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Nutrition and Temperature Regulate the Expression of Heart-Type Fatty Acid Binding Protein Gene in Golden Pompano Larvae (*Trachinotus ovata*, Limmaeus, 1758)

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Abstract

This study examined the regulation of nutrition and temperature on the expression of heart-type Fatty acid binding protein (H-FABP) gene in golden pompano to understand importance of protein development in fish larvae. Firstly, H-FABPs expression during ontogeny of larvae in the first 18 days after hatching was tested, and then the responses of H-FABPs to temperature (23, 26 and 29°C) on 12-days post hatching (DPH) and 18 DPH were compared. On 18 DPH, the response of H-FABPs to the manipulation of nutrients was evaluated. The expression of H-FABPs increased after transition to endogenous feeding, and was significantly affected by temperature. The expression of H-FABP at 23 and 26°C was higher than at 29°C on 12 DPH, and the expression of H-FABP at 29°C was higher than at other temperatures on 18 DPH, suggesting that H-FABP expression is determined by both temperature and fish age. The expression of H-FABP on 18 DPH was highest when Algamac 3080 enriched *Artemia nauplii* were fed to fish and lowest when fish were fed with *Nannochloropsis* sp. enriched *Artemia nauplii*. The correlation analysis between the expression of H-FABP and fatty acids shows that the ratio of fatty acids is more important than the relative quantity of single fatty acid in regulating the expression of H-FABP at the early stage of fish larvae. This study indicates that changes in environmental temperature and feed type influence H-FABP expression, growth and survival of golden pompano in early life.

Keywords: FABP; Nutrition; Temperature; Gene expression; Fish larvae

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Introduction

Most fishes rely on lipids as a main energy source and teleost fish show the concentration of plasma lipoproteins twice higher than mammals, indicating differential use of lipid among animals [1]. In an excessive lipid supply, fish usually accumulate lipids in the liver and affect the normal liver function [2]. As a main player in fatty acid oxidation and energy provision, fatty acid-binding proteins (FABPs) are of a major interest to understand the pattern of fish growth in aquaculture.

FABPs are divided into 14 different tissue-specific subfamilies in teleost fishes and have tissue-specific expressions [3-5].These proteins have a common clamshell tertiary structure and present in tissues with great capacities to oxidize or to store lipids [6,7]. With a highly conserved gene organisation,the FABPs are composed of four exons separated by three introns, but little is known on their gene expression in fish [4,8-10]. Being able to bind long-chain fatty acids and other hydrophobic molecules , FABPs as carrier proteins are important for intracellular lipid transport, metabolism and homeostasis [3]. From cytoplasm to nucleus,

those carrier proteins transport, and then release the lipids to a variety of nuclear receptors [11]. However, little is known about the regulation of FABP gene expressions by temperature and fatty acids in either fish or terrestrial animals [12].

FABPs subfamilies play various roles in fatty acid metabolism [13]. The heart-type FABPs (H-FABPs) subfamily contains the muscle and heart FABPs that have a high binding affinity for C16-C20 fatty acids [14]. Despite wide distribution in the heart, skeletal and smooth muscle, mammary epithelial cells, aorta, distal tubules of the kidney, lung, brain, placenta and ovary, H-FABP is mainly expressed in the brain and heart with a predominant expression in muscle cells [15]. H-FABPs in mammals have a role to play with the intramuscular fat accretion [16]. However, in previous studies on shark, catfish and lamprey, FABPs in the liver are clearly related to H-FABPs but not to liver-FABPs (L-FABPs) [17-19]. H-FABPs are mainly expressed in the liver of aquatic organisms and play a role in fatty acid mitochondrial β-oxidation [12].

Several studies on the FABPs genes in fish have explored the gene structure and expression in nurse shark and elephant fish , Antartic teleost fishes, Japanese sea bass, rainbow trout and zebrafish [2,3,20,21]. Recent studies on zebrafish and crustaceans show that the H-FABPs mRNA is present mainly in the liver and ovaries, suggesting the possible role in ovary growth and development [22-24]. Zhang et al. [7] found that H-FABPs concentration was highest at the primary growth stage of oocyte followed by a significant decrease at the mature oocyte stage. According to several studies on FABPs in fish liver, the change in expression patterns of H-FABPs between mammals and fish is likely due to an evolutional divergence in transcriptional regulation and highlight the difference of the fish liver function and lipid metabolism [2-4,18,19,21]. The golden pompano *Trachinotus ovatus* (Linnaeus, 1758) is widely distributed in Asia-Pacific regions and has become a fish of interest for commercial aquaculture in recent years [25,26]. In particular, its fast growth, high flesh quality and suitability for cage culture make golden pompano an easy species to maintain in aquaculture [27]. Although golden pompano's best conditions of culture have been well explored at the juvenile and adult stages, fish survival at larval stage has been recognised as an important bottleneck limiting development of aquaculture industry [28-31]. The pompano larvae require a high amount of fatty acids especially highly unsaturated fatty acids during early ontogeny, and the unbalanced supply of dietary unsaturated fatty acids can reduce growth and increase body malformations [27,32]. In order to understand the requirement of fatty acids for this fish, knowledge on the biochemical and molecular mechanisms underlying the requirement of essential fatty acids is essential. The aim of this study was to evaluate the impact of temperature and nutrition on the expression of H-FABPs gene during the development of golden pompano larvae. The expression of H-FABPs genes was firstly tested during at different temperatures (23, 26 and 29°C) and then the gene expression was examined under three diets with different fatty acid enrichment. The results of this study would provide new understanding on the role of H-FABPs in regulating fish growth and as an indicator for fish development in hatcheries at different environmental conditions and nutrition supply.

