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*)

Xiaozhong Zheng[a], Bente E. Torstensen[b], Douglas R. Tocher[a,*], James R. Dick[a], R. James Henderson[a], J. Gordon Bell[a]

[a]*Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK*

[b]*Institute of Nutrition and Seafood Research, N-5804 Bergen, Norway*

* Corresponding author. Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland. Tel.: +44-1786-467996; fax.: +44-1786-472133.

E-mail address: d.r.tocher@stir.ac.uk

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Abbreviations FO, fish oil; HUFA, highly unsaturated fatty acids (carbon chain length ≥ C_{20} with ≥ 3 double bonds); VO, vegetable oil.

Abstract

Highly unsaturated fatty acid (HUFA) synthesis in Atlantic salmon (*Salmo salar*) was known to be influenced by both nutritional and environmental factors. Here we aimed to test the hypothesis that both these effectors involved similar molecular mechanisms. Thus, HUFA biosynthetic activity and the expression of fatty acyl desaturase and elongase genes were determined at various points during an entire two year production cycle in salmon fed diets containing either 100% fish oil or diets in which a high proportion (75% and 100%) of fish oil was replaced by C_{18} polyunsaturated fatty acid-rich vegetable oil. The results showed that HUFA biosynthesis in Atlantic salmon varied during the growth cycle with peak activity around seawater transfer and subsequent low activities in seawater. Consistent with this, gene expression of Δ6 desaturase, the rate-limiting step in the HUFA biosynthetic pathway, was highest around the point of seawater transfer and lowest during the seawater phase. In addition, the expression of both Δ6 and Δ5 desaturase genes was generally higher in fish fed the vegetable oil-substituted diets compared to fish fed fish oil, particularly in the seawater phase. Again, generally consistent with this, the activity of the HUFA biosynthetic pathway was invariably higher in fish fed diets in which fish oil was substituted by vegetable oil compared to fish fed only fish oil. In conclusion, these studies showed that both nutritional and environmental modulation of HUFA biosynthesis in Atlantic salmon involved regulation of fatty acid desaturase gene expression.

1. Introduction

Fish are the only major dietary source of n-3 highly unsaturated fatty acids (HUFA) for humans [1] and, with declining fisheries, aquaculture supplies an increasing proportion of the fish in the human diet [2-4]. However, the current high use of fish oils, derived from marine feed-grade fisheries, in aquaculture feeds is not sustainable in the longer term, and will constrain continued growth of aquaculture activities [5]. The only sustainable alternative to fish oils are vegetable oils, which can be rich in C_{18} polyunsaturated fatty acids (PUFA) such as linoleic (18:2n-6) and α-linolenic (18:3n-3) acids, but devoid of the n-3HUFA, eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids, that are abundant in fish oils [6]. The extent to which fish can convert C_{18} PUFA to $C_{20/22}$ HUFA varies with species, and is associated with their capacity for fatty acyl desaturation and elongation [7]. In this context, it is essential to determine what regulates HUFA biosynthesis, and how it can be optimised to enable fish to make effective use of dietary vegetable oil.

Recently, a variety of fatty acid desaturases and elongases, critical enzymes in the pathways for the biosynthesis of the long-chain $C_{20/22}$ HUFA from shorter chain C_{18} PUFA, have been cloned from a range of freshwater and marine teleosts [8-10]. In particular, three cDNAs encoding enzymes in the HUFA biosynthetic pathway have been cloned from Atlantic salmon [11,12]. Heterologous expression in the yeast *Saccharomyces cerevisiae* showed that the cDNAs encoded a Δ6 desaturase responsible for the conversion of 18:3n-3 to 18:4n-3 [12], a Δ5 desaturase responsible for the conversion of 20:4n-3 to 20:5n-3 [11] and a PUFA elongase with high 18:4n-3 to 20:4n-3 activity, but also capable of elongating 20:5n-3 to 22:5n-3 and further to 24:5n-3 [11].

Several studies have shown that the activity of the HUFA biosynthesis pathway in Atlantic salmon was increased in fish in which dietary fish oil was replaced with vegetable oils [13-16]. The underlying cause of the increase in activity in fish fed vegetable oil was unclear, but thought to be mainly due to a

reduction in the suppression of enzyme activity by n-3HUFA abundant in fish oil [17]. The precise mechanism of suppression of activity was not known but, subsequently, the expression of both desaturase and elongase genes were demonstrated to be under nutritional regulation in salmon, being up regulated in a graded manner in the livers of fish fed diets in which graded increments of 18:3n-3-rich linseed oil replaced fish oil [18]. HUFA biosynthetic activity has also been shown to be under environmental regulation in Atlantic salmon, being increased during the period of parr-smolt transformation with peak activities around seawater transfer [19,20]. Although it is known that smoltification is largely controlled by photoperiod with water temperature playing a secondary role, the mechanism whereby these environmental triggers could regulate fatty acid metabolism are unknown [20]. However, feeding fish oil during the period of parr-smolt transformation was shown to attenuate the pre-adaptive rise in HUFA biosynthetic activity and reduce the peak activity at seawater transfer in comparison to fish fed vegetable oil [19,20], suggesting similar biochemical or molecular mechanisms may be involved in both the nutritional and environmental regulation of HUFA biosynthesis in Atlantic salmon.

In the present study we aimed to test the hypothesis that both the nutritional and environmental regulation of HUFA biosynthesis in Atlantic salmon involved a similar molecular mechanism, specifically changes in expression of key genes in the biosynthetic pathway. Thus, HUFA biosynthetic activity and the expression of fatty acyl desaturase and elongase genes were determined at various points during an entire two year production cycle in Atlantic salmon. In addition, the effects of diet on the natural cycle of activity was investigated by feeding the fish diets containing either 100% fish oil or diets in which a high proportion (75% and 100%) of the fish oil was replaced by a vegetable oil blend, formulated to mimic fish oil in saturated and monounsaturated fatty acid content, but with C_{18} PUFA replacing the n-3HUFA.

2. Materials and methods

2.1. Animals and diets

The effect of replacing fish oil with vegetable oil at two replacement levels (75 and 100%) was investigated in Atlantic salmon in a trial conducted over an entire two year production cycle. As the trial was both large scale and long term, it was carried out as a collaboration between the Institute of Aquaculture, University of Stirling, Scotland, and the National Institute of Nutrition and Seafood Research, Bergen, Norway, with the 75% replacement diet in Scotland and the 100% replacement tested in Norway, with the control FO diet replicated at each site. The diets were fed to triplicate tanks/cages and the experiments were performed using identical culture conditions other than the obvious environmental differences such as the slight differences in ambient water temperature at the two sites. In Scotland, the trial was carried out at Marine Harvest Ltd. facilities at Invergarry (freshwater) and Loch Duich, Lochalsh (seawater), and in Norway, the entire trial was conducted at the Nutreco Aquaculture Research Centre, Lerang Research Station, Stavanger. At each site, Atlantic salmon fry were distributed randomly into 6 tanks (3m x 3m, depth 0.5m) at a stocking level of 3000/tank, and weaned onto extruded feeds containing 20% added oil which was either fish oil (FO; capelin oil) or a vegetable oil blend (VO), containing rapeseed, palm and linseed oils in a 3.7 : 2 : 1 ratio, replacing 75% or 100% of the FO. Fish were fed the diets described above for around one year until sea water transfer, at which point fish (average weight ~ 50g) were transferred into 5m x 5m net pens at 700 fish/pen. The fish were fed the same diet in seawater as in freshwater although the dietary oil levels were increased to 25% (3mm pellet) rising to 32% (9mm pellets) through the year long seawater phase. The diets aimed to be practical, and were formulated and manufactured by Nutreco ARC, Stavanger, Norway according to current practices in the salmon feed industry. All diets were formulated to satisfy the nutritional requirements of salmonid fish [21]. The measured proximate and

fatty acid compositions of the diets are given in Table 1. The only significant difference in final weights was that the fish fed 100%VO (2.69 ± 0.07 Kg) were slightly larger than fish fed the FO diet (Norway) (2.36 ± 0.13 Kg) and 75% VO (2.37 ± 0.13 Kg). The final weight of fish fed the FO diet in Scotland was not different to any other treatment (2.54 ± 0.14 Kg).

2.2. Proximate composition of diets

Dry matter in the diets was measured gravimetrically after freeze-drying of homogenised samples for 48 hours. Total nitrogen was determined on homogenised, freeze-dried samples using a nitrogen determinator (LECO, FP-428 system 601-700-500; Perkin Elmer Coop., Ct, USA). Protein was calculated as N x 6.25. Total lipid of the diets was measured gravimetrically after ethyl acetate extraction and acid hydrolysis.

