

Molecular cloning and gene expression of desaturases and elongases in embryos, larvae, and adults of bay snook *Petenia splendida* (Cichliformes: Cichlidae)



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The bay snook (*Petenia splendida*) belongs to the family Cichlidae, distributed in the southeastern region of Mexico and Central America, and it is a very important cichlid at a commercial level. Despite their potential for aquaculture, little information exists on their metabolic pathways related to physiology and nutrition. This study focuses on the expression of the fatty acid desaturases (*fads2*) and elongases (*elovl4b* and *elovl5*) genes in embryos 0 days post-hatching (dph) and larvae of 5, 10, 15, 20, 25, and 30 dph. Additionally, the differential expression of these genes was analyzed in the liver, intestine, kidney, muscle, heart, testes, gills, stomach, pancreas, brain, and adipose tissue in adult fish. Our study utilized specific qPCR primers designed for *fads2*, *elovl4b*, and *elovl5* to detect the expression of these genes. We found the highest expression were observed in larvae at 20 dph and adults, predominantly in the liver and intestine of the three genes tested. Importantly, our results showed that the desaturase and elongase genes are differentially expressed in the tissues of adults of *P. splendida*, and their expression fluctuates during larval development.

Keywords: Desaturase, Early ontogeny, Elongases, Gene expression, *Petenia splendida*.

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La mojarra tenguayaca (*Petenia splendida*) pertenece a la familia Cichlidae, se distribuye en la región sureste de México y Centroamérica y es un cíclido de gran importancia a nivel comercial. A pesar de su potencial para la acuicultura, existe poca información sobre sus vías metabólicas relacionadas con la fisiología y la nutrición. Este estudio se centra en la expresión de los genes de las desaturasas de ácidos grasos (*fads2*) y elongasas (*elovl4b* y *elovl5*) en embriones de 0 días post-eclosión (dpe) y larvas de 5, 10, 15, 20, 25 y 30 dpe. Adicionalmente, se analizó la expresión diferencial de estos genes en hígado, intestino, riñón, músculo, corazón, testículos, branquias, estómago, páncreas, cerebro y tejido adiposo en peces adultos. En nuestro estudio, utilizamos oligonucleóticos específicos diseñados para *fads2*, *elovl4b* y *elovl5* para determinar la expresión relativa a través de qPCR. Descubrimos que la expresión más alta se observó en larvas a los 20 dph y en adultos, predominantemente en el hígado y el intestino de los tres genes analizados. Es importante destacar que nuestros resultados mostraron que los genes de la desaturasa y la elongasa se expresan de manera diferencial en los tejidos de los adultos de *P. splendida* y su expresión fluctúa durante el desarrollo larvario.

Palavras-chave: Desaturase, Elongasas, Expresión de genes, Ontogenia temprana, *Petenia splendida*.

INTRODUCTION

The bay snook (*Petenia splendida* Günther, 1862) belongs to the Cichlidae family, distributed in the southeastern region of Mexico and Central America (Álvarez-González *et al.*, 2008). This species has piscivorous habits, although it also feeds on plant remains and insects, inhabiting freshwater bodies, and some studies indicate that it has a tremendous reproductive capacity and high fecundity (Álvarez-González *et al.*, 2013). Additionally, *P. splendida* is accepted in local markets and has high economic value and great potential for aquaculture. Our research, therefore, can significantly contribute to this field, potentially aiding aquaculture and conservation efforts (Álvarez-González *et al.*, 2010). In this sense, there are several studies describing different aspects of *P. splendida*, such as its biology and physiology (Jiménez-Martínez *et al.*, 2009; Treviño *et al.*, 2011), taxonomy and ecology (Méndez-García, 2010), aquaculture technology (Pérez-Sánchez, Páramo-Delgadillo, 2008; Vidal-López *et al.*, 2009), cytogenetics (Arias-Rodríguez *et al.*, 2008), molecular biology (Castillo-Collado *et al.*, 2022), and nutrition and digestive physiology (Álvarez-González *et al.*, 2008; Uscanga-Martínez *et al.*, 2011; Rodríguez-Estrada *et al.*, 2020).

Studies on the nutritional and energetic needs of aquatic organisms are crucial to maximizing growth in less time and improving the profitability of the culture. Optimizing the use of nutrients, particularly dietary lipids, is fundamental to the metabolic functions of these organisms (Lee *et al.*, 2003). In this context, lipids are divided into saturated (no double bond) and unsaturated fatty acids that have one or more double bonds (unsaturation) along the carboxylic chain, and they can be divided into monosaturated (MSFA) and polyunsaturated fatty acids (PUFA) (Fahy *et al.*, 2011).

Long-chain polyunsaturated fatty acids (LC-PUFA) are essential and contain more than 20 carbons in their molecular structure with multiple double bonds. They can be classified as n-3 and n-6 according to their final double-bond position with the final methyl group (Monroig *et al.*, 2011a), particularly LC-PUFA, play a crucial role in the fluidity of the membrane structure and signaling processes, which are relevant in many physiological functions such as growth, neurological functions, inflammation, immune response, and reproduction (Colombo *et al.*, 2017; Ayisi *et al.*, 2018). LC-PUFA requirements, such as arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are essential for fish, mainly marine fish (Takeuchi, 1997; Mejri *et al.*, 2021), due to the lack of desaturases and elongases genes, many marine species are not able to synthesize these kinds of PUFA (Monroig *et al.*, 2022). Considering the above, molecular studies of the enzymes involved in lipid synthesis have recently been developed, revealing a diversity of biosynthesis routes in different nonaquatic organisms. In contrast, studies in fish have shown a great diversity of adaptations in lipid metabolic pathways (Morais *et al.*, 2015).

