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**Long-chain polyunsaturated fatty acid biosynthesis in the euryhaline  
herbivorous teleost *Scatophagus argus*: Functional characterization, tissue  
expression and nutritional regulation of two fatty acyl elongases**

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29    **Abstract:**

30       Both the spotted scat *Scatophagus argus* and rabbitfish *Siganus canaliculatus* belong to the few  
31   cultured herbivorous marine teleost, however, their fatty acyl desaturase (Fad) system involved in  
32   long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis are different. The *S. argus* has a  $\Delta 6$   
33   Fad, while the rabbitfish has  $\Delta 4$  and  $\Delta 6/\Delta 5$  Fad, which were the first report in vertebrate and  
34   marine teleost, respectively. In order to compare the characteristics of elongases of very long-chain  
35   fatty acids (Elovl) between them, two Elovl cDNAs were cloned from *S. argus* in the present study.  
36   One has 885 bp of open read fragment (ORF) encoding a protein with 294 amino acid (aa) showing  
37   Elovl5 activity functionally characterized by heterologous expression in yeast, which was primarily  
38   active for the elongation of C18 and C20 PUFA. The other has 915 bp of ORF coding for a 305 aa  
39   protein showing Elovl4 activity, which was more efficient in the elongation of C20 and C22 PUFA.  
40   Tissue distribution analyses by RT-PCR showed that *elovl5* was highly expressed in liver compared  
41   to other tissues determined, whereas *elovl4* transcripts were only detected in eye. The expression of  
42   *elovl5* and *elovl4* were significantly affected by dietary fatty acid composition, with highest  
43   expression of mRNA in liver and eye of fish fed a diet with an 18:3n-3/18:2n-6 ratio of 1.7:1. These  
44   results indicated that the *S. argus* has a similar Elovl system in the LC-PUFA biosynthetic pathway  
45   to that of rabbitfish although their Fad system was different, suggesting that the diversification of  
46   fish LC-PUFA biosynthesis specificities is more associated with its Fad system. These new insights  
47   expand our knowledge and understanding of the molecular basis and regulation of LC-PUFA  
48   biosynthesis in fish.

49   **Key words:** Elovl5, Elovl4, LC-PUFA biosynthesis, *Scatophagus argus*

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51

## 52 **Introduction**

53 The long-chain (C20-24) polyunsaturated fatty acids (LC-PUFA), particularly  
54 eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids found primarily in  
55 fish and seafood, are regarded as beneficial in a series of human pathologies including metabolic  
56 disorders, cardiovascular, inflammatory and neurological diseases (Muhlhausler & Ailhaud, 2013;  
57 Delgado-Lista et al., 2012; Awada et al., 2013; Campoy et al., 2012). Comparing to freshwater and  
58 salmonids species, marine fish are generally thought to have limited capability or inability for *de*  
59 *novo* LC-PUFA biosynthesis (Sargent et al., 2002; Tocher, 2010). As marine fish are a major source  
60 of n-3 LC-PUFA for humans, the biosynthesis and metabolic regulation of these key nutrients have  
61 become areas of considerable research in recent years (Tocher, 2010; Xie et al., 2014; Zhang et al.,  
62 2014; Kabeya et al., 2015).

63 The biosynthesis of LC-PUFA from the C18 precursors  $\alpha$ -linolenic acid (LNA, 18:3n-3) and  
64 linoleic acid (LA, 18:2n-6) consists of sequential reactions catalyzed by a series of fatty acyl  
65 desaturase (Fads) and elongase of very long-chain fatty acid (Elovl) enzymes such as  $\Delta 6$  Fad,  $\Delta 5$   
66 Fad,  $\Delta 6/\Delta 5$  Fad,  $\Delta 6/\Delta 8$  Fad,  $\Delta 4$  Fad, Elovl5, Elovl4 and Elovl2 (Torstensen & Tocher, 2010; Li et  
67 al., 2010; Monroig et al., 2011a; Fonseca-Madriral et al., 2014; Castro et al., 2016). Differences or  
68 absence in the activity of enzymes in one or more steps of the pathways result in differential  
69 LC-PUFA biosynthetic capability in fish (Castro et al., 2016). Currently, existing data show that the  
70 capability for LC-PUFA biosynthesis in marine fish is more diverse than that in other vertebrates  
71 (Fonseca-Madriral et al., 2014) and the diversity was primarily associated with differences in the  
72 compliment of enzymes/activities in the pathway of LC-PUFA biosynthesis. Among marine fish,  $\Delta 6$   
73 Fad and Elovl5 cDNAs have been identified in more than a dozen species (Monroig et al., 2011b;  
74 Castro et al., 2016). However, Elovl4 has been investigated to a lesser extent but was reported in  
75 cobia (*Rachycentron canadum*) (Monroig et al., 2011c), rabbitfish (*Siganus canaliculatus*)  
76 (Monroig et al., 2012), Nibe croaker (*Nibea mitsukurii*) (Kabeya et al., 2015) and orange-spotted  
77 grouper (*Epinephelus coioides*) (Li et al., 2015).  $\Delta 4$  Fad has been identified in two marine fish,  
78 rabbitfish and Senegalese sole (*Solea senegalensis*, 1858) (Li et al., 2010; Morais et al., 2012),  
79 while  $\Delta 6/\Delta 5$  Fad was found only in rabbitfish (Li et al., 2010) among the marine teleost. Thus, to  
80 date, rabbitfish is the only marine teleost in which Fad and Elovl enzymes that possess all the  
81 activities required for the production of LC-PUFA from C18 PUFA have been found.

With respect to the differences in the capability for LC-PUFA biosynthesis among marine fish, Castro et al. (2012) hypothesized that the losses and diversifications of crucially important genes in the LC-PUFA biosynthetic pathway during fish evolution might be linked to habitat-specific food web characteristics, such as LC-PUFA availability, in different environments. More recently, other confounding factors including “trophic ecology” and diadromy have been proposed (Morais et al., 2012; Monroig et al., 2013). Herbivorous rabbitfish has a wide distribution in the coral reefs of the Indo-Pacific region (Woodland, 1983) and can also live in brackish water (Li et al., 2008), and feeds on a range of macroalgae including *Enteromorpha prolifera* and *Gracilaria lemaneiformis* (You et al., 2014).

Both the spotted scat (*Scatophagus argus*) and rabbitfish are economically important cultured teleost. The spotted scat has similar habitat (euryhaline) and feeding (herbivore) habits to rabbitfish, and is distributed widely in freshwater, brackish and marine habitats of the Indo-Pacific, South and South East Asia (Barry & Fast, 1992; Gandhi, 2002; Yoshimura et al., 2003). In order to know whether *S. argus* has an enzymatic complement for LC-PUFA biosynthesis similar to that of rabbitfish, we aimed to clone and functionally characterize all the genes involved in LC-PUFA biosynthesis in this species. Our previous study showed that the Fads2 of *S. argus* was a monofunctional  $\Delta 6$  desaturase enzyme ( $\Delta 6$  Fad), which is in contrast to the more diverse enzymatic complement ( $\Delta 4$  Fad and  $\Delta 6/\Delta 5$  Fad) found in rabbitfish (Li et al., 2010), suggesting that the above mentioned diversification also exists within marine herbivorous fish (Xie et al., 2014). Besides, two elongases including Elovl4 and Elovl5 were identified in rabbitfish (Oscar et al., 2012). In order to compare the characteristics of Elovl system between spotted scat and rabbitfish, and provide basis for fully understanding the LC-PUFA biosynthetic capability of *S. argus*, the present study reports the cloning, functional characterization, tissue expression and nutritional regulation of two Elovl cDNAs encoding putative Elovl4 and Elovl5, key enzymes with well-demonstrated roles in LC-PUFA biosynthesis in fish (Castro et al., 2016).

## Materials and Methods

### *Experimental fish and sampling*

Juvenile *S. argus* (body mass around 4.3 g) were purchased from a commercial hatchery in Zhuhai (Guangdong, China). Six isoproteic and iso-lipidic experimental diets (D1-D6) were

112 formulated with 32 % crude protein and 8 % crude lipid (soybean oil, perilla oil or fish oil as lipid  
113 source). Diet D2 contained fish oil (FO) as control, and diets D1, D3-D6 contained different  
114 proportions of soybean oil and perilla oil, which resulted in LNA: LA ratios of 0.14, 0.57, 0.84, 1.72  
115 and 2.85, respectively. The detailed dietary formulations, proximate and fatty acid compositions  
116 were shown in [Xie et al. \(2014\)](#).

