Up-regulated Desaturase and Elongase Gene Expression Promoted Accumulation of Polyunsaturated Fatty Acid (PUFA) but Not Long-Chain PUFA in *Lates calcarifer*, a Tropical Euryhaline Fish, Fed a Stearidonic Acid- and γ-Linoleic Acid-Enriched Diet

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ABSTRACT: The limited activity of Δ6 fatty acid desaturase (FAD6) on α-linolenic (ALA, 18:3n-3) and linoleic (LA, 18:2n-6) acids in marine fish alters the long-chain ($\geq C_{20}$) polyunsaturated fatty acid (LC-PUFA) concentration in fish muscle and liver when vegetable oils replace fish oil (FO) in aquafeeds. Echium oil (EO), rich in stearidonic acid (SDA, 18:4n-3) and γ-linoleic acid (GLA, 18:3n-6), may enhance the biosynthesis of n-3 and n-6 LC-PUFA by bypassing the rate-limiting FAD6 step. Nutritional and environmental modulation of the mechanisms in LC-PUFA biosynthesis was examined in barramundi, *Lates calcarifer*, a tropical euryhaline fish. Juveniles were maintained in either freshwater or seawater and fed different dietary LC-PUFA precursors present in EO or rapeseed oil (RO) and compared with FO. After 8 weeks, growth of fish fed EO was slower compared to the FO and RO treatments. Irrespective of salinity, expression of the FAD6 and elongase was up-regulated in fish fed EO and RO diets, but did not lead to significant accumulation of LC-PUFA in the neutral lipid of fish tissues as occurred in the FO treatment. However, significant concentrations of eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (ARA, 20:4n-6), but not docosahexaenoic acid (DHA, 22:6n-3), appeared in liver and, to a lesser extent, in muscle of fish fed EO with marked increases in the phospholipid fraction. Fish in the EO treatment had higher EPA and ARA in their liver phospholipids than fish fed FO. Endogenous conversion of dietary precursors into neutral lipid LC-PUFA appears to be limited by factors other than the initial rate-limiting step. In contrast, phospholipid LC-PUFA had higher biosynthesis, or selective retention, in barramundi fed EO rather than RO.

KEYWORDS: *Echium plantagineum*, fatty acid, fish oil replacement, rapeseed oil, stearidonic acid

■ INTRODUCTION

Marine teleost fish have a lower ability to endogenously convert dietary α-linolenic (ALA, 18:3n-3) and linoleic (LA, 18:2n-6) acids into long-chain ($\geq C_{20}$, ≥ 2 double bonds) poly-unsaturated fatty acids (LC-PUFA) compared with freshwater fish species.[1] This distinction was previously ascribed to the lack of specific fatty acid desaturase (FAD) gene activity in marine fish[2,3] and has been recently linked with the natural diet and environment.[4] As the ancestor species of freshwater fish originally moved from the ocean into freshwater ecosystems, they evolved the ability to convert significant amounts of C_{18} PUFA to LC-PUFA. Marine fish remained in an environment rich in dietary LC-PUFA, where such conversion was less advantageous.[5,6] Similarly, changes in temperature and diet duplicated Δ9-CoA desaturase in modern freshwater fish from their ancient ancestors.[7,8] The Δ5 and Δ6 fatty acid desaturases (FAD5, FAD6) belong to the group of Δ6-like desaturases and are membrane-bound enzymes present in animals.[9,10] The functional significance of FAD6 in fish species that do not readily convert C_{18} PUFA to LC-PUFA is not understood. However, retaining a functional FAD6 gene in marine and diadromous fish implies an, as yet unknown, biochemically and nutritionally important function.[5,11]

Functional characterization of the zebrafish, *Danio rerio*, desaturase showed the enzyme to have both FAD5 and FAD6

activities, whereas in Atlantic salmon, *Salmo salar*, FAD5 and FAD6 share >95% nucleic acid identity.[12,13] The bifunctional zebrafish gene and the multiple subfunctionalized salmon genes have enabled these species, which spend all or part of their life cycle in LC-PUFA-poor riverine environments, to synthesize eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). In contrast, nutritional modulation did not stimulate LC-PUFA accumulation in some marine fish, such as the sea bream, *Sparus aurata*.[14] Heterologously expressed fatty acid elongases (FAE) from fish exhibited the ability to chain lengthen n-3 and n-6 PUFA and monounsaturated fatty acid (MUFA) substrates from C_{18} to C_{22}.[1,15] Despite requiring FAD5 activity in LC-PUFA biosynthesis, there is no clear evidence that this enzymatic activity exists in some euryhaline fish such as barramundi, *Lates calcarifer*.[16] Barramundi spends its early development stages in estuaries and freshwater before migrating to marine environments as an adult. Furthermore, juveniles are euryhaline and grow normally at salinities ranging from zero to full seawater. Consequently, it was of interest to investigate

whether salinity affects the FAE and FAD6 gene expression and LC-PUFA biosynthesis in muscle and liver of barramundi as it does in their whole body.[17]

Echium oil (EO), extracted from the seeds of *Echium plantagineum*, is a rich source of stearidonic acid (SDA, 18:4n-3) and γ-linolenic acid (GLA, 18:3n-6) compared with other terrestrial vegetable oils.[18] Dietary SDA has been evaluated as a source of an n-3 LC-PUFA precursor superior to ALA and increased EPA content in different tissues in humans,[19−21] dogs,[22] mice,[23] and poultry.[24] When EO was fed to some fish species, SDA was converted to eicosatetraenoic acid (ETA, 20:4n-3), but not to

Table 1. Ingredients and Lipid Composition of Experimental Diets (EO, Echium Oil; RO, Rapeseed Oil; FO Fish Oil) Fed to Barramundi

	EO	FO	RO
ingredient composition (g/kg^{-1} dry matter)			
fishmeal (defatted)	690	690	690
echium oil	170	0	0
fish oil	0	170	0
rapeseed oil	0	0	170
gelatinized starch	59	59	59
vitamin mix[a]	15	15	15
mineral mix[b]	15	15	15
vitamin C[c]	20	20	20
choline chloride	10	10	10
CMC[d]	10	10	10
sodium monophosphate	10	10	10
yttrium oxide[e]	1	1	1
chemical composition			
dry matter	824.9	856.2	858.2
crude protein	605.5	599.7	599.1
total lipid	218.9	224.9	215.6
energy (MJ kg^{-1} DM)	20.71	21.18	21.21
FA			
neutral lipids[f] (mg g^{-1})			
total SFA	24.8	39.1	19.9
total MUFA	36.7	95.5	104.8
18:3n-3	45.2	1.4	12.6
18:4n-3	18.2	2.9	0.6
20:5n-3	0.8	11.9	0.9
22:6n-3	0.6	13.9	0.8
total n-3 PUFA	65.1	34.6	15.3
18:2n-6	32.8	4.4	31.6
18:3n-6	16.1	0.5	0.3
20:4n-6	0.1	1.2	0.3
total n-6 PUFA	49.4	6.5	32.6
total PUFA	114.9	42.1	48.6
total FA	177.1	176.6	174.0
phospholipids (mg kg^{-1})			
total SFA	201.6	189	167.3
total MUFA	976.5	843.1	850.8
18:3n-3	17.0	3.0	7.0
18:4n-3	24.8	15.4	0.0
20:5n-3	303.8	302.8	331.8
22:6n-3	689.0	683.7	648.4
total n-3 PUFA	1038.8	1026.4	1008.1

Table 1. Continued

	EO	FO	RO
18:2n-6	27.4	41.4	24.9
18:3n-6	0.0	4.4	7.9
20:4n-6	58.6	38.0	43.6
total n-6 PUFA	89.4	90.2	83.9
total PUFA	1129.2	1119.6	1112.0
total FA	2292.3	2211.7	2138.1

[a] Vitamin mix (mg kg^{-1}): vitamin A (22.5), vitamin D (27), Rovimix E50 (450), menadone sodium bisulfate (9), riboflavin (18), calcium D-pantothenate (97.83), nicontinic acid (45), vitamin B-12 (0.045), d-biotin (2.025), folic acid (4.5), thiamin HCl (5.04), pyridoxine HCl (16.47), *myo*-inositol (1350), and α-cellulose (2452.59) as a filler. [b] Mineral mix (mg kg^{-1}): CuSO$_4$·5H$_2$O (106.111), FeSO$_4$·7H$_2$O (1633.95), MnSO$_4$·H$_2$O (276.84), Na$_2$SeO$_3$ (2.97), ZnSO$_4$·7H$_2$O (593.73), KI (6.48), CoSO$_4$·7H$_2$O (42.93), and α-cellulose (1836.99) as a filler. [c] Rovimix Stay-C 35. [d] CMC, carboxymethyl cellulose. [e] Digestibility marker, results for digestibility not shown. [f] SFA, saturated fatty acid; MUFA; monounsaturated fatty acid.

EPA and DHA.[25,26] Although substantially lower than in the fish oil (FO) treatment, DHA concentrations in freshwater fish fed SDA were higher than the concentrations in fish fed ALA-rich diets.[27,28] Previous research suggests that fish from different ecosystems fed the Δ6 desaturated fatty acid (SDA) bypassed the rate-limiting step involving FAD6, but varied in their ability to biosynthesize and accumulate further downstream products depending on their evolutionary history. On the basis of this environment-regulated lipid metabolism, we investigated the biochemical capacity of barramundi to synthesize and accumulate n-3 and n-6 LC-PUFA in their muscle and liver when raised in either freshwater (FW) or seawater (SW). The capacity of barramundi to biosynthesize these compounds in neutral and phospholipids from an SDA- and GLA-containing EO-based diet was compared with a rapeseed oil (RO) diet as a negative control and with a FO diet containing preformed n-3 LC-PUFA as a positive control.

