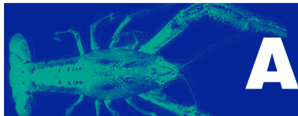
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# Molecular cloning, tissue distribution and nutritional regulation of a fatty acyl *elovl5-like* elongase in large yellow croaker, *Larimichthys crocea*

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## Abstract

In this study, the full-length cDNA of fatty acyl *elovl5-like* elongase was first cloned from large yellow croaker, *Larimichthys crocea*. The cDNA of *elovl5-like* was 1551 bp, including a 5′-terminal untranslated region (UTR) of 120 bp, a 3′-terminal UTR of 546 bp and an open reading frame (ORF) of 885 bp encoding a polypeptide of 294 amino acid residues. Sequence comparison showed that the predicted protein revealed a high percentage identity (>80%) with Elovl5 from other marine fish species. Tissue distribution analysis revealed that the *elovl5-like* was expressed highly in liver, brain and gill, and at much lower levels in stomach, intestine, heart and spleen. Quantity polymerase chain reaction showed that hepatic *elovl5-like* transcription decreased significantly with the increase in dietary n-3LC-PUFA ($P < 0.05$). The mRNA levels of *elovl5-like* in the liver of fish fed diets with 0.15%, 0.60% and 0.98% n-3LC-PUFA were up-regulated by 1.77-fold, 1.41-fold and 1.41-fold than that with 2.25% n-3LC-PUFA respectively. No significant differences were observed in the hepatic mRNA levels of *elovl5-like* in response to the increased ratio of dietary DHA/EPA ($P > 0.05$). These results demonstrate for the first time the presence and nutritional modulation of *elovl5-like* cDNA in large yellow croaker. This could contribute to better understanding the process of n-3LC-PUFA biosynthesis in this fish species.

**Keywords:** large yellow croaker, *elovl5-like*, nutritional regulation, n-3 LC-PUFA, DHA/EPA

## Introduction

Large yellow croaker, *Larimichthys crocea*, is an important carnivorous marine fish species that is being widely cultured in southeast China. Recently, low retention of n-3 LC-PUFA has been observed after large proportion of fish oil was replaced by vegetable oil, which seriously affected fillet quality (Duan, Mai, Shentu, Ai, Zhong, Jiang, Zhang, Zhang & Guo 2014). This was largely attributed to their less capacity to biosynthesize LC-PUFA from linolenic acid (18:3n-3, LNA) and linoleic acid (18:2n-6, LA) compared with the fresh water counterparts (Ghioni, Tocher, Bell, Dick & Sargent 1999; Torstensen, Bell, Rosenlund, Henderson, Graff & Tocher 2005). Thus, there is great interest in elucidating the LC-PUFA biosynthesis pathway and regulation mechanism in this fish species.

Two categories of rate-limiting enzymes, fatty acid desaturase (Fads) and elongase (Elovl), have been found to be involved in LC-PUFA biosynthesis of fish species. Among the fatty acid elovl proteins, Elovl5 showed high activity towards $C_{16}$, $C_{18}$ and $C_{20}$ substrates, but a low activity towards $C_{22}$ LC-PUFA. Elovl2, which could elongate $C_{22}$ LC-PUFA to $C_{24}$ counterparts, has been found to exist in freshwater rather than marine fish species. Elovl4 has been found to show activity towards LC-PUFA with chain lengths ≥24 carbons in several freshwater and marine fish species (Monroig, Webb, Ibarra-Castro, Holt & Tocher 2011). Up to date, *elovl5* have been successfully cloned from a variety of teleost fish species, such as Atlantic salmon (*Salmo salar*) (Hastings, Agaba, Tocher, Zheng, Dickson, Dick & Teale 2005; Morais, Monroig,

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Zheng, Leaver & Tocher 2009), common carp (*Cyprinus carpio*) (Ren, Yu, Xu & Tang 2012), bluefin tuna (*Thunnus maccoyii*) (Gregory, See, Gibson & Schuller 2010), meagre (*Argyrosomus regius*) (Monroig, Tocher, Hontoria & Navarro 2013) and rainbow trout (*Oncorhynchus mykiss*) (Gregory & James 2014). Also, *elovl2-like* have been recently reported in Atlantic salmon (Morais *et al*. 2009), *elovl2* in rainbow trout (Gregory & James 2014), *elovl4 in* Atlantic salmon (Carmona-Antoñanzas, Monroig, Dick, Davie & Tocher 2011), and *elovl4-like* in cobia (*Rachycentron canadum*) (Monroig *et al*. 2011) and white-spotted rabbitfish (*Siganus canaliculatus*) (Monroig, Wang, Zhang, You, Tocher & Li 2012).

Transcription of *elovl* and *fads* could be regulated by developmental stage (Ishak, Tan, Khong, Jaya-Ram, Enyu, Kuah & Shu-Chien 2008; Monroig, Rotllant, Sánchez, Cerdá-Reverter & Tocher 2009; Monroig, Rotllant, Cerdá-Reverter, Dick, Figueras & Tocher 2010; Tan, Chung & Shu-Chien 2010; Morais, Mourente, Ortega, Tocher & Tocher 2011), environmental factors (salinity and water temperature) (Zheng, Torstensen, Tocher, Dick, Henderson & Bell 2005; Fonseca-Madrigal, Pineda-Delgado, Martinez-Palacios, Rodriguez & Tocher 2012) as well as dietary lipid and fatty acid, especially LC-PUFA according to previous studies on teleosts (Zheng *et al*. 2005; Ling, Kuah, Sifzizul, Muhammad, Kolkovski & Shu-Chien 2006; Jaya-Ram, Kuah, Lim, Kolkovski & Shu-Chien 2008; Morais *et al*. 2011). Also, hepatic *elovl5* could be regulated by two nuclear receptor, liver X receptors (LXRs) and the transcription factors sterol regulatory element binding proteins (Qin, Dalen, Gustafsson & Nebb 2009). Furthermore, dietary n-3 LC-PUFA could reduce Δ6-FAD promoter activity by increasing the methylation rate of the promoter region of this gene, and thus suppress LC-PUFA synthesis in Atlantic salmon (Zheng, Leaver & Tocher 2009) and Japanese seabass (Xu, Dong, Ai, Mai, Xu, Zhang & Zuo 2014).

During the past decade, numerous studies have been conducted to investigate nutrient requirement, metabolism and nutritional immunology in juvenile large yellow croaker (Ai, Mai, Tan, Xu, Duan, Ma & Zhang 2006; Ai, Mai, Zhang, Tan, Zhang, Xu & Li 2007; Ai, Zhao, Mai, Xu, Tan, Ma & Liufu 2008; Ai, Xu, Mai, Xu, Wang & Zhang 2011; Zuo, Ai, Mai, Xu, Wang, Xu, Liufu & Zhang 2012a,b; Zuo, Ai, Mai & Xu 2013). However, as far as we know, little information was available about the molecular basis of LC-PUFA biosynthesis in large yellow croaker. Recently, a *Δ6-fad-like* has been successfully cloned from large yellow croaker (Zuo, Mai, Xu, Dong & Ai 2014). Basic information about Fads and Elovl is not only of high relevance in advancing our understanding of molecular basis of LC-PUFA biosynthesis and regulation in this fish but also provides possibility for applying transgenic technology in the future (Alimuddin, Yoshizaki, Kiron, Satoh & Takeuchi 2005, 2007; Alimuddin, Kiron, Satoh, Takeuchi & Yoshizaki 2008; Kabeya, Takeuchi, Yamamoto, Yazawa, Haga, Satoh & Yoshizaki 2014). Thus, this study was conducted to investigate cDNA, tissue distribution and mRNA profile of *elovl5* in response to dietary fatty acid composition. It was aimed to find more essential preliminary clues to better understand LC-PUFA biosynthetic process and potential regulation mechanism in large yellow croaker.

