Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation and the effects of dietary linseed and rapeseed oils.

D. R. Tocher[1], J. G. Bell[1], J.R. Dick[1], R.J. Henderson[1], F. McGhee[1], D. Mitchell[2] and P. C. Morris[3]

[1]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland; [2]Marine Harvest McConnell Ltd., Blar Mhor Industrial Estate, Fort William PH33 7PT, Highland, Scotland; [3]Trouw Aquaculture, Renfrew Mill, Wright Street, Renfrew, Renfrewshire PA4 8BF, Strathclyde, Scotland

Running title; PUFA metabolism in salmon during smoltification.

Correspondence to; Dr. Douglas R. Tocher, Tel. + 44 1786 467996; Fax. + 44 1786 472133; E-mail, d.r.tocher@stir.ac.uk

Key words; salmon, PUFA, desaturase, smoltification, parr-smolt transformation, prostaglandin, osmoregulation.

Abbreviations: AA, arachidonic acid; BHT, butylated hydroxytoluene; DHA, docosahexaenoic acid; EFA, essential fatty acid; EPA, eicosapentaenoic acid; FAF-BSA, fatty acid free bovine serum albumin; FO, fish oil; HBSS, Hanks balanced salt solution; HUFA, highly unsaturated fatty acids acids (carbon chain length ≥ C_{20} with ≥ 3 double bonds); LO, linseed oil; PGF, prostaglandin F; PL, polar lipid; PUFA, polyunsaturated fatty acids; RO, rapeseed oil; TLC, thin-layer chromatography.

Abstract

Duplicate groups of Atlantic salmon parr were fed diets containing either fish oil (FO), rapeseed oil (RO), linseed oil (LO) or linseed oil supplemented with arachidonic acid (20:4n-6; AA) (LOA) from October (week 0) to seawater transfer in March (week 19). From March to July (weeks 20-34) all fish were fed a fish oil-containing diet. Fatty acyl desaturation and elongation activity in isolated hepatocytes incubated with [1-^{14}C]18:3n-3 increased in all dietary groups, peaking in early March about one month prior to seawater transfer. Desaturation activities at their peak were significantly greater in fish fed the vegetable oils, particularly RO, compared to fish fed FO. Docosahexaenoic acid (22:6n-3:DHA) and AA in liver and gill polar lipids (PL) increased in all dietary groups during the freshwater phase whereas eicosapentaenoic acid (20:5n-3;EPA) increased greatly in all groups after seawater transfer. The AA/EPA ratio in tissue PL increased up to seawater transfer and then decreased after transfer. AA levels and the AA/EPA ratio in gill PL were generally higher in the LOA group. The levels of 18:3n-3 in muscle total lipid were increased significantly in the LO, LOA and, to a lesser extent, RO groups prior to transfer but were reduced to initial levels by the termination of the experiment (week 34). In contrast, 18:2n-6 in muscle total lipid was significantly increased after 18 weeks in fish fed the diets supplemented with RO and LO, and was significantly greater in the FO and RO groups at the termination of the experiment. Gill PGF production showed a large peak about two months after transfer to seawater. The production of total PGF post-transfer was significantly lower in fish previously fed the LOA diet. However, plasma chloride concentrations in fish subjected to a seawater challenge at 18 weeks were all lower in fish fed the diets with vegetable oils. This effect was significant in the case of fish receiving the diet with LOA, compared to those fed the diet containing FO. The present study showed that during parr-smolt transformation in Atlantic salmon there is a pre-adaptive increase in hepatocyte fatty acyl desaturation/elongation activities that is controlled primarily by environmental factors such as photoperiod and temperature but that can also be significantly modulated by diet. Feeding salmon parr diets supplemented with rapeseed or linseed oils prevented inhibition of the desaturase activities that is induced by feeding parr diets with fish oils and thus influenced the smoltification process by altering tissue PL fatty acid compositions and eicosanoid production. These effect, in turn, had a beneficial effect on the ability of the fish to osmoregulate and thus adapt to salinity changes.

Introduction

The seaward migration of anadromous salmonids, such as Atlantic salmon (*Salmo salar*), juveniles or parr is accompanied by profound changes in morphology, behaviour and physiology. Collectively, these developmental and physiological changes are known as parr-smolt transformation or smoltification (Folmar and Dickoff 1980). The process involves activation of neuroendocrine and endocrine systems which result in biochemical and molecular reorganisations within the tissues which prepare the fish, while still in freshwater, for life in the marine environment (Hoar 1976; Wedermeyer et al. 1980). Studies with steelhead trout (*Salmo gairdnerii*) and masu salmon (*Oncorhynchus masou*) have shown that tissue fatty acid compositions change from a typical freshwater pattern, which is relatively low in polyunsaturated fatty acids (PUFA), to a more typical marine pattern which is relatively rich in long-chain PUFA. The latter are particularly rich in the n-3 highly unsaturated fatty acids (HUFA) viz., eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3;DHA), and the elevation of n-3 HUFA is preadaptive response to seawater entry (Sheridan et al. 1985; Li and Yamada 1992). Other studies have observed a significantly elevated level of arachidonic acid (20:4n-6; AA) in the tissues of wild parr and smolts compared to their cultured counterparts, whereas the cultured parr and smolts have all contained considerable amounts of dietary-derived 18:2n-6, which was apparently not desaturated and elongated to AA (Ackman and Takeuchi 1986; Bolgova and Shchurov 1987; Bell 1996). These studies on fatty acid compositions in parr and smolts have led to the suggestion that there may be changes in fatty acid desaturase/elongation reactions in salmon undergoing smoltification (Ackman and Takeuchi 1986). Changes in membrane long-chain PUFA composition have been found to affect systems involved in adaptation to salinity such as the activity of ion pumps and membrane bound enzymes, including Na^+,K^+-ATPase (Spector and Yorek 1985; Gerbi et al. 1994). Furthermore, essential fatty acid-deficiency in fish has led to reduced ionic permeability in trout brush border membranes (DiConstanzo et al. 1983) and morphological changes in the gills of turbot (Bell et al. 1985). Prostaglandins, metabolites of C_{20} PUFA, are known to mediate fluid and electrolyte fluxes in fish gill and kidney and are, therefore, important in the adaptation to changes in salinity (Brown and Bucknall 1986; Mustafa and Srivastava 1989).