■ MATERIALS AND METHODS

Diets, Animals, and Sample Collection. Three identical diets, differing only in their lipid source, were formulated and pelleted to compare EO provided as Crossential SA14 (Croda Chemicals) with RO (Woolworths Ltd.) and South American anchovy FO (Skretting Australia) (Table 1). Fishmeal was defatted overnight with a mixture of hexane and ethanol (2:1 v/v, 400 mL per 100 g of fishmeal). Juvenile barramundi (5 ± 0.3 g) were used in this study (Animal Ethics Committee, University of Tasmania, A0010171) and were randomly stocked into 24 tanks at 10 fish per tank in semi-flow-through systems. Fresh water (0−2 ppt salinity) or seawater (30−32 ppt salinity) was recirculated through 12 tanks.[17] Water was treated identically in each system through UV and trickle biofilters; 40% of the system water was replaced every 2 days with preheated water at each salinity.[17] For each salinity, four tanks of fish were hand-fed equal rations of each of the three experimental diets for 8 weeks. Fish were maintained at a constant 30 °C and 24 h light photoperiod.[17] Fish were weighed at the start and end of the experiment. Six initial fish from the whole population and two final fish from each tank were sampled. Livers and skeletal muscle collected below the dorsal fin were split into two subsamples: one was frozen at −20 °C for lipid analysis and the other preserved in RNA preservative reagent (25 mM sodium citrate, 10 mM EDTA, 10 M ammonium sulfate, pH 5.2) and kept at −20 °C for RNA extraction and analysis of gene expression.

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Table 2. Nucleotide Sequences of Primer Pairs Used in qPCR Assay and GenBank Accession Numbers of the Sequence Used as Reference for Primer Design and Amplification Product Size

gene		sequence	accession no.	amplicon (bp)
Ubq	F	5′-AAA TGT CAA GGC TAA GAT C-3′	GQ507428	114
	R	5′-TGG ATG TTG TAG TCA GAC-3′		
EF1-α	F	5′-TAC ATC AAG AAG ATC GGC TAC-3′	GQ507427	82
	R	5′-CCT CCA GCA TGT TGT CTC-3′		
β-actin	F	5′-GAC CTC ACA GAC TAC CTC-3′	GU188683	97
	R	5′-GCT TCT CCT TGA TGT CAC-3′		
FAE	F	5′-TGT TAT GAA CTG GAT ACC-3′	GQ214180	88
	R	5′-GCC GTA ATA AGA ATA CAT C-3′		
FAD6	F	5′-TGT TGA GCG TCT TTG TAG-3′	GU047383	92
	R	5′-ATA CTG GTG TTG GTG ATT-3′		

Chemical Composition and Fatty Acid (FA) Analysis. Standard methods were used to determine the chemical composition of diet, whole carcass, and tissue samples: dry matter by drying at 135 °C for 2 h, crude protein (nitrogen × 6.25) determined by micro-Kjeldahl,[29] and total lipid (TL) content by a modified Bligh and Dyer protocol.[17,30] TL was applied to 1 g of silica gel (activated at 100 °C for 1 h; pore size 60 Å, 70−230 mesh, Sigma-Aldrich) in a chromatography column and separated into neutral lipids, glycolipids, and phospholipids in a stepwise elution of chloroform (10 mL), acetone (20 mL), and methanol (10 mL), respectively, with separation confirmed by TLC-FID analysis for each class. Aliquots of the neutral and phospholipids were trans-methylated in methanol/chloroform/hydrochloric acid (10:1:1, v/v/v) for 1 h at 100 °C to obtain fatty acid methyl esters (FAME). FAME were made up to a known volume with an internal injection standard (19:0 FAME) added of known concentration and analyzed using a GC (Agilent Technologies 7890A) equipped with an Supelco Equity-1 fused silica capillary column (15 m × 0.1 mm internal diameter, 0.1 μm film thickness). Samples were injected in splitless mode at 120 °C using an Agilent Technologies 7683B injector with helium as carrier gas. After injection, oven temperature was raised to 270 °C at 10 °C/min and finally to 310 °C at 5 °C/min. Acquired peaks were quantified with Agilent Technologies ChemStation software (Palo Alto, CA). Initial peak identifications were by comparison of retention time data with purchased and laboratory standards (Nu-Chek Prep, Inc., Elysian, MN). GC−mass spectrometric (GC-MS) analyses confirmed component identifications and were performed separately for representative samples (n = 3 for each tissue type) on a Finnigan GCQ Plus GC-MS (Finnigan Corp., San Jose, CA) ion trap fitted with on-column injection set at 45 °C as mentioned before.[17,31,32] Briefly, samples were injected using an AS2000 autosampler onto a retention gap attached to a HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d. × 0.17 μm film thickness). The initial temperature of 45 °C was kept for 1 min, followed by a programmed rate of temperature increase of 30 °C/min to 140 °C and then at 3 °C/min to 310 °C, at which it was kept for 12 min. Helium was used as the carrier gas. Mass spectrometer operating conditions were as follows: electron impact energy, 70 eV; emission current, 250 μA; transfer line, 310 °C; source temperature, 240 °C; scan rate, 0.8 scans/s; and mass range, 40−650 Da. Mass spectra were acquired and processed with Xcalibur (Finnigan Corp.) software. No evidence of PUFA degradation was observed for the GC and GC-MS analyses.

RNA Extraction and Real-Time Quantitative PCR. Eight muscle and liver samples per treatment (two fish per tank) were collected, and their total RNA was isolated and purified using TRI Reagent (Molecular Research Center, USA) including DNA-*free* DNase treatment (Ambion, USA) to remove any possible genomic DNA contamination. RNA was recovered in nuclease-free water (Ambion, USA); its yield (A_{260}) and purity ($A_{260/230}$ and $A_{260/280}$) were assessed spectrophotometrically, and its integrity was estimated on a 1% agarose gel. Five micrograms of total RNA was reverse transcribed into cDNA using a Bioscript One-Step RT-PCR kit (Bioline, Australia) with oligo $(dT)_{18}$ priming. Reactions started at 65 °C for 10 min and then at 47 °C for 50 min before inactivating the reverse transcriptase enzyme at 75 °C for 15 min. First-strand cDNA reactions (20 μL) were diluted to 100 μL using nuclease-free water and stored at −80 °C until real-time quantitative PCR (qPCR) was performed. qPCR primers (Table 2) for FAD6 and FAE were designed using gene sequences available in GenBank (http://www.ncbi.nlm.nih.gov). qPCR used SYBR green chemistry on an iQ5 real-time PCR detection system (Bio-Rad). Each reaction (20 μL) contained primers (400 nM each), 10 μL of SensiMixPlus SYBR and Fluorescein PCR master mix (Quantace), and 2 μL of cDNA. All samples were analyzed for each gene in duplicate with no-template controls and a five-step, 4-fold cDNA dilution series for PCR efficiency calculation on the same plate. The thermal cycling protocol consisted of 10 min at 95 °C to activate the heat-activated DNA polymerase followed by 40 cycles of 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 25 s. At the end of the 40 cycles, a melt curve analysis was performed to test the specificity of reaction to generate a single product and no primer−dimer artifacts. Relative mRNA expression level of FAE and FAD6 in each sample was normalized using the geometric mean of three stably expressed reference housekeeping genes in barramundi: polyubiquitin, elongation factor 1-α, and β-actin. Automated analysis of qPCR data was performed by qBasePLUS (Biogazelle, Belgium) software.

Statistical Analysis. Data were expressed as mean values with standard error of the means (SEM). Percentage values were arcsine-transformed when needed before analysis. Differences between FA concentrations, growth performance, and gene expression collected after 8 weeks of feeding on each experimental diet were tested for homogeneity of variance and then compared between treatments by two-way analysis of variance (ANOVA) followed by multiple comparisons using Tukey's honestly significant difference at $P < 0.05$ using PASW Statistics v. 18 (SPSS Inc., Chicago, IL).

■ **RESULTS**

Biometry and Chemical Composition. Neither the interaction between diet and salinity nor salinity alone significantly

Table 3. Growth Performance and Chemical Composition of Barramundi Fed Experimental Diets with Echium Oil (EO), Fish Oil (FO), and Rapeseed Oil (RO) (Mean ± SEM, n = 8)[a]

| | initial | | | treatment | | | | | | | | | effect[b] | | |
				FO			EO			RO			interaction	salinity	diet
parameter[c]															
W_i (g)				4.8	±	0.1	4.7	±	0.1	4.9	±	0.1			
W_f (g)				89.4	±	5.3b	67.5	±	6.1a	80.6	±	4.7ab	ns	ns	*
WG (g)				84.6	±	5.1b	61.7	±	6.0a	75.6	±	2.8ab	ns	ns	*
FC (g)				84.7	±	3.1	80.4	±	2.4	79.8	±	3.8	ns	ns	ns
FER (g g^{-1} DM)				1.0	±	0.1b	0.7	±	0.0a	0.9	±	0.1b	ns	ns	*
chemical composition[d]															
DM (mg g^{-1} wet wt)	217.1	±	0.2	269.3	±	0.1	253.4	±	0.1	228.3	±	0.2	ns	ns	ns
CP (mg g^{-1} DM)	160.3	±	5.3	145.5	±	2.7	150.5	±	2.3	150.8	±	2.7	ns	ns	ns
TL (mg g^{-1} DM)	42.7	±	1.6	77.8	±	1.9	67.6	±	2.1	83.2	±	1.4	ns	ns	ns

[a] Means in a row followed by different letters are significantly different (Tukey's test). [b] Significant effects are reported at $P < 0.05$ and denoted by an asterisk; ns, not significant. [c] W_i, initial wet weight; W_f, final wet weight; WG, weight gain; FC, total amount of feed consumed by individual through the experiment period; FER, feed efficiency ratio (g g^{-1}) = total mass gain (g) × FC^{-1} (g). [d] DM, dry matter; CP, crude protein; TL, total lipid.

affected growth performance or chemical composition of the whole body (Table 3). Food consumption was not different between treatments. Consequently, intakes of the three different oils were not different. Diet significantly affected the final weight, weight gain, and feed efficiency ratio, which all were highest in fish fed FO and lowest in fish fed EO. Diet did not significantly change the chemical composition of fish.

