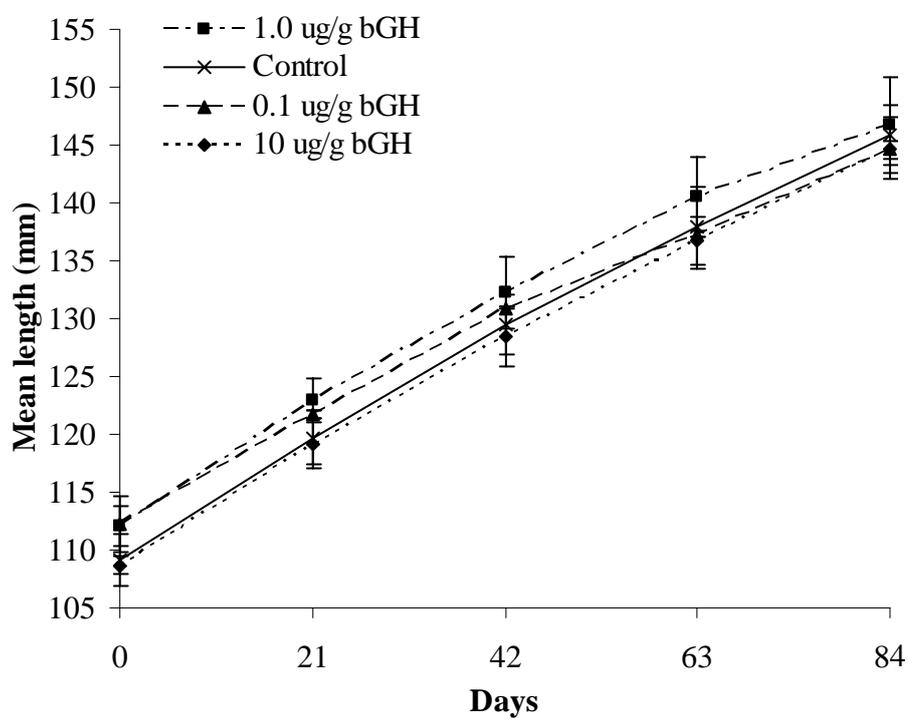


Fig. 2b

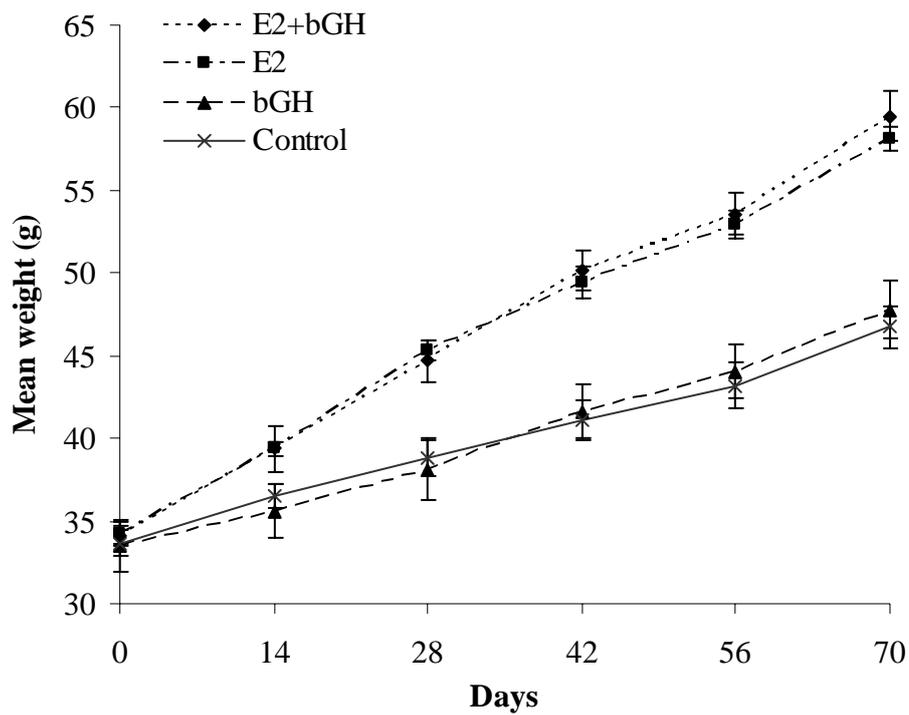


Fig. 3a

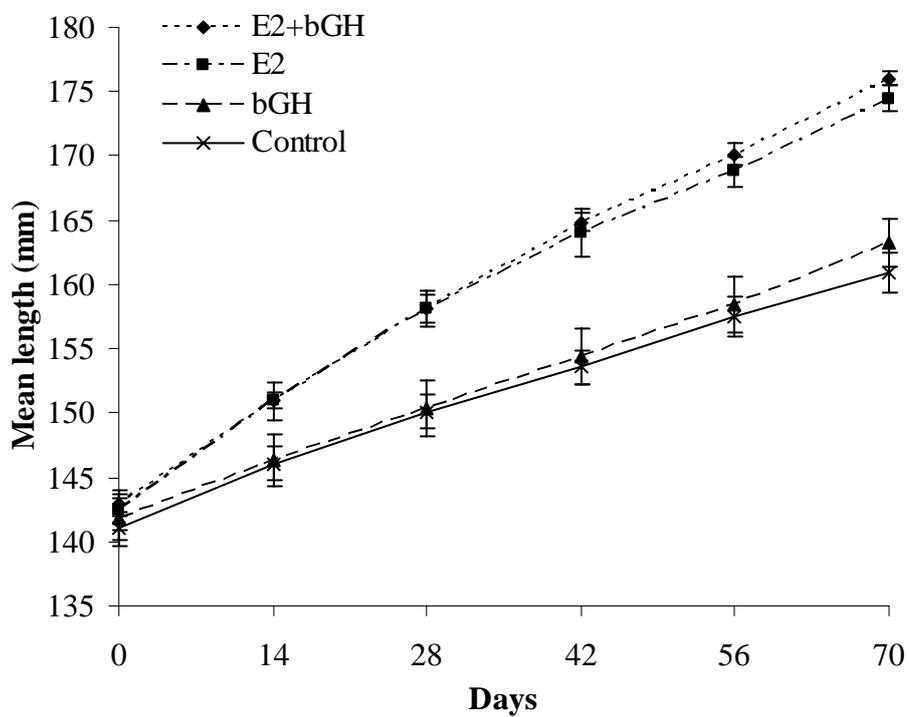


Fig. 3b

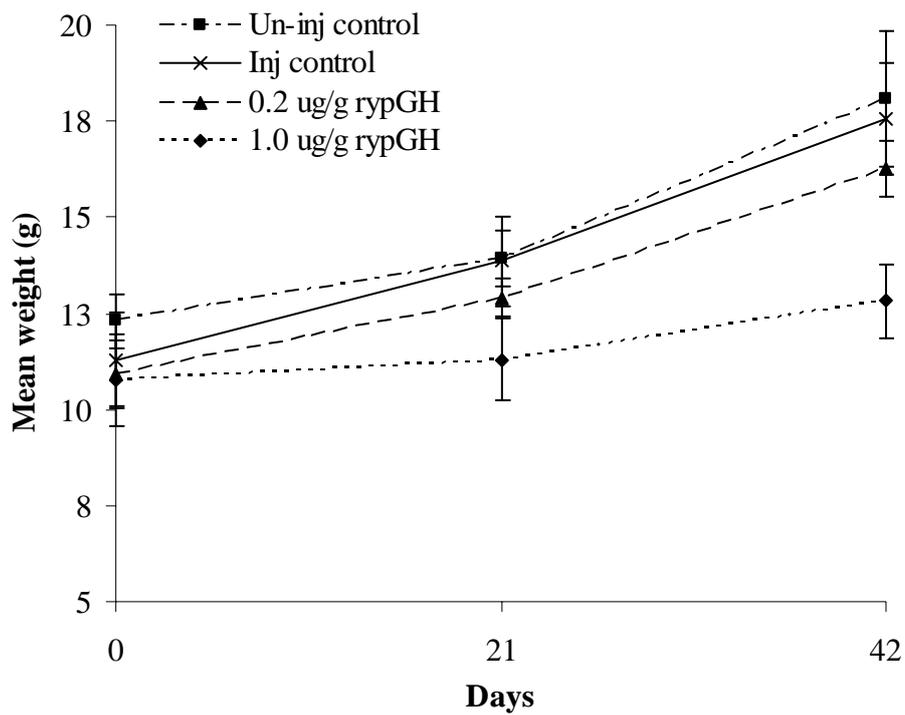


Fig. 4a

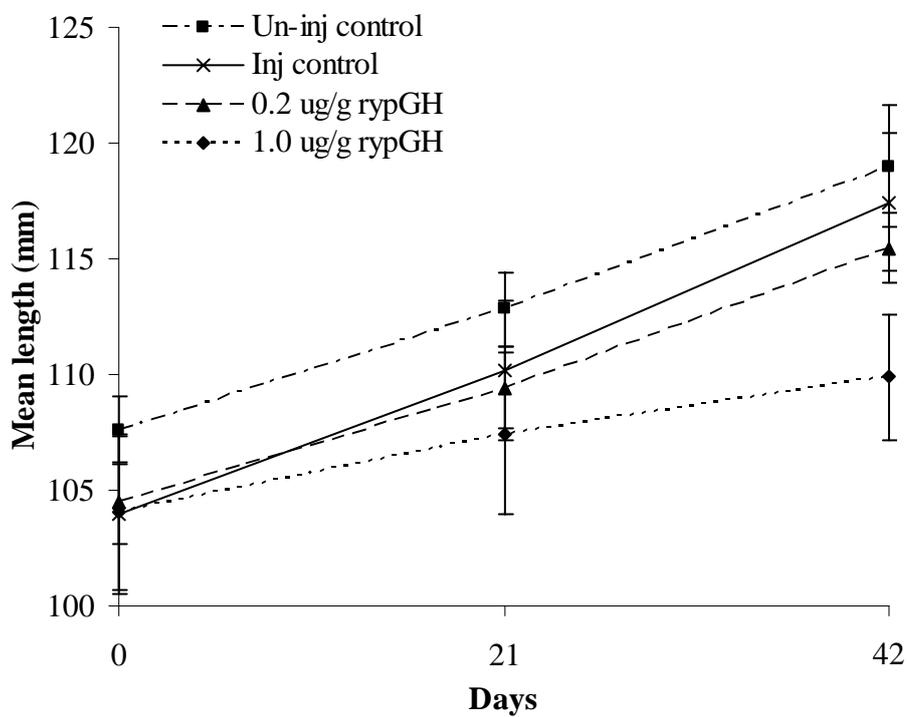


Fig. 4b

Treatment	IGF-I (ng/ml) 12 h post-inj.	IGF-I (ng/ml) 24 h post-inj.
Control Male	4.78 ± 0.46	1.13 ± 0.12
Control Female	4.55 ± 0.38	1.50 ± 0.31
E ₂ Male	5.47 ± 0.98	1.67 ± 0.37
E ₂ Female	6.18 ± 1.76	1.87 ± 0.32
bGH Male	4.23 ± 0.55	2.10 ± 0.50
bGH Female	5.30 ± 1.19	1.75 ± 0.15
E ₂ + bGH Male	4.10 ± 0.49	1.90 ± 0.64
E ₂ + bGH Female	6.30 ± 1.31	1.17 ± 0.09

Table 1: IGF-I serum levels (ng/ml) 12 and 24 h post bGH injection in yellow perch males and females. Data represent $\bar{x} \pm \text{SEM}$, 12 h post injection $n = 6$, and 24 h post injection $n = 3$.

There were no differences between treatments.