Materials and Methods

Larval rearing of golden pompano

These fish specimens were obtained from a previous feeding trial [33]. In brief, fertilized eggs of golden pompano 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. Eggs hatched in 500-L fiberglass incubators at 26.5°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 pore size) from the bottom of each tank through upwelling with a daily exchange rate of 200% tank volume. Two air stones were used in each tank to maintain dissolved oxygen close to saturation. Light intensity was maintained at 2400 lux and the light regime was controlled at 14 h light and 10 h dark. The salinity was maintained at 33 ± 0.8% and water temperature was 26.5 ± 1.0°C throughout the experiment. Rotifers (*Brachionus rotundiformis*) were fed to fish larvae from 2 DPH to 10 DPH at a density of 10-20 ind/mL. *Artemia nauplii* were added into the rearing tank from 10 DPH until the experiment was completed. Both rotifers and *Artemia nauplii* were enriched with docosahexaenoic acid (DHA) Protein Selco (INVE Aquaculture, Salt Lake City, USA) according to the manufacturer's instruction. On 0, 1, 2, 3, 4, 5, 12 and 18 DPH, approximately 300 mg (wet weight) fish larvae were sampled from each rearing tank in triplicate to study the ontogenetic expression of the H-FABP. On 18 DPH, a total of 100 individuals were collected in triplicate and examined under a dissecting microscope for tissue expression analysis.

Response of H-FABP gene to rearing temperature

Fertilized eggs of the same batch were obtained from Lingshui, Hainan Province. Upon arrival, all eggs were transferred into 500 L incubators and hatched at 26.5°C. The experimental design included three constant temperatures 23, 26 and 29°C with three replicates each. In this study, 26°C was used as the control temperature. On 2 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^{-1}. Except for the rearing temperature, all the feeding protocols and rearing conditions were the same as described in the previous section. Approximately 50 individuals were collected in triplicate on 12 DPH and 18 DPH to analyze the response of H-FABP gene to rearing temperature.

Response of H-FABP gene to nutrition manipulation

This present study was derived from the same feeding trial in our previous study [32]. The nutritional manipulation experiment included three dietary treatments with three replicates each. *Artemia nauplii* were treated in three methods and each diet differed in fatty acid composition: (1) enriched with instant microalgal paste (*Nannochloropsis* sp., Qingdao Hong Bang Biological Technology Co., Ltd, Qingdao, China), (2) enriched with Algamac 3080® (Aquafauna, USA), and (3) without any enrichment

as control. For each treatment, three replicate tanks were used in this study. Approximately 50 individuals were collected in triplicate on 18 DPH for nutrition manipulation analysis.

Total RNA extraction and reverse transcription

Total RNA was extracted using TRIzol (Invitrogen, USA). RNA integrity was verified by electrophoresis on a formaldehyde-agarose gel (1.2%). The RNA concentration was measured by absorbance at 260 nm and the purity was determined at the ratio of absorbance at 260 nm and 280 nm (260/280) after 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.

Cloning of the gene cDNA and real-time PCR

Based on a preliminary test on golden pompano transcriptome sequences in our laboratory (Illumina HiSeq2000, annotated by NR, KOG, kegg and SwissProt), the genes cloning primers were designed **(Table 1)** with Primer 5.0 (Premier Biosoft International, Palo Alto, CA, USA). The PCR reaction 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) and 37.5 μL of ddH_2O were mixed in a total volume of 50 μL. The PCR conditions were 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 sequenced.