117 All juvenile *S. argus* were reared in floating cages (0.6×0.6×3.0 m) located on the coast near  
118 Nan'ao Marine Biological Station (NAMBS), Shantou University, and fed an equal mix of the six  
119 experimental diets for two weeks before the start of feeding trial. The feeding trial was conducted in  
120 18 cages at ambient temperature, salinity and photoperiod, with each cage containing 25 fish that  
121 were allocated randomly. Fish in triplicate cages were fed one of the experimental diets twice a day  
122 (at 9:00 and 16:00 h) for 8 weeks. At the end of the feeding trial, fish were anaesthetized with  
123 0.01% 2-phenoxyethanol (Sigma-Aldrich Inc., USA), and livers of 54 fish (3 fish per replicate cage)  
124 were collected, frozen in liquid nitrogen and stored at -80 °C prior to the analysis of *elovl* mRNA  
125 expression by quantitative PCR (qPCR). In order to determine the tissue distribution of *elovl5* and  
126 *elovl4* transcripts, eye, brain, liver, muscle, heart, gills, spleen, kidney and intestine were collected  
127 from wild *S. argus* (50–60 g) captured from the coast near NAMBS, after fish were anaesthetized  
128 with 0.01% 2-phenoxyethanol. Tissue samples were frozen in liquid nitrogen immediately after  
129 collection and stored at -80 °C until RNA extraction.

### 130 ***Molecular cloning of elovl5 and elovl4 cDNAs***

131 Total RNA was extracted from *S. argus* liver and eye using Trizol reagent (Invitrogen, USA)  
132 and reverse transcribed into cDNA using random primers and an appropriate RT-PCR kit  
133 (Invitrogen, USA). For *elovl5*, degenerate primers (E5F1 and E5R1, Table1) were designed on the  
134 basis of alignment of fish *elovl5* including rabbitfish (GU597350), cobia (FJ440239), zebrafish  
135 (*Danio rerio*) (NM\_200453) and rainbow trout (*Oncorhynchus mykiss*) (AY605100), and used for  
136 amplifying partial fragments of putative *elovl5* cDNA from *S. argus* by polymerase chain reaction  
137 (PCR). For *elovl4*, degenerate primers (E4F1 and E4R1, Table1) were designed on the basis on the  
138 alignment of several fish *elovl4* including cobia (HM026361), rabbitfish (JF320823), and zebrafish  
139 *Elov14a* (NM\_200796) and *Elov14b* (NM\_199972), and used for amplifying partial fragments of  
140 putative *elovl4* cDNA fragment by PCR. For both *elovl* cDNAs, PCR (RT-PCR kit, Invitrogen, USA)  
141 consisted of an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at

142 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 1 min, followed by a final  
143 extension at 72 °C for 10 min. For both genes, the PCR fragments of the expected size were  
144 subsequently cloned into the pMD18-T vector (Takara, Dalian, China) and sequenced (Sangon,  
145 Shang Hai, China). Gene-specific primers were then designed to produce the full-length cDNA by  
146 5' (primers E5R2/E5R3 and E4R2/E4R3 for *elovl5* and *elovl4*, respectively) and 3' (E5F2 and E5F3,  
147 E4F2 and E4F3) rapid amplification of cDNA ends (RACE) PCR (Gene Racer <sup>TM</sup> Kit, Invitrogen,  
148 USA) (Table1).

#### 149 ***Sequence and phylogenetic analysis of Elovl5 and Elovl4***

150 The deduced amino acid (aa) sequences of the newly cloned elongases were aligned with their  
151 corresponding orthologues from rabbitfish (Elovl5, ADE34561; Elovl4, ADZ73580), Nibe croaker  
152 (Elovl5, ACR47973; Elovl4, AJD80650), cobia (Elovl5, ACJ65150; Elovl4, ADG59898), Atlantic  
153 salmon (Elovl5a, AAO13175; Elovl5b, ACI62499; Elovl4, ADJ95235) and zebrafish (Elovl5,  
154 NP\_956747; Elovl4b, NP956266) using ClustalW2 (Higgins & Sharp, 1989). The aa sequence  
155 identities between deduced Elovl proteins from *S. argus* and other vertebrate homologues were  
156 compared by the EMBOSS Needle Pairwise Sequence Alignment tool ([http://www.  
157 ebi.ac.uk/Tools/psa/emboss\\_needle/](http://www.ebi.ac.uk/Tools/psa/emboss_needle/)). A phylogenetic tree comparing the aa sequence similarities of  
158 different types of elongases (Elovl2, Elovl4 and Elovl5) from a variety of vertebrate lineages was  
159 constructed using the neighbor-joining method (Saitou & Nei, 1987). Confidence in the resulting  
160 phylogenetic tree branch topology was measured through bootstrapping through 1000 iterations.

#### 161 ***Functional characterization of cloned elongase genes in yeast***

162 Functional characterization of the *S. argus* putative elongase genes was conducted by expressing  
163 their open reading frame (ORF) in yeast *Saccharomyces cerevisiae*. Expression primers listed in  
164 Table 1 (*elovl5*: E5F4 and E5R4, *elovl4*: E4F4 and E4R4) containing restriction sites *Bam*HI and  
165 *Xba*I were designed for amplification of the *elovl5* and *elovl4* ORFs from liver and eye cDNA using  
166 high-fidelity DNA polymerase (TianGen, Beijing, China) under the following conditions: initial  
167 denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing  
168 at 58 °C for 45 s and extension at 72 °C for 3 min, and a final extension at 72 °C for 10 min. The  
169 DNA fragments were purified and digested with the corresponding restriction endonucleases (New  
170 England Biolabs, UK) and ligated into the yeast episomal plasmid pYES2 (Invitrogen). The  
171 recombinant plasmids (pYES2-*elovl5* or pYES2-*elovl4*) were transformed into *S. cerevisiae* (strain

172 INVSc1, Invitrogen) using the S.C. Easy Comp Transformation kit (Invitrogen).

173 A single colony expressing either the *elovl5* or *elovl4* ORF was grown on *S. cerevisiae*  
174 minimal medium minus uracil (SCMM<sup>-uracil</sup>). Stearidonic acid (18:4n-3),  $\gamma$ -linolenic acid (18:3n-6),  
175 EPA (20:5n-3), arachidonic acid (ARA, 20:4n-6), docosapentaenoic acid (DPA, 22:5n-3) or  
176 docosatetraenoic acid (DTA, 22:4n-6) were used as substrates for testing the elongase activity of the  
177 *S. argus elovl5* and *elovl4*. All the fatty acids were purchased from Cayman Chemicals Co (Ann  
178 Arbor, MI, USA). The PUFA substrates were added at final concentrations of 0.5 (C18), 0.75 (C20)  
179 and 1.0 (C22) mM (Li et al., 2010). After two days culture, yeast cells were harvested and washed  
180 as described previously (Li et al., 2010).

#### 181 ***Lipid extraction and fatty acid analysis***

182 Yeast samples were homogenized in chloroform/methanol (2:1, v/v) containing 0.01 % BHT as  
183 antioxidant, and total lipid extracted according to the Folch method (Folch et al., 1957). Fatty acid  
184 methyl esters (FAME) were prepared by transesterification with boron trifluoride etherate (ca. 48 %,   
185 Acros Organics, NJ, USA) as described previously (Li et al., 2008; Xie et al., 2014). FAME were  
186 purified by TLC, resuspended in hexane (Berry, 2004), and separated using a gas chromatograph  
187 (GC2010-plus, Shimadzu, Japan) as described in detail previously (Li et al., 2010). The activity of  
188 elongase was calculated as the proportion of substrate fatty acid converted to elongated FA products  
189 as follows:  $100 \times [\text{individual product area} / (\text{all product areas} + \text{substrate area})]$  (Li et al., 2010).