## Materials and methods

### Experimental designs and diets

First, five large yellow croaker (246.5 ± 4.8 g) were bought from a commercial farm in Xiangshan bay, Ningbo, China and used for *elovl5* gene isolation and tissue-specificity expression detection. After being anaesthetized with eugenol (1:10 000; Shanghai Reagent, China), seven tissues (brain, kidney, spleen, liver, stomach, intestine, and gill) from five fish were separately collected, flash-frozen in liquid nitrogen and then stored at −80°C for analyzing tissue distribution of *elovl5*.

Then, two feeding experiments were conducted to investigate the hepatic mRNA profiles of *elovl5* in response to graded dietary n-3 LC-PUFA (0.15%, 0.60%, 0.98%, 1.37%, 1.79% and 2.25% dry weight) (Experiment 1, Tables 1 and 2) and DHA/EPA (0.61, 1.54, 2.17, 3.04 and 3.88) (Experiment 2, Tables 3 and 4). Feeds formulation, pellets producing procedures and experimental conditions have been described in detail in our previous studies (Zuo *et al*. 2012a,b). Generally, six diets with graded n-3 LC-PUFA and five diets with graded DHA/EPA were formulated by adding different amounts of DHA-enriched oil, EPA-enriched oil and palmitin. After 2 weeks of acclimation to the experimental conditions and feeds, fish of similar sizes (9.8 ± 0.6 g; mean ± SEM) were distributed into 33 sea cages (1 m × 1 m × 1.5 m), and each cage was stocked with 60 fish. Each diet was randomly allocated to triplicate cages of fish. Fish were hand-fed twice daily (05:00 and 17:00 hours) to apparent satiation for

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**Table 1** Formulation and proximate analysis of the experimental diets with graded levels of n-3 LC-PUFA (% dry weight)

| | Dietary n-3 LC-PUFA (% dry weight) | | | | | |
|---|---|---|---|---|---|---|
| **Ingredients (%)** | **0.15** | **0.60** | **0.98** | **1.37** | **1.79** | **2.25** |
| Defatted white fish meal* | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Soybean meal | 32.00 | 32.00 | 32.00 | 32.00 | 32.00 | 32.00 |
| Casein† | 12.00 | 12.00 | 12.00 | 12.00 | 12.00 | 12.00 |
| Wheat meal | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 |
| Mineral premix‡ | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Vitamin premix§ | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Attractant | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Mold inhibitor | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Lecithin | 2.60 | 2.60 | 2.60 | 2.60 | 2.60 | 2.60 |
| DHA enriched oil¶ | 0.05 | 0.77 | 1.48 | 2.18 | 2.93 | 3.62 |
| EPA enriched oil** | 0.00 | 0.45 | 0.90 | 1.36 | 1.79 | 2.26 |
| Palmitin†† | 7.45 | 6.28 | 5.12 | 3.96 | 2.78 | 1.62 |
| ARA enriched oil‡‡ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |
| Proximate analysis (*n* = 3) | | | | | | |
| Crude protein (%) | 41.27 | 41.21 | 40.99 | 42.08 | 41.42 | 41.36 |
| Crude lipid (%) | 11.37 | 11.37 | 11.29 | 11.03 | 11.18 | 10.98 |
| n-3 LC-PUFA (% dry weight) | 0.15 | 0.60 | 0.98 | 1.37 | 1.79 | 2.25 |

*Defatted fish meal: 79.1% crude protein and 1.6% crude lipid; white fish meal were defatted with ethanol (fish meal:ethanol = 1:2 (w:v)) at 37°C for three times.

†Casein: 93% crude protein and 1% crude lipid, Alfa Aesar, Avocado Research Chemicals, UK.

‡Mineral premix (mg or g kg$^{-1}$ diet): $CuSO_4 \cdot 5H_2O$, 10 mg; $Na_2SeO_3$ (1%), 25 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $FeSO_4 \cdot H_2O$, 80 mg; Ca $(IO_3)_2$, 180 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; zeolite, 18.35 g.

§Vitamin premix (mg or g kg$^{-1}$ diet): vitamin D, 5 mg; vitamin K, 10 mg; vitamin $B_{12}$, 10 mg; vitamin $B_6$, 20 mg; folic acid, 20 mg; vitamin $B_1$, 25 mg; vitamin A, 32 mg; vitamin $B_2$, 45 mg; pantothenic acid, 60 mg; biotin, 60 mg; niacin acid, 200 mg; α-tocopherol, 240 mg; inositol, 800 mg; ascorbic acid, 2000 mg; microcrystalline cellulose, 16.47 g.

¶DHA enriched oil: DHA content, 270.3 mg g$^{-1}$ oil; EPA content, 6.5 mg g$^{-1}$ oil; both in the form of methylester; Hubei Youzhiyou Biotechnology, China.

**EPA enriched oil: EPA content, 301.2 mg g$^{-1}$ oil; DHA content, 157.8 mg g$^{-1}$ oil; both in the form of triglyceride; HEBEI HAIYUAN Health Biological Science and Technology, China.

††Palmitin: Palmitic acid content, 99.3% of TFA, in the form of methylester; Shanghai Dinghua Chemical, China.

‡‡ARA enriched oil: ARA content, 348.1 mg g$^{-1}$ oil; in the form of ARA-methylester; Hubei Youzhiyou Biotechnology, China.

58 days. During the experimental period, the water temperature, salinity and dissolved oxygen were measured daily during the experimental period. The water temperature ranged from 21.5 to 30.0°C, and salinity from 32‰ to 36‰. The dissolved oxygen was approximately 7 mg L$^{-1}$. At the termination of the experiment, the fish were fasted for 24 h before harvest. Liver from five fish in each cage were sampled and pooled together into 1.5 mL tube (RNAase-Free, Axygen, Tewksbury, MA, USA), frozen in liquid nitrogen and then stored at −80°C for the analysis of expression profiles of Elovl5 in response to dietary n-3 LC-PUFA and DHA/EPA.

## RNA extraction and cDNA synthesis

Total RNA from all samples above was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) and electrophoresed on a 1.2% denaturing agarose gel to test the integrity. Then, RNA was treated with RNA-Free DNase (Takara, Dalian, China) to remove DNA contaminant and reverse transcribed to cDNA by PrimeScript™ RT reagent Kit (Takara) following the instructions.