During LC-PUFA biosynthesis from C18 PUFA, two families of enzymes of great importance participate in fatty acyl desaturases (*fads*) and very long chain fatty acid elongation (*elovl*). The first enzyme is responsible for introducing a new double bond (unsaturation) between the carboxyl terminus of a fatty acyl chain and a pre-existing double bond. At the same time, *elovl* is responsible for catalyzing the initial rate-limiting condensation reaction of chain elongation fatty acyl. According to various works, *fads1* has been lost in modern teleost, with a predominance of *fads2* presenting a great $\Delta 4$, $\Delta 6$, and $\Delta 5$ diversification, mainly in freshwater fish (Fonseca-Madrigal *et al.*, 2014; Ferraz *et al.*, 2019; Li *et al.*, 2019; Lopes-Marques *et al.*, 2018; Garrido *et al.*, 2019, 2021). In the case of *Elovl*, three isoforms are recorded in teleost *elovl2*, *elovl4b*, and *elovl5*, which are involved in the processes of LC-PUFA biosynthesis (Castro *et al.*, 2016; Zhu *et al.*, 2018).

Despite the extensive research on lipid metabolism, the function of the genes that code for the desaturase and elongase enzymes and their conversions remains a mystery, particularly in this species of cichlid, such as *P. splendida*. For this reason, this study presents a groundbreaking approach by aiming to clone the partial sequences, functional characterization, and tissue distribution of *fads2*, *elovl4*, and *elovl5* involved in the metabolic pathways of LC-PUFA biosynthesis in adults and on the initial ontogeny of *P. splendida*.

MATERIAL AND METHODS

Fish acquisition. A total of 20 adult *P. splendida* specimens were obtained from the Laboratory of Physiology in Aquatic Resources (LAFIRA) in the División Académica de Ciencias Biológicas at the Universidad Juárez Autónoma de Tabasco, Southeast Mexico (catalog number ECOSC 14897 of *Petenia splendida* included in the ichthyological collection of the Colegio de la Frontera Sur (ECOSUR) in San Cristóbal de las Casas, Chiapas, Mexico). Fish were kept in circular 2000-L polyethylene tanks and were fed with a rainbow trout diet (45% protein and 16% fat, El Pedregal® Silver Cup, Toluca, Mexico) at apparent satiation three times per day (7:00, 13:00 and 19:00 h) with

particle diameters ranging between 5.5 and 9.0 mm. Water parameters were constantly assessed with a YSI 85® Meter, YSI Inc., Yellow Springs, OH (temperature ($28.0 \pm 0.7^\circ\text{C}$), dissolved oxygen ($5.9 \pm 0.6\text{ mg/L}$), and pH (7.1 ± 0.3)). Embryos and larvae were obtained from spontaneous spawning from the broodstock in the same facility. Six females and three males were transferred from holding tanks to a 2000-L breeding tank. Six acrylic sheets (one side smooth, one side rough) were placed in each tank to provide shelter and egg-laying surfaces (rough side). Right after hatching, larvae were separated from the adults. After 3 days, 150 larvae per tank were placed in three 70-L oval tanks with constant aeration ($\sim 95\%$ air saturation) connected to an open system with water changes (80%) every two or three days. Larvae were fed satiety with brine shrimp (*Artemia* sp.) nauplii five times per day (8:00, 11:00, 13:00, 15:00, and 18:00 h) for 7 days (until 10 days post-hatching, dph). From 11 to 13 dph, larvae were provided with a co-feeding of brine shrimp nauplii and trout feed (Silver Cup, Nelson and Sons, Inc; proximate composition: 45% proteins, 16% lipids, 21% carbohydrates, 9–12% ashes) and from the 14 dph, larvae were only provided with trout feed until 30 dph. Food was provided at apparent satiation, and particle size was adjusted according to larval growth (250–500, 500–750, and $> 750\text{ }\mu\text{m}$). During larviculture, water parameters were constantly assessed with a YSI 85® Meter, YSI Inc., Yellow Springs, OH (temperature ($28.1 \pm 0.5^\circ\text{C}$), dissolved oxygen ($5.8 \pm 0.5\text{ mg/L}$), and pH (7.0 ± 0.2)).

Sampling. Captive *P. splendida* male adults were euthanized by cold thermal shock (-4°C) after 24 h of fasting and dissected for the harvesting collection of 11 organs: liver, intestine, kidney, muscle, heart, testis, gill, stomach, pancreas, brain, adipose tissue. Larvae were sampled on different days after hatching (10 larvae per tank) before first feeding, starting from the embryo (considered as 0 dph) and 5, 10, 15, 20, 25, and 30 dph. Larvae were removed from each tank, rinsed in distilled water, and transferred to Eppendorf tubes with 1.5 mL of RNA Later and stored at -80°C .

RNA extraction and cDNA synthesis. RNA extraction was performed from tissues and pooled larvae (10 individuals) using the Trizol Reagent (Invitrogen, Carlsbad, CA) method under the manufacturer's indications. RNA concentration and purity (through the 260/280 ratios) were measured with a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The integrity of RNA was confirmed after observing two discrete bands for 28s and 18s ribosomal RNA in a native agarose electrophoresis (1.5%). The First strand cDNA synthesis process using the iScript™ Select cDNA Synthesis Kit 170–8,896 (Bio-Rad, Hercules, CA). Reverse transcription reactions were performed following the kit manufacturer's protocol. First-strand cDNA was synthesized by incubating a 20 μL total reaction mix containing 2 μL random primer, 4 μL 5x iScript select reaction mix, 1 μL reverse transcriptase, 1 μg total RNA, and nuclease-free water at 25°C for 5 min and then at 42°C for 30 min. Inactivation of reverse transcriptase was performed by incubating the reaction mix at 85°C for 5 min and the product was stored at -20°C for use in PCR amplification. Subsequently, 1 μL of cDNA was used for the end-point Polymerase Chain Reaction (PCR). The cDNA samples were run in a 96-well thermocycler using the Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA) to obtain the partial sequences of fatty acyl desaturase (*fads2*) and fatty acyl elongases (*elovl4b* and *elovl5*). Amplification

was conducted under the following conditions: 10 min at 95°C, followed by 35 cycles at 95°C for 30 s, 58°C for 30 s, and 72°C for 50 s with a 5 min extension at 72°C using specific oligonucleotides previously determined from alignment (using Clustal-W software, Infobiogen) of corresponding sequences available in the library from different species for the *fads2* gene, the sequences of fish such as Nile tilapia (*Oreochromis niloticus* KF268464.1), princess cichlid (*Neolamprologus pulcher*, XM 006789228.2), flameback (*Pundamilia nyererei*, XM 005730101.1) and Eastern happy (*Astatotilapia calliptera*, XM 026175749.1) while for the gene *elovl4* sequences of the fish were taken from *Neolamprologus pulcher* (XM 006797273.1), *Astatotilapia calliptera* (XM 026143115.1), *Oreochromis niloticus* (XM 003440621.5) and *Stegastes partitus* (008302422.1). Finally, for the gene *elovl5*, the sequences were obtained from *Oreochromis niloticus* (NM 001279460.1), *Neolamprologus pulcher* (XM 006800731.2), *Maylandia zebra* (XM 023153084.2), and *Astatotilapia burtoni* (XM 005915537.3) (Tab. 1).