Muscle Lipid and FA. FA composition in muscle was, generally, not significantly affected by the interaction of salinity and diet or by salinity (Table 4). The muscle FA profile for both neutral and phospholipid fractions differed in response to diet without a significant effect of salinity or the interaction between diet and salinity (Tables 4 and 5). Fish fed FO had significantly higher neutral lipid saturated fatty acid (SFA) and the lowest concentration of phospholipid SFA. Muscle neutral lipid MUFA was higher in fish fed FO and RO than in fish fed EO. There were no observed differences in phospholipid MUFA concentration among dietary treatments.

Fish fed EO had greater total n-3 PUFA in both phospholipid and neutral lipid fractions of muscle compared with the RO-fed fish, but concentrations were significantly less than for FO treatment fish. The neutral lipid n-3 PUFA profile was mainly composed of EPA and DHA in fish fed FO and of ALA and SDA in the EO treatment. Although fish fed EO and RO diets had less n-3 LC-PUFA in their muscle neutral lipid, the EO diet elevated the concentrations of DPA and DHA incorporated in phospholipid to significantly higher concentrations of these FA than occurred in the RO treatment, although still markedly less than in FO-treated fish. Fish fed EO and RO had higher n-6 PUFA content in their muscle neutral and phospholipid; these were mainly LA and GLA in the EO treatment, which also elevated the dihomo-γ-linolenic acid (DGLA, 20:3n-6) concentrations to higher than observed for the other two dietary treatments. Arachidonic acid (ARA, 20:4n-6) accumulation in muscle of EO and RO fish was lower when compared to fish fed FO. Total PUFA were greater in fish fed EO than in FO- and RO-fed fish and comprised mainly C$_{18}$ PUFA in both the phospholipids and neutral lipids. This increase led to a significantly higher n-3:n-6 ratio in the neutral lipid fraction for fish from the EO treatment compared with RO-fed fish, although the ratio was lower than in the FO treatment. The n-3:n-6 PUFA ratio in phospholipids was

higher in muscle for fish fed the FO diet than for fish fed EO and RO. Total FA concentrations in neutral lipid were generally not affected by diet, whereas the concentrations in phospholipid were significantly higher in muscle of fish fed EO.

Liver Lipid and FA. A similar trend was observed in liver as in muscle in that only diet affected fatty acid profile, with no effects due to salinity or the interaction between salinity and diet (Tables 6 and 7). Fish fed EO had significantly higher SFA, both in neutral and phospholipids, in their livers. Neutral lipid MUFA were significantly higher in both the FO and RO treatments, and phospholipid MUFA was highest in liver of fish fed EO compared with fish fed the other diets.

Although dominated by ALA and SDA, the liver of fish fed EO accumulated phospholipid n-3 PUFA at a higher concentration than did FO-fed fish, with neutral lipid n-3 PUFA at a comparable concentration to FO-fed fish. Phospholipids of the liver of EO-fed fish contained significantly higher concentrations of EPA than found in fish fed the other diets, with more DHA also present than for RO fish. EO-fed fish had concentrations of total neutral lipid n-6 PUFA in their livers comparable to those in the RO treatment. Concentrations of n-6 PUFA were significantly lower in fish fed FO, whereas livers of EO-fed fish accumulated phospholipid n-6 PUFA at higher concentrations than in the other two treatments. LA and GLA were the major n-6 PUFA in fish fed EO and RO, with neutral lipid ARA lower than in FO-fed fish. Fish fed EO contained phospholipid ARA at significantly higher concentrations than the fish fed FO or RO. This led to significantly higher total PUFA content in neutral and phospholipids in liver of fish fed the EO diet. Consequently, the n-3:n-6 ratio was significantly lower in the liver of fish fed EO and RO compared to the FO treatment.

Gene Expression. The magnitude of mRNA of FAD6 and FAE abundance measured in the liver by qPCR was generally higher than in muscle and was affected only by diet, irrespective of the salinity or the interaction between salinity and diet in barramundi (Figure 1). FO-fed fish had the lowest expression levels of the FAD6 and FAE genes in both tested tissues compared with the EO and RO treatments. In addition, FAD6 showed lower expression in tissues of fish fed the EO diet rich in SDA and GLA than in fish fed the RO diet lacking these two C$_{18}$ precursors. Relative expressions of FAE were comparable in the RO and EO treatments in each tissue.

Table 4. Lipid Content and Neutral Lipid FA Composition (Milligrams per Gram Dry Weight) in Muscle of Barramundi Fed Echium Oil (EO), Fish Oil (FO), and Rapeseed Oil (RO) (Mean ± SEM, $n = 4$)[a]

FA	initial	±	FO FW	±	FO SW	±	EO FW	±	EO SW	±	RO FW	±	RO SW	±	effect[b] diet	salinity	interaction
lipid content	70.2	3.7	47.4	8.2	39.6	5.4	45.2	10.2	38.6	4.2	48.0	7.0	44.8	3.6	ns	ns	ns
FA																	
14:0	0.0	0.0	0.5	0.1a	0.4	0.1a	0.0	0.0b	0.0	0.0b	0.1	0.0b	0.1	0.0b	*	ns	ns
16:0	3.4	0.8	5.5	0.7a	5.1	1.1a	2.9	0.2b	3.1	0.3b	2.7	0.2b	3.0	0.4b	*	ns	ns
18:0	1.7	0.6	1.8	0.2ab	1.7	0.2ab	1.9	0.1a	2.1	0.1a	1.4	0.1b	1.4	0.1b	*	ns	ns
20:0	0.1	0.0	0.1	0.0a	0.1	0.0a	0.1	0.0a	0.1	0.0a	0.2	0.0b	0.3	0.1b	*	ns	ns
total SFA	5.4	1.4	8.3	1.2a	7.6	1.6a	5.1	0.4b	5.5	0.5b	4.6	0.4b	5.0	0.6b	*	ns	ns
16:1n-7	0.3	0.1	1.5	0.3a	1.3	0.5a	0.2	0.0b	0.1	0.0b	0.2	0.0b	0.3	0.0b	*	ns	ns
18:1n-7	0.6	0.2	1.4	0.2a	1.3	0.3a	0.5	0.0b	0.5	0.0b	1.3	0.1a	1.4	0.2a	*	ns	ns
18:1n-9	4.7	1.0	7.8	1.2b	7.3	1.8b	4.1	0.4c	4.3	0.4c	11.3	1.4a	12.6	2.1a	*	ns	ns
20:1n-9	0.2	0.0	2.3	0.4a	2.1	0.6a	0.2	0.0b	0.2	0.0b	0.3	0.0b	0.3	0.0b	*	ns	ns
22:1n11	0.0	0.0	0.7	0.1a	0.6	0.2a	0.0	0.0b	0.0	0.0b	0.0	0.0b	0.0	0.0b	*	ns	ns
24:1n-9	0.2	0.1	0.4	0.0a	0.4	0.0a	0.2	0.0b	0.3	0.0b	0.2	0.0b	0.2	0.0b	*	ns	ns
total MUFA	6.0	1.3	15.0	2.5a	13.9	3.5a	5.3	0.5b	5.6	0.5b	13.6	1.6a	15.0	2.4a	*	ns	ns
18:3n-3	0.1	0.0	0.2	0.0b	0.2	0.1b	2.9	0.3a	2.7	0.3a	1.2	0.2c	1.5	0.3c	*	ns	ns
18:4n-3	0.0	0.0	0.3	0.1b	0.3	0.1b	1.1	0.1a	1.0	0.1a	0.1	0.0b	0.1	0.0b	*	ns	ns
20:4n-3	0.0	0.0	0.2	0.0a	0.2	0.1a	0.1	0.0b	0.1	0.0b	0.0	0.0b	0.0	0.0b	*	ns	ns
20:5n-3	0.6	0.3	1.8	0.2a	1.7	0.3a	0.4	0.0b	0.4	0.0b	0.5	0.0b	0.5	0.1b	*	ns	ns
22:5n-3	0.3	0.1	0.6	0.1a	0.6	0.1a	0.1	0.0b	0.2	0.0b	0.2	0.0b	0.2	0.0b	*	ns	ns
22:6n-3	1.8	0.8	3.9	0.3a	3.9	0.4a	0.7	0.0b	0.9	0.0b	0.9	0.1b	1.0	0.1b	*	ns	ns
total n-3	2.8	1.2	7.0	0.8a	6.8	1.0a	5.3	0.5b	5.3	0.5b	2.8	0.2c	3.3	0.4c	*	ns	ns
18:2n-6	2.5	0.7	0.7	0.1c	0.7	0.2c	3.7	0.3b	3.8	0.3b	4.5	0.5a	5.1	0.8a	*	ns	ns
18:3n-6	0.1	0.0	0.0	0.0c	0.0	0.0c	1.4	0.1a	1.4	0.1a	0.2	0.0b	0.2	0.0b	*	ns	ns
20:3n-6	0.3	0.1	0.0	0.0b	0.1	0.0b	0.4	0.0a	0.4	0.0a	0.1	0.0b	0.1	0.0b	*	ns	ns
20:4n-6	0.3	0.1	0.4	0.0b	0.4	0.0b	0.1	0.0b	0.1	0.0b	0.1	0.0b	0.1	0.0b	*	ns	ns
total n-6	3.0	0.9	1.4	0.2b	1.3	0.3b	5.3	0.4a	5.5	0.4a	4.9	0.5a	5.6	0.8a	*	ns	ns
total PUFA	5.8	2.0	8.4	1.0c	8.1	1.3c	10.6	0.9ab	10.8	0.9ab	7.7	0.7bc	8.9	1.2bc	*	ns	ns
n-3:n-6	0.8	0.2	5.2	0.1a	5.6	0.3a	1.0	0.0b	1.0	0.0b	0.6	0.0c	0.6	0.0c	*	ns	ns
total FA	17.3	4.8	31.6	4.6	29.6	6.4	21.0	1.7	21.9	1.8	25.9	2.8	28.8	4.1	ns	ns	ns

[a] Means in a row followed by different letters are significantly different (Tukey's test). [b] Significant effects are reported at $P < 0.05$ and denoted by an asterisk; ns, not significant.

