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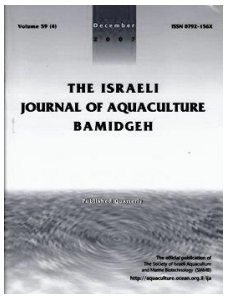

Molecular Cloning and Response to Water Temperature and Nutrient Manipulation of Insulin-like Growth Factor (IGF) Genes in Golden Pompano *Trachinotus ovatus* (Linnaeus 1758) Larvae

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Keywords: Insulin-like growth factors; gene expression; temperature; enrichment; golden pompano *Trachinotus ovatus*.

Abstract

In this study, insulin-like growth factor I (IGF I) and IGF II in golden pompano larvae were cloned and analyzed. In the first trial, IGF expression during ontogeny of larvae in the first 18-days of their life was explored, and then the response of IGFs to water temperature (23, 26, and 29°C) on 12 day post hatching (DPH) and 18 DPH were compared. On 28 DPH, the response of IGFs to the manipulation of nutrients was evaluated. The expression of IGF I increased with the increase of fish age, and was not significantly affected by water temperature. The expression of IGF II was affected by water temperature on 12 DPH and 18 DPH. The expression of IGF II at 23°C was significantly higher than at 26°C and 29°C. The expression of IGFs in fish larvae on 28 DPH was not concomitant with nutrient manipulation. This study detected the gene expression of IGFs at the early stage of golden pompano larvae. The time dependent expression of IGF genes in fish larvae is important to understand the ontogenetic development and growth of fish larvae in early life.

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Introduction

Insulin-like growth factors (IGFs) represent a group of polypeptide hormones that affect varietal cellular processes such as metabolic regulation, cell growth, organ differentiation, and basic metabolism (Saltiel and Kahn, 2001; Taniguchi et al., 2006). The functionality of IGFs is mediated through complex intracellular signaling pathways that depend on the binding of peptides to their corresponding receptors and the activation of intrinsic receptor tyrosine kinases (Ozaki et al., 2013). IGFs are mainly produced in the liver which is the center for IGF circulation (Reinecke and Collet, 1998; Schmid et al., 1999), and are an indicator for growth performance (Duan, 1998; Moriyama et al., 2006; Reinecke et al., 2005). As growth regulation is a major concern for fish production in aquaculture (Reinecke and Collet, 1998), the relationship between the amount of IGFs and fish growth has been intensively investigated (Tanaka et al., 1998; de Jesus et al., 2002; Kaneko et al., 2011), but the mechanism underlying IGF regulation of growth at the molecular level in fish is still poorly understood. Studies on the role of IGFs have expanded to encompass molecular biology, physiology, and biochemistry of various fish species (Duan, 1998; Reinecke et al., 2005; Schmid et al., 1999). Cloning and expression analysis of IGFs have been conducted on several fish species such as rainbow trout Oncorhynchus mykiss (Perrot and Funkenstein, 1999), Japanese ell *Anguilla japonica* (Moriyama et al., 2006), torafugu *Takifugu rubripes* (Kaneko et al., 2011), *Sarotherodon melanotheron* (Fan and Li, 2011) gilthead seabream *Sparus aurata* (Duguay et al., 1996), Japanese flounder *Paralichthys olivaceus* (Tanaka). et al., 1998), sea bass *Dicentrarchus labrax* (Terova et al., 2007), barramundi *Lates calcarifer* (Stahlbom et al., 1999), and milkfish *Chanos chanos* (de Jesus et al., 2002). Existing literature indicates that the level of IGFs in fish is affected by nutrition, developmental stage, and seasonal changes (Duan, 1998; Moriyama et al., 2000; Perrot and Funkenstein, 1999), and it plays an important role in the regulation of development, somatic growth, reproduction, and osmoregulation (Duan, 1998; Moriyama et al., 2000; Moriyama et al., 2006; Reinecke et al., 2005).

IGF I is a highly conserved 70-amino-acid polypeptide presenting a structural sequence similarity to both IGF II and insulin. It plays a central role in regulating development and growth through metabolic and mitogenic activity (Patruno et al., 2008; Reinecke and Collet, 1998). In mammals, IGF I is an important mediator of growth hormone (GH) activity during postnatal life (Froesch et al., 1985), but IGF I shows little dependence on GH and is more related to fetal growth (Daughaday and Rotwein, 1989). In fish, both IGF I and IGF II are detected in liver, brain, eye, gills, heart, gastrointestinal tract, pancreatic islets, kidney, skeletal muscle, spleen, and gonads (Ayson et al., 2002; Vong et al., 2003), and act not only as a growth factor but also as a metabolic hormone in fish (Reinecke et al., 2005).

Golden pompano belongs to the family of Carangidae and is a good candidate species for aquaculture due to its rapid growth and suitability to cage culture. Currently, information on the IGFs in golden pompano is rare. As it is the primary growth related gene, an understanding of IGF expression during fish ontogeny would improve our understanding of fish growth. This study was designed to explore the expression of IGFs during ontogeny of golden pompano larvae in the first 18 days post-hatch (DPH), the response of IGFs to water temperature at 12 and 18 DPH, and nutrient manipulation at 28 DPH. The expression pattern of IGFs would provide essential information on the growth of golden pompano larvae. Such knowledge can improve understanding of general development of golden pompano, and may provide possible indicators for growth measurement of fish larvae.

Materials and Methods

Experiment 1: Expression of IGFs in the first 18 days of golden pompano larvae. Fertilized eggs of golden pompano were obtained from Guanghui Aquaculture Hatchery, Hainan Provence, P.R. China, and were transported to Lingshui Town and hatched in 500 L fiberglass incubators at 26.5^{o}C with a hatching rate of 97.5 ± 1.5% (mean ± SD). On 2 DPH, larvae were stocked into three 1000 L larval rearing tanks. Larval rearing tanks were supplied with filtered seawater (5 μm pores) from the bottom of each tank

with a daily exchange rate of 200% tank volume. Water was discharged through an outlet screen (300 μm) on the upper side of each tank, and the screen was cleaned daily to reduce clogging. Two air stones were used in each tank to maintain dissolved oxygen close to saturation. Light intensity was maintained at 2,400 lux, and the photoperiod was 14 h light:10 h dark. Salinity was maintained at 33 ± 0.8‰ and rearing temperature was 26.5 ± 1.0°C throughout the experiment.