The amplification products were separated in 1.5% agarose gel stained with ethidium bromide. Observed bands under UV light (Bio-Rad® Model Universal Hood II, Hercules, CA) were cut from the gel and purified using the PureLink® PCR Purification Kit (Invitrogen). The purified bands were sent to the Synthesis and Sequencing Unit of the Institute of Biotechnology of the Universidad Nacional Autónoma de México (UNAM) to be sequenced.

Sequence analysis. Obtained partial sequences were edited and analyzed using ExPASy translation software to search for the open reading frame (ORF). Once the ORF was identified, it was translated to amino acid (AA) sequences using standard genetic codes. The nucleotide sequence was compared with DNA sequences from other fish available in the GenBank database network service at NCBI (<https://blast.ncbi.nlm.nih.gov/>). Protein sequence alignments were performed by the multiple sequence alignment software BioEdit 7.2 (www.mbio.ncsu.edu/bioedit/bioedit.html). A phylogenetic tree was generated using neighbor-joining (NJ) methods based on the AA sequence using MEGA 7.0 software.

Quantitative polymerase chain reaction (qPCR). The resulting cDNA from adult tissues, embryos, and larvae were diluted in 200 µL of distilled water. The quantitative polymerase chain reactions (qPCRs) were performed in a 96-well thermocycler CFX96 Real-Time System Thermal Cycle (Model C1000, CA). The reaction mixture included 10 µL of Eva Green, 2 µL cDNA, and 0.2 µL of each primer (shown in Tab. 1). The

TABLE 1 | Oligonucleotides used for sequencing and real-time polymerase Chain Reaction in *Petenia splendida*.

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')	Size, pb	Step
<i>fads2</i>	ctggtttgtgtgggtkactb	tytatrgagatatgcacca	317	RT-PCR
	cgagaccactgttttccaa	cccagagggttttcagtgacg	154	qPCR
<i>elovl4b</i> <i>elovl5</i> <i>actb</i>	ctggacacggctdctwcat	bggtmggtctgtagtaaaa	377	RT-PCR
	cagcgttcggacaaaagatc	aggatgatgtacgtgacggc	178	qPCR
	tactccaga achctcacng	aggvtgscgggatggctgg	285	RT-PCR
	cacctctaccaccacgctag	gatggctggaatggctgaga	150	qPCR
	gagctatgagctgcctgatgg	gtggtctcatgaatgccacagg	119	qPCR

thermal program included 2 min at 95°C, followed by 38 cycles at 95°C for 10 s, 60°C for 30 s, and extension at 70°C for 5 s. Duplicates performed all reactions. To normalize the gene expression of the evaluated genes, it is important to mention that three reference genes were used: *actb*, *ef1*, and *18S rRNA*; however, *actb* was considered the best for the larvae/tissue samples since it complied with a constitutive and stable expression according to Jiménez-Martínez *et al.*, 2022. A standard curve for each pair of primers was generated to estimate amplification efficiencies based on known amounts of cDNA (four serial dilutions corresponding to cDNA transcribed from 100 to 0.1 ng of total RNA), obtaining values between 97 and 99%. The melting curve analysis determined that the melting temperature peak varied between 81.5 and 83 °C, which corresponded to the product obtained by these primers. In addition, the absence of primer dimers and nonspecific ties was confirmed. Relative gene expression of tissues and larval growth stages was calculated using the delta-delta copy threshold (CT) method (Pfaffl, 2001).

Statistical analysis. The relative expression of the gene fatty acyl desaturase *fads2* and fatty acyl elongases *elovl4b* and *elovl5* between the different tissues of *P. splendida* adult and the comparison between embryos and larvae at different ages (dph) were analyzed using the Kruskal-Wallis test. A posteriori Nemenyi test was performed to determine significant differences between tissues (adults) and developmental time (embryos and larvae) ($P \leq 0.05$). All statistical analyses were performed using the software Statistica TM v. 7.0 (Statsoft, Tulsa, OK).

RESULTS

PCR amplification and sequencing analysis. A meticulously obtained partial sequence for *fads2* of 317 bp that encodes 105 AA was registered in the GenBank accession number ON 142679 (Fig. 1A). Similarly, the gene *elovl4b* was sequenced with utmost precision, obtaining a partial sequence of 377 bp that encodes 120 AA with accession number ON720978 (Fig. 1B). Finally, for the *elovl5* gene, a carefully obtained partial sequence of 285 pb that encodes 95 AA with accession number ON142677 (Fig. 1C).

The phylogenetic tree revealed intriguing evolutionary relationships. We observed that *P. splendida fads1* forms a clade with a high bootstrap of 99% with Nile tilapia, indicating a close evolutionary relationship, and a bootstrap of 48% with *Hippoglossus stenolepis* (Fig. 2A). Similarly, *elovl4b* forms a clade with a strong bootstrap of 100% between *P. splendida* and Nile tilapia, suggesting a recent common ancestor, and a bootstrap of 31% with *Astatotilapia burtoni* (Fig. 2B). Finally, *elovl5* forms a clade with a robust bootstrap of 100% between *P. splendida* and Nile tilapia, and a bootstrap of 58% with *Neolamprogus pulcher* (Fig. 2C).