2.3. Lipid extraction and fatty acid analysis

Fish were not fed during the 24 h prior to sampling. Intact livers were dissected from 18 fish (pooled into 6 samples of 3 livers each) per dietary treatment at each sampling point and immediately frozen in liquid nitrogen. Total lipid was extracted from livers by homogenization in chloroform/methanol (2:1, v/v) containing 0.01 % butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch et al. [22]. Fatty acid methyl esters (FAME) were prepared from total lipid by either acid-catalyzed transesterification, with FAME extracted and purified as described previously [23] (Scotland) or by boron trifluoride following saponification essentially as described by Lie et al. [24] (Norway). FAME were separated and quantified by gas-liquid chromatography with on-column injection either using a Thermo Finnegan, Trace 2000 GC (Thermoquest, Hemel Hempstead U.K.) using a fused silica

capillary column (CP wax 52CB; 30m x 0.32 mm id.; Chrompak Ltd., London, U.K) with hydrogen as carrier gas and temperature programming from 50ºC to 150ºC at 40ºC/min and then to 195ºC at 1.5ºC/min (Scotland) then to 195ºC at 0.5ºC/min and finally to 220ºC for 2 min, or using a Thermo Finnegan Trace 2000 GC equipped with a fused silica capillary column (CP-sil 88; 50m x 0.32 mm id.; Chrompak Ltd.) with temperature programming of 60°C for 1 minute, 160°C for 28 minutes, 190 °C for 17 minute, and finally 220°C for 10 minutes with all intervening temperature ramps being at 25 ºC/min (Norway). Individual methyl esters were identified by comparison to known standards and by reference to published data [25]. Data were collected and processed using the Chromcard for Windows (version 1.19) computer package (Thermoquest Italia S.p.A., Milan, Italy) (Scotland) or using the Totalchrom software (ver. 6.2, Perkin Elmer) (Norway).

2.4. RNA extraction and real-time quantitative-PCR (rtqPCR)

Three to six liver samples per dietary treatment and time point were collected, frozen immediately in liquid nitrogen and stored at –80 ºC prior to extraction. Total RNA was isolated from 100 - 500 μg of liver tissue by the standard TRIzol extraction method (Invitrogen Ltd, Paisley, UK) and recovered in 100 μl molecular biology grade water. In order to remove any possible genomic DNA contamination, the total RNA samples were pre-treated using DNA-*free*™ DNase treatment and removal reagents kit (Ambion Inc.,Austin, TX, USA) following the manufacture's protocol. Different rtqPCR systems were used at the two laboratories and so methodologies varied accordingly.

Scotland

Five μg of total RNA was reverse transcribed into cDNA using M-MLV reverse transcriptase first strand cDNA synthesis kit (Promega UK, Southampton, UK). Expression of target genes was normalised using β-Actin as a housekeeping gene. The PCR primers were designed according to the

published salmon Δ6 desaturase (accession no. AY458652), salmon Δ5 desaturase (accession no: AF478472), salmon elongase (accession no: AY170327) and salmon β-actin (accession no: AF012125) cDNA sequences. For the Δ6 desaturase, the forward primer was 5'-CCCCAGACGTTTGTGTCAG-3', and the reverse primer was 5'-CCTGGATTGTTGCTTTGGAT-3'. For the Δ5 desaturase, the forward primer was 5'-GTGAATGGGGATCCATAGCA-3', and the reverse primer was 5'-AAACGAACGGACAACCAGA-3'. For the elongase, the forward and reverse primers were 5'-TGATTTGTGTTCCAAATGGC-3' and 5'-CTCATGACGGGAACCTCAAT-3', respectively. For β-actin, 5'-ACATCAAGGAGAAGCTGTGC -3' and 5'-GACAACGGAACCTCTCGTTA-3' were the forward and reverse primers, respectively. PCR product sizes were 181, 192, 219 and 141bp, respectively. The linearised plasmid DNA containing the target sequence for each gene was quantified to generate a standard curve of known copy number. Amplification of cDNA samples and DNA standards was carried out using the SYBR Green PCR Kit (Qiagen, Crowley, West Sussex, UK) in the following conditions: denaturation for 15 min at 95 ºC followed by 45 cycles of 15 s at 94 ºC, 15 s at 55 ºC and 30 s at 72 ºC, followed by product melting to check purity of PCR product. Thermal cycling and fluorescence detection were conducted using the Rotor-Gene 3000 system (Corbett Research, Cambridge, UK). The copy number of the target gene in the sample was obtained and the ratio of copy numbers between this gene and β-actin was calculated.

Norway

RT- PCR was performed using a modified TaqMan reverse transcription protocol (Applied Biosystems, California, USA), before subjecting to rtqPCR (TaqMan universal PCR master mix, Applied Biosystems). RT-PCR efficiency was monitored using two fold dilution curves of RNA. Expression of target genes was normalised using elongation factor α (E1α) as a housekeeping gene. Primers and probes (TaqManMGB) were constructed based on exon-exon boundaries, and designed using Assay by Design (Applied Biosystems). The PCR primers and TaqManMGB probes were designed according to

the published salmon Δ6 and Δ5 desaturases (as above) and elongation factor alpha (E1α) (accession no: AF321836). For Δ6 desaturase, the forward primer was 5'-GGGATTTAATCCATCGCATATTAACT-3', the reverse primer was 5'-CGTCACAACAAAATACAGCATCTG-3', and the probe was 5'FAM-TGTGAACAGAGATAGTTTCCCCAGACGTTTG-MGBNFQ. For the Δ5 desaturase, the forward primer was 5'-GGAACCACAAACTGCACAAGT-3', the reverse primer was 5'-GTGCTGGAAGTGACGATGGT-3', and the probe was 5'FAM-CAGAGGCACCCTTTAGGTG-MGBNFQ, respectively. For the elongation factor alpha (E1 α), the forward and reverse primers and probe were 5'-CCCCTCCAGGACGTTTACAAA -3', 5'-CACACGGCCCACAGGTACA-3', and 5'-FAM-ATCGGTGGTATTGGAAC-MGBNFQ-3', respectively. For the two fold dilution curve four concentrations were used (from 250 ng total RNA). For analysis of gene expression (separate 96 well plates) 3 parallels were used at a total RNA concentration at 125 ng (± 5 %). The following conditions were used for amplification of cDNA: 2 min at 50ºC followed by denaturation for 10 min at 95ºC followed by 50 cycles of 15 s at 95ºC and 1 min at 60ºC. Thermal cycling and fluorescence detection was done using ABI Prism 7000 Sequence Detection System (Applied Biosystems, Oslo, Norway). QGene was used for normalisation, and calculation of relative expression data [26]. QGene takes into account the PCR efficacy, calculated on the basis of two fold dilution curves, obtaining normalised expression data.

2.5. Assay of hepatocyte fatty acyl desaturation/elongation activities

Fish were killed by a blow to the head and the livers dissected immediately. Enriched hepatocyte preparations were prepared by collagenase treatment and sieving as described in detail previously [17]. Five ml of each hepatocyte suspension were dispensed into a 25 cm^2 tissue culture flask and the cells

incubated at 20 ºC for 2 h with 0.25 μCi (~ 1 μM) [1-^{14}C] 18:3n-3, added as a complex with fatty acid-free bovine serum albumin (FAF-BSA) in phosphate buffered saline prepared as described previously [27]. After incubation, the cell suspension was transferred to glass conical test tubes and centrifuged at 500 g for 2 min. The supernatant was discarded and the hepatocyte cell pellet washed twice with 5 ml of ice-cold Hank's balanced salt solution without Ca^{2+} and Mg^{2+} (HBSS) with the first wash also containing 1% FAF-BSA. Total lipid was extracted from the washed cell pellets using ice-cold chloroform/methanol (2:1, v/v) containing 0.01% (w/v) BHT as described in detail previously [28]. Total lipid was transmethylated and FAME prepared as described above. The methyl esters were redissolved in 100 μl isohexane containing 0.01% BHT and applied as 2.5 cm streaks to TLC plates impregnated by spraying with 2 g silver nitrate in 20 ml acetonitrile and pre-activated at 110 ºC for 30 min. Plates were fully developed in toluene/acetonitrile (95:5, v/v) [29]. Autoradiography was performed with Kodak MR2 film for 6 days at room temperature. Areas of silica containing individual PUFA were scraped into scintillation mini-vials containing 2.5 ml of scintillation fluid (Ecoscint A, National Diagnostics, Atlanta, Georgia) and radioactivity determined in a TRI-CARB 2000CA scintillation counter (United Technologies Packard, U.K.). Results were corrected for counting efficiency and quenching of ^{14}C under exactly these conditions. Protein concentrations in isolated hepatocyte suspensions were determined according to the method of Lowry et al. [30] after incubation with 0.4 ml of 0.25% (w/v) SDS/1M NaOH for 45 min at 60ºC.

