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INSULIN-LIKE GROWTH FACTOR-I AS A GROWTH INDICATOR IN TILAPIA

*Eleventh Work Plan, Fish Nutrition and Feed Technology Research 4 (11FNFR4)
Final Report*

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ABSTRACT

A set of studies was carried out on the expression of the IGF-I gene in juvenile tilapia, using fish approximately of the initial size that is stocked for growout in commercial aquaculture in the Philippines (~1–1.5 g). IGF-I is a mitogenic polypeptide that is an important regulator of growth in fish. The potential of IGF-I mRNA abundance as an instantaneous growth indicator in juvenile Nile tilapia, *Oreochromis niloticus*, was evaluated. Hepatic IGF-I cDNA was isolated and cloned and partially cloned. The partial sequence having 539 bp was found to code for the signal peptide (44 amino acids), mature protein (68 aa) and a portion of the E domain (19 aa). The deduced 68 aa sequence for mature IGF-I showed 84–90% and 77–79% sequence identity with fish and mammalian counterparts, respectively, confirming the highly conserved sequence homology among species. The B and A domains were even more highly conserved with respect to the deduced amino acid sequence (90–96%). Based on the mature IGF-I peptide, a sensitive TaqMan real time qRT-PCR assay for *O. niloticus* was developed for measures of hepatic IGF-I mRNA levels. Hepatic IGF-I mRNA levels were found to be significantly correlated with growth rate of individual juvenile fish reared under different feeding regimes and temperature conditions. Higher feed consumption and water temperature produced faster growing fish and increased hepatic IGF-I mRNA expression. These findings suggest that hepatic IGF-I plays a key role in controlling growth in *O. niloticus* and indicates IGF-I mRNA measures could prove useful to assess current growth rate in this species.

INTRODUCTION

Increased and efficient fish production demands fast growing strains, efficient feeds and feeding protocols, and optimization of the culture environment and other parameters known to impact the growth and health of fish. Evaluation of the effect of a particular parameter on growth usually requires numerous and costly production trials. Aside from the considerable expense involved, research progress is also limited by the length of time required to see gross changes in body weight or length for a specific growth trial (for example, see Brown et al., 2000). Normally this involves controlled laboratory or research scale tests followed by large-scale farm trials; both approaches require proper controls and replication for statistical validity. To save time and money, there is a need, therefore, for the development of a means for rapid and direct assessment of growth of fish over short

periods of exposure to the parameter being tested.

Somatic growth in fish, as in other vertebrates, is regulated by a variety of growth factors/hormones acting through endocrine, paracrine and/or autocrine modes. A central step in this hormonal network is the growth hormone (GH) – insulin-like growth factor (IGF) axis (Duan, 1997, 1998). The IGF system is composed of ligands (IGF-I and IGF-II), receptors, and IGF binding proteins (IGFBPs). Of the components of the IGF system, IGF-I is the most promising candidate as an instantaneous growth indicator in fish. IGF-I is a naturally produced molecule of approximately 70 amino acid residues with structural homology to proinsulin and is an important regulator of growth and differentiation (Meton et al., 2000; Degger et al., 2000). The IGF-I prepropeptide has S-, B-, C-, A-, D-, and E-domains. The S- and E-domains are removed by proteolytic processing of the propeptide to yield

mature IGF-I, which is released into the serum (Chen et al., 2000). Pituitary GH secreted into the blood stream stimulates the production of the mitogenic peptide IGF-I in the liver (Duan, 1998; Kajimura et al., 2001; Pierce et al., 2004). IGF-I is also produced in several tissues and acts locally in a paracrine and/or autocrine manner (McRory and Shewood, 1994; Duan, 1997; Plisetskaya, 1998). IGF-I mediates the growth promoting actions of GH as well as regulates excessively high production of GH through a negative feedback mechanism (Rotwein et al., 1994; Duan, 1997; Fruchtmann et al., 2000). Most of the hepatic-derived circulating IGF-I is bound to a number of IGF binding proteins (IGFBPs) which regulate their availability to target tissues, prolong their half-lives and prevent their insulin-like activity (Duan, 1997; Hwa et al., 1999; Degger et al., 2000; Kelly et al., 2001). Upon release from these IGFBPs, the majority of IGF-I actions are mediated through the type I IGF receptor (IGF-IR) (LeRoith et al., 1995).