Relative expression in adult fish tissues. The relative expression in the tissues of *P. splendida* adults showed that the *fads2* gene had the highest expression in the liver, indicating its crucial role in this organ's function. It is followed by the intestine, suggesting its involvement in digestion. Lower levels of expression were detected in adipose tissue, brain, pancreas, stomach, gill, muscle kidney, testis, and heart, indicating

A Accession ON 142679 delta-6-desaturasa (*fads2*), partial
Petenia splendida.

gatg aat cac ata ccg atg gac atc gat cat gaa aag cac agg gac tgg gtg acc atg cag 61
M N H I P M D I D H E K H R D W V T M Q 20
tta caa tcc acc tgt aat atc gag cag tcc ttc aac gac tgg ttc agt gga cac ctc 121
L Q S T C N I E Q S F F N D W F S G H L 40
aac ttt caa atc gag cac cac tgg ttt cca atg atg ccg cgg cac aac tac cac ctg gtg 181
N F Q I E H H L F P M M P R H N Y H L V 60
gcc ccg cag gtc cgt gcg ctc tgc gag aaa cac ggg att cct tac cag gag aaa act ttg 241
A P Q V R A L C E K H G I P Y Q E K T L 80
tgg cga ggc ttc gct gat att gtc acg tca ctg aaa acc tct ggg gac ctc tgg ctg gat 301
W R G F A D I V T S L K T S G D L W L D 100
gca tat ctc cat aaa t 317
A Y L H K 105

B Accession ON720978 fatty acid elongase 4 (*elovl4b*),
partial *Petenia splendida*.

tcatg agg aag aag ttt aat cag gtc agc ttc ctg cac gtc tat cac cac tgc aca atg ttc 62
M R K K F N Q V S F L H V Y H H C T M F 20
atc ctg tgg tgg atc ggc atc aaa tgg gtt cct ggc gga caa tct ttc ttt ggc gct acg 122
I L W W I G I K W V P G G Q S F F G A T 40
att aat tca ggc att cat gtg ctg atg tat ggc tac tat ggc ctg gca gcg ttc gga cca 182
I N S G I H V L M Y G Y Y G L A A F G P 60
aag atc cag aag tac ctg tgg tgg aag aaa tac ctc aca att att cag atg atc cag ttc 242
K I Q K Y L W W K K Y L T I I Q M I Q F 80
cac gtc acc att ggc cac gcc gct cat tct ctc tac acg ggc tgc cca ttc cca gcc tgg 302
H V T I G H A A H S L Y T G C P F P A W 100
atg cag tgg gct ctg att ggc tac gcc acg tac atc atc ctg ttt gcc aac ttc tac 362
M Q W A L I G Y A V T Y I I L F A N F Y 120
tac cag aca tac cga 377
Y Q T Y R

C Accession ON142677 fatty acid elongase 5 (*elovl5*),
partial *Petenia splendida*.

tgca gag gaa gcg gat att aag gtt ata aat gtc ctc tgg tgg tac tac ttc tcc aag ctc 61
A E E A D I K V I N V L W W Y Y F S K L 20
atc gag ttc atg gac acc aac ttc ttc ata cta cga aaa aat aat cac cag atc acg ttc 121
I E F M D T N F F I L R K N N H Q I T F 40
ctg cac ctc tac cac cac gct agc atg ctg aat atc tgg tgg ttt gtt atg aac tgg ata 181
L H L Y H H A S M L N I W W F V M N W I 60
ccc tgt ggt cat tgc tac ttc ggt gcc tca cta aac agc ttc atc cac gtc gtg atg tat 241
P C G H S Y F G A S L N S F I H V V M Y 80
tcg tac tat ggc ctc tca gcc att cca gcc atc cgg cca tac ct 285
S Y Y G L S A I P A I R P Y L 95

FIGURE 1 | Partial sequence of nucleotides and amino acids (AA) encoding delta-6-desaturase (*fads2*) (A), fatty acid elongase 4 (*elovl4b*) (B), fatty acid elongase 5 (*elovl5*) (C), and from *Petenia splendida* taken from Gen Bank to design specific oligonucleotides for qPCR.