Rotifers *Brachionus rotundiformis* at a density of 10-20 ind/ml were used to feed the larvae from 2 DPH to 10 DPH. The rotifers, fed with baker's yeast, were enriched with DHA protein Selco (INVE Aquaculture, Salt Lake City, UT, USA) for 12 h before they were added into the larval rearing tanks. Instant microalgal paste (*Nannochloropsis* sp.) was also added into larval fish tanks to create a green-water background. *Artemia* nauplii were first introduced at 0.1 nauplii/mL on 10 DPH, and were then added at daily increments of 90%. After five days co-feeding, *Artemia* nauplii were gradually phased out at a daily reduction of 20% until the co-feeding period ended. *Artemia* nauplii were enriched with DHA Protein Selco (INVE Aquaculture, Salt Lake City, UT, USA) following manufacturer's instructions.

Experiment 2: Temperature trial. Fertilized eggs of the same batch were obtained from Lingshui, Hainan Province, and transported to the Tropical Fisheries Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Xincun Town. Upon arrival, all eggs were transferred into 500 L incubators and hatched at 26°C. The experimental design included three constant temperatures 23, 26, and 29°C with three replicates each. On 2 days post hatch (DPH), yolk sac larvae were acclimatized at each desired temperature for 5 h, and then stocked in 500 L fiberglass tanks at a density of 60 fish/L. Apart from the rearing temperature, all feeding protocols and rearing conditions were the same as in experiment I.

Experiment 3: Nutritional manipulation trial. This present study was derived from the same feeding trial as in our previous study (Yang et al. 2015). In brief, the same batch of fertilized eggs of golden pompano hatched in 500 L fiberglass incubators at 26°C with a hatch rate of 97.1 ± 1.9%. On 2 DPH, larvae were stocked into four 1000 L larval rearing tanks at a density of 60 fish/L. All rearing conditions were the same as experiment I. Rotifers *Brachionus rotundiformis* at a density of 10-20 rotifers/mL were used to feed fish larvae from 2 DPH to 12 DPH. The feeding protocol was the same as in the temperature trial. On the morning of 11 DPH, fish larvae were restocked into nine 500 L larval rearing tanks at a density of 20 fish/L.

The nutritional manipulation experiment included three dietary treatments with three replicates each. *Artemia* nauplii were treated according to three methods: (1) enriched with instant microalgal paste (*Nannochloropsis* sp., Qingdao Hong Bang Biological Technology Co., Ltd, Qingdao, China; (2) enriched with Algamac 3080® (Aquafauna, USA); (3) the Control, with no enrichment. For each treatment, three replicate tanks were used in this study. *Artemia* cysts were produced from the Great Salt Lake, UT, USA (INVE Aquaculture). *Artemia* nauplii instar II were enriched for 12 hours at 25°C following manufacturer's instruction before being fed to fish. After harvest, pre-washed *Artemia* nauplii were fed directly to fish larvae. *Artemia* nauplii were fed to fish from 11 DPH to 27 DPH. On 11 DPH, *Artemia* nauplii were first introduced at 200 nauplii/L, and then added at daily increments of 90%. In each treatment, *Artemia* nauplii were enriched with each product following manufacturer's instructions. Each tank bottom was siphoned daily to remove dead fish, uneaten food, and feces. For the analysis of gene expression, three replicates were used in this study.

Growth and survival measurement. For the temperature trial, 10 fish per tank were sampled for size measurements at 1, 3, 5, 9, 12, 18 DPH. In the nutritional manipulation trial, 10 fish per tank were sampled for size measurement at 0, 12 and 28 DPH. Fish were anesthetized with AQUI-S® (New Zealand Ltd., Lower Hutt, New Zealand) and were measured on a stereo microscope with a micrometer at 10× magnification to the nearest 0.01 mm. Growth was determined by specific growth rate (SGR) as %/day using the following equation (Hopkins, 1992): $SGR = 100 (LnSLf - LnSLi) / \Delta t$, where SLf and SLi were the final and initial fish standard lengths (mm), respectively, and Δt was the time

between sampling intervals. At the end of each experiment, the remaining fish larvae from each rearing tank were harvested for survival calculation.

Total RNA extraction and reverse transcription. On 0, 1, 2, 3, 4, 5, and 12 DPH, approximately 300 mg (wet weight) fish larvae were sampled from rearing tanks in triplicate. Approximately 50 individuals were collected in triplicate on 18 DPH and 28 DPH. Total RNA was extracted using TRIzol (Invitrogen, USA). RNA integrity was verified by electrophoresis on formaldehyde-agarose gel (1.2%). RNA concentration was measured by absorbance at 260 nm and purity was determined at OD 260/280 ratio ($1.7 < OD260/OD280 < 2.0$), OD 260/230 ratio ($2.0 < OD260/OD230 < 2.5$) and agarose gel electrophoresis. RNA was reverse-transcribed to cDNA with oligo (dT) primers using a PrimeScript 1st strand cDNA synthesis kit (TaKaRa Biotechnology, Dalian Co., Ltd). The cDNA was used as a template in subsequent PCR. The cDNAs for quantitative real-time PCR were synthesized from one microgram of the total RNA of each sample using the PrimeScriptTM RT reagent kit with gDNA Eraser (TaKaRa).

