

Betancor M, Howarth FJE, Glencross BD & Tocher DR (2014)  
Influence of dietary docosahexaenoic acid in combination with  
other long-chain polyunsaturated fatty acids on expression of  
biosynthesis genes and phospholipid fatty acid compositions in  
tissues of post-smolt Atlantic salmon (*Salmo salar*),  
*Comparative Biochemistry and Physiology - Part B:  
Biochemistry and Molecular Biology*, 172-173, pp. 74-89.

**This is the peer reviewed version of this article**

*NOTICE: this is the author's version of a work that was accepted for publication in Comparative Biochemistry and Physiology - Part B: Biochemistry and Molecular Biology. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was published in Comparative Biochemistry and Physiology - Part B: Biochemistry and Molecular Biology, [VOL 172-173 (2014)] DOI: <http://dx.doi.org/10.1016/j.cbpb.2014.04.007>*

**Influence of dietary docosahexaenoic acid in combination with other long-chain  
polyunsaturated fatty acids on expression of biosynthesis genes and phospholipid fatty acid  
compositions in tissues of post-smolt Atlantic salmon (*Salmo salar*)**

Mónica B. Betancor<sup>a\*</sup>, Fraser J.E. Howarth<sup>a</sup>, Brett D. Glencross<sup>b</sup> and Douglas R. Tocher<sup>a</sup>

<sup>a</sup>*Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland*

<sup>b</sup>*CSIRO Food Futures Flagship, GPO Box 2583, Brisbane, QLD 4001, Australia*

**Running title:** Effects of DHA and other LC-PUFA in Atlantic salmon post-smolts.

**ms. has 41 pages, 5 figures, 7 tables**

\*Corresponding author:

Dr. Mónica B. Betancor

Institute of Aquaculture, School of Natural Sciences, University of Stirling

Stirling FK9 4LA

Scotland

Tel: +44-1786-467993

m.b.betancor@stir.ac.uk

## 23    **Abstract**

24    To investigate interactions of dietary LC-PUFA, a dose-response study with a range of  
25    docosahexaenoic acid (DHA; 22:6n-3) levels (1g kg<sup>-1</sup>, 5 g kg<sup>-1</sup>, 10 g kg<sup>-1</sup>, 15 g kg<sup>-1</sup> and 20 g kg<sup>-1</sup>)  
26    was performed with post-smolts (111 ± 2.6 g; mean ± S.D.) over a nine-week feeding period.  
27    Additional diets included 10 g kg<sup>-1</sup> DHA in combination with 10 g kg<sup>-1</sup> of either eicosapentaenoic  
28    acid (EPA; 20:5n-3) or arachidonic acid (ARA; 20:4n-6), and a diet containing 5g kg<sup>-1</sup> each of  
29    DHA and EPA. Liver, brain, head kidney and gill were collected at the conclusion of the trial and  
30    lipid and fatty acid compositions determined as well as expression of genes of LC-PUFA  
31    biosynthesis. Total lipid content and class composition were largely unaffected by changes in  
32    dietary LC-PUFA. However, phospholipid (PL) fatty acid compositions generally reflected that of  
33    the diet, although the response varied between tissues. Liver most strongly reflected diet, followed  
34    by head kidney. In both tissues increasing dietary DHA led to significantly increased DHA in PL  
35    and inclusion of EPA or ARA led to higher levels of these fatty acids. Brain showed the most  
36    conserved composition and gene expression profile, with increased dietary LC-PUFA resulting in  
37    only minor changes in PL fatty acids. Dietary LC-PUFA significantly affected the expression of  $\Delta 6$   
38    and  $\Delta 5$  desaturases, Elovl 2, 4 and 5, and SREBPs although this varied between tissues with  
39    greatest effects observed in liver followed by head kidney, similar to PL fatty acid compositions.

40

41    **Key words:** Atlantic salmon, polyunsaturated fatty acid, DHA, ARA, EPA, composition, LC-PUFA  
42    biosynthesis, liver, brain, head kidney, gill, muscle

43

44

45

46

## 1. Introduction

It is now widely appreciated that fish, particularly oily species such as Atlantic salmon (*Salmo salar*), herring (*Clupea harengus*) and mackerel (*Scomber scombrus*), represent a rich and almost unique source of n-3 long-chain ( $\geq C_{20}$ ) polyunsaturated fatty acids (LC-PUFA) in the human diet (Bell et al., 2001; Tocher, 2009; Monroig et al., 2010). The beneficial health effects of these fatty acids are well established through the roles they play in cardiovascular disease (Calder, 2004), inflammatory and autoimmune diseases (Simopoulos, 2002) and neurological disorders (Dyall & Michael-Titus, 2008). At the same time, there are concerns surrounding the accumulation of contaminants in fish and the perceived health risks these may pose to the human consumer, although scientific evidence is lacking and risks have yet to be defined or quantified (Bell & Waagbø, 2008; Tocher, 2009). However, the most urgent issue is that worldwide demand for aquatic food products continues to grow beyond the sustainable limits of global capture fisheries (Sargent & Tacon, 1999). This has resulted in significant growth of the aquaculture sector in recent decades and, coupled with changes in public attitude towards the sustainability of the industry, the continued production of high-quality, n-3 LC-PUFA-rich fish faces a number of challenges (Subasinghe et al., 2009).

Atlantic salmon represents one of the most economically important species for aquaculture worldwide but, as a carnivorous species, it also presents somewhat of a paradox. The aquafeeds used to rear Atlantic salmon have traditionally relied upon high proportions of fish oils derived from small, pelagic marine fish on the basis that they provide an excellent source of n-3 LC-PUFA (Sargent & Tacon, 1999; Bendiksen et al., 2011). However, the majority of world stocks for these forage fish are considered to be either fully or over-exploited (FAO, 2012), and the limited supply of fish oil is only exacerbated by competition for inclusion in human nutritional supplements and agricultural feeds (Bell et al. 2001; Naylor et al. 2009). Consequently, much of the research in recent years has focussed on sustainable alternatives to fish oils, principally vegetable oils (reviewed by Nasopoulou & Zabetakis, 2012). Numerous feeding trials have revealed that growth,

73 feed conversion and survival of Atlantic salmon are largely unaffected when fish oil is partially  
74 replaced by vegetable oil (Tortensen et al., 2000; Bell et al., 2001; Rosenlund et al., 2001;  
75 Bransden et al., 2003). However, vegetable oils are notable for their lack of LC-PUFA, indicating  
76 that high replacement of fish oils cannot be accomplished without compromising product quality  
77 through reduced flesh n-3 LC-PUFA content (Bell et al., 2003; Menoyo et al., 2005; Tocher, 2010).  
78 Therefore, the reputation of farmed Atlantic salmon as a health promoting food seems reliant upon  
79 a better understanding of the functional requirements and metabolism of LC-PUFA.

80 All vertebrates including fish require three key fatty acids for normal growth and  
81 development: docosahexaenoic acid (DHA; 22:6n-3), eicosapentaenoic acid (EPA; 20:5n-3) and  
82 arachidonic acid (ARA; 20:4n-6) (Sargent et al., 1997; Bell, 1998; Sargent et al., 1999). These  
83 biologically active fatty acids play important roles in cell membrane structure and function  
84 (Sargent et al. 2002), the regulation of reproduction (Bell & Sargent, 2003) and the modulation of  
85 immune responses (Waagbø, 1994). Salmonids can synthesise these LC-PUFA *de novo* from their  
86 C<sub>18</sub> precursors  $\alpha$ -linolenic acid (LNA; 18:3n-3) and linoleic acid (LOA; 18:2n-6), though their  
87 capacity for this is limited (Castell et al., 1972; Bell et al., 1993; Tocher et al., 2000). The enzymes  
88 involved in the bioconversion of both n-3 and n-6 PUFA to LC-PUFA are the  $\Delta$ -6 and  $\Delta$ -5 fatty  
89 acyl desaturases (FADS2D6 and FADS2D5 respectively) and two fatty acyl elongases (ELOVL2  
90 and ELOVL5). The  $\Delta$ 6 desaturation of both C<sub>18</sub> and C<sub>24</sub> PUFA is likely required for the  
91 biosynthesis of DHA in salmon, although there are three functional FADS2D6 in Atlantic salmon  
92 that may indicate differential regulation of these desaturation steps (Monroig et al., 2010).  
93 Similarly, two functional ELOVL5 (a and b) have been identified in salmon (Morais et al., 2009).  
94 Functional studies suggest that ELOVL5 is mainly involved in the elongation of C<sub>18</sub>→C<sub>20</sub> PUFA,  
95 with residual C<sub>20</sub>→C<sub>22</sub> activity, whereas ELOVL2 elongates C<sub>20</sub>→C<sub>22</sub> but not C<sub>18</sub>→C<sub>20</sub> (Morais et  
96 al., 2009). The activity of the LC-PUFA biosynthesis pathway relies on the presence of substrates  
97 but also transcription factors (TF) such as sterol regulatory element binding protein (SREBP) 1

98 and 2 or liver X receptor (LXR), which may be involved in gene regulation (Carmona-Antoñanzas  
99 et al., 2014).

100 To understand performance characteristics at the level of the organism in greater detail, it is  
101 necessary to evaluate the different roles that LC-PUFA play within individual tissues. Thus, liver is  
102 considered an important site for LC-PUFA synthesis and lipid metabolism in Atlantic salmon  
103 (Monroig et al., 2010). Neural tissues like brain and retina are characteristically rich in DHA  
104 (Tocher & Harvie, 1988; Bell & Tocher, 1989), and thus, DHA-deficient diets lead to impaired  
105 visual performance (Bell et al., 1995). Head kidney is of interest because it forms a key component  
106 of the fish immune system (Tort et al. 2003), the functions of which are known to be influenced by  
107 dietary LC-PUFA (Waagbø, 1994; Lall, 2000). Specifically, LC-PUFA are considered essential for  
108 the production of eicosanoids such as leukotrienes, prostaglandins and thromboxanes, substances  
109 that act as key mediators between immune cell membranes and inflammatory responses in fish  
110 (Rowley et al. 1995; Martinez-Rubio et al., 2013). The gills are another tissue susceptible to  
111 dietary changes in PUFA and this is of specific interest because, on top of respiration, the gills play  
112 vital roles in osmoregulation and ion balance (Bell et al., 1992; 1996).

113 Phospholipids (PL), major constituents of cell lipids, tend to be fairly constant in composition  
114 under normal physiological conditions, enabling functional associations to be drawn between  
115 different organs/tissues (Christie, 2003a). In addition, LC-PUFA are preferentially deposited in PL  
116 over triacylglycerol (TAG) (Sargent et al., 2002). It was hypothesised that the PL fatty acid  
117 compositions of liver, brain, head kidney and gill tissues of Atlantic salmon would respond  
118 differently to altered dietary LC-PUFA based on their individual fatty acid requirements and  
119 functional roles in lipid and fatty acid metabolism. The present study therefore aimed to examine  
120 the different tissue specificities for DHA, in addition to potential interactions with either EPA or  
121 ARA in post-smolts fed diets containing varying levels of these essential LC-PUFA. Furthermore,  
122 the study sought to evaluate the influence dietary LC-PUFA may have on total lipid contents and

123 compositions of the different tissues, in addition to understanding the molecular mechanisms  
124 involved in the control and regulation of LC-PUFA metabolism.

125

## 126 **1. Materials and Methods**

### 127 *2.1. Experimental diets*

128 A single basal diet was formulated to provide protein and lipid at 460 g kg<sup>-1</sup> and 200 g kg<sup>-1</sup>  
129 diet at a gross energy level of 22.0 MJ kg<sup>-1</sup> (estimated digestible protein and energy of 440 g kg<sup>-1</sup>  
130 and 19.5 MJ kg<sup>-1</sup>, respectively). A total of eight experimental diets were produced by vacuum  
131 coating the dry basal extruded pellets with custom, pre-mixed oil blends as follows. To investigate  
132 the effect of DHA concentration, a series of five DHA inclusion levels (1 g kg<sup>-1</sup>, 5 g kg<sup>-1</sup>, 10 g kg<sup>-1</sup>  
133 <sup>1</sup>, 15 g kg<sup>-1</sup> and 20 g kg<sup>-1</sup>, named D1, D5, D10, D15 and D20 respectively) were created using a  
134 blend of oils that included an algal DHA source derived from *Crypthecodinium* sp. (HuaTai  
135 BioPharm Inc., Deyang, Sichuan, China) along with a combination of clarified butterfat and olive  
136 oil as a lipid base (Table 1). To examine additional effects of EPA and ARA inclusion, three  
137 further treatments were created. Two EPA diets containing either 10 g kg<sup>-1</sup> (D10E) or 5 g kg<sup>-1</sup>  
138 (D5E) each of EPA and DHA were formulated using anchovy oil that contained EPA and DHA in  
139 equal amounts. A single ARA treatment (D10A) was formulated using fungal-derived concentrate  
140 (HuaTai BioPharm Inc., Deyang, Sichuan, China) to include 10 g kg<sup>-1</sup> each of ARA and DHA. For  
141 full compositional analysis of experimental diets see Table 2 and for additional information on diet  
142 manufacture refer to Glencross et al. (2014).

143

### 144 *1.2. Fish and husbandry*

145 Prior to experimental work, Atlantic salmon smolts were sourced from Howietoun hatchery  
146 (Bannockburn, Scotland) and transferred to the Marine Environmental Research Laboratory  
147 (Machrihanish, Argyll, Scotland) where they were on-grown to 110.9 ± 2.61 g (mean ± S.D.) post-

148 smolts in two 10,000 L seawater tanks. All fish were anesthetized using benzocaine prior to  
149 handling. The fish were weighed on an electronic toploading balance to 0.5 g accuracy and 20 fish  
150 allocated to each of 24 x 500 L tanks. The experimental system comprised a flow-through,  
151 ambient water temperature, 500 L x 24-tank array. Water temperature was  $14.0 \pm 0.82^{\circ}\text{C}$  (mean  $\pm$   
152 S.D.) and dissolved oxygen was at  $7.8 \pm 0.60 \text{ mg L}^{-1}$  (mean  $\pm$  S.D.) for the duration of the 9-week  
153 experiment. All eight treatments were fed in triplicate (three tanks of 20 fish each). Experimental  
154 feeds were delivered on a restricted pair-wise feeding regime to eliminate feed intake variability,  
155 and feed rations were increased incrementally over the duration of the study. Further details of  
156 feeding regime are provided elsewhere (Glencross et al., 2014).

157

### 158 *1.3. Sample collection and management*

159 At the end of the feeding trial, a total of six fish (two per tank) from each treatment were  
160 randomly sampled and euthanized by benzocaine overdose. Samples of liver, brain, gill and head  
161 kidney tissue were collected from each fish and immediately frozen in liquid nitrogen prior to  
162 storage at  $-70^{\circ}\text{C}$ . Approximately 100 mg of each individual tissue was sampled for total RNA  
163 extraction, whereas paired samples from each tank were pooled to form individual replicates by  
164 treatment and tissue type for total lipid extraction.

165

### 166 *1.4. Total lipid extraction*

167 Lipid was extracted from tissue samples using a modified method of Folch et al. (1957).  
168 Briefly, liver and gill samples were homogenized in 16 ml of chloroform/methanol (2:1, v/v) using  
169 an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK), while brain and head  
170 kidney samples were homogenized using a glass-barrel homogenizer in the same volume of  
171 solvent. Non-lipid impurities were isolated by washing with 4 ml of 0.88% aqueous KCl (w/v).  
172 The upper aqueous layer was removed by aspiration and the lower solvent layer containing the



lipid extract dried under oxygen-free nitrogen. Total lipid content was determined gravimetrically after overnight desiccation *in vacuo*.