Circulating IGF-I is predominantly derived from the liver (Schwander et al., 1983; Duan et al., 1994; Reinecke et al., 1997; Duan, 1998; Pierce et al., 2004). Both homologous and heterologous IGF-I's have been shown to promote growth in teleosts (McCormick et al., 1992; Negatu and Meier, 1995; Chen et al., 2000; Degger et al., 2000). Several studies indicated a significant and positive correlation between circulating IGF-I and growth rates (Jones and Clemmons, 1995; Beckman et al., 1998; Shimizu et al., 2000; Larsen et al., 2001; Kajimura et al., 2001). The relationship of IGF levels and growth rate is more consistent than that of GH with growth rate. Growth hormone levels can become dissociated with growth rate under some conditions (e.g. malnutrition; prolonged starvation), under which the correlation of IGF-I with growth persists (Duan and Plisetskaya, 1993; Duan, 1997, 1998). For these reasons, the detection of IGF-I is gaining more appeal as a possible indicator of growth rate in fishes (Shimizu et al., 2000; Larsen et al., 2001). In the present study, we cloned a portion of *O. niloticus* IGF-I cDNA and developed a sensitive TaqMan real time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assay for measures of IGF-I mRNA abundance. We report the use of hepatic IGF-I mRNA as an instantaneous growth indicator in Nile tilapia, with respect to investigations on growth control by temperature and feeding regimen.

METHODS AND MATERIALS

Fish

Juvenile Nile tilapia (~1-1.5g) obtained from Aquasafra, Inc., Bredenton, Florida were maintained in circular (1.7 m³, 1.7 m diameter) tanks at the Marine Biology Laboratory, Florida International University. This size is comparable to the optimal stocking size for juvenile tilapia to be used for growout (see Final technical report, Investi-

gation 11FNFR2). Fish were reared in fresh water under natural photoperiod and fed a commercial (AquaMax) pelleted diet. Studies were conducted under the authority of Florida International University's Animal Care and Use Committee (FIU IACUC protocol number 02-018)

Cloning of IGF-I cDNA

RNA Isolation

Liver was collected from juvenile Nile tilapia, immediately frozen on dry ice, and stored at -80°C. Total RNA was isolated according to Chomczynski and Sacchi (1987). Excess glycogen was removed during RNA extraction via lithium chloride (Puissant and Houdebine, 1990).

Oligonucleotide Design

Primers for the polymerase chain reaction (PCR) (InvitrogenTM, Carlsbad, California, USA) were designed based on the previously cloned sequence for IGF-I in *O. mossambicus* (NCBI Genbank accession no: AF033796). The forward primer for standard PCR was 5'-TTCTC-CAAACGAGCCTGCG-3' (5'-promoter region) and the reverse primer was 5'-TCTGCTACTAACCTTGGGTGC-3' (E domain).

Reverse-Transcription and Cloning

First-strand cDNA was synthesized from 1 µg of total RNA using the gene-specific reverse primer (1 µM) described above, 1X buffer RT, 0.5 mM of each dNTP, 10-units RNase inhibitor, and 4-units reverse transcriptase in a total reaction volume of 20 µl (OmniscriptTM RT kit, Qiagen Inc., Valencia, California, USA). The reaction was incubated for 60 minutes at 37°C, inactivated by heating the reaction to 93°C for 5 minutes, and stored at -20°C.

The PCR was carried out in a Hybaid thermal cycler (Thermo Electron Corp., Waltham, Massachusetts, USA) using the HotStar TaqTM PCR Kit (Qiagen Inc.). For PCR reactions, 2 µl of the reverse-transcription reaction (~100 ng template DNA), 1X PCR buffer (1.5 mM MgCl₂), 200 µM of each dNTP, 0.2 µM gene-specific forward and reverse primers, and 2.5 units of HotStarTaq DNA polymerase were mixed for a total reaction volume of 50 µl. Thermal cycling conditions were as follows: initial activation at 95°C for 15 minutes, 30-PCR cycles at 94°C for 1 minute, 62.5°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The PCR above was repeated to generate a greater amount of the amplicon using the product above as the template. PCR products were electrophoresed on a 1% agarose gel. A distinct band estimated at ~540 nucleotides was generated. This band was extracted from the agarose gel and purified using a QIAquick Gel Extraction Kit (Qiagen

Inc.). The product was ligated into pCR[®]II vector and resulting plasmids were transformed into INVaF⁺ One Shot[®] competent cells for 'blue/white screening' (TA Cloning[®] Kit, Invitrogen[™]). Six white colonies were selected for culture in Luria Bertani/ampicillin media and grown for ~18 hours in a shaking incubator at 37°C. Plasmid DNA was purified using the QIAprep[®] miniprep kit (Qiagen Inc.). An aliquot (5-8 µl) of each plasmid DNA sample was digested with EcoR I + Buffer H to verify insert size. A clone containing an insert of the predicted size was selected for sequencing at the University of Chicago, Cancer Research Center-DNA Sequencing Facility. The sequence was obtained using M13/pUC universal forward primers. Resulting sequences were compared to those for other species at the National Center for Biotechnology Information (NCBI) website using the BLAST search service. Sequence alignments and comparisons to other IGF-I sequences were performed using MacVector[™] 7.0 software (Genetics Computer Group, Oxford, Molecular, Madison, Wisconsin, USA).