## Cloning and sequencing of elovl cDNA fragment

Two degenerate primers (*elovl* 01 and *elovl* 02, Table 5) were designed to clone the middle fragment by polymerase chain reaction (PCR). Liver cDNA was used as a template for amplification. PCR was performed using primers *elovl* 01 and *elovl* 02, with 1 cycle of denaturation at 94°C for 3 min, 35 cycles of 94°C for 30 s, 52.6°C annealing for 30 s and 72°C for 60 s, followed by a 10 min extension at 72°C. All PCR products

**Table 2** Fatty acid composition of the experimental diets with graded levels of n-3 LC-PUFA (% total fatty acids)*

| | Dietary n-3 LC-PUFA (% dry weight) | | | | | |
|---|---|---|---|---|---|---|
| Fatty acid | 0.15 | 0.60 | 0.98 | 1.37 | 1.79 | 2.25 |
| 14:0 | 0.57 | 1.03 | 1.79 | 2.23 | 2.80 | 3.53 |
| 16:0 | 70.55 | 64.25 | 60.40 | 48.67 | 39.77 | 31.16 |
| 18:0 | 2.71 | 2.67 | 2.58 | 2.75 | 2.87 | 2.91 |
| 20:0 | 0.43 | 0.48 | 0.49 | 0.66 | 0.73 | 0.84 |
| ∑SFA | 74.26 | 68.43 | 65.26 | 54.30 | 46.18 | 33.43 |
| 16:1 | 0.77 | 0.73 | 0.78 | 0.77 | 0.82 | 0.86 |
| 18:1 | 5.99 | 6.15 | 6.34 | 6.90 | 7.14 | 7.60 |
| ∑MUFA | 6.76 | 6.88 | 7.12 | 7.67 | 7.96 | 8.46 |
| 18:2n-6 | 12.27 | 12.74 | 12.87 | 13.81 | 13.99 | 14.53 |
| 20:4n-6 | 3.37 | 3.65 | 3.46 | 4.23 | 4.39 | 4.61 |
| ∑n-6 PUFA† | 15.64 | 16.40 | 16.33 | 18.04 | 18.38 | 19.14 |
| 18:3n-3 | 1.27 | 1.31 | 1.34 | 1.47 | 1.50 | 1.64 |
| 20:5n-3 | 0.48 | 1.71 | 2.79 | 4.86 | 6.32 | 8.05 |
| 22:6n-3 | 0.91 | 3.54 | 5.00 | 9.44 | 12.68 | 15.71 |
| ∑n-3 PUFA‡ | 2.66 | 6.56 | 9.14 | 15.78 | 20.51 | 25.39 |
| n-3/n-6PUFA | 0.17 | 0.40 | 0.56 | 0.87 | 1.11 | 1.33 |
| n-3LC-PUFA§ | 1.39 | 5.25 | 7.79 | 14.31 | 19.01 | 23.75 |
| DHA/EPA¶ | 1.90 | 2.02 | 1.93 | 1.94 | 2.01 | 1.95 |

*Some fatty acids, of which the contents are minor, trace amount or not detected, such as C22:0, C24:0, C14:1, C20:1n-9, C22:1n-11, C20:2n-6, C20:3n-6, C22:5n-3, were not listed in the table.
†n-6 PUFA: n-6 poly-unsaturated fatty acids.
‡n-3 PUFA: n-3 poly-unsaturated fatty acids.
§n-3 LC-PUFA: n-3 highly unsaturated fatty acids.
¶DHA/EPA: 22:6n-3/20:5n-3.
SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids.

were cloned into pEASY-T1 simple cloning vector (Beijing TransGen Biotech, Beijing, China) and sequenced in BioSune (Shanghai, China).

## Rapid amplification of cDNA ends

Based on the partial sequence of *elovl*, the 3′ and 5′ ends were obtained by rapid amplification of cDNA ends (RACE) approaches. The 3′ end RACE PCR reaction was performed with liver cDNA template using the gene-specific primer *elovl* 03 and the adaptor primer RIP (Table 5) following the instructions of a 3′-Full RACE Core Set Ver.2.0 kit (cat. no. D314; TaKaRa). The PCR was carried out according to the programme of 94°C for 3 min, 35 cycles of 94°C for 30 s, 57.5°C annealing for 30 s and 72°C for 60 s, followed by a 10 min extension at 72°C.

The 5′ end of *elovl* cDNA was obtained strictly following the instructions of SMARTerTM RACE cDNA Amplification Kit (cat. no. 634923; Clontech, Mountain View, CA, USA). One specific reverse primer, *elovl* 04 was designed based on the partial sequence amplified by degenerated primers (Table 5). The PCR amplification was performed using universal primer A mix (UPM) and *elovl* 04

**Table 3** Formulation and proximate analysis of the experimental diets with graded ratios of DHA/EPA (% dry weight)

| | Dietary DHA/EPA ratio | | | | |
|---|---|---|---|---|---|
| Ingredients | 0.61 | 1.54 | 2.17 | 3.04 | 3.88 |
| White fish meal* | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 |
| Soybean meal* | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 |
| Wheat meal* | 25.50 | 25.50 | 25.50 | 25.50 | 25.50 |
| Mineral premix† | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Vitamin premix‡ | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Attractant§ | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Mold inhibitor¶ | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Lecithin | 2.60 | 2.60 | 2.60 | 2.60 | 2.60 |
| DHA enriched oil** | 0.10 | 1.15 | 1.78 | 2.20 | 2.52 |
| EPA enriched oil†† | 1.68 | 1.05 | 0.67 | 0.42 | 0.25 |
| Palmitin‡‡ | 3.82 | 3.40 | 3.15 | 2.98 | 2.83 |
| ARA enriched oil§§ | 1.40 | 1.40 | 1.40 | 1.40 | 1.40 |
| Proximate analysis (n = 3) | | | | | |
| Crude protein (%) | 41.40 | 41.31 | 41.19 | 41.08 | 41.42 |
| Crude lipid (%) | 11.22 | 11.27 | 11.19 | 11.03 | 11.38 |
| DHA/EPA ratio | 0.61 | 1.54 | 2.17 | 3.04 | 3.88 |
| n-3 LC-PUFA (%) | 1.07 | 1.04 | 1.02 | 1.04 | 1.03 |

*White fish meal: crude protein 74.3% dry matter, crude lipid 6.6% dry matter; soybean meal: crude protein 49.4% dry matter, crude lipid 0.9% dry matter; wheat meal: crude protein 16.4% dry matter, crude lipid 1.0% dry matter.
†Mineral premix (mg or g $kg^{-1}$ diet): $CuSO_4 \cdot 5H_2O$, 10 mg; $Na_2SeO_3$ (1%), 25 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $FeSO_4 \cdot H_2O$, 80 mg; Ca $(IO_3)_2$, 180 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; zeolite, 18.35 g.
‡Vitamin premix (mg or g $kg^{-1}$ diet): vitamin D, 5 mg; vitamin K, 10 mg; vitamin $B_{12}$, 10 mg; vitamin $B_6$, 20 mg; folic acid, 20 mg; vitamin $B_1$, 25 mg; vitamin A, 32 mg; vitamin $B_2$, 45 mg; pantothenic acid, 60 mg; biotin, 60 mg; niacin acid, 200 mg; α-tocopherol, 240 mg; inositol, 800 mg; ascorbic acid, 2000 mg; microcrystalline cellulose, 16.47 g.
§Attractant: glycine and betaine.
¶Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.
**DHA enriched oil: DHA content, 270.3 mg $g^{-1}$ oil; EPA content, 6.5 mg $g^{-1}$ oil; both in the form of DHA-methylester; Hubei Youzhiyou Biotechnology, China.
††EPA enriched oil: EPA content, 301.2 mg $g^{-1}$ oil; DHA content, 157.8 mg $g^{-1}$ oil; both in the form of triglyceride; HEBEI HAIYUAN Health Biological Science and Technology, China.
‡‡Palmitin: Palmitic acid content, 99.3% of total fatty acids, in the form of methylester; Shanghai Dinghua Chemical, China.
§§ARA enriched oil: ARA content, 348.1 mg $g^{-1}$ oil; in the form of ARA-methylester; Hubei Youzhiyou Biotechnology, China.