Gene cloning. Based on unpublished golden pompano transcriptome sequences (Illumina HiSeq2000, annotated by NR, KOG, Kegg, and Swissprot), the genes cloning primers were designed (shown in Table 1) with Primer 5.0 (Premier Biosoft International, Palo Alto, CA, USA) based on data of golden pompano sequence measured previously in our laboratory. The PCR reactions systems were as follows: 1 μL of golden pompano larval cDNA, 1 μL of gene-specific forward primer (F), 1 μL of gene-specific reverse primer(R), 0.5 μL of ExTaq, 5 μL of PCR buffer, 4 μL of dNTP mixture (2.5 μM), 37.5 μL of ddH_2O, in a total volume of 50 μL. The PCR conditions were as follows: denaturation at 94°C for 1 min, 35-cycles of 94°C for 30 s, annealing temperature of each gene for 30 s, 72°C for 4 min, followed by a 10 min extension at 72°C. The PCR products were cloned into the PMD-19T vector (TAKARA, Japan), and were then sequenced.

Table 1. Summary of genes cloning primers used in this study

Primers	*Sequence (5′ - 3′)*	*Amplicon sizes (bp)*
IGF I-F	TCCTGTTCGCTAAATCTCACTT	
IGF I-R	TGTCCATTCGCTCCTTCC	742
IGF II-F	TCCAACCAAATAACCCC	
IGF II-R	GACAAAGCTATAATCCCCTAG	768

Gene expression analysis by quantitative real-time PCR. Quantitative real-time PCR (qPCR) was used to analyze the expression levels of IGF genes in golden pompano larvae. Gene specific primer pairs for IGF genes (Table 2) were amplified in LightCycler480 II (Roche, Switzerland). EF-1α was used as the internal reference and amplified. The cycling conditions for IGF genes and EF1α were as follows: 1 min at 95°C, followed by 40-cycles 95°C for 15 s, and 60°C for 1min. Dissociation curves were employed to ensure that only one single PCR product was amplified in each gene reaction. For each test, three replicates were performed. The relative quantification (RQ) was calculated using ΔΔCT (comparative threshold cycle) method (ΔCT = CT of target gene - CT of EF-1α, ΔΔCT = ΔCT of any sample - ΔCT of calibrator sample). The efficiencies of the primers (E) were EIGF I = 0.997, EIGF II = 1.004.

Table 2. Summary of quantitative real-time PCR primers used in this study

Primers	*Sequence (5′ - 3′)*	*Amplicon sizes (bp)*
IGF I-qF	CGCAATGGAACAAAGTCGG	
IGF I-qR	AGGAGATACAGCACATCGCACT	198
IGF II-qF	GCAAAGACACGGACCCCACT	
IGF II-qR	CGAGGCCATTTCCACAACG	142

Statistical analysis. The data were all expressed as mean ± SD, and compared with one way ANOVA (PASW Statistics 18.0, Chicago, SPSS Inc.). Tukey's test was used for multiple range comparisons with the level of significant difference set at $P < 0.05$. All data were tested for normality, homogeneity and independence to satisfy the assumptions of ANOVA.

Results

Cloning and sequencing of golden pompano IGF I and IGF II cDNA. IGF I obtained from golden pompano cDNA contained a total of 742 bp, and an open reading frame of 558 bp encoding 185 amion acid (aa) residues (Fig. 1). The deduced IGF I protein constituted the signal peptide (1-43aa) and the pro-IGF I (44-185aa) and was composed of domains B (29aa), C (10aa), A (21aa), D (8aa) and E (74aa). The six characteristic cysteine residues (Cys50, Cys62, Cys88, Cys89, Cys93, and Cys102) involved in the formation of the disulfide bonds were conserved.

The IGF II obtained from golden pompano cDNA consisted of 768 bp, and contained an open reading frame of 648 bp encoding 215 aa residues (Fig. 2). The deduced IGF II protein constituted the signal peptide (1-47aa), and the mature protein included domains B (32aa), C (11aa), A (21aa), D (6aa), and the final E domain (98aa). The six characteristic cysteine residues (Cys56, Cys68, Cys96, Cys97, Cys101, and Cys110) involved in the formation of the disulfide bonds were conserved.

Deduced amino acid sequence comparisons of golden pompano IGF I and IGF II with those from other vertebrates, revealed a high sequence identity (Fig. 3). In the present study, the deduced amino acid sequences of golden pompano IGF I cDNA was aligned with the IGF I cDNAs in other vertebrate species, and the comparative analysis indicated that the degree of homology was high among the teleosts. The deduced amino acid sequence of golden pompano IGF-I gene had 48.7-95.7% identity with those of other vertebrate species, and exhibited the highest identity to *Lateolabrax japonicas* (95.7%) and *Sparus aurata* (95.7%), while the lowest identity is to *Homo sapiens* (48.7%) and *Mus musculus* (48.7%). Furthermore, the deduced amino acid sequences from golden pompano, *Lateolabrax japonicus* and *Sparus aurata* IGF I contained extra amino acid sequences (KVSTAGHKVDKGTERRTAQ QQDKTKNK) in the E domain compared to other vertebrate species.