2.6. Materials

[1-^{14}C]18:3n-3 (50-55 mCi/mmol) was obtained from NEN (DuPont (U.K.) Ltd., Stevenage, U.K.). HBSS, collagenase (type IV), FAF-BSA, BHT and silver nitrate were obtained from Sigma Chemical Co. (Poole, U.K.). Thin-layer chromatography (TLC) plates, precoated with silica gel 60 (without

fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fisher Scientific UK, Loughborough, England.

2.7. Statistical analysis

All the data are presented as means ± SD (n = 3, 4 or 6 as stated). The effects of oil composition on dietary fatty acid compositions and the effects of diet on salmon liver fatty acid compositions were analyzed by one-way ANOVA, followed where appropriate by Tukey's post test to determine significant differences between treatments. Percentage data and data which were identified as non-homogeneous (Bartlett's test) were subjected to either arcsine or log transformation before analysis. The significance of effects of diet and time of sampling on hepatocyte HUFA biosynthetic activity and hepatic expression of genes of HUFA biosynthesis were determined by two-way ANOVA with Bonferroni post-tests. Differences were reported as significant when $P < 0.05$ [31].

3. Results

3.1. Dietary fatty acid compositions

In freshwater, the control diet (FO), formulated with 100% FO, contained approximately 20% total saturates, mainly 16:0 and 14:0, almost 60% total monounsaturated fatty acids over half of which were the long chain monoenes, 20:1 and 22:1, 4.4% n-6 fatty acids predominantly 18:2n-6, and 15% n-3 fatty acids, predominantly the n-3HUFA, 20:5n-3 and 22:6n-3 in approximately equal amounts, and less than 1% 18:3n-3 (Table 1). Replacement of FO with the vegetable oil blend resulted in increased percentages of 18:3n-3, 18:2n-6 and 18:1n-9 with concomitant decreased proportions of n-3HUFA, 20:1, 22:1 and total monoenes. These effects were quantitatively greater in the diet with the higher

level of FO replacement, diet VO100. The FO diet in seawater was characterized by having lower 20:1 and higher 22:6n-3 compared to the freshwater phase due to seasonal variation in batches of capelin oil. However, replacement of FO with the VO blend had similar effects in seawater diets as in the freshwater diets other than the fact that total monoene levels were unchanged by substitution (Table 1). The vegetable oil (VO) blend was formulated to mimic FO in total saturated, monounsaturated and polyunsaturated fatty acid content and this was largely achieved, particularly in the seawater diets.

3.2. Effects of diet on liver fatty acid compositions

The principal effects of feeding the diets containing the VO blend on liver fatty acid composition were very much a reflection of the changes in fatty acid compositions of the diets described above. Therefore, substituting FO with VO resulted in increased proportions of 18:1n-9, 18:2n-6 and 18:3n-3, and reduced proportions of 20:5n-3, 22:6n-3, 20:1 and 22:1 in total lipid of livers (Tables 2 & 3). Percentages of desaturated and elongated products of 18:2n-6 including 20:3n-6 and 20:4n-6, and the dead-end product 20:2n-6 were also increased by feeding the diets containing VO, with the highest percentages of these fatty acids in fish fed diet VO100 (Tables 2 & 3). In contrast, the percentages of desaturated and elongated products of 18:3n-3, such as 18:4n-3 and 20:4n-3, were generally not significantly increased in liver by feeding the diets containing VO. Generally, there was no major effect of time of feeding on the fatty acid composition of liver total lipid. Thus, the levels of 18:1n-9, 18:2n-6, 18:3n-3, 20:5n-3 and 22:6n-3 observed in fish fed VO after 16 (Norway) or 20 (Scotland) months feeding were not very different to the percentages observed at 6 (Norway) and 9 (Scotland) months of feeding. Some variations observed over the time scale were due to batch variation in the various dietary oils used.

3.3. Effects of diet on activity of the hepatocyte HUFA biosynthesis pathway

In the Scottish trial, the activity of the HUFA biosynthetic activity in isolated hepatocytes was determined at eight points throughout the growth cycle. The data clearly showed that the activity of the pathway increased to peak activity around the point of seawater transfer and declined again after transfer (Fig.1). HUFA biosynthetic capacity was always greater in fish fed the VO75 diet, significantly so in the period approaching seawater transfer and also at most points in seawater despite the lower activities in general. The activity of the HUFA biosynthetic pathway in isolated hepatocytes was also measured at three selected time points in the Norwegian trial, specifically 6, 9 and 12 months that corresponded to the 9, 12 and 20-month samples in the Scottish trial. Again at all time points, the fish fed the VO100 blend showed significantly higher HUFA synthesis activity compared to fish fed the FO diet (Fig. 2). The temporal pattern of activity was very similar to that in the Scottish trial, with highest activities observed at 9 months, just prior to seawater transfer, and the lowest activities at 17 months in seawater. The results of two-way ANOVA confirmed that HUFA biosynthesis in Atlantic salmon was significantly affected both by the substitution of dietary FO with VO and also by the time of sampling in the growth cycle irrespective of trial location (Table 4).

3.4. Effects of diet on liver fatty acid desaturase and elongase gene expression

The expression of Δ6, Δ5 desaturase and fatty acid elongase genes was determined by rtqPCR at selected time points during the growth cycle in the Scottish trial. The expression of all the genes examined was significantly affected by time of sampling (Table 4). The expression of Δ6 desaturase, the enzyme regarded as the rate-limiting step in the pathway, was highest at the 12 and 12.75-month time points, either side of seawater transfer, and lowest at the later point in seawater, 20 months (Fig. 3). A similar temporal pattern, although quantitatively lesser, was observed with Δ5 desaturase expression but elongase expression was highest at the last sampling point in seawater (Fig.3). The

expression of both desaturase genes was always greater in fish fed VO compared to fish fed FO, significantly so at 9 and 20 months (Fig.3). In contrast, fatty acid elongase expression was greater in fish fed FO at the latter time point. The expression of Δ6 and Δ5 desaturase genes was also significantly affected by time of sampling in the Norwegian trial (Table 4). In particular, the expression of the Δ6 desaturase gene peaked just prior to seawater transfer and was low in seawater (Fig. 4). In contrast, Δ5 desaturase showed higher levels of expression early in the freshwater phase and then at the final sample point in seawater. Both desaturases showed significant effects of diet with the expression of both being significantly higher in fish fed VO at all points in seawater (Fig.4), although this was masked in the two-way ANOVA for Δ6 by the fact that the expression was higher in fish fed FO just prior to transfer (Table 4).

4. Discussion

The primary aim of this study was to determine if similar molecular mechanisms were involved in both the nutritional and environmental regulation of HUFA synthesis in Atlantic salmon. The results show that the mechanisms whereby HUFA synthesis is regulated by both nutritional and environmental factors involved modulation of gene expression of fatty acyl desaturases, but not of elongase. This is the first report in fish, and vertebrates in general, showing the regulation of the expression of the genes of HUFA synthesis (Δ6 and Δ5 desaturases) in response to an ultimately environmental cue. Regulation of Δ6 and Δ5 desaturase gene expression by nutritional factors has been reported previously. Recently, in a study where salmon were fed diets in which the FO was replaced in a graded manner by linseed oil, the expression of fatty acid Δ5 desaturase and fatty acid elongase in liver were increased in a graded manner by increasing dietary linseed oil [18]. Expression of both genes was positively and negatively correlated with dietary 18:3n-3 and n-3HUFA, respectively. Similarly, increased transcript level in liver of a putative desaturase was reported in rainbow trout fed linseed oil

compared to trout fed fish oil [32]. In addition, transcript level of a putative desaturase was highly expressed in livers from sea bream fed a HUFA-free diet and only slightly expressed in livers from fish fed a HUFA-rich diet [33]. Both the trout and sea bream putative desaturases were later confirmed as Δ6 desaturases [10]. Nutritional regulation of fatty acid desaturase gene expression has also been reported in mammals. The levels of hepatic mRNA for both Δ6 and Δ5 desaturases in rats fed either safflower oil (18:2n-6) or fish oil (n-3HUFA) were only 25% of those in rats fed a fat-free diet or a diet containing triolein (18:1n-9) [34]. Similarly, in mice fed an EFA-deficient diet (triolein), the mRNA abundance of hepatic Δ6 desaturase was double that in mice fed corn oil rich in 18:2n-6 [35].

