Molecular characterization, spatiotemporal expression patterns of fatty acid elongase (*elovl8*) gene, and its transcription changes in response to different diet stimuli in yellow catfish (*Pelteobagrus fulvidraco*)

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Elongase of very long-chain fatty acid 8 (Elovl8) is a new member identified in the Elovl family that is involved in the synthesis of highly unsaturated fatty acids (HUFAs). However, the evolutionary and physiological roles of this enzyme are still largely unknown. In the present study, the *elovl8* gene was identified and characterized from yellow catfish *Pelteobagrus fulvidraco*, and then its evolutionary and molecular characteristics as well as transcriptional changes in response to various nutritional status were determined. Results showed that the open reading frame (ORF) of *elovl8* was 795 bp in length, encoding a protein of 264 amino acids. Multiple sequences alignment showed that the yellow catfish Elovl8 was highly conserved with other homologs in teleosts, sharing similar structural characteristics (including six conserved transmembrane α-helical domains, four conserved elongase motifs, and three highly conserved cysteine residues). Meanwhile, comparisons of genetic synteny confirmed that the *elovl8* gene identified from the yellow catfish was the homolog of *elovl8b* in other teleosts, and thus, the *elovl8a* gene was lost in the genome of the yellow catfish. Gene structure analysis revealed that the *elovl8b* gene contained eight exons and seven introns, which was highly conserved in teleosts, implying the functional conservation among various fish species. Tissue distribution analysis detected by

real-time quantitative PCR (RT-qPCR) showed that the *elovl8* gene was extensively expressed in all detected tissues except eyes, with high expression levels in the intestine and liver. Temporal expression analysis revealed that the expression level of *elovl8* was stably expressed in the early 12 h after fertilization, and then dramatically decreased at 24, 48, 72, and 96 h after fertilization, implying that *elovl8* is required for HUFA biosynthesis in the early development stages. Functional experiments showed that the expression of the *elovl8* gene was stimulated after feeding with egg yolk but was not obviously affected after feeding with halogenated worms, indicating that diets full of HUFAs can inhibit the expression of *elovl8* in yellow catfish. Our findings will help us to better understand the evolutionary and functional characteristics of *elovl8* in teleosts, and lay a solid basis for investigating the regulation mechanism of HUFA biosynthesis.

Introduction

Highly unsaturated fatty acids (HUFAs) are a series of straight-chain fatty acids with three or more double bonds and 20 or more carbon atoms, which play important roles in maintaining cell membrane fluidity, regulating fat metabolism, enhancing immunity, and reducing inflammation (Zhang et al., 2019; Ri et al., 2022). Notably, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are the most prominent HUFAs that play critical roles involved in promoting growth and development, and preventing cardiovascular and neurodevelopmental disorders (De Giuseppe et al., 2014; Zárate et al., 2017).

Usually, vertebrates possess the ability to biosynthesize HUFAs by using essential polyunsaturated fatty acids as precursors with two critical rate-limiting enzymes including elongases of very long chain fatty acids (Elovls) and fatty acid desaturases (Fads) (Oboh et al., 2017). The former can elongate the carbon chain of polyunsaturated fatty acids while the latter can introduce a double bond at a specific position on the carbon chain to produce desaturation (Nakamura and Nara, 2004; Guillou et al., 2010). In teleosts, it is commonly accepted that freshwater and migratory fishes have obvious capacity to biosynthesize HUFAs while seawater fishes usually have lower or no capacity to achieve this goal because they are surrounded by an environment with abundant HUFAs in diets (Li et al., 2010; Jaya-Ram et al., 2011). Meanwhile, it seems that food habitats also have effects on the HUFA biosynthesis in teleosts. For example, herbivorous or omnivorous freshwater fishes such as grass carp (Du et al., 2006) and zebrafish (Ishak et al., 2008) are proved to have the ability to synthesize arachidonic acid (AA), EPA, and DHA with linoleic acid (LA) and linolenic acid (LNA) as precursors. Differently, carnivorous fishes such as groupers (Li et al., 2016) and gilthead seabream (Ganga et al., 2005) lack this kind of capacity or have a weak capacity, and thus higher levels of dietary HUFAs

are required to meet their requirements for growth, development, and reproduction. However, more studies are still required to investigate the exact mechanisms involved in HUFA biosynthesis in teleosts.

Thus far, seven members named ELOVL1–ELOVL7 in the ELOVL family were identified in mammals, and these members showed different substrate specificities for various fatty acid substrates (Jakobsson et al., 2006; Ohno et al., 2010). Overall, ELOVL1, ELOVL3, ELOVL6, and ELOVL7 primarily extend saturated fatty acids as well as monounsaturated fatty acids; ELOVL2, ELOVL4, and ELOVL5 mainly catalyze the elongation reactions of polyunsaturated fatty acids, while Elovl4 prolongs both very-long-chain saturated fatty acids and very-long-chain polyunsaturated fatty acids (Agbaga et al., 2008; Guillou et al., 2010). Recently, a novel teleost-specific member of the Elovl family, named Elovl8, had been identified in a marine rabbitfish (*Siganus canaliculatus*) (Li et al., 2020). Genetic synteny and gene phylogeny revealed that this new member commonly contains two isoforms, and it seemed that the *elovl8a* gene has been lost in some fish genomes (Li et al., 2020). Further functional experiments indicated that Elovl8b but not Elovl8a possesses the capacity to biosynthesize HUFAs (Li et al., 2020). Consistently, a single *elovl8b* gene was identified in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂), and functional experiments revealed that diets full of HUFAs can inhibit *elovl8b* transcription in liver, suggesting that a negative feedback regulation of the HUFA synthetic pathway existed (Wu et al., 2022). However, the evolutionary and functional characteristics of this new member are still largely unclear, and much more studies are required to illustrate these issues.