Recently, we showed that there was a preadaptive increase in the fatty acyl desaturation/ elongation activities in salmon hepatocytes during the period up to seawater transfer (Bell et al. 1997). The increased desaturation of 18:3n-3 and 18:2n-6 was much greater in fish fed a vegetable oil diet containing a blend of rapeseed oil and linseed oil and formulated to more closely mimic the fatty acid composition of freshwater aquatic insects that make up the diet of wild salmon parr, than a diet containing fish oil (Bell et al. 1997). The HUFA levels in tissue polar lipids also changed significantly during the period of parr-smolt transformation and, in combination, the results suggested that feeding salmon parr diets containing fish oil partially inhibited the natural preadaptive increase in hepatic fatty acid desaturase activities with possible detrimental effects on tissue fatty acid compositions. Perhaps reflecting this, prostaglandin (PG) F production by gill cells was significantly reduced in fish fed the diet containing fish oil in comparison with the diet containing the blend of linseed and rapeseed oils (Bell et al. 1997). In addition, fish fed the diet with the vegetable oil blend appeared to be better adapted for seawater transfer as they displayed significantly lower plasma chloride values compared to fish fed the diet with fish oil in a seawater tolerance test a month prior to seawater transfer. Overall, the results suggested that feeding salmon parr diets containing vegetable oils to could be beneficial in affecting the smoltification process.

In the previous study, the diet containing the blend of rapeseed and linseed oils had an 18:2n-6/18:3n-3 ratio of 1 (Bell et al. 1997). In the present study, we aimed to determine the relative importance of 18:2n-6 and 18:3n-3 in the diets by feeding the oils individually so as to give ratios of dietary 18:2n-6/18:3n-3 of 2 and 0.5 for rapeseed and linseed oils, respectively. A further diet containing an increased percentage of AA was used to determine the importance of supplying preformed AA on the production of PGF, and on the ability of the salmon to osmoregulate and successfully transfer to seawater. Therefore, duplicate groups of Atlantic salmon parr were fed diets containing either rapeseed (RO) or linseed (LO) oil or linseed oil supplemented with an AA-rich fungal oil (ARASCO,) (LOA). Control fish received a diet containing a Northern hemisphere fish oil (FO), since commercial feeds used in the salmon farming industry are usually supplemented with this lipid source. All groups of fish were switched to a diet containing FO after transfer to seawater. Fish were sampled on a regular basis from five months before seawater transfer to 4 months after seawater transfer. At each sampling time, fatty acyl desaturation and elongation activity was determined in isolated hepatocytes, and prostaglandin F was measured in isolated gill

cells that were stimulated with the calcium ionophore A23187. Samples of liver, gill and muscle were also collected for lipid compositional analyses.

Materials and Methods

Animals and diets

Four thousand Atlantic salmon parr supplied by Marine Harvest McConnell Ltd.(MHM) were distributed randomly into eight 2m x 2m tanks at the MHM freshwater unit at Kinlochmoidart, Invernesshire, Scotland in October 1997. Each tank contained 1500 L and was supplied with freshwater at a rate of 45 L/min. The entire experiment (both freshwater and seawater phases) was performed under natural photoperiod. The diets were fed to duplicate tanks of fish and all fish were fed their prescribed diets to satiation using automatic feeders. Experimental diets were prepared by Trouw Aquaculture (Renfrew, Scotland). The feed components and proximate compositions of the diets are shown in Table 1. The methods of proximate analysis (moisture, oil, protein and ash) were performed as described by Woyewoda et al. (1986).The freshwater diets contained approximately 49% protein and 19% lipid while the post-transfer diet contained 55% protein and 19% lipid. The fatty acid compositions of the diets are shown in Table 2. All diets were formulated to satisfy the nutritional requirements of salmonid fish (US National Research Council 1993).

In late March 1998 (week 19), 500 fish per treatment which had undergone smoltification (i.e. those which had changed to silver colouration) were selected randomly and transferred to the MHM research unit at Inverailort, Invernesshire, Scotland. Fish from the different freshwater treatments were distributed into four 3m diameter circular tanks of 3000 L capacity. Fish were initially acclimated in fresh water for a few days before salinity was gradually increased by switching to seawater, supplied at 50L/min. All fish were then switched to the post-transfer seawater diet which was fed to satiation by automatic feeders. The temperature range during the experimental period varied from 2.5 ºC in January to 15.0 ºC in July. Six fish per dietary treatment were sampled at the initiation of the experiment in late October (week 0) and at regular intervals (weeks 7, 14 and 18 in freshwater and weeks 20, 28 and 34 in seawater) until late July 1998, with seawater transfer in week 19.

Lipid extraction and phospholipid fatty acid analysis

Intact gill arches and liver were dissected from three fish per dietary treatment at each time point and immediately frozen in liquid nitrogen. Samples of white muscle were taken at selected time points; i.e. at the beginning of the experiment, just prior to seawater transfer and at the termination of the experiment. Samples were stored at -20ºC prior to lipid extraction. Total lipid was extracted from gill, liver, muscle and diet samples by the method of Folch et al. (1957). For diets and muscle samples, fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalysed transesterification using 2 ml of 1% H_2SO_4 in methanol plus 1 ml toluene as described by Christie (1982). For gill and liver samples, total polar lipid (PL) was prepared by separating neutral lipids from PL by thin-layer chromatography (TLC) using hexane/diethylether/acetic acid (80:20:2, by vol.) as the developing solvent. The PL retained on the origin was scraped and subjected to acid-catalysed transesterification, directly on the silica, as above. FAME were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Fisons Ltd., Crawley, U.K.) using a 30m x 0.32 mm capillary column (CP wax 52CB; Chrompak Ltd., London, U.K). Hydrogen was used as the carrier gas and temperature programming was from 50ºC to 150ºC at 40ºC/min and then to 230ºC at 2.5ºC/min. Individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman 1980).

Preparation of isolated hepatocytes and gill cells

Fish were killed by a blow to the head and the liver and gill arches were dissected immediately. The gall bladder was removed carefully from the liver and the main blood vessels were removed. The liver was perfused via the hepatic vein with solution A (calcium and magnesium-free Hanks balanced salt solution (HBSS) + 10 mM HEPES + 1 mM EDTA), using a syringe fitted with a 23g needle, to clear blood from the tissue. The liver was chopped finely with scissors and incubated with 20 ml of solution A containing 0.1% (w/v) collagenase in a 25ml “Reacti-flask” held in a shaking water bath at 20ºC for 45 min. The digested liver was filtered through 100 µm nylon gauze and the cells were collected by centrifugation at 1000 x g for 5 min. The cell pellet was washed with 20 ml of solution A containing 1% w/v fatty acid-free bovine serum albumin (FAF-BSA) and

re-centrifuged. The hepatocytes were resuspended in 10 ml of Medium 199 containing 10 mM HEPES, 2 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin. One hundred μl of cell suspension were mixed with 400 μl of Trypan Blue and hepatocytes were counted and their viability assessed using a haemocytometer. One hundred μl of the cell suspension were retained for protein determination.