#### 190 ***Tissue distribution of elovl5 and elovl4 mRNA***

191 In order to determine the distribution of elongase mRNA in *S. argus*, total RNA (1  $\mu$ g) from eye,  
192 brain, liver, muscle, heart, gills, spleen, kidney and intestine was reverse transcribed into cDNA  
193 (Cloned AMV First-Strand cDNA Synthesis Kit, Invitrogen). RT-PCR was carried out with an  
194 initial denaturing step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s,  
195 annealing at 58 °C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 5 min.  
196 The expression of the housekeeping gene *18S rRNA* was used as internal control to check the  
197 efficiency of cDNA synthesis and cDNA integrity. The primer pairs used for RT-PCR are given in  
198 Table 1.

#### 199 ***Expression of elovl5 and elovl4 mRNA in liver and eye in response to diets with different*** 200 ***18:3n-3/18:2n-6 ratios***

201 The levels of *elovl5* and *elovl4* mRNA were measured by quantitative real-time PCR (qPCR)  
202 in liver and eye, respectively, from fish fed on the experimental diets D1-D6. QPCR primers were



203 E5qF/E5qR and E4qF/E4qR for *elovl5* and *elovl4*, respectively (Table 1). One  $\mu$ g total RNA from  
204 liver and eye was reverse transcribed into cDNA according to manufacturers instruction (Takara).  
205 The 20  $\mu$ L reaction system consisted of 2  $\mu$ L diluted cDNA, 0.4  $\mu$ L for each primer (10  $\mu$ mol), 10  
206  $\mu$ L SYBR Premix, and 7.2  $\mu$ L sterile double distilled water. PCR amplifications were carried out  
207 using a Lightcycler 480 real-time PCR detection system (Roche, Switzerland) with an initial  
208 denaturing step at 95 °C for 30s, followed by 40 cycles of denaturation at 95 °C for 5s, with a final  
209 step at 60 °C for 31 s. The mRNA levels of *elovl5* and *elovl4* in the liver and eye of *S. argus* in each  
210 dietary groups were normalized relative to the expression of *18S rRNA* calculated by the  
211 comparative threshold cycle (Ct) method (Whelan et al., 2003).

## 212 **Statistics**

213 The *elovl* mRNA expression data were presented as means  $\pm$  standard error of mean (n = 9).  
214 Differences in the expression of *elovl5* and *elovl4* (tissue distribution and nutritional regulations  
215 experiments) were analyzed by one-way ANOVA followed by Tukey's multiple comparison. All  
216 analyses were conducted using SPSS v17.0 (SPSS Inc., Chicago, IL, USA).

## 217 **Results**

### 218 **3.1. Sequence and phylogenetic analyses of *S. argus elovl5* and *elovl4* cDNAs**

219 The newly cloned *S. argus* elongase cDNAs were 1390 bp (*elovl5*) and 1484 bp (*elovl4*) in  
220 full-length, and deposited in the GenBank database with the accession numbers KF029625 and  
221 KF029624, respectively. The *elovl5*-like cDNA had a 885 bp ORF encoding a peptide of 294 aa,  
222 whereas the *elovl4*-like cDNA had a 918 bp ORF encoding a protein of 304 aa. When compared to  
223 other teleost Elov15 and Elov14 sequences, the *S. argus* Elov15 was 71-85 % identical to teleost  
224 Elov15 including zebrafish, Atlantic salmon, rabbitfish, Nibe croaker and cobia, while *S. argus*  
225 Elov14 shares aa sequence identities of 84-97 % to Elov14 from teleosts including zebrafish, Atlantic  
226 salmon, Nibe croaker, cobia and rabbitfish.

227 Similar to other teleost Elov1-like proteins, both *S. argus* Elov15 and Elov14 deduced proteins  
228 possessed the histidine box motif (HXXHH) conserved in the elongase family (Fig. 1) (Jakobsson et  
229 al., 2006). They have lysine or arginine residues at the carboxyl terminus, more specifically  
230 KXRXX in Elov15 and RXKXX in Elov14, regarded as putative endoplasmic reticulum (ER)  
231 retrieval signals. Five putative transmembrane-spanning regions containing hydrophobic aa  
232 stretches were predicted by comparison with other vertebrate ELOVL proteins (Fig. 1).

233 A neighbor-joining phylogenetic tree was constructed based on the deduced elongase aa  
234 sequences from Elovl2, Elovl4 and Elovl5 retrieved from fish and other vertebrate genomes. Our  
235 results showed that sequences from the same type of elongase (Elovl2, Elovl4 and Elovl5) clustered  
236 together regardless of the vertebrate lineage considered. Thus, the herein characterized *S. argus*  
237 Elovl4 and Elovl5 grouped with other orthologues from other fish and more distantly other  
238 vertebrates (Fig. 2).

### 239 **3.2. Functional characterization**

240 The two putative Elovl isolated from *S. argus* were functionally characterized by heterologous  
241 expression in yeast *S. cerevisiae* grown in the presence of the following FA substrates: 18:4n-3,  
242 18:3n-6, 20:5n-3, 20:4n-6, 22:5n-3 or 22:4n-6. The FA composition of control yeast transformed  
243 with the empty pYES2 vector was 16:0, 16:1 isomers (16:1n-9 and 16:1n-7), 18:0 and 18:1n-9, as  
244 well as any exogenously added PUFA substrate (data not shown). This was consistent with the  
245 earlier observations that *S. cerevisiae* lacks PUFA elongase activity (Agaba et al., 2004; Monroig et  
246 al., 2012). Interestingly, yeast transformed with pYES2-*elovl5* were able to convert C18 to C22  
247 PUFA substrates to corresponding elongated products (Fig. 3). As shown in Table 2, *S. argus* Elovl5  
248 had an apparent preference for C18 and C20 over C22 FA substrates. Moreover, n-3 PUFA were  
249 elongated to a greater extent compared to their corresponding n-6 isomers with, for example, almost  
250 73% of added 18:4n-3 was elongated whereas only 40% of added 18:3n-6 was elongated.

251 When the *S. argus* Elovl4 cDNA was expressed in the yeast cells, evidence of elongation of all  
252 fatty acids was observed (Fig. 4, Table 2). Moreover, *S. argus* Elovl4 was more effectively convert  
253 both C20 and C22 PUFA substrates to C24 products with no obvious preference in terms of fatty  
254 acyl chain length (C20 vs C22) or FA series (n-3 vs n-6).

### 255 **3.3. Tissue expression of Elovl4 and Elovl5**

256 RT-PCR was used to analyze the expression of *elovl5* and *elovl4* in *S. argus* tissues (Fig. 5).  
257 The transcript of *elovl5* was detected in all tissues, with apparent higher expression levels in liver  
258 compared to eye, intestine and brain. However, the expression of *elovl4* was only detected in eye.  
259 As expected, the housekeeping gene *18S rRNA* was expressed in all tissues analyzed (Fig. 5).

### 260 **3.4. Effects of dietary fatty acid composition on the mRNA expression level of *elovl4* and *elovl5***

261 Nutritional regulation of the newly cloned Elovl5 was analyzed by qPCR in liver (*elovl5*) and  
262 eye (*elovl4*). Compared to fish fed the control diet based on FO (D2), livers from fish fed vegetable  
263 oil-based diets showed higher ( $P<0.05$ ) expression of *elovl5* except for fish fed diet D3, which had

with the same LNA/LA ratio as D2 (Fig. 6B). The highest expression level of *elovl5* was detected in fish fed diet D5 with a dietary LNA/LA ratio of 1.72 (Fig. 6A). A similar pattern was observed for the expression of *elovl4* in eye. Thus, fish fed all vegetable oil-based diets, except D3, showed a significantly higher expression of *elovl4* compared to fish fed FO. Like *elovl5* in liver, the highest expression of *elovl4* in eye was observed in fish fed diet D5 (Fig. 6B).

269

## 270 Discussion

Elovl enzymes account for the condensation step of the elongation reaction resulting in the addition of 2 carbon atoms to the pre-existing FA substrate (Jakobsson et al., 2006; Guillou et al., 2010). Investigations in many fish species have demonstrated that Elovl5 preferentially elongates C18 and C20 PUFA, with residual conversion toward C22 substrates (Agaba et al., 2004, 2005; Monroig et al., 2012). On the other hand, Elovl4 has been regarded as participating in the biosynthesis of very long-chain (> C24) PUFA, although studies in fish have revealed a role in the biosynthetic pathways of long-chain (C20-24) PUFA (Castro et al., 2016). To date, *elovl5* cDNAs have been identified in numerous fish species, while Elovl4 has been studied in a lesser number of species (Castro et al., 2016). In the present study, we provide evidence for the existence of both Elovl5 and Elovl4 encoding cDNAs and demonstrate their role in the biosynthesis of long-chain PUFA in the euryhaline teleost *S. argus*.