Yellow catfish (*Pelteobagrus fulvidraco*) belongs to family Bagridae, order Siluriformes, and is an omnivorous freshwater fish that is widely cultured in China due to its rapid growth, good taste, and valuable nutrition values (Guo et al., 2023; Wei et al.,

2023). Thus far, two key genes involved in HUFA biosynthesis including *fad2* and *elovl5* have been identified in yellow catfish (Song et al., 2015; Qin et al., 2017), but their exact roles have not been well investigated. In the present study, we identified a teleost-specific *elovl8* gene from yellow catfish, and then its evolutionary and molecular characteristics, spatiotemporal expression patterns, and functional traits were determined for the first time. Our findings will provide not only a novel insight into mechanisms involved in HUFA biosynthesis in yellow catfish, but also valuable data for better understanding the evolutionary and functional traits of *elovl8* in teleosts.

Materials and methods

Fish sampling

Yellow catfish (weight = 32.6 ± 3.1 g) used in this study were purchased from a fishery farm in Sichuan province of China, and fishes were transported to the laboratory and temporarily reared in 1 m × 1 m × 1 m net boxes set up in a 16-m^2 microfluidic pond. Fishes were reared for 2 weeks in a natural light condition (12 L/12 D), and water temperature was maintained at 24–26°C. Fishes were fed with commercial feed (approximately 3%–4% of body weight) at 19:00 every day as described in our previous studies (Da et al., 2022). Experimental fishes showed normal feeding and activity during the accumulation period. After that, five fishes were randomly selected and used for tissue distribution studies. Fishes were anesthetized with MS-222 before decapitated, and then tissues including adipose, brain, barbel, eye, gill, gonad, heart, intestine, kidney, liver, muscle, spleen, and stomach were collected. Tissue samples were immediately frozen in liquid nitrogen, and subsequently stored at −80°C until further utilization.

Meanwhile, fertilized eggs of yellow catfish used in this study were obtained by artificial breeding in our laboratory. During incubation, the development status of yellow catfish embryos was observed with a microscope, and five embryos and larvae were respectively sampled at each time point including 0 h, 3 h, 6 h, 12 h, 24 h, 36 h, 48 h, 72 h, and 96 h after fertilization in the early developmental stages. Similarly, samples were frozen rapidly with liquid nitrogen and then stored at −80°C for further utilization.

Diets with different content of HUFAs, including *Artemia nauplii* and egg yolk, were separately used as larval stage feeds to investigate the potential effects of various diets on transcription of *elovl8* in yellow catfish. Meanwhile, approximately 200 yellow catfish larvae were randomly selected and fed with different diets on day 4 after hatching, and three parallel experiments were designed for each group. During the experiment, 25% water changes were carried out daily, the oxygenation pump was kept continuously oxygenated, and the dissolved oxygen in the water was maintained above 6 mg/L. Fishes were fed at 08:00, 12:00, 16:00 and 20:00 to ensure sufficient diet supplement in the experimental tanks. The experiment lasted for 3 days, and five fishes from each tank were collected at 24, 48, and 72 h after feeding. Finally, samples were immediately frozen in liquid nitrogen and stored at −80 °C for further utilization.

Identification of the *elovl8* gene in yellow catfish

Protein similarity BLAST was conducted to identify the genomic and transcriptomic sequences from yellow catfish genome data in NCBI database and transcriptome database using several valid fish Elovl8 protein sequences as queries. Subsequently, potential sequences were verified and confirmed by NCBI-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Meanwhile, specific primers used for cloning experiment were designed with the Primer Premier 5.0 software (www.PremierBiosoft.com) (Supplementary Table 1).

PCR was performed to amplify the aimed *elovl8* gene fragment with liver cDNA as template. Then, PCR products were tested by agarose gel electrophoresis and isolated and purified using the TaKaRa gel recovery kit. Finally, PCR products were cloned into the pMD-19T vector (TaKaRa, Dalian, China) and then sequenced at Sangon Co. Ltd. (Guangzhou, China) to ensure the accuracy of the potential *elovl8* gene in yellow catfish.

Bioinformatics analysis

The nucleotide sequence of *elovl8* was obtained from yellow catfish using BLAST in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST). After validation, the nucleotide sequences were translated into protein sequences using the Primer Premier 5.0 software. Multiple Elovl8 protein sequence alignment was conducted by using Clustal X software as described in our previous studies (Li et al., 2020). Meanwhile, comparative analyses of genetic synteny and gene structure were conducted to determine the evolutionary pattern of *elovl8* genes in teleosts. Additionally, a phylogeny was constructed on the basis of the protein sequences of various teleosts to investigate the evolutionary history of the *elovl8* gene family. Elovl8 protein sequences used for phylogeny were downloaded from the NCBI or Ensembl databases. After calculation, JTT + G was selected as the best model, and the neighbor-joining (NJ) method was chosen to construct the phylogenetic tree. Finally, the robustness of the tree topology was assessed by a nonparametric bootstrap analysis with 1,000 resampling replicates. Details of the selected Elovls family proteins are given in Supplementary Table 2.

RNA extraction and quantitative PCR

Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol, and then the concentration and quality of total RNA were confirmed by a spectrophotometer (Nano Drop 2000, Thermo Scientific, USA) as described in our previous studies (Wen et al., 2020; Wen et al., 2021). Subsequently, 1 μg of total RNA was reverse transcribed to cDNA using the QuantiTect® Reverse Transcription kit (Takara Biotech, Dalian, China). Quantitative real-time PCR (qPCR) was conducted to measure the mRNA level of rabbitfish *elovl8* on a Light Cycler Real-Time system with a final volume of 20

µL. Meanwhile, the relative expression level of mRNA was normalized with *β-actin* after assessing the stability of five reference genes. Finally, the relative transcription level of *elovl8* was calculated using the Pfaffl method (Yang et al., 2018; Wen et al., 2019). Sequences of the specific primers used for qPCRs are provided in Supplementary Table 1.

Statistical analysis

SPSS 22.0 (IBM, Armonk, NY, USA) and GraphPad Prism 7.0 (GraphPad, Prism Software Inc., San Diego, California) were used for statistical analysis. All data were shown as mean normalized values ± standard error of the mean (SEM). Significant differences were determined using one-way analysis of variance (ANOVA) followed by Tukey's test, and differences were significant when $p < 0.05$.