Quantitative real-time PCR was used to analyze the level of H-FABP gene expression in golden pompano larvae. Gene specific primer pairs for the H-FABP gene **(Table 1)** were amplified in LightCycler480 II (Roche, Switzerland). EF-1α was used as the internal reference for amplification. The cycling conditions for H-FABP genes and EF-1α were as follows: 1 min at 95°C, followed by 40 cycles 95°C for 15 s and 60°C for 1 min. Dissociation curves was used to guarantee 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 the ΔΔCT (comparative threshold cycle) method, i.e., 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 E H-FABP=0.1003.

Sequences and phylogenic analysis

The H-FABP gene cDNA sequences were analyzed by BLAST at the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The complete ORF regions and amino acid sequences were deduced with ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The molecular weight (Mw) and isoelectronic point (pI) of deduced amino acids were computed by the pI/Mw tool of ExPASy (http://web.expasy.org/compute_pi/). Protein domains were predicted using SMART (http://smart.embl-heidelberg.de/). Multiple sequence alignments of amino acids were performed by ClustalX 2.1. The phylogenetic tree was constructed by the neighbor-joining (NJ) method in MEGA 6.0 and the bootstrap values were replicated 1000 times to derive the confidence value for the analysis [34]. Pairwise deduced amino acids sequence identity and similarity matrices of the H-FABP family sequences from various species were performed using Matgat 2.02 [35]. The three-dimensional structures of golden pompano H-FABP were obtained by homology modeling (http://swissmodel.expasy.org/workspace/index.php).

Statistical analysis

The data were 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 H-FABP gene cDNA

The length cDNA sequence of golden pompano H-FABP gene (GenBank accession: MF034870) was 743 bp, including a 5'-untranslated region (UTR) of 47 bp, a 3'-UTR of 294 bp and an open reading frame (ORF) of 402 bp, which encoded a polypeptide of 133 amino acids (aa) with a calculated molecular weight (Mw) of 14.95 kDa and theoretical isoelectric point (pI) of 5.92.

The bioinformatics analysis deduced that the polypeptide sequence contains a lipocalin domain **(Figure 1)**. The molecular modeling of golden pompano H-FABP **(Figure 2)** is composed of 10 anti-parallel β-strands that set out a barrel with a clamshell-like structure and a barrel cap composed of a pair of α-helices beta sheets in N-terminal amino acid that enclosed the cavity forming a hydrophobic pocket. The golden pompano H-FABP sequence shared 72.31% identity with the bovine heart-type fatty acid binding protein (PDB ID: 1bwyA).

Multiple sequence alignments and phylogenetic analysis

Multiple sequence alignment of the deduced amino acid sequences of H-FABP genes with some known H-FABP family amino acid sequences from various species is shown in **Table 2**. The predicted amino acid sequence of H-FABP genes from golden pompano showed the greatest similarity and identity to the Japanese rice fish *Oryzias latipes* (respectively 96.2% and 87.2%, XP_004074237.1) and the mummichog *Fundulus heteroclitus*

Table 1 Sequences of primers used in this study.