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**Table 4** Fatty acid composition of the experimental diets with graded ratios of DHA/EPA (% total fatty acids)*

| | Dietary DHA/EPA ratio | | | | |
|---|---|---|---|---|---|
| **Fatty acid** | **0.61** | **1.54** | **2.17** | **3.04** | **3.88** |
| 14:0 | 1.55 | 1.89 | 2.66 | 2.85 | 3.29 |
| 16:0 | 51.98 | 42.98 | 44.30 | 43.01 | 42.83 |
| 18:0 | 3.43 | 3.60 | 3.55 | 3.68 | 3.57 |
| 20:0 | 1.07 | 1.21 | 1.00 | 1.04 | 0.99 |
| ∑SFA | 58.03 | 49.68 | 51.51 | 50.58 | 50.68 |
| 16:1 | 2.02 | 1.70 | 1.78 | 1.85 | 1.94 |
| 18:1 | 9.71 | 9.54 | 9.27 | 9.34 | 8.86 |
| ∑MUFA | 11.73 | 11.24 | 11.05 | 11.19 | 10.80 |
| 18:2n-6 | 13.30 | 13.33 | 13.45 | 13.45 | 13.56 |
| 20:4n-6 | 4.58 | 5.51 | 5.18 | 5.25 | 5.29 |
| ∑n-6 PUFA† | 17.88 | 18.84 | 18.63 | 18.70 | 18.85 |
| 18:3n-3 | 1.41 | 1.45 | 1.48 | 1.45 | 1.57 |
| 20:5n-3 | 7.51 | 5.06 | 3.60 | 2.90 | 2.39 |
| 22:6n-3 | 4.58 | 7.79 | 7.82 | 8.81 | 9.27 |
| ∑n-3 PUFA‡ | 13.50 | 14.30 | 12.90 | 13.16 | 13.23 |
| n-3/n-6PUFA | 0.76 | 0.76 | 0.69 | 0.70 | 0.70 |
| n-3LC-PUFA§ | 12.09 | 12.85 | 11.42 | 11.71 | 11.66 |
| ARA/EPA¶ | 0.61 | 1.09 | 1.44 | 1.81 | 2.21 |
| DHA/EPA** | 0.61 | 1.54 | 2.17 | 3.04 | 3.88 |

*Some fatty acids, of which the contents are minor, trace amount or not detected, such as C22:0, C24:0, C14:1, C20:1n-9, C22:1n-11, C20:2n-6, C20:3n-6, C22:5n-3, were not listed in the table.

†n-6 PUFA: n-6 poly-unsaturated fatty acids.

‡n-3 PUFA: n-3 poly-unsaturated fatty acids.

§n-3 LC-PUFA: n-3 highly unsaturated fatty acids.

¶ARA/EPA: 20:4n-6/20:5n-3.

**DHA/EPA: 22:6n-3/20:5n-3.

SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids.

according to the programme of 94°C for 3 min, 35 cycles of 94°C for 30 s, 62.4°C annealing for 30 s and 72°C for 60 s, followed by a 10 min extension at 72°C. PCR products were gel-purified, cloned, and sequenced as described above.

### Sequence analysis and phylogenetic analysis

The cDNA sequence of *elovl* was analyzed for similarity with other known sequences using the BLAST program at web servers of NCBI (http://www.ncbi.nlm.nih.gov/BLAST/). The deduced *elovl* amino acid sequence was analyzed with DNAstar. Alignment of multiple sequences was performed using the CLUSTALW program at the European Bioinformatics Institute (http://www.ebi.ac.uk/clustalw/) and Multiple Alignment show (http://www.bio-soft.net/sms/index.html). SMART program (http://smart.emblheidelberg.de/) and PROSITE program (http://kr.expasy.org/prosite/) were used to predict the functional sites or domains in the amino acid sequence. Phylogenetic and molecular evolutionary analyses were conducted according to the amino acid sequences of the selected HSP90s by programmes of CLUSTAL X1.83 and MEGA 4.0 (Tamura, Dudley, Nei & Kumar 2007). An unrooted phylogenetic tree among these species was determined using the neighbour -joining distance method. The relative importance of branching order was evaluated by the bootstrapping method (1000 replications).

### Real-time PCR analysis of elovl5-like expression

Real-time PCR was applied to evaluate tissue distribution of *elovl5-like* in seven different tissues (brain, kidney, spleen, liver, stomach, intestine, and gill) of five fish. Also, hepatic mRNA levels of *elovl5-like* in response to graded dietary n-3 LC-PUFA and DHA/EPA was detected by real-time PCR. First strand cDNA was synthesized as described in section 2.2 and then diluted by 4 times using sterilized double-distilled water. Two primers, *elovl5-like* QF and *elovl5-like* QR (Table 5), were used to amplify a fragment of 98 bp from the liver cDNA and the PCR product was sequenced to verify the specificity of RT-PCR. Two β–actin primers, β–actin-F and β–actin-R (Table 5) were used to amplify a 107 fragment as an internal control to calibrate the cDNA template for corresponding samples.

Real-time RT-PCR was carried out in a quantitative thermal cycler (Mastercycler ep realplex, Eppendorf, Hamburg, Germany). The amplification was performed in a total volume of 25 μL, containing 1 μL of each primer (10 μM), 1 μL of the diluted first strand cDNA product, 12.5 μL of 2× SYBR Premix Ex Taq™II (Takara) and 9.5 μL of sterilized double-distilled water. The real-time PCR program was as follows: 95°C for 2 min, followed by 35 cycles of 95°C for 10 s, 58.7°C for 10 s, and 72°C for 20 s. At the end of each PCR reaction, melting curve analysis was performed to confirm that only one PCR product was present in these reactions. Standard curves were made with five different dilutions (in triplicate) of the cDNA samples and amplification efficiency was analyzed according to the following equation $E = 10^{(-1/\mathrm{Slope})} - 1$. The primer amplification efficiency was 1.100 for *elovl5-like*, 1.073 for β–actin. The abosolute ΔCT value (*elovl5-like*-β–actin) of the slope is

**Table 5** Sequences of PCR primers utilized in this study

| Primers | Sequences (5′–3′) | Annealing temperature (°C) | Sequence information |
|---|---|---|---|
| *elovl 01* | GATRGGTCCCMGAGATCA | 52.6 | RT primer |
| *elovl 02* | CTAACRCRCTACAGTGAG | 52.6 | RT primer |
| *elovl 03* | ACCAGAACGGCTCTCCTGTA | 57.5 | 3′RACE primer |
| *elovl 04* | TGTTTAGGGAGGCACCGAAGTACGAAT | 62.4 | 5′RACE primer |
| RIP | CGCGGATCCTCCACTAGTGATTTCACTATAGG | – | 3′RACE primer |
| UPM (short) | CTAATACGACTCACTATAGGGC | – | 5′RACE primer |
| UPM (long) | CTAATACGACTCACTATAGGGCAAGCAGTGGT ATCAACGCAGAGT | – | 5′RACE primer |
| elovl-like QF | ATCACCTTCCTTCACATCTATCACC | 58.7 | qPCR primer |
| elovl-like QR | GAGGCACCGAAGTACGAATGG | 58.7 | qPCR primer |
| β-actin F | TTATGAAGGCTATGCCCTGCC | 57.5 | Inner control |
| β-actin R | TGAAGGAGTAGCCACGCTCTGT | 57.5 | Inner control |

0.027, which is close to zero and indicate that ΔΔCT calculation for the relative quantification of target genes can be used. To calculate the expression of *elovl5-like*, the comparative CT method ($2^{-\Delta\Delta t}$ method) was used as described by Yao, Kong, Wang, Ji, Cai, Liu and Han (2008).