### 1.5. Lipid class composition

Lipid classes were separated by double-development, high-performance thin-layer chromatography (HPTLC) using 10 x 10 cm plates (VWR, Lutterworth, UK) according to Henderson & Tocher (1992). Total lipid samples (1-2 µg) were applied as 3 mm origins and the plates developed in methyl acetate/isopropanol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by vol.) to 5.2 cm. Excess solvent was evaporated via air drying and vacuum desiccation and plates developed to 9.5 cm using a solvent mixture containing iso-hexane/diethyl ether/acetic acid (80:20:1, by vol.) before termination and drying as above. Lipid classes were visualized by spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid and charring plates at 160 °C for 20 min. Lipid classes were quantified by densitometry using a CAMAG-3 TLC scanner (version Firmware 1.14.16; CAMAG, Muttenz, Switzerland) with winCATS software (Planar Chromatography Manager, version 1.2.3).

### 1.6. Phospholipid fatty acid composition

Phospholipids were isolated using thin-layer chromatography (TLC) by loading 2 mg of total lipid onto 2.5 cm origins on 20 x 20 cm TLC plates (VWR, Lutterworth, UK) and running in a solvent mixture comprising isohexane/diethyl ether/acetic acid (80:20:1, by vol.). Plates were sprayed with 1% (w/v) 2',7'-dichlorofluorescein in 97% (v/v) methanol containing 0.05% (w/v) BHT and visualized under UV light (UVGL-58 Minerallight® Lamp, Ultraviolet Prod. Inc., Calif., USA). Total polar lipids were scraped into test tubes and fatty acid methyl esters (FAME) were prepared by acid-catalyzed transmethylation according to the method of Christie (2003b). FAME were separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m × 0.32 mm i.d.  $\mu$ m-25B -wax column

199 (Phenomenex, Cheshire, UK), on-column injector and a flame ionization detector. Hydrogen was  
200 used as the carrier gas in constant flow mode at  $2.5 \text{ ml min}^{-1}$ , with an initial oven thermal gradient  
201 from  $50 \text{ }^{\circ}\text{C}$  to  $150 \text{ }^{\circ}\text{C}$  at  $40 \text{ }^{\circ}\text{C min}^{-1}$  to a final temperature of  $230 \text{ }^{\circ}\text{C}$  at  $2 \text{ }^{\circ}\text{C min}^{-1}$ . Data were  
202 collected and processed using Chromcard for Windows (version 2.01; Thermoquest Italia S.p.A.,  
203 Milan, Italy). Individual FAME were identified by comparison to known standards and published  
204 data (Ackman, 1980; Tocher & Harvie, 1988). Selected FAME were confirmed by gas  
205 chromatography-mass spectrometry (GC-MS) using a gas chromatograph (GC8000) coupled to a  
206 MD800 mass spectrometer (ThermoFisher Scientific, Hemel Hempstead, UK).

207

#### 208 *1.7. RNA extraction and quantitative real time PCR (qPCR)*

209 Five of the eight experimental treatments were chosen for the gene expression study. These  
210 diets were chosen to represent a low DHA level (D1), an “optimum” level according to previous  
211 studies (D10; Glencross et al., 2014), and the three combinations of LC-PUFA (D10A, D10E and  
212 D5E). Liver, brain, gill and head kidney samples from six individual fish per treatment ( $n = 2$  fish  
213 per tank) were homogenized in TriReagent<sup>®</sup> (Sigma-Aldrich, Dorset, UK) RNA extraction buffer  
214 following the manufacturer’s instructions. Quantity and quality of isolated total RNA were  
215 determined by spectrophotometry with an ND-1000 Nanodrop (Labtech Int., East Sussex, UK) and  
216 electrophoresis using 500 ng of total RNA in a 1% agarose gel. cDNA was synthesized using 2  $\mu\text{g}$   
217 of total RNA and random primers in 20  $\mu\text{l}$  reactions and the High capacity reverse transcription kit  
218 without RNase inhibitor according to the manufacturer’s protocol (Applied Biosystems,  
219 Warrington, UK). The resulting cDNA was diluted 20-fold with milliQ water.

220 For qPCR, primers for fatty acyl desaturases and elongases, and TF involved in their  
221 regulation, were used (see Table 3). The efficiency of the primers for each gene was previously  
222 evaluated to ensure that it was close to 100%. In addition, two reference genes, cofilin-2 and  
223 elongation factor-1 $\alpha$  were quantified. qPCR was performed using a Biometra TOptical  
224 Thermocycler (Analytik Jena, Goettingen, Germany) in 96-well plates in duplicate 20  $\mu\text{l}$  reaction

volumes containing 10 µl of SYBR Green RT-PCR Master Mix (Applied Biosystems, Paisley, UK), 1 µl of the primer corresponding to the analyzed gene (10 pmol), 3 µl of molecular biology grade water and 5 µl of cDNA, with the exception of the reference genes, which were determined using 2 µl of cDNA. In addition amplifications were carried out with a systematic negative control (NTC-non template control) containing no cDNA. Standard amplification parameters contained an initial activation step at 95°C for 15 min, followed by 35 cycles: 15 s at 95°C, 30 s at the annealing T<sub>m</sub> and 30 s at 72°C. A calibrator sample was included within each plate in order to compare the gene expression among the different tissues/plates.

233

#### 234 1.8. Statistical Analysis

All data are means ± S.D. (*n* = 3) unless otherwise specified. Percentage data for total lipid content, lipid class composition and polar lipid fatty acid composition were all subjected to arcsin square-root transformation prior to analyses. Effects of DHA inclusion level (diets D1-D20) were examined with regression analysis. Additionally, effects of EPA and ARA inclusion were examined against equivalent levels of DHA. Specifically, the 10 g kg<sup>-1</sup> diets D10 and D5E were examined by one-way analysis of variance (ANOVA), while the 20 g kg<sup>-1</sup> diets D20, D10A and D10E were examined by one-way ANOVA followed by a Tukey-Kramer HSD multiple comparison of means. All statistical analyses were performed using Minitab (version 16.1.0; Minitab Inc., State college, PA).

Gene expression results were analyzed using the relative expression software tool (REST 2008; <http://www.gene-quantification.info/>), which employs a pairwise fixed reallocation randomization test (10,000 randomizations) with efficiency correction (Pfaffl et al., 2002) to determine the statistical significance of expression ratios (gene expression fold changes) between two treatments. In addition, a supervised hierarchical clustering was applied employing the relative gene expression ratio for each gene based on the PCR efficiency and Ct of sample compared to the

control, according to Pfaffl's mathematical model (Pfaffl, 2001). Tree View software (Page, 1996) was used to generate visual representations of the classification.

## 2. Results

### 3.1. Total lipid content

In general lipid content was fairly constant across all eight dietary treatments (Tables 4-7). Brain tissue had the highest mean lipid content (7.7%), followed by liver (4.8%) and head kidney (4.2%), while gill tissue contained on average just 1.7% lipid (Tables 4-7). Increasing inclusion of DHA in the diet (D1-D20) had no significant effect on lipid content in any of the tissues examined. The lipid content of head kidney from fish fed diet D20 was lower than that of fish fed diet D10E (Table 5), and brain lipid content was higher in fish fed diet D5E compared with those fed diet D10 (Table 7). Other than these differences, the mixed LC-PUFA diets had no significant effects on tissue lipid contents.

### 2.2. Lipid class composition

In general the dietary treatments had little effect on the lipid class compositions of liver, brain, head kidney and gill. The proportions of phosphatidylcholine (PC) and cholesterol were slightly, but significantly, increased and the proportion of phosphatidylinositol (PI) decreased, in liver with increasing dietary DHA (data not shown). Increasing dietary DHA had no significant effect on the lipid class composition of head kidney, gill or brain. The level of TAG in brain was highly variable between fish fed the different diets although only significant in fish fed diet D10E, which had higher TAG than fish fed diets D20 or D10A (data not shown). Other than this, the diets with combinations of LC-PUFA had no significant effects on the lipid class compositions of salmon tissues.

### 2.3. Fatty acid compositions of liver and head kidney phospholipids

The predominant fatty acids in liver PL were DHA, 18:1n-9 and 16:0, though overall fatty acid composition was readily influenced by that of the diet (Tables 2, 4 & 5). Relative concentrations of DHA, EPA and ARA in liver PL were always considerably higher than those of the diet, while total saturated fatty acids (SFA) and total monounsaturated fatty acids (MUFA) were consistently lower than those of the diet. Increased inclusion of DHA in the diet resulted in a highly significant increase (10.4 %) in the concentration of DHA in the liver ( $P<0.001$ ) and correspondingly increased total n-3 PUFA and LC-PUFA (Table 4). The relative contents of EPA and docosapentaenoic acid (DPA; 22:5n-3) in the liver tended to decrease with increasing DHA, while levels of ARA and 22:4n-6 were largely unaffected. Total SFA content of liver PL remained fairly stable, but MUFA content decreased significantly across diets D1-D20, reflecting the dietary levels of these fatty acid groups. The concentration of 22:5n-6 in liver PL increased significantly in fish fed diets D1 through to D20 reflecting the increasing level of this fatty acid in these diets (Tables 2 & 4).

Inclusion of other LC-PUFA (EPA and ARA) in the diet also influenced the fatty acid composition of liver PL (Table 5). Inclusion of EPA (diets D5E & D10E) resulted in DHA levels similar to those found in liver PL from fish fed equivalent diets containing DHA alone (D10 and D20) (Tables 2 & 5). EPA inclusion also tended to increase the levels of both EPA and DPA found in liver PL, with the higher inclusion of EPA resulting in highly significant increases ( $P<0.001$ ) in these fatty acids relative to diets D20 and D10A (Table 5). Furthermore, diets D5E and D10E resulted in the lowest levels of ARA in liver PL. Inclusion of ARA (D10A) resulted in significantly higher levels of ARA and 22:4n-6 in liver PL compared with DHA alone (D20) (Table 5). Diet D10A also resulted in the lowest level of EPA in liver PL out of all the diets. Inclusion of EPA or ARA also clearly altered the EPA/ARA ratio in their favour, but there were no significant effects of EPA or ARA inclusion on the relative amount of SFA or MUFA.

300 The fatty acid composition of head kidney PL differed from that of liver in that it contained  
301 slightly more SFA and slightly less PUFA (Tables 4 & 5). Additionally, head kidney contained a  
302 small percentage of dimethyl acetals (DMA; 2.6 % total fatty acids), derived from plasmalogen  
303 PL, not observed in the liver. However, the effects of increased dietary DHA were broadly the  
304 same as those described for liver PL, although the mean increase in relative DHA content in head  
305 kidney was not as defined as in liver (5.6 % vs. 10.4 % total fatty acids) (Table 4). Effects of EPA  
306 or ARA inclusion on the fatty acid composition in head kidney PL were also very similar to those  
307 observed in liver PL (Table 5).

#### 308 *2.4. Fatty acid compositions of brain and gill phospholipids*

309 The fatty acid composition of brain PL showed a number of general differences to that of liver  
310 PL. Brain PL contained a notable proportion (> 7% total fatty acids) of DMA (Tables 6 & 7). In  
311 addition, the relative content of EPA was higher, and that of ARA lower in brain PL compared to  
312 liver PL. Furthermore, the relative MUFA content of brain PL (due to higher 24:1n-9, not shown)  
313 was noticeably higher than that of the liver (39.6 vs. 23.3 % total fatty acids) (Tables 6 & 7).  
314 Increasing DHA inclusion resulted in only a small, but significant, increase (1.7 %) in the relative  
315 DHA content of brain PL (Table 6). Relative SFA contents increased slightly and relative MUFA  
316 contents decreased in fish fed diets D1 through D20, reflecting dietary compositions (Tables 2 &  
317 6). EPA and DPA both declined in brain PL in fish fed diets D1 through D20, although the effects  
318 were subtle in comparison to the liver (Table 6). Diets D1-D20 had no effect on the relative  
319 amounts of ARA and 22:4n-6 in brain PL, whereas 22:5n-6 increased in line with dietary levels  
320 (Table 6). Effects of EPA and ARA inclusion on the fatty acid composition of brain PL also  
321 followed the same overall patterns as seen in the liver, but the magnitude of these effects was  
322 generally much lower (Table 7). It was notable that the EPA/ARA ratio was always positive, even  
323 when fish were fed diet D10A with increased ARA. The fatty acid composition of gill PL  
324 contained slightly higher percentages of ARA and 22:4n-6 and slightly lower percentages of EPA  
325 and DPA than liver PL, and total PUFA content was consistently lower (Tables 6 & 7). Relative

326 SFA and MUFA contents were slightly higher than that of liver PL. Like brain, increasing DHA  
327 inclusion did not have the same clear effect on relative DHA content in gill PL as observed in liver,  
328 and the relationship was non-significant ( $P = 0.057$ ) (Table 6). Effects of EPA or ARA inclusion  
329 were similar to those for liver but less pronounced (Table 7).

## 330 2.5. Tissue expression profile of lipid metabolism genes

331 Differing dietary LC-PUFA contents affected the expression of the studied genes, although the  
332 effects varied between the tissues. Therefore, brain showed the more stable expression pattern  
333 whereas, in contrast, liver showed the highest number of altered genes, followed by head kidney  
334 (Fig. 1). Increased dietary DHA content from 1 to 10 g kg<sup>-1</sup> significantly reduced the mRNA  
335 abundance of desaturases *fads2d6b*, *fads2d6c* and *fads2d5*, as well as *elovl2* and *elovl4* in liver  
336 (Fig. 2), but no dietary effect was observed in brain (Fig. 3). Reduced expression of both *fads2d6b*  
337 and *fads2d5* was also observed in head kidney and gill, respectively, although statistically non-  
338 significant (Figs. 4 & 5). Generally, fish fed diet D10A displayed the lowest expression of all the  
339 fatty acyl desaturases and elongases genes studied in head kidney and liver, though this tendency  
340 was not observed in brain and gill. Both *elovl5a* and *5b* showed variable expression in all tissues  
341 with the only statistically significant difference found in brain where fish fed diet D10A showed  
342 highest expression of *elovl5b*.

343 Regarding TF, *lxr* gene expression did not differ among fish fed any of the diets in any of the  
344 tissues. In contrast, *srebp1* expression was up-regulated in liver (Fig. 2) and gill (Fig. 5) of fish fed  
345 diet D1, head kidney of fish fed D10E (Fig. 3), and brain of fish fed D10A (Fig. 4). Gene  
346 expression of *srebp2* was only significantly regulated only in liver, with lower expression in fish  
347 fed D10A (Fig. 2).

348

## 349 3. Discussion

350        Increasing dietary DHA and additional EPA and ARA to Atlantic salmon had clear effects on  
351        PL fatty acid compositions of all tissues although the precise nature and magnitude of the effects  
352        varied markedly between the tissues. Liver, brain, head kidney and gill were chosen in the present  
353        study based on the different roles they play in LC-PUFA metabolism and vice versa, the varied role  
354        LC-PUFA play in the functions of the tissues. In this context, the liver was of interest because of its  
355        important roles in LC-PUFA biosynthesis and overall body lipid homeostasis in Atlantic salmon  
356        (Tocher et al., 2003; Monroig et al., 2010; Martinez-Rubio et al., 2013). In contrast, in common  
357        with other higher vertebrates, brain of fish is characteristically enriched in DHA (Tocher & Harvie,  
358        1988; Bell & Tocher, 1989) and so was of particular interest in the present study where dietary  
359        DHA was the primary variable. Head kidney in fish forms an integral component of the immune  
360        system (Tort et al., 2003; Gjøen et al., 2007), and so was of interest because dietary lipid and PUFA  
361        content are known to influence immune function and thus health status of fish (Waagbø, 1994; Lall,  
362        2000; Martinez-Rubio et al., 2013). Finally, previous work had shown that gill phospholipid fatty  
363        acid compositions significantly alter during the smoltification process and that these changes can be  
364        influenced by diet (Bell et al., 1997; Tocher et al., 2000).