Table 5. Phospholipid FA Composition (Micrograms per Gram Dry Weight) in Muscle of Barramundi Fed Echium Oil (EO), Fish Oil (FO), and Rapeseed Oil (RO) (Mean ± SEM, $n = 4$)[a]

		FO		EO		RO		effect[b]		
FA	initial	FW	SW	FW	SW	FW	SW	interaction	salinity	diet
16:0	294.5 ± 53.1	76.0 ± 9.9b	86.4 ± 12.7b	111.7 ± 16.2a	143.4 ± 20.0a	127.0 ± 18.4a	± 12.1a	ns	ns	*
18:0	525.7 ± 67.9	64.3 ± 10.3b	74.8 ± 14.3b	76.5 ± 3.2a	116.6 ± 9.5a	73.6 ± 7.1b	± 3.0b	ns	ns	*
total SFA	878.7 ± 131.1	154.5 ± 9.8c	179.8 ± 22.5c	194.4 ± 32.1b	297.1 ± 17.0b	212.0 ± 23.5a	± 14.9a	ns	ns	*
18:1n-9	353.5 ± 26.5	95.9 ± 8.5c	108.9 ± 14.8c	148.9 ± 17.6a	192.7 ± 4.6a	163.7 ± 14.1b	± 7.0b	ns	ns	*
18:1n-7	96.1 ± 12.6	25.2 ± 3.3a	27.6 ± 5.3a	15.3 ± 6.7a	25.8 ± 2.6a	17.4 ± 2.8b	± 0.3b	ns	ns	*
20:1n-9	27.6 ± 2.3	5.9 ± 0.7b	7.2 ± 0.7b	5.4 ± 5.2a	20.0 ± 0.3a	6.0 ± 0.6b	± 1.9b	ns	ns	*
24:1n-9	17.1 ± 1.7	37.2 ± 4.4b	35.3 ± 8.8b	32.0 ± 7.1a	51.7 ± 6.2a	28.4 ± 3.1b	± 1.4b	ns	ns	*
total MUFA	508.5 ± 35.8	185.0 ± 51.2	272.5 ± 39.0	128.1 ± 21.4	301.0 ± 15.4	227.1 ± 38.7	± 80.2	ns	ns	ns
18:3n-3	8.7 ± 0.9	0.5 ± 0.2c	1.1 ± 0.3c	17.6 ± 6.9a	46.0 ± 5.6a	14.3 ± 2.6b	± 1.6b	ns	ns	*
18:4n-3	0.3 ± 0.1	0.2 ± 0.2b	0.0 ± 0.0b	0.0 ± 0.9a	4.8 ± 1.3a	0.1 ± 0.0b	± 0.1b	ns	ns	*
20:4n-3	0.8 ± 0.1	0.0 ± 0.0b	0.3 ± 0.3b	0.2 ± 1.3a	5.6 ± 0.6a	0.3 ± 0.1b	± 0.1b	ns	ns	*
20:5n-3	66.3 ± 7.5	20.0 ± 1.4a	24.9 ± 2.3a	8.7 ± 2.2b	17.0 ± 0.7b	8.2 ± 3.4c	± 2.7c	ns	ns	*
22:5n-3	40.1 ± 4.7	6.1 ± 0.9a	6.0 ± 1.2a	0.0 ± 0.6b	1.8 ± 0.0b	0.1 ± 0.0c	± 0.1c	ns	ns	*
22:6n-3	360.2 ± 49.7	134.8 ± 10.4a	144.6 ± 14.5a	18.7 ± 9.0b	53.0 ± 7.1b	17.6 ± 2.7c	± 2.3c	ns	ns	*
total n-3	476.5 ± 60.2	161.5 ± 7.7a	176.9 ± 15.6a	45.3 ± 1.4b	128.2 ± 2.8b	40.7 ± 8.7c	± 3.5c	ns	ns	*
18:2n-6	309.0 ± 85.7	8.9 ± 1.0c	9.4 ± 1.8c	52.1 ± 7.8a	99.3 ± 10.5a	52.6 ± 13.4b	± 20.6b	ns	ns	*
18:3n-6	4.0 ± 1.0	0.1 ± 0.1b	0.0 ± 0.0b	0.3 ± 2.8a	23.1 ± 1.8a	0.4 ± 0.2b	± 0.1b	ns	ns	*
20:2n-6	0.5 ± 0.2	0.2 ± 0.2b	0.0 ± 0.0b	0.4 ± 4.2a	27.3 ± 0.8a	0.4 ± 0.0b	± 0.2b	ns	ns	*
20:3n-6	8.1 ± 1.8	0.1 ± 0.1b	0.0 ± 0.0b	1.7 ± 1.6a	5.0 ± 1.0a	1.8 ± 0.3b	± 0.3b	ns	ns	*
20:4n-6	52.0 ± 6.7	10.6 ± 0.9a	12.3 ± 1.8a	0.1 ± 2.7b	6.1 ± 1.2b	0.0 ± 0.1c	± 0.0c	ns	ns	*
total n-6	406.4 ± 87.3	32.4 ± 0.6c	38.2 ± 6.8c	66.4 ± 3.9a	182.1 ± 13.7a	65.2 ± 11.3b	± 24.4b	ns	ns	*
total PUFA	882.9 ± 129.1	193.8 ± 8.3b	215.1 ± 22.3b	111.7 ± 5.3a	310.2 ± 10.9a	105.9 ± 19.9c	± 27.9c	ns	ns	*
n-3:n-6	1.2 ± 0.2	5.0 ± 0.1a	4.7 ± 0.4a	0.7 ± 0.0b	0.7 ± 0.1b	0.7 ± 0.0b	± 0.2b	ns	ns	*
total FA	2270.1 ± 292.1	533.3 ± 39.3b	667.5 ± 23.8b	453.2 ± 47.7a	908.3 ± 39.3a	545.0 ± 38.3b	± 81.0b	ns	ns	*

[a] Means in a row followed by different letters are significantly different (Tukey's test). [b] Significant effects are reported at $P < 0.05$ and denoted by asterisk. ns: not significant.

Table 6. Lipid Content and Neutral FA Compositiont (Milligrams per Gram Dry Weight) in Liver of Barramundi Fed Echium Oil (EO), Fish Oil (FO), and Rapeseed Oil (RO) (Mean ± SEM, $n = 4$)[a]