The gills were blotted on tissue to remove blood clots and water after which they were chopped with scissors and incubated in 30 ml solution A containing 0.1% collagenase as described above. The incubation was continued in a shaking water bath at 20ºC for 45 min as described above. The material was filtered through 100 μm nylon gauze and the cells were collected by centrifugation at 1000 x g for 5 min. The gill cells were washed with 20 ml of HBSS and finally they were resuspended in 1 ml of HBSS containing 2mM $CaCl_2$. Fifty μl were removed for protein determination.

Assay of hepatocyte fatty acyl desaturation/elongation activities

Five ml of each hepatocyte suspension were dispensed into two 25 cm^2 tissue culture flasks. Hepatocytes were incubated with 0.25 μCi of [1-^{14}C] 18:3n-3, added as a complex with FAF-BSA. Briefly, 25 μCi of fatty acid (0.5 μmol) in ethanol were placed in a reaction vial, and then the solvent was evaporated under a stream of nitrogen and 100 μl of 0.1M KOH were added. The mixture was stirred for 10 min at room temperature before 5 ml of 50 mg/ml FAF-BSA in HBSS containing 10 mM HEPES buffer were added and subsequently the mixture was stirred for 45 min at 20 ºC. After addition of isotope, the flasks were incubated at 20 ºC for 2 h. After incubation, the cell layer was dislodged by gentle rocking, then the cell suspension was transfered to glass conical test tubes and the flasks were washed with 1 ml of ice-cold HBSS containing 1% FAF-BSA. The cell suspensions were centrifuged at 300 g for 2 min. Thereafter, the supernatant was discarded and the cell pellets were washed with 5 ml of ice-cold HBSS/FAF-BSA. The supernatant was discarded and the tubes were placed upside down on paper towels to blot for 15 sec before extraction of total lipid using ice-cold chloroform/methanol (2:1, v/v) containing 0.01% (w/v) butylated hydroxy toluene (BHT) essentially as described by Folch et al. (1957) and as described in detail previously (Tocher et al. 1988).

Total lipid was transmethylated and FAME were prepared as described above. The methyl esters were redissolved in 100 µl hexane 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. The plates were fully developed in toluene/acetonitrile (95:5, v/v) (Wilson and Sargent 1992). Autoradiography was performed with Kodak MR2 film for 6 days at room temperature. Silica corresponding to individual PUFA was scraped into scintillation mini-vials containing 2.5 ml of scintillation fluid (Ecoscint A, National Diagnostics, Atlanta, Georgia) and the radioactivity was determined in a TRI-CARB 2000CA scintillation counter (United Technologies Packard, U.K.). The results were corrected for counting efficiency and quenching of ^{14}C under exactly these conditions.

Ionophore stimulation, eicosanoid extraction and measurement

Gill cell suspensions were incubated for 10 min at 20 ºC in a shaking water bath before the addition of A23187 (2 µl in dimethylsulphoxide, 10 µM final concentration). The incubation was continued for 15 min after which the cells were removed by centrifugation (12 000 x g, 2 min) and then the supernatant was transferred to tubes containing 150 µl ethanol and 50 µl 2 M formic acid. Samples were frozen immediately in liquid nitrogen and stored at -20ºC until extraction and measurement of eicosanoids. The frozen gill cell supernatants were thawed and centrifuged (12 000 x g, 2 min) to remove any precipitate. The supernatants were extracted using octadecyl silica (C18) ‘Sep-Pak’ minicolumns (Millipore (UK), Watford) as described in detail by Bell et al. (1994). The final extract was dissolved in 0.1 ml methanol and stored at –20 ºC prior to analysis by enzyme immunoassay. Samples were dried under nitrogen and redissolved in immunoassay buffer. Measurement of total PGF was performed using an enzyme immunoassay kit for $PGF_{2\alpha}$ according to the manufacturer’s protocols (SPI-Bio, Massy, France).

Seawater challenge and plasma chloride determination

In mid March (week 18 of trial), twenty fish per dietary treatment that were still in freshwater were subjected to a seawater challenge test to assess their ability to osmoregulate. The fish were

transferred to a tank containing standardised seawater at 35 parts per thousand in a static tank, with aeration, for a period of 24 h. Artificial seawater was produced by adding the appropriate volume of "Seamix" sea salts (Peacock Salt Ltd., Glasgow, Scotland) to freshwater. After 24 h the fish were euthanased and blood was removed via the caudal vein into heparinised tubes. The tubes were mixed, centrifuged immediately, and then the plasma was removed and stored at -20ºC. Plasma chloride concentrations were measured using a Jenway Chloride meter (Model PCLM3) using the method described in the operating manual.

Protein determination

Protein concentrations in isolated gill cell and hepatocyte suspensions were determined according to the method of Lowry et al. (1951) after incubation with 0.25 ml of 0.25% (w/v) SDS/1M NaOH for 45 min at 60ºC.

Materials

[1-^{14}C]18:3n-3 (50-55 mCi/mmol) was obtained from NEN (DuPont (U.K.) Ltd., Stevenage, U.K.). HBSS, Medium 199, HEPES buffer, glutamine, penicillin, streptomycin, collagenase (type IV), FAF-BSA, calcium ionophore A23187, 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.

Statistical analysis

All the data are presented as means ± SD (n=3) unless otherwise stated. The significance of treatment effects on tissue fatty acid compositions, hepatocyte fatty acid desaturation and gill prostaglandin production due to both time and diet and their interaction were determined by two-way analysis of variance (ANOVA). The effects of diet on growth and the effects of time on muscle fatty acid composition were also analysed by one-way ANOVA and, where appropriate, the differences between means were determined by Tukey's post-test. Similarly, the effects of diet on hepatocyte desaturation and gill PGF production at each individual time point were also determined

by one-way ANOVA and, where appropriate, differences between means were determined by Tukey's post-test. Percentage data and data which were identified as non-homogeneous (Bartlett's test) were subjected to either arcsine, square root or log transformation before analysis. Differences were regarded as significant when $P < 0.05$.