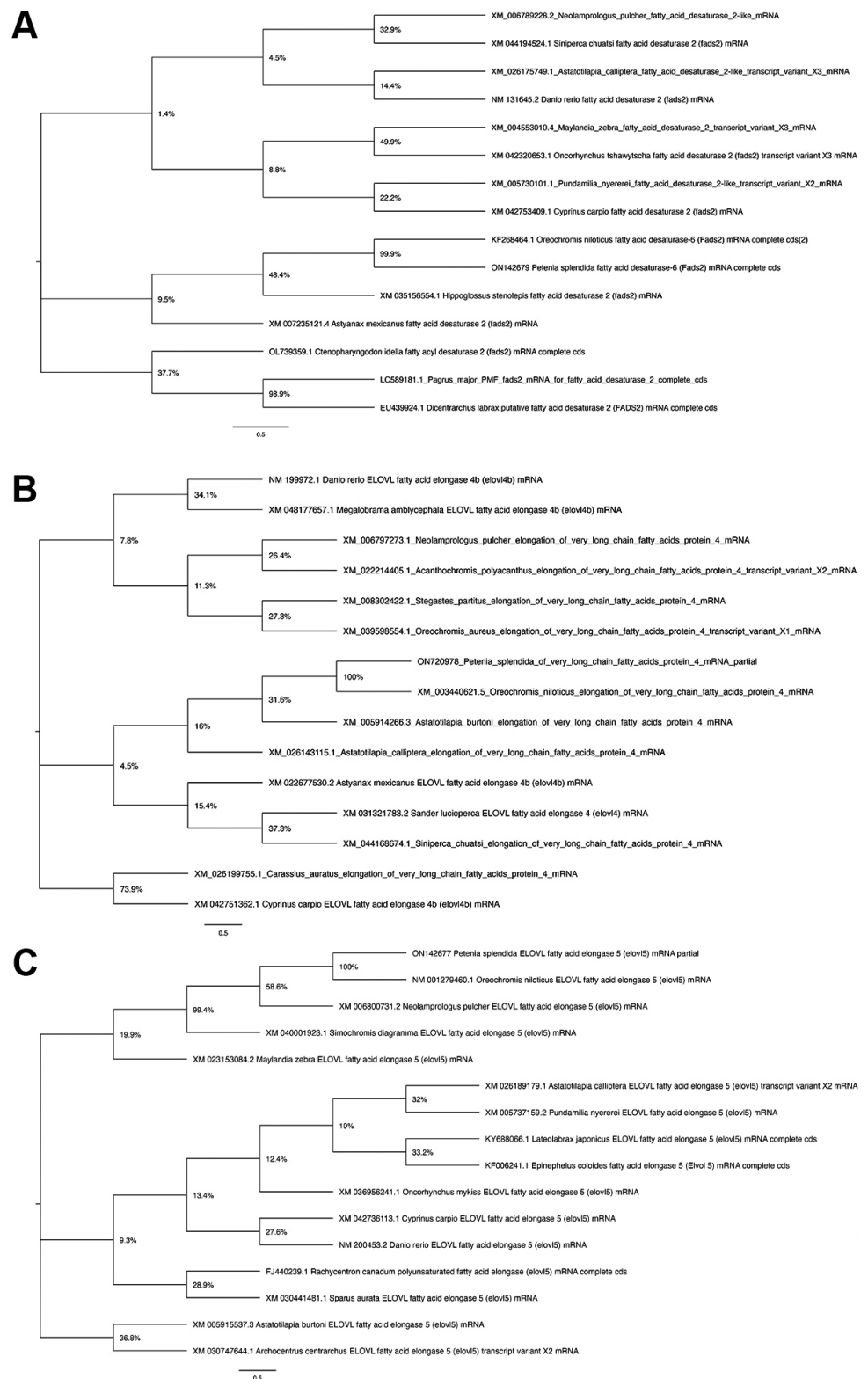


FIGURE 2 | Phylogenetic tree based on the sequence of delta-6-desaturase (*fads2*) (A), fatty acid elongase 4 (*elovl4b*) (B), fatty acid elongase 5 (*elovl5*) (C) from *Petenia splendida* and other teleosts using the neighbor-joining (NJ) method. Values at branch points represent percentage frequencies for tree topology after 1,000 interactions.

its presence in these tissues as well ($P \leq 0.05$) (Fig. 3A). The highest expression level of *elovl4b* was detected in the testis, suggesting its role in reproductive functions, followed by the intestine with low expression detected in the other tissues analyzed ($P \leq 0.05$) (Fig. 3B). Concerning *elovl5*, the highest expression was detected in liver, followed by the intestine and lower levels of expression were detected in the brain, kidney, pancreas, muscle, heart, testis, gills and stomach in *P. splendida* adults, indicating its widespread presence in various organs ($P \leq 0.05$) (Fig. 3C).

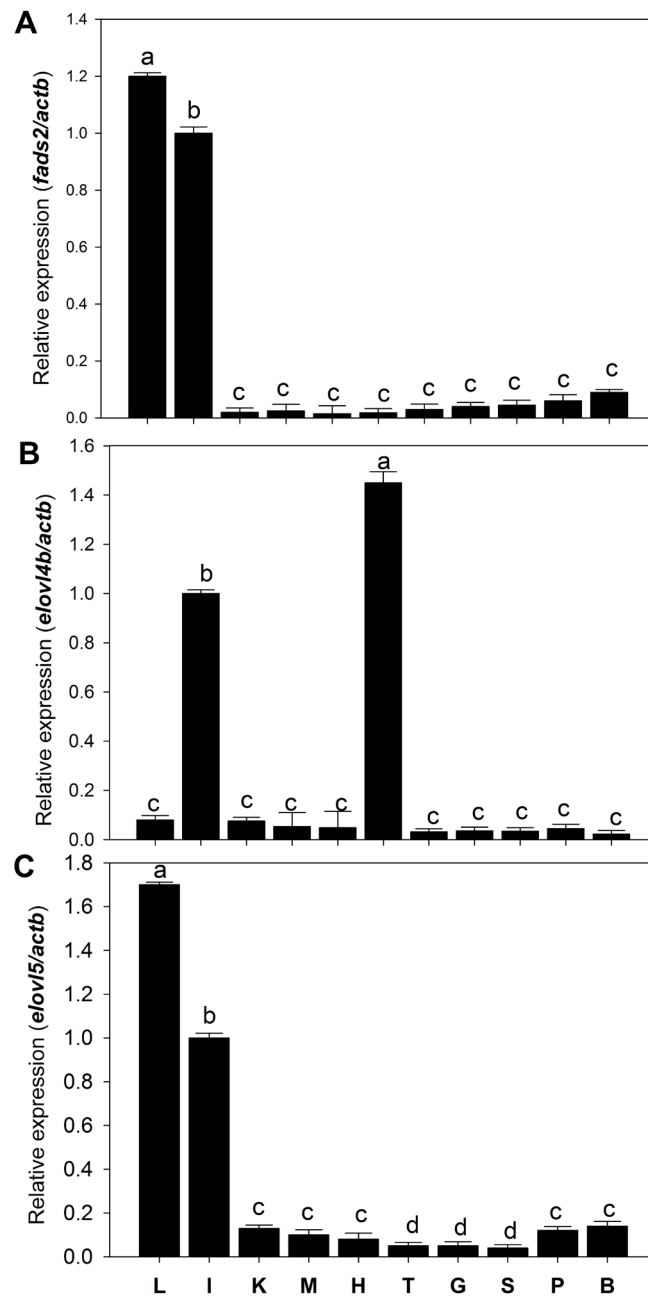


FIGURE 3 | Relative expression of delta-6-desaturase (*fads2*) (A), fatty acid elongase 4 (*elovl4b*) (B), fatty acid elongase 5 (*elovl5*) (C) in the liver (L), intestine (I), kidney (K), muscle (M), heart (H), testis (T), gill (G), stomach (S), pancreas (P), brain (B), adipose tissue (AT) of adult *Petenia splendida* (mean $\pm$ SEM; $n = 3$). Lowercase letters indicate significant differences between the tissue expression levels ($p < 0.05$).