Results

Molecular characteristics of the *elovl8* gene in yellow catfish

In the present study, we identified the *elovl8* gene from yellow catfish for the first time. NCBI-Blast revealed that the identified *elovl8* gene is the homolog of *elovl8b* gene in teleost. The open reading frame (ORF) of yellow catfish *elovl8* was 795 bp in length and encoded a protein with 264 amino acids. Multiple Elovl8b protein sequence alignment showed that Elovl8b is highly conserved (protein similarity > 84.4%) in teleosts and that they shared similar structural features, containing six conserved transmembrane α-helix structural domains, four conserved elongase motifs, and three highly conserved cysteine residues (Figure 1).

Phylogenetic analysis

To better understand the evolutionary relationships of the *elovl8* gene family in teleosts, a phylogenetic tree was constructed using the NJ method with MEGA-X software. Results showed that the *elovl8* family was divided into two clades, namely, *elovl8a* and *elovl8b* subfamilies, and the yellow catfish *elovl8* was clustered into the *elovl8b* clade (Figure 2). Notably, red-bellied piranha (*P. nattereri*), chum salmon (*O. keta*), zebrafish (*D. rerio*), Roho labeo (*L. rohita*), fathead minnows (*P. promelas*), grass carp (*C. idella*), cachama (*C. macropomum*), and delta smelt (*H. transpacificus*) contained two *elovl8* paralogs. Differently, north African catfish (*C. gariepinus*), channel catfish (*I. punctatus*), yellow catfish (*P. fulvidraco*), freshwater shark (*P. hypophthalmus*), southern catfish (*S. meridionalis*), Mexican tetra (*A. mexicanus*), the sharp-headed submarginal fish (*X. texanus*), and the common carp (*C. carpio*) only contained the *elovl8b* subtype (Figure 2). Moreover, yellow catfish *elovl8b* shared a close relationship with channel catfish and African catfish *elovl8b* (Figure 2).

Genetic synteny

Comparative genomics were conducted to further explore the exact evolutionary status of *elovl8* genes in eight representative fishes, namely, *L. rohita*, *C. macropomum*, *C. idella*, *A. mexicanus*, *P. hypophthalmus*, *I. punctatus*, *C. gariepinus*, and *P. fulvidraco* (Figure 3). Results showed that three species, namely, *L. rohita*, *C. macropomum*, and *C. idella*, contained the *elovl8a* gene, and a conserved gene cluster *tesk2-toe1-selenop2-elovl8a-zswim5-urod* was identified in these fishes (Figure 3A). Notably, the *elovl8a* gene had been lost in the other five fish species, namely, *P. fulvidraco*, *I. punctatus*, *C. gariepinus*, *P. hypophthalmus*, and *A. mexicanus* (Figure 3A). Moreover, a highly conserved gene cluster *dmap1-guk1b-armh1-mutyh-elovl8b* was identified in all the representative fish genomes (Figure 3B).
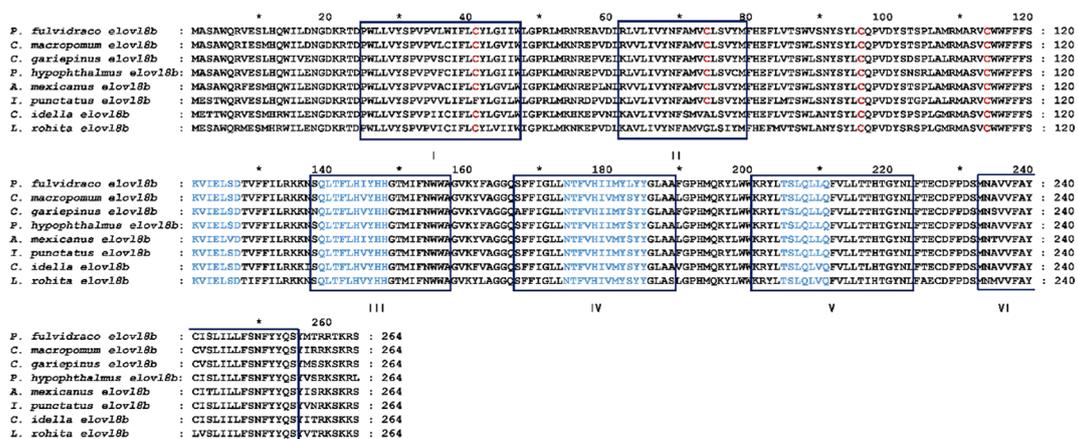

FIGURE 1

Multiple protein sequence alignment of Elovl8b between yellow catfish and other representative teleosts. Six conserved transmembrane α-helix structural domains are labeled with I–VI. The four conserved elongase motifs are labeled in blue font. Cysteines are shown in red. C-terminal ER retrieval signals are marked with dashed boxes. Asterisks (*) indicate the amino acids conserved among all members of the proteins.
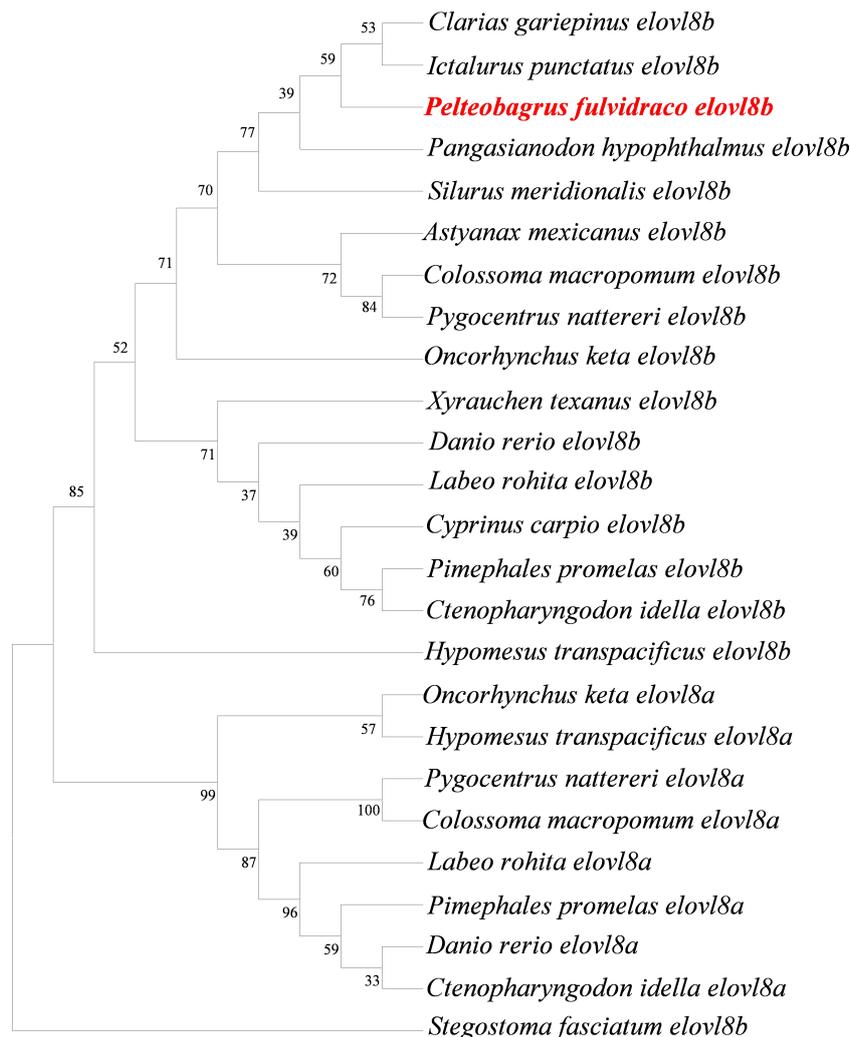
FIGURE 2
Phylogeny reveals the evolutionary history of the *elovl8* gene family in teleosts. Protein sequences were used to construct a phylogenetic tree with the NJ method using MEGA-X software. Numbers up the clades represent the bootstrap percentages from 1,000 replicates and the yellow catfish *elovl8* is labeled in red color. The leopard shark (*Stegostoma fasciatum*) was used as the outgroup.