Results

Effects of time

Tissue fatty acid compositions. The fatty acid compositions of the polar lipid from the liver and gills varied very significantly over the 9 month time-course of the experiment (Table 3). In the liver there was a clear trend for the proportions of both AA and DHA to increase in the polar lipid up to seawater transfer and then to decrease after transfer (Fig.1). In contrast, percentages of EPA declined initially following transfer of the fish to the experimental diets. Subsequently, the proportions of EPA in liver polar lipid were relatively constant up to the time of seawater transfer and then they increased dramatically during the seawater residency period. Gill fatty acid compositions showed more variability to the extent that there were no statistically significant temporal effects on AA composition. By contrast, trends were found for increasing proportions of DHA prior to seawater transfer and for increasing EPA after seawater transfer (Table 3; Fig.2). Both liver and gill polar lipid AA/EPA ratios varied significantly over the time course of the study and peak values were evident at, or just prior to, seawater transfer (Table 3; Fig.3). The fatty acid composition of muscle (flesh) total lipid was also altered significantly ($P < 0.0001$) over the time-course of the experiment but these differences were highly dependent upon diet as evinced by the very highly significant values for the interaction, particularly for 18:2n-6 and 18:3n-3 ($P < 0.0001$) (Table 3; Fig.4).

Hepatocyte fatty acid desaturation/elongation activities. The hepatocyte desaturation/elongation activity, determined using [1-^{14}C]18:3n-3 as the substrate, displayed a highly significant variation during the time-course of the experiment (Table 3). The total amount of 18:3n-3 desaturated increased during the first part of the freshwater phase and showed peak levels of activity at 14 weeks, about 1 month prior to seawater transfer (Fig.5). The activity of the desaturation/elongation

pathway declined just prior to seawater transfer and remained low during the seawater phase of the experiment. The peak in total desaturation products was also observed at 14 weeks with the products of the individual desaturation steps in the pathway. Thus, the tetraene products (18:4n-3 and 20:4n-3), pentaene products (20:5n-3 and 22:5n-3) and the major hexaene product (22:6n-3) all showed peak production at 14 weeks and declined prior to seawater transfer (Fig.6).

Gill prostaglandin production. Total prostaglandin F production by gill cells in response to calcium ionophore A23187 stimulation was very significantly affected over the time-course of the experiment showing a large peak in production at week 28, approximately 2 months after transfer to seawater (Table 3; Fig.7).

Effects of diet

Growth. The diets containing vegetable oils had no significant effect on growth compared to the diet containing fish oil during the freshwater phase when the diets were actually being fed (Table 4). However, after transfer to seawater, when all groups were fed the same FO diet, the fish that had previously been fed diets containing LO showed slightly, but statistically significant lower final mean weights and they tended to have lower SGR at the termination of the experiment.

Seawater adaptation. In a seawater tolerance test carried out at week 18, the mean plasma chloride concentration for fish fed the LOA diet was significantly lower than that of fish fed the FO diet (Table 4). The mean plasma chloride concentrations for fish fed the other diets containing vegetable oils were lower than the mean value noted for fish fed the FO diet although the differences were not statistically significant.

Tissue fatty acid compositions. There were some highly significant dietary effects observed in the tissue fatty acid compositions (Table 3). The proportion of EPA in liver polar lipids was lower in fish fed the diets containing vegetable oils compared to the FO diet whereas the proportion of AA tended to be lower in fish fed the FO diet compared to the diets containing vegetable oil (Fig.1). In gill polar lipids, only the proportion of DHA was significantly affected by diet with the gill polar lipids from fish fed the diets containing LO tending to show higher proportions (Fig.2). The

AA/EPA ratios in tissue polar lipids was significantly affected by diet (Table 3). In liver, the AA/EPA ratio tended to be lower in the FO group whereas in gills the AA/EPA ratio was generally higher in the LOA group (Fig. 3). The levels of 18:2n-6 and 18:3n-3 in muscle total lipid were significantly influenced by diet with 18:3n-3 being greatly increased in fish fed the diets containing linseed oil (LO and LOA) and, to a lesser extent, rapeseed oil, after 18 weeks of feeding (Table 3; Fig.4). The level of 18:3n-3 in muscle total lipid was similar to initial levels by the termination of the experiment in all groups of fish after 15 weeks on a diet containing only fish oil. Similarly, the level of 18:2n-6 in the flesh was significantly increased in the RO and LO groups, but not the LOA group (Fig.4). However, the level of 18:2n-6 in muscle total lipid at the termination of the experiment was significantly greater in the fish previously fed the FO and RO diets compared to fish previously fed diets containing linseed oil (Fig.3). Only the RO diet significantly reduced the level of total n-3HUFA in flesh total lipid after 18 weeks on the diet (Fig.4).

Hepatocyte fatty acid desaturation/elongation activities. Hepatocyte fatty acid desaturation/elongation activities were very significantly affected by diet with the amount of [1-^{14}C]18:3n-3 desaturated at the time of peak activity (14 weeks) being highest in the fish fed the RO diet and lowest in the fish fed the FO diet (Table 3; Fig.5). There was no significant difference in hepatocyte desaturation/elongation activity between the groups of fish at the termination of the experiment (Fig.5). The production of tetraene, pentaene and hexaene products at the time of peak activity were all greatest in fish fed the RO diet and lowest in fish fed the FO diet (Fig.6).

Gill prostaglandin production. All groups of fish showed the same peak of gill total prostaglandin F production 9 weeks after seawater transfer. However, the level of PGF in fish previously fed the LOA diet was lower than the levels observed for the other groups of fish; significantly so in the case of fish previously fed the FO and LO diets (Table 3; Fig.7).

Discussion

The present study confirmed our earlier results that during parr-smolt transformation in Atlantic salmon there is a pre-adaptive increase in hepatocyte fatty acyl desaturation/elongation activities

(Bell et al. 1997). Also as noted previously, dietary fatty acid composition was shown to have very significant modulating effects particularly in terms of the magnitude of the changes. However, the present study has reinforced the idea that the primary regulation of the fatty acyl desaturation/elongation activities during this period involves powerful environmental and genetic controls. It is fairly well established that the primary stimulus for parr-smolt transformation is photoperiod (Duston and Saunders 1990). Therefore, it is likely that the increase in hepatocyte fatty acyl desaturation activity as measured in vitro is stimulated by increased photoperiod just as previously observed for gill $Na^{+}K^{+}$ ATPase activity (Duston and Saunders 1990).