The deduced aa sequences of *S. argus* Elovl5 and Elovl4, containing all the main structural features common for Elovl protein family members (Jakobsson et al., 2006), shared high similarity to other fish orthologues. Consistent with this, the functional characterization of the newly cloned Elovl-like encoding cDNAs showed similar substrate specificities as those described in rabbitfish. Thus the *S. argus* Elovl5 has high activity towards C18 and C20 PUFA substrates, and relatively low activity towards C22 PUFA. In addition to rabbitfish (Monroig et al., 2012), these results are consistent with previously reported activities in other Elovl5 proteins characterized in cobia (Zheng et al., 2009), southern bluefin tuna (*Thunnus maccoyii*) (Gregory et al., 2010), Asian sea bass (*Lates calcarifer*) (Mohd-Yusof et al., 2010), Atlantic bluefin tuna (*Thunnus thynnus* L.) (Morais et al., 2011), rabbitfish (Monroig et al., 2012), Nibe croaker (Kabeya et al., 2015). In contrast to the Elovl5, *S. argus* Elovl4 may effectively elongate the tested C20 and C22 PUFA, generating products up to C24 in length. However, previously reported Elovl4 from zebrafish, Atlantic salmon, cobia, rabbitfish and orange-spotted grouper Elovl4, have the ability to catalyze the conversion of

295 C20 and C22 PUFA up to C36 PUFA (Monroig et al., 2010; Carmona-Antoñanzas et al., 2011;  
 296 Monroig et al., 2011c, 2012; Li et al., 2015). The capability of the *S. argus* Elovl4 for elongation of  
 297 C22 and C24 PUFA substrates up to C36 products was not found, and a recent investigation on the  
 298 Nibe croaker Elovl4 (Kabeya et al., 2015) reported similar activities as those obtained for *S. argus*  
 299 Elovl4, i.e. elongation products up to C24. Although elongation products longer than C24 could  
 300 have been produced in yeast at amounts below the detection level, this results were similar to  
 301 those reported in other fish species (Monroig et al., 2011b) and mammals (Agbaga et al., 2008).  
 302 Taken together, it is still reasonable to believe that the *S. argus* Elovl4 plays a prominent role in the  
 303 biosynthesis of VLC-PUFA. This is consistent with the mRNA tissue distribution showing that the *S.*  
 304 *argus elovl4* was highly expressed in eye, a major metabolic site for VLC-PUFA (Agbaga et al.,  
 305 2010) where these compounds accumulate in photoreceptor cell phospholipids (Avelaño, 1988;  
 306 Agbaga et al., 2010; Harkewicz et al., 2012).

307 The restricted pattern of *elovl4* mRNA found in *S. argus* tissues is largely consistent with that of  
 308 rabbitfish, although brain also showed expression of *elovl4* in the latter (Monroig et al., 2012). In  
 309 contrast, more widespread distribution of *elovl4* mRNA has been observed in the marine teleosts  
 310 cobia and orange-spotted grouper, where *elovl4* transcripts were detected in eye, brain, testis, liver,  
 311 kidney, muscle and stomach (Monroig et al., 2011c; Li et al., 2015). Although further studies are  
 312 required to draw a firm conclusion, the difference in the distribution pattern of *elovl4* mRNAs of *S.*  
 313 *argus* and rabbitfish compared to other teleost fish may be linked to their feeding habits. For *S.*  
 314 *argus elovl5* mRNA, a wide spread distribution pattern was obtained, with greatest expression level  
 315 in liver, eye, intestine and brain. This is similar to the tissue expression pattern obtained from  
 316 rabbitfish, in which the expression of an *elovl5* was greatest in liver, followed by intestine and brain.  
 317 In contrast, studies on carnivorous marine fish, including cobia, Asian sea bass, Nibe croaker,  
 318 meagre, Japanese eel and Northern pike, showed that the expression of *elovl5* transcript was  
 319 substantially higher in brain than other tissues (Zheng et al., 2009; Mohd-Yusof et al., 2010;  
 320 Yamamoto et al., 2010; Monroig et al., 2013; Carmona-Antoñanzas et al., 2013; Wang et al., 2014).

321 From the functional and phylogenetic analysis, and tissue distribution of *elovl5* and *elovl4*  
 322 transcripts, it is possible to conclude that the elongase complement involved in LC-PUFA  
 323 biosynthesis in *S. argus* is similar to that characterized in rabbitfish (Monroig et al., 2012). While it  
 324 is unclear if phylogeny and/or feeding habits can partly explain such similarity between *S. argus*  
 325 and rabbitfish elongation capability, it is clear that such resemblance does not extend to Fads

326 complement. Thus, the sole Fads2 found in *S. argus* (Xie et al., 2014), as observed in many fish  
327 species, such as cobia, Nibe croaker, Japanese eel (*Anguilla japonica*) and common carp (*Cyprinus*  
328 *carpio* var. Jian) (Zheng et al., 2009; Yamamoto et al., 2010; Wang et al., 2014; Kabeya et al., 2015;  
329 Ren et al., 2013), was characterized with  $\Delta 6$ -desaturase activity but no  $\Delta 5$ - and  $\Delta 4$ -desaturase (Xie  
330 et al., 2014). In contrast, the rabbitfish possess at least two Fads2 desaturases, a dual  $\Delta 6/\Delta 5$   
331 desaturase and a  $\Delta 4$  desaturase, the latter being the first record of a  $\Delta 4$  desaturation activity in  
332 vertebrates (Li et al., 2010). Additionally, the distribution of *S. argus fads2* mRNA, with highest  
333 expression in liver, followed a pattern typically found in freshwater/salmonid species in contrast to  
334 carnivorous marine species whereby brain has shown the highest levels of *fads2* transcription  
335 (Monroig et al., 2011b). These results further confirm the enormous diversification of fish  
336 LC-PUFA biosynthesis specificities that has been previously hypothesized to be associated with  
337 factors including habitat, trophic level and ecology, as well as species-specific evolutionary history  
338 (Fonseca-Madrigal et al., 2014).

339 The ability of fish to regulate LC-PUFA biosynthesis has been extensively investigated in  
340 commercially important species in order to understand the metabolic impact of replacing FO by VO  
341 devoid of LC-PUFA in aquafeed (Ling et al., 2006; Jordal et al., 2005; Li et al., 2008; Thanuthong  
342 et al., 2011; Navarro-Guillen et al., 2014; Xie et al., 2014, 2015; Kuah et al., 2015). We herein  
343 showed that dietary lipid resource also affected the expression of *elovl5* and *elovl4* in *S. argus*.  
344 Generally speaking, both *elovl4* and *elovl5* were up-regulated in *S. argus* in response to low dietary  
345 LC-PUFA (high VO) input. While nutritional regulation of *elovl5* in liver has often been reported  
346 (Ling et al., 2006; Morais et al., 2009; Yamamoto et al., 2010; Thanuthong et al., 2011;  
347 Navarro-Guillen et al., 2014; Xie et al., 2015; Kuah et al., 2015), regulatory mechanisms of  
348 LC-PUFA in eye have less been investigated despite eye accumulating large amounts of LC-PUFA  
349 in some species (Aveldaño, 1988; Agbaga et al., 2010; Harkewicz et al., 2012). Li et al. (2015)  
350 recently described an up-regulation of *elovl4* in viscera of orange-spotted grouper larvae. Although  
351 the specific mechanism remains to elucidated, it is largely accepted that the increased expression of  
352 key enzymes including *elovl* and *fads* involved in LC-PUFA biosynthesis pathway stimulated by  
353 dietary VO is a biochemical/molecular mechanism that can at least partially compensate dietary  
354 essential fatty acid deficiencies (Tocher et al., 2003).