Gene structure analysis

Gene structure comparison was conducted to reveal the structural and functional differentiation of the *elovl8b* gene in eight representative fish species. Results showed that the *elovl8b* gene of seven selected fish species, namely, *P. fulvidraco, A. mexicanus, C. gariepinus, C. idella, C. macropomum, L. rohita*, and *P. hypophthalmus*, had similar gene structures, containing eight exons and seven introns (Figure 4). Differently, the gene structure of *I. punctatus elovl8b* contained nine exons and eight introns, but showed a conserved coding sequence (CDS) region with the seven other fish *elovl8b* genes (Figure 4).

Tissue distribution pattern of *elovl8* in yellow catfish

The distribution pattern of *elovl8* in yellow catfish was determined by real-time quantitative PCR. Results showed that

the *elovl8* gene of yellow catfish was widely expressed in most selected tissues including adipose, barbel, brain, gill, gonad, heart, intestine, kidney, liver, muscle, spleen, and stomach, and relative high expression levels were observed in intestine and liver tissues (Figure 5). Differently, no expression was detected in the eyes (Figure 5).

Expression pattern of the *elovl8* gene at early developmental stages in yellow catfish

Quantitative PCR was conducted to detect the expression level of the *elovl8* gene in the early developmental stages of yellow catfish. Results showed that the expression level was not significantly changed at 0, 3, 6, and 12 h, but dramatically decreased at 24, 36, 48, and 72 h, and reached the lowest expression level at 96 h after fertilization (Figure 6).
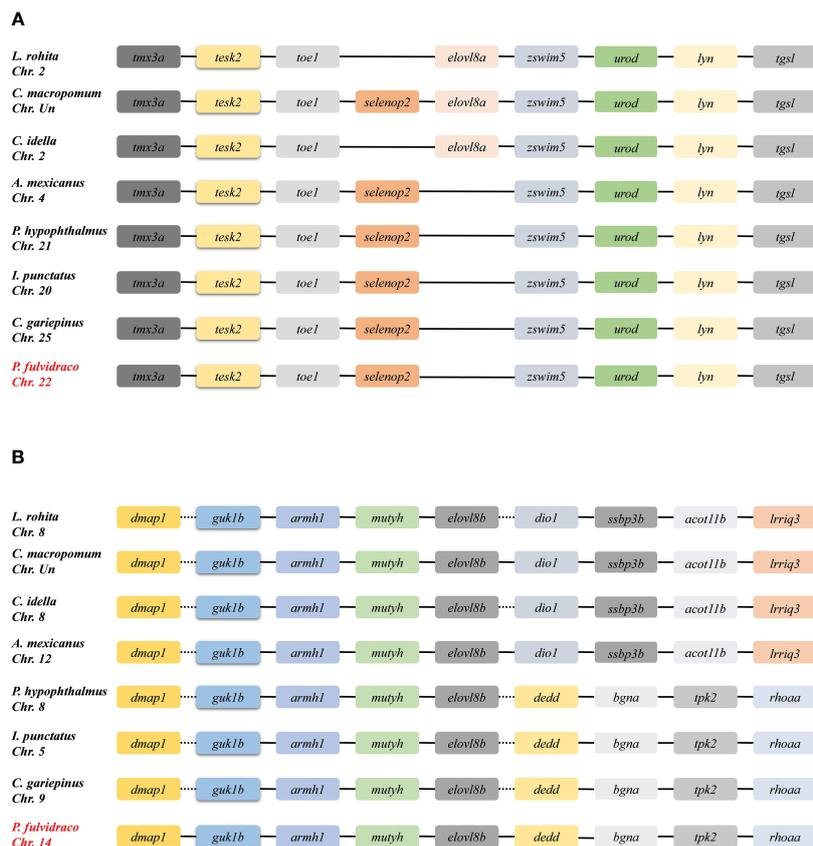
FIGURE 3
Genetic synteny of *elovl8a* **(A)** and *elovl8b* **(B)** in representative fish genomes. The colorful blocks represent different genes. The solid lines represent intergenic regions. The target species in the present study are marked in red front.

Effect of different diets on the expression of the *elovl8* gene in yellow catfish

Transcriptional change patterns of the yellow catfish *elovl8b* gene in response to larval stage diets with different HUFA content were determined (Figure 7). Results showed that the expression level of the *elovl8b* gene was slightly increased but not significantly changed at 1, 2, and 3 days after feeding with *A. nauplii* as in yellow catfish (Figure 7A). Differently, the expression level of the *elovl8b* gene was dramatically increased at 2 days and then significantly decreased at 3 days after feeding with egg yolk in yellow catfish (Figure 7B).