Primers	Sequence (5'-3')	Amplicon sizes (bp)
H-FABP -F	TCCACACTACCGCAGACA	532
H-FABP -R	GCAGCAGCCATACAAGGC	
H-FABP- qF	ACCTCAAGGAAAGCGAGAAGTT	140
H-FABP- qR	GCTCTGGGTCTTCACCGTCA	
EF-1α-qF	CCCCTTGGTCGTTTTGCC	101
EF-1α-qR	GCCTTGGTTGTCTTTCCGCTA	

```
  1 CTCTCTCTCTCTCTCTCTCCCTGATTCCTCCACACTACCGCAGACatggtcgaggctt 60
  1                                              M  V  E  A  F  5
 61 tcgtcggcacatggaacctcaaggaaagcgagaagtttgatgactacatgaaggagctgg 120
  6   V  G  T  W  N  L  K  E  S  E  K  F  D  D  Y  M  K  E  L  G 25
121 gtgtgggctttgctacacgtaaggtgggcaacatgaccaaacccaccactgttatctcag 180
 26   V  G  F  A  T  R  K  V  G  N  M  T  K  P  T  T  V  I  S  V 45
181 tggatggcgacaaggtgacggtgaagacccagagcaccattaagaacacagagctcagct 240
 46   D  G  D  K  V  T  V  K  T  Q  S  T  I  K  N  T  E  L  S  F 65
241 tcaagataggagaggagtttgacgagaccaccgccgatgacaggaaggttaagtccattg 300
 66   K  I  G  E  E  F  D  E  T  T  A  D  D  R  K  V  K  S  I  V 85
301 taaaaatcgaggatgggaagatggtgcacatacagaaatgggacggcaaagagaccagtc 360
 86   K  I  E  D  G  K  M  V  H  I  Q  K  W  D  G  K  E  T  S  L 105
361 tggtcagggaagtcaacggaaacgccctcacactaacacttacacttggacaagttgttt 420
106   V  R  E  V  N  G  N  A  L  T  L  T  L  T  L  G  Q  V  V  C 125
421 gcacacgtcgctacgagaaggcagagtaaAACCCAACACTGGATCCCTGCATCATTTACT 480
126   T  R  R  Y  E  K  A  E  *                                  133
481 TGCATCATCTAAAGCCCTGAACCACAAACTTCCTAACACCACTTCAACCCAGGTCAAGCA 540
541 GACTGCCTTGTATGGCTGCTGCAGATTTCCCAAAATATCCCCTCATACTGTACTCTCAGT 600
601 ATTAACATTGCTGCTGACTGTGACCTCCATTTTTCTCCCTGTCTTTCACGTTATACTGTT 660
661 GTTCCTTCTACTGTAAGAAACACTGAATAAATTCCAATGATTTGTTGTTGTTTTTTCTCT 720
721 CTAAAAAAAAAAAAAAAAAAAAA                                     743
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Figure 1 Nucleotide sequence and deduced animo acid of heart-type fatty acid binding protein (*H-FABP*) gene from golden pompano *Trachinotus ovatus* (Linnaeus 1758).

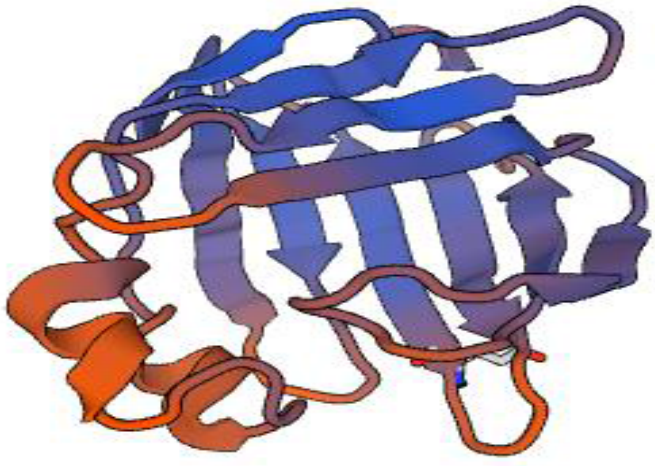

Figure 2 Predicted tertiary structure of golden pompano H-FABP.

(respectively 93.2% and 86.5%, NP_001296909.1). Golden pompano H-FABP has an amino acid identity between 75.9 and 87.2% with eight fish species and between 63.2 and 69.2% with other vertebrates. Its similarity is around 80.5 and 96.2% cumulatively with eight fish and six other vertebrates **(Table 2)**.

The phylogenetic tree of hedgehogs comprised two main clusters: fish clusters and bird and mammal clusters **(Figure 3)**. The deduced H-FABP amino acid sequences of nine fish species and other vertebrates contained the lipocalin domain and all of them showed high identity and similarity **(Figure 4)**.

Ontogenetic expression of H-FABP gene in golden pompano larvae

The expression of H-FABP gene in golden pompano larvae at hatch was relative high and reached the highest level on 1 DPH **(Figure 5)**. Subsequently, the expression of H-FABP reduced significantly and reached the lowest level on 3 DPH when fish commenced exogenous feeding. Staring from 4 DPH, the expression of H-FABP increased and remained at a similar level until the experiment was completed **(Figure 5)**.

Tissue expression of H-FABP gene in golden pompano