### Statistical analysis

All data were subjected to a one-way ANOVA and differences between means were tested by Tukey's multiple range test. The level of significance was chosen at $P < 0.05$ and the results were presented as means ± SEM. All statistical analyses were performed by SPSS 16.0 (SPSS Incorporation, Chicago, IL, USA) for Windows.

## Results

### Sequence analyses of elovl cDNAs

The cDNA fragment of 884b was amplified by the degenerated primers *elovl* 01 and *elovl* 02 and its nucleotide sequence was homologous to other known *elovl5* from fish. Two end fragments were amplified by 3′-RACE and 5′-RACE PCR respectively. The complete cDNA sequence of this gene was obtained by overlapping these fragments mentioned above. The full-length sequence of *elovl* was deposited in Genbank under the accession no. JQ320377. The complete sequence of *elovl* mRNA and the deduced amino acids were shown in Figure 1. The full-length cDNA sequence of *elovl* was shown to be 1551 bp with a 5′-UTR of 120 bp and 3′-UTR of 546 bp. Analyses by DNAstar indicated that cDNA included an open reading frame of 885 bp, which encoded a polypeptide of 294 amino acids with predicted molecular mass of 35.06 kDa and theoretical isoelectric point of 9.19.

### Multiple sequences alignment and phylogenetic analysis

The deduced amino acid sequences of *elovl* were analyzed using BLAST and results showed that it shared higher homologies with other fatty acid Elovl proteins from marine fish, such as *Nibea mitsukurii* (99%), *Argyrosomus regius* (99%), *Dicentrarchus labrax* (95%), *Thunnus thynnus* (94%), *Rachycentron canadum* (93%), *Sparus aurata* (93%), *Solea senegalensis* Elovl5 (85%), *Oreochromis niloticus* Elovl5 (84%), *Salmo salar* Elovl5 (83%), more than 60% identity with *Homo sapiens* Elovl5 isoform-1 (70%) and isoform-2 (64%), but less than 60% identity with other elongase families, such as *Homo sapiens* Elovl1 (38%), Elovl2 (59%), Elovl3 (29%), Elovl4 (43%), Elovl6 (32%) and Elovl7 (45%) (Fig. 2).

To reveal the phylogeny of *elovl* cloned in this study, a tree was constructed using the programmes of CLUSTAL X 1.83 and MEGA 4.0. The Elovl belongs to the branch of Elovl5, which were separated from branches of other Elovl sub-class in the tree. In the branch of Elovl5, all marine fish species and freshwater fish species were clustered together and formed a sister group to the branch of other vertebrates, including frog and some mammal species such as rat and pig (Fig. 3).

### Tissue expression of Elovl5-like

The constitutive expressions of *elovl5*-like in seven tissues of five fish were separately confirmed by

```
1       ACATGGGGACACACACACATATACACACAAACACACACACACACACACACACGCAGCTGGATCTGAAACATTTCTCTCTGTACCTCTA
91      AGAAGCCGCTGGGTGACTTTATGGTGACAAATGGAGACCTTCAATCATAAACTGAACACTTACTTAGAGTCATGGATGGGTCCCAGAGAT
                                        M  E  T  F  N  H  K  L  N  T  Y  L  E  S  W  M  G  P  R  D
181     CAGCGTGTGCGGGGATGGCTACTGCTCGACAACTACCCACCAACCTTTGCACTCACAGTCATGTACCTTGTGATCGTGTGGATGGGGCCC
21       Q  R  V  R  G  W  L  L  L  D  N  Y  P  P  T  F  A  L  T  V  M  Y  L  V  I  V  W  M  G  P
271     AAGTACATGAAACACAGGCAGCCATACTCCTGCAGGGGCCTCCTGGTGCTCTACAATCTGGGCCTCACACTCTTGTCCTTCTACATGTTC
51       K  Y  M  K  H  R  Q  P  Y  S  C  R  G  L  L  V  L  Y  N  L  G  L  T  L  L  S  F  Y  M  F
361     TATGAGCTTGTTACCGCTGTGTGGCATGGTGGCTACAACTTCTACTGCCAGGACATTCACAGTGCACAGGAAGTGGATAATAAGATCATA
81       Y  E  L  V  T  A  V  W  H  G  G  Y  N  F  Y  C  Q  D  I  H  S  A  Q  E  V  D  N  K  I  I
451     AATGTCCTGTGGTGGTACTACTTCTCCAAGCTCATCGAGTTCATGGACACCTTTTTCTTCATACTACGAAAGAATAATCACCAGATCACC
111      N  V  L  W  W  Y  Y  F  S  K  L  I  E  F  M  D  T  F  F  F  I  L  R  K  N  N  H  Q  I  T
541     TTCCTTCACATCTATCACCATGCTAGTATGCTGAACATCTGGTGGTTTGTTATGAACTGGGTACCCTGCGGCCATTCGTACTTCGGTGCC
141      F  L  H  I  Y  H  H  A  S  M  L  N  I  W  W  F  V  M  N  W  V  P  C  G  H  S  Y  F  G  A
631     TCCCTAAACAGCTTCGTCCACGTCGTGATGTATTCTTATTACGGCCTCTCGGCCATCCCAGCCATGCGGCCGTACCTTTGGTGGAAGAAG
171      S  L  N  S  F  V  H  V  V  M  Y  S  Y  Y  G  L  S  A  I  P  A  M  R  P  Y  L  W  W  K  K
721     TACATCACACAGTTACAGCTGACCCAGTTCTTTTTAACCATGTCCCAGACTTTGTGCGCAGTCGTTTGGCCATGTGGCTTCCCCATGGGA
201      Y  I  T  Q  L  Q  L  T  Q  F  F  L  T  M  S  Q  T  L  C  A  V  V  W  P  C  G  F  P  M  G
811     TGGCTGTACTTCCAAATAAGCTACATGGTCACGCTTATTTTCCTTTTCTCAAACTTCTACGTTCAGACTTACAAGAAGCACAGTGTGTCT
231      W  L  Y  F  Q  I  S  Y  M  V  T  L  I  F  L  F  S  N  F  Y  V  Q  T  Y  K  K  H  S  V  S
901     CTAAAGAAGGAGCACCAGAACGGCTCTCCTGTATCAACAAATGGACATGCAAATGGGACGCCATCTTTGGAGCACGCTGCACACAAGAAA
261      L  K  K  E  H  Q  N  G  S  P  V  S  T  N  G  H  A  N  G  T  P  S  L  E  H  A  A  H  K  K
991     CTGAGGGTGGATTGACATTTGAGAAACCGTGCACACAATTCTCACTGTAGCGCGTTAGCTAATGCTGCTAGGAGGTATATGTATCTTATC
291      L  R  V  D
1081    TAGAATAGTCTTGCACTTGAGATGAAAAATAAGCCATAGCCTCATACATCCAGAGACTTTCCATGTTTTTACACACGTTCCTACTCATAG
1171    GTATTGAATTATTCAATTAATATAGTTAAAGGAGAAGATTATTGTAGTATGGTTGACACTGCACAATATTGCCTCCCATAACCTCTAGGG
1261    GAAATTCACTCCGAAGTAAAAATAAAAAAATCTCTTTCTTGCTACCAGCAAACAAACAAACAAACAAACAAACAAACAAACAAACAAACA
1351    CACATTCTGACTCATTCAATGATGCTTTACGCACAGAAGGTCCAAAGATGAGCTCCAGTGAGTGTGTTGGCAGCTGAAGGTTCCATCACA
1441    CTCGATGACATCTCAACATCATGTCTCAGCAACACATGGGACAAATAAAATAACACAGCAGCTTACTATGATCCTTAATGTGTAACTAGA
1531    AAAAAAAAAAAAAAAAAAAAA
```