365        The results of the present study showed that lipid content of the brain was the highest of all the  
366        tissues examined (~ 7.7% wet weight), which was comparable with the value of 7.1% reported for  
367        Atlantic salmon by Stoknes et al. (2004). There was a clear trend for lipid content in brain to  
368        increase when EPA or ARA was added to the diets although this was only significant in fish fed  
369        D5E. The increased lipid content was driven by increased TAG content but the reason for this was  
370        unclear. It may be that the relative level of DHA was lower when EPA and ARA was present and so  
371        not all uptake was able to be processed for PL synthesis/turnover. Liver and head kidney lipid  
372        content did not vary significantly, perhaps because the lipid and energy content was fairly stable  
373        across all dietary treatments (Martinez-Rubio et al., 2013), while analysis of gill revealed the lowest  
374        lipid content of any of the tissues examined, on average just 1.65% wet tissue weight, which is



375 similar to the low gill lipid content (range 0.6 – 1.4%) found for turbot, *Scophthalmus maximus*  
376 (Castell et al., 1994).

377 The fatty acid profiles of the four tissues analyzed showed different patterns likely related to  
378 their physiological functions. The fatty acid composition of liver PL largely reflected that of the  
379 diet, which is consistent with other studies on Atlantic salmon (Brodtkorb et al., 1997; Bell et al.,  
380 2003; Bransden et al., 2003). The relative DHA content of liver PL was always considerably higher  
381 than that of the diet, indicating the important role of this fatty acid in cell membranes (Sargent et al.,  
382 2002). In contrast, relative levels of SFA and MUFA in liver PL were noticeably lower than those  
383 of the feed, suggesting selective discrimination and/or preferential  $\beta$ -oxidation of these fatty acids  
384 (Henderson & Sargent, 1985; Turchini & Francis, 2009). The fatty acid composition of brain PL  
385 was much more conserved and less affected by diet than the liver, consistent with other studies  
386 examining these tissues in Atlantic salmon (Bell et al., 1990; Brodtkorb et al., 1997) and turbot  
387 (Bell et al., 1999). This implied a slower turnover of lipids and fatty acids in the brain, although the  
388 relatively short duration of the experiment may have limited the ability to detect greater differences  
389 between dietary groups.

390 Ruyter et al. (2000) showed that increasing inclusion of DHA and EPA in the diets of Atlantic  
391 salmon fry led to increased percentages of these fatty acids in liver PL. Similarly, in the present  
392 study, increasing dietary DHA through diets D1-D20 led to a highly significant increase in the  
393 percentage of DHA in liver PL. However, this contrasted with the results of Bell et al. (1989) who  
394 found no significant differences in the relative amount of DHA in liver PL when Atlantic salmon  
395 post-smolts were fed diets containing either fish oil or a combination of corn oil and lard, despite  
396 the former diet containing three times more DHA. Regression analysis showed that increasing  
397 dietary DHA led to only a small increase in the relative DHA content of brain PL, probably  
398 reflecting the fact that brain PL fatty acid composition is much more tightly controlled rather than  
399 any particular control on DHA uptake. Indeed, work on juvenile Atlantic salmon by Brodtkorb et al.

400 (1997) found no effects of increasing dietary DHA content on the fatty acid composition or on DHA  
401 levels within individual lipid classes of the brain. However, the diets used in the previous study  
402 contained much higher levels of DHA (range 6.3 – 17.9 % total fatty acids) representing dietary  
403 contents far above any reported requirement levels compared with the present study that included  
404 levels of DHA that may be more limiting (range 0.5 – 7.6 % total fatty acids). Interestingly, the  
405 significance of the response in brain in the present study was removed when the lowest DHA  
406 treatment (D1) was excluded from analysis ( $P = 0.454$ ), supporting the view that only very low  
407 DHA affected brain PL fatty acid compositions, and not dietary DHA above requirement levels.

408 Previous studies on the replacement of fish oil with soybean oil in the diets of Atlantic salmon  
409 showed a reduction in the percentage of DHA in head kidney total lipid, consistent with the reduced  
410 DHA content of vegetable oil-based diets (Gjøen et al., 2004). Similarly, the present study showed  
411 that increasing inclusion of DHA in the diet was reflected in head kidney PL with increased  
412 percentages of DHA, though the magnitude of effect was only half that observed in liver. The head  
413 kidney contains high numbers of immune cells and so changes in dietary fatty acid composition has  
414 the potential to alter cell membrane physiology and immune function of these cells (Mourete et al.,  
415 2007). In contrast, increasing dietary DHA did not significantly affect the percentage of DHA in gill  
416 PL, although variation observed between replicates in D10-fed fish may have influenced this.  
417 However, there was still no obvious trend and so the effect, if any, was subtle in comparison to the  
418 liver. Overall, this may suggest more selective uptake of DHA in gill PL, or more conserved  
419 composition, as observed in brain PL.

420 Given that the impact of dietary DHA was most clearly observed in the liver, it is likely that  
421 liver in Atlantic salmon plays an important role in the initial selectivity for DHA, as reported in  
422 other vertebrates (Polozova & Salem, 2007). Despite very low levels of DPA in the diet, its higher  
423 content in liver PL may suggest that endogenous synthesis of DHA from LNA and/or EPA may  
424 have occurred in at least the lower inclusion of DHA (D1). Consistent with this, *fads2d6b*, *fadsd26c*

425 and *fads2d5* expression were higher in fish fed 1 g kg<sup>-1</sup> of DHA denoting an upregulation of the LC-  
426 PUFA biosynthesis pathway as previously described (Moya-Falcón et al., 2005; Thomassen et al.,  
427 2012). The elongation of EPA to DPA appears to be very active in Atlantic salmon liver  
428 (Thomassen et al., 2012), which is in agreement with the present study, where high expression of  
429 both *elovl2* and *elovl5* was observed together with high levels of DPA. However, only statistical  
430 differences were found in *elovl2*, suggesting a greater role of this enzyme compared to *elovl5* in  
431 elongation of C<sub>20</sub> PUFA. Similarly, heterologous expression in yeast showed that salmon *elovl5*  
432 elongated C<sub>18</sub> and C<sub>20</sub> PUFA, with low activity towards C<sub>22</sub>, whereas *elovl2* elongated C<sub>20</sub> and C<sub>22</sub>  
433 PUFA with lower activity towards C<sub>18</sub> (Morais et al., 2009). Interestingly, *elovl4* expression was  
434 also upregulated in liver of fish fed 1 g kg<sup>-1</sup> of DHA. ELOVL4 is involved in the synthesis of very  
435 long-chain PUFA (> C<sub>24</sub>) in mammals, but it was recently shown that Atlantic salmon *elovl4* open  
436 reading frame (ORF) was able to elongate both EPA and ARA (15.4 % and 11.5% conversion,  
437 respectively) indicating that it was also involved in LC-PUFA biosynthesis (Carmona-Antoñanzas  
438 et al., 2011). These data are consistent with the results in the present study, where high  
439 concentrations of DPA could be due to combined activity of the different fatty acid elongases. In  
440 contrast, the highest inclusion of DHA (D20) was at the upper end of the documented requirement  
441 for LC-PUFA in salmonids (Ruyter et al., 2000) and this probably suppressed further endogenous  
442 synthesis of DHA in the liver, gill and head kidney (Bell & Sargent, 2003; Zheng et al., 2004).

443 Dietary inclusion of EPA or ARA typically resulted in increased levels of these fatty acids in  
444 the PL of all studied tissues, reflecting the preferential incorporation of LC-PUFA into cell  
445 membranes (Sargent et al., 2002). These two fatty acids had inverse reciprocal effects on their  
446 respective levels in tissue PL such that inclusion of one reduced the relative amount of the other,  
447 highlighting their strong biological link in fatty acid metabolism (Bell et al., 1989). In addition, the  
448 tissue proportion of DHA was increased in fish fed both diets containing EPA, regardless of total  
449 LC-PUFA content, whereas inclusion of ARA appeared to have the opposite effect in that it  
450 lowered the relative amount of DHA and also EPA present in liver, head kidney and gill PL. In

451 contrast, DHA levels in the brain were not affected by dietary EPA or ARA denoting once again the  
452 importance of this fatty acid for neural functions. It was noteworthy that EPA did not appear to  
453 influence the activity of fatty acyl desaturases, as the EPA level in diet D1 was similar to the other  
454 diets, but fish fed diet D1 displayed an up-regulation in these enzymes, especially in liver, head  
455 kidney and gill. This may indicate a key role for DHA in the regulation of desaturase expression as  
456 has been suggested previously (Thomassen et al., 2012). However, results obtained from a previous  
457 *in vitro* study showed inhibition of desaturation and elongation of 18:3n-3 when EPA or DHA were  
458 added to the cell medium (Zheng et al., 2009). This earlier study was performed on an established  
459 cell line where cells reflect the fatty acid composition of the foetal bovine serum present in the  
460 medium, thus the response to LC-PUFA may vary when compared to an *in vivo* model (Tocher et  
461 al., 1988).

462 Interestingly, brain PL consistently maintained a positive EPA/ARA ratio, even when fish were  
463 fed diet D10A, which contained much more ARA than EPA (5.1% vs 0.6% total fatty acids). A  
464 similar effect was seen on a whole-body mass basis in a study with Asian seabass (*Lates calcarifer*)  
465 when they were fed diets similar to the D10A and D10E used in the present study (Glencross et al.,  
466 2011). Both EPA and ARA are well known to compete as substrates for eicosanoid synthesis in  
467 vertebrates (Bell et al., 1994; Calder, 2006). This suggests a preferential incorporation of EPA over  
468 ARA in brain PL. Inclusion of EPA and ARA in the diet was of particular relevance to the head  
469 kidney because of the key role of eicosanoids in immune/inflammatory responses (Martinez-Rubio  
470 et al., 2013). In this sense, the addition of ARA to the diet resulted in significantly higher ARA in  
471 head kidney PL, which may have increased inflammatory potential in these fish. This may have in  
472 part contributed to the lower survival of this dietary group reported previously (Glencross et al.,  
473 2014). On the other hand, inclusion of EPA in the diet resulted in an increased percentage of EPA in  
474 head kidney PL, and this would presumably increase the availability of anti-inflammatory  
475 eicosanoids. Indeed, recent trials using functional feeds containing both reduced lipid content and  
476 increased EPA have been shown to reduce the intensity of inflammatory responses associated with

477 Atlantic salmon reovirus-induced HSMI (heart and skeletal muscle inflammatory disease)  
478 (Martinez-Rubio et al., 2012). Another trial modulating the inclusion of EPA and DHA in the diet  
479 of Asian seabass found acutely contrasting effects on a range of both clinical and sub-clinical  
480 inflammation markers (Glencross et al., 2011). Interestingly, the inclusion of EPA in the present  
481 study still only resulted in an EPA/ARA ratio of 1.1 in gill PL, reaffirming the bias toward ARA  
482 over EPA in this tissue. Extreme dietary alterations in these fatty acids might therefore compromise  
483 osmoregulatory function and overall health of the fish.

484 Regulation of lipid metabolism is complex and controlled by several TF including SREBPs and  
485 LXR. In mammals, SREBP1 is involved in activation of genes that participate in fatty acid  
486 metabolism and *de novo* lipogenesis whereas SREBP2 is more selective for genes involved in  
487 cholesterol homeostasis (Horton et al., 2004). Furthermore, n-3 and n-6 fatty acids can induce  
488 transcription of *lxr* through DR1 elements (Tobin et al., 2002) and regulate the expression of *srebp1*  
489 (Joseph et al., 2002), which is a major regulator of lipogenesis in mammals (Davidson, 2006). In the  
490 present study the diet with highest LC-PUFA content (D10A; 13.5 %) was found to down-regulate  
491 the expression of *srebp1* in liver, whereas fish fed diet D1, with only 1% LC-PUFA, showed the  
492 highest expression. This is in agreement with previous studies in Atlantic salmon both *in vitro*  
493 (Minghetti et al., 2011) and *in vivo* (Morais et al., 2011) where *srebp1* expression was reduced by  
494 LC-PUFA supplementation, denoting a similar nutritional regulation to mammals (Davidson et al.,  
495 2006; Caputo et al., 2010). Similarly, this lower expression profile was reflected in lower  
496 expression levels of some SREBP1 target genes such as *fads2d6c* and *fads2d5*. However, this  
497 pattern of expression was not observed in all tissues and so, in contrast, *srebp1* expression in brain  
498 was highest in D10A-fed fish. Conversely, *lxr* gene expression was not affected by dietary PUFA  
499 content. The explanation to this could be that LXR is activated by a variety of sterols, including  
500 intermediates in the synthesis of cholesterol, and adequate levels of cholesterol were present in all  
501 of the diets, which could also explain why *srebp2* expression was unaffected.

502 In summary, the present study demonstrated that manipulation of dietary LC-PUFA directly  
503 affected the fatty acid profile of tissue PL and gene expression of key metabolic tissues in post-  
504 smolt Atlantic salmon. Liver displayed the greatest response to dietary DHA, accumulating this  
505 fatty acid in higher amounts than any other tissue, with increased expression of key enzymes  
506 involved in LC-PUFA synthesis in fish fed the lowest DHA diet. A qualitatively similar but  
507 quantitatively lower effect was observed in head kidney. In contrast, PL fatty acid profile and gene  
508 expression was more conserved in brain and less affected by dietary treatment, and a similar  
509 response to diet was observed in gill. The tissue variation observed most likely reflected the unique  
510 functions of each tissue.

## 511 References

512 Ackman, R.G., 1980. Fish Lipids. In: Connell, J.J. (Ed.), Advances in Fish Science and  
513 Technology, Fishing News Books, Farnham, pp. 87-103.

514  
515 Bell, J.G., Youngson, A., Mitchel, A.I., Cowey, C.B., 1989. The effect of enhanced intake of  
516 linoleic acid on the fatty acid composition of tissue polar lipids of post-smolt Atlantic salmon  
517 (*Salmo salar*). Lipids 24, 240-242.

518  
519 Bell, J.G., Sargent, J.R., Raynard, R.S., 1992. Effects of increasing dietary linoleic acid on  
520 phospholipid fatty acid composition and eicosanoid production in leucocytes and gill cells of  
521 Atlantic salmon (*Salmo salar*). Prostaglandins Leukot. Essent. Fatty Acids 45, 197-206.

522  
523 Bell, J.G., Dick, J.R., McVicar, A.H., Sargent, J.R., Thompson, K.D., 1993. Dietary  
524 sunflower, linseed and fish oils affect phospholipid fatty acid composition, development of cardiac  
525 lesions, phospholipase activity and eicosanoid production in Atlantic salmon (*Salmo salar*).  
526 Prostaglandins Leukot. Essent. Fatty Acids 49, 665-673.

527  
528 Bell, J.G., Tocher, D.R., Sargent, J.R., 1994. Effect of supplementation with 20:3(*n* - 6),  
529 20:4(*n* - 6) and 20:5(*n* - 3) on the production of prostaglandins E and F of the 1-, 2- and 3-series  
530 in turbot (*Scophthalmus maximus*) brain astroglial cells in primary culture. Lipids and Lipid  
531 Metabolism 1211, 335-342.