On 18 DPH, the highest expression level of H-FABP was observed in the heart of larval golden pompano, followed by stomach and liver **(Figure 6)**. The expression of H-FABP can be detected at brain, eye, gill, head-kidney, muscle, spleen, and intestine, but the expression levels were low compared to that observed in the heart ($P<0.05$, **Figure 6**).

Table 2 Identity and similarity between golden pompano *H-FABP* with *H-FABP* from other species homologue.

Species	Accession No.	AA	Similarity (%)	Identity (%)
Trachinotus ovatus	Present study	133	-	-
Oryzias latipes	XP_004074237.1	133	96.2	87.2
Fundulus heteroclitus	NP_001296909.1	132	93.2	86.5
Lateolabrax japonicus	AOW69621.1	133	92.5	85
Sparus aurata	AFV39808.1	133	93.2	85.7
Salmo salar	ACI69211.1	133	88.7	75.9
Oncorhynchus mykis	NP_001118185.1	133	87.2	76.7
Danio rerio	NP_694493.1	133	87.2	69.2
Bos taurus	NP_776738.1	133	83.5	71.4
Gallus gallus	AFP43354.1	133	84.2	71.4
Ovis aries	NP_001254813.1	133	82	69.9
Homo sapiens	NP_004093.1	133	82.7	67.7
Mus musculus	NP_034304.1	133	80.5	63.2
Rattus norvegicus	NP_077076.1	133	81.2	64.7

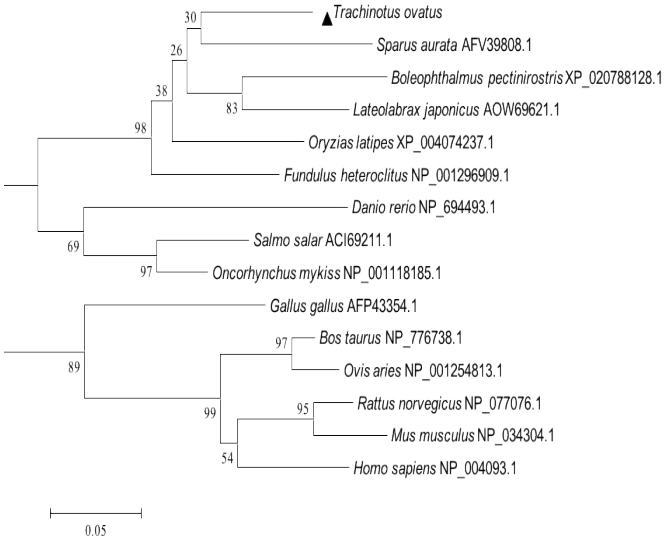

Figure 3 Phylogenetic tree of heart-type fatty acid binding protein. The numbers represent the frequencies with which the tree topology presented here was replicated after 1000 bootstrap iterations

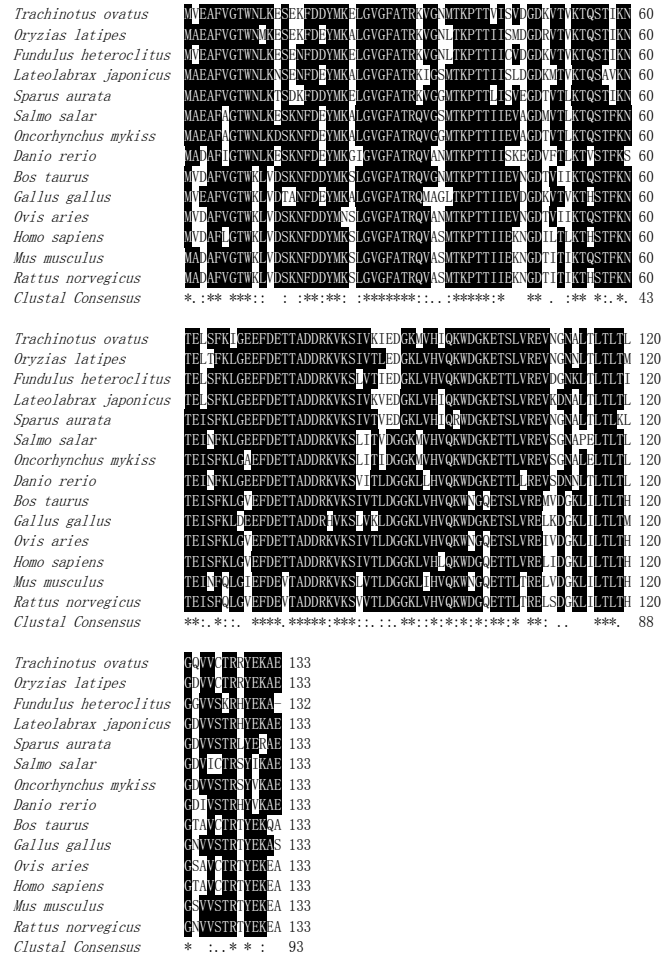

Figure 4 Multiple sequence alignment of the deduced amino acid sequence of H-FABP with other known homologous H-FABP amino acid sequence.

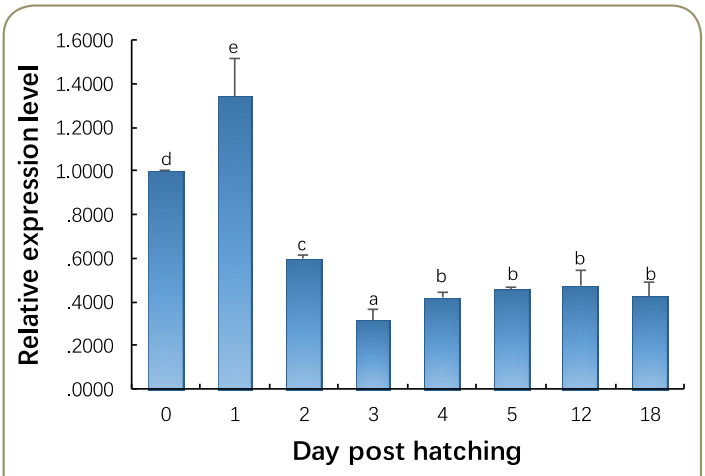

Figure 5 Ontogenetic expression of *H-FABP* in golden pompano larvae.

Response of H-FABP gene to water temperature

In this study, the expression of H-FABP was significantly affected by the rearing temperature (P<0.05, **Figure 7**). On 12 DPH, the highest expressions of H-FABP were observed when fish were at 23°C and 26°C and lowest expression occurred in fish at 29°C (P<0.05). On 18 DPH, the highest expression of H-FABP was observed in fish at 29°C (**Figure 7**), and the expression level of H-FABP was not significantly different between fish at 23°C and 26°C (P>0.05).

Response of H-FABP gene to nutrition manipulation

Dietary fatty acid composition was presented in **Appendix 1**. On 18 DPH, the expression level of H-FABP was significantly affected by nutrition manipulation (P<0.05, **Figure 8**). The highest expression of H-FABP was observed in fish fed Algamac 3080 enriched *Artemia nauplii* and the lowest expression of H-FABP was found in fish fed *Nannochloropsis* sp. enriched *Artemia nauplii*. The correlation coefficients between fatty acids and H-FABP expression are presented in **Table 3**. The expression of H-FABP was negatively correlated with docosahexaenoic acid/eicosapentaenoic acid (DHA/EPA; r=-0.75, P<0.05) and positively correlated with eicosapentaenoic acid/arachidonic acid (EPA/ARA; r=0.67, P<0.05, **Table 3**).

Discussion

The present study cloned and analyzed the expression of H-FABP genes during the early development of golden pompano. The H-FABP cDNA from golden pompano included a 5'-UTR, a 3'-UTR and an ORF and were composed of a lipocalin domain encoding a 133 aa polypeptide. The deduced protein sequence had a conserved domain of iLFBPs in the lipocalin superfamily. Moreover, the characteristic of an intracellular protein is confirmed by the absence of predicted signal peptide sequences and transmembrane domains in golden pompano H-FABP. The