Nutritional regulation of desaturase gene expression, via specific transcription factors, may be triggered indirectly via nutritionally modulated hormones such as insulin, or directly via nutrients such as dietary fatty acids, particularly unsaturated fatty acids and HUFA or their derivatives such as eicosanoid-like molecules [36,37]. Transcription factors (trans elements) involved in regulating PUFA desaturases are beginning to be determined with the two most studied being sterol response element binding proteins (SREBPs) and peroxisome proliferator activated receptors (PPARs). Dual regulation of mouse Δ6 and Δ5 desaturase gene expression by SREBP-1c and PPARα has been demonstrated, with over expression of SREBP-1 increasing transcription, and disruption of SREBP-1 gene reducing transcription of both desaturase genes [38]. In addition, fibrate, a peroxisome proliferator (PP) and pharmacological ligand for PPARα, also significantly increased the expression of both Δ6 and Δ5 desaturase [38]. This is paradoxical because SREBP-1c transactivates genes of fatty acid synthesis such as fatty acid synthetase and acetyl-CoA carboxylase (and also cholesterol synthesis), whereas PPARs induce enzymes for fatty acid oxidation [39,40]. Indeed, desaturases are unique in that they are the only genes whose transcription is activated by both SREBP and PPARs [39,40]. It has been suggested that the effect of peroxisome proliferators and PPARs on desaturase activity are not direct, but rather a secondary compensatory response due to PPARα switching on β-oxidation resulting in an

increased demand for HUFA [39,40]. However, other studies have shown direct effects of PPARs on desaturase gene transcription. Wy-14,643, a PPARα activator, increased transcription of the human Δ6 desaturase gene by 500%, an effect that was dependent upon PPARα expression [41]. Most interestingly, the natural endogenous activators of PPARs include specific unsaturated fatty acids and eicosanoid-like fatty acid derivatives [42]. Factors that affect desaturase gene expression via SREBP-1c include insulin and oxysterols (via LXR) that stimulate, and HUFA, glucagon, adrenalin and steroid hormones that inhibit. The mechanism of membrane HUFA suppression of SREBP-1c includes the inhibition of maturation and release of mature SREBP, and also an increased rate of decay [39,40]. HUFA may also have inhibitory effects on desaturase expression by inhibiting the effects of oxysterols or NF-Y [39,40]. The cis-elements for SREBPs and PPARs are also beginning to be identified in mammals, and sterol response elements (SREs) and peroxisome proliferator response elements (PPREs) have been identified in the promoter region for human Δ6 desaturase [41,43].

In contrast, there are no data on environmental factors affecting expression of fatty acid Δ6 and Δ5 desaturase genes. However, regulation of other fatty acid desaturase genes by environmental factors is well established, specifically in relation to homeoviscous adaptation. The expression of stearoyl-CoA (Δ9) desaturase, responsible for the conversion of 18:0 to 18:1n-9, has been shown to be cold-induced in carp (*Cyprinus carpio*) [44]. Subsequently, it was demonstrated that there were two Δ9 isoforms in carp, Cds1 and Cds2, which showed differential expression in response to diet (induced by high saturated fat) and temperature, respectively [45]. The primary environmental cue for smoltification is photoperiod (day length) and a large number of endocrine systems are switched on during this period including melatonin, growth hormone, thyroid hormones, insulin-like growth factor (IGF) and prolactin [46-48]. Surprisingly, little is known about the possible interaction of these hormones and their tissue receptors with transcription factors especially in fish [49]. Therefore, the involvement of specific transcription factors and the mechanism of signal transduction are unclear, but there are many potential

mechanisms whereby photoperiod could affect hepatic fatty acid desaturase gene expression.

An obvious likely outcome of changes in gene expression is a change in protein function such as enzymatic activity. In the present study, the changes in gene expression observed in response to dietary and environmental factors were related to changes in enzyme activities. Thus, the increased expression of Δ6 desaturase gene expression, irrespective of diet, observed around the point of seawater transfer in both Scottish and Norwegian trials, was reflected in increased activity of the HUFA biosynthesis pathway. The increased activity in the pathway around seawater transfer has been observed previously [19,20,50], but this is the first report linking this to increased expression of a desaturase gene. Furthermore, the lowest levels of Δ6 desaturase expression were obtained in seawater and this was reflected in the lowest activities of the HUFA biosynthetic pathway. In contrast, although Δ5 desaturase and fatty acid elongase gene expression also showed temporal variation, neither correlated with the changes in HUFA biosynthetic pathway activity. These data were consistent with Δ6 desaturase being the rate-limiting step in the pathway [51], and suggest that the mechanism of regulation of HUFA biosynthesis in response to seasonal effects, including smoltification, were primarily due to regulation of Δ6 desaturase expression.

The altered expression of genes of HUFA biosynthesis in response to dietary oil was largely reflected in altered activity of the HUFA biosynthetic pathway. In both the Scottish and Norwegian trials, replacing FO with VO consistently resulted in increased activity of the HUFA biosynthetic pathway in isolated hepatocytes, and this has also been observed in a number of trials from a variety of fish species including salmonids [13-16,19,20,52,53]. Although not always attaining statistical significance, the expression of Δ6 desaturase in fish fed VO exceeded that in fish fed FO in all samples other than at 9 months in the Norwegian trial. Furthermore, in all samples obtained from fish in seawater irrespective of location, the expression of both Δ6 and Δ5 desaturases were significantly higher in fish fed VO compared to fish fed FO. Previously, we had shown that the expression of liver

Δ5 desaturase in salmon was increased by dietary linseed oil [18]. Therefore, the data indicate that both Δ6 and Δ5 desaturase gene expression are under nutritional regulation, consistent with the findings of earlier biochemical studies [51]. However, in the previous trial with linseed oil, fatty acid elongase gene expression was also increased in response to increased dietary linseed oil, and correlated with the activity of the HUFA biosynthetic pathway [18]. In contrast, in the present trial, elongase gene expression was not increased by feeding the VO blend and did not correlate with HUFA biosynthetic pathway activity. The reason for the differential response in elongase gene expression to the different VO diets is unclear. Furthermore, there are few other studies to compare in which gene expression and enzyme activity have both been measured. However, in mice fed an 18:1n-9-rich, EFA-deficient diet, the mRNA abundance and hepatic Δ6 desaturase activity were, respectively, 50% and 70% higher than in mice fed a diet rich in 18:2n-6 [35].

The end result of the dietary input combined with endogenous metabolism is the tissue fatty acid composition. The present trial showed that liver desaturase gene expression and activity of the HUFA biosynthetic pathway in hepatocytes were both increased in fish fed VO and some indications of these effects could be observed in the liver fatty acid compositions. The levels of n-6 fatty acids such as 20:2n-6 and 20:3n-6 were increased in fish fed VO indicating metabolism of 18:2n-6. Furthermore, the levels of 18:2n-6 and 18:3n-3 decreased in the sample just prior to seawater transfer (months 12 and 9 in Scotland and Norway) coinciding with the peak in HUFA pathway activity. However, no increase in metabolites of 18:3n-3 were observed in fish fed VO indicating that the reduced dietary input of 18:4n-3, 20:4n-3, 20:5n-3 and 22:6n-3 far outweighs the effects of increased HUFA synthesis. Therefore, although the reduced level of 20:5n-3 and 22:6n-3 in the VO diets was largely balanced by increased 18:3n-3, thus providing sufficient 18:3n-3 for potential conversion to 20:5n-3 and 22:6n-3, feeding high levels of VO to salmon resulted in tissues with increased C_{18} PUFA and reduced n-3HUFA. This was despite the increase in gene expression, and increased activity of the HUFA biosynthetic pathway.

Increased C_{18} PUFA and reduced n-3HUFA in salmon fed high levels of VO have been observed previously [13,14,16,17,50,52,54,55].

In conclusion this study has shown that both nutritional and environmental modulation of HUFA biosynthesis in Atlantic salmon involved regulation of fatty acid desaturase gene expression. Specifically, we show for the first time that the increase in HUFA biosynthesis observed in Atlantic salmon during parr-smolt transformation involved increased fatty acid Δ6 desaturase gene expression, and that both Δ6 and Δ5 desaturase genes were up-regulated in response to feeding diets in which high levels of FO were replaced by VO.