532  
533 Bell, M.V., Batty, R.S., Dick, J.R., Fretwell, K., Navarro, J.C., Sargent, J.R., 1995. Dietary  
534 deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring  
535 (*Clupea harengus* L.). Lipids 30, 443-449.

536  
537 Bell, J.G., Farndale, B.M., Dick, J.R., Sargent, J.R., 1996. Modification of membrane fatty  
538 acid composition, eicosanoid production, and phospholipase a activity in Atlantic Salmon (*Salmo*  
539 *salar*) gill and kidney by dietary lipid. Lipids 31, 1163-1171.

540

- Bell, J.G., Tocher, D.R., Farndale, B.M., Cox, D.I., McKinney, R.W., Sargent, J.R., 1997. The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation. *Lipids* 32, 515-525.
- Bell, J.G., 1998. Current aspects of lipid nutrition in fish farming. In: Black K.D., Pickering A.D. (Eds.), *Biology of Farmed Fish*, Academic Press, Sheffield, pp. 114-145.
- Bell, J.G., Tocher, D.R., Farndale, B.M., McVicar, A.H., Sargent, J.R., 1999. Effects of essential fatty acid-deficient diets on growth, mortality, tissue histopathology and fatty acid compositions in juvenile turbot (*Scophthalmus maximus*). *Fish Physiol. Biochem.* 20, 263-277.
- Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R., 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *J. Nutr.* 131, 1535-1543.
- Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. *Aquaculture* 218, 491-499.
- Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R., Crampton, V.O., 2003. Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *J. Nutr.* 133, 2793-2801.
- Bell, J.G., Waagbø, R., 2008. Safe and nutritious aquaculture produce: benefits and risks of alternative sustainable aquafeeds. In: Holmer M., Black K.D., Duarte C.M., Marba N., Karakassi I. (Eds.), *Aquaculture in the Ecosystem*, Springer Verlag BV, London, pp.185-225.
- Bendiksen, E.Å., Johnsen, C.A., Olsen, H.J., Jobling, M., 2011. Sustainable aquafeeds: Progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture* 314, 132-139.
- Brandsen, M.P., Carter, C.G., Nichols, P.D., 2003. Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth performance, tissue fatty acid composition and disease resistance. *Comp. Biochem. Physiol. B* 135, 611-625.
- Brodtkorb, T., Rosenlund, G., Lie, Ø., 1997. Effects of dietary levels of 20:5n-3 and 22:6n-3 on tissue lipid composition in juvenile Atlantic salmon, *Salmo salar*, with emphasis on brain and eye. *Aquacult. Nutr.* 3, 175-187.
- Calder, P.C., 2004. n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin. Sci.* 107, 1-11.
- Calder, P.C., 2006. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am. J. Clin. Nutr.* 83, 1505S-1519S.
- Caputo, M., Zirpoli, H., Torino, G., Tecce, M.F., 2010. Selective regulation of UGT1A1 and SREBP-1c mRNA expression by docosahexaenoic, Eicosapentaenoic and Arachidonic acids. *J. Cell. Physiol.* 226, 187-193.
- Carmona-Antoñanzas, G., Monroig, O., Dick, J.R., Davie, A., Tocher, D.R. 2011. Biosynthesis of very long-chain fatty acids (C>24) in Atlantic salmon: cloning, functional characterisation, and tissue distribution of and Elovl4 elongase. *Comp. Biochem. Physiol. B* 159, 122-129.

Carmona-Antoñanzas, G., Tocher, D.R., Martínez-Rubio, L., Leaver, M.J., 2014. Conservation of lipid metabolic gene transcriptional regulatory networks in fish and mammals. *Gene* 534, 1-9.

Castell, J.D., Lee, D.J., Sinnhuber, R.O., 1972. Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): lipid metabolism and fatty acid composition. *J Nutr.* 102, 93-100.

Castell, J.D., Bell, J.G., Tocher, D.R., Sargent, J.R., 1994 Effects of purified diets containing different combinations of arachidonic and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (*Scophthalmus maximus*). *Aquaculture* 128, 315-333.

Christie, W.W., 2003a. Lipids: their structures and occurrence. In: Christie W.W. (Ed), *Lipid Analysis: Isolation, Separation and Structural Analysis of Lipids*, 3<sup>rd</sup> edition, Oily Press, Somerset, pp. 3-36.

Christie, W.W., 2003b. Preparation of derivatives of fatty acids. In: Christie W.W. (Ed), *Lipid Analysis: Isolation, Separation and Structural Analysis of Lipids*, 3<sup>rd</sup> edition, Oily Press, Somerset, pp. 205-225.

Davidson, M.H. 2006. Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids. *Am. J. Cardiol.* 98, 27i-33i.

Dyall, S.C., Michael-Titus, A.T., 2008. Neurological benefits of omega-3 fatty acids. *Neuromol. Med.* 10, 219-235.

FAO, 2012. Food and Agriculture Organisation (FAO), 2009. *The State of World Fisheries and Aquaculture*. FAO, Rome.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497-509.

Gjøen, T., Obach, A., Røsjø, C., Helland, B.G., Rosenlund, G., Hvattum, E., Ruyter, B., 2004. Effect of dietary lipids on macrophage function, stress susceptibility and disease resistance in Atlantic salmon (*Salmo salar*). *Fish Physiol. Biochem.* 30, 149-161.

Gjøen, T., Kleiveland, E.J., Moya-Falcón, C., Frøystad, M.K., Vegusdal, A., Hvattum, E., Berge, R.K., Ruyter, B., 2007. Effects of dietary thia fatty acids on lipid composition, morphology and macrophage function of Atlantic salmon (*Salmo salar* L.) kidney. *Comp. Physiol. Biochem. B* 148, 103-111.

Glencross, B.D., Rutherford, N., 2011. A determination of the quantitative requirements for docosahexaenoic acid for juvenile barramundi (*Lates calcarifer*). *Aquacult. Nutr.* 17, e536-e548.

Glencross, B.D., Tocher, D.R., Matthew, C., Bell, J.G., 2014. Interactions between dietary docosahexaenoic acid and other long-chain polyunsaturated fatty acids on performance and fatty acid retention in post-smolt Atlantic salmon (*Salmo salar*). *Fish Physiol. Biochem.*, in press.

Henderson, R.J., Sargent, J.R., 1985. Chain-length specificities of mitochondrial and peroxisomal  $\beta$ -oxidation of fatty acids in livers of rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol. B* 82, 79-85.



644 Henderson, R.J., Tocher, D.R., 1992. Thin-layer chromatography. In: Hamilton R.J., Hamilton  
645 S. (Eds.), *Lipid Analysis: A Practical Approach*, Oxford University Press, Oxford, pp. 65-111.  
646

647 Horton, J.D., Shah, N.A., Warrington, J.A., Anderson, N.N., Park, S.W., Brown, M.S.,  
648 Goldstein, J.L. 2004. Combined analysis of oligonucleotide microarray data from transgenic and  
649 knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci.* 100, 12027-12032.  
650

651 Joseph, S.B., Laffitte, B.A., Patel, P.H., Watson, M.A., Matsukuma, K.E., Walczak, R.,  
652 Collins, J.L., Osborne, T.F., Tontonoz, P., 2002. Direct and indirect mechanisms for regulation of  
653 fatty acid synthase gene expression by liver X receptors. *J. Biol. Chem.* 277, 11019-11025.  
654

655 Lall, S.P., 2000. Nutrition and health of fish. In: L.E. Cruz-Suárez, D. Ricque-Marie, M.  
656 Tapia-Salazar, M.A. Olvera-Novoa, & R. Civera-Cerecedo, eds, *Avances en Nutrición Acuicola V.*  
657 *Memorias del V Simposium Internacional de Nutrición Acuicola*. 19-22 Novembre, 2000. Mérida,  
658 Yucatán, Mexico, 13-23.  
659

660 Martínez-Rubio, L., Morais, S., Evensen, Ø., Wadsworth, S., Vecino, J.L.G., Bell, J.G.,  
661 Tocher, D.R., 2012. Functional feeds dampen heart inflammation and pathology in Atlantic salmon  
662 (*Salmo salar* L.) with experimentally induced heart and skeletal muscle inflammation (HSMI).  
663 *PLoS ONE* 7, e40266.

664 Martínez-Rubio, L., Morais, S., Evensen, Ø., Wadsworth, S., Vecino, J.L.G., Ruohonen, K.,  
665 Bell, J.G., Tocher, D.R., 2013. Effect of functional feeds on fatty acid and eicosanoid metabolism  
666 in liver and head kidney of Atlantic salmon (*Salmo salar* L.) with experimentally induced Heart  
667 and Skeletal Muscle Inflammation. *Fish Shellfish Immun* 34, 1533-1545.  
668

669 Menoyo, D., López-Bote, C.J., Obach, A., Bautista, J.M., 2005. Effect of dietary fish oil  
670 substitution with linseed oil on the performance, tissue fatty acid profile, metabolism, and  
671 oxidative stability of Atlantic salmon. *J. Anim. Sci.* 83, 2853-2862.  
672

673 Minghetti, M., Leaver, M.J., Tocher, D.R., 2011. Transcriptional control mechanisms of genes  
674 of lipid and fatty acid metabolism in the Atlantic salmon (*Salmo salar* L.) established cell line,  
675 SHK-1. *Biochim. Biophys. Acta* 1811, 194-202.  
676

677 Monroig, Ó. Zheng, X., Morais, S., Leaver, M.J., Taggart, J.B., Tocher, D.R., 2010. Multiple  
678 genes for functional  $\Delta 6$  fatty acyl desaturases (Fad) in Atlantic salmon (*Salmo salar* L.): Gene and  
679 cDNA characterization, functional expression, tissue distribution and nutritional regulation.  
680 *Biochim. Biophys. Acta* 1801, 1072-1081.  
681

682 Morais, S., Monroig, Ó. Zheng, X., Leaver, M.J., Tocher, D.R., 2009. Highly unsaturated fatty  
683 acid synthesis in Atlantic salmon: characterization of ELOVL5- and ELOVL2- like elongases.  
684 *Mar. Biotechnol.* 11, 627-639.  
685

686 Morais, S., Pratoomyot, J., Taggart, J.B., Bron, J.E., Guy, D.R., Bell, J.G., Tocher, D.R., 2011.  
687 Genotype-specific responses in Atlantic salmon (*Salmo salar*) subject to dietary fish oil replacement  
688 by vegetable oil: a liver transcriptomic analysis. *BMC Genomics* 12, 255-272.

689 Mourente, G., Good, J.E., Thompson, K.D., Bell, J.G., 2007. Effects of partial substitution of  
690 dietary fish oil with blends of vegetable oils, on blood leucocyte fatty acid compositions, immune  
691 function and histology in European sea bass (*Dicentrarchus labrax* L.). *Brit. J. Nutr.* 98, 770-779.  
692

693 Moya-Falcón, C., Thomassen, M.S., Jacobsen, J.V., Ruyter, B., 2005. Effects of dietary  
694 supplementation of rapeseed oil on metabolism of 1-14C 18:1n-9, 1-14C 20:3n-6 and 1-14C in  
695 Atlantic salmon hepatocytes. *Lipids* 40, 709-717.

696

697 Nasopoulou, C., Zabetakis, I., 2012. Benefits of fish oil replacement by plant originated oils in  
698 compounded fish feeds. A review. *LWT-Food Sci. Technol.* 47, 217-224.

699

700 Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I.,  
701 Gatlin, D.M., Goldburg, R.J., Hua, K., Nichols, P.D., 2009. Feeding aquaculture in an era of finite  
702 resources. *Proc. Natl. Acad. Sci. U.S.A.* 106, 15103-15110.

703

704 Page, R.D., 1996. TREEVIEW: An application to display phylogenetic trees on personal  
705 computers. *Comput. Appl. Biosci.* 12, 357-358.

706

707 Pfaffl, M.W. (2001) A new mathematical model for relative quantification in real-time RT-  
708 PCR. *Nucleic Acid Research* 29(9), e45.

709

710 Pfaffl, M.W., Morgan, G.W., Dempfle, L., 2002. Relative expression software tool (REST)  
711 for group-wise comparison and statistical analysis of relative expression results in real-time PCR.  
712 *Nucleic Acids Res.* 30, e36.

713

714 Polozova, A., Salem, N. Jr., 2007. Role of liver and plasma lipoproteins in selective transport  
715 of n-3 fatty acids to tissues: a comparative study of <sup>14</sup>C-DHA and <sup>3</sup>H-oleic acid tracers. *J. Mol.*  
716 *Neurosci.* 33, 56-66.

717

718 Rosenlund, G., Obach, A., Sandberg, M.G., Standal, H., Tveit, K., 2001. Effect of alternative  
719 lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar* L.).  
720 *Aquacult. Res.* 32, 323-328.

721

722 Rowley, A.F., Knight, J., Lloyd-Evans, P., Holland, J.W., Vickers, P.J., 1995. Eicosanoids  
723 and their role in immune modulation in fish—a brief overview. *Fish and Shellfish Immun.* 5, 549-  
724 567.

725

726 Ruyter, B., Røsjø, C., Einen, O., Thomassen, M.S., 2000. Essential fatty acids in Atlantic  
727 salmon: effects of increasing dietary doses of n-6 and n-3 fatty acids on growth, survival and fatty  
728 acid composition of liver, blood and carcass. *Aquacult. Nutr.* 6, 119-127.

729

730 Sargent, J.R. McEvoy, L.A., Bell, J.G., 1997. Requirements, presentation and sources of  
731 polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture* 155, 117-127.

732

733 Sargent, J.R., Tacon, A.G., 1999. Development of farmed fish: a nutritionally necessary  
734 alternative to meat. *Proc. Nutr. Soc.* 58, 377-383.

735

736 Sargent, J.R., Bell, J.G., McEvoy, L.A., Tocher, D.R., Estevez, A., 1999. Recent  
737 developments in the essential fatty acid nutrition of fish. *Aquaculture* 177, 191-199.

738

739 Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The Lipids. In: Halver J.E., Hardy R.W., (Eds.),  
740 *Fish Nutrition*, 3rd edition, Academic Press, San Diego, pp. 181-257.

741

742 Simopoulos, A.P., 2002. Omega-3 fatty acids in inflammation and autoimmune diseases. *J.*  
743 *Am. Coll. Nutr.* 21, 495-505.

744

745 Stoknes, I.S., Økland, H.M.W., Falch, E., Synnes, M., 2004. Fatty acid and lipid class  
 746 composition in eyes and brain from teleosts and elasmobranchs. *Comp. Biochem. Physiol. B* 138,  
 747 183-191.

748 Subasinghe, R., Soto, D., Jia, J., 2009. Global aquaculture and its role in sustainable  
 749 development. *Rev. Aquaculture* 1, 2-9.

751 Thomassen, M.S., Rein, D., Berge, G.M., Østbye, T-K., Ruyter, B., 2012. High dietary EPA  
 752 does not inhibit  $\Delta 5$  and  $\Delta 6$  desaturases in Atlantic salmon (*Salmo salar* L.) fed rapeseed oil diets.  
 753 *Aquaculture* 360, 78-85.

755 Tobin, K.A.R., Steineger, H.H., Alberti, S., Spydevold, O., Auwerx, J., Gustafsson, J.A., Nebb,  
 756 H.I., 2002. Cross-talk between fatty acid and cholesterol metabolism mediated by liver X receptor-  
 757 alpha. *Mol. Endocrinol.* 14, 741-752.