Experiments

IGF-I and Growth Modulation Using Different Feeding Regimes

A feeding regime experiment was conducted to evaluate the relationship of IGF-I level to growth of the fish. Tilapia fingerlings from a holding tank were distributed randomly into 12, 64-liter aquaria at a density of 15 fish per aquarium. The aquaria were divided into three blocks and the following treatments were assigned randomly in each block: (i) daily feeding (DF, 6% of BW); (ii) alternate-day feeding (ADF, 6% of BW); (iii) daily feeding to satiation (SAT); and (iv) feeding at restriction level (RLF, 1% of BW). Fish in the first two treatments (DF and ADF) were supposed to be fed following a feeding guide based on their body weight but the amount of feed consumed by fish at satiation level was lower than amount based on the feeding guide. This resulted in the reduction of the amount of food equal to that given at satiation level. Thus the DF group was essentially fed to satiation daily just as the SAT group. Fish in the RLF group were fed at 16% of the satiation level. Fish were fed once daily except for ADF. Fish in each aquarium were bulk weighed at the start and end of the study. The experiment was terminated after four weeks and two fish per aquarium or group (3 replicate aquaria/group) were anesthetized in tricaine methanesulfonate (MS-222) and the liver was rapidly removed and snap frozen on liquid nitrogen prior to RNA extraction.

IGF-1 mRNA Levels and Growth Modulation Using Different Water Temperatures

The fish were fasted in an experimental tank for a period of one month (fed at 1% of BW, given thrice a week

for the first 15 days and food-deprived for the last 15 days) prior to the start of the experiment. Fingerlings were distributed randomly into three 64-liter aquaria at a density of 10 fish per aquarium. Aquaria were assigned randomly to the following treatments: (i) water temperature manipulated, higher or equal to 28°C, (ii) non-manipulated, natural water temperature and (iii) water temperature manipulated, lower or equal to 24°C. The water temperature fluctuations in the experimental groups are presented in Figure 1. Fish were fed once daily, to satiation. Fish in each temperature condition were bulk weighed at the start and end of the study. The experiment was terminated after three weeks and four fish per aquaria were anesthetized in MS-222, individually weighed, and the livers were rapidly dissected and snap frozen in liquid nitrogen to RNA extraction.

RNA Isolation and Processing

Liver tissues were collected from experimental fish at time zero and upon termination of the experiment. Total RNA was isolated following the extraction method in the manufacturer's protocol (Trizol[®], Invitrogen[™]). High salt precipitation solution was used for glycogen removal in the samples. RNA was treated with DNA-free[™] (Ambion[®], Austin, Texas, USA) in two separate reactions to remove any possible genomic DNA contamination. RNA was quantified and purity was assessed by spectrophotometry (NanoDrop[®] ND-1000 spectrophotometer). The A260/A280 values ranged from 1.7–2.0 with most samples having values from 1.9–2.0.

Reverse Transcription

First strand cDNA was synthesized in 20 µl RT reactions with 1 µg total RNA template, Omniscript[®] reverse transcriptase, 10x RT buffer, 5 µM dNTP, 10 µM oligo-dT primer (Promega[®], Madison, Wisconsin, USA), and RNase inhibitor (RNasin[®], Promega[®]). Samples were reverse transcribed by incubation at 37°C for 60 minutes.

Quantification of IGF-1

TaqMan[®] qRT-PCR was performed on GeneAmp[®] 5700 Sequence Detector (Applied Biosystems, Foster City, California, USA), using the standard cycling conditions recommended by the manufacturer (50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 s, 60°C for 1 minute). Gene specific primers and FRET probes (Biosource, Camarillo, California, USA) for qRT-PCR were designed with Primer Express[®] program (Applied Biosystems). The forward primer was 5'-GTCTGTGGAGAGCGAGGCTTT-3' (5'-preproIGF-I region) and the reverse primer was 5'-CACGTGACCGCCTTGCA-3' (E domain). The sequence of the probe was 5' TTTCAATAAACCAACAGGCTATGGCCCC 3'. The selected reporter and quencher dyes for the probe were FAM and TAMRA, respectively. Reactions for each

sample were done in triplicate with each well containing 25 μ l PCR mixture (10 ng cDNA template, 1x TaqMan[®] universal PCR master mix, 900 nM forwards and reverse primers and 250 nM probe). No template controls (NTC) were used to confirm that reagents were not contaminated with carryover PCR products. No amplification controls (NAC) were included in triplicate to test for any possible genomic DNA contamination in RNA preparations. Cycle threshold (CT) values corresponded to the number of cycles at which monitored fluorescence emission in qRT-PCR exceeded the threshold limit. A serial dilution of pooled sample cDNA was run to generate a standard curve of IGF-1 (0.01 -100 ng) and to assess PCR efficiency. The amount of IGF mRNA was determined by comparison with the generated standard curve and normalized to total RNA as suggested by Bustin (2000, 2002). Values of IGF-I mRNA were then expressed relative the lowest sample level measured within an experiment (assigned an arbitrary value of 1).