In the previous study, the peak activities were observed at the time of seawater transfer and declined rapidly thereafter. As the fish in the previous study were fed a fish oil-containing diet upon seawater transfer, it was not possible to fully discriminate possible environmental and genetic cues for this decline from the inhibitory effects on fatty acyl desaturation of dietary long chain PUFA (contained in the dietary fish oil) such as EPA and DHA. However, the fact that fish being fed a fish oil diet throughout the parr stage also showed a decline in desaturation activities upon seawater transfer had suggested that environmental/genetic regulation was a factor. In the present study the peak of desaturation/elongation activity actually occurred about 1 month prior to the time that the fish were transferred to seawater and the activities had decreased prior to transfer. The latter occurred while the fish were still being fed the parr diets and before they were switched to the fish oil-containing marine ongrowing or post-smolt diet. This clearly demonstrates the importance of environmental/genetic factors in the regulation of the desaturation/elongation activities. Ignoring the actual point of seawater transfer, which is an artificially imposed time-point (albeit based on well established observations of appearance, behaviour, and plasma chloride data obtained from seawater tolerance tests), in terms of date, the hepatocte fatty acyl desaturation activities peaked in early March in the present study compared to late April in the previous trial (Bell et al. 1997). This was a very significant finding of the present study because it strongly suggested that another environmental cue, other than photoperiod, had an important controlling effect on hepatocyte desaturase activity, namely, temperature. The winter of 1997-98 was exceptionally mild especially during January and February with average temperatures of 4.9 ± 1.3 °C and 6.8 ± 1.8 °C in those months respectively, compared to 3.7 ± 1.6 °C and 5.0 ± 0.8 °C in the previous study. Based on the criteria described above, the fish had undergone parr-smolt transformation (i.e. were ready for seawater transfer) at least one month earlier, in late March as opposed to late April,

in the present study and compared to the previous study. Indeed, the plasma chloride values obtained in week 18 indicated that all groups of fish were well-adapted smolts. Therefore, we suggest that, due to the mild winter and warmer temperatures experienced by the fish, parr-smolt transformation was advanced compared to the previous study and that the peak in *in vitro* hepatocyte desaturation/elongation activites was advanced similarly. Temperature has been reported previously to influence the smolting and desmolting processes in salmonids including Atlantic salmon (Wagner 1974; Duston and Saunders 1990; Gaigon and Quemener 1992; McCormick 1995). Specifically, increases in temperature are known to be an important cue for the completion of smolting in salmon (Duston and Saunders 1990). The hepatocyte fatty acyl desaturation data suggest that the fish in the present study were ready for seawater transfer earlier than the time at which they were actually transferred. This was supported by anecdotal evidence from staff responsible for fish husbandry, but caution is required in over-interpreting the differences in the data from the two studies. This is because it is not possible to determine which pattern, either peak of desaturation and elongation activities exactly at seawater transfer or peak prior to transfer, is the one that would be found in wild fish. However, there was no evidence from the fatty acid compositional data (i.e reversion to a "freshwater", lower HUFA pattern, that would suggest the decline in desaturase activities prior to transfer in the present study was a sign of a "desmoltification" process induced by being in freshwater longer than optimal as has been described previously (Li and Yamada 1992).

We have suggested that the effects observed on in vitro hepatocyte fatty acyl desaturation activities over the two studies are evidence that environmental temperature is an important factor in determining the timing of the changes in desaturation activities. This tends to imply that the mechanism for such an effect is based on temperature being an environmental cue for the processes of parr-smolt transformation in general and that increased desaturation activity is dependent on other physiological and hormonal stimuli stimulated by temperature changes. The evidence appears to support this. Whereas the earlier increase in desaturation activity in the present trial could simply be the result of a direct kinetic effect of increased temperature on enzyme activity, it would not be able to explain the subsequent very large decrease in activity. Furthermore, there are precendents with other enzyme activities for environmental temperature to be regarded as an environmental cue. For instance, gill $Na^{+}K^{+}$ATPase activity in smolts maintained in freshwater

decreased more rapididly in fish maintained at higher temperatures (Duston and Saunders 1990; McCormick 1995).

It is clear, therefore, that in the period of parr-smolt transformation in Atlantic salmon, there is an environmentally/genetically driven increase in hepatocyte fatty acyl desaturase/elongation activities that reaches a peak before declining again and that, qualitatively at least, these changes are independent of dietary effects. However, our previous study in which we compared dietary fish oil and vegetable oil (a 1:1 rapeseed/linseed oil blend), showed that diet could influence the smoltification process (Bell et al. 1997). Specifically, we showed that the increased hepatocyte fatty acyl desaturation activities were inhibited in parr fed fish oil compared to parr fed the vegetable oil blend and that the blend also had beneficial effects on salinity adaptation. Therefore, a primary aim of the present study was to discriminate between the relative efficacies of linseed oil, with an 18:3n-3/18:2n-6 ratio of 2, and rapeseed oil, with an 18:3n-3/18:2n-6 ratio of 0.5, in their ability to influence the process of parr-smolt transformation. In this respect, and as expected, both vegetable oil diets had very significant effects on hepatocyte fatty acyl desaturation, with fish fed the diets containing vegetable oils showing much greater increases in activity than those fed the FO diet, and with the activities being significantly higher in fish fed the RO diet compared to those fed the LO diet. These effects were observed at all steps of the desaturase/elongase pathway. The mechanism for these effects can probably be attributed entirely to the much reduced levels of HUFA in the RO and LO diets compared to the FO diet in which the 3-fold higher levels of EPA and DHA would exert a powerful feedback (product) inhibition on $\Delta 6$ and $\Delta 5$ desaturase enzymes (Brenner 1981; Tocher et al. 1997).