Relative expression in larvae. The *fads2*, *elovl4b*, and *elovl5* transcripts were detected from the embryo, decreased at day 5, 10, and 15 dph, and the highest expression was detected on days 20, followed by 25 and 30 dph (Figs. 4A–C). This gene expression pattern suggests that these genes may be a specific role of these genes in the larval stage.

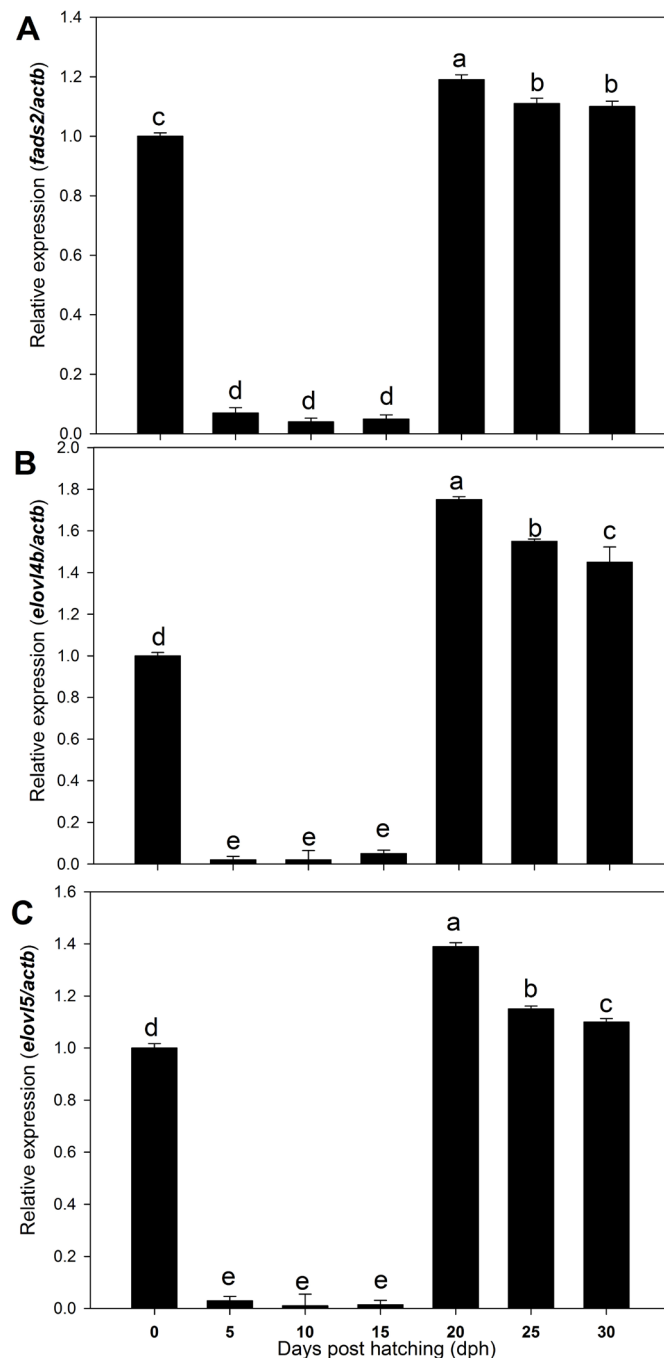


FIGURE 4 | Relative expression of delta-6-desaturase (*fads2*) (A), fatty acid elongase 4 (*elovl4b*) (B), and fatty acid elongase 5 (*elovl5*) (C) during the early ontogeny of *Petenia splendida*. Lowercase letters indicate significant differences in the expression as a function of developmental time ($p < 0.05$).

DISCUSSION

Characterization and expression of *fads2*, *elovl4b*, and *elovl5* in tissues from *P. splendida* adults. In our study, we cloned, characterized, and evaluated the relative expression of two genes of the desaturase group (*fads*) and one of the elongases (*elovl*) in adults of *P. splendida*, which are essential for the biosynthesis of low-chain polyunsaturated fatty acids (LC-PUFA) from linolenic acid (LA) and α -linolenic acid (ALA). In this regard, desaturases (FADS) incorporate double bonds in the fatty acid chain, and elongases (ELOVL) elongate the carbon chains of fatty acids, adding two-carbon atom units (Monroig et al., 2018; Galindo et al., 2021). Teleost (modern) fishes have lost the *fads1* gene, presenting exclusively *fads2*, which has diversified to fulfill a bifunctional function such as $\Delta 6\Delta 5$ (Castro et al., 2012, 2016), as shown in Nile tilapia (*O. niloticus*) striped snakehead (*Channa striata*), Japanese medaka (*Oryzias latipes*) Osteoglossomorpha (*Pantodon buchholzi*), zebrafish (*Danio rerio*) and tambaqui (*Colossoma macropomum*), white rabbitfish (*Siganus canaliculatus*), silverfish (*Chirostoma estor*), tench fish (*Tinca tinca*), where this enzyme is responsible for the desaturation of PUFA to LC-PUFA (Tanomman et al., 2013; Fonseca-Madrigras et al., 2014; Kuah et al., 2015; Oboh et al., 2017; Lopes-Marques et al., 2018; Ferraz et al., 2019; Garrido et al., 2019, 2020; Li et al., 2019). In contrast, $\Delta 4$ *fads2* has been reported in several teleost species (Morais et al., 2012, 2015; Fonseca-Madrigras et al., 2014; Kuah et al., 2015; Oboh et al., 2017; Garrido et al., 2019). However, it is essential to emphasize that $\Delta 4$ Fads2 is reported in the pituitaries of cichlids, including *Oreochromis niloticus*, *Maylandia zebra*, *Haplochromis burtoni*, and *Pundamilia nyererei* (Oboh et al., 2017; Li et al., 2020). In fish liver, $\Delta 6$ -desaturase catalyzes the conversion of fatty acids precursors into n-6 and n-3 series PUFAs. These PUFAs are especially important for fish because they influence the composition of lipids in tissues by incorporating them into phospholipids in cell membranes, affecting their fluidity and permeability, as well as for the development of tissues and organs and the regulation of energy metabolism among other metabolic processes involved in reproduction and tolerance to environmental changes (Xu et al., 2014). Finally, $\Delta 8$ desaturases are reported as an intrinsic feature among teleost fishes (Monroig et al., 2011b; Garrido et al., 2019). In the case of elongases, three *elovls* (*elovl2*, *elovl4*, and *elovl5*) have been reported (Castro et al., 2016). Records of *elovl2* expression are studied in Atlantic salmon (*Salmo salar*) and *Danio rerio*, and the enzyme *elovl2* was cloned and functionally characterized for Atlantic salmon (Hastings et al., 2001; Morais et al., 2009) and later zebrafish *Danio rerio* (Monroig et al., 2009) and *O. mykiss* (Gregory, James, 2014). In the case of *elovl4* in zebrafish, two isoforms, *elovl4a*, and *elovl4b*, are recorded; the first is expressed mainly in the brain and the second in the gonads. The *elovl4a* in zebrafish *Danio rerio* (Monroig et al., 2010) and *elovl4b* in cobia (*R. canadum*), Atlantic salmon (*S. salar*), and rabbitfish (*S. canaliculatus*) (Carmona-Antoñanzas et al., 2011; Monroig et al., 2011a; Monroig et al., 2012a). Finally, *elovl5* has been cloned and characterized in species such as Atlantic salmon (*Salmo salar*) (Morais et al., 2009), Nile tilapia (*Oreochromis niloticus*), cobia (*Rachycentron canadum*) (Zheng et al., 2009), black seabream (*Acanthopagrus schlegelii*) (Kim et al., 2012), meagre (*Argyrosomus regius*) (Monroig et al., 2013), rainbow trout (*O. mykiss*) (Gregory, James, 2014), Nibe croaker *Nibea mitsukurii* (Kabeya et al., 2015).