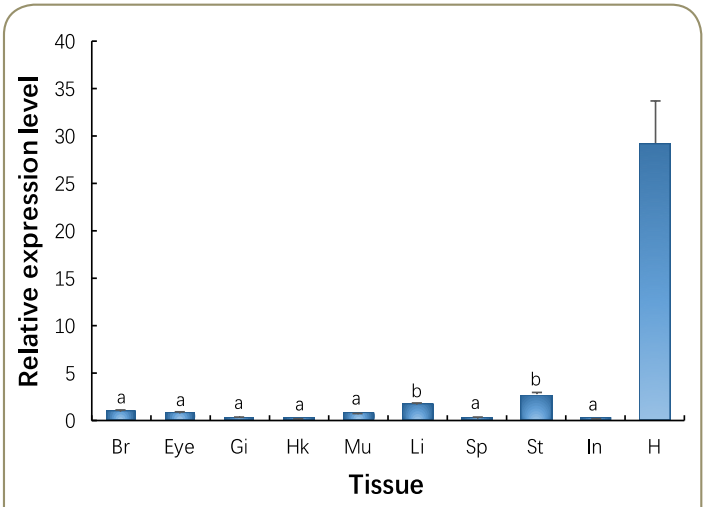

Figure 6 Expression of H-FABP in golden pompano larvae after 18 DPH in different tissues.
Br: Brain; Gi: Gill; Hk: Head-kidney; Mu: Muscle; Li: Liver; Sp: Spleen; St: Stomach; In: Intestine; H: Heart
«a» group data are significantly different to « b » group data. Expression of H-FABP in heart is significantly different compared to « a » and « b » group data

predicted H-FABP three-dimensional structure reinforced the highly conserved structure of the FABP family [15].

The multi-sequence alignment revealed that the H-FABP of golden pompano shared high identity to the orthologs of other teleost (69.2%-87.2% for the listed species in **Figure 1**) and other vertebrates (63.2%-71.4%). The phylogenetic tree **(Figure 3)** showed that the golden pompano H-FABP clustered closest to the Japanese rice fish (*Oryzias latipes*) and the mummichog (*Fundulus heteroclitus*) as opposed to the zebra fish (*Danio rerio*) which is furthest but still relatively close (with 87.2% of similarity and 69.2% of identity). Several other studies have identified H-FABP in fishes such as lamprey, mummichog, chimaera, zebra fish [19,20,36]. Sequence analysis comparisons suggest that FABP gene is highly conserved across some fish species [1]. Our data suggest that these H-FABPs have diverged from a common ancestor, thus having most likely similar role in the organisms. This indicates that H-FABPs functions in golden pompano are comparable with those reported H-FABPs in other teleost and vertebrates. Highlighting the divergence from a common ancestor before the fish and mammals ramification, the phylogenetic tree also provided new evidence for the hypothesis that the individual members of the subfamilies branched out after fish and mammalian divergence.

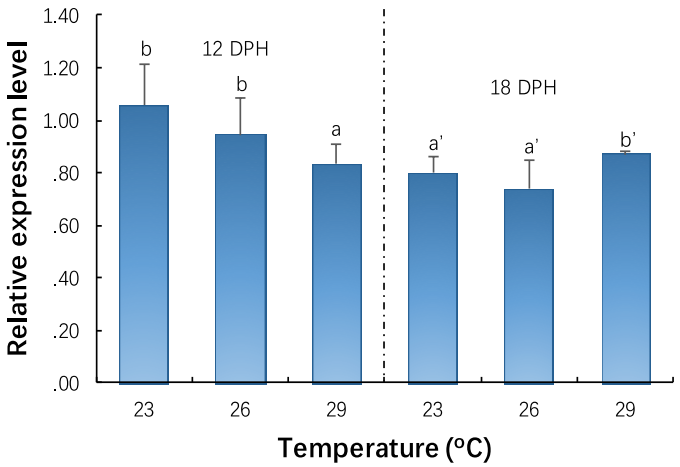

Figure 7 Expression of *H-FABP* on 12 and 18 DPH at different water temperature.

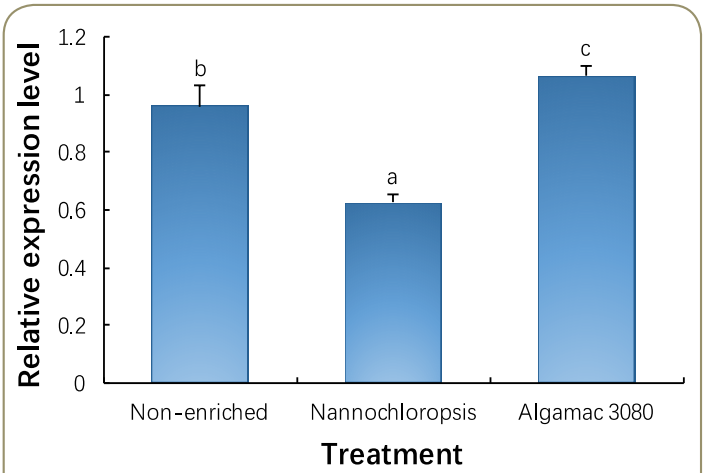

Figure 8 Response of H-FABP to different nutrition enhancements in golden pompano larvae after 18 DPH.

As described by Ma et al. [30], the developmental period of golden pompano larvae, like most of the marine fishes, is subdivided into 3-stages depending on its morphological characteristics. Stage 1 starts at hatching and ends just before the beginning of exogenous feeding on 2 DPH. At the end of the stage 1, the mouth is opened and the gut is well developed to accept exogenous food. Stage 2 starts from first exogenous feeding on 3 DPH and finishes by the formation of gastric glands. This is a crucial period for the fish survival because of the need to develop exogenous feeding skills to prevent starvation. Stage 2 corresponds to a period of mixed nutrition with a gradual transfer from endogenous nutrition to sole exogenous nutrition. During this phase, golden pompano digestive system undergoes an important structural and functional development to adapt to exogenous feeding before the depletion of yolk-sac reserves. The stage 3 starts with the appearance of gastric glands and the pyloric caeca in the stomach. From hatching to 18 DPH, the expression of H-FABP was assessed and was highest on 1 DPH. This period corresponds to the highest energy consumption, which decreases sharply until 3 DPH, in line with the start of exogenous feeding. This suggests that H-FABP is highly required for larvae to start exogenous feeding [37].