The different dietary oils had significant effects on liver and gill polar lipid fatty acid compositions. These were perhaps fewer than expected, although they varied between the tissues. For instance, AA and EPA were significantly affected by diet in the liver but not the gill whereas the opposite was true for DHA. However, the present study showed that the proportions of AA and DHA in the liver and gill both increased up to seawater transfer and then decreased after transfer. This pattern is consistent with the observed changes in fatty acyl desaturation/elongation activities and was very similar to the pattern observed previously in gills (Bell et al. 1997). However in the previous study, the levels of AA and DHA in the liver also increased after seawater transfer. In that study the dietary levels of AA and DHA also increased after transfer as the seawater diet had 24% lipid compared to only 19% lipid in the freshwater diets, and so it is possible that the levels of

AA and DHA in the liver were affected by the increased intake of AA and DHA by the fish in seawater. The present study has shown that salmon fed diets with equal lipid content during freshwater and seawater residency do not exhibit any increase in liver AA and DHA post transfer. In contrast, the very large increase in the proportion of EPA in both liver and gill polar lipids after seawater transfer, as observed previously, was reproduced exactly in the present study (Bell et al. 1997). As before, the changes in polar lipid EPA level post transfer were independent of previous dietary history in that the fish fed FO prior to transfer showed exactly the same increase in EPA as observed with fish fed the RO, LO and LOA diets despite the fact that there was no difference in the EPA content of the FO diet and the seawater diet. This supports our previously expressed view that increased tissue polar lipid EPA was not due to differences in lipid contents or compositions of the diets and that there appears to be a physiological requirement for increased polar lipid EPA in post-smolts (Bell et al. 1997). However, the mechanism underpinning the increased tissue polar lipid EPA content at a time when there is no increased intake of EPA (as in the FO fed fish) is unclear but may reflect increased retention and decreased oxidation of dietary EPA and, possibly, retroconversion of dietary DHA although this is all speculative.

Important commercial factors in the feeding of diets containing vegetable oils to Atlantic salmon relate to the effects they may have on growth performance and on muscle (flesh) lipid fatty acid composition. It was therefore noteworthy that the diets containing the rapeseed oil and linseed oil did not adversley affect the growth of the Atlantic salmon in freshwater. However, despite being fed the same diet in the post-smolt ongrowing phase, the fish previously fed either FO or RO diets had significantly higher final weights compared to those fed either of the LO-containing diets. This probably relates to the increased levels of monounsaturated fatty acids present in the FO and RO diets (44 & 50% of total dietary fatty acids, respectively) compared to those in the LO-containing diets (25 & 26%, respectively). The monounsaturated fatty acids in the FO and RO diets would be stored in the tissue triacylglycerols during the freshwater phase for subsequent catabolism in the rapid growth phase post-seawater transfer. The more complex catabolic pathways required to oxidise PUFA means that they generate less metabolic energy during catabolism than either monounsaturated or saturated fatty acids (Sargent et al. 1989). Consequently, the higher levels of monounsaturated fatty acids in the freshwater diets formulated with FO and RO allowed for protein sparing during the seawater phase resulting in increased growth.

The ingestion of the RO and LO diets for 19 weeks affected the flesh fatty acid compositions of the juvenile Atlantic salmon when measured just prior to transfer. For instance, percentages of 18:2n-6 were increased in the muscle of fish fed the diets with RO and LO and 18:3n-3 was increased more than 5-fold in the fish fed the LO diet relative to those fed the FO diet. It was noteworthy however that the inclusion of AA to the linseed oil diet significantly reduced the level of 18:3n-3 in the muscle and substantially lowered 18:2n-6 compared to the LO diet. The level of flesh 18:3n-3 was also increased by the RO diet but much less so than the LO diet, as expected, but perhaps more importantly, it was the only diet that reduced flesh n-3 HUFA levels prior to transfer. These changes in muscle fatty acid composition would not be desirable if they persisted when fish were in seawater, but 15 weeks of feeding the seawater diet containing fish oil resulted in complete normalisation of the flesh 18:3n-3 levels in fish on all diets and 18:2n-6 in the linseed oil-containing diets. In contrast, the level of 18:2n-6 in the flesh was not decreased by feeding the seawater diet in fish previously fed the RO diet, and actually significantly increased in fish previously fed the FO diet. Therefore, there was a clear difference between the fish previously fed the FO and RO diets and the fish previously fed the linseed oil diets with the FO and RO fish displaying significantly higher 18:2n-6 levels in the muscle than the linseed oil fish. Coincidentally, this biochemical difference correlated with the growth data but the physiological significance, if any, is unclear.

However, another important consequence of altered tissue polar lipid fatty acid compositions are effects on eicosanoid metabolism. This study showed that the AA/EPA ratios in the polar lipid of both gills and liver peaked around the time of transfer and that diet significantly affected this parameter, as observed previously (Bell et al. 1997). In particular, the addition of AA to the linseed oil diet (diet LOA) increased the AA content of gill polar lipids and the gill AA/EPA ratio suggesting that gill AA is an important factor in successful seawater adaptation/osmoregulation. However, paradoxically it was the LOA diet that resulted in the significantly lowest peak of gill total PGF production post transfer. The peak of gill PGF production post transfer was interesting for two reasons. It occurred a relatively long time after transfer (9 and 8 weeks in the present and previous trials, respectively) although this was dependent upon the time of sampling chosen and on the dietary history prior to transfer as it only occurred in fish fed vegetable oil in the previous study, and there were significant differences between the dietary groups in the present study. In addition, an important difference to the previous study was observed, in that there was no

significant peak in gill PGF production prior to transfer with any of the diets in the present study whereas there was a peak of gill PGF production prior to transfer in fish fed the linseed /rapeseed blend (Bell et al. 1997). These differences may relate to differences in time of sampling relative to stage of parr-smolt transformation, which was more advanced in the present study.

The physiological significance of the peak in gill PGF production post transfer was unclear but eicosanoids have diverse physiological functions in fish including roles in reproductive function, hormone release, cardiovascular function, neural function and osmoregulation (Mustafa and Srivastava 1989). In particular, prostaglandins are modulators of ion and electrolyte balance in the kidney (Brown and Bucknall 1986; Bell et al. 1993), gills and opercular epithelium (Wales 1988) and operate synergistically with prolactin, a hyperosmoregulatory hormone, in the regulation of plasma Na^+ and Ca^{2+} concentrations (Van Pragg et al. 1987). Prostaglandins may also affect electrolyte balance at a number of sites including the Na^+/Cl^- co-transporter, the Na^+K^+ATPase, the apical chloride conductance, or the basolateral K^+ conductance (Van Praag et al. 1987). In the present study, a seawater challenge performed at 18 weeks, a week prior to seawater transfer, showed that all the groups of fish fed the vegetable oils and especially the LOA group, had lower concentrations of plasma chloride compared to those fed FO. Therefore, it was noteworthy that the diet that most affected gill AA levels and AA/EPA ratio, as well as gill PGF production, was also the diet that most significantly affected plasma chloride levels. Therefore, the ability of the fish to osmoregulate was directly related to their diet and this may be mediated through changes in tissue polar lipid fatty acid compositions, in particular AA and EPA levels and AA/EPA ratios, and the resultant prostaglandin production.