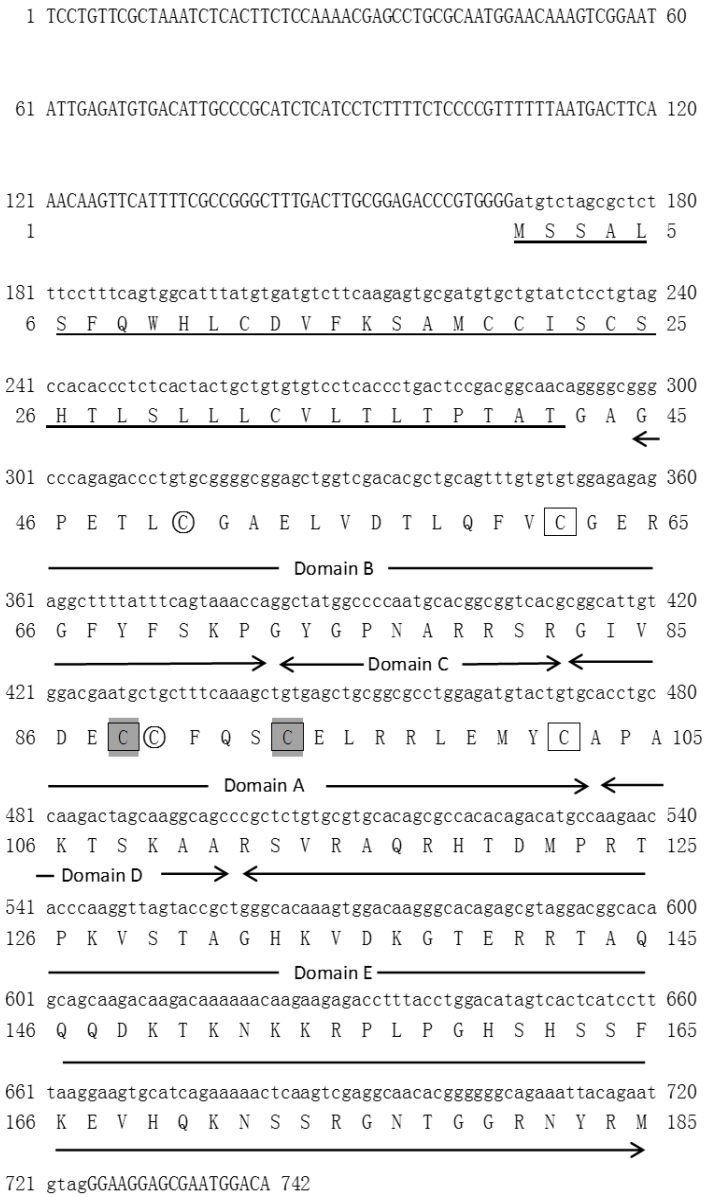

Fig. 1 Nucleotide and deduced amino acid sequence of golden pompano IGF I. The domains B, C, A, and D are marked. The three disulfide bonds formed by the six cysteine residues are shown as circles, rectangles, and shaded rectangles. The disulfide bonds are formed between symbols. The stop codon is denoted by an asterisk.

In this study, the deduced amino acid sequences of golden pompano IGF II cDNA was aligned with the IGF II cDNAs in 12 other vertebrate species (Fig. 3). Comparative analysis revealed that the degree of homology was high among the teleosts. The deduced amino acid sequence of golden pompano IGF II gene had 44.9-92.6% identity with those of other vertebrate species, and exhibited the highest identity to *Lates calcarifer* and *Lateolabrax japonicas* (92.6%), while the lowest identity is to *Bos taurus* (44.9%).

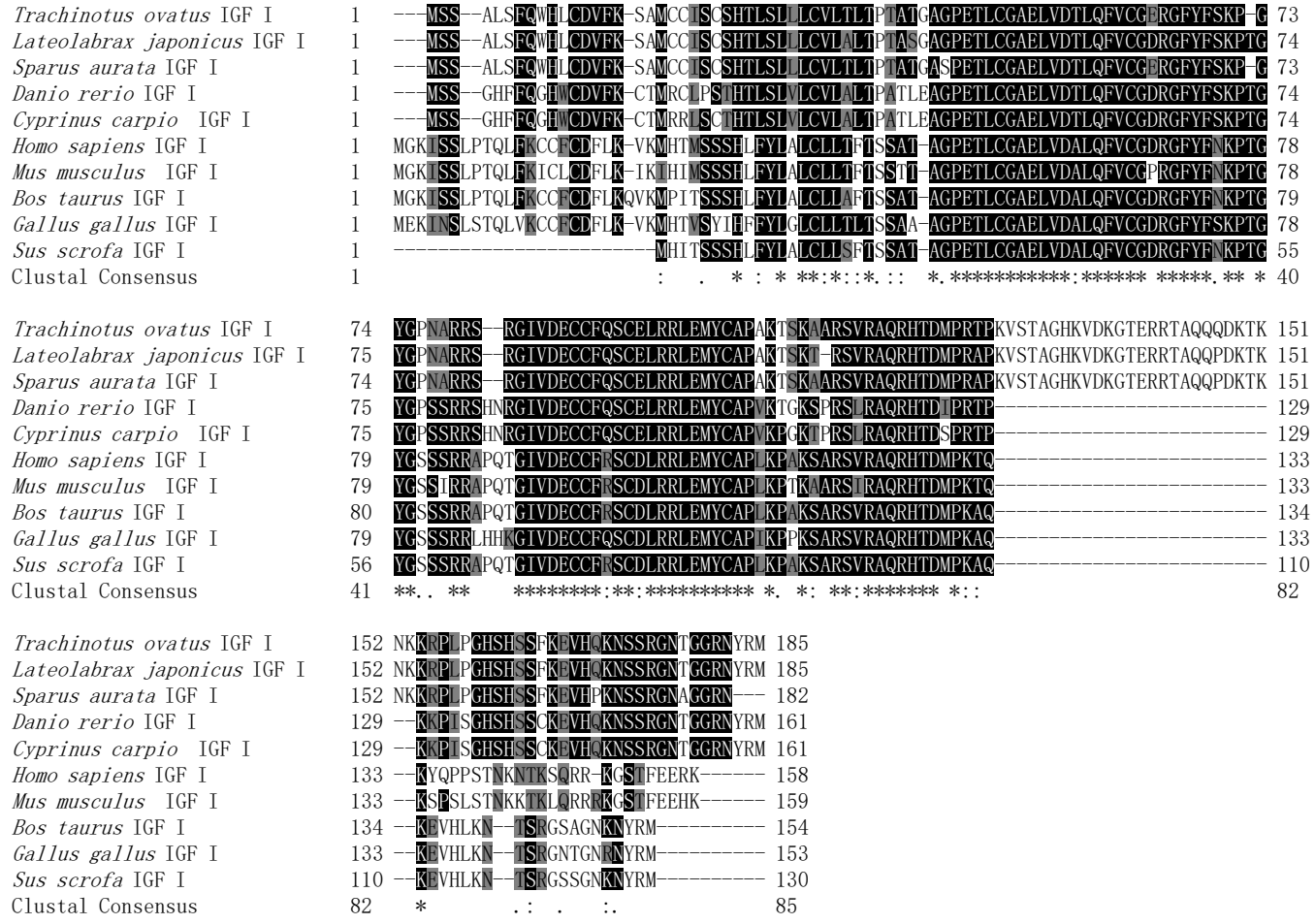

Fig. 2 Nucleotide and deduced amino acid sequence of golden pompano IGF I. The domains B, C, A, and D are marked. The six cysteine residues are marked with rectangles. The disulfide bonds are formed between each symbol. The stop codon is denoted by an asterisk.