According to our study, the intestine was the organ in which the sequences of the desaturase *fasd2*, *elovl4b*, and *elovl5* elongase genes were obtained in adults of *P. splendida*. This tissue was selected due to its lipid metabolic capabilities to obtain the partial sequences and was analyzed through phylogenetic trees. The phylogenetic tree indicated a large percentage of identity between the amino acid sequences with the tilapia species (*O. niloticus*), possibly due to the high phylogenetic relationship that both freshwater cichlids that influences the biosynthetic capacity of LC-PUFA of teleost (Schmitter-Soto, 2007; Galindo *et al.*, 2021; Castillo-Collado *et al.*, 2022). This is consistent with various studies investigating the amino acid sequences of *fads* and *elovl* in teleost fish and cichlids such as *O. niloticus*, *M. zebra*, and *H. burtoni*, where they mention that these fish have a long-chain PUFA (LC-PUFA) biosynthesis capacity that is very different from other vertebrates (Obloh *et al.*, 2017; Garrido *et al.*, 2019). This is due to the evolutionary changes that these genes have undergone, such as: conservation of ancestral function, pseudogenization, sub functionalization, and neofunctionalization, causing the modification of amino acids that alter the specificity, affinity, or kinetic properties of the enzymes, as well as the establishment of new protein interactions due to changes in the coding sequence or subcellular localization (Fonseca-Madrigal *et al.*, 2014; Obloh *et al.*, 2017). In the analysis of the expression levels of *fasd2* desaturase and *elovl4b* and *elovl5* elongases in the tissues of *P. splendida* adults, the *fasd2* and *elovl5* showed the highest level of expression in the liver, followed by the intestine and brain. In the case of *elovl4b*, the highest expression level was recorded in the testis, where long chain fatty acids (PUFAs) are essential components of phospholipids, which are part of cell membranes, it is closely related to several vital functions such as the formation and maturation of sperm, maintaining the integrity and fluidity of the cell membranes of germ cells. This enzyme plays an important role in the regulation of spermatogenesis (Engel *et al.*, 2020), followed by the intestine, suggests that locally synthesized PUFAs could have specific functions related to intestinal modulation in the cell membrane properties, facilitating dietary lipids absorption and modulate the immune response. Their synthesis in the intestine could be essential to maintain intestinal homeostasis and protect against pathogens and their local synthesis is necessary for growth and renewal of intestinal cells (Xu *et al.*, 2016) due to elongases and desaturases are key enzymes involved in lipoproteins and cholesterol metabolism (Panserat *et al.*, 2009). The expression of *elovl4b* could vary in response to different diets, allowing fish to adjust the composition of PUFAs in their tissues to adapt to different food sources (Benedito-Palos *et al.*, 2014). Our results are consistent with the studies found in various freshwater fishes such as *D. rerio* (Monroig *et al.*, 2009), pike silverside (*Chirostoma estor*) (Fonseca-Madrigal *et al.*, 2014), and tench (*Tinca tinca*) (Garrido *et al.*, 2020). In the case of marine fish, the opposite is reported. The highest expression of these genes is recorded in the brain of vertebrate species (Castro *et al.*, 2016). In this sense, the increased expression of *fasd2* and *elovl5* is reflected in the liver because it is the main organ responsible for metabolizing lipids, while the intestine plays a vital role in the absorption and biosynthesis of LC-PUFA (Monroig *et al.*, 2012b; Garrido *et al.*, 2020; Rodríguez *et al.*, 2022). Several studies in fish mention that fatty acid synthesis in enterocytes was regulated by nutritional factors, precisely the fatty acid composition of the diet, in a manner generally like that of hepatocytes (Fonseca, Madrigal *et al.*, 2006; Monroig *et al.*, 2012a; Morais *et al.*, 2015). Similarly, in