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Table legends:

Table 1. Proximate compositions (percentage of diet weight) and fatty acid compositions (percentage by weight of total fatty acids) of represenatative diets used in freshwater (3mm) and seawater (9 mm pellet). Results are means ± SD (n=3 for proximates and n = 2 for fatty acids). [1]totals include 15:0 present at up to 0.5%;[2]also contains n-9 isomer; [3]also contains n-11 and n-7 isomers; [4]also contains n-9 and n-7 isomers; [5]totals include 18:3n-6, 20:2n-6 and 22:5n-6 present at up to 0.2%; [6]totals include 20:3n-3 present at up to 0.1%; 7totals include C_{16} PUFA present at up to 1.1% in FO diets and up to 0.5% in VO diets.

Table 2. Effect of feeding diets containing vegetable oil on the fatty acid composition (percentage of weight) of total lipid from livers of Atlantic salmon (*Salmo salar*) at selected freshwater stages from Scotland (S) and Norway (N).

Results are means ± SD (n=4). [1]totals include 15:0 present at up to 0.3%; [2]also contains n-9 isomer; [3]also contains n-11 and n-7 isomers; [4]also contains n-9 isomer; [5]totals include 18:3n-6 and 22:5n-6 present at up to 0.3%; [6]totals include 20:3n-3, present at up to 0.3%; [7]Totals include C_{16} PUFA, present at up to 0.3%. FO, fish oil diet; VO75 and VO100, diets with 75% and 100% of fish oil replaced by vegetable oil.

Table 3. Effect of feeding diets containing vegetable oil on the fatty acid composition (percentage of weight) of total lipid from livers of Atlantic salmon (*Salmo salar*) at selected seawater stages from Scotland (S) and Norway (N).

Results are means ± SD (n=4). [1]totals include 15:0 present at up to 0.3%; [2]also contains n-9 isomer; [3]also contains n-11 and n-7 isomers; [4]also contains n-9 isomer; [5]totals include 18:3n-6 and 22:5n-6

present at up to 0.6%; [6]totals include 20:3n-3 present at up to 0.6%; [7]totals include C_{16} PUFA present at up to 0.5%. FO, fish oil diet; VO75 and VO100, diets with 75% and 100% of fish oil replaced by vegetable oil.

Table 4. Significance of combined effects of diet (fish oil, FO v. vegetable oil, VO) and time (sampling point) as determined by two-way ANOVA analyses of the data in Figures 2 and 3 for highly unsaturated fatty acid (HUFA) biosynthesis activity and gene expression.
Results of Boneferroni post-tests for significance of differences between values for fish fed FO compared to fish fed VO are shown on Figs. 2 and 3.

Legends to Figures:

Fig. 1. Effect of feeding dietary vegetable oil on the activity of the HUFA biosynthetic pathway in isolated hepatocytes from Atlantic salmon at the Scottish location. Results are means ± S.D. (n = 6) and represent the rate of conversion (pmol.h^{-1}.mg $protein^{-1}$) of [1-^{14}C]18:3n-3 to all desaturated products (sum of radioactivity recovered as 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3). The dotted line indicates the time of seawater transfer. An asterisk indicates that the value at a particular time point for fish fed the VO diet was significantly different to the value for fish fed FO as determined by two-way ANOVA with Bonferroni post-tests. FO, fish oil; VO, vegetable oil.

Fig. 2. Effect of feeding dietary vegetable oil on the activity of the HUFA biosynthetic pathway in isolated hepatocytes from Atlantic salmon at the Norwegian location. Results are means ± S.D. (n = 3) and represent the rate of conversion (pmol.h^{-1}.mg $protein^{-1}$) of [1-^{14}C]18:3n-3 to all desaturated products (sum of radioactivity recovered as 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3). An

asterisk indicates that the value at a particular time point for fish fed the VO diet was significantly different to the value for fish fed FO as determined by two-way ANOVA with Bonferroni post-tests. FO, fish oil; VO, vegetable oil.

Fig. 3. Effect of feeding dietary vegetable oil on the expression of fatty acid Δ6 desaturase, Δ5 desaturase and elongase genes in liver of Atlantic salmon at the Scottish location. Transcript (mRNA) copy number was determined by real-time quantitative PCR (rtqPCR) as described in the Materials and Methods Section. Results were calculated as the ratio of copy numbers between the target gene and β-actin and are means ± S.D. (n = 6). An asterisk indicates that the expression of a specific gene at a particular time point for fish fed the VO diet was significantly different ($P < 0.05$) to the gene expression in fish fed FO as determined by two-way ANOVA with Bonferroni post-tests. FO, fish oil; VO, vegetable oil.

Fig. 4. Effect of feeding dietary vegetable oil on the expression of fatty acid Δ6 and Δ5 desaturase genes in liver of Atlantic salmon at the Norwegian location. Transcript (mRNA) copy number was determined by real-time quantitative PCR (rtqPCR) as described in the Materials and Methods Section. Results were calculated as the ratio of copy numbers between the target gene and E1α and are means ± S.D. (n = 3). The dotted line indicates the time of seawater transfer. An asterisk indicates that the expression of a specific gene at a particular time point for fish fed the VO diet was significantly different ($P < 0.05$) to the gene expression in fish fed FO as determined by two-way ANOVA with Bonferroni post-tests. FO, fish oil; VO, vegetable oil.

Table 1. Proximate compositions (percentage of diet weight) and fatty acid compositions (percentage by weight of total fatty acids) of represenatative diets used in freshwater (3mm) and seawater (9 mm pellet).

	Freshwater			Seawater		
	FO	VO75	VO100	FO	VO75	VO100
Proximate composition						
Protein	46.5 ± 0.4	45.8 ± 0.3	46.7 ± 0.2	42.1 ± 0.2	41.2 ± 0.4	40.1 ± 0.3
Lipid	19.6 ± 0.1	18.2 ± 0.2	18.8 ± 0.1	30.2 ± 0.8	32.8 ± 0.3	32.5 ± 0.1
Moisture	8.4 ± 0.0	6.8 ± 0.1	6.6 ± 0.0	7.0 ± 0.3	6.4 ± 0.2	6.5 ± 0.1
Ash	7.8 ± 0.0	7.9 ± 0.0	7.5 ± 0.0	7.0 ± 0.1	7.1 ± 0.0	6.6 ± 0.1
Fatty acid composition						
14:0	6.1 ± 0.1	2.7 ± 0.1	1.1 ± 0.1	6.2 ± 0.1	2.2 ± 0.2	0.6 ± 0.1
16:0	12.4 ± 0.2	15.5 ± 0.5	16.9 ± 0.4	14.5 ± 0.4	16.1 ± 0.2	15.3 ± 0.3
18:0	1.5 ± 0.0	2.4 ± 0.1	2.7 ± 0.0	2.4 ± 0.6	3.0 ± 0.5	2.7 ± 0.0
Total saturated[1]	20.3 ± 0.3	20.8 ± 0.6	21.8 ± 0.4	23.6 ± 0.9	21.5 ± 0.5	19.4 ± 0.3
16:1n-7[2]	7.9 ± 0.1	3.2 ± 0.0	1.1 ± 0.0	4.9 ± 0.2	1.8 ± 0.3	0.5 ± 0.1
18:1n-9	11.9 ± 0.4	30.6 ± 0.7	40.4 ± 0.5	13.2 ± 0.4	35.2 ± 0.0	43.0 ± 0.2
18:1n-7	3.3 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.5 ± 0.2	2.4 ± 0.0
20:1n-9[3]	19.9 ± 0.4	7.4 ± 0.1	2.7 ± 0.1	11.1 ± 1.0	3.8 ± 0.3	1.3 ± 0.1
22:1n-11[4]	15.8 ± 0.3	6.4 ± 0.1	2.3 ± 0.1	16.5 ± 1.9	5.1 ± 0.4	0.8 ± 0.0
24:1n-9	0.7 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
Total monoenes	59.4 ± 1.3	50.6 ± 0.8	50.2 ± 0.5	48.8 ± 2.5	48.6 ± 0.6	48.2 ± 0.5
18:2n-6	3.9 ± 0.1	11.7 ± 0.3	13.5 ± 0.2	3.6 ± 0.6	12.7 ± 1.2	17.1 ± 0.2
20:4n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.2 ± 0.1	0.0 ± 0.0
Total n-6 PUFA[5]	4.4 ± 0.1	12.2 ± 0.3	13.7 ± 0.2	4.6 ± 0.7	13.0 ± 1.1	17.1 ± 0.2
18:3n-3	0.6 ± 0.0	6.8 ± 0.3	8.0 ± 0.2	1.2 ± 0.1	9.0 ± 0.7	13.4 ± 0.5
18:4n-3	1.9 ± 0.1	1.0 ± 0.1	0.4 ± 0.1	2.5 ± 0.1	0.8 ± 0.0	0.2 ± 0.0
20:4n-3	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.7 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
20:5n-3	5.8 ± 0.6	3.6 ± 0.3	2.1 ± 0.0	6.5 ± 0.2	2.4 ± 0.5	0.6 ± 0.0
22:5n-3	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.9 ± 0.2	0.3 ± 0.2	0.0 ± 0.0
22:6n-3	5.9 ± 0.6	4.2 ± 0.4	3.4 ± 0.2	10.0 ± 0.6	3.7 ± 1.1	1.0 ± 0.0
Total n-3 PUFA[6]	14.9 ± 1.4	15.9 ± 1.1	14.3 ± 0.6	21.8 ± 0.8	16.5 ± 1.1	15.2 ± 0.5
Total PUFA[7]	20.3 ± 1.6	28.6 ± 1.3	28.0 ± 0.8	27.6 ± 1.6	29.8 ± 0.1	32.3 ± 0.7