	initial	FO		EO		RO		effect[b]		
FA		FW	SW	FW	SW	FW	SW	interaction	salinity	diet
lipid content	105.7 ± 20.7	487.7 ± 25.0	425.1 ± 32.5	466.2 ± 38.4	442.4 ± 48.4	436.8 ± 23.3	458.9 ± 31.4	ns	ns	ns
FA										
14:0	0.4 ± 0.2	4.0 ± 1.0a	3.0 ± 0.8a	1.2 ± 0.3b	0.9 ± 0.3b	0.9 ± 0.3b	0.8 ± 0.2b	ns	ns	*
16:0	22.2 ± 1.3	53.9 ± 5.6a	48.8 ± 5.8a	42.9 ± 5.3a	46.0 ± 7.5a	27.8 ± 2.2b	30.6 ± 1.8b	ns	ns	*
18:0	8.2 ± 2.8	14.0 ± 1.0b	13.8 ± 1.3b	25.7 ± 2.1a	26.9 ± 4.2a	12.3 ± 0.9b	12.3 ± 0.4b	ns	ns	*
20:0	0.3 ± 0.3	1.2 ± 0.2b	1.2 ± 0.2b	0.8 ± 0.2b	1.0 ± 0.4b	2.7 ± 0.3a	2.5 ± 0.3a	ns	ns	*
total SFA	32.2 ± 8.6	75.9 ± 8.0a	71.2 ± 8.8a	72.0 ± 7.8a	76.7 ± 12.1a	44.9 ± 3.1b	47.6 ± 2.5b	ns	ns	*
16:1n-7	2.0 ± 0.3	15.9 ± 2.4a	14.5 ± 1.7a	3.1 ± 0.6b	4.1 ± 0.5b	2.6 ± 0.3b	2.7 ± 0.4b	ns	ns	*
18:1n-7	3.1 ± 0.8	13.7 ± 1.4a	13.4 ± 1.5a	5.4 ± 0.6b	5.7 ± 0.9b	14.1 ± 1.3a	14.0 ± 1.2a	ns	ns	*
18:1n-9	30.2 ± 2.9	83.9 ± 9.6b	79.3 ± 9.3b	72.3 ± 8.6b	78.1 ± 13.0b	159.2 ± 16.0a	168.2 ± 15.9a	ns	ns	*
20:1n-9	1.0 ± 0.3	24.4 ± 3.0a	25.2 ± 2.8a	3.2 ± 0.6b	2.9 ± 0.4b	3.3 ± 0.5b	3.4 ± 0.3b	ns	ns	*
22:1n11	0.3 ± 0.2	7.3 ± 1.0a	8.6 ± 1.1a	0.3 ± 0.1b	0.6 ± 0.1b	0.3 ± 0.1b	0.1 ± 0.1b	ns	ns	*
24:1n-9	0.8 ± 0.3	3.2 ± 0.2a	3.6 ± 0.3a	1.4 ± 0.2b	1.4 ± 0.1b	1.2 ± 0.1b	1.1 ± 0.1b	ns	ns	*
total MUFA	38.1 ± 3.1	158.1 ± 18.8a	154.8 ± 17.9a	87.5 ± 10.8b	94.7 ± 15.1b	183.6 ± 18.2a	192.3 ± 17.9a	ns	ns	*
18:3n-3	0.8 ± 0.5	0.8 ± 0.3c	0.4 ± 0.2c	25.6 ± 6.7a	20.8 ± 7.1a	9.8 ± 1.7b	9.6 ± 1.7b	ns	ns	*
18:4n-3	0.2 ± 0.1	1.5 ± 0.3b	1.6 ± 0.3b	10.1 ± 3.0a	6.6 ± 2.6a	1.2 ± 0.2b	1.2 ± 0.2b	ns	ns	*
20:4n-3	0.2 ± 0.1	1.1 ± 0.3a	1.6 ± 0.3a	0.6 ± 0.4b	0.2 ± 0.2b	0.2 ± 0.1b	0.1 ± 0.1b	ns	ns	*
20:5n-3	2.2 ± 0.8	7.5 ± 1.1a	8.7 ± 1.0a	0.7 ± 0.3b	0.6 ± 0.3b	1.7 ± 0.2b	1.3 ± 0.4b	ns	ns	*
22:5n-3	1.1 ± 0.1	2.7 ± 0.5a	3.3 ± 0.5a	0.1 ± 0.1b	0.0 ± 0.0b	0.3 ± 0.1b	0.3 ± 0.1b	ns	ns	*
22:6n-3	6.3 ± 0.4	8.6 ± 1.2a	10.2 ± 1.4a	1.7 ± 0.3b	1.2 ± 0.2b	2.3 ± 0.3b	1.6 ± 0.1b	ns	ns	*
total n-3	10.8 ± 0.9	22.3 ± 3.5bc	26.2 ± 3.3bc	37.9 ± 4.7a	28.6 ± 3.2ab	15.4 ± 2.2c	14.1 ± 2.3c	ns	ns	*
18:2n-6	13.4 ± 1.6	5.9 ± 0.8b	6.1 ± 0.6b	34.6 ± 6.3a	32.0 ± 5.7a	37.9 ± 4.8a	38.9 ± 5.0a	ns	ns	*
18:3n-6	0.5 ± 0.1	0.1 ± 0.1c	0.1 ± 0.1c	13.7 ± 3.2a	11.0 ± 3.6a	4.5 ± 0.4b	3.8 ± 0.3b	ns	ns	*
20:4n-6	0.9 ± 0.7	1.0 ± 0.2a	1.3 ± 0.3a	0.0 ± 0.0b	0.0 ± 0.0b	0.1 ± 0.1b	0.1 ± 0.1b	ns	ns	*
total n-6	15.6 ± 1.1	7.4 ± 1.0b	7.8 ± 0.8b	48.6 ± 9.6a	43.4 ± 7.2a	43.1 ± 4.9a	43.3 ± 5.3a	ns	ns	*
total PUFA	26.4 ± 2.0	29.7 ± 4.5bc	34.0 ± 4.0bc	86.5 ± 10.3a	71.9 ± 12.4a	58.6 ± 7.1ab	57.4 ± 7.6ab	ns	ns	*
n-3:n-6	0.5 ± 0.2	3.0 ± 0.2a	3.4 ± 0.3a	0.7 ± 0.1b	0.6 ± 0.0b	0.3 ± 0.0b	0.3 ± 0.0b	ns	ns	*
total FA	96.7 ± 9.7	263.6 ± 30.7	260.0 ± 30.0	245.9 ± 27.6	243.4 ± 38.9	287.0 ± 27.2	297.2 ± 19.6	ns	ns	ns

[a] Means in a row followed by different letters are significantly different (Tukey's test). [b] Significant effects are reported at $P < 0.05$ and denoted by an asterisk; ns, not significant.

Table 7. Phospholipid FA Composition (Micrograms per Gram Dry Weight) in Livers of Barramundi Fed Echium Oil (EO), Fish Oil (FO), and Rapeseed Oil (RO) (Mean ± SEM, $n = 4$)[a]

FA	initial	FO FW	FO SW	EO FW	EO SW	RO FW	RO SW	interaction	salinity	diet
16:0	34.3 ± 6.1	10.1 ± 1.8b	11.2 ± 2.1b	43.1 ± 16.6a	38.0 ± 7.5a	0.3 ± 0.1c	0.7 ± 0.3c	ns	ns	*
18:0	815.5 ± 43.7	245.3 ± 15.1b	215.8 ± 21.7b	693.7 ± 53.5a	603.5 ± 65.2a	231.4 ± 37.0b	224.3 ± 25.0b	ns	ns	*
total SFA	872.7 ± 133.2	273.8 ± 0.7b	257.9 ± 36.0b	863.5 ± 59.4a	687.9 ± 64.3a	330.5 ± 22.1b	326.7 ± 19.3b	ns	ns	*
18:1n-9	1209.0 ± 127.5	315.0 ± 52.5c	313.2 ± 78.1c	922.9 ± 79.5a	870.6 ± 27.2a	435.5 ± 26.1b	466.2 ± 32.9b	ns	ns	*
18:1n-7	149.5 ± 24.9	41.9 ± 7.3c	46.1 ± 7.8c	137.4 ± 34.0a	154.1 ± 9.9a	65.8 ± 11.9b	62.0 ± 5.0b	ns	ns	*
20:1n-9	6.9 ± 1.7	100.0 ± 3.6a	110.9 ± 11.8a	41.5 ± 6.2b	31.7 ± 5.5b	123.0 ± 1.6a	126.8 ± 7.3a	ns	ns	*
24:1n-9	29.8 ± 5.0	136.9 ± 24.0a	129.4 ± 19.8a	86.6 ± 8.4c	76.8 ± 2.3c	106.1 ± 3.7b	96.5 ± 14.7b	ns	ns	*
total MUFA	2257.7 ± 477.1	1043.5 ± 79.4b	1070.2 ± 226.6b	2198.1 ± 35.8a	1524.9 ± 10.1a	1242.6 ± 2.1b	1178.8 ± 70.2b	ns	ns	ns
18:3n-3	3.4 ± 1.2	23.9 ± 3.4b	17.6 ± 4.5b	257.2 ± 16.3a	289.1 ± 29.9a	22.0 ± 2.1b	23.3 ± 3.0b	ns	ns	*
18:4n-3	0.0 ± 0.0	0.0 ± 0.0b	0.0 ± 0.0b	21.9 ± 7.3a	19.7 ± 5.5a	0.0 ± 0.0b	0.0 ± 0.0b	ns	ns	*
20:4n-3	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	ns	ns	*
20:5n-3	2.6 ± 0.5	56.7 ± 9.0b	58.8 ± 1.5b	71.7 ± 2.2a	69.7 ± 8.9a	5.7 ± 0.8c	5.1 ± 1.1c	ns	ns	*
22:5n-3	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	ns	ns	*
22:6n-3	155.5 ± 31.5	158.3 ± 20.1a	180.6 ± 7.6a	48.2 ± 7.4b	44.1 ± 1.1b	28.2 ± 1.1b	29.0 ± 2.8b	ns	ns	*
total n-3	161.5 ± 33.2	239.0 ± 7.8b	257.1 ± 13.6b	399.0 ± 14.4a	402.8 ± 39.9a	57.9 ± 4.1c	67.5 ± 4.5c	ns	ns	*
18:2n-6	290.0 ± 21.7	50.7 ± 5.5c	65.8 ± 6.8c	320.7 ± 5.9a	262.8 ± 31.9a	168.7 ± 6.3b	156.8 ± 9.6b	ns	ns	*
18:3n-6	0.0 ± 0.0	0.0 ± 0.0c	0.0 ± 0.0c	134.1 ± 3.7a	138.4 ± 10.2a	23.2 ± 5.0b	19.6 ± 3.6b	ns	ns	*
20:2n-6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	ns	ns	*
20:3n-6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	ns	ns	*
20:4n-6	3.0 ± 0.6	20.8 ± 7.2b	23.2 ± 7.3b	30.2 ± 2.0a	30.1 ± 4.0a	8.9 ± 1.3c	7.6 ± 1.5c	ns	ns	*
total n-6	293.1 ± 21.5	71.5 ± 1.7c	89.1 ± 14.1c	485.0 ± 7.7a	431.3 ± 25.7a	201.1 ± 0.1b	194.2 ± 11.7b	ns	ns	*
total PUFA	454.5 ± 39.7	310.5 ± 6.1b	346.1 ± 0.5b	884.1 ± 22.1a	834.2 ± 65.6a	257.0 ± 4.0c	268.7 ± 7.2c	ns	ns	*
n-3:n-6	0.6 ± 0.1	3.3 ± 0.2a	3.0 ± 0.6a	0.8 ± 0.0b	0.9 ± 0.0b	0.3 ± 0.0b	0.3 ± 0.0b	ns	ns	*
total FA	3584.8 ± 246.5	1627.8 ± 56.3c	1674.3 ± 30.1c	3945.7a ± 45.7	3046.9 ± 38.8a	1830.1 ± 26.0b	1804.2 ± 36.7b	ns	ns	*