759 Tocher, D.R., Harvie, D.G., 1988. Fatty acid compositions of the major phosphoglycerides  
 760 from fish neural tissues; (n-3) and (n-6) polyunsaturated fatty acids in rainbow trout (*Salmo*  
 761 *gairdneri*) and cod (*Gadus morhua*) brains and retinas. *Fish Physiol Biochem* 5, 229-239.

762 Tocher, D.R., Bell, J.G., Dick, J.R., Henderson, R.J., McGhee, F., Michell, D., Morris, P.C.,  
 763 2000. Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-  
 764 smolt transformation and the effects of dietary linseed and rapeseed oils. *Fish Physiol. Biochem.*  
 765 23, 59-73.

767 Tocher, D.R., Bell, J.G., McGhee, F., Dick, J.R., Fonseca-Madriral, J. 2003. Effects of dietary  
 768 lipid level and vegetable oil on fatty acid metabolism in Atlantic salmon (*Salmo salar* L.) over the  
 769 whole production cycle. *Fish Physiol. Biochem.* 29, 193-209.

771 Tocher, D.R., 2009. Issues surrounding fish as a source of omega-3 long-chain  
 772 polyunsaturated fatty acids. *Lipid Tech.* 21, 13-16.

774 Tocher, D.R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish.  
 775 *Aquacult. Res.* 41, 717-732.

777 Torstensen, B.E., Lie, Ø., Frøyland, L., 2000. Lipid metabolism and tissue composition in  
 778 Atlantic salmon (*Salmo salar* L.)—effects of capelin oil, palm oil, and oleic acid-enriched  
 779 sunflower oil as dietary lipid sources. *Lipids* 35, 653-664.

781 Tort, L., Balasch, J.C., Mackenzie, S., 2003. Fish immune system. A crossroads between  
 782 innate and adaptive responses. *Immunologia* 22, 277-286.

784 Turchini, G.M., Francis, D.S., 2009. Fatty acid metabolism (desaturation, elongation and  $\beta$ -  
 785 oxidation) in rainbow trout fed fish oil or linseed oil-based diets. *Brit. J. Nutr.* 102, 69-81.

787 Waagbø, R., 1994. The impact of nutritional factors on the immune system in Atlantic  
 788 salmon, *Salmo salar* L.: a review. *Aquacult. Res.* 25, 175-197.

790 Zheng, X., Tocher, D.R., Dickson, C.A., Bell, J.G., Teale, A.J., 2004. Effects of diets  
 791 containing vegetable oil on expression of genes involved in highly unsaturated fatty acid  
 792 biosynthesis in liver of Atlantic salmon (*Salmo salar*). *Aquaculture* 236, 467-483.

794

795 Zheng, X., Leaver, M.J., Tocher, D.R., 2009. Long-chain polyunsaturated fatty acid synthesis  
796 in fish: comparative analysis of Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus morhua*  
797 L.)  $\Delta$ 6 fatty acyl desaturase gene promoters. Comp. Biochem. Physiol. B 154, 255-263.

798

## 799 Legends

### 800 **Figure 1. Heat map of the eleven target genes analyzed based on qPCR gene data.**

801 Columns represent mean data values of the five different dietary treatments analyzed in the four  
802 tissues and rows represent single genes. Expression level of each gene was squared-root  
803 normalized in relation to a single sample, so that comparisons could be made in any sense. Means  
804 are depicted by a colour scale, green indicating low (green), neutral (black) or high (red) relative  
805 expression levels, as indicated by the colour bar on the left. *fads2d6a*, delta-6 fatty acyl desaturase  
806 isoform a; *fads2d6b*, delta-6 fatty acyl desaturase isoform b; *fads26c*, delta-6 fatty acyl desaturase  
807 isoform c; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*, fatty acyl elongase 2; *elovl5a*, fatty acyl  
808 elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform b; *elovl4*, fatty acyl elongase 4; *lxr*, liver  
809 X receptor; *srebp*, sterol regulatory element binding protein.

### 810 **Figure 2. Expression of transcription factors and LC-PUFA biosynthesis pathway genes**

811 **in Atlantic salmon liver after nine weeks of feeding.** Results are normalized expression ratios  
812 (average +SE, n = 6) of the expression of these genes in fish fed the different diets in relation to  
813 fish fed D5 diet. Diets contain either 5 g kg<sup>-1</sup> DHA (D5), 10 g kg<sup>-1</sup> DHA (D10) and DHA+EPA  
814 (D5E) or 20 g kg<sup>-1</sup> of DHA+ARA (D10A) and DHA+EPA (D10E). *fads2d6a*, delta-6 fatty acyl  
815 desaturase isoform a; *fads2d6b*, delta-6 fatty acyl desaturase isoform b; *fads26c*, delta-6 fatty acyl  
816 desaturase isoform c; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*, fatty acyl elongase 2; *elovl5a*,  
817 fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform b; *elovl4*, fatty acyl elongase  
818 4; *lxr*, liver X receptor; *srebp*, sterol regulatory element binding protein.

### 819 **Figure 3. Expression, measured by qPCR, of transcription factors and LC-PUFA**

820 **biosynthesis pathway genes in Atlantic salmon brain after nine weeks of feeding.** Results are

821 normalized expression ratios (average +SE, n = 6) of the expression of these genes in fish fed the  
822 different diets in relation to fish fed D5 diet. Diets contain either 5 g kg<sup>-1</sup> DHA (D5), 10 g kg<sup>-1</sup>  
823 DHA (D10) and DHA+EPA (D5E) or 20 g kg<sup>-1</sup> of DHA+ARA (D10A) and DHA+EPA (D10E).  
824 *fads2d6a*, delta-6 fatty acyl desaturase isoform a; *fads2d6b*, delta-6 fatty acyl desaturase isoform  
825 b; *fads26c*, delta-6 fatty acyl desaturase isoform c; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*,  
826 fatty acyl elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform  
827 b; *elovl4*, fatty acyl elongase 4; *lxr*, liver X receptor; *srebp*, sterol regulatory element binding  
828 protein.

829 **Figure 4. Expression, measured by qPCR, of transcription factors and LC-PUFA**  
830 **biosynthesis pathway genes in Atlantic salmon head kidney after nine weeks of feeding.**

831 Results are normalized expression ratios (average +SE, n = 6) of the expression of these genes in  
832 fish fed the different diets in relation to fish fed D5 diet. Diets contain either 5 g kg<sup>-1</sup> DHA (D5),  
833 10 g kg<sup>-1</sup> DHA (D10) and DHA+EPA (D5E) or 20 g kg<sup>-1</sup> of DHA+ARA (D10A) and DHA+EPA  
834 (D10E). *fads2d6a*, delta-6 fatty acyl desaturase isoform a; *fads2d6b*, delta-6 fatty acyl desaturase  
835 isoform b; *fads26c*, delta-6 fatty acyl desaturase isoform c; *fads2d5*, delta-5 fatty acyl desaturase;  
836 *elovl2*, fatty acyl elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase  
837 isoform b; *elovl4*, fatty acyl elongase 4; *lxr*, liver X receptor; *srebp*, sterol regulatory element  
838 binding protein.

839 **Figure 5. Expression, measured by qPCR, of transcription factors and LC-PUFA**  
840 **biosynthesis pathway genes in Atlantic salmon gill after nine weeks of feeding.**

841 Results are normalized expression ratios (average +SE, n = 6) of the expression of these genes in fish fed the  
842 different diets in relation to fish fed D5 diet. Diets contain either 5 g kg<sup>-1</sup> DHA (D5), 10 g kg<sup>-1</sup>  
843 DHA (D10) and DHA+EPA (D5E) or 20 g kg<sup>-1</sup> of DHA+ARA (D10A) and DHA+EPA (D10E).  
844 *fads2d6a*, delta-6 fatty acyl desaturase isoform a; *fads2d6b*, delta-6 fatty acyl desaturase isoform  
845 b; *fads26c*, delta-6 fatty acyl desaturase isoform c; *fads2d5*, delta-5 fatty acyl desaturase; *elovl2*,

846 fatty acyl elongase 2; *elovl5a*, fatty acyl elongase 5 isoform a; *elovl5b*, fatty acyl elongase isoform  
847 b; *elovl4*, fatty acyl elongase 4; *lxr*, liver X receptor; *srebp*, sterol regulatory element binding  
848 protein.

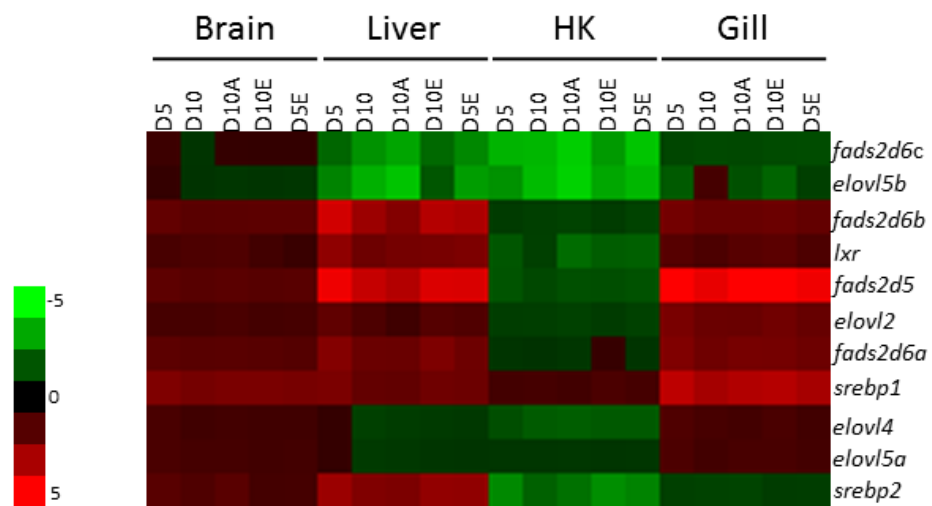


Figure 1.

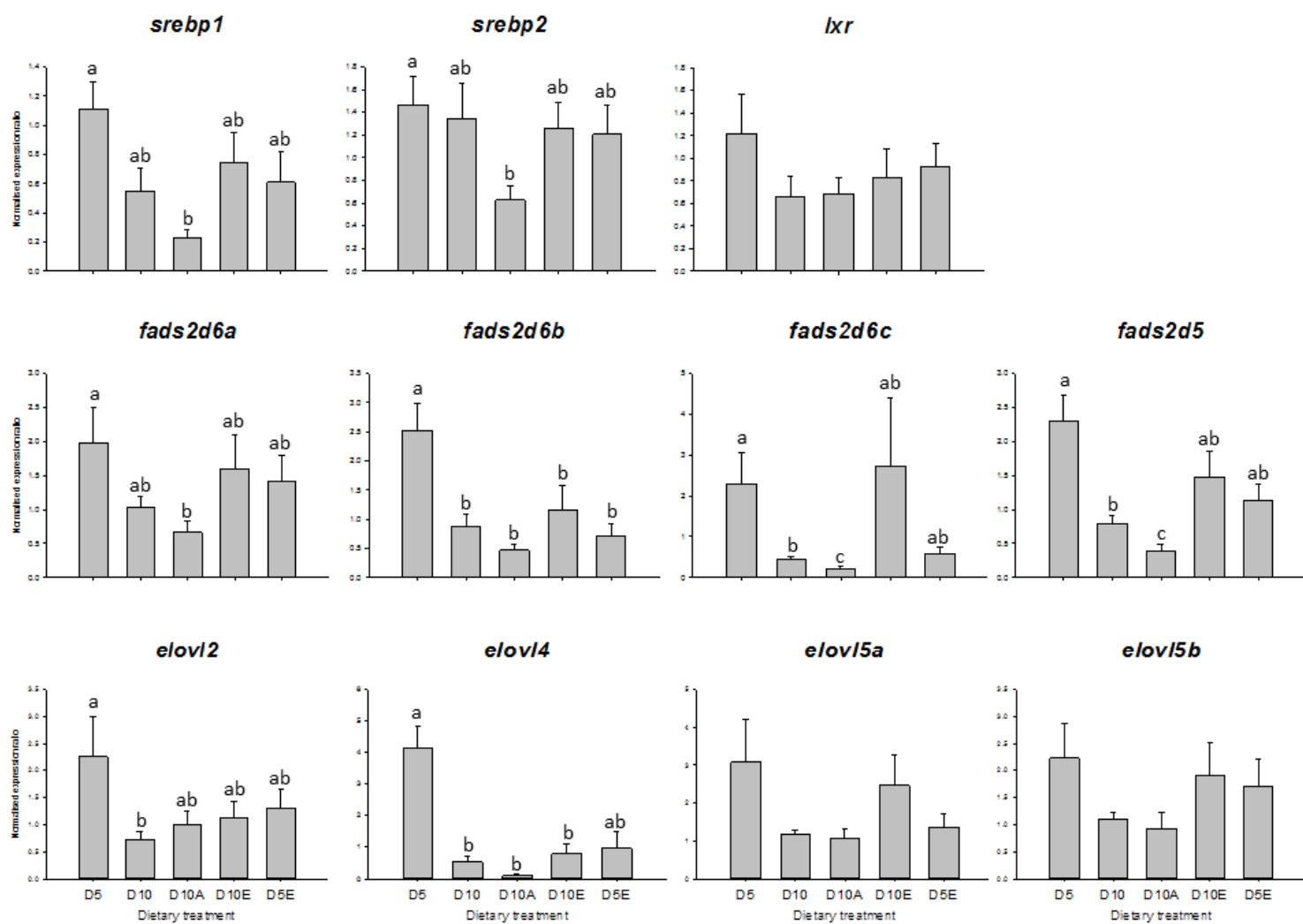


Figure 2.