FABPs are involved in modulation of cell growth, proliferation, antioxidant activities, inflammation, and neurodegenerative disorders and also participate in immune pathways mediated by fatty acids [38-41]. H-FABP family shows the widest range of tissue-specific distribution of the iLBPs multigene family. Mammalian H-FABP is found in heart, skeletal and muscle, brain, kidney, stomach, lactating mammary gland, ovary, testis and placenta, but is absent in the liver, white fat and intestine. In fish on the other hand, H-FABP is mainly expressed in the liver and ovaries [22-24]. Golden pompano H-FABP was mainly expressed in the stomach and liver but was barely expressed in brain, eyes, gill, head-kidney, muscles, spleen and intestine. Vayda et al. previously described a similar expression pattern in four Antarctic teleost fish species exhibiting a high mRNA level in the heart, muscle and brain, but not in the liver [21]. Such results suggest that H-FABP gene expression is likely species-specific. The difference in expression patterns of H-FABPs between mammals and fish is likely due to evolutional divergence in transcription regulation [4]. This present study may provide new evidence of divergence between groups of fish species leading to altered patterns of gene expression [12].

Temperature is an important factor regulating the vital rate of marine fish during early life history [42,43]. Changes in temperature can affect enzyme activity, metabolism, growth and development in fish [44]. In aquaculture, temperature influences the size of fish larvae at hatch, yolk absorption, growth, feeding and digestion [45]. In this study, the relative expression of H-FABP was different between temperature treatments and dates post hatch. Indeed, the 12 DPH larvae expressed significantly more H-FABP at 23 and 26°C than at 29°C. On the contrary, larvae at 18

Table 3 Spearman rank correlation coefficients among the response variables (*P<0.05; **P<0.01).

	Treatment	DHA	EPA	ARA	DHA/EPA	EPA/ARA	DHA/ARA	*H-FABP*
DHA		1.00	0.66	-0.64	0.63	0.68*	0.95**	-0.03
EPA			1.00	-0.81**	-0.15	0.99**	0.82**	0.65
ARA				1.00	-0.02	-0.89**	-0.83**	-0.62
DHA/EPA					1.00	-0.11	0.41	-0.75*
EPA/ARA						1.00	0.86**	0.67*
DHA/ARA							1.00	0.24
H-FABP								1.00

Abbreviations: DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid; ARA: Arachidonic Acid

DPH expressed more H-FABP at 29°C. At both 12 DPH and 18 DPH, golden pompano larvae were able to take exogenous feed. The difference of H-FABP expression at 12 DPH and 18 DPH between temperature treatments may be due to improved food ingestion and digestive function of larvae after 15 DPH. Ma et al. [30] found that the goblet cells and gastric glands formed in the gut of golden pompano larvae after 15 DPH at 27-29°C. The cytoplasmic lipid binding proteins transfer lipidic ligands into nuclei to initiate transcriptional activity and provide lipid signaling pathways for lipid catabolism and storage [46]. A change in temperature could lead to a reduction in H-FABP expression. The influence of temperature on the expression of H-FABP would impact lipid transport and storage in tissues [31].