The present study has shown that during parr-smolt transformation in Atlantic salmon there is a pre-adaptive increase in hepatocyte fatty acyl desaturation/elongation activities that is primarily controlled by environmental factors such as photoperiod and temperature but that can also be significantly modulated by diet. The feeding of diets supplemented with vegetable oils such as rapeseed and linseed oils to parr can prevent dietary inhibition of the desaturase activities induced by feeding diets containing fish oils at this stage. Also, this strategy can influence the smoltification process by altering the tissue polar lipid fatty acid compositions and eicosanoid production and it appears to have a beneficial effect on the ability of the fish to osmoregulate and thus allow more successful adaptation to the marine environment. Diets containing either linseed oil or rapeseed oil

displayed the above beneficial effects but neither oil on its own nor linseed oil supplemented with AA were more effective than a blend of the two oils that was used in a previous study.

Acknowledgements

We would like to thank Marine Harvest McConnell Ltd. for supplying fish and rearing facilities and Trouw Aquaculture for preparing the experimental diets. We are also grateful to the staff of Marine Harvest McConnell Ltd. at Kinlochmoidart and Lochailort for their expertise in fish husbandry.

References

Ackman, R.G. 1980. Fish lipids, part 1. *In*: Advances in Fish Science and Technology. pp. 87-103. Edited by J.J. Connell. Fishing News Books, Farnham, U.K.

Ackman, R.G. and Takeuchi, T. 1986. Comparison of fatty acids and lipids of smolting hatchery-fed and wild Atlantic salmon *Salmo salar*. Lipids 21: 117-120.

Bell, J.G. 1996. Influences of dietary polyunsaturated fatty acid composition and eicosanoid production in Atlantic salmon (*Salmo salar*). Doctor of Philosophy Thesis, University of Stirling, Scotland, U.K.

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

Bell, J.G., Tocher, D.R., Farndale, B.M., Cox, D.I., McKinney, R.W. and Sargent, J.R. 1997. The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation. Lipids 32: 515-525.

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

Bell, M.V., Henderson, R.J., Pirie, B.J.S. and Sargent, J.R. 1985. Effects of dietary polyunsaturated fatty acid deficiencies on mortality and gill structure in the turbot *Scophthalmus maximus*. J. Fish Biol. 26: 181-191.

Bolgova, O.M. and Shchurov, I.L. 1987. Adaptive changes in fatty acid Spectra in tissue lipids of wild and cultivated salmon *Salmo salar* during the parr-smolt period. Zh. Evol. Biokhim. Fiziol. 23: 211-215.

Brenner, R.R. 1981. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog. Lipid Res. 20: 41-47.

Brown, J.A. and Bucknall, R.M. 1986. Antidiuretic and cardiovascular actions of PGE_2 in the rainbow trout *Salmo gairdneri*. Gen. Comp. Endocrinol. 61: 330-337.

Christie, W.W. 1982. Lipid Analysis 2nd Edition. Pergamon Press, Oxford. pp. 207.

Di Constanzo, G., Duportail, G., Florents, A. and Leray, C. 1983. The brush border membrane of trout intestine: Influence of its lipid composition on ion permeability, enzyme acitvity and membrane fluidity. Mol. Physiol. 4: 279-290.

Duston, J. and Saunders, R.L. 1990. Control of the timing of smoltification in Atlantic salmon: Endogenous rhythms and environmental factors. In Proc. Of Canada-Norway Finfish Aquaculture Workshop. Pp. 99-105. Edited by R.L. Saunders. Can. Tech. Rep. Fish. Aquat. Sci., Canada.

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

Folmar, L.C. and Dickhoff, W.W. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids: A review of selected literature. Aquaculture 21: 1-37.

Gaignon, J.-L. and Quemener, L. 1992. Influence of early thermic and photoperiodic control on growth and smoltification in Atlantic salmon (*Salmo salar*). Aquat. Living Resour. 5: 185-195.

Gerbi, A., Zerouga, M., Debray, M., Durand, G., Chanez, C. and Bourre, J-M. 1994. Effect of fish oil diet on fatty acid composition of phospholipids of brain membranes and on kinetic properties of Na^+, K^+-ATPase isoenzymes of weaned and adult rats. J. Neurochem. 62: 1560-1569.

Hoar, W.S. 1976. Smolt transformation: Evolution, behaviour and physiology. J. Fish Res. Board Can. 33: 1234-1252.

Li, H.-O. and Yamada, J. 1992. Changes of the fatty acid composition in smolts of masu salmon (*Oncoryhnchus masou*) , associated with desmoltification and seawater transfer. Comp. Biochem. Physiol. 103A: 221-226.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. J. Biol Chem. 193: 265-275.

McCormick, S.D. 1995. Loss of smolt characteristics in hatchery- and stream-reared Atlantic salmon. In High Performance Fish. pp. 51-56. Edited by D.D. Mackinlay. American Fisheries Soc.

Mustafa, T. and Srivastava, K.C. 1989. Prostaglandins (eicosanoids) and their role in ectothermic organisms. Adv. Comp. Environ. Physiol. 5: 157-207.

Sargent, J.R., Henderson, R.J. and Tocher, D.R. 1989. The lipids. *In*: Fish Nutrition. pp. 154-218. Edited by J. E. Halver. Academic Press, New York.

Sheridan, M.A., Allen, W.V. and Kerstetter, T.H. 1985. Changes in the fatty acid composition of steelhead trout, S*almo gairdnerii* Richardson associated with parr-smolt transformation. Comp. Biochem. Physiol. 80B: 671-676.

Spector, A.A. and Yorek, M.A. 1985. Membrane lipid composition and cellular function. J. Lipid Res. 26: 1015-1035.

Tocher, D.R., Bell, J.G., Dick, J.R. and Sargent, J.R. 1997. Fatty acyl desaturation in isolated hepatocytes from Atlantic salmon (*Salmo salar*): Stimulation by dietary borage oil containing γ-linolenic acid. Lipids 32: 1237-1247.