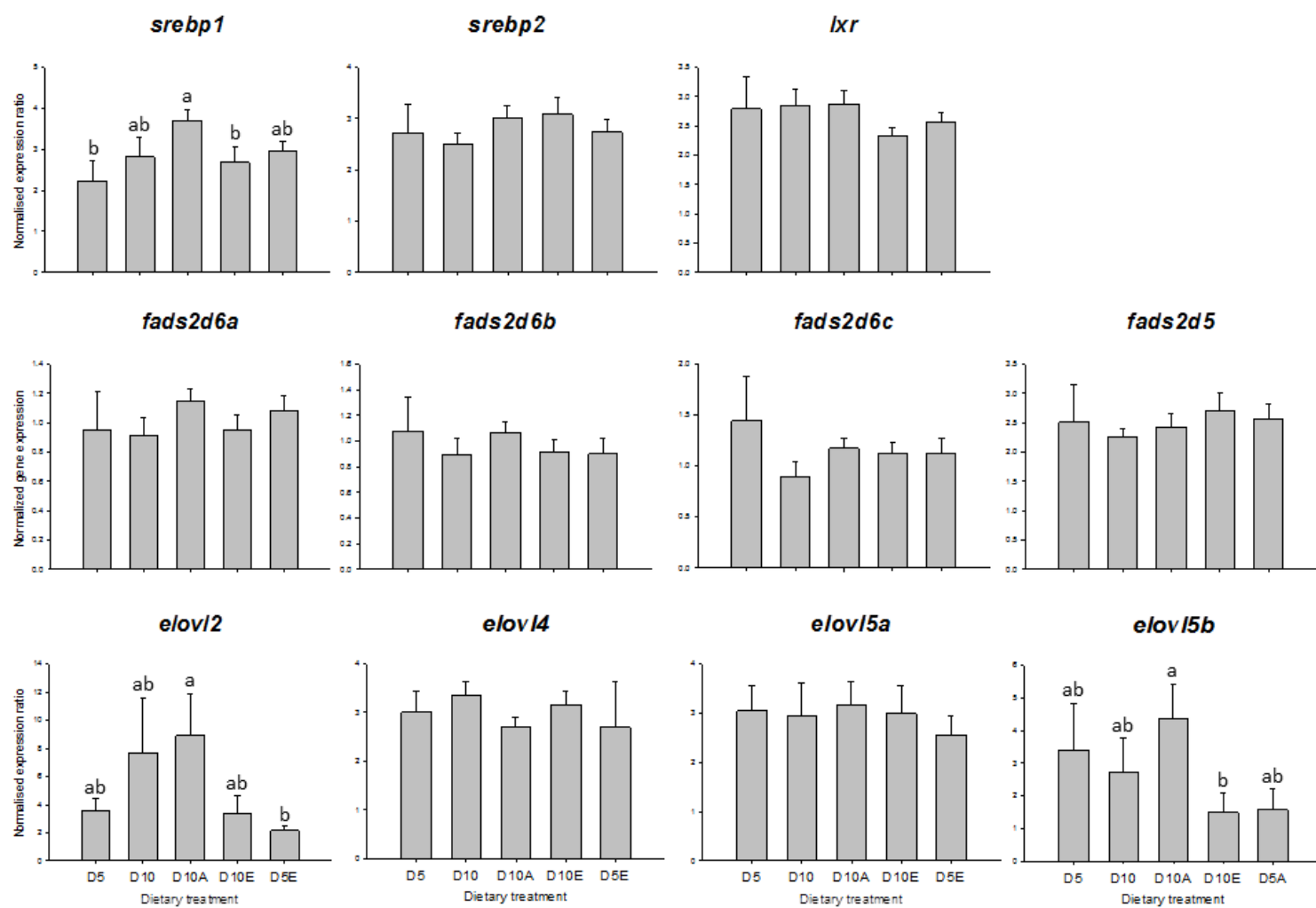


Figure 3.

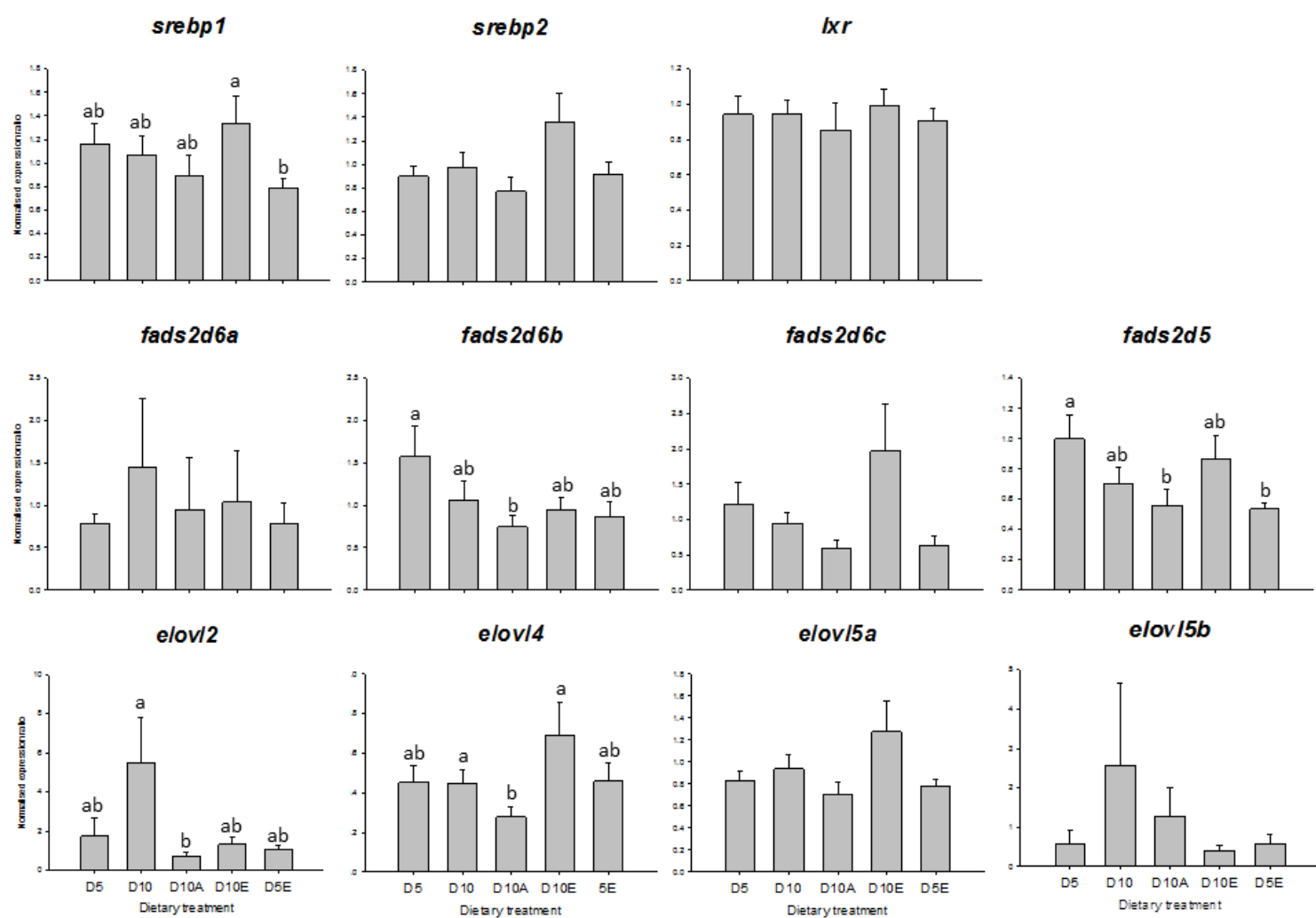


Figure 4.

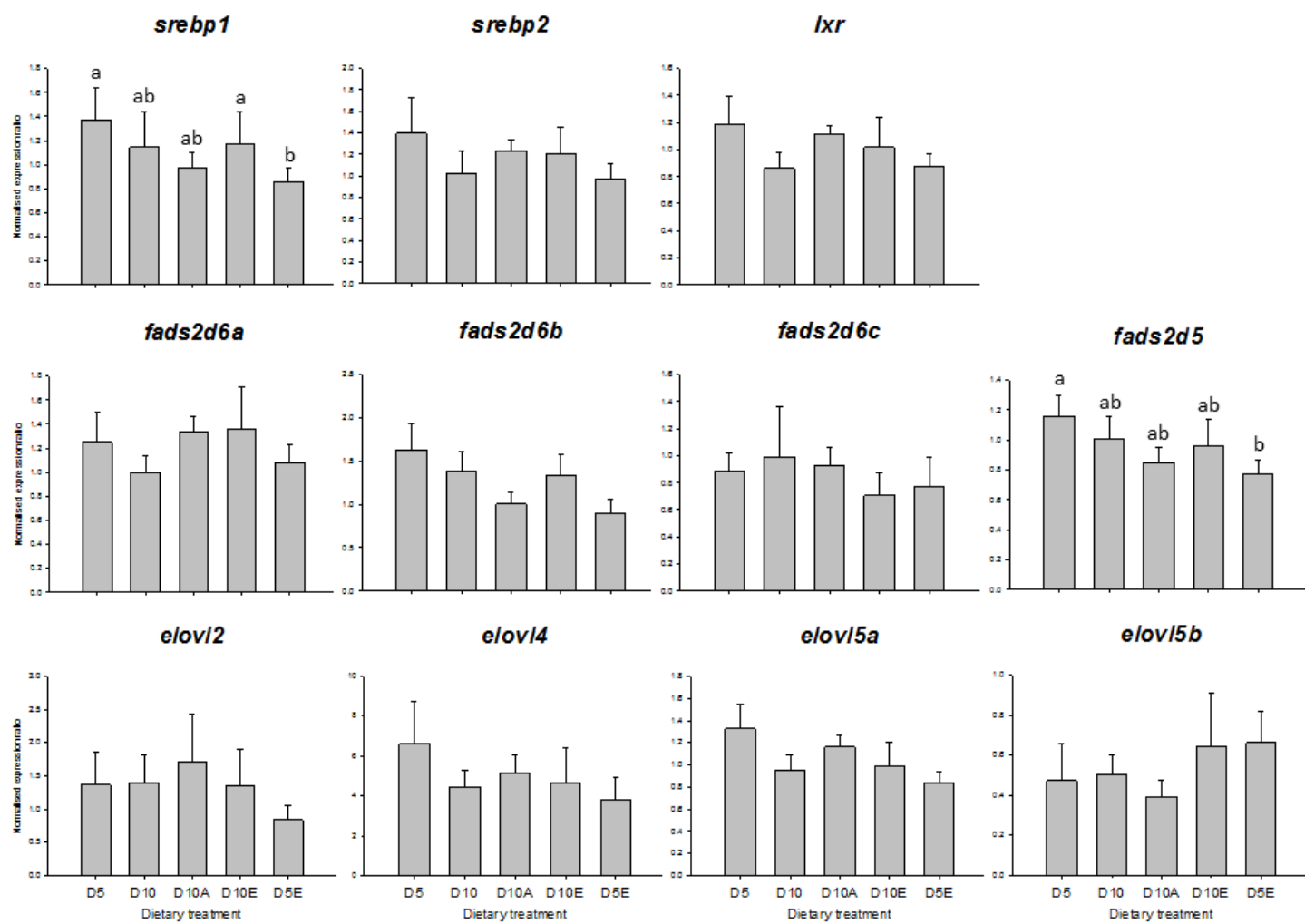


Figure 5.

Table 1. Formulations of experimental diets (all values are g kg<sup>-1</sup>).

Ingredient	D1	D5	D10	D15	D20	D10A	D10E	D5E
Defatted fish meal <sup>a</sup>	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0
Pregelised starch <sup>b</sup>	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Wheat gluten <sup>b</sup>	60.0	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Wheat flour <sup>b</sup>	155.0	155.0	155.0	155.0	155.0	155.0	155.0	155.0
Soy protein isolate <sup>c</sup>	221.0	221.0	221.0	221.0	221.0	221.0	221.0	221.0
Fish oil <sup>a</sup>	0.0	0.0	0.0	0.0	0.0	0.0	75.0	30.0
Olive oil <sup>d</sup>	92.5	88.3	82.0	77.8	71.5	68.3	55.0	77.5
DHASCO <sup>e</sup>	0.0	8.4	21.0	29.4	42.0	21.0	0.0	0.0
ARASCO <sup>e</sup>	0.0	0.0	0.0	0.0	0.0	27.5	0.0	0.0
Butter fat <sup>f</sup>	92.5	88.3	82.0	77.8	71.5	68.3	55.0	77.5
L-Histidine <sup>g</sup>	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
L-Lysine <sup>g</sup>	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-Methionine <sup>g</sup>	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
L-Threonine <sup>g</sup>	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Yttrium oxide <sup>h</sup>	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CaPO <sub>4</sub> <sup>g</sup>	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamins/minerals <sup>i</sup>	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

<sup>a</sup> Fish meal (prior to being defatted): Chilean anchovy meal and oil, Skretting Australia, Cambridge, TAS, Australia. <sup>b</sup> Wheat gluten, wheat flour and pregelatinised starch: Manildra, Auburn, NSW, Australia. <sup>c</sup> Soy protein isolate: ADM, Decatur, IL, USA. <sup>d</sup> Refined olive oil: Conga Foods, Coburg North, VIC, Australia. <sup>e</sup> DHASCO and ARASCO oils: HuaTai BioPharm Inc, Deyang, Sichuan, China. <sup>f</sup> Butterfat: Woolworths Dairies, Bella Vista, NSW, Australia. <sup>g</sup> Amino acids and monocalcium phosphate: BEC Feed Solutions, Carole Park, QLD, Australia. <sup>h</sup> Yttrium oxide: Stanford Materials, Aliso Viejo, California, United States. <sup>i</sup>\* Vitamin and mineral premix includes (IU kg<sup>-1</sup> or g kg<sup>-1</sup> of premix): Vitamin A, 2.5MIU; Vitamin D3, 0.25 MIU; Vitamin E, 16.7 g; Vitamin K<sub>3</sub>, 1.7 g; Vitamin B1, 2.5 g; Vitamin B2, 4.2 g; Vitamin B3, 25 g; Vitamin B5, 8.3; Vitamin B6, 2.0 g; Vitamin B9, 0.8; Vitamin B12, 0.005 g; Biotin, 0.17 g; Vitamin C, 75 g; Choline, 166.7 g; Inositol, 58.3 g; Ethoxyquin, 20.8 g; Copper, 2.5 g; Ferrous iron, 10.0 g; Magnesium, 16.6 g; Manganese, 15.0 g; Zinc, 25.0 g.

Table 2. Nutrient composition of experimental diets (adapted from Glencross et al. 2014).

Ingredient	D1	D5	D10	D15	D20	D10A	D10E	D5E
Dry matter (g/kg)	958	967	952	961	943	921	946	944
Protein (g/kg DM)	525	526	511	513	521	519	517	518
Fat (g/kg DM)	181	176	204	205	204	178	186	182
Carbohydrate (g/kg DM)	186	239	230	253	206	214	194	213
Ash (g/kg DM)	82	72	68	69	71	86	82	74
Gross energy (kJ/g)	22.3	22.4	23.1	22.7	22.1	22.7	22.5	23.0
Protein:Energy (g/MJ)	23.5	23.5	22.1	22.6	23.6	22.9	23.0	22.5
<i>All fatty acid data are %TFA</i>								
14:0	6.2	6.0	6.7	6.8	7.4	5.8	7.3	6.8
16:0	21.5	20.6	21.5	22.9	23.7	20.7	21.9	22.5
18:0	8.4	7.7	7.3	7.7	7.2	7.4	6.9	7.8
∑ saturated	36.7	34.4	35.9	37.3	38.7	34.9	36.6	37.4
16:1n-7	1.4	1.3	1.8	1.9	2.1	1.4	5.1	3.2
18:1n-9	49.7	48.2	44.3	42.5	39.2	38.8	35.5	43.5
18:1n-7	4.0	3.9	3.7	3.7	3.7	3.1	3.8	4.0
∑ monounsaturated	56.0	54.1	51.1	49.1	46.0	44.1	45.8	51.7
18:2n-6	5.8	6.7	5.9	5.3	4.9	6.5	5.6	6.1
20:4n-6	0.1	0.1	0.1	0.1	0.1	5.1	0.4	0.1
22:5n-6	0.0	0.6	1.3	1.5	1.6	1.3	0.0	0.0
∑ n-6	5.9	7.7	7.7	6.9	6.8	13.8	6.3	6.2
18:3n-3	0.5	0.8	0.6	0.5	0.5	0.6	0.7	0.6
20:5n-3	0.4	0.6	0.5	0.4	0.4	0.6	4.8	2.0
22:5n-3	0.0	0.1	0.2	0.0	0.0	1.9	0.6	0.0
22:6n-3	0.5	2.0	3.6	5.7	7.6	4.1	3.9	1.7
∑ n-3	1.4	3.8	5.3	6.6	8.5	7.1	11.3	4.7
∑ LC-PUFA	1.0	3.4	5.8	7.8	9.8	13.5	10.0	3.8
n-3/n-6	0.24	0.49	0.69	0.96	1.25	0.52	1.78	0.76

%TFA = percentage of total fatty acids. LC-PUFA = long chain polyunsaturated fatty acids.

Table 3. Details of PCR primers used in the present study for real-time quantitative PCR (qPCR), The data include sequences and annealing temperatures (Ta) for primer pairs, amplicon sizes and accession numbers.