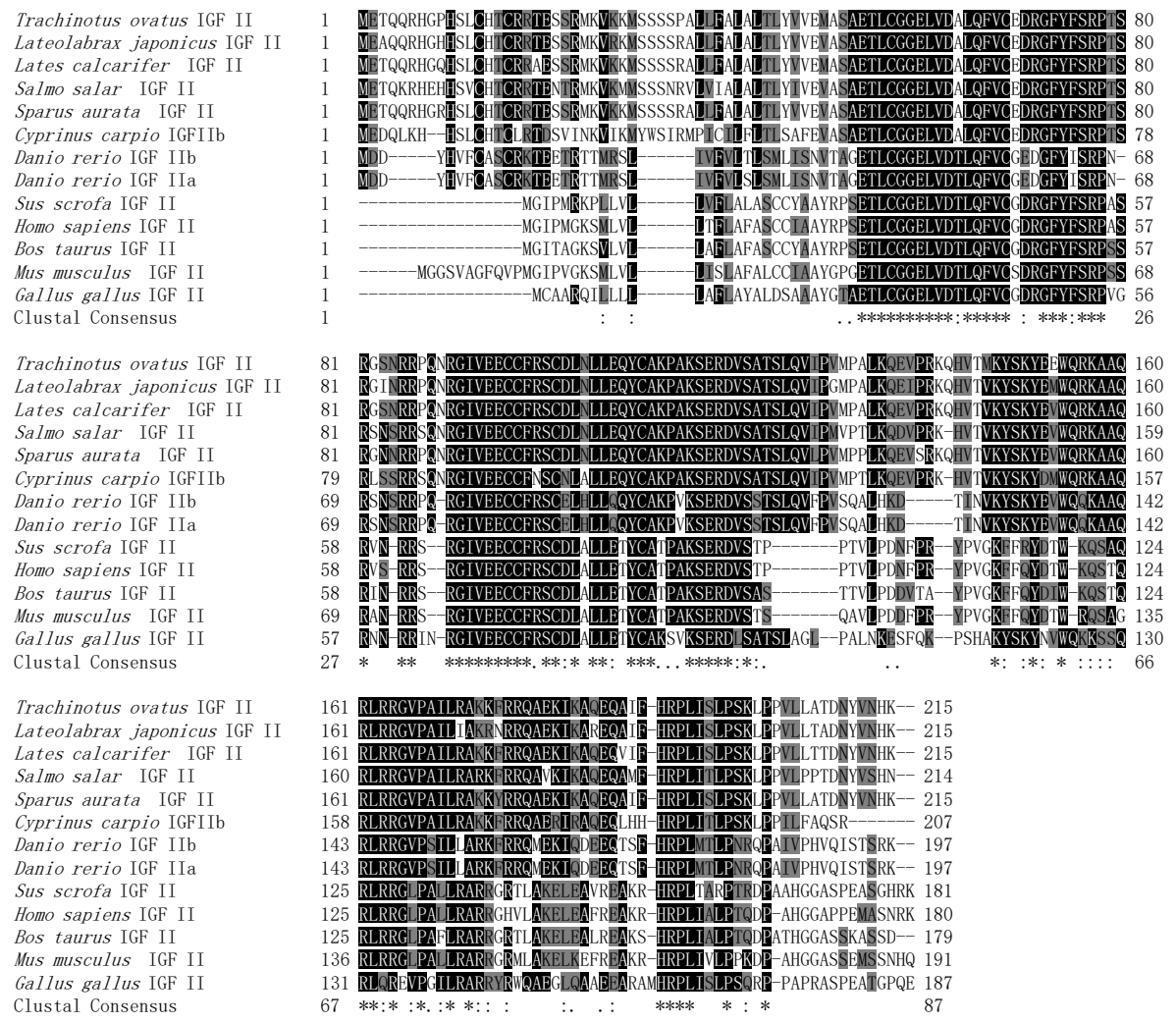

Fig. 3 Amino acid sequence comparisons of both golden pompano IGF I and IGF II with those from other vertebrate revealed a high sequence identity. The IGF I and IGF II sequences from other species were downloaded from GenBank (http://www.ncbi.nih.gov). The references for IGF I and IGF II amino acid sequences are as follows: Homo sapiens (IGF I：NP_001104753.1 , IGF II: NP_000603.1), *Bos taurus* (IGF I：NP_001071296.1 , IGF II: NP_776512.2), *Mus musculus* (IGF I ：NP_034642.2 , IGF II: NP_034644.2), *Sus scrofa* (IGF I: NP_999421.1, IGF II: NP_999048.1), *Gallus gallus* (IGF I：NP_001004384.1 , IGF II: NP_001025513.1), *Lateolabrax japonicus* (IGF I：AEX60712.2 , IGF II: AEX60713.2), *Cyprinus carpio* (IGF I：ABQ08938.1 , IGF II: ADQ44896.1), Sparus aurata (IGF I：ABQ52656.1 , IGF II: AAY46224.1), *Danio rerio* (IGF I：NP_571900.1 , IGF IIa: NP_571508.1 , IGF IIb: AAL06080.1), *Lates calcarifer* (IGF II: AAB64195.1), *Salmo salar* (IGF II: NP_001117119.1)

A phylogenetic tree was constructed by the neighbor-joining method to investigate the phylogenetic relationships of IGF genes between golden pompano and other vertebrate species (Fig. 4). The results showed that these species fell into two distinct lineages, one was composed of IGF I, while another was composed of IGF II. In each distinct lineage, the branches of the teleost species clustered together. The results of the phylogenetic analysis were almost identical with the established phylogeny. The deduced amino acid sequences of golden pompano IGFs cDNA were aligned with the IGFs cDNAs in other vertebrate species. Comparative analysis revealed that the degree of homology was high among teleosts. The deduced amino acid sequence of golden pompano IGF I had 48.7-95.7% identity with that of other vertebrate species, and exhibited the highest identity to *Lateolabrax japonicus* (95.7%), while the lowest identity was to Homo sapiens (48.7%).