[a] Means in a row followed by different letter are significantly different (Tukey's test). [b] Significant effects are reported at $P < 0.05$ and denoted by an asterisk; ns, not significant.
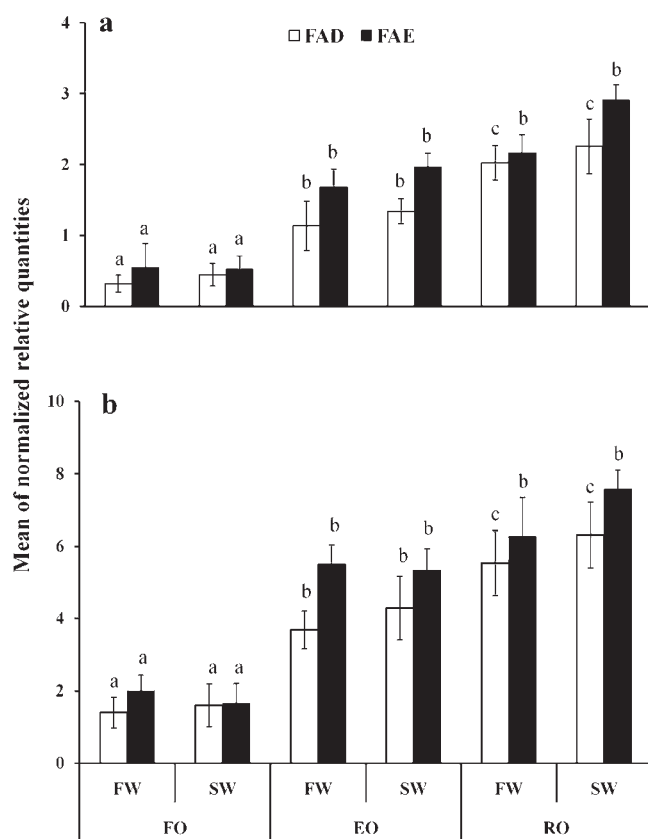
Figure 1. Differential gene expression of fatty acid Δ6 desaturase (FAD) and elongase (FAE) in (a) muscle and (b) liver of barramundi fed diets containing echium oil (EO), fish oil (FO), or rapeseed oil (RO) reared in either freshwater (FW) or seawater (SW). Different letters above bars of the same gene indicate significant effect ($P < 0.05$) for the diet as determined by Tukey's test.

DISCUSSION

Differences in the lipid metabolism of fish species reflect differences in the available dietary fatty acids.[4,5] Aquatic organisms adapt to dietary and environmental factors that influence their fatty acid metabolism. There is considerable research on the effects of temperature on fatty acid biosynthesis, depots, and their unsaturation levels,[33−35] with less attention directed to understanding the effects of salinity.[17,36] Freshwater fish are capable of endogenously converting ALA and LA to LC-PUFA, whereas marine species have limited capacity for such biosynthesis due to lower fatty acid desaturase enzyme activity.[1,2] The diadromous Atlantic salmon had a comparable biosynthesis yield of LC-PUFA in seawater and freshwater when fed the same diet,[32,37] whereas the euryhaline rabbitfish had higher conversion of ALA and LA to LC-PUFA in low salinity than in high salinity.[36] In comparison, the euryhaline barramundi was similar to Atlantic salmon and demonstrated similar patterns of fatty acid metabolism at both biochemical and molecular levels irrespective of ambient salinity. Marine fish, and diadromous species in marine ecosystems, utilize dietary LC-PUFA but not necessarily at optimal ratios. This may explain the retention of a functional FAD6 to manipulate membrane EPA/DHA ratios in these species.[5]

When sufficient enzyme activity exists, endogenous synthesis of n-3 LC-PUFA from their precursors is regulated by substrate concentration and competition for existing enzymes independent of changes in the expression of the synthetic enzymes or their functional regulatory genes.[38] This finding prioritizes nutritional factors in LC-PUFA biosynthesis compared to the availability of the pathway enzymes or the expression of their encoding genes. In apparent cellular homeostasis, ALA and LA promote, whereas LC-PUFA suppress, the expression of FAD and FAE.[39,40] High concentrations of dietary SDA and GLA from EO had a similar influence on the expression of LC-PUFA biosynthesis genes; the less unsaturated C_{18} PUFA were elevated, accompanied by reduced long-chain products compared to Atlantic salmon fed the FO diet.[31] However, studies on other fish showed increasing LC-PUFA accumulation in vitro.[25,41] We showed here that a diet rich in SDA and GLA did not stimulate significant accumulation of LC-PUFA in the neutral lipid fraction of barramundi muscle and liver. However, phospholipid LC-PUFA were observed in EO-fed barramundi liver to the extent that they exceeded the content when fish were fed FO. This appearance may be explained by increased biosynthesis from dietary precursors, whereas selective retention for some LC-PUFA can also be considered as a likely explanation.

In the present study, dietary LC-PUFA were reduced to low concentrations in fishmeal before the experimental oils were added. Concentrations of the neutral lipid LC-PUFA in barramundi tissues therefore were largely the result of endogenous conversion. Nonetheless, fish fed EO did not produce the high concentrations of neutral lipid n-3 LC-PUFA that occurred in fish fed FO. Concentrations of the LC-PUFA in neutral lipid in EO-fed fish tissues were similar to those found for the RO, which did not contain SDA or GLA. High expression of FAD and FAE genes in tissues from EO-fed fish did not result in as much accumulation of products as hypothesized with the salinity changes across the life history. It is believed that FAD and FAE have major roles in the maintenance of optimal levels of brain DHA by converting dietary DPA and EPA[5] and in tuning LC-PUFA synthesis in the liver, a major organ of lipid metabolism. Fatty acid apparent metabolism in the whole body of barramundi indicated a limited efficiency for LC-PUFA biosynthesis from their C_{18} dietary precursors.[17] It has been defined that Δ6 desaturation is a rate-limiting step in the formation of LC-PUFA in vertebrates;[40,42] hence, EO was considered a better vegetable oil to provide dietary precursors, thereby bypassing this first step and enhancing the downstream biosynthesis of LC-PUFA. Juvenile barramundi fed vegetable oils can attain high growth in salinity ranging from freshwater to seawater, whereas FO is still necessary for higher growth rates. Although having different contents of LC-PUFA precursors, EO and RO diets contained very similar and low concentrations of the essential fatty acids, EPA, DHA, and ARA, within the neutral lipids and phospholipids. These differences had profound effects on lipid metabolism in barramundi, but are not predicted to be the primary reason for growth differences. Under the experimental conditions employed, the EO diet lacking n-3 and n-6 LC-PUFA blended with defatted fishmeal appears to compromise growth and FER compared to the FO treatment. Using EO or any other SDA-rich oil in aquafeeds is presently restricted due to the limited availability of this oil and its high cost.[17,37] At this stage, EO can be considered as a potential partial replacement rather than a complete alternative to FO in aquafeed.

Variations in biosynthesis efficiency from dietary SDA and GLA were influenced by age,[43] ambient conditions,[37] and dietary regimen,[44] whereas varied levels of accumulation were shown in

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the same tissue from the same organism.[24] In the present study, we showed varied appearance of LC-PUFA in different lipid fractions of barramundi. In liver, phospholipid EPA and ARA appeared following feeding on SDA and GLA, respectively, and exceeded levels occurring in the neutral lipid fraction. Likewise, dietary SDA and GLA were bioconverted and appeared in neutral lipid as ETA and DGLA, respectively, but were not further converted to LC-PUFA in muscle of fish fed EO. This finding indicates that barramundi have either a high ability for LC-PUFA retention in their phospholipid fraction or limited endogenous capacity to produce LC-PUFA from $\Delta 6$ desaturated C_{18} PUFA even if they are supplied in the diet in relatively high levels. It was demonstrated recently that neither ALA nor SDA serves as a substrate for the efficient production of EPA and DHA in some temperate and coldwater marine fish species.[45] Differential expression of FAD6 in the diadromous Atlantic salmon showed the highest levels in brain, then liver,[46] whereas it was the opposite in marine fish.[47] This suggested that FAD6 in marine fish maintains optimal DHA in neural tissues by converting dietary EPA.[5] Barramundi, a representative member of a large group of tropical euryhaline fish, was less efficient in converting and accumulating LC-PUFA dietary precursors to EPA and DHA in neutral lipid even when they were supplied with precursors beyond the reputed initial rate-limiting step. In view of these observations and results for the RO treatment, LC-PUFA biosynthesis appears to be tightly controlled in barramundi by factors other than the initial rate-limiting $\Delta 6$ desaturation step. A lack of activity or low activity of FAD5 can also be considered as another rate-limiting step in LC-PUFA biosynthesis pathway in barramundi and needs to be further investigated.

Dietary vegetable oil, lacking EPA, modulates ARA metabolism.[48] This was evident in the phospholipid from liver of fish fed EO rather than in the neutral lipid fraction in liver or muscle. It is known that total polar lipid FA composition and concentrations are influenced by the dietary lipid source in coldwater[49,50] and warmwater fish[51,52] and are always higher in liver following feeding on a vegetable oil-based diet compared to FO. Our results concur with these previous findings and suggest that various vegetable oils with different FA profiles can also affect the levels of polar lipid FA accumulation and biosynthesis, particularly in the liver. Relatively high biosynthesis of LC-PUFA in phospholipid from their dietary precursors occurred in fish tissues, compared with levels observed in the neutral lipid fraction; this preference could be driven by the priority of incorporating LC-PUFA into cellular membranes to sustain their structure, fluidity, and functionality. Ectotherms from coldwater ecosystems require higher cell membrane fluidity, reflected by increased desaturation and LC-PUFA incorporation into their membranes[33,51] than those living in temperate and tropical regions like barramundi. Fish fed EO grew more slowly than fish given the other treatments, and they may have therefore retained more phospholipids in their tissues compared to neutral lipids. However, as the EO diet contained virtually no LC-PUFA, the concentrations of ARA and EPA appearing in the phospholipid in tissues of EO-fed fish that exceeded those in the fish fed FO can be explained only as a result of endogenous biosynthesis. Dietary lipids are important for the integrity of lipid structure in cell membranes. Although DHA is a very physically active and flexible molecule,[53] it was postulated that it is not the sole contributor to the high degree of membrane fluidity,[54] which varies depending on tissue and cell type.[55] Phospholipid raft domains within the cellular membrane were shown to be rich in ARA,[56] whereas EPA

was confirmed to be a major modifier for membrane caveolae structure and function.[57] These LC-PUFA can provide sufficient flexibility and functionality in cell membranes even with reduced concentrations of DHA. Changes in cell membrane PUFA composition can be brought about by altered PUFA metabolism, by variation in diet, or by altered PUFA incorporation into or out of membrane phospholipid.[58] We have shown here that dietary lipid, but not an environmental factor, salinity, manipulates the phospholipid PUFA profile in barramundi liver and muscle, with endogenous conversion of dietary SDA and GLA occurring in the phospholipid fraction. With feeding on EO, an alternate remodeling mechanism could have led to selective retention of EPA and ARA in the phospholipid fraction as a consequence of normal lipid turnover and needs further investigation. LC-PUFA precursors (SDA and GLA) derived from an EO diet were efficiently incorporated, with tissue-specific biosynthesis efficiency increased mainly in the liver, the major lipid metabolism organ, and to a lesser extent in muscle.