Transcript	Primer sequence (5'-3')	Amplicon (bp)	Ta (°C)	Accession No.
<i>Fads2d6a</i>	F: CCCCAGACGTTTGTGTCTAG R: CCTGGATTGTTGCTTTGGAT	180	56	AY458652 <sup>a</sup>
<i>Fads2d6b</i>	F: ATAGAGGGTTTATATAGTAGGGCC R: GGTGGGACGCTAGAAAGTTAA	204	58	NM_001172281.1 <sup>a</sup>
<i>Fads2d6c</i>	F: CCCACCCCATCTTAAACT R: CTGGGGTCCAAACAAGGTTA	171	60	NM_001171780.1 <sup>a</sup>
<i>Fads2d5</i>	F: GTGAATGGGGATCCATAGCA R: AAACGAACGGACAACCAGA	192	56	AF478472 <sup>a</sup>
<i>Elovl2</i>	F: CGGGTACAAAATGTGCTGGT R: TCTGTTTGCCGATAGCCATT	145	60	TC91192 <sup>b</sup>
<i>Elovl5a</i>	F: ACAAGACAGGAATCTCTTTCAGATTAA R: TCTGGGGTTACTGTGCTATAGTGATC	137	60	AY170327 <sup>a</sup>
<i>Elovl5b</i>	F: ACAAAAAGCCATGTTTATCTGAAAGA R: AAGTGGGTCTCTCTGGGGCTGTG	141	60	DW546112 <sup>a</sup>
<i>Elovl4</i>	F: TTGTCAAATTGGTCCTGTGC R: TTAAGGCCCTTTGGGATGA	191	61	HM208347 <sup>a</sup>
<i>Srebp1</i>	F: GCCATGCGCAGGTTGTTTCTTCA R: TCTGGCCAGGACGCATCTCACACT	151	63	TC148424 <sup>b</sup>
<i>Srebp2</i>	F: GACAGGCACAACACAAGGTG R: CAGCAGGGGTAAGGGTAGGT	215	60	DY733476 <sup>a</sup>
<i>Lxr</i>	F: GCCGCCGCTATCTGAAATCTG R: CAATCCGGCAACCAATCTGTAGG	210	58	FJ470290 <sup>a</sup>
<i>Cofilin-2</i>	F: AGCCTATGACCAACCCACTG R: TGTTACAGCTCGTTTACCG	224	60	TC63899 <sup>b</sup>
<i>elf-1α</i>	F: CTGCCCCTCCAGGACGTTTACAA R: CACCGGGCATAGCCGATTCC	175	60	AF321836 <sup>a</sup>

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

<sup>b</sup>Atlantic salmon Gene Index (<http://compbio.dfci.harvard.edu/tgi/>)

Table 4. Fatty acid compositions (percentage of total fatty acids) of liver and head kidney polar lipids of Atlantic salmon post-smolts fed diets containing increasing levels of DHA.

Fatty acid	D1	D5	D10	D15	D20	R <sup>2</sup>	P-value
<b>Liver</b>							
Lipids % (wet wt.)	4.9 ± 1.2	4.9 ± 1.2	5.1 ± 0.9	5.1 ± 0.4	5.0 ± 0.5	0.001	0.761
∑ saturated	26.8 ± 0.8	28.7 ± 0.9	28.2 ± 0.8	28.1 ± 0.4	26.7 ± 0.2	0.019	0.625
∑ MUFA	28.7 ± 0.9	24.5 ± 1.9	23.2 ± 1.5	22.7 ± 1.3	20.5 ± 0.5	0.780	0.000
18:2n-6	3.8 ± 0.2	3.6 ± 0.2	3.2 ± 0.6	3.0 ± 0.2	2.7 ± 0.1	0.728	0.000
20:2n-6 <sup>1</sup>	1.4 ± 0.1	1.1 ± 0.2	1.0 ± 0.0	0.9 ± 0.2	0.8 ± 0.0	0.708	0.000
20:3n-6	4.5 ± 0.6	3.1 ± 0.9	2.3 ± 0.6	1.5 ± 0.4	1.1 ± 0.1	0.869	0.000
20:4n-6	5.2 ± 0.7	4.1 ± 0.4	4.0 ± 0.3	4.2 ± 0.6	4.8 ± 0.4	0.037	0.495
22:4n-6	0.4 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.179	0.116
22:5n-6	1.1 ± 0.2	3.7 ± 0.6	5.1 ± 1.8	6.7 ± 1.1	7.9 ± 0.4	0.848	0.000
∑ n-6 PUFA <sup>2</sup>	16.6 ± 0.3	16.0 ± 1.9	15.9 ± 1.9	16.6 ± 1.7	17.5 ± 0.5	0.057	0.393
18:3n-3	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.061	0.374
20:5n-3	3.1 ± 0.1	3.0 ± 1.5	3.1 ± 1.4	2.2 ± 0.9	1.7 ± 0.2	0.304	0.033
22:5n-3	1.5 ± 0.2	1.0 ± 0.4	0.9 ± 0.4	0.7 ± 0.3	0.5 ± 0.0	0.601	0.001
22:6n-3	22.3 ± 0.7	26.2 ± 2.7	28.0 ± 2.6	29.4 ± 1.3	32.7 ± 0.5	0.816	0.000
∑ n-3 PUFA <sup>3</sup>	27.3 ± 0.6	30.4 ± 4.7	32.4 ± 4.1	32.6 ± 2.5	35.2 ± 0.7	0.502	0.003
DMA	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
∑ n-3 LC-PUFA <sup>4</sup>	27.0 ± 0.6	30.3 ± 4.6	32.2 ± 4.0	32.4 ± 2.5	35.0 ± 0.6	0.514	0.003
EPA/ARA	0.6 ± 0.1	0.7 ± 0.4	0.8 ± 0.4	0.5 ± 0.2	0.4 ± 0.1	0.164	0.135
<b>Head kidney</b>							
Lipids % (wet wt.)	5.3 ± 2.8	4.3 ± 1.2	3.7 ± 0.9	3.8 ± 0.7	3.4 ± 1.1	0.182	0.105
∑ saturated	30.4 ± 0.2	29.8 ± 0.6	30.5 ± 0.3	30.8 ± 0.7	30.6 ± 0.6	0.123	0.199
∑ MUFA	26.5 ± 0.5	24.8 ± 0.6	24.7 ± 0.8	22.4 ± 0.4	21.0 ± 1.1	0.868	0.000
18:2n-6	3.6 ± 0.2	3.2 ± 0.2	2.9 ± 0.1	2.5 ± 0.1	2.4 ± 0.0	0.883	0.000
20:2n-6 <sup>1</sup>	1.0 ± 0.0	0.8 ± 0.1	0.7 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.935	0.000
20:3n-6	2.7 ± 0.2	2.0 ± 0.3	1.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.0	0.940	0.000
20:4n-6	5.3 ± 0.1	4.2 ± 0.4	4.4 ± 0.1	5.0 ± 0.3	5.1 ± 0.7	0.009	0.741
22:4n-6	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.576	0.001
22:5n-6	0.9 ± 0.1	3.3 ± 0.3	5.4 ± 0.1	6.1 ± 0.1	7.2 ± 0.1	0.885	0.000
∑ n-6 PUFA <sup>2</sup>	13.8 ± 0.5	13.6 ± 0.5	14.7 ± 0.0	15.1 ± 0.3	16.0 ± 0.6	0.774	0.000
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.049	0.427
20:5n-3	3.6 ± 0.2	3.5 ± 0.5	2.2 ± 0.3	2.4 ± 0.3	2.1 ± 0.4	0.641	0.000
22:5n-3	0.9 ± 0.0	0.7 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.879	0.000
22:6n-3	21.5 ± 0.5	24.4 ± 0.6	24.5 ± 0.5	25.8 ± 0.8	27.1 ± 1.0	0.830	0.000
∑ n-3 PUFA <sup>3</sup>	26.4 ± 0.7	29.1 ± 1.0	27.4 ± 0.7	29.0 ± 1.1	29.9 ± 1.3	0.429	0.008
DMA	2.7 ± 0.1	2.5 ± 0.4	2.5 ± 0.1	2.6 ± 0.4	2.5 ± 0.3	0.024	0.578
∑ n-3 LC-PUFA <sup>4</sup>	26.1 ± 0.7	28.9 ± 1.0	27.2 ± 0.6	28.8 ± 1.1	29.7 ± 1.3	0.444	0.007
EPA/ARA	0.7 ± 0.0	0.8 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.633	0.000

Data expressed as means ± S.D. (*n* = 3). Diets D1-D20 represent feeds with increasing levels of DHA as described in the Materials and Methods section. Statistical differences were determined by regression analysis (*P* < 0.05). DHA, docosahexaenoic acid; DMA, dimethyl acetal; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids. <sup>1</sup> Includes trace amounts of 20:3n-9; <sup>2</sup> Totals include 18:3n-6; <sup>3</sup> Totals include 18:4n-3, 20:3n-3 and 20:4n-3; <sup>4</sup> Totals include 20:3n-3 and 20:4n-3.

Table 5. Fatty acid compositions (percentage of total fatty acids) of liver and head kidney polar lipids of Atlantic salmon post-smolts fed diets containing various combinations of DHA, ARA and EPA.

Fatty acid	10 g kg <sup>-1</sup> diets		20 g kg <sup>-1</sup> diets		
	D10	D5E	D20	D10A	D10E
<b>Liver</b>					
Lipids % (wet wt.)	5.1 ± 0.9	4.9 ± 0.8	5.0 ± 0.5	4.4 ± 1.0	4.1 ± 0.0
∑ saturated	28.2 ± 0.8	26.7 ± 1.6	26.7 ± 0.2	27.9 ± 1.4	28.0 ± 1.1
∑MUFA	23.2 ± 1.5	25.7 ± 0.7	20.5 ± 0.5	20.1 ± 0.9	21.3 ± 1.4
18:2n-6	3.2 ± 0.6	3.2 ± 0.1	2.7 ± 0.1	1.9 ± 0.6	2.4 ± 0.1
20:2n-6 <sup>1</sup>	1.0 ± 0.0	1.1 ± 0.1	0.8 ± 0.0 <sup>a</sup>	0.6 ± 0.1 <sup>b</sup>	0.7 ± 0.1 <sup>ab</sup>
20:3n-6	2.3 ± 0.6	2.8 ± 0.7	1.1 ± 0.1	1.4 ± 0.1	1.3 ± 0.3
20:4n-6	4.0 ± 0.3	3.3 ± 0.6	4.8 ± 0.4 <sup>b</sup>	13.8 ± 0.6 <sup>a</sup>	2.9 ± 0.1 <sup>c</sup>
22:4n-6	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0 <sup>b</sup>	1.5 ± 0.2 <sup>a</sup>	0.1 ± 0.0 <sup>c</sup>
22:5n-6	5.1 ± 1.8	0.6 ± 0.0*	7.9 ± 0.4 <sup>a</sup>	6.9 ± 0.7 <sup>a</sup>	0.5 ± 0.1 <sup>b</sup>
∑ n-6 PUFA <sup>2</sup>	15.9 ± 1.9	11.2 ± 0.3*	17.5 ± 0.5 <sup>b</sup>	26.1 ± 1.0 <sup>a</sup>	8.0 ± 0.3 <sup>c</sup>
18:3n-3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
20:5n-3	3.1 ± 1.4	4.7 ± 0.6	1.7 ± 0.2 <sup>b</sup>	1.1 ± 0.2 <sup>b</sup>	6.6 ± 0.8 <sup>a</sup>
22:5n-3	0.9 ± 0.4	1.5 ± 0.1	0.5 ± 0.0 <sup>b</sup>	0.7 ± 0.1 <sup>b</sup>	2.1 ± 0.2 <sup>a</sup>
22:6n-3	28.0 ± 2.6	29.6 ± 2.0	32.7 ± 0.5 <sup>a</sup>	23.9 ± 0.9 <sup>b</sup>	33.4 ± 0.4 <sup>a</sup>
∑ n-3 PUFA <sup>3</sup>	32.4 ± 4.1	36.2 ± 2.6	35.2 ± 0.7 <sup>b</sup>	25.8 ± 0.9 <sup>c</sup>	42.6 ± 1.0 <sup>a</sup>
∑ DMA	n.d.	n.d.	n.d.	n.d.	n.d.
∑ n-3 LC-PUFA <sup>4</sup>	32.2 ± 4.0	36.0 ± 2.5	35.0 ± 0.6 <sup>b</sup>	25.7 ± 0.9 <sup>c</sup>	42.3 ± 1.0 <sup>a</sup>
EPA/ARA	0.8 ± 0.4	1.5 ± 0.1	0.4 ± 0.1 <sup>b</sup>	0.1 ± 0.0 <sup>c</sup>	2.3 ± 0.2 <sup>a</sup>
<b>Head kidney</b>					
Lipids % (wet wt.)	3.7 ± 0.9	4.2 ± 0.7	3.4 ± 1.1	3.7 ± 0.1	5.1 ± 0.2
∑ saturated	30.5 ± 0.3	31.5 ± 0.2*	30.6 ± 0.6	31.5 ± 1.3	32.3 ± 0.5
∑MUFA	24.7 ± 0.8	25.6 ± 0.5	21.0 ± 1.1	22.1 ± 0.9	22.4 ± 1.3
18:2n-6	2.9 ± 0.1	2.8 ± 0.2	2.4 ± 0.0 <sup>a</sup>	2.3 ± 0.0 <sup>b</sup>	2.2 ± 0.0 <sup>c</sup>
20:2n-6 <sup>1</sup>	0.7 ± 0.0	0.6 ± 0.0*	0.5 ± 0.0 <sup>a</sup>	0.3 ± 0.0 <sup>b</sup>	0.4 ± 0.0 <sup>b</sup>
20:3n-6	1.2 ± 0.1	1.6 ± 0.1*	0.7 ± 0.0	0.9 ± 0.1	0.7 ± 0.1
20:4n-6	4.4 ± 0.1	3.4 ± 0.3*	5.1 ± 0.7 <sup>b</sup>	14.6 ± 0.5 <sup>a</sup>	3.7 ± 0.3 <sup>c</sup>
22:4n-6	0.1 ± 0.0	0.1 ± 0.0*	0.1 ± 0.0 <sup>b</sup>	0.8 ± 0.1 <sup>a</sup>	0.1 ± 0.0 <sup>b</sup>
22:5n-6	5.4 ± 0.1	0.4 ± 0.0*	7.2 ± 0.1 <sup>a</sup>	4.0 ± 0.3 <sup>b</sup>	0.4 ± 0.0 <sup>c</sup>
∑ n-6 PUFA <sup>2</sup>	14.7 ± 0.0	8.9 ± 0.1*	16.0 ± 0.6 <sup>b</sup>	22.9 ± 1.1 <sup>a</sup>	7.5 ± 0.1 <sup>c</sup>
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:5n-3	2.2 ± 0.3	5.3 ± 0.5*	2.1 ± 0.4 <sup>b</sup>	1.1 ± 0.2 <sup>b</sup>	6.9 ± 1.8 <sup>a</sup>
22:5n-3	0.5 ± 0.0	1.3 ± 0.2*	0.4 ± 0.0 <sup>b</sup>	0.5 ± 0.0 <sup>b</sup>	1.5 ± 0.1 <sup>a</sup>
22:6n-3	24.5 ± 0.5	24.0 ± 0.7	27.1 ± 1.0 <sup>a</sup>	19.1 ± 1.0 <sup>b</sup>	26.3 ± 0.6 <sup>a</sup>
∑ n-3 PUFA <sup>3</sup>	27.4 ± 0.7	31.1 ± 0.4*	29.9 ± 1.3 <sup>b</sup>	20.9 ± 1.2 <sup>c</sup>	35.1 ± 2.1 <sup>a</sup>
∑ DMA	2.5 ± 0.1	2.7 ± 0.1	2.5 ± 0.3	2.7 ± 0.1	2.6 ± 0.4
∑ n-3 LC-PUFA <sup>4</sup>	27.2 ± 0.6	30.9 ± 0.5*	29.7 ± 1.3 <sup>b</sup>	20.7 ± 1.2 <sup>c</sup>	35.0 ± 2.1 <sup>a</sup>
EPA/ARA	0.5 ± 0.1	1.6 ± 0.1*	0.4 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>c</sup>	1.8 ± 0.3 <sup>a</sup>

Data expressed as means ± S.D. ( $n = 3$ ). Diets represent feeds containing 10 g kg<sup>-1</sup> DHA (D10) or DHA+EPA (D5E), and feeds containing 20 g kg<sup>-1</sup> DHA (D20), DHA+ARA (D10A) or DHA+EPA (D10E). Asterisks denote statistical differences between the 10 g kg<sup>-1</sup> diets as determined by one-way ANOVA ( $P < 0.05$ ). Different superscript letters within a row represent significant differences between the 20 g kg<sup>-1</sup> diets as determined by one-way ANOVA with Tukey's comparison test ( $P < 0.05$ ). DHA, docosahexaenoic acid; DMA, dimethyl acetate; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids. <sup>1</sup> Includes trace amounts of 20:3n-9; <sup>2</sup> Totals include 18:3n-6; <sup>3</sup> Totals include 18:4n-3, 20:3n-3 and 20:4n-3; <sup>4</sup> Totals include 20:3n-3 and 20:4n-3.