355 In addition to the dietary lipid source (FO v. VO), the ratio of dietary LNA and LA also  
356 influenced the expression of key enzymes involved in LC-PUFA biosynthesis. Functional

357 characterization of Fads and Elovl isolated from various teleosts species have often revealed a  
358 higher activity towards n-3 compared to n-6 PUFA substrates (Morais et al., 2009; Zheng et al.,  
359 2009; Li et al., 2010; Monroig et al., 2012; Monroig et al., 2013; Xie et al., 2014; Kabeya et al.,  
360 2015). Previous studies have revealed that Fad gene expression and enzymatic activity varied with  
361 dietary LNA/LA ratio. For example, up-regulation of  $\Delta 6$  *fads2* gene expression was measured in  
362 rabbitfish, Murray cod (*Maccullochella peelii peelii*), rainbow trout and *S. argus* fed high dietary  
363 ratios of LNA/LA (Li et al., 2008; Senadheera et al., 2011; Thanuthong et al., 2011; Xie et al.,  
364 2014). An excess of LNA in the diet could also block  $\Delta 6$  *fads* gene expression (Izquierdo et al.,  
365 2008; Xie et al., 2014). Unlike desaturases, few studies have reported the influence of dietary  
366 LNA/LA ratio on elongase gene expression. In the present study, the mRNA expression of *elovl5*  
367 and *elovl4* was highest in liver and eye of *S. argus* fed a diet with an LNA/LA ratio of around 1.72.  
368 As prior to the elongase activity, the  $\Delta 6$  desaturase acting on LNA and LA increased with the elevation  
369 of the dietary ALA/LA ratio (Thanuthong et al., 2011; Xie et al., 2014). In our previous study, the  
370 highest  $\Delta 6$  *fads* mRNA expression was detected in liver of *S. argus* fed a diet with an LNA/LA ratio of  
371 1.72 (Xie et al., 2014), which means the high level Elovl substrate are alviable to Elvol5 and Elovl4 in  
372 the dietary treatment with an LNA/LA ratio of 1.72. Therefore, it appears that dietary ratio of  
373 LNA/LA can influence the expression of *fads* and *elovl* and LC-PUFA biosynthesis efficiency  
374 could be optimized with particular dietary levels of C18 PUFA.

375 In summary, the present study showed that *S. argus* has at least two Elovl with high sequence,  
376 function and distribution homology to Elovl4 and Elovl5 reported previously in another herbivorous  
377 species, the rabbitfish. However, the Fads complement of *S. argus* and rabbitfish is remarkably  
378 different and this suggests that the diversification of fish LC-PUFA biosynthesis specificities is  
379 highly varied. Replacing FO with a VO blend with a dietary ratio of LNA/LA of 1.72 resulted in  
380 highest expression of *elovl5* and *elovl4* in liver and eye of *S. argus*, respectively. These discoveries  
381 will expand our knowledge in understanding the molecular basis and regulation of LC-PUFA  
382 biosynthesis in fish.

383

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546

547

548 Table 1

549 Primers used for cDNA cloning or determining gene expression of *Scatophagus argus* elongases

Aim	Primer	Primer sequence	Accession No <sup>1</sup>
First fragment cloning	E5F1	5'-GGTACTACTTCTCCAAGCTCAT-3'	KF029625
	E5R1	5'-GTGATGTATCTCTTCCACC-3'	
	E4F1	5'-GTCTACAACTTCAGCATGGTG-3'	
	E4R1	5'-GGAACCTGGATCATCTGAATAA-3'	
3'RACE	E5F2	5'-ACAGCTTCGTCCACGTCGTGATGTA-3'	KF029625
	E5F3	5'-TTCGTTATGAACTGGCAACCCTGTG-3'	
	E4F2	5'-TGGCAGCCTTGGGACCTCAG-3'	
	E4F3	5'-GTGGATTGGCATCAAATGGGTC-3'	
5'RACE	E5R2	5'-TTCAGCATGGTAGCGTGGTGGTAGA-3'	KF029625
	E5R3	5'-TGTTTATGGCGGCACCGAAGTATGA-3'	
	E4R2	5'-GCGAGGGATGTAAGGGTTTCTTCAGAC-3'	
	E4R3	5'-GTGGATGGAAGAGTTGATGGTTGC-3'	
ORF cloning	E5F4	5'-CCC <u>AAGCTT</u> CAAATGGAGACCATCAATC-3'	KF029625
	E5R4	5'-CCG <u>CTCGAGT</u> CAATCCATCCTCAGCTT-3'	
	E4F4	5'-CCC <u>AAGCTT</u> GCCATGGAGGTTGTAACAC-3'	
	E4R4	5'-CCG <u>CTCGAGT</u> TACTCTCTTTTGCTCT-3'	
RT-PCR and qPCR	E5qF	5'-ATGAACTGGCAACCCTGTGG-3'	KF029625
	E5qR	5'-ATATGGCTGCACACATCGTCTG-3'	
	E4qF	5'-TAGCAGACAAGAGGGTGGAGAA-3'	
	E4qR	5'-CTATGAGGGTCTTCCTGAGTGTA-3'	
	18SF	5'-CGCCGAGAAGACGATCAAAC-3'	
	18SR	5'-TGATCCTTCCGCAGGTTTCAC-3'	

550 <sup>1</sup> GenBank(<http://www.ncbi.nlm.nih.gov/>)

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563 Table 2  
 564 Functional characterization of the *Scatophagu argus* elongases in *Saccharomyces cerevisiae* yeast.  
 565 Results are expressed as a percentage of total substrate fatty acid converted to elongated products.

Elongase	Substrate fatty acid	Product	%Conversion	Activity
Elov15	18:3n-6	20:3n-6	43.9	C18→20
		22:3n-6	10.3	C20→22
	18:4n-3	20:4n-3	72.9	C18→20
		22:4n-3	20.8	C20→22
	20:4n-6	22:4n-6	32.0	C20→22
		24:4n-6	6.9	C22→24
	20:5n-3	22:5n-3	35.8	C20→22
		24:5n-3	10.1	C22→24
	22:4n-6	24:4n-6	7.5	C22→24
	22:5n-3	24:5n-3	11.5	C22→24
Elov14	18:3n-6	20:3n-6	7.6	C18→20
	18:4n-3	20:4n-3	12.3	C18→20
	20:4n-6	22:4n-6	37.3	C20→22
		24:4n-6	19.3	C22→24
	20:5n-3	22:5n-3	35.2	C20→22
		24:5n-3	21.6	C22→24
	22:4n-6	24:4n-6	26.5	C22→24
	22:5n-3	24:5n-3	34.8	C22→24