Barramundi has been a model animal for nutrition,[59,60] physiology,[61,62] and food chemistry[63,64] research. Our findings suggest this species is also a useful vertebrate model for investigating LC-PUFA biosynthesis with distinct genetic and metabolic responses to nutritional factors. Dietary oils lacking LC-PUFA up-regulated key LC-PUFA biosynthetic genes in the muscle and liver of this euryhaline fish, but did not increase concentrations of EPA and DHA. Precursor FA were converted into EPA and ARA in liver phospholipid, and to a lesser extent in muscle, with selective retention of phospholipid LC-PUFA likely contributing to the observed tissue composition of barramundi fed EO. Adaptive physiological and behavioral mechanisms in ectothermic euryhaline animals are normally synchronized with changes in lipid metabolism and LC-PUFA biosynthesis following changes in environmental parameters as observed. Considerable information exists on coldwater marine species, but little is available for tropical fish. This research improves our understanding of the basic nutritional biochemistry of barramundi. These findings are pertinent for molecular and biochemical research on tropical fish and more broadly in animal nutrition and can be extended to human nutrition due to the increasing production of land plants containing SDA oils.

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■ REFERENCES

(1) Leaver, M. J.; Bautista, J. M.; Bjornsson, B. T.; Jonsson, E.; Krey, G.; Tocher, D. R.; Torstensen, B. E. Towards fish lipid nutrigenomics: current state and prospects for fin-fish aquaculture. Rev. Fish. Sci. 2008, 16, 73–94.

(2) Tocher, D. R. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.* **2003**, *11* (2), 107–184.

(3) Vagner, M.; Santigosa, E. Characterization and modulation of gene expression and enzymatic activity of Δ-6 desaturase in teleosts: a review. *Aquaculture* **2011**, *315*, 131–143.

(4) Li, Y.; Monroig, O.; Zhang, L.; Wang, S.; Zheng, X.; Dick, J. R.; You, C.; Tocher, D. R. Vertebrate fatty acyl desaturase with Δ4 activity. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107* (39), 16840–16845.

(5) Bell, M.; Tocher, D. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In *Lipids in Aquatic Ecosystems*; Kainz, M., Brett, M., Arts, M., Eds.; Springer: Berlin, Germany, 2009; pp 211–236.

(6) Cossins, A.; Crawford, D. Fish as models for environmental genomics. *Nat. Rev. Genet.* **2005**, *6* (4), 324–333.

(7) Evans, H.; De Tomaso, T.; Quail, M.; Rogers, J.; Gracey, A.; Cossins, A.; Berenbrink, M. Ancient and modern duplication events and the evolution of stearoyl-CoA desaturases in teleost fishes. *Physiol. Genomics* **2008**, *35* (1), 18.

(8) Polley, S.; Tiku, P.; Trueman, R.; Caddick, M.; Morozov, I.; Cossins, A. Differential expression of cold- and diet-specific genes encoding two carp liver Δ9-acyl-CoA desaturase isoforms. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2003**, *284* (1), R41.

(9) Shanklin, J.; Guy, J. E.; Mishra, G.; Lindqvist, Y. Desaturases: emerging models for understanding functional diversification of diiron-containing enzymes. *J. Biol. Chem.* **2009**, *284* (28), 18559–18563.

(10) Gormaz, J. G.; Rodrigo, R.; Videla, L. A.; Beems, M. Biosynthesis and bioavailability of long-chain polyunsaturated fatty acids in non-alcoholic fatty liver disease. *Prog. Lipid Res.* **2010**, *49* (4), 407–419.

(11) Zheng, X.; Seiliez, I.; Hastings, N.; Tocher, D.; Panserat, S.; Dickson, C.; Bergot, P.; Teale, A. Characterization and comparison of fatty acyl Δ6 desaturase cDNAs from freshwater and marine teleost fish species. *Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol.* **2004**, *139* (2), 269–279.

(12) Hastings, N.; Agaba, M. K.; Tocher, D. R.; Zheng, X.; Dickson, C. A.; Dick, J. R.; Teale, A. J. Molecular cloning and functional characterization of fatty acyl desaturase and elongase cDNAs involved in the production of eicosapentaenoic and docosahexaenoic acids from α-linolenic acid in Atlantic salmon (*Salmo salar*). *Mar. Biotechnol.* **2005**, *6* (5), 463–474.

(13) Hastings, N.; Agaba, M.; Tocher, D. R.; Leaver, M. J.; Dick, J. R.; Sargent, J. R.; Teale, A. J. A vertebrate fatty acid desaturase with Δ5 and Δ6 activities. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98* (25), 14304–14309.

(14) Seiliez, I.; Panserat, S.; Corraze, G.; Kaushik, S.; Bergot, P. Cloning and nutritional regulation of a Δ6-desaturase-like enzyme in the marine teleost gilthead seabream (*Sparus aurata*). *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* **2003**, *135* (3), 449–460.

(15) Agaba, M. K.; Tocher, D. R.; Zheng, X.; Dickson, C. A.; Dick, J. R.; Teale, A. J. Cloning and functional characterisation of polyunsaturated fatty acid elongases of marine and freshwater teleost fish. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* **2005**, *142* (3), 342–352.

(16) Mohd-Yusof, N.; Monroig, O.; Mohd-Adnan, A.; Wan, K. L.; Tocher, D. Investigation of highly unsaturated fatty acid metabolism in the Asian sea bass, *Lates calcarifer*. *Fish Physiol. Biochem.* **2010**, *36* (4), 827–843.

(17) Alhazzaa, R.; Bridle, A. R.; Nichols, P. D.; Carter, C. G. Replacing dietary fish oil with Echium oil enriched barramundi with C$_{18}$ PUFA rather than long-chain PUFA. *Aquaculture* **2011**, *312* (1–4), 162–171.

(18) Whelan, J.; Rust, C. Innovative dietary sources of n-3 fatty acids. *Annu. Rev. Nutr.* **2006**, *26*, 75–103.

(19) James, M. J.; Ursin, V. M.; Cleland, L. G. Metabolism of stearidonic acid in human subjects: comparison with the metabolism of other n-3 fatty acids. *Am. J. Clin. Nutr.* **2003**, *77* (5), 1140–1145.

(20) Surette, M. E.; Edens, M.; Chilton, F. H.; Tramposch, K. M. Dietary echium oil increases plasma and neutrophil long-chain (n-3) fatty acids and lowers serum triacylglycerols in hypertriglyceridemic humans. *J. Nutr.* **2004**, *134* (6), 1406–1411.

(21) Harris, W. S.; Lemke, S. L.; Hansen, S. N.; Goldstein, D. A.; DiRienzo, M. A.; Su, H.; Nemeth, M. A.; Taylor, M. L.; Ahmed, G.; George, C. Stearidonic acid-enriched soybean oil increased the omega-3 index, an emerging cardiovascular risk marker. *Lipids* **2008**, *43* (9), 805–811.

(22) Harris, W. S.; DiRienzo, M. A.; Sands, S. A.; George, C.; Jones, P. G.; Eapen, A. K. Stearidonic acid increases the red blood cell and heart eicosapentaenoic acid content in dogs. *Lipids* **2007**, *42* (4), 325–333.

(23) Ishihara, K.; Komatsu, W.; Saito, H.; Shinohara, K. Comparison of the effects of dietary α-linolenic, stearidonic, and eicosapentaenoic acids on production of inflammatory mediators in mice. *Lipids* **2002**, *37* (5), 481–486.

(24) Kitessa, S. M.; Young, P. Echium oil is better than rapeseed oil in enriching poultry meat with *n*-3 polyunsaturated fatty acids, including eicosapentaenoic acid and docosapentaenoic acid. *Br. J. Nutr.* **2009**, *101* (5), 709–715.

(25) Bell, J. G.; Strachan, F.; Good, J. E.; Tocher, D. R. Effect of dietary echium oil on growth, fatty acid composition and metabolism, gill prostaglandin production and macrophage activity in Atlantic cod (*Gadus morhua* L.). *Aquacult. Res.* **2006**, *37* (6), 606–617.

(26) Tocher, D. R.; Dick, J. R.; MacGlaughlin, P.; Bell, J. G. Effect of diets enriched in Δ6 desaturated fatty acids (18:3n-6 and 18:4n-3), on growth, fatty acid composition and highly unsaturated fatty acid synthesis in two populations of Arctic charr (*Salvelinus alpinus* L.). *Comp. Biochem. Physiol. B* **2006**, *144* (2), 245–253.

(27) Bharadwaj, A. S.; Hart, S. D.; Brown, B. J.; Li, Y.; Watkins, B. A.; Brown, P. B. Dietary source of stearidonic acid promotes higher muscle DHA concentrations than linolenic acid in hybrid striped bass. *Lipids* **2010**, *45* (1), 21–27.