Growth and survival of golden pompano larvae. On 18 DPH, the growth and survival of golden pompano larvae were affected by water temperature. The SGR of fish reared at 29°C was significantly higher than fish reared at 23°C and 26°C ($P < 0.05$, Table 3). The survival of golden pompano larvae reared at 26 and 29°C was significantly higher than the survival of fish larvae reared at 23°C ($P < 0.05$, Table 3). In the present study, the survival rates of fish from all the treatments were very low in all replicates.

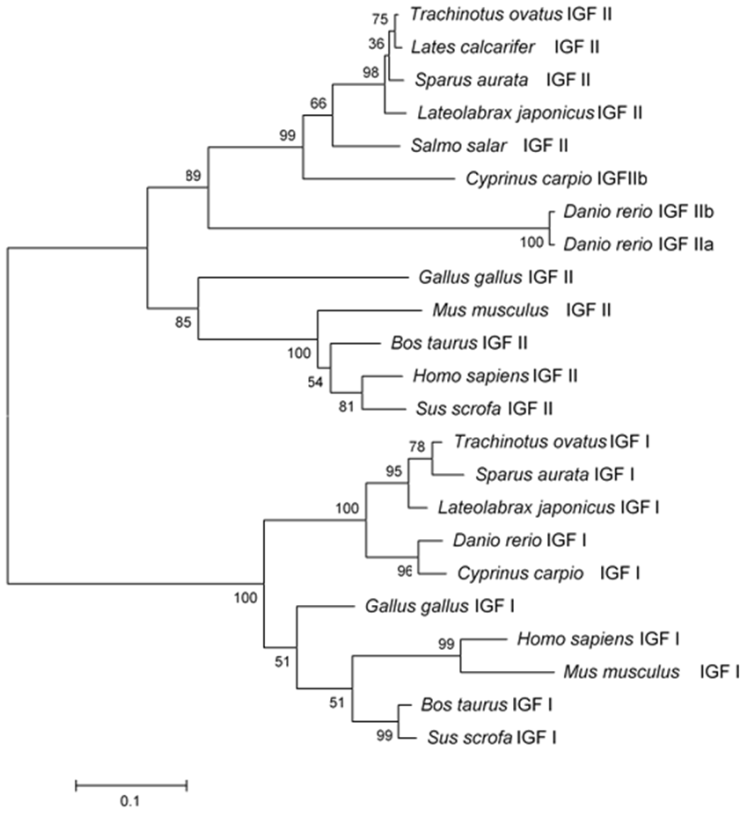

Fig. 4 Phylogenetic tree of IGF I and IGF II constructed by the neighbor-joining method.

Table 3. Specific growth rate and survival of golden pompano larvae on 18 DPH and 28 DPH

	23°C	*26°C*	*29°C*
12 DPH Specific growth rate (%/day)	2.06 ± 0.93[a]	2.68 ± 0.85[a]	3.47 ± 0.33[b]
18 DPH Specific growth rate (%/day)	2.56 ± 0.28[a]	2.70 ± 0.42a	3.64 ± 0.20b
Survival on 18 DPH (%)	12.38 ± 0.16a	15.30 ± 0.41b	16.36 ± 1.08b
	Non-enriched	*Nannochloropsis*	*Algamac3080*
28 DPH Specific growth rate (%/day)	5.68 ± 0.22[a]	6.25 ± 0.08[b]	6.49 ± 0.5[c]
Survival on 28 DPH (%)	31.83± 8.60[b]	29.33± 5.32[b]	10.33 ± 0.90[a]

Different letters in the same row represent significant difference ($P < 0.05$).

On 28 DPH, nutrient manipulations significantly affected the growth and survival of golden pompano larvae ($P < 0.05$, Table 3). The highest SGR was observed in fish fed Algamac3080 enriched with *Artemia* nauplii, and the lowest SGR was found in fish fed non-enriched *Artemia* nauplii. The highest survival was achieved in the treatment of un-enriched *Artemia* nauplii and *Nanochloropsis* enriched *Artemia* nauplii ($P < 0.05$, Table 3), and the lowest survival was observed when fish were fed with Algamac 3080 enriched *Artemia* nauplii ($P < 0.05$).

Expressions of IGF I and IGF II during ontogenetic development. At hatching, the relative expression of IGF I in fish larvae was not significantly different on 1 DPH ($P > 0.05$, Fig. 5). Starting from 1 DPH to 3 DPH, the relative expression of IGF I in fish larvae increased rapidly. The relative expression of IGF I in fish larvae was similar from 3 to 5

DPH, and increased sharply on 12 DPH. The relative expression of IGF II in fish larvae was significantly lower than the expression observed on 1 DPH ($P < 0.05$, Fig. 5). Starting from 1 DPH, the relative expression of IGF II in fish larvae increased sharply, and peaked on 3 DPH. On 4 DPH and 5 DPH, the relative expressions of IGF II in fish larvae were not significantly different, but were significantly lower than the expression on 12 DPH and 18 DPH.