**Figure 1** Nucleotide and deduced amino acid sequences of *elovl5-like*. The nucleotides and amino acids are numbered along the left margin respectively. The start (ATG) and stop (TGA) codons are marked in bold. Highly conserved motifs are boxed, and the ER retention signal is bold shaded.

quantitative real-time RT-PCR using the specific primers (*elovl5-like* QF/QR and β-actin F/R, Table 5) and β-actin was used as an internal control. The transcription of *elovl5-like* was detected in liver, brain, intestine, gill, heart, stomach and spleen. Expression of *elovl5-like* was strongly observed in liver, brain and gill, and weakly in spleen, intestine, heart and stomach. The highest transcriptional levels of the detected gene were observed in liver and brain, more than 80 folds than the corresponding values in spleen, intestine and heart ($P < 0.01$) (Fig. 4).

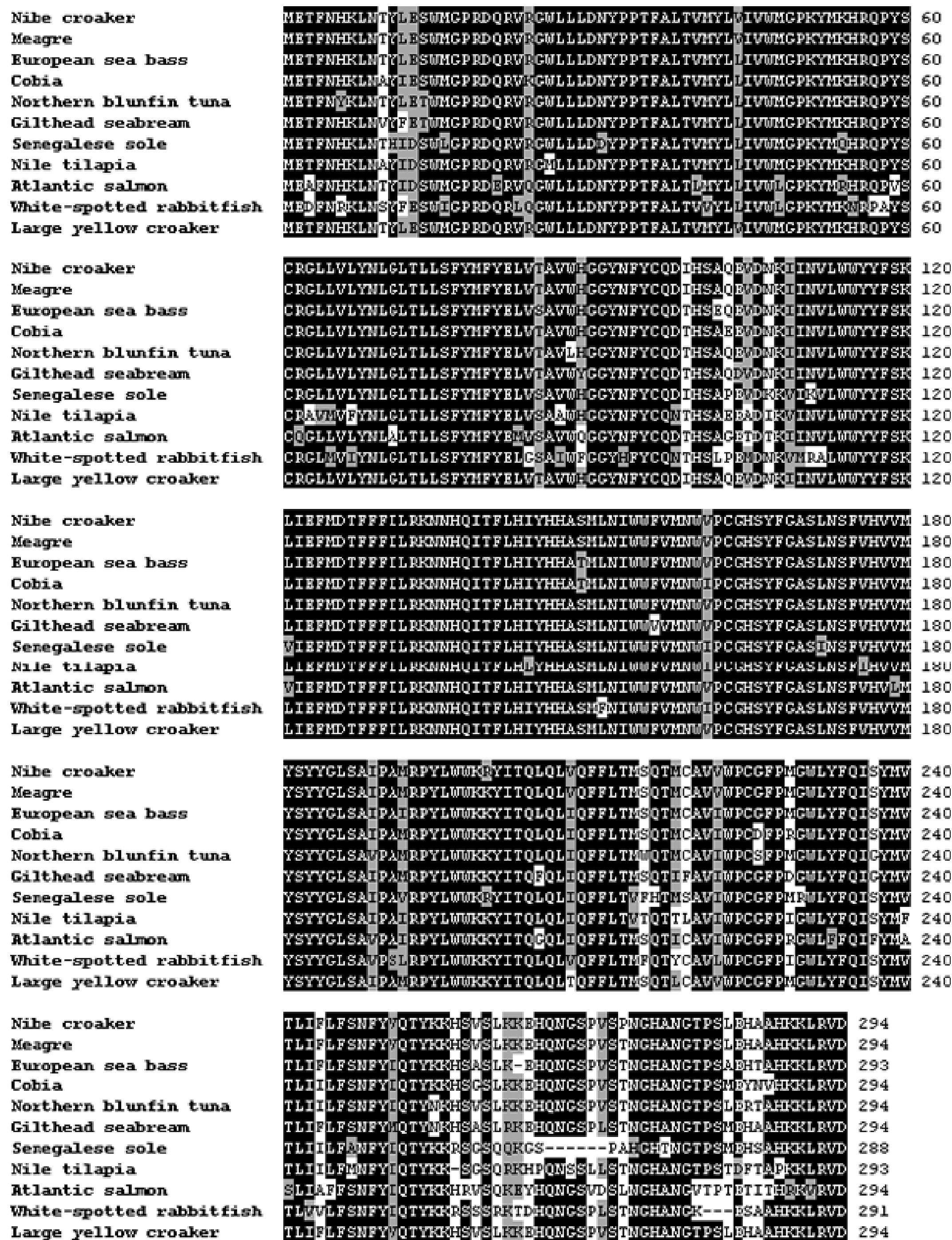

**Figure 2** A deduced amino acid sequence comparison of the *elovl5-like* from large yellow croaker (*Larimichthys crocea*, AFB81415) with fatty acyl elongases from Nibe croaker (*Nibea mitsukurii*, ACR47973), Meagre (*Argyrosomus regius*, AGG69479.1) European seabass (*Dicentrarchus labrax*, CBX53576), Cobia (*Rachycentron canadum*, ACJ65150), Northern bluefin tuna (*Thunnus thynnus*, ADX62355), Gilthead seabream (*Sparus aurata*, AAT81404), Senegalese sole (*Solea senegalensis*, AER58183), Nile tilapia (*Oreochromis niloticus*, XP_003441040), Atlantic salmon (*Salmo salar*, NP_001117039), White-spotted rabbitfish (*Siganus canaliculatus*, ADE34561). The amino acid sequences were aligned using ClustalW Multiple alignment. Identity/similarity shading was based on the BLOSUM62 matrix, and the cutoff for shading was 75%.