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581	SaE5	METINLKLNAQLETWIGPR---DQRVRGWLLLDNYPPTFALTVIYLLIVWMGPKYMKYRQPYSRGLLVFNGLTLL	75
582	ScE5	MEDFNRKLSNFESWIGPR---DQLRQGWLLEDNYPPTFALTVVYLLIVVLGPVKMKNRPAYSCRGLMVINYNLGLTLL	75
583	RcE5	METFNHKLNAVIESWMGPR---DQRVGWLLLDNYPPTFALTVMYLLIVWMGPKYMKHRQPYSRGLLVLYNLGLTLL	75
584	NmE5	METFNHKLNTYLESWMGPR---DQRVRGWLLLDNYPPTFALTVMYLVIVWMGPKYMKHRQPYSRGLLVLYNLGLTLL	75
585	SsE5a	METFNYKLNMYIDSWMGPR---DERVQGWLLLDNYPPTFALTVMYLLIVVLGPVKYMRHRQPVSCRGLLL VYNLGLTIL	75
586	SsE5b	MEAFNHKLNTYIDSWMGPR---DERVQGWLLLDNYPPTFALTLMYLLIVVLGPVKYMRHRQPVSCQGLLVLYNLAL TLL	75
587	DrE5	METFSHRVNSYIDSWMGPR---DLRVTGWFLDDYIPTFI FTVMYLLIVWMGPKYMKNRQAYS CRALLVPYNLC LTLL	75
588	SaE4	MEVVTHFVN DTFEFYKWSLT IADKRVEKWPMSSPLPTLAISCLYL LFLWAGPRYMQDRQPCTLRKTLIVYNFS MVVL	78
589	ScE4	MEVVTHFVN DTFEFYKWSLT IADKRVEKWPMSSPLPTLAISCLYL LFLWAGPRYMQDRQPFTLRKTLIVYNFS MVVL	78
590	RcE4	MEVVTHFVN DTFEFYKWSLT IADKRVENWPMASPLPTLAISCLYL LFLWVGPRYMQDRQPYTLRR TLIVYNFS MVVL	78
591	NmE4	MEAVTHFVN DTFEFYKWGLTIADKRVENWPMSSPLPTLAISCLYL LFLWAGPRYMQDRQPFTLRKTLIVYNFS MVVL	78
592	SsE4	MEAVTHFMNDTFEFYRWSLTIADKRVEKWPMSSPAPT LAISCLYL LFLWAGPKYMQNREPFQLRKTLIVYNFS MVIL	78
593	DrE4b	METVVHLMNDSVEFYKWSLT IADKRVEKWPMSSPLPTLGISVLYL LFLWAGPLYMQNREPFQLRKTLIVYNFS MVLL	78
594		** .        : *   ..   .:.:::   * *:   * ::   ** :   ::   **:::*   **   **:   *. :   : :   **:   .:.:..*	
595		I <span style="float:right">II</span>	
596	SaE5	SFYMFYELVTAVWYG GYNFYCQNS-HSAEEADNKIMNVLWWWYFSKLIEFMDTFFFILRKNNHQISFLHVYHHATMLN	150
597	ScE5	SFYMFYELGSAIWFGGYHFYCQNT-HSLEPDNKMVRALWWWYFSKLIEFMDTFFFILRKNNHQITFLHIYHHASMFN	150
598	RcE5	SFYMFYELVTAVWHGGYNFYCQDT-HSAEEVDNKIINVLWWWYFSKLIEFMDTFFFILRKNNHQITFLHIYHHATMLN	150
599	NmE5	SFYMFYELVTAVWHGGYNFYCQDI-HSAQEVDNKIINVLWWWYFSKLIEFMDTFFFILRKNNHQITFLHIYHHASMLN	150
600	SsE5a	SFYMFYEMVASAVWHGDYNFYCQDT-HSAGETDKIINVLWWWYFSKLIEFMDTFFFILRKNNHQITFLHIYHHASMLN	150
601	SsE5b	SFYMFYEMVASAVWQGGYNFYCQDT-HSAGETDKIINVLWWWYFSKVIEFMDTFFFILRKNNHQITFLHIYHHASMLN	150
602	DrE5	SLYMFYELVMSVYQGGYNFFCQNT-HSGGDADNRMMNVLWWWYFSKLIEFMDTFFFILRKNNHQITFLHVYHHATMLN	150
603	SaE4	NFYIAKELLGSRAAGSYLCQPVNYSNDVNEVRIASALWWWYISKGEFLDTVFFILRKKFNQVSFLHVYHHCTMFI	154
604	ScE4	NFYIAKELLGSRAAGSYLCQPVNYSNDVNEVRIASALWWWYISKGEFLDTVFFILRKKFNQVSFLHVYHHCTMFI	154
605	RcE4	NFYIAKELLIATRAAGSYLCQPVNYSNDVNEVRIASALWWWYISKGEFLDTVFFILRKKFNQVSFLHVYHHCTMFI	154
606	NmE4	NFYIAKELLGSRAAGSYLCQPVNYSNDVNEVRIASALWWWYISKGEFLDTVFFIMRKKFNQVSFLHVYHHCTMFI	154
607	SsE4	NFYIAKELLGARAAGSYLCQPVSYSDVNEVRIASALWWWYISKGEYLDTVFFILRKKINQVSFLHVYHHCTMFI	154
608	DrE4b	NFYICKELLGSRAAGSYLCQPVNYSNDVNEVRIASALWWWYISKGEFLDTVFFIMRKKFNQVSFLHVYHHCTMFI	154
609		:::: * :   .   ..* : **   :*   : ::   .**** ::* :   *:.*.***:**:   :*:***:**. *: :	
610		II	
611	SaE5	IWWFVMNWPCGHSYFGAAINSFVHVVMYSYYGLSAI-PGIRPYLWKKYITQLQMIQFFLTMCQTMCAAIWPCGV PV	225
612	ScE5	IWWFVMNWIPC GHSYFGASLNSFVHVVMYSYYGLSAV-PSLRPYLWKKYITQLQLVQFFLTMTFQTYCAVLWPCGFPI	225
613	RcE5	IWWFVMNWIPC GHSYFGASLNSFVHVVMYSYYGLSAI-PAMRPYLWKKYITQLQLIQFFLTMSQTMC A VIWPCDFPR	225
614	NmE5	IWWFVMNWVPCGHSYFGASLNSFVHVVMYSYYGLSAI-PAMRPYLWKKRYITQLQLVQFFLTMSQTMC AVVWPCGFPM	225
615	SsE5a	IWWFVMNWVPCGHSYFGASLNSFIHVL MYSY YGLSAV-PALRPYLWKKYITQGQLIQFFLTMSQTICAVIWPCGFPR	225
616	SsE5b	IWWFVMNWVPCGHSYFGASLNSFVHVL MYSY YGLSAV-PAIRPYLWKKYITQGQLIQFFLTMSQTICAVIWPCGFPR	225
617	DrE5	IWWFVMNWVPCGHSYFGATFNSFIHVL MYSY YGLSAV-PALRPYLWKKYITQGQLVQFVLTMTFQTSCAVVWPCGFPM	225
618	SaE4	LWWIGIKWVPGGQSFFGATINSSIHL MYGY YGLAALGPQM KYL WKKYLTIIQMIQHVTIGHAGHS LYTGC PFPA	229
619	ScE4	LWWIGIKWVPGGQSFFGATINSSIHL MYGY YGLAALGPQM KYL WKKYLTIIQMIQHVTIGHAGHS LYTGC PFPA	229
620	RcE4	LWWIGIKWVPGGAFFGATINSSIHL MYGY YGLAALGPQM KYL WKKYLTIIQMIQHVTIGHAGHS LYTGC PFPC	229

621	NmE4	LWWIGIKWVPGGQSFFGATINSSIHVLMYGYGLAALGPQMQLWKKYLTIIQMIQFHTIGHAGHSlyTGCPFPA	229
622	SsE4	LWWIGIKWVPGGQSFFGAGINSSIHVLMYGYGLAAGPKIQKFLWKKYLTIIQMIQFHTIGHAGHSlyTGCPFPA	229
623	DrE4b	LWWIGIKWVPGGQSFFGATINSGIHVLMYGYGLAAGPKIQKFLWKKYLTIIQMIQFHTIGHAAHSlyTGCPFPA	229
624		:** ::* . *::** :*: **:** ***:*. * : : *****:* * *:** *: : : : * . *	
625		----- III ----- IV ----- V	
626	SaE5	GWLYLQIGYTITLMIFFLNfyfQTYKKRSPS-RQK---EHMNGSPVSTNGHANGTPSMEHSG-----HKKL RMD	294
627	ScE5	GWLYFQISYMTLVVLFsnfyIQTYKKRSSS-RKT---DHQNGSPLSTNGHANGK---ESAA-----HKKL RVD	291
628	RcE5	GWLYFQISYMTLIILFSNfyIQTYKKHSGS-LKK---EHQNGSPVSTNGHANGTPSMEYNV-----HKKL RVD	294
629	NmE5	GWLYFQISYMTLIFLFSNfyVQTYKKHSVS-LKK---EHQNGSPVSPNGHANGTPSLEHAA-----HKKL RVD	294
630	SsE5a	GWLYFQIFVVTLIALFSNfyIQTYKKHLVSQKKE---CHQNGSVASLNGHVNGVTP TETIT-----HRKVRGD	295
631	SsE5b	GWLFFQIFYMASLIAFFSNfyIQTYKKHRVS-QKE---YHQNGSVDSLNGHANGVTP TETIT-----HRKVRVD	294
632	DrE5	GWLYFQISYMTLILLFSNfyIQTYKKRSGS-RKS---DYPNGS---VNGHTNGVMSSEKIK-----HRKARAD	291
633	SaE4	WMQWALIGYAVTFIILFANFYHAYRRKPSSMQKGDKP--VANGTSTVTNG-HSKVEEVDNDK-KRQKKGRAKRE	305
634	ScE4	WMQWALIGYAVTFIILFANFYHAYRRKPSSGQKGGKP--VTNGTSTVTNG-HSKVEEEE---KRQKKGRAKRE	302
635	RcE4	WMQWALIGYAVTFIILFANFYHAYRGKPSSSQKGGKP--IANGTSVVTNG-HSKVEEVEDNG-KRQKKGRAKRE	305
636	NmE4	WMQWALIGYAVTFIILFANFYHAYRRKPSSAQKGGKP--AVNGTSMVTNG-HSKAEEVEDNG-KRQKKGRAKRE	305
637	SsE4	WMQWALIGYAVTFIILFGNfyQTYRRTPRSAHKVAKP--VTNGVSMATNG-YNKLQDVEENGLKQKKGRAKRE	306
638	DrE4b	WMQWALIGYAVTFIILFANFYQTYRRQPR--LKTAKS--AVNGVSMSTNG-TSKTAEVTENG-KKQKKGKGKHD	303
639		:.. :* * :.. :* *** :*:.. ** ** :. : :	
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Figure 1. Alignment of the deduced amino acid (aa) sequences of elongases Elov15 (E5) and Elov14 (E4) isolated from *Scatophagus argus* (Sa) with their corresponding orthologues, including rabbitfish (*Siganus canaliculatus*, Sc) Elov15 (ADE34561) and Elov14 (ADZ73580), cobia (*Rachycentron canadum*, Rc) Elov15 (ACJ65150) and Elov14 (ADG59898), Nibe croaker (*Nibea mitsukurii*, Nm) Elov15 (ACR47973) and Elov14 (AJD80650), Atlantic salmon (*Salmo salar*, Ss) Elov15a (AAO13175), Elov15b (ACI62499) and Elov14 (ADJ95235), zebrafish (*Danio rerio*, Dr) Elov15 (NP\_956747) and Elov14b (NP956266). Deduced aa sequences were aligned using ClustalW2. Identical and similar residues are marked with ‘\*’ and ‘:’, respectively. The conserved histidine box HXXHH is shaded grey, five putative transmembrane domains are dash-underlined, and the putative ER retrieval signal is solid underlined.