Tocher, D.R., Sargent, J.R. and Frerichs, G.N. 1988. The fatty acid compositions of established fish cell lines after long-term culture in mammalian sera. Fish Physiol. Biochem. 5: 219-227.

U.S. National Research Council 1993. Nutrient Requirements of Fish, Washington D.C. National Academy Press.

Van Praag, D., Farber, S.J., Minkin, E. and Primor, N. 1987. Production of eicosanoids by the killifish gills and opercular epithelia and their effect on active transport of ions. Gen. Comp. Endocrinol. 67: 50-57.

Wagner, H.H. 1974. Photoperiod and temperature regulation of smolting in steelhead trout (*Salmo gairdneri*). Can. J. Zool. 52: 219-234.

Wales, N.A.M. 1988. Hormone studies in myxine glutinosa: effects of the eicosanoids arachidonic

acid, prostaglandin E_1, E_2, A_2, $F_{2\alpha}$, thromboxane B_2 and of indomethacin on plasma cortisol, blood pressure, urine flow and electrolyte balance. J. Comp. Physiol. 158B: 621-626.

Wedermeyer, G.A., Saunders, R.L. and Clarke, W.C. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev. 42: 1-14.

Wilson, R. and Sargent, J.R. 1992. High resolution separation of polyunsaturated fatty acids by argentation thin-layer chromatography. J. Chromatogr. 623: 403-407.

Woyewoda, A.D., Shaw, S.J., Ke, P.J. and Burns, B.G. 1986. Recommended laboratory methods for assessment of fish quality. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1448. 143 pages.

Legends to Figures:

FIG. 1 Levels of the highly unsaturated fatty acids 20:4n-6, 20:5n-3, and 22:6n-3 in polar lipids of liver from Atlantic salmon juveniles fed diets containing either fish oil (FO; ●), rapeseed oil (RO; ▲), linseed oil (LO; ■) or linseed oil supplemented with 20:4n-6 (LOA; □) up to seawater transfer. Values are % weight of total fatty acids and are means ± SD for three replicate samples. The dotted line represents the time of transfer from freshwater to seawater.

FIG. 2 Levels of the highly unsaturated fatty acids 20:4n-6, 20:5n-3, and 22:6n-3 in polar lipids of gill from Atlantic salmon juveniles fed diets containing either fish oil (FO; ●), rapeseed oil (RO; ▲), linseed oil (LO; ■) or linseed oil supplemented with 20:4n-6 (LOA; □) up to seawater transfer. Values are % weight of total fatty acids and are means ± SD for three replicate samples. The dotted line represents the time of transfer from freshwater to seawater.

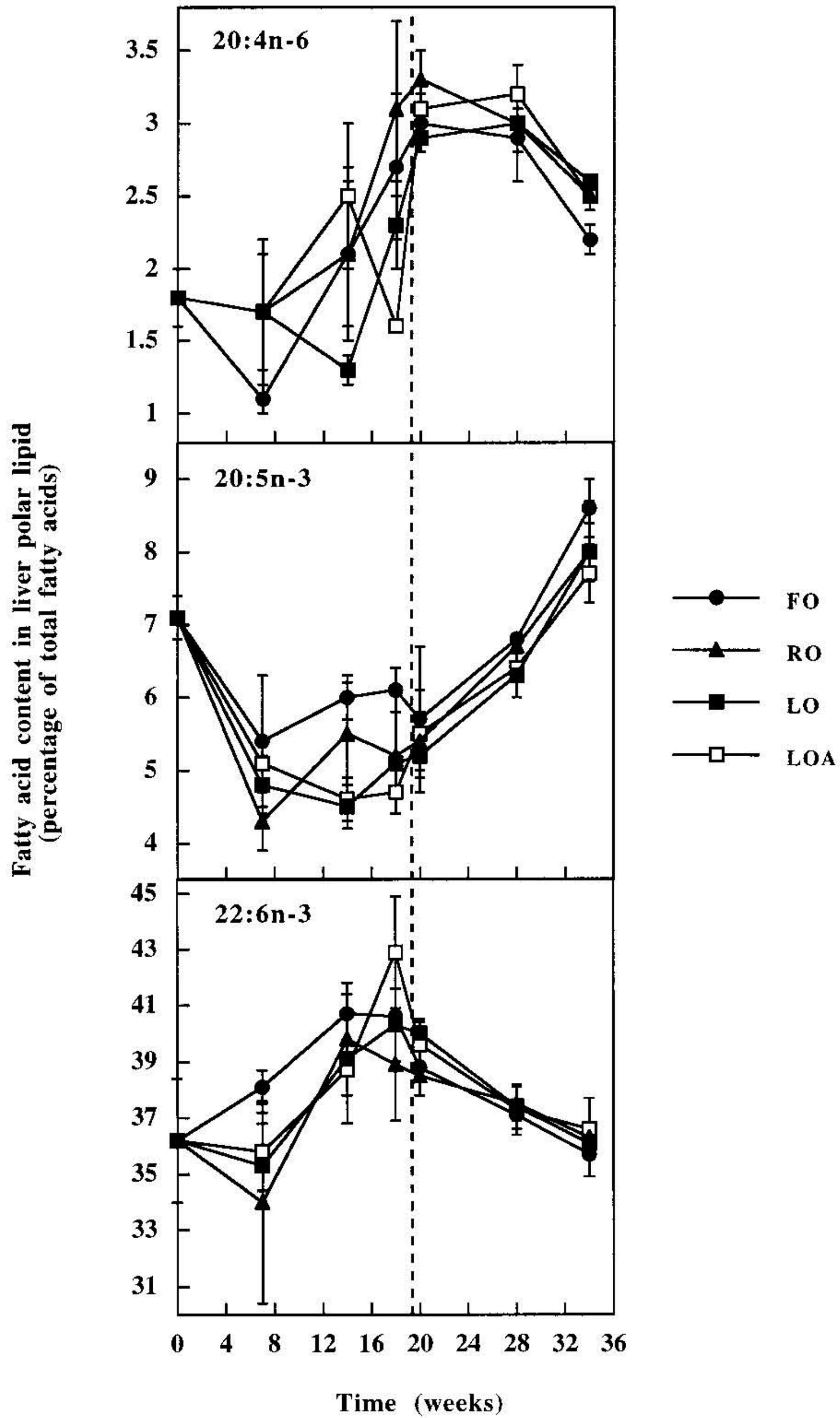
FIG. 3. The ratio of 20:4n-6/20:5n-3 (AA/EPA) in the polar lipids of