Statistical Analyses

Data were analyzed using one-way analysis of variance (ANOVA). The Least Significant Difference test was used to determine significant difference between means. Linear relationship between gain in weight and relative abundance of IGF-I mRNA was assessed using linear regression and Pearson correlation coefficient. All statistical analyses were done using version 11 SPSS software.

RESULTS

Sequence of IGF-I cDNA

The amplicon generated for *O. niloticus* using two primers localized in the 5' preproIGF-I and E domain was 539 nucleotides (Figure 2). The identified sequence contains the signal peptide, B, C, A, D domains and portion of the E domain. The sequence exhibits the six (2 in B domain and 4 in A domain) conserved cysteine residues. BLAST search results demonstrated that the amplicon showed high sequence homology to IGF-I in other fishes. Identities between *O. niloticus* IGF-I and the corresponding region in fish species including *O. mossambicus* ranged from 75-99%, whereas those between *O. niloticus* IGF-I and other vertebrates ranged from 70-80%. The mature IGF-I peptide (B-D domain) was 100% identical to that of *O. mossambicus*. Comparison of the deduced 68 amino acid sequence of IGF-I (B-D domain) shows 84 to 90% sequence identity with fish counterparts and 77 to 79% with the corresponding mammalian hormones (Figure 3). The expressed mature IGF-I sequence was found to be two amino acids shorter at its C domain than the comparable domain in mammalian and other fish species.

Effect of Feeding Regimes on Growth

The mean weight gains of the fish are presented in Figure 4. Feeding to satiation (SAT) led to the highest weight gain ($2.56g \pm 0.06$), which was not significantly different compared to the daily feeding (DF) group ($2.25g \pm 0.18$). Not unexpectedly, fish on the restricted diet (RLF) showed the lowest weight gain of $0.56g \pm 0.10$, which was significantly different ($P < 0.01$) compared to all treatments. ADF ($1.78g \pm 0.15$) was also significantly different from all treatments.

Increased Growth Related to Increased IGF-1 Mrna Levels in Liver

Mean IGF-I mRNA levels in DF, ADF, SAT and RLF fish were elevated 4.1-fold, 3.8-fold, 4.6-fold and 2.3-fold, respectively compared to that of time 0 fish. Statistical analysis, however, showed no significant difference among treatments, which may be attributed to the wide variation of the relative abundance of IGF-I mRNA within treatment. A very high positive correlation ($r^2 = 0.98$, $P < 0.01$) however was observed between mean weight gain and relative abundance of hepatic IGF-I mRNA.

Higher Temperature Dramatically Increased IGF-I mRNA

Mean (of 10 fish) weight gains of fish reared in low, non-manipulated, and high temperatures were 1.25g, 8.68g and 10.11g, respectively. Compared to fasted fish, mean IGF mRNA levels increased 13-fold, 60-fold and 269-fold in fish reared at low, non-manipulated and high temperatures, respectively. This indicates a dramatic elevation of IGF-I mRNA expression as growth rate increases with increased water temperature. Comparison of individual gain in weight to relative IGF-I mRNA abundance showed a significant and positive correlation ($r = 0.64$, $P < 0.05$; Figure 6).

DISCUSSION

Sequence of IGF-I cDNA

The partial *O. niloticus* IGF-I cDNA sequence of 539 nucleotides, as shown in Fig. 2, encodes for 132 amino acid (aa) residues. The identified sequence contains a signal peptide, beginning with the first ATG, (132 bp; 44 aa), B domain (87 bp; 29 aa), C domain (30 bp; 10 aa), A domain (63 bp; 21 aa), D domain (24 bp; 8 aa), and partial E domain (60 bp; 20 aa). Mature IGF-I (B-D domain) in turbot, *Psetta maxima*, (Duval et al., 2002), flounder, *Paralichthys olivaceus* (Tanaka et al., 1998), barramundi, *Lates calcarifer* (Stahlbom et al., 1999), rabbitfish, *Siganus guttatus* (Ayson et al., 2002), coho salmon, *Oncorhynchus kisutch* (Cao et al., 1989), common carp, *Cyprinus carpio* (Liang et al., 1996), Goldfish, *Carassius auratus* (Kermouni et al., 1998), chicken, *Gallus gallus* (Kajimoto and Rotwein, 1989), rat, *Rattus norvegicus* (Roberts et al., 1987) and human (Rinderknecht and Humbel, 1978; Jansen et al.,