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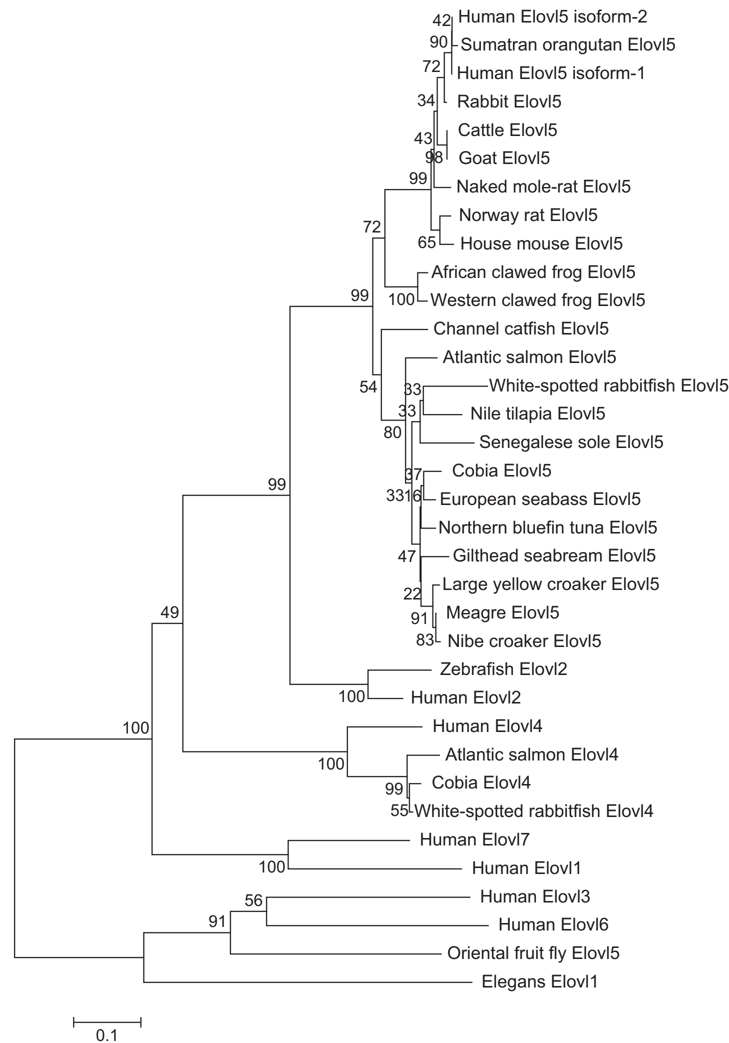


**Figure 3** Phylogenetic relationship between the amino acid sequences of Elovl5-like and 30 available Elovls. The amino acid sequences were derived from the GenBank under the following accession numbers (in parentheses): Human (*Homo sapiens*): Elovl1 (NP_073732), Elovl2 (NP_060240), Elovl3 (NP_689523), Elovl4 (NP_073563), Elovl5 isoform-1 (NP_068586), Elovl5 isoform-2 (NP_001229757), Elovl6 (NP_076995), Elovl7 (NP_079206); Zebrafish (*Danio rerio*): Elovl2 (AAI34116); Elegans (*Caenorhabditis elegans*): Elovl1 (CAA92958); Sumatran orangutan (*Pongo abelii*): Elovl5 (NP_001127147); Rabbit (*Oryctolagus cuniculus*): Elovl5 (XP_002714555); Cattle (*Bos Taurus*): Elovl5 (NP_001040062); Goat (*Capra hircus*): Elovl5 (BAF49682); Norway rat (*Rattus norvegicus*): Elovl5 (ADP36858); House Mouse (*Mus musculus*): Elovl5 (NP_599016); Naked mole-rat (*Heterocephalus glaber*): Elovl5 (EHB10085); African clawed frog (*Xenopus laevis*): Elovl5 (NP_001089883); Western clawed frog (*Xenopus* (*Silurana*) *tropicalis*): Elovl5 (NP_001011248); Channel catfish (*Ictalurus punctatus*): Elovl5 (NP_001188041); Atlantic salmon (*Salmo salar*): Elovl5 (NP_001117039), Elovl4 (NP_001182481); Nile tilapia (*Oreochromis niloticus*): Elovl5 (XP_003441040); Senegalese sole (*Solea senegalensis*): Elovl5 (AER58183); White-spotted rabbitfish (*Siganus canaliculatus*): Elovl5 (ADE34561), Elovl4 (ADZ73580); Northern bluefin tuna (*Thunnus thynnus*): Elovl5 (ADX62355); Cobia (*Rachycentron canadum*): Elovl5 (ACJ65150), Elovl4 (ADG59898); European seabass (*Dicentrarchus labrax*): Elovl5 (CBX53576); Gilthead seabream (*Sparus aurata*): Elovl5 (AAT81404); Large yellow croaker (*Larimichthys crocea*): Elovl5 (AFB81415); Nibe croaker (*Nibea mitsukurii*): Elovl5 (ACR47973); Meagre (*Argyrosomus regius*): AGG69479.1; Oriental fruit fly (*Bactrocera dorsalis*): Elovl5 (ADM65956). Sequences were aligned using the CLUSTAL W algorithm and were analyzed by phylogenetic analysis using the neighbour -joining distance method.

### Nutritional regulation of elovl5-like

The *elovl5-like* transcriptional levels in liver decreased significantly with the increase in dietary n-3LC-PUFA. The hepatic *elovl5-like* expression levels in fish fed diets with 0.15–0.98% n-3LC-PUFA were significantly higher than those fed high level of n-3LC-PUFA (1.79–2.25%; $P < 0.05$). The *elovl5-like* transcript levels were up-regulated by 1.77-fold, 1.41-fold, and 1.41-fold in the level of 0.15%, 0.60% and 0.98% treatments compared with the treatment of 2.25% n-3LC-PUFA respectively (Fig. 5a).

The relative hepatic mRNA expression of *elovl5-like* showed increasing tendency with the increase in dietary DHA/EPA. However, no significant differences were observed in the mRNA levels of *elovl5-like* among dietary treatments ($P > 0.05$) (Fig. 5b).

## Discussion

In this study, a full-length cDNA of *elovl5-like* was first obtained from large yellow croaker. The KXXEXXDT, QXXXLHXYHH (which contains the histidine box), NXXXHXXNYXYY and TXXQXXQ motifs are highly conserved in all PUFA elongases cloned up to now (Meyer, Kirsch, Domergue, Abbadi, Sperling, Bauer, Cirpus, Zank, Moreau, Roscoe, Zahringer & Heinz 2004). The deduced 294 amino acids showed high identity with elongases of some marine fish species, particularly Nibe croaker (*Nibea mitsukurii*, 99%), Meagre (*Argyrosomus regius*, 99%), European seabass (*Dicentrarchus labrax*, 95%), Atlantic bluefin tuna (*Thunnus thynnus*, 94%), gilthead sea bream (*Sparus aurata*, 93%), cobia (*Rachycentron canadum*, 93%), Senegalese sole (*Solea senegalensis*, 85%). The phylogenetic sequence analyses showed that Elovl5 clustered most closely with mammalian Elovl5 and Elovl2 while more distantly with human Elovl1, Elovl3, Elovl4, Elovl6 and Elovl7. This was consistent with the findings of some previous studies (Agaba, Tocher, Zheng, Dickson, Dick & Teale 2005; Gregory *et al.* 2010; Monroig *et al.* 2013).

According to previous studies, brain and liver usually had a high transcription of *elovl5*. However, expression of this gene in other tissues (intestine, stomach, gill, spleen and skin) varied among different fish species (Tocher, Zheng, Schlechtriem, Hastings, Dick & Teale 2006). In this study, tissue expression study showed that *elovl5*-like was constitutively expressed in all detected tissues, with the highest expression level in liver, followed by brain, gill and stomach, and the lowest level in heart, intestine and spleen. In Atlantic cod, transcription of PUFA elongase was highest in brain and liver, and lowest in stomach and heart (Tocher *et al.* 2006). In salmon, the transcriptional levels of PUFA elongase were highest in intestine, liver and brain (Zheng *et al.* 2005). Intestine is now acknowledged as a site of significant fatty acid metabolism, at least in salmonids (Bell, Dick & Porter 2003). However, high transcription of *elovl5.-like* in brain as observed in this and some previous studies could imply the importance of this enzyme in maintaining normal LC-PUFA levels of cell membranes, especially those in the neuronal tissues (Zheng, Ding, Xu, Monroig, Morais & Tocher 2009). It is well known that neural tissue phospholipids of vertebrates are rich in DHA, which plays a critical role in visual and learning processes (Neuringer, Connor, van Petten & Bastad 1984; Neuringer, Anderson & Connor 1988; Rodríguez, Pérez, Díaz, Izquierdo, Fernández-Palacios &