(28) Miller, M. R.; Nichols, P. D.; Carter, C. G. Replacement of dietary fish oil for Atlantic salmon parr (*Salmo salar* L.) with a stearidonic acid containing oil has no effect on omega-3 long-chain polyunsaturated fatty acid concentrations. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* **2007**, *146* (2), 197–206.

(29) AOAC. *Official Methods of Analysis of AOAC International*, 18th ed., rv 3; AOAC International: Gaithersburg, MD, 2010.

(30) Bligh, E.; Dyer, W. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37* (8), 911–917.

(31) Miller, M. R.; Bridle, A. R.; Nichols, P. D.; Carter, C. G. Increased elongase and desaturase gene expression with stearidonic acid enriched diet does not enhance long-chain (n-3) content of seawater Atlantic salmon (*Salmo salar* L.). *J. Nutr.* **2008**, *138* (11), 2179–2185.

(32) Codabaccus, M. B.; Bridle, A. R.; Nichols, P. D.; Carter, C. G. Effect of feeding Atlantic salmon (*Salmo salar* L.) a diet enriched with stearidonic acid from parr to smolt on growth and n-3 LC-PUFA biosynthesis. *Br. J. Nutr.* **2011**, *105*, 1772–1782.

(33) Guschina, I. A.; Harwood, J. L. Mechanisms of temperature adaptation in poikilotherms. *FEBS Lett.* **2006**, *580* (23), 5477–5483.

(34) Guderley, H. Metabolic responses to low temperature in fish muscle. *Biol. Rev. Camb. Philos. Soc.* **2004**, *79* (2), 409–427.

(35) Buda, C.; Dey, I.; Balogh, N.; Horvath, L. I.; Maderspach, K.; Juhasz, M.; Yeo, Y. K.; Farkas, T. Structural order of membranes and composition of phospholipids in fish brain cells during thermal acclimatization. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91* (17), 8234–8238.

(36) Li, Y. Y.; Hu, C. B.; Zheng, Y. J.; Xia, X. A.; Xu, W. J.; Wang, S. Q.; Chen, W. Z.; Sun, Z. W.; Huang, J. H. The effects of dietary fatty acids on liver fatty acid composition and Δ^{6}-desaturase expression differ with ambient salinities in *Siganus canaliculatus*. *Comp. Biochem. Physiol. B* **2008**, *151* (2), 183–190.

(37) Miller, M. R.; Nichols, P. D.; Carter, C. G. *n*-3 oil sources for use in aquaculture — alternatives to the unsustainable harvest of wild fish. *Nutr. Res. Rev.* **2008**, *21* (2), 85–96.

(38) Tu, W. C.; Cook-Johnson, R. J.; James, M. J.; Mühlhäusler, B. S.; Gibson, R. A. Omega-3 long chain fatty acid synthesis is regulated more by substrate levels than gene expression. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2010**, *83* (2), 61–68.

(39) Jakobsson, A.; Westerberg, R.; Jacobsson, A. Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog. Lipid Res.* **2006**, *45* (3), 237–249.

(40) Skrzypski, J.; Bellenger, S.; Bellenger, J.; Sinclair, A.; Poisson, J. P.; Tessier, C.; Rialland, M.; Narce, M. Revisiting Δ-6 desaturase regulation by C18 unsaturated fatty acids, depending on the nutritional status. *Biochimie* **2009**, *91* (11–12), 1443–1449.

(41) Díaz-López, M.; Pérez, M. J.; Acosta, N. G.; Jerez, S.; Dorta-Guerra, R.; Tocher, D. R.; Lorenzo, A.; Rodríguez, C. Effects of dietary fish oil substitution by Echium oil on enterocyte and hepatocyte lipid metabolism of gilthead seabream (*Sparus aurata* L.). *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* **2010**, *155* (4), 371–379.

(42) Nakamura, M. T.; Nara, T. Y. Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases. *Annu. Rev. Nutr.* **2004**, *24*, 345–376.

(43) Yang, Q.; O'Shea, T. M. Dietary Echium oil increases tissue (n-3) long-chain polyunsaturated fatty acids without elevating hepatic lipid concentrations in premature neonatal rats. *J. Nutr.* **2009**, *139* (7), 1353–1359.

(44) Díaz-López, M.; Pérez, M. J.; Acosta, N. G.; Tocher, D. R.; Jerez, S.; Lorenzo, A.; Rodríguez, C. Effect of dietary substitution of fish oil by Echium oil on growth, plasma parameters and body lipid composition in gilthead seabream (*Sparus aurata* L.). *Aquacult. Nutr.* **2009**, *15* (5), 500–512.

(45) Tocher, D. R.; Francis, D. S.; Coupland, K. n-3 Polyunsaturated fatty acid-rich vegetable oils and blends. In *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*; Turchini, G. M., Ng, W.-K., Tocher, D. R., Eds.; CRC Press: Boca Raton, FL, 2010; pp 209–244.

(46) Zheng, X.; Torstensen, B. E.; Tocher, D. R.; Dick, J. R.; Henderson, R. J.; Bell, J. G. Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo salar*). *Biochim. Biophys. Acta* **2005**, *1734* (1), 13–24.

(47) Tocher, D.; Zheng, X.; Schlechtriem, C.; Hastings, N.; Dick, J.; Teale, A. Highly unsaturated fatty acid synthesis in marine fish: cloning, functional characterization, and nutritional regulation of fatty acyl Δ6 desaturase of Atlantic cod (*Gadus morhua* L.). *Lipids* **2006**, *41* (11), 1003–1016.

(48) Tocher, D. R. Glycerophospholipid metabolism. In *Biochemistry and Molecular Biology of Fishes*; Hochachka, P. W., Mommsen, T. P., Eds.; Elsevier: Amsterdam, The Netherlands, 1995; Vol. 4, pp 119–157.

(49) Tocher, D. R.; Bell, J. G.; MacGlaughlin, P.; McGhee, F.; Dick, J. R. Hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition of liver in salmonids: effects of dietary vegetable oil. *Comp. Biochem. Physiol. B* **2001**, *130* (2), 257–270.

(50) Yang, X. W.; Dick, T. A. Arctic char (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*) differ in their growth and lipid metabolism in response to dietary polyunsaturated fatty acids. *Can. J. Fish. Aquat. Sci.* **1994**, *51* (6), 1391–1400.

(51) Hazel, J. R. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annu. Rev. Physiol.* **1995**, *57*, 19–42.

(52) Tocher, D. R.; Agaba, M.; Hastings, N.; Bell, J. G.; Dick, J. R.; Teale, A. J. Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis niloticus*). *Fish Physiol. Biochem.* **2001**, *24* (4), 309–320.

(53) Stillwell, W.; Wassall, S. R. Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chem. Phys. Lipids* **2003**, *126* (1), 1–27.

(54) Hulbert, A. J. Membrane fatty acids as pacemakers of animal metabolism. *Lipids* **2007**, *42* (9), 811–819.

(55) Hashimoto, M.; Hossain, S.; Yamasaki, H.; Yazawa, K.; Masumura, S. Effects of eicosapentaenoic acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells. *Lipids* **1999**, *34* (12), 1297–1304.

(56) Pike, L. J.; Han, X.; Chung, K. N.; Gross, R. W. Lipid rafts are enriched in arachidonic acid and plasmenylethanolamine and their composition is independent of caveolin-1 expression: a quantitative electrospray ionization/mass spectrometric analysis. *Biochemistry* **2002**, *41* (6), 2075–2088.

(57) Li, Q.; Zhang, Q.; Wang, M.; Zhao, S.; Ma, J.; Luo, N.; Li, N.; Li, Y.; Xu, G.; Li, J. Eicosapentaenoic acid modifies lipid composition in caveolae and induces translocation of endothelial nitric oxide synthase. *Biochimie* **2007**, *89* (1), 169–177.

(58) Peet, M.; Shah, S.; Selvam, K.; Ramchand, C. Polyunsaturated fatty acid levels in red cell membranes of unmedicated schizophrenic patients. *World J. Biol. Psychiatry* **2004**, *5* (2), 92–99.

(59) Harpaz, S.; Hakim, Y.; Slosman, T.; Eroldogan, O. T. Effects of adding salt to the diet of Asian sea bass *Lates calcarifer* reared in fresh or salt water recirculating tanks, on growth and brush border enzyme activity. *Aquaculture* **2005**, *248* (1–4), 315–324.

(60) Nankervis, L.; Southgate, P. Enzyme and acid treatment of fish meal for incorporation into formulated microbound diets for barramundi (*Lates calcarifer*) larvae. *Aquacult. Nutr.* **2009**, *15* (2), 135–143.

(61) Katersky, R. S.; Carter, C. G. Growth and protein synthesis of barramundi, *Lates calcarifer*, fed lupin as a partial protein replacement. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* **2009**, *152* (4), 513–517.

(62) Glencross, B.; Rutherford, N. A determination of the quantitative requirements for docosahexaenoic acid for juvenile barramundi (*Lates calcarifer*). *Aquacult. Nutr.* **2010**, *17* (2), e536–e548 (doi: 10.1111/j.1365-2095.2010.00795).

(63) Fuller, S. C.; Frank, D. C.; Fitzhenry, M. J.; Smyth, H. E.; Poole, S. E. Improved approach for analyzing bromophenols in seafood using stable isotope dilution analysis in combination with SPME. *J. Agric. Food Chem.* **2008**, *56* (18), 8248–8254.

(64) Frank, D.; Poole, S.; Kirchhoff, S.; Forde, C. Investigation of sensory and volatile characteristics of farmed and wild barramundi (*Lates calcarifer*) using gas chromatography–olfactometry mass spectrometry and descriptive sensory analysis. *J. Agric. Food Chem.* **2009**, *57* (21), 10302–10312.

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