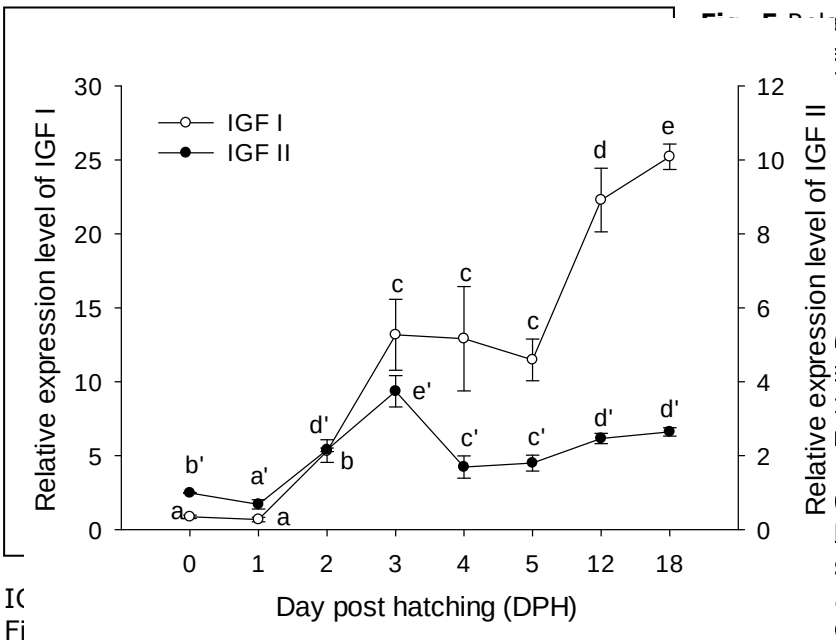

Fig. ative expressions of IGFs in the elopmental stage of golden *rachinotus ovatus* larvae.

of IGFs to water ire. On 12 DPH and 18 DPH, ession of IGF I was not ly affected by water ire ($P > 0.05$, Fig. 6). the expression of IGF I on as significantly higher than 2 DPH in all temperature s ($P < 0.05$). In contrast to ater temperature ($P < 0.05$, was significantly higher than in fish at 26°C and 29°C ($P < 0.05$), and the expression of IGF II was not significantly different in fish at 26°C and 29°C ($P > 0.05$). Similarly, the expression of IGF II in fish at 23°C was significantly higher than at 26°C and 29°C ($P < 0.05$) on 18 DPH.

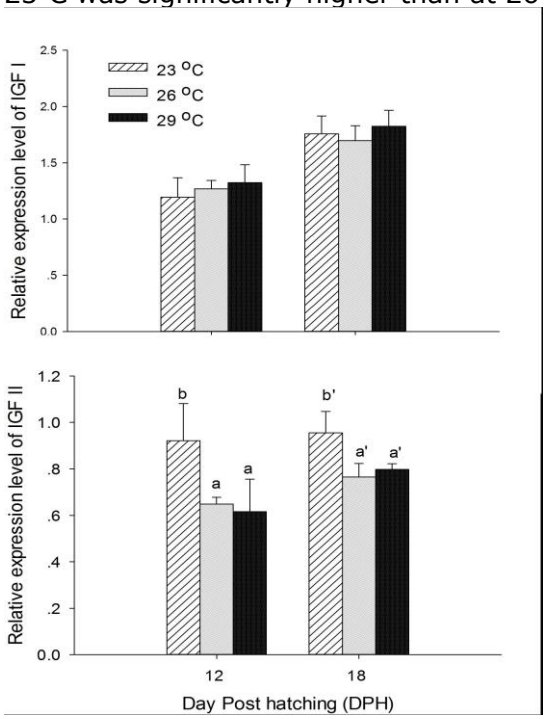

Fig. 6 Response of IGF I and IGF II to the water temperature of 23, 26, and 29°C on 12 DPH and 18 DPH in golden pompano *Trachinotus ovatus* larvae. Data with * or with different letters were significantly different ($P < 0.05$).

Response of IGFs to nutrient manipulation. In this study, the expressions of both IGF I and IGF II in golden pompano larvae were not sensitive to current nutrient manipulation. On 28 DPH, the expression of both IGF I and IGF II of fish fed non-

enriched, *Nannochloropsis* enriched, and Algamac 3080 enriched *Artemia* nauplii were not significantly different ($P > 0.05$, Fig. 7).

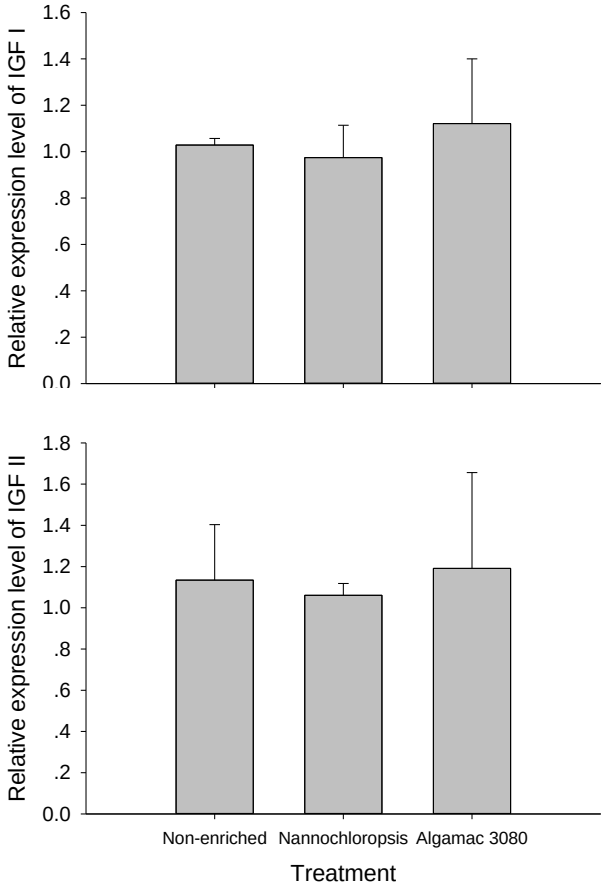

Fig. 7 Response of IGF I and IGF II to nutrient manipulation of golden pompano *Trachinotus ovatus* larvae on 28 DPH.

Discussion

The present study cloned and analyzed the expression of IGF I and IGF II genes during the early development of golden pompano. The IGF I and IGF II cDNA obtained from golden pompano included six cysteine residues in the mature peptide region, and were composed of five domains. The deduced amino acid sequence of golden pompano IGF I and IGF II revealed high identity to other fish species. The cysteine residues existed in A and B domains of IGF I in golden pompano. Similar to other species, the A and B domains play an important role in maintaining the tertiary structure (Fukada et al., 2012; Reinecke et al., 1997), and are important regions for binding to its receptors and binding proteins (Fan and Li, 2011; Duguay et al., 1996).