1983) differ from tilapia IGF-I by 7, 7, 9, 9, 9, 10, 11, 15, 16, and 16 amino acids, respectively (77-90% homology; Figure 3). Comparison of the nucleotide sequences for fish and non-human mammals shows high evolutionary conservation. The homology between IGF-Is indicates that amino acid substitutions are relatively few, and the IGF-I mature peptide sequence is highly conserved during phylogeny. Greater conservation of the amino acid composition can be observed in the B and A domains, while considerably less conservation is found in C and D domains. It is in the B and A domains where the conserved cysteine residues are located which are responsible for maintenance of tertiary structure (Reinecke et al., 1997). The importance of this high sequence identity of B and A domains in different species can be ascribed to the functional roles of these regions in the binding of IGF-I with its receptor and IGFBP (Duval et al., 2002). This degree of structural conservation during vertebrate evolution reflects the importance of the biological actions of IGF-I (Upton et al., 1998).

Feeding Regimes, Growth, and IGF Levels

Growth of juvenile fish was significantly affected by the feeding regime as varied in our first experiment. Fish that consumed greater amount of food generally had faster growth rate. The absence of significant difference on weight gain between DF and SAT may be attributed to the reduction of the ration in DF, which was equal to that of SAT. It is interesting to note that feeding on alternate days resulted in weight gain only slightly reduced in comparison to feeding daily, or feeding daily to satiation (see Figure 4). The efficiency and hence the economics of heavy daily feeding have been called into question by recent studies that indicate that Nile tilapia thrive on diets reduced by half through alternate-day feeding (Brown et al., 2004; Bolivar et al., submitted).

The wide variation of the relative abundance of IGF-I mRNA within treatments observed in this study might be explained by the observed variation of sizes of fish within the same treatment during the end of the study. Probably because of the limited space available, there was an establishment of hierarchies in feeding among the fish. Dominant individuals within a population have consumed more food and have grown faster than submissive individuals. Dominant individuals were observed chasing the submissive individuals during feeding periods, which may have reduced the amount of food consumed by the latter. The individual weight of fish was, however, not recorded in this study.

Nutritional status affects production of hepatic and circulating IGF-I in several species of fish (Duan and Plisetskaya, 1993; Perez-Sanchez et al., 1995; Larsen et al., 2001). In the present study, manipulation of growth rates by using different feeding regimes (varying amount of ration and interval of feeding) resulted in a good correla-

tion between hepatic IGF-I mRNA levels and growth. Perez-Sanchez et al. (1995) reported positive correlation between circulating IGF-I level and growth rates in gilthead sea bream by regulating nutrition using different feeding and dietary protein levels. In *O. mossambicus*, hepatic IGF-I mRNA levels were significantly increased after rbGH and Posilac® injections (Kajimura et al., 2001). In the same study, significant correlation was observed between circulating IGF-I levels, body length and mass. This similarity in the changes of mRNA levels in both the plasma and the liver of *O. mossambicus*, infer that a similar phenomenon might occur in *O. niloticus*. Significant correlation between circulating IGF-I level and growth rate was also observed by Beckman et al. (1998) in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in spring. Faster growing channel catfish (*Ictalurus punctatus*) had higher circulating IGF-I levels than slower growing groups (Silverstein et al., 2000).

Exogenous treatment with IGF-I can also stimulate growth and protein synthesis (Negatu and Meier, 1995). McCormick et al. (1992) found that injection of rbIGF-I stimulated growth in coho salmon (*O. kisutch*); similarly, Chen et al. (2000) observed enhanced growth of juvenile tilapia (*O. mossambicus*) following injection of recombinant tilapia IGF-I. The relationship of IGF-I levels and growth rate is more consistent than that of GH and growth rate. Under some conditions, GH levels can become dissociated with growth rate, under which the correlation of IGF-I with growth still persists (Duan, 1997, 1998). In coho salmon, prolonged starvation resulted in cessation of growth but significantly increased level of circulating GH (Duan and Plisetskaya, 1993). This starvation-induced elevation of circulating GH level is associated with significant decrease in the hepatic binding sites for GH (Gray et al., 1992), suggesting GH resistance at tissue level. The subsequent low levels of circulating IGF-I resulted in an alleviation of the negative feedback on GH secretion. In contrast to IGF-I, circulating insulin and thyroxine levels did not show any clear relationship to body growth of yearling Coho salmon (Larsen et al., 2001). These findings suggest that IGF-I, both hepatic and circulating, plays a key role in controlling somatic growth in several species of fish and indicates that it can be used as an index for growth.