the case of *elov4b*, the highest expression was recorded in the testicle, followed by the intestine. This high expression agrees with the work carried out in the species *Danio rerio*, *Gadus morhua*, and *Clarias gariepinus*, where the highest expression of *elov4b* is reported in the gonads and retina (Monroig, 2010; Xue et al., 2014; Oboh et al., 2017; Ferraz et al., 2019, 2020; Morais et al., 2020). In this sense, *elov4b* participates in the biosynthesis of VLC-PUFA through consecutive elongations of a varied range of shorter-chain PUFA precursors; these lipids are usually synthesized in the retina and gonads (Oboh et al., 2017). According to our results, the high expression of *elov4b* in the testis may be because the testis contains large amounts of VLC-PUFA, essential for phototransduction and male fertility, indicating that males are ready for reproduction (Poulos, 1995; Oboh et al., 2017). In this way, VLC-PUFAs are lipids that are usually synthesized in the retina and testes; this explains why the high expression of *elov4b* in the testis may indicate that males are ready for reproduction (Ferraz et al., 2020). The expression of *elov4b* was presented in the intestine as well as other species, such as *Sparus aurata* and *Solea senegalensis*, where it mentions that adult fishes, both *elov4a* and *elov4b* are distributed in all the tissues analyzed, being greater its expression in the brain, retina, and gonads, which are relevant in the synthesis of the LG-PUFA (Zhao et al., 2019; Morais et al., 2020).

Expression of *fasd2*, *elov4b* and *elov5* of *P. splendida* larvae. According to the results obtained in our study, the levels of *fasd2* desaturase, *elov4b*, and *elov5* elongases during the initial ontogeny of *P. splendida* expression were detected from day 0 DAH. This high expression can be attributed to two factors: the first factor may be maternal mRNA, this type of transcript is active in the oocyte maturation process and remains until the early stages of embryogenesis (Fujimura, Okada, 2007; Burggren, Blank, 2009; Treviño et al., 2011; Lubzens et al., 2017; Ahi et al., 2018), the second factor may be due to the different nutrients found in eggs (lipids, fatty acids, free amino acids, minerals, and vitamins) and lipids are found in a higher concentration of neutral lipids stored in oil droplets, while in yolk we find phospholipids and lipovitellins necessary for their structural functions and for the maintenance of metabolic pathways (Ospina-Robles, 2020; Burggren, Blank et al., 2009; Monroig et al., 2010; Castillo-Collado et al., 2022). In the case of *fasd2* desaturase and *elov4b* and *elov5* elongases, the highest level of expression was detected at 20 dph followed by days 25 and 30 dph; our results agree with the study by Uscanga-Martínez et al. (2011) where they mention that the lipase enzyme activity in the initial ontogeny of *Petenia splendida* lipase was high at the time of hatching and increased to 15 and 20 dph, after which it gradually decreases due to the lipid inclusions throughout development reflect a high level of lipids contained in the supplied diets (trout diet). These lipids administered in the commercial diet possibly activate the pathway of genes involved in lipid metabolism, associated with morphophysiological processes such as the arrangement between hepatocyte cords and pancreatic acinar tissue, development of the brain, retina, and gonads (Jiménez-Martínez et al., 2019; Zhao et al., 2019; De la Cruz et al., 2021). Similarly, at day 20 dph, the larvae are fully formed with all their functional organs, such as the intestine, liver, brain, eyes, and retina tissues, related to the synthesis of fatty acids coinciding with the same pattern of development as other species of cichlids *Oreochromis niloticus*, *C. urophthalmus*, *Amphilophus trimaculatus*, (Morrison et al., 2001; Monroig et al.,

2011b; Cuenca-Soria *et al.*, 2013; Li *et al.*, 2020; Frías-Quintana *et al.*, 2021; Hilerio-Ruiz *et al.*, 2021). Also, in some morphological studies during early ontogeny, lipid accumulation is recorded in the intestinal mucosa, liver, and pancreas, which has been observed in *P. splendida* larvae when fed a high lipid (16%) trout diet, indicating that although the fish were able to digest and absorb them, they were accumulated in the organisms, so that this diet does not meet the nutritional requirements of early juveniles of this species (Treviño *et al.*, 2011).

Finally, in our applied study of *P. splendida* in captive organisms, the composition of the diet plays an important role on the expression levels of *fasd2* and the elongases *elovl4b* and *elovl5* as reported by Zhao *et al.* (2023) where the HUFA content in the diet plays an important role in the functional and expression analyses of these genes in different tissues in adults and larvae of common carp (*Cyprinus carpio*). In addition to the diet, it is important to mention that the expression of *fasd2* and the elongases *elovl4b* and *elovl5* can be modified by the habitat, developmental stage, reproductive status, environmental factors, temperature and salinity (Tocher *et al.*, 2015; Garrido *et al.*, 2019; De La Cruz-Alvarado *et al.*, 2021). These factors are not only reflected in captive fish species but are also observed in wild species which are subjected to environmental stress due to anthropogenic pollution mainly by compounds called obesogens in their habitat causing an effect on the decrease of LC-PUFA content in organisms and an effect on transcription factors such as: response element binding proteins (SREBP) and peroxisome proliferator-activated receptors (PPAR) genes that regulate lipid metabolism and the genetic expression of the main pathways for LC-PUFA biosynthesis influencing oxidative stress and chemical toxicity (Zheng *et al.*, 2005; Martins *et al.*, 2012; Liao *et al.*, 2019; Capitão *et al.*, 2017; Koelmel *et al.*, 2020; Gladyshev *et al.*, 2022; Rigaud *et al.*, 2023).

In conclusion, the maximum expression of *fasd2* and the *elovl4b* and *elovl5* elongases was detected at 20 dph, followed by 25 and 30 dph days when organogenesis is complete (20 dph), especially in the liver and intestine. Similarly, in adults, *P. splendida* is expressed mainly in the liver and intestine, organs considered the main metabolic sites for the Novo LC-PUFA biosynthesis in these types of fish. The expression of these genes is relevant for to improve the design of diets and feeds that specifically match the fatty acid biosynthesis capabilities of the *P. splendida* species.

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ETHICAL STATEMENT

Animals were handled in compliance with the Norma Oficial Mexicana NOM-062-ZOO-1999 from the Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, the Mexican standard for good welfare practices for laboratory animals.

COMPETING INTERESTS

The authors declare no competing interests.

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