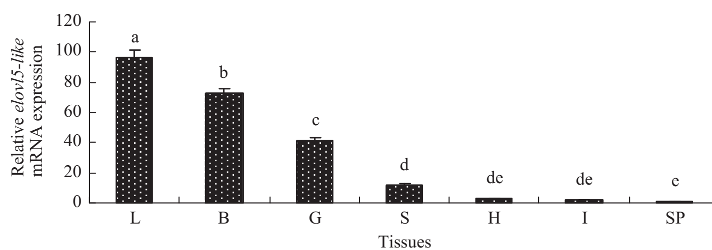


**Figure 4** Relative expression of *elovl5-like* in different tissues of *Larimichthys crocea*. The transcriptional levels of LycElovl5 in brain (B), stomach (S), spleen (SP), gill (G), heart (H), intestine (I) and liver (L) were normalized to that of spleen (SP). Values are means ± SEM ($n = 5$). Bars bearing with different letters are significantly different by Tukey's test ($P < 0.05$).

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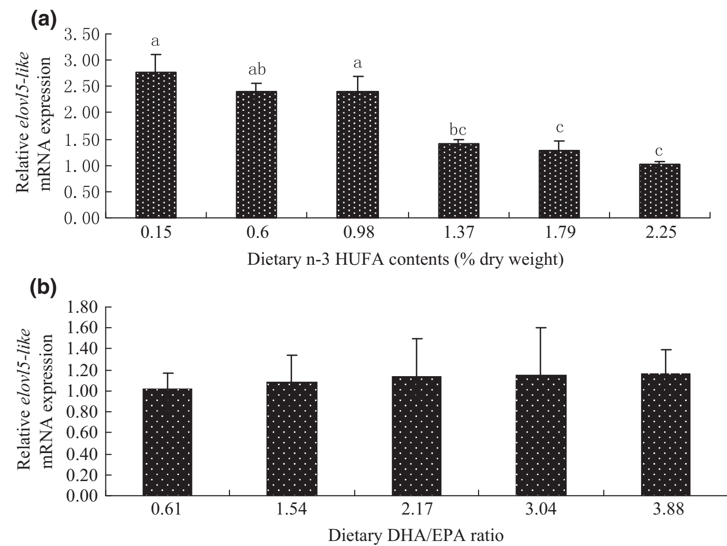


**Figure 5** Relative hepatic *elovl5-like* mRNA expression of large yellow croaker, *Larimichthys crocea* fed diets with graded levels of dietary n-3 LC-PUFA (a) and ratios of DHA/EPA (b). Values are means ± SEM ($n$ = 3). Bars bearing with different letters are significantly different by Tukey's test ($P$ < 0.05).

Lorenzo 1997). A diet lacking DHA or with a low ratio of DHA/EPA could result in the visual development problems which would then lead a decrease in hunting efficiency and consequently a reduction in growth rate of marine fish larvae (Watanabe, Izquierdo, Takeuchi, Satoh & Kitajima 1989; Mourente & Tocher 1993; Rodríguez *et al.* 1997) and juveniles such as striped jack (Watanabe, Takeuchi, Arakawa, Imaizumi, Sekiya & Kitajima 1989), red seabream (Takeuchi, Toyota, Satoh & Watanabe 1990), grouper (Wu, Ting & Chen 2002) and barramundi (Glencross, Rutherford & Jones 2011).

Previous studies have showed that most Elovl5 had high elongation efficiency towards $C_{16}$, $C_{18}$ and $C_{20}$ substrates, but a low activity towards $C_{22}$ PUFA (Agaba *et al.* 2005; Zheng, Ding *et al.* 2009; Gregory *et al.* 2010; Morais *et al.* 2009, 2011; Monroig, Wang *et al.* 2012; Monroig, Guinot, Hontoria, Tocher & Navarro 2012; Monroig *et al.* 2013). In this study, fish fed diets with the lowest content (0.15%) of n-3 LC-PUFA had the lowest growth performance but highest transcription of *elovl5*, which was consistent with the findings of some previous studies (Ling *et al.* 2006; Tocher *et al.* 2006; Jaya-Ram *et al.* 2008). This indicated that large yellow croaker could not or not efficiently synthesize n-3 LC-PUFA from $C_{18}$ fatty acids. Among marine fish species, only Elovl5 from rabbitfish (*Siganus canaliculatus*), a marine herbivorous fish, has been found to have some capacity to convert 22:5n-3 to 24:5n-3 (Monroig, Wang *et al.* 2012). Thus, it is possible that *elovl5-like*. from large yellow croaker did not possess the activity of elongating fatty acid substrates with carbon chain equal to or above 22, just like other marine carnivorous fish. During the synthesis of DHA, elongation of $C_{22}$ LC-PUFA to $C_{24}$ LC-PUFA was the last but essential step. Unlike mammals, marine teleosts appear to lack elovl2, an enzyme that elongates $C_{20}$ and $C_{22}$ LC-PUFA including 22:5n-3 to 24:5n-3 (Monroig *et al.* 2011). In the second study, no significance was observed in the transcription of Elovl5-like as dietary DHA/EPA increased. However, it should be noted that total n-3 LC-PUFA was constant at 1.0% total dry diet as dietary DHA/EPA increased. Thus, it was reasonable that transcription of *elovl5-like* was not statistically different among the five dietary treatments. This further indicated that Elovl5-like from large yellow croaker could possess no activity towards $C_{22}$ LC-PUFA substrates.

However, marine fish may have other Elovl enzymes which could have partially compensated functions in the last elongation steps during the biosynthetic pathway of LC-PUFA (Monroig *et al.* 2011). Recently, *elovl4* have been successfully cloned from some fish species, such as zebrafish (Monroig *et al.* 2010), Atlantic salmon (Carmona-Antoñanzas *et al.* 2011), cobia (Monroig *et al.* 2011) and white-spotted rabbitfish (Monroig, Wang *et al.* 2012), which has been proven to possess some capacity of elongating substrates with chain lengths ≥24 carbons in yeast heterologous expression system. The clone and functional analysis of these crucial enzymes definitely pro-

mote better understanding of the LC-PUFA biosynthesis pathway. More importantly, these initial and preliminary information make it possible for researchers to find practical means of elevating the expression levels and potentially activities of crucial enzymes through possible perspectives, such as promoters (Zheng, Leaver *et al*. 2009a; Xu *et al*. 2014), transcription factors (e.g. sterol regulatory element-binding protein and peroxisome proliferator-activated receptor) involved in LC-PUFA metabolism (Li, Mai, He, Ai, Zhang, Xu, Wang, Liufu, Zhang & Zhou 2013) or transgenic technology (Alimuddin *et al*. 2007, 2008; Kabeya *et al*. 2014).

To conclude, a fatty acid *elovl5-like* was first cloned from large yellow croaker. This *elovl5-like* was broadly expressed with the highest level in liver, followed by brain, gill and stomach, and lowest level in heart, intestine and spleen. The hepatic expression of *elovl5-like* decreased significantly with the increase in dietary n-3 LC-PUFA while was unaffected by dietary DHA/EPA. Future studies should be emphasized to determine the categories and functions of other critical enzymes involved in LC-PUFA biosynthesis, and elucidate mechanisms of nutritional regulation on these enzymes in large yellow croaker.

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