Temperature, Growth and IGF Levels

Fish used in the study were fasted for one-month prior to start of the experiment. Fasting reduced the energy source and the fish displayed suppressed growth. When given sufficient nutrition and ideal environment, fasted fish may undergo rapid compensatory growth (Ali et al., 2003; Picha et al., 2004; Skalski et al., submitted). Growth of fish in the second study was predominantly influenced by temperature. Warm-reared ($\geq 28^{\circ}\text{C}$) fish grew steadily throughout the experiment, whereas fish reared under relatively cooler condition ($\leq 24^{\circ}\text{C}$) had

limited growth. This may be explained in part because food consumption of fish was influenced by temperature. The optimum rearing temperature for this species of fish is from 26-32°C (Balarin and Haller, 1982). It was observed that fish reared under cold condition consumed only small amount of food (around 19%) compared to the amount consumed by fish reared at high temperatures. The low temperature suppressed the appetite of the fish and large portion of the food consumed apparently was used for maintenance, as opposed to growth. The fish reared under non-manipulated temperature consumed around 13% less than the total amount of food consumed by fish reared at high temperature. The appetite of fish reared under non-manipulated temperature condition was reduced during the cooler periods.

The growth rates of refeed fasted fish reared under different temperature conditions may partly be attributed to the degree of alterations in hepatic IGF-I mRNA production. In this study higher hepatic IGF-I mRNA expression was observed in faster growing fish reared at higher temperatures. Refeeding increased mean IGF mRNA levels 13-fold, 60-fold and 269-fold in fish reared at low, non-manipulated and high temperatures, respectively. This effect of temperature on growth and IGF-I levels is in agreement with the findings of Beckman et al. (1998) that Chinook salmon reared under warmer conditions in spring had higher growth rates and circulating IGF-I levels than those reared under relatively cooler conditions. Similar results were observed in channel catfish reared in 21.7 or 26°C (Silverstein et al., 2000); in Atlantic salmon (*Salmo salar*) reared at 2-3 or 10°C (McCormick et al., 2000); and in coho salmon reared at 2.5 or 10°C (Larsen et al., 2001). The increase in hepatic IGF-I mRNA levels after refeeding was also reported by Duan and Plietskaya (1993), in salmon. Chauvigne et al. (2003) also observed dramatic increase of IGF-I mRNA expression, but in this case in muscle, from refeed rainbow trout (*O. mykiss*). Refeeding and provision of ideal environmental conditions, therefore, induces both systemic and local IGF-I productions.

CONCLUSIONS

A distinct relationship of IGF-I gene expression to growth in individual juvenile *O. niloticus* was confirmed. IGF-I cDNA from this species was cloned and the high sequence identity among related species was confirmed. Greater conservation of the amino acid composition was observed in the B and A domains, demonstrating the highly conservative evolution of these functionally important domains. Based on the partial 539 nucleotide sequence, which spans from the 5'-pre-proIGF-I region to the E domain, a sensitive TaqMan real time qRT-PCR assay was developed. Nutritional and environmental (temperature) regulation of IGF-I expression was observed in the liver of juvenile *O. niloticus*.

Using the assay, hepatic IGF-I mRNA level was found to be significantly correlated with growth manipulated through both alterations in temperature and feed regimen. Our findings suggest that hepatic IGF-I plays a key role in controlling growth in *O. niloticus* and indicate that it could prove useful as an instantaneous indicator for growth in this species.

ANTICIPATED BENEFITS

This work has been prepared for publication in an international journal but has not yet been accepted. Our project has demonstrated a means of ascertaining growth rate using just a small number of fish at any particular point in time. We believe the instantaneous assessment of growth offers a huge advantage over costly and time-consuming determinations of growth on a commercial scale. Farmers and scientists will be able to test environmental and nutritional effects on fish growth in a much more cost-effective manner, allowing the pace of refinement of culture technology to move faster.

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Figure 1. Water temperature of experimental groups in study 2. (◆) manipulated; $\geq 28^{\circ}\text{C}$; (n) non-manipulated; (s) manipulated; $\leq 24^{\circ}\text{C}$.