Expression of IGFs from 0 DPH to 18 DPH. Insulin-like growth factors (IGF) are peptide hormones that play a critical role in somatic growth of vertebrates (Kaneko et al., 2011). IGF I and IGF II are the major isoforms in IGF, and share a high sequence similarity. In teleosts, the functions of IGF I in association with somatic growth are conserved or partly conserved (Moriyama et al., 2000; Wood et al., 2005). IGF II can promote postnatal growth in response to nutritional states in teleosts (Fox et al., 2010; Shamblott et al., 1995;). In *Oncorhynchus kisutch*, fast-growing phenotype of fish are accompanied with elevated expression levels of IGF I and IGF II. In the present study, the expression levels of IGF I in golden pompano larvae increased with fish age, and maintained a relatively high level upon completion of the experiment. Increased expression levels of IGF I in golden pompano larvae during the ontogenetic development are consistent with the rapid growth of this species (Ma et al., 2014). In the present study, the expression levels of IGF II increased with fish age, and peaked on 3 DPH. With onset of first feeding, the expression levels of IGF II in fish larvae dropped significantly, and maintained relatively low levels until 18 DPH. The changes of the expression of IGF II may be caused by changes of nutrient supplement, as prior to 3 DPH most fish rely on indigenous nutrient supplements and after 3 DPH fish rely on exogenous nutrient supplements (Fox et al., 2010; Shamblott et al., 1995).

Response of IGFs to water temperature. In the present study, the expression of IGF I in golden pompano larvae did not correspond to water temperature on 12 DPH and 18 DPH. The expression levels of IGF I in fish larvae on 18 DPH were significantly higher than on 12 DPH in all temperature treatments. In this study, fish were given a mixed feeding phase whereby both rotifers and *Artemia* nauplii were present in the diet on 12 DPH, while fish were fed solely with *Artemia* nauplii on 18 DPH. The increase of IGF I expression associated with growth acceleration may be due to an improvement in feed intake at advanced developmental stages as seen in other species (Duan et al., 2010;

Martins et al., 2014). Evidence indicates that the expression of IGF I can be affected by the rearing temperature (Hevrøy et al., 2012). However, in the present study, the expression of IGF I was not significantly affected by water temperature. This result was similar to findings reported by Hevrøy et al. (2013) and Hevrøy et al. (2015). This may suggest that temperatures in the present study are within the suitable range for fish growth, and expression of IGF I is not sensitive to these temperatures.

IGF II precipitates the growth process, and expression of IGF II is related to hypertrophy, hyperplasia, and differentiation of muscle cells (Duan et al., 2010). IGF II expression is more pronounced than IGF I during fish growth (Peterson et al., 2004). Previous studies have demonstrated that the expression of IGF II in fish can be regulated by temperature and salinity (Cnaani and Hulata, 2011; Martins et al., 2014). In the present study, temperature significantly affected expression of IGF II on 12 and 18 DPH. On both 12 and 18 DPH, the expressions of IGF II at 23^{o}C were significantly higher than that at 26 and 29^{o}C. In contrast, expression of IGF II in *Lophiosilurus alexaxdri* tends to increase with increasing water temperature (Martins et al., 2014). Existing evidence indicates that expression of IGF II tends to be higher in faster growing fish (Peterson et al., 2004). In the present study, the expression of IGF II was not correlated to growth of golden pompano larvae under the tested water temperature. Although higher growth rate was observed in fish reared at 29^{o}C, expression of IGF II at 29^{o}C was significantly lower than at 23^{o}C. The level of IGF II was not directly regulated by temperature, but by nutritional status (Gabillard et al. 2005). Temperature does not seem to directly regulate the level of IGF II, which instead tends to reflect the nutritional status of fish. Since the metabolic level of fish at low temperatures tends to be low (Wohlschlag, 1960), relative nutrient preservation may be higher in fish at high temperatures. This may explain why expression level of IGF II in fish at 23^{o}C was higher that at 26 and 29^{o}C.

Responses of IGFs to nutrient manipulations. The expression of IGFs plays an important role in fish somatic growth. The expression of IGF I corresponds to nutritional conditions in species such as yellowtail *Seriola quinqueradiata* (Fukada et al., 2012), hybrid striped bass *Mornone chrysops* x *Morone saxatilis* (Picha et al., 2008), channel catfish *Ictalurus punctatus* (Peterson and Waldbieser, 2009), and rainbow trout *Oncorhynchus mykiss* (Gabillard et al., 2006). Most of these studies focused on the response of IGF I to changes in nutritional conditions (e.g. fasting and re-feeding). Expression of IGFs in fish can be altered by nutrients such as vitamins, lipids, and fatty acids (Fernández et al., 2011; Villeneuve et al., 2006). Additionally, concomitant relationships between IGF expression and nutrients may depend on the developmental stage of fish (Chen et al., 2000). In this study, although both growth and survival of golden pompano larvae were significantly affected by nutrient manipulations, the expression of IGF I and IGF II in fish larvae on 28 DPH did not correspond to the nutrient treatments. This may suggest that both IGF I and IGF II in fish larvae on 28 DPH were not sensitive to nutrient enhancement used in the present study. Since survival rates of all replicates were low in this study it is unclear if low survival rates have any negative impact on the expression of IGFs. This may need further investigation.

In summary, IGF I and IGF II cDNA of golden pompano larvae were cloned and analyzed in this study. Results from the present study indicate that the expression of IGF II in golden pompano larvae were significantly affected by water temperature, but expression of IGFs in golden pompano did not correspond to nutrient manipulation. The time dependent expression of IGF genes in fish larvae is important in understanding the ontogenetic development and growth of fish larvae in early life. Measures of IGF genes in golden pompano larvae may serve as useful growth biomarkers in the field and in aquaculture, leading to rapid assessment of environmental conditions and nutrient status affecting fish growth.

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