Temperature influences feed intake, nutrient utilization and fish growth [28,43]. Tutman et al. [28] demonstrated that feed intake in golden pompano is linked to water temperature. Juveniles of same size showed significantly higher growth rate at 23.7-29.4°C compared to that in cold-water (<15°C). Variations in temperature greatly affect fish development, which in turn, impacts pelagic larval duration and time exposure to predators [42]. Additionally, at high temperature, while the yolk-sac absorption is faster, the period of endogenous feeding becomes shortened [45]. Therefore, our results may serve as an indicator of temperature adaptation in early life for golden pompano for growth and survival in aquaculture.

This study assessed the influence of three feed types on H-FABP expression. During larval stage, the food uptake follows a precise feeding behavior to control the quantity and type of food ingestion [31]. Good knowledge of the larval nutritional requirements would contribute to improving feeding efficiency and fish quality [47]. Unlike adult fish, feeding success depends on development of physiological functions of the digestive system [31]. In this study, the expression of H-FABP in fish larvae on 18 DPH was affected by nutrient manipulation. The expression level was highest when Algamac 3080 enriched *Artemia nauplii* were used. It was lowest when fish were fed with *Nannochloropsis* sp. enriched *Artemia nauplii*. This may suggest that H-FABP in fish larvae on 18 DPH was sensitive to nutrient enhancement Feeding of Algamac 3080 enriched *Artemia nauplii* is likely the most suitable feeding regime according to the level of H-FABP expression. Considering the importance of lipid accumulation during larval development, it is likely that Algamac 3080 enriched *Artemia nauplii* could lead to the highest expression of H-FABP and therefore lipids transport, storage and accumulation would support better good larval development [31]. Moreover, correlation analysis between the expression of H-FABP and fatty acids showed that only DHA/EPA (negatively correlated) and EPA/ARA (positively correlated) ratios affected significantly the expression of H-FABP in larval fish. DHA and ARA are major components in the brain and because they cannot be dissolved in water, but need to bind with FABPs within the cytoplasm for transportation to various tissues [48-50]. Our results suggest that fatty acids elements such as DHA or EPA by itself do not have a significant effect on the H-FABP expression in larval fish.

Conclusion

In this study, we examined the regulation of nutrition and temperature on the expression of H-FABP gene in golden pompano to understand importance of protein development in fish larvae. Results from this study indicate that changes in environmental temperature and feed type influence H-FABP expression, growth and survival of golden pompano in early life. The results of this study provided new understanding on the role of H-FABPs in regulating fish growth and as an indicator for fish development in hatcheries at different environmental conditions and nutrition supply.

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