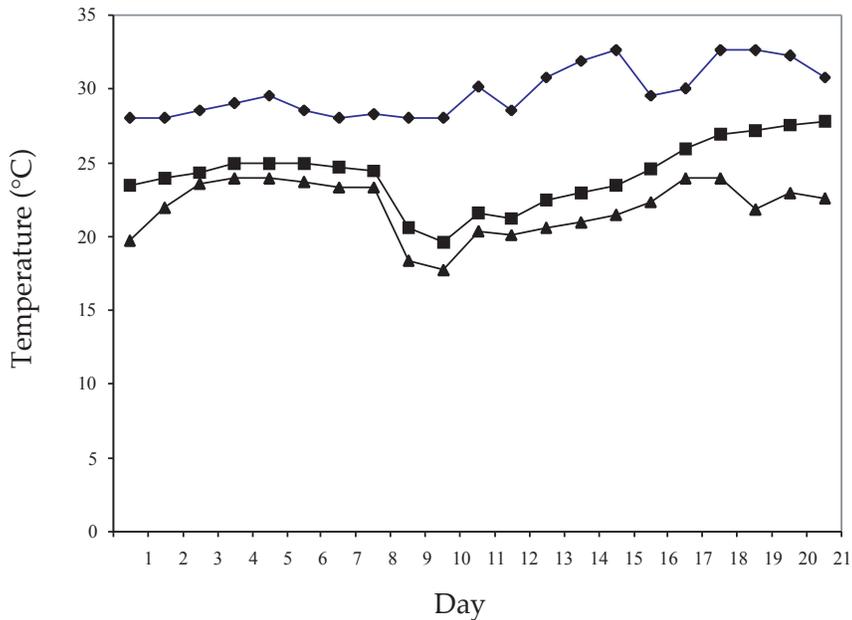


Figure 2. Partial nucleotide sequence for IGF-I cDNA in the Nile tilapia, *Oreochromis niloticus*. The 539 nucleotide sequence spans from the 5'-preproIGF-I region to the E domain. Predicted amino acid sequence is shown below the nucleotide sequence. The mature peptide contains 68 aa. Conserved cysteines are highlighted.

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1   TTCTCCAAAACGAGCCTGCGCAATGGAACAAAGTCGGAATATTGAGATGTGACATCGCC   59
60  CGCATCTCATCCTCTTTCTCCCTGTTTTAATGACTTTAAACAAGTTCATTTTCGTGCGGGC   119

      Signal Peptide (144-275) →
120  TTTGTCTTGTGGAGACCCGTGGGGATGTCTAGCGCTTTTTCCCTTCAGTGGCATTATGT   179
      M S S A F S L Q W H L C
180  GATGTCTTCAAGAGTGCATGTGCTGTATCTCCTGTAGCCACACCCTTCACTACTGCCG   239
      D V F K S A M C C I S C S H T L S L L P
      B domain (276-362) →
240  TGCGTCTCACCCCTGACTCCGACGGCAACAGGGGCGGGCCCTGAGACCCTGTGCGGGGCG   299
      C V L T L T P T A T G A G P E T L C G A
300  GAGCTGGTGCACACGCTGCAGTTTGTCTGTGGAGAGCGAGGCTTTTATTTCAATAAACCA   359
      E L V D T L Q F V C G E R G F Y F N K P
      C domain (363-392) →           A domain (393-455) →
360  ACAGGCTATGGCCCCAGTGCAAGGCGGTCACGTGGCATCGTGGACGAGTGCTGCTTCCAA   419
      T G Y G P S A R R S R G I V D E C C F Q
      D domain (456-479) →
420  AGCTGTGAGCTGCAGCGCCTTGAGATGTACTGTGCACCTGTCAAGACTCCCAAGATTCT   479
      S C E L R Q L E M Y C A P V K T P K I S
      E domain (480-539) →
480  CGCTCTGTGCGTTACAGCGGCACACAGACATGCCAAGAGCACCCAAGGTTAGTAGCAGA   539
      R S V R S Q R H T D M P R A P K V S S R

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Figure 3. Amino acid sequence comparison of tilapia (*O. niloticus*), turbot, flounder, rabbitfish, common carp, goldfish, coho salmon, barramundi, chicken, rat and human IGF-I. Numbering is according to longest amino acid sequence. Identical residues compared to tilapia are indicated as dashes (-). Hash (#) symbols were used to maximize the sequence of alignment. Comparison with tilapia amino acid sequence is expressed in percentage identity, rounded to the nearest whole number.