Discussion

Elovls are key rate-limiting enzymes to extend the carbon chain of polyunsaturated fatty acids by catalyzing condensation reaction, which play important roles involved in the biosynthesis of HUFAs in animals (Nugteren, 1965). Thus far, *elovl1–elovl7* are widely studied both in vertebrates and in invertebrates, but less is known about *elovl8*, the newly identified member of the *elovl* family (Ohno et al., 2010; Naganuma et al., 2011; Gregory and James, 2014; Yan et al., 2018). In this study, the *elovl8* gene of the yellow catfish was identified and characterized; it contained a 795- bp-long ORF that encodes a protein of 264 amino acids. Similar results were also
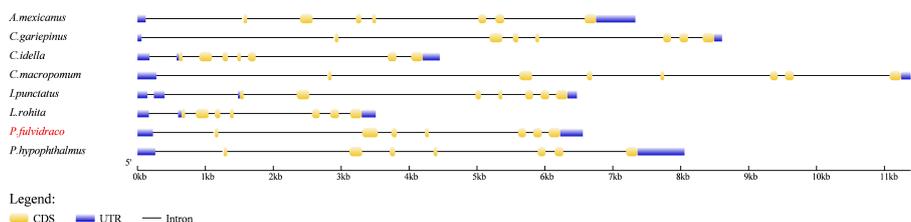

FIGURE 4
Comparison of the gene structure of *elovl8b* in eight representative teleosts. The colorful blocks and solid lines represent the exons and introns, respectively. UTR and CDS are marked with blue and yellow blocks, respectively.
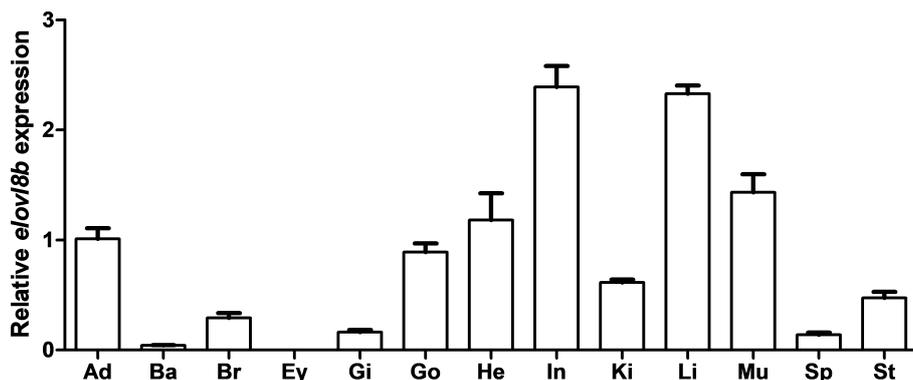
FIGURE 5
Tissue distribution of the *elovl8* gene in yellow catfish. Adipose (Ad); Barbel (Ba); Brain (Br); Eye (Ey); Gill (Gi); Gonad (Go); Heart (He); Intestine (In); Kidney (Ki); Liver (Li); Muscle (Mu); Spleen (Sp); Stomach (St). *β-actin* was selected as the internal reference gene. Data are expressed as mean ± standard error (*n* = 5).

observed in rabbitfish (*Siganus canaliculatus*) (Li et al., 2020) and hybrid grouper (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂) (Wu et al., 2022), suggesting that the *elovl8* genes are conserved among different fishes.

Multiple protein sequence comparison showed that yellow catfish Elovl8 possessed all the characteristics of ELOVL protein family members, including four conserved regions (KXXEXXDT, QXXFLHXYHH, NXXXHXXMYXYY, and TXXQXXQ), endoplasmic reticulum (ER)-resident signals, six transmembrane regions, and a histidine cluster (HXXHH) that is involved in fatty acid elongation processes (Jakobsson et al., 2006). The secondary characteristics are similar to that of Elovl8b in the rabbitfish (Li et al., 2020) and hybrid grouper (Wu et al., 2022), implying the evolutionary and functional conservation of the Elovl8b among various teleosts.

Phylogenetic analysis showed that the *elovl8* gene family was clustered into two branches of *elovl8a* and *elovl8b* subfamilies, which was consistent with the findings in previous studies, suggesting that teleost-specific elovl8 family may commonly contain two paralogs in teleost genomes, and this phenomenon may be caused by an additional teleost-specific genome duplication (TSGD) event (Kuraku and Meyer, 2009; Sun et al., 2021). Genetic

synteny further supported teleost lineage, which usually contains *elovl8a* and *elovl8b* isoforms, such as fishes that belong to the orders Cyprinodontiformes, Salmoniformes, and Osmeriformes. However, it seemed that the *elovl8a* gene had been lost in Siluriformes teleosts, including *C. gariepinus*, *P. hypophthalmus*, *I. punctatus*, and *P. fulvidraco*, suggesting that *elovl8b* should be more conserved than *elovl8a*, and thus, the former but not the latter may play important roles in HUFA biosynthesis (Li et al., 2020). To our knowledge, the *elovl8a* gene lost in some teleost genomes may be caused by functional redundancy between *elovl8a* and *elovl8b* isoforms in the early period of evolution. In addition, the structure of the *elovl8b* gene commonly contained eight exons and seven introns in teleosts, suggesting that this gene was highly conserved and might play similar functions among various teleosts.