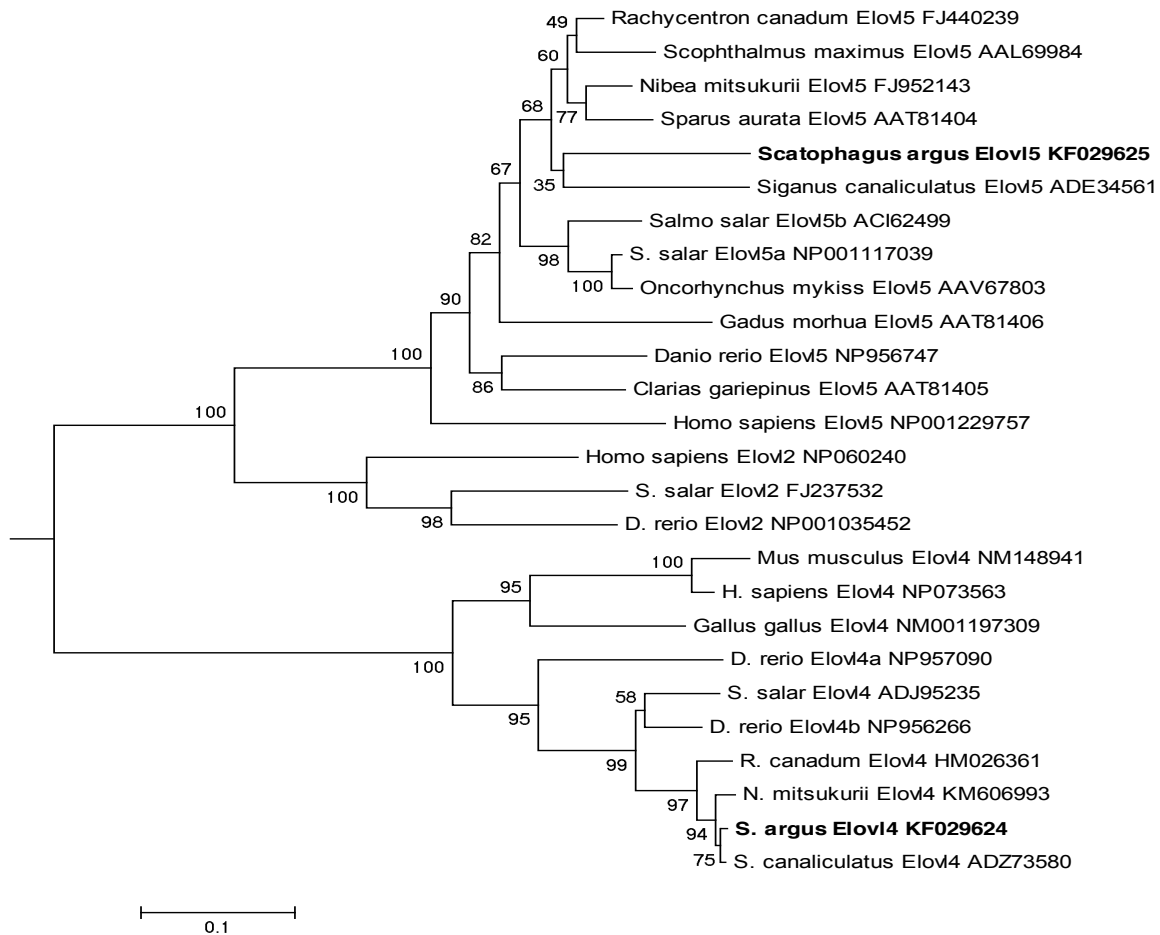


Figure 2. Phylogenetic tree comparing the deduced amino acids of *Scatophagus argus* Elov14 and Elov15 with other Elov1 members from fish and mammals. The tree was constructed using the neighbor-joining method (Saitou and Nei,1987 ) with MEGA6. The horizontal branch length is proportional to the substitution rate per site. Numbers represent the frequencies with which the tree topology presented was replicated after 1000 bootstrap iterations.

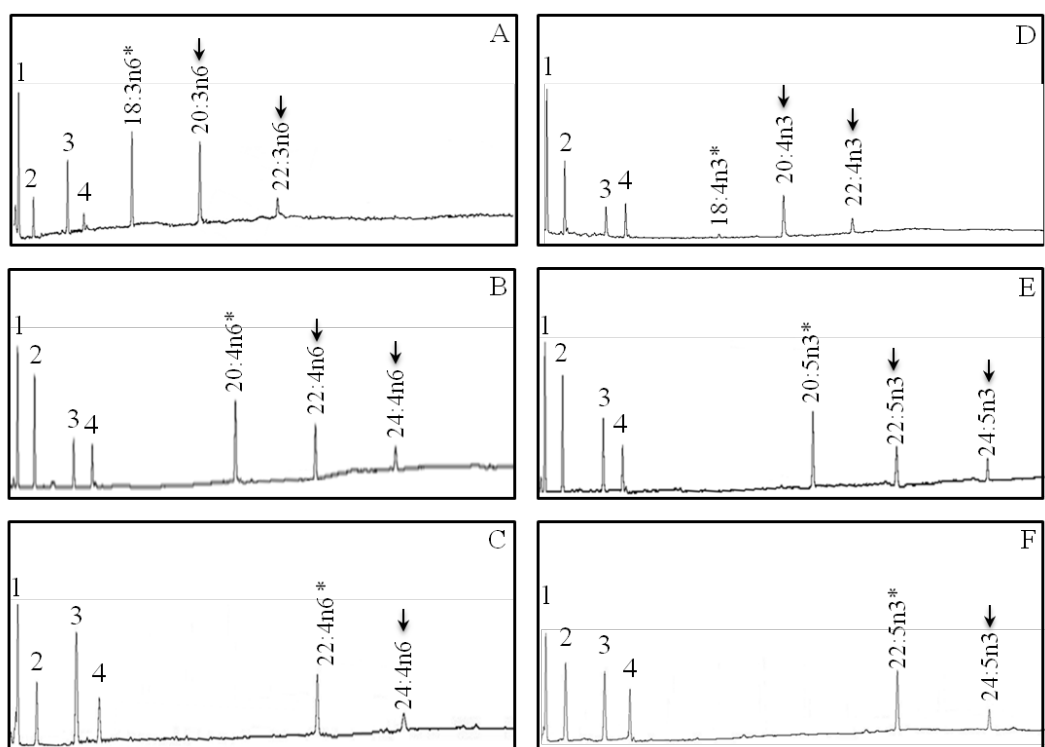
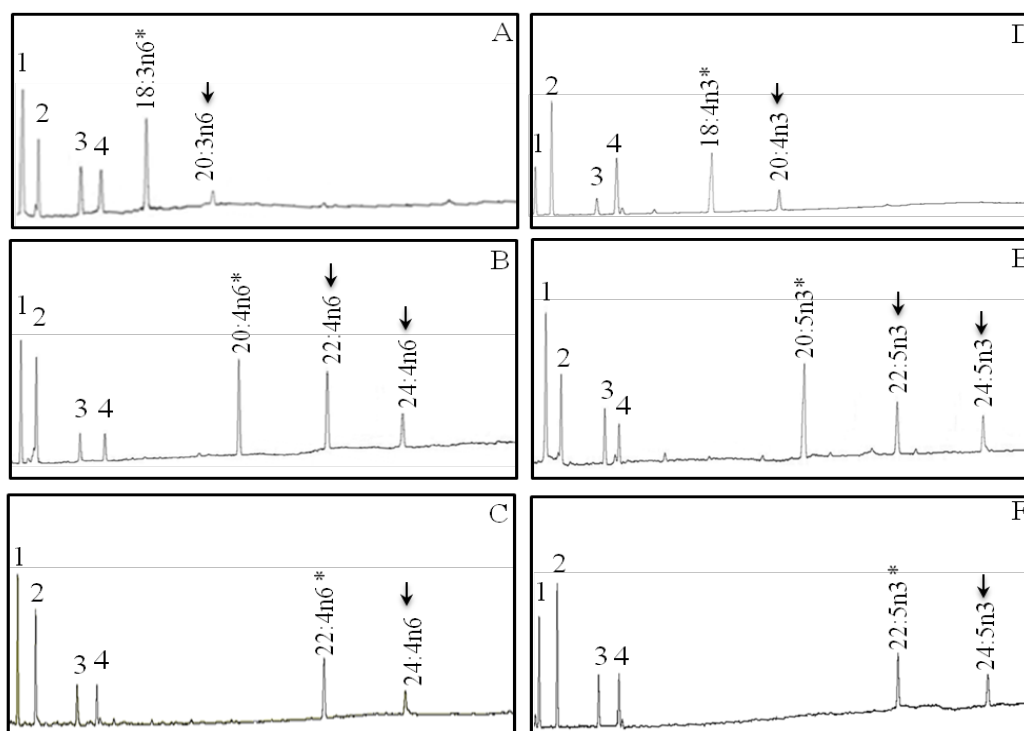