Results are means ± SD (n=3 for proximates and n = 2 for for fatty acids). [1]totals include 15:0 present at up to 0.5%; [2]also contains n-9 isomer; [3]also contains n-11 and n-7 isomers; [4]also contains n-9 and n-7 isomers; [5]totals include 18:3n-6, 20:2n-6 and 22:5n-6 present at up to 0.2%; [6]totals include 20:3n-3 present at up to 0.1%; [7]totals include C_{16} PUFA present at up to 1.1% in FO diets and up to 0.5% in VO diets.

Table 2. Effect of feeding diets containing vegetable oil on the fatty acid composition (percentage of weight) of total lipid from livers of Atlantic salmon (*Salmo salar*) at selected freshwater stages from Scotland (S) and Norway (N).

	Month 9 (S) / Month 6 (N)			Month 12 (S) / Month 9 (N)		
	FO	VO75	VO100	FO	VO75	VO100
14:0	3.4 ± 0.1 [a]	1.3 ± 0.2 [b]	1.0 ± 0.2 [b]	3.0 ± 0.4 [a]	1.0 ± 0.2 [b]	0.8 ± 0.0 [b]
16:0	16.3 ± 0.3 [b]	18.1 ± 0.3 [a]	13.3 ± 0.4 [c]	19.0 ± 0.1 [a]	19.7 ± 1.7 [a]	14.7 ± 0.5 [b]
18:0	2.8 ± 0.3 [c]	3.7 ± 0.3 [b]	4.5 ± 0.2 [a]	3.0 ± 0.3 [c]	4.6 ± 0.2 [b]	5.2 ± 0.1 [a]
Total saturated[1]	22.8 ± 0.2 [a]	23.4 ± 0.4 [a]	19.4 ± 0.3 [b]	25.3 ± 0.4 [a]	25.5 ± 1.6 [a]	20.9 ± 0.6 [b]
16:1n-7[2]	4.1 ± 0.2 [a]	2.1 ± 0.1 [b]	1.3 ± 0.3 [c]	4.0 ± 0.8 [a]	1.7 ± 0.2 [b]	1.7 ± 0.1 [b]
18:1n-9	12.7 ± 0.4 [c]	19.1 ± 0.6 [b]	24.2 ± 0.5 [a]	13.1 ± 2.4 [c]	19.9 ± 2.6 [b]	29.6 ± 1.8 [a]
18:1n-7	3.1 ± 0.1 [a]	2.5 ± 0.2 [b]	2.4 ± 0.2 [b]	3.0 ± 0.3 [a]	2.1 ± 0.1 [b]	2.1 ± 0.2 [b]
20:1n-9[3]	9.8 ± 0.6 [a]	4.0 ± 0.2 [b]	3.6 ± 0.4 [b]	8.7 ± 1.5 [a]	2.4 ± 0.4 [b]	2.6 ± 0.3 [b]
22:1n-11[4]	3.5 ± 0.6 [a]	1.3 ± 0.1 [b]	0.5 ± 0.1 [b]	2.5 ± 1.1 [a]	0.5 ± 0.1 [b]	0.3 ± 0.0 [b]
24:1n-9	1.3 ± 0.2 [a]	1.4 ± 0.2 [a]	0.1 ± 0.0 [b]	1.0 ± 0.2 [a]	0.7 ± 0.1 [a]	0.1 ± 0.0 [b]
Total monoenes	34.6 ± 0.8 [a]	30.3 ± 1.0 [b]	33.2 ± 2.6 [a]	32.6 ± 5.8	27.5 ± 3.4	37.3 ± 2.6
18:2n-6	2.4 ± 0.1 [b]	6.9 ± 0.3 [a]	7.1 ± 0.4 [a]	2.3 ± 0.3 [c]	5.7 ± 0.4 [b]	6.8 ± 0.2 [a]
20:2n-6	0.4 ± 0.1 [c]	0.9 ± 0.1 [b]	1.4 ± 0.1 [a]	0.3 ± 0.0 [b]	0.5 ± 0.1 [b]	0.9 ± 0.1 [a]
20:3n-6	0.3 ± 0.1 [c]	0.7 ± 0.1 [b]	1.1 ± 0.1 [a]	0.4 ± 0.1 [b]	1.9 ± 0.4 [a]	2.3 ± 0.2 [a]
20:4n-6	1.4 ± 0.1 [b]	1.2 ± 0.1 [b]	2.4 ± 0.1 [a]	1.3 ± 0.2 [b]	1.6 ± 0.1 [b]	2.3 ± 0.3 [a]
Total n-6 PUFA[5]	4.7 ± 0.1 [c]	9.9 ± 0.1 [b]	12.0 ± 0.5 [a]	4.7 ± 0.1 [c]	10.2 ± 0.3 [b]	12.3 ± 0.2 [a]
18:3n-3	0.4 ± 0.1 [b]	2.8 ± 0.2 [a]	2.6 ± 0.1 [a]	0.3 ± 0.1 [c]	1.5 ± 0.1 [b]	1.8 ± 0.1 [a]
18:4n-3	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.0
20:4n-3	0.8 ± 0.1 [b]	1.0 ± 0.1 [a]	0.8 ± 0.0 [b]	0.6 ± 0.1 [b]	0.8 ± 0.1 [a]	1.0 ± 0.0 [a]
20:5n-3	5.7 ± 0.3 [a]	4.1 ± 0.1 [b]	3.7 ± 0.2 [b]	4.6 ± 1.0	3.7 ± 0.5	3.5 ± 0.3
22:5n-3	1.1 ± 0.1 [a]	0.6 ± 0.1 [b]	0.7 ± 0.0 [b]	1.0 ± 0.1	0.9 ± 0.3	0.8 ± 0.1
22:6n-3	29.2 ± 0.8 [a]	27.3 ± 1.3 [ab]	26.3 ± 1.1 [b]	30.4 ± 5.3 [a]	28.9 ± 1.8 [a]	21.6 ± 1.8 [b]
Total n-3 PUFA[6]	37.6 ± 0.6 [a]	36.4 ± 1.4 [ab]	34.3 ± 0.9 [b]	37.2 ± 6.1	36.5 ± 2.1	29.1 ± 2.1
Total PUFA[7]	42.6 ± 0.7 [a]	46.3 ± 1.2 [b]	46.3 ± 1.4 [b]	42.1 ± 6.0	47.0 ± 2.0	41.4 ± 2.3

Results are means ± SD (n=4). [1]totals include 15:0 present at up to 0.3%; [2]also contains n-9 isomer; [3]also contains n-11 and n-7 isomers; [4]also contains n-9 isomer; [5]totals include 18:3n-6 and 22:5n-6 present at up to 0.3%; [6]totals include 20:3n-3, present at up to 0.3%; [7]Totals include C_{16} PUFA, present at up to 0.3%. FO, fish oil diet; VO75 and VO100, diets with 75% and 100% of fish oil replaced by vegetable oil.

Table 3. Effect of feeding diets containing vegetable oil on the fatty acid composition (percentage of weight) of total lipid from livers of Atlantic salmon (*Salmo salar*) at selected seawater stages from Scotland (S) and Norway (N).