Tissue distribution experiments showed that *elovl8b* was widely expressed in various tissues with high expression levels in liver and intestine, which was different with rabbitfish and hybrid grouper (Li et al., 2020; Wu et al., 2022), indicating that the tissue distribution pattern of *elovl8b* is species-specific and it may play diverse roles in various teleosts. Indeed, this phenomenon may be caused by the different ability to synthesize HUFAs in different fishes (Gong et al., 2014). Notably, the *elovl8b* was highly expressed in liver in most
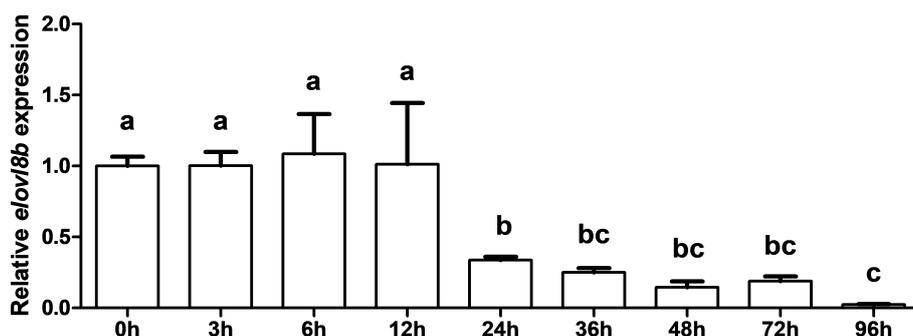

FIGURE 6
Gene expression pattern of the *elovl8b* gene in the early developmental stage of yellow catfish. *β-actin* was selected as the internal reference gene. Data are expressed as mean ± standard error (*n* = 5). Groups that differ significantly are indicated by different lowercase letters above bars.

FIGURE 7
Effect of dietary *A nauplii* **(A)** and egg yolk **(B)** on *elovl8* gene expression in yellow catfish larvae. *β-actin* was selected as the internal reference gene. Data are expressed as mean $\pm$ standard error (n = 5). Groups that differ significantly are indicated by different lowercase letters above bars.
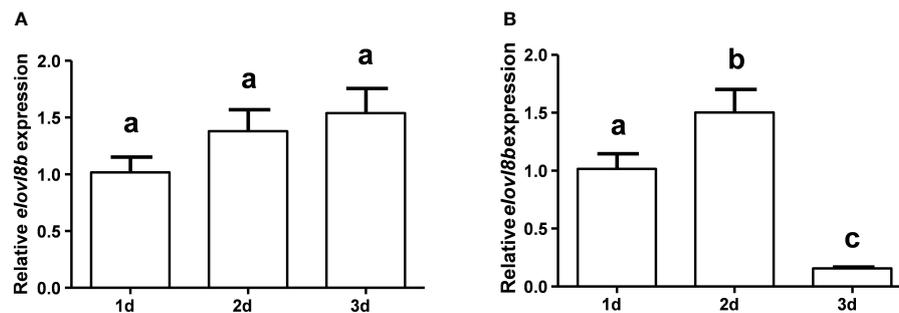
teleosts, suggesting that this gene may also play similar roles in HUFA biosynthesis because liver is the major organ that is involved in HUFA production.

The gene expression pattern of the *elovl8b* gene during the early development stage of yellow catfish was also determined, and results showed that *elovl8b* was stably expressed within 12 h after fertilization, which was slightly different with other HUFA biosynthesis-related genes in rabbitfish (You et al., 2017), suggesting that HUFA biosynthesis is very important for the early development of embryo despite the fact that the pattern may be different among various species. However, the expression level of yellow catfish *elovl8* was significantly decreased from 24 h to 96 h. It has been shown that during the early stage of embryonic development in yellow catfish, the PUFAs in yolk are involved in metabolism and catabolism to provide energy preparation for embryonic development, with C18:2n-6, C20:4n-3, and DHA dominating (Yao et al., 2009). Interestingly, the *elovl8b* gene was shown to have the ability to prolong C18 (18:2n-6, 18:3n-3, and 18:4n-3) and C20 (20:4n-6 and 20:5n-3) HUFAs to long-chain polyunsaturated fatty acids (LC-HUFAs) in rabbitfish (Li et al., 2020). This expression pattern suggests that yellow catfish *elovl8* is also involved in the synthesis of long-chain polyunsaturated fatty acids and plays an important role in early embryonic development.

Previous studies have shown that diets full of HUFAs could inhibit the transcriptional expression in rabbitfish and hybrid grouper (Li et al., 2020; Wu et al., 2022). In the present study, functional experiments showed that the transcription of *elovl8* was not significantly affected by feeding with *A. salina*, whereas it was inhibited after feeding with egg yolk in yellow catfish. It is shown that the lack of HUFAs in *A. salina* might explain why dietary *A. salina* slightly increased *elovl8* gene expression (Tocher et al., 2003; Garcia et al., 2008). Meanwhile, dietary egg yolk induced but then inhibited *elovl8* expression, which may be caused by the high content of phospholipid in egg yolk. Our findings further supported the idea that a compensatory mechanism also existed in yellow catfish, which was similar to previous reports and could demonstrate a key role for *elovl8b* in LC-PUFA biosynthesis (Cho et al., 1999; Seiliez et al., 2001).

In summary, an *elovl8* gene was identified and characterized from yellow catfish for the first time. Multiple protein sequence alignment, genetic synteny, gene structure comparisons, and phylogeny revealed that *elovl8a* had been lost in Siluriformes, and *elovl8b* was highly conserved among various teleosts. Moreover, spatiotemporal expression analyses suggested that *elovl8* was widely distributed, and it could play important roles in the early development stage in yellow catfish. Finally, functional experiments showed that a diet full of HUFAs may inhibit the transcription of *elovl8* in yellow catfish. Our findings will help us to better understand the evolutionary and functional characteristics of *elovl8* in teleosts, and lay a solid basis for investigating the regulation mechanism of HUFA biosynthesis.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The animal study was approved by Neijiang Normal University Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

W-HZ: Formal Analysis, Investigation, Writing – original draft. X-YW: Writing – original draft, Formal Analysis, Investigation, Software. WQ: Writing – original draft, Investigation. C-JQ: Writing – review & editing. QS: Writing – review & editing. SG: Writing – review & editing, Formal Analysis, Software. PP: Software, Writing – review & editing. SZ: Writing – review & editing, Funding acquisition, Investigation. PF: Writing – review & editing, Formal Analysis, Investigation. WH: Methodology, Supervision, Writing – review & editing. H-WY: Supervision, Writing – review & editing. Z-YW: Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1270776/full#supplementary-material

References

Agbaga, M. P., Brush, R. S., Mandal, M. N., Henry, K., Elliott, M. H., and Anderson, R. E. (2008). Role of Stargardt-3 macular dystrophy protein (ELOVL4) in the biosynthesis of very long chain fatty acids. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12843–12848. doi: 10.1073/pnas.0802607105