Figure 3 Functional characterization of *Scatophagus argus* putative Elovl5 in yeast *Saccharomyces cerevisiae*. FAMES were extracted from yeast transformed with the pYES2-*elovl5*, and grown in the presence of PUFA substrates 18:3n-6 (A), 18:4n-3 (B), 20:4n-6 (C), 20:5n-3 (D), 22:4n-6 (E) and 22:5n-3 (F). Based on retention times, substrates (\*) and their corresponding elongated products (↓) are indicated accordingly. Peaks 1–4 represent the main endogenous FAs of *S. cerevisiae*, namely 16:0, 16:1 isomers, 18:0 and 18:1n-9, respectively. Vertical axis, FID response; horizontal axis, retention time.



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Figure 4 Functional characterization of *Scatophagus argus* putative Elovl4 in yeast *Saccharomyces cerevisiae*. FAME were extracted from yeast transformed with the pYES2-*elovl4*, and grown in the presence of PUFA substrates 18:3n-6 (A), 18:4n-3 (B), 20:4n-6(C), 20:5n-3 (D), 22:4n-6 (E) and 22:5n-3 (F). Based on retention times, substrates (\*) and their corresponding elongated products (↓) are indicated accordingly. Peaks 1–4 represent the main endogenous FAs of *S. cerevisiae*, namely 16:0, 16:1 isomers, 18:0 and 18:1n-9, respectively. Vertical axis, FID response; horizontal axis, retention time.

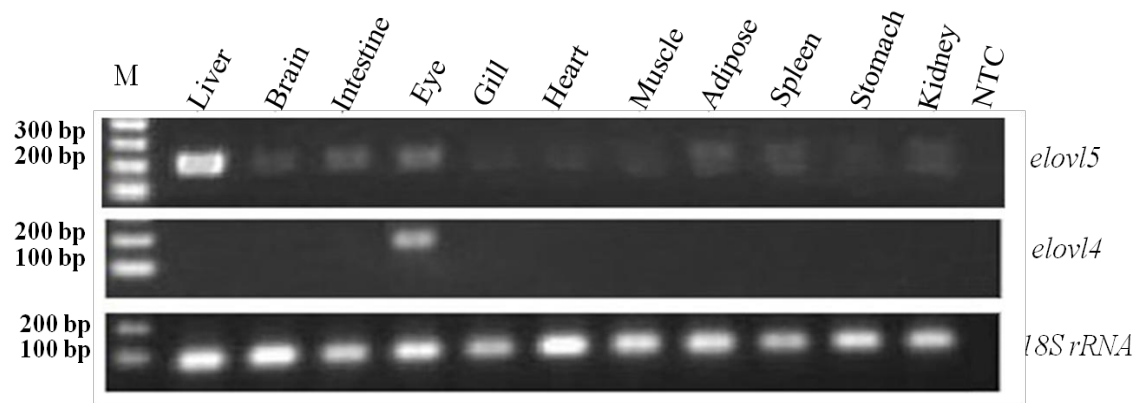
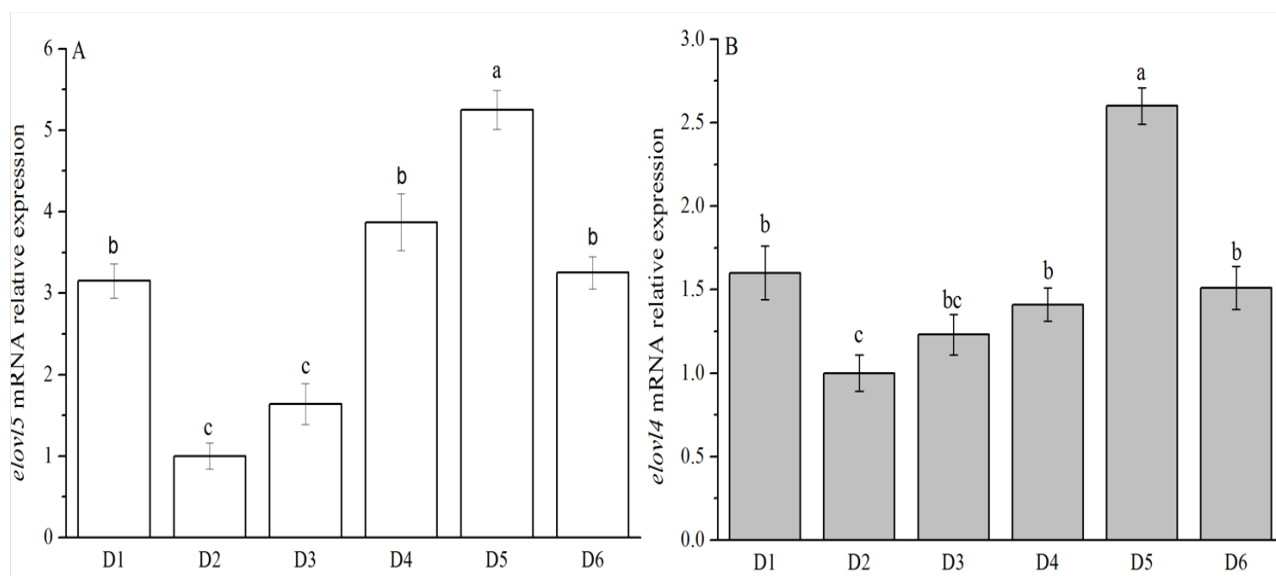


Figure 5. Tissue-specific expression of *elovl5* and *elovl4* from *S. argus*. Expression of the housekeeping gene *18S rRNA* is also shown. NTC: no template control.



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Figure 6. Relative expression levels of *elovl5* (A) and *elovl4* (B) in livers and eyes collected from *S. argus* fed six experimental diets. Expression values were normalized to those of *18S rRNA*. Data are means  $\pm$  SEM (n = 6). Bars with different superscripts are significantly different (P < 0.05, one-way ANOVA and Tukey's tests). D2: control diet with fish oil as lipid source; D1, D3–D6: diets with blended vegetable oils as lipid source with dietary LNA/LA ratios of 0.14, 0.57, 0.84, 1.72, and 2.85, respectively.