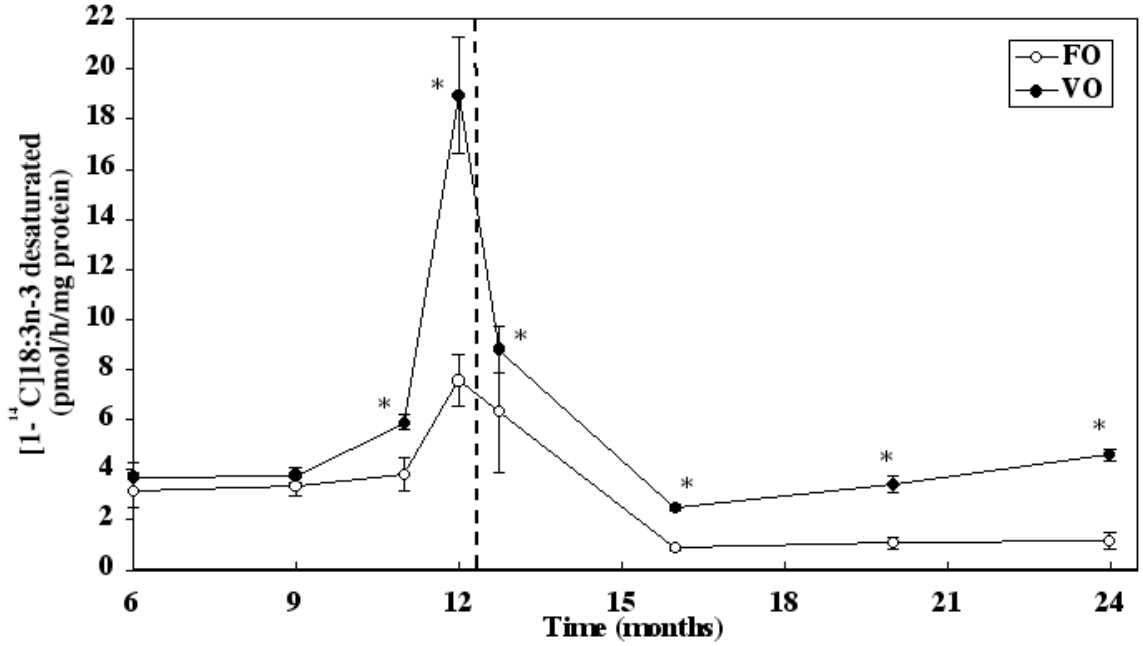
	Month 12.75 (S) / Month 14 (N)			Month 20 (S) / Month 16 (N)		
	FO	VO75	VO100	FO	VO75	VO100
14:0	2.3 ± 0.2 [a]	1.1 ± 0.1 [b]	0.6 ± 0.0 [c]	2.3 ± 0.3 [a]	1.0 ± 0.1 [b]	0.5 ± 0.0 [c]
16:0	20.7 ± 1.0 [a]	20.2 ± 0.6 [a]	13.3 ± 1.0 [b]	14.4 ± 0.7	13.2 ± 0.7	14.3 ± 0.7
18:0	4.2 ± 0.2 [c]	4.9 ± 0.2 [b]	5.8 ± 0.1 [a]	5.0 ± 0.3 [c]	5.6 ± 0.3 [b]	6.6 ± 0.1 [a]
Total saturated[1]	27.5 ± 1.2 [a]	26.4 ± 0.7 [a]	20.3 ± 1.1 [b]	21.9 ± 0.8 [a]	20.0 ± 0.9 [b]	21.9 ± 0.7 [a]
16:1n-7[2]	3.7 ± 0.4 [a]	1.9 ± 0.1 [b]	0.8 ± 0.1 [c]	3.3 ± 0.3 [a]	2.2 ± 0.3 [b]	0.5 ± 0.0 [c]
18:1n-9	11.9 ± 0.9 [c]	20.8 ± 0.8 [b]	26.9 ± 2.0 [a]	17.7 ± 0.9 [c]	27.8 ± 2.3 [b]	23.4 ± 1.7 [a]
18:1n-7	3.1 ± 0.2 [a]	2.2 ± 0.2 [b]	2.1 ± 0.1 [b]	2.7 ± 0.1 [a]	2.3 ± 0.1 [b]	1.7 ± 0.0 [c]
20:1n-9[3]	5.9 ± 0.8 [a]	2.6 ± 0.1 [b]	2.8 ± 0.4 [b]	7.7 ± 0.5 [a]	4.5 ± 0.4 [b]	2.0 ± 0.1 [c]
22:1n-11[4]	1.8 ± 0.3 [a]	0.8 ± 0.1 [b]	0.0 ± 0.0 [c]	3.8 ± 0.5 [a]	1.0 ± 0.1 [b]	0.0 ± 0.0 [c]
24:1n-9	1.0 ± 0.1 [a]	0.8 ± 0.1 [b]	0.2 ± 0.0 [c]	0.8 ± 0.1 [a]	0.6 ± 0.1 [a]	0.2 ± 0.1 [b]
Total monoenes	27.3 ± 2.8 [b]	29.2 ± 0.9 [b]	33.2 ± 2.6 [a]	36.0 ± 2.1 [a]	38.4 ± 3.2 [a]	28.2 ± 1.9 [b]
18:2n-6	2.3 ± 0.1 [c]	6.5 ± 0.3 [b]	8.5 ± 0.2 [a]	1.7 ± 0.1 [c]	6.0 ± 0.4 [b]	8.5 ± 0.3 [a]
20:2n-6	0.3 ± 0.1 [c]	0.6 ± 0.0 [b]	1.5 ± 0.2 [a]	0.5 ± 0.1 [b]	1.5 ± 0.2 [a]	1.5 ± 0.1 [a]
20:3n-6	0.5 ± 0.1 [a]	1.6 ± 0.2 [a]	1.2 ± 0.1 [b]	0.2 ± 0.1 [c]	0.6 ± 0.2 [b]	1.5 ± 0.1 [a]
20:4n-6	1.8 ± 0.2 [b]	1.6 ± 0.1 [b]	2.3 ± 0.1 [a]	2.1 ± 0.3 [b]	1.6 ± 0.2 [c]	3.3 ± 0.2 [a]
Total n-6 PUFA[5]	5.2 ± 0.1 [c]	10.9 ± 0.4 [b]	13.6 ± 0.2 [a]	5.1 ± 0.4 [c]	10.1 ± 0.4 [b]	14.8 ± 0.2 [a]
18:3n-3	0.3 ± 0.1 [c]	1.7 ± 0.1 [b]	3.8 ± 0.1 [a]	0.4 ± 0.1 [c]	2.5 ± 0.4 [b]	3.2 ± 0.2 [a]
18:4n-3	0.4 ± 0.1 [b]	1.1 ± 0.2 [a]	0.2 ± 0.0 [b]	0.3 ± 0.1 [a]	0.1 ± 0.1 [b]	0.2 ± 0.1 [a]
20:4n-3	0.8 ± 0.1 [b]	0.9 ± 0.1 [b]	1.1 ± 0.0 [a]	1.1 ± 0.1 [a]	0.8 ± 0.1 [b]	0.8 ± 0.1 [b]
20:5n-3	4.8 ± 0.6 [a]	3.2 ± 0.3 [b]	4.8 ± 0.5 [a]	8.2 ± 0.5 [a]	5.2 ± 0.5 [c]	6.5 ± 0.1 [b]
22:5n-3	1.3 ± 0.2 [a]	1.0 ± 0.2 [b]	1.2 ± 0.1 [a]	2.5 ± 0.2 [a]	1.5 ± 0.1 [b]	1.7 ± 0.0 [b]
22:6n-3	32.2 ± 2.6 [a]	25.2 ± 1.5 [b]	21.5 ± 1.3 [b]	23.8 ± 1.7	20.6 ± 2.3	22.3 ± 1.4
Total n-3 PUFA[6]	39.8 ± 3.3 [a]	33.3 ± 1.4 [b]	32.6 ± 1.7 [b]	36.4 ± 2.2 [a]	31.5 ± 2.6 [b]	34.7 ± 1.3 [b]
Total PUFA[7]	45.2 ± 3.3	44.4 ± 1.6	46.2 ± 1.9	42.0 ± 2.4 [b]	41.6 ± 2.7 [b]	49.5 ± 1.5 [a]