Cho, H. P., Nakamura, M., and Clarke, S. D. (1999). Cloning, expression, and fatty acid regulation of the human delta-5 desaturase. *J. Biol. Chem.* 274, 37335–37339. doi: 10.1074/jbc.274.52.37335

Da, F., Wen, Z., Wang, X.-D., and Luo, Y. (2022). Molecular identification, tissue distribution, and effects of fasting and refeeding on the transcription of uncoupling protein 2 in yellow catfish, *Pelteobagrus vachelli. Pak J. Zool.* 54, 1539–1547. doi: 10.17582/journal.pjz/20200316080346

De Giuseppe, R., Roggi, C., and Cena, H. (2014). n-3 LC-PUFA supplementation: effects on infant and maternal outcomes. *Eur. J. Nutr.* 53, 1147–1154. doi: 10.1007/s00394-014-0660-9

Du, Z. Y., Clouet, P., Zheng, W. H., Degrace, P., Tian, L. X., and Liu, Y. J. (2006). Biochemical hepatic alterations and body lipid composition in the herbivorous grass carp (*Ctenopharyngodon idella*) fed high-fat diets. *Br. J. Nutr.* 95, 905–915. doi: 10.1079/bjn20061733

Ganga, R., Bell, J. G., Montero, D., Robaina, L., Caballero, M. J., and Izquierdo, M. S. (2005). Effect of dietary lipids on plasma fatty acid profiles and prostaglandin and leptin production in gilthead seabream (*Sparus aurata*). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 142, 410–418. doi: 10.1016/j.cbpb.2005.09.010

Garcia, A. S., Parrish, C. C., and Brown, J. A. (2008). Growth and lipid composition of Atlantic cod (*Gadus morhua*) larvae in response to differently enriched *Artemia franciscana. Fish. Physiol. Biochem.* 34, 77–94. doi: 10.1007/s10695-007-9149-2

Gong, Y., Wan, X., Jiang, M., Hu, C., Hu, H., and Huang, F. (2014). Metabolic engineering of microorganisms to produce omega-3 very long-chain polyunsaturated fatty acids. *Prog. Lipid Res.* 56, 19–35. doi: 10.1016/j.plipres.2014.07.001

Gregory, M. K., and James, M. J. (2014). Rainbow trout (*Oncorhynchus mykiss*) Elov15 and Elov12 differ in selectivity for elongation of omega-3 docosapentaenoic acid. *Biochim. Biophys. Acta* 1841, 1656–1660. doi: 10.1016/j.bbalip.2014.10.001

Guillou, H., Zadravec, D., Martin, P. G., and Jacobsson, A. (2010). The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. *Prog. Lipid Res.* 49, 186–199. doi: 10.1016/j.plipres.2009.12.002

Guo, S., Zeng, M., Gao, W., Li, F., Wei, X., Shi, Q., et al. (2023). Toll-like receptor 3 in the hybrid yellow catfish (*Pelteobagrus fulvidraco* ♀ × *P. vachelli* ♂): Protein structure, evolution and immune response to exogenous *Aeromonas hydrophila* and Poly (I:C) stimuli. *Animals* 13 (2), 288. doi: 10.3390/ani13020288

Ishak, S. D., Tan, S. H., Khong, H. K., Jaya-Ram, A., Enyu, Y. L., Kuah, M. K., et al. (2008). Upregulated mRNA expression of desaturase and elongase, two enzymes involved in highly unsaturated fatty acids biosynthesis pathways during follicle maturation in zebrafish. *Reprod. Biol. Endocrinol.* 6, 56. doi: 10.1186/1477-7827-6-56

Jakobsson, A., Westerberg, R., and Jacobsson, A. (2006). Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog. Lipid Res.* 45, 237–249. doi: 10.1016/j.plipres.2006.01.004

Jaya-Ram, A., Ishak, S. D., Enyu, Y. L., Kuah, M. K., Wong, K. L., and Shu-Chien, A. C. (2011). Molecular cloning and ontogenic mRNA expression of fatty acid desaturase in the carnivorous striped snakehead fish (*Channa striata*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 158, 415–422. doi: 10.1016/j.cbpa.2010.11.018

Kuraku, S., and Meyer, A. (2009). The evolution and maintenance of Hox gene clusters in vertebrates and the teleost-specific genome duplication. *Int. J. Dev. Biol.* 53 (5-6), 765–773. doi: 10.1387/ijdb.072533km

Li, Y., Monroig, O., Zhang, L., Wang, S., Zheng, X., Dick, J. R., et al. (2010). Vertebrate fatty acyl desaturase with Δ4 activity. *Proc. Natl. Acad. Sci. U.S.A.* 107, 16840–16845. doi: 10.1073/pnas.1008429107

Li, Y., Wen, Z., You, C., Xie, Z., Tocher, D. R., Zhang, Y., et al. (2020). Genome wide identification and functional characterization of two LC-PUFA biosynthesis elongase (*elovl8*) genes in rabbitfish (*Siganus canaliculatus*). *Aquaculture* 522, 735127. doi: 10.1016/j.aquaculture.2020.735127

Li, S., Yuan, Y., Wang, T., Xu, W., Li, M., Mai, K., et al. (2016). Molecular cloning, functional characterization and nutritional regulation of the putative elongase *elovl5* in the orange-spotted grouper (*Epinephelus coioides*). *PloS One* 11, e0150544. doi: 10.1371/journal.pone.0150544

Naganuma, T., Sato, Y., Sassa, T., Ohno, Y., and Kihara, A. (2011). Biochemical characterization of the very long-chain fatty acid elongase ELOVL7. *FEBS Lett.* 585, 3337–3341. doi: 10.1016/j.febslet.2011.09.024

Nakamura, M. T., and Nara, T. Y. (2004). Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annu. Rev. Nutr.* 24, 345–376. doi: 10.1146/annurev.nutr.24.121803.063211

Nugteren, D. H. (1965). The enzymic chain elongation of fatty acids by rat-liver microsomes. *Biochim. Biophys. Acta* 106, 280–290. doi: 10.1016/0005-2760(65)90036-6

Oboh, A., Navarro, J. C., Tocher, D. R., and Monroig, O. (2017). Elongation of very Long-Chain (>C24) Fatty Acids in *Clarias gariepinus*: Cloning, Functional Characterization and Tissue Expression of elovl4 Elongases. *Lipids* 52, 837–848. doi: 10.1007/s11745-017-4289-3

Ohno, Y., Suto, S., Yamanaka, M., Mizutani, Y., Mitsutake, S., Igarashi, Y., et al. (2010). ELOVL1 production of C24 acyl-CoAs is linked to C24 sphingolipid synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18439–18444. doi: 10.1073/pnas.1005572107

Qin, C.-J., Wen, Z.-Y., Yuan, D.-Y., Shao, T., and Gong, Q. (2017). Molecular cloning and expression of fatty acid desaturase and elongase genes in darkbarbel (*Pelteobagrus vachellii*). *J. Oceanol. Limnol.* 48, 884–893.