	B-Domain	% homology	
	10 20 .		
Tilapia	GPETLCGAELVDTLQFVCGERGFYFNKPT	-	
Turbot	-----S---	97	97
Flounder	-----S---	97	
Rabbitfish	-----S---	9	7
Common carp	-----D-----S---	93	
Goldfish	-----D-----S---	93	
Salmon	-----S---		97
Barramundi	-----S--#	93	
Chicken	-----A-----D-----S---	90	
Rat	-----A-----P-----	93	
Human	-----A-----D-----	93	
	C-Domain		
	30 40		
Tilapia	GYGPSARRS##R	-	
Turbot	----N-----		90
Flounder	----N-----	90	
Rabbitfish	----NS--P---	70	
Common carp	----S---HN-		75
Goldfish	----NS---HN-	67	
Salmon	----S---HN-		75
Barramundi	---SN-----	80	
Chicken	---S-S--LHHK	50	
Rat	---S-I--APQT	50	
Human	---S-S--APQT	50	
	A-Domain		
	50 60.		
Tilapia	GIVDECCFQSCELRQLEMYCA	-	
Turbot	-----W-----		95
Flounder	-----R-----		95
Rabbitfish	-----R-----	95	
Common carp	-----R-----	95	
Goldfish	-----R-----		95
Salmon	-----R-----		95
Barramundi	-----R-----	95	
Chicken	-----D--R-----	90	
Rat	-----R--D--R-----		86
Human	-----R--D--R-----		86
	D-Domain		
	70		
Tilapia	PVKTPKIS	-	
Turbot	-A--S-AA		50
Flounder	-A--S-AA	50	
Rabbitfish	-A--S-AA	50	
Common carp	---PG-TP		50
Goldfish	---PG-TP	50	
Salmon	---SG-AA		50
Barramundi	-P--#-AA	50	
Chicken	-I-P--SA	50	
Rat	-L-PT-SA	38	
Human	-L-PA-SA	38	

Figure 4. Mean weight gain of *O. niloticus* under different feeding regimes. DF – daily feeding; ADF – alternate-day feeding; SAT – daily feeding to satiation; RLF – feeding at restriction level. Bars indicate standard error of mean (N = 6). Significantly different means ($P < 0.05$) are indicated by different letters.

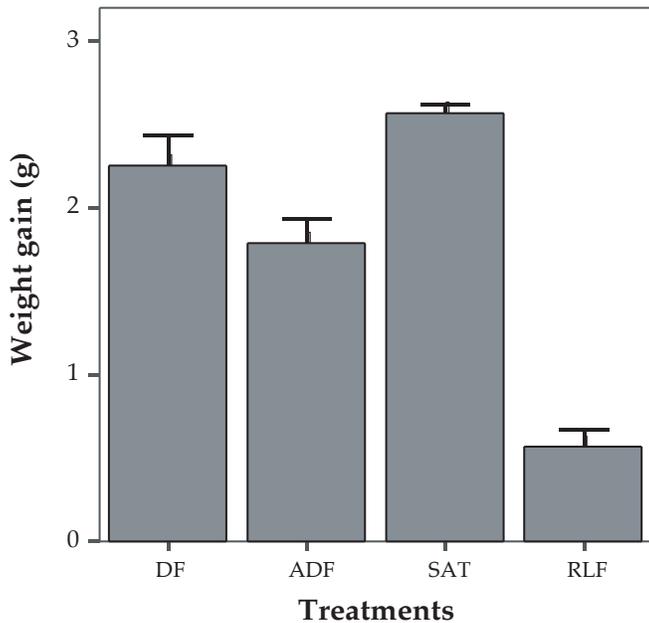


Figure 5. IGF-I mRNA quantification in liver from *O. niloticus* fed under four feeding regimes. Bars indicate standard errors of means. Values of IGF-I mRNA were expressed relative to the time 0 level (assigned an arbitrary value of 1). No significant difference was observed between means.

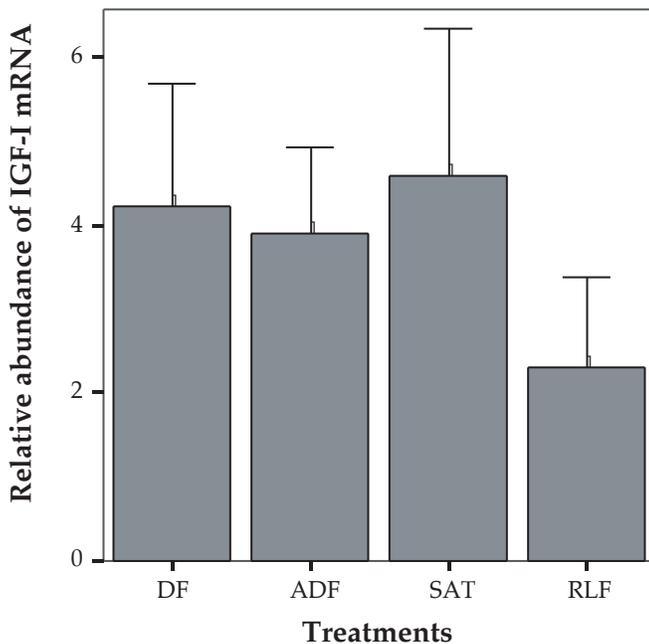


Figure 6 Correlation of relative IGF-I mRNA abundance and individual weight gain in the temperature study (N = 12), $r = 0.64$ ($P < 0.05$)