Table 6. Fatty acid compositions (percentage of total fatty acids) of head kidney polar lipids of Atlantic salmon post-smolts fed diets containing increasing levels of DHA.

Fatty acid	D1	D5	D10	D15	D20	R <sup>2</sup>	P-value
<b>Brain</b>							
Lipids % (wet wt.)	7.5 ± 0.4	7.6 ± 0.7	7.4 ± 0.7	7.4 ± 0.3	7.0 ± 0.2	0.15	0.149
∑ saturated	23.6 ± 0.6	24.5 ± 0.6	24.1 ± 0.6	24.9 ± 0.4	25.0 ± 0.4	0.457	0.006
∑ MUFA	40.8 ± 0.8	39.1 ± 0.9	40.1 ± 1.5	38.8 ± 0.7	38.5 ± 1.1	0.329	0.025
18:2n-6	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.737	0.000
20:2n-6 <sup>1</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
20:3n-6	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
20:4n-6	1.3 ± 0.1	1.3 ± 0.0	1.3 ± 0.1	1.4 ± 0.0	1.4 ± 0.0	0.494	0.003
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.054	0.405
22:5n-6	0.1 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.836	0.000
∑ n-6 PUFA <sup>2</sup>	2.3 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	0.782	0.000
18:3n-3	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
20:5n-3	4.4 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.0 ± 0.0	4.0 ± 0.0	0.839	0.000
22:5n-3	1.8 ± 0.2	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.0	1.6 ± 0.0	0.412	0.010
22:6n-3	19.0 ± 0.6	20.3 ± 0.6	19.7 ± 1.0	20.3 ± 0.7	20.7 ± 0.7	0.304	0.033
∑ n-3 PUFA <sup>3</sup>	25.3 ± 0.5	26.4 ± 0.6	25.6 ± 1.0	26.1 ± 0.6	26.4 ± 0.8	0.127	0.192
DMA	7.8 ± 0.3	7.4 ± 0.3	7.4 ± 0.2	7.3 ± 0.3	7.2 ± 0.2	0.411	0.010
∑ n-3 LC-PUFA <sup>4</sup>	25.3 ± 0.5	26.4 ± 0.6	25.6 ± 1.0	26.1 ± 0.6	26.4 ± 0.8	0.127	0.192
EPA/ARA	3.4 ± 0.2	3.3 ± 0.1	3.1 ± 0.2	2.9 ± 0.1	2.8 ± 0.0	0.736	0.000
<b>Gill</b>							
Lipids % (wet wt.)	1.6 ± 0.4	2.2 ± 0.5	1.5 ± 0.1	1.6 ± 0.3	1.8 ± 0.5	0.001	0.787
∑ saturated	31.6 ± 0.3	31.6 ± 0.2	32.6 ± 2.1	33.4 ± 0.6	32.4 ± 0.2	0.195	0.100
∑ MUFA	29.0 ± 0.6	28.2 ± 1.3	28.7 ± 1.4	27.2 ± 1.3	25.7 ± 0.4	0.535	0.002
18:2n-6	3.0 ± 0.1	2.5 ± 0.3	2.3 ± 0.1	2.1 ± 0.2	2.0 ± 0.1	0.818	0.000
20:2n-6 <sup>1</sup>	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.789	0.000
20:3n-6	2.2 ± 0.1	1.5 ± 0.2	1.0 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.934	0.000
20:4n-6	5.8 ± 0.6	4.6 ± 0.1	4.9 ± 0.3	5.0 ± 0.2	5.2 ± 0.2	0.034	0.509
22:4n-6	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.043	0.458
22:5n-6	0.9 ± 0.1	3.1 ± 0.3	4.7 ± 0.5	5.3 ± 0.2	6.2 ± 0.2	0.863	0.000
∑ n-6 PUFA <sup>2</sup>	13.1 ± 0.8	12.7 ± 0.7	13.8 ± 0.8	13.9 ± 0.5	14.7 ± 0.2	0.530	0.002
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.001	0.907
20:5n-3	2.3 ± 0.3	2.0 ± 0.2	1.6 ± 0.3	1.6 ± 0.2	1.7 ± 0.1	0.521	0.002
22:5n-3	0.7 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.797	0.000
22:6n-3	18.8 ± 0.3	20.6 ± 1.3	19.0 ± 2.2	19.9 ± 0.8	21.8 ± 0.5	0.251	0.057
∑ n-3 PUFA <sup>3</sup>	22.1 ± 0.5	23.4 ± 1.3	21.2 ± 2.6	22.0 ± 0.6	23.9 ± 0.4	0.047	0.440
DMA	4.0 ± 0.2	4.1 ± 0.3	3.6 ± 0.1	3.4 ± 0.2	3.2 ± 0.1	0.711	0.000
∑ n-3 LC-PUFA <sup>4</sup>	21.9 ± 0.5	23.2 ± 1.3	21.0 ± 2.5	21.9 ± 0.6	23.8 ± 0.4	0.054	0.403
EPA/ARA	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.438	0.007

Data expressed as means ± S.D. ( $n = 3$ ). Diets D1-D20 represent feeds with increasing levels of DHA as described in the Materials and Methods section. Statistical differences were determined by regression analysis ( $P < 0.05$ ). DHA, docosahexaenoic acid; DMA, dimethyl acetal; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids.<sup>1</sup> Includes trace amounts of 20:3n-9; <sup>2</sup> Totals include 18:3n-6; <sup>3</sup> Totals include 18:4n-3, 20:3n-3 and 20:4n-3; <sup>4</sup> Totals include 20:3n-3 and 20:4n-3.

Table 7. Fatty acid compositions (percentage of total fatty acids) of brain and gill polar lipids of Atlantic salmon post-smolts fed diets containing various combinations of DHA, ARA and EPA.

Fatty acid	10 g kg <sup>-1</sup> diets		20 g kg <sup>-1</sup> diets		
	D10	D5E	D20	D10A	D10E
<b>Brain</b>					
Lipids % (wet wt.)	7.4 ± 0.7 <sup>b</sup>	8.3 ± 0.6 <sup>a</sup>	7.0 ± 0.2	7.8 ± 0.2	8.5 ± 1.5
∑ saturated	24.1 ± 0.6	23.9 ± 0.8	25.0 ± 0.4 <sup>a</sup>	23.9 ± 0.1 <sup>b</sup>	24.9 ± 0.2 <sup>a</sup>
∑ MUFA	40.1 ± 1.5	40.5 ± 1.4	38.5 ± 1.1	40.2 ± 0.5	39 ± 0.7
18:2n-6	0.2 ± 0.0	0.5 ± 0.4	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:2n-6 <sup>1</sup>	n.d.	n.d.	n.d.	n.d.	n.d.
20:3n-6	n.d.	n.d.	n.d.	n.d.	n.d.
20:4n-6	1.3 ± 0.1	1.0 ± 0.1*	1.4 ± 0.0 <sup>b</sup>	2.6 ± 0.2 <sup>a</sup>	1.0 ± 0.0 <sup>c</sup>
22:4n-6	0.1 ± 0.0	0.1 ± 0.0*	0.1 ± 0.0 <sup>b</sup>	0.2 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>c</sup>
22:5n-6	0.7 ± 0.0	0.1 ± 0.0*	0.8 ± 0.0 <sup>a</sup>	0.6 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>b</sup>
∑ n-6 PUFA <sup>2</sup>	2.6 ± 0.1	2.0 ± 0.3*	2.8 ± 0.1 <sup>b</sup>	3.8 ± 0.2 <sup>a</sup>	1.5 ± 0.1 <sup>c</sup>
18:3n-3	n.d.	n.d.	n.d.	n.d.	n.d.
20:5n-3	4.1 ± 0.1	4.6 ± 0.2*	4.0 ± 0.0 <sup>b</sup>	3.8 ± 0.0 <sup>c</sup>	4.8 ± 0.1 <sup>a</sup>
22:5n-3	1.6 ± 0.1	1.9 ± 0.0*	1.6 ± 0.0 <sup>b</sup>	1.5 ± 0.0 <sup>b</sup>	2.0 ± 0.1 <sup>a</sup>
22:6n-3	19.7 ± 1.0	18.9 ± 1.2	20.7 ± 0.7 <sup>a</sup>	18.7 ± 0.2 <sup>b</sup>	20.0 ± 0.3 <sup>a</sup>
∑ n-3 PUFA <sup>3</sup>	25.6 ± 1.0	25.7 ± 1.3	26.4 ± 0.8 <sup>a</sup>	24.2 ± 0.1 <sup>b</sup>	27.0 ± 0.6 <sup>a</sup>
∑ DMA	7.4 ± 0.2	7.9 ± 0.8	7.2 ± 0.2 <sup>b</sup>	7.8 ± 0.1 <sup>a</sup>	7.5 ± 0.2 <sup>ab</sup>
∑ n-3 LC-PUFA <sup>4</sup>	25.6 ± 1.0	25.7 ± 1.3	26.4 ± 0.8 <sup>a</sup>	24.2 ± 0.1 <sup>b</sup>	27.0 ± 0.6 <sup>a</sup>
EPA/ARA	3.1 ± 0.2	4.6 ± 0.2*	2.8 ± 0.0 <sup>b</sup>	1.5 ± 0.1 <sup>c</sup>	5.0 ± 0.1 <sup>a</sup>
<b>Gill</b>					
Lipids % (wet wt.)	1.5 ± 0.1	1.5 ± 0.0	1.8 ± 0.5	1.5 ± 0.1	1.6 ± 0.1
∑ saturated	32.6 ± 2.1	31.8 ± 0.9	32.4 ± 0.2	32.8 ± 1.1	34.0 ± 1.7
∑ MUFA	28.7 ± 1.4	28.9 ± 0.8	25.7 ± 0.4	25.2 ± 0.3	26.9 ± 1.4
18:2n-6	2.3 ± 0.1	2.5 ± 0.1*	2.0 ± 0.1 <sup>a</sup>	1.7 ± 0.1 <sup>b</sup>	1.9 ± 0.1 <sup>ab</sup>
20:2n-6 <sup>1</sup>	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0 <sup>a</sup>	0.4 ± 0.0 <sup>b</sup>	0.4 ± 0.0 <sup>b</sup>
20:3n-6	1.0 ± 0.0	1.4 ± 0.1*	0.6 ± 0.0	0.6 ± 0.1	0.7 ± 0.1
20:4n-6	4.9 ± 0.3	4.2 ± 0.1*	5.2 ± 0.2 <sup>b</sup>	11.6 ± 1.0 <sup>a</sup>	4.1 ± 0.3 <sup>c</sup>
22:4n-6	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0 <sup>b</sup>	1.5 ± 0.2 <sup>a</sup>	0.3 ± 0.0 <sup>b</sup>
22:5n-6	4.7 ± 0.5	0.5 ± 0.0*	6.2 ± 0.2 <sup>a</sup>	3.8 ± 0.1 <sup>b</sup>	0.5 ± 0.0 <sup>c</sup>
∑ n-6 PUFA <sup>2</sup>	13.8 ± 0.8	9.5 ± 0.1*	14.7 ± 0.2 <sup>b</sup>	19.6 ± 1.4 <sup>a</sup>	7.8 ± 0.3 <sup>c</sup>
18:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:5n-3	1.6 ± 0.3	4.0 ± 0.6*	1.7 ± 0.1 <sup>b</sup>	1.0 ± 0.0 <sup>c</sup>	4.7 ± 0.2 <sup>a</sup>
22:5n-3	0.4 ± 0.1	1.1 ± 0.2*	0.4 ± 0.0 <sup>b</sup>	0.4 ± 0.0 <sup>b</sup>	1.2 ± 0.1 <sup>a</sup>
22:6n-3	19.0 ± 2.2	20.9 ± 1.1	21.8 ± 0.5 <sup>a</sup>	17.1 ± 0.5 <sup>b</sup>	21.7 ± 2.6 <sup>a</sup>
∑ n-3 PUFA <sup>3</sup>	21.2 ± 2.6	26.2 ± 0.4*	23.9 ± 0.4 <sup>a</sup>	18.6 ± 0.5 <sup>b</sup>	27.9 ± 2.7 <sup>a</sup>
∑ DMA	3.6 ± 0.1	3.5 ± 0.3	3.2 ± 0.1 <sup>b</sup>	3.8 ± 0.1 <sup>a</sup>	3.4 ± 0.1 <sup>b</sup>
∑ n-3 LC-PUFA <sup>4</sup>	21.0 ± 2.5	26.0 ± 0.4*	23.8 ± 0.4 <sup>a</sup>	18.4 ± 0.5 <sup>b</sup>	27.7 ± 2.7 <sup>a</sup>
EPA/ARA	0.3 ± 0.1	0.9 ± 0.1*	0.3 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>c</sup>	1.1 ± 0.1 <sup>a</sup>

Data expressed as means ± S.D. (*n* = 3). Diets represent feeds containing 10 g kg<sup>-1</sup> DHA (D10) or DHA+EPA (D5E), and feeds containing 20 g kg<sup>-1</sup> DHA (D20), DHA+ARA (D10A) or DHA+EPA (D10E). Asterisks denote statistical differences between the 10 g kg<sup>-1</sup> diets as determined by one-way ANOVA (*P* < 0.05). Different superscript letters within a row represent significant differences between the 20 g kg<sup>-1</sup> diets as determined by one-way ANOVA with Tukey's comparison test (*P* < 0.05). DHA, docosahexaenoic acid; DMA, dimethyl acetal; EPA, eicosapentaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids.<sup>1</sup> Includes trace amounts of 20:3n-9; <sup>2</sup> Totals include 18:3n-6; <sup>3</sup> Totals include 18:4n-3, 20:3n-3 and 20:4n-3; <sup>4</sup> Totals include 20:3n-3 and 20:4n-3.