Ri, K., Lee-Okada, H. C., and Yokomizo, T. (2022). Omega-6 highly unsaturated fatty acids in Leydig cells facilitate male sex hormone production. *Commun. Biol.* 5, 1001. doi: 10.1038/s42003-022-03972-y

Seiliez, I., Panserat, S., Kaushik, S., and Bergot, P. (2001). Cloning, tissue distribution and nutritional regulation of a Delta6-desaturase-like enzyme in rainbow trout. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 130, 83–93. doi: 10.1016/s1096-4959(01)00410-9

Song, Y. F., Luo, Z., Pan, Y. X., Zhang, L. H., Chen, Q. L., and Zheng, J. L. (2015). Three unsaturated fatty acid biosynthesis-related genes in yellow catfish *Pelteobagrus fulvidraco*: Molecular characterization, tissue expression and transcriptional regulation by leptin. *Gene* 563 (1), 1–9. doi: 10.1016/j.gene.2014.12.014

Sun, S., Wang, Y., Goh, P. T., Lopes-Marques, M., Castro, L. F. C., Monroig, Ó., et al. (2021). Evolution and functional characteristics of the novel *elovl8* that play pivotal roles in fatty acid biosynthesis. *Genes* 12 (8), 271. doi: 10.3390/genes12081287

Tocher, D. R., Bell, J. G., Dick, J. R., and Crampton, V. O. (2003). Effects of dietary vegetable oil on Atlantic salmon hepatocyte fatty acid desaturation and liver fatty acid compositions. *Lipids* 38 (7), 723–732. doi: 10.1007/s11745-003-1120-y

Wei, X. Y., Wang, J., Guo, S. T., Lv, Y. Y., Li, Y. P., Qin, C. J., et al. (2023). Molecular characterization of a teleost-specific toll-like receptor 22 (tlr22) gene from yellow catfish (*Pelteobagrus fulvidraco*) and its transcriptional change in response to poly I:C and *Aeromonas hydrophila* stimuli. *Fish. Shellfish Immunol.* 134, 108579. doi: 10.1016/j.fsi.2023.108579

Wen, Z. Y., Liu, T., Qin, C. J., Zou, Y. C., Wang, J., Li, R., et al. (2021). MRAP2 interaction with melanocortin-4 receptor in snakehead (*Channa argus*). *Biomolecules* 11 (3), 481. doi: 10.3390/biom11030481

Wen, Z. Y., Qin, C. J., Wang, J., He, Y., Li, H. T., Li, R., et al. (2020). Molecular characterization of two leptin genes and their transcriptional changes in response to fasting and refeeding in Northern snakehead (*Channa argus*). *Gene* 736, 144420. doi: 10.1016/j.gene.2020.144420

Wen, Z. Y., Wang, J., Bian, C., Zhang, X., Li, J., Peng, Y., et al. (2019). Molecular cloning of two kcnk3 genes from the Northern snakehead (*Channa argus*) for quantification of their transcriptions in response to fasting and refeeding. *Gen. Comp. Endocrinol.* 281, 49–57. doi: 10.1016/j.ygcen.2019.05.016

Wu, Q., Zheng, Z., Wang, C., Wang, Y., Sun, Y., and Gao, Y. (2022). Molecular characterization, tissue distribution and differential nutritional regulation of three n-3 LC-PUFA biosynthesis-related genes in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). *Animals* 12 (3), 234. doi: 10.3390/ani12030234

Yan, J., Liang, X., Cui, Y., Cao, X., and Gao, J. (2018). Elovl4 can effectively elongate C18 polyunsaturated fatty acids in loach Misgurnus anguillicaudatus. *Biochem. Biophys. Res. Commun.* 495, 2637–2642. doi: 10.1016/j.bbrc.2017.12.123

Yang, S., Wen, Z. Y., Zou, Y. C., Qin, C. J., Wang, J., Yuan, D. Y., et al. (2018). Molecular cloning, tissue distribution, and effect of fasting and refeeding on the expression of neuropeptide Y in *Channa argus*. *Gen. Comp. Endocrinol.* 259, 147–153. doi: 10.1016/j.ygcen.2017.11.017

Yao, J., Zhao, H. Y., Li, C., He, D., and Hu, C. (2009). Changes in Fatty Acid Composition During Early Embryonic Development of Yellow Catfish *Pelteobagrus fiulvidraco*. *Fish. Sci.* 28, 644–647.

You, C., Miao, S., Lin, S., Wang, S., Waiho, K., and Li, Y. (2017). Expression of long-chain polyunsaturated fatty acids (LC-PUFA) biosynthesis genes and utilization of fatty acids during early development in rabbitfish *Siganus canaliculatus*. *Aquaculture* 479, 774–779. doi: 10.1016/j.aquaculture.2017.07.028

Zárate, R., El Jaber-Vazdekis, N., Tejera, N., Pérez, J. A., and Rodríguez, C. (2017). Significance of long chain polyunsaturated fatty acids in human health. *Clin. Transl. Med.* 6, 25. doi: 10.1186/s40169-017-0153-6

Zhang, T. T., Xu, J., Wang, Y. M., and Xue, C. H. (2019). Health benefits of dietary marine DHA/EPA-enriched glycerophospholipids. *Prog. Lipid Res.* 75, 100997. doi: 10.1016/j.plipres.2019.100997