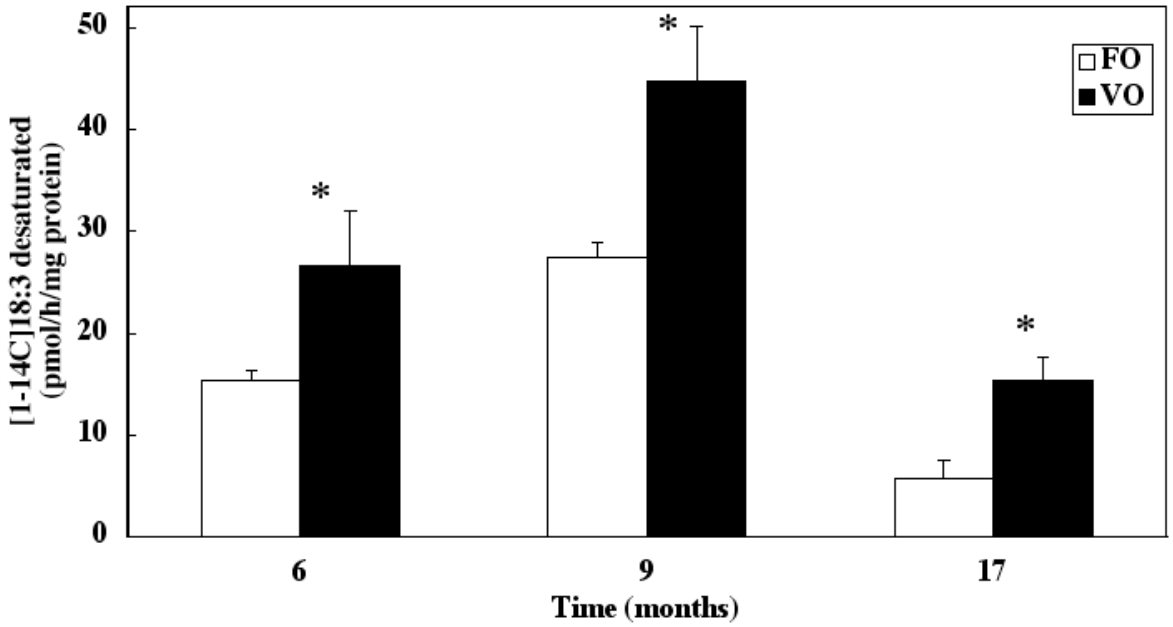
Results are means ± SD (n=4). [1]totals include 15:0 present at up to 0.3%; [2]also contains n-9 isomer; [3]also contains n-11 and n-7 isomers; [4]also contains n-9 isomer; [5]totals include 18:3n-6 and 22:5n-6 present at up to 0.6%; 6totals include 20:3n-3 present at up to 0.6%; [7]totals include C_{16} PUFApresent at up to 0.5%. FO, fish oil diet; VO75 and VO100, diet with 75% and 100% of fish oil replaced by vegetable oil.

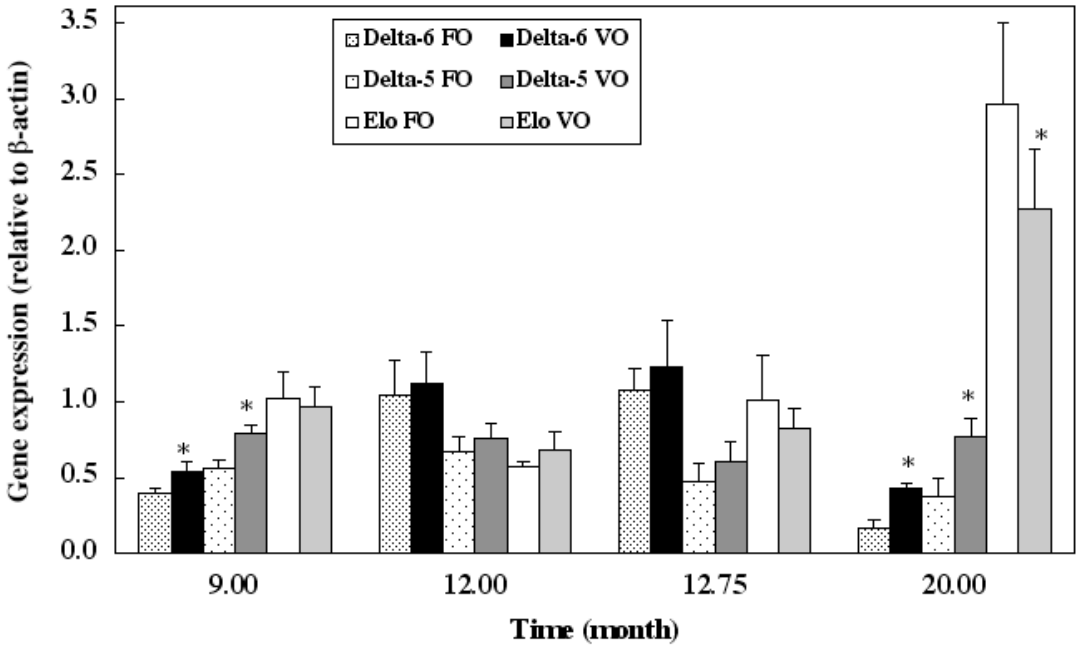
Table 4. Significance of combined effects of diet (fish oil, FO v. vegetable oil, VO) and time (sampling point) as determined by two-way ANOVA analyses of the data in Figures 2 and 3 for highly unsaturated fatty acid (HUFA) synthesis and gene expression.

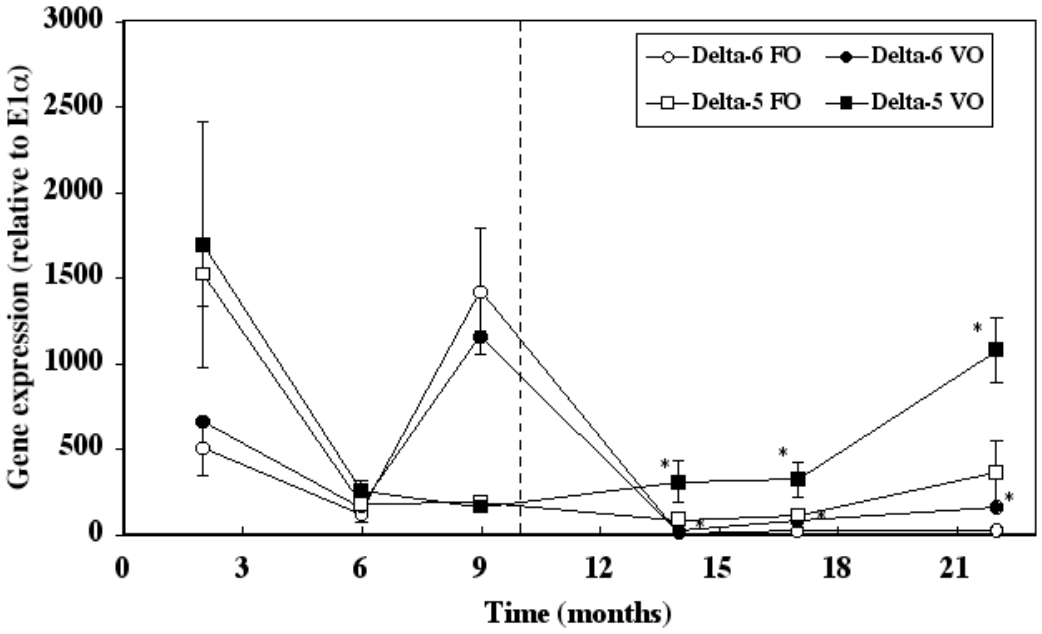
	Diet		Time		Interaction	
Data	p value	F value	p value	F value	p value	F value
HUFA synthesis						
Fig. 2A (Scotland)	<0.0001	227.40	0.0001	179.80	0.0001	37.94
Fig. 3A (Norway)	<0.0001	62.68	<0.0001	85.96	0.1518	2.22
Δ6 desaturase expression						
Fig. 2B (Scotland)	0.0017	11.28	<0.0001	81.32	0.5689	0.68
Fig. 3B (Norway)	0.4968	0.4714	<0.0001	140.4	0.0177	3.178
Δ5 desaturase expression						
Fig. 2B (Scotland)	<0.0001	48.20	0.0003	7.73	0.0041	5.17
Fig. 3B (Norway)	0.0017	11.51	<0.0001	47.87	0.0558	2.40
Elongase expression						
Fig. 2B (Scotland)	0.0137	6.64	<0.0001	129.20	0.0067	7.70

Results of Boneferroni post-tests for significance of differences between values for fish fed FO compared to fish fed VO are shown on Figs. 2 and 3.


FO
VO
[1-14C]18:3n-3 desaturated (pmol/h/mg protein)
Time (months)
0
2
4
6
8
10
12
14
16
18
20
22
6
9
12
15
18
21
24
*


50
40
30
20
10
0
[1-14C]18:3 desaturated
(pmol/h/mg protein)
*
*
*
FO
VO
6
9
17
Time (months)


Delta-6 FO
Delta-6 VO
Delta-5 FO
Delta-5 VO
Elo FO
Elo VO
Gene expression (relative to β-actin)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
9.00
12.00
12.75
20.00
Time (month)


Delta-6 FO
Delta-6 VO
Delta-5 FO
Delta-5 VO
Gene expression (relative to E1α)
Time (months)
3000
2500
2000
1500
1000
500
0
0
3
6
9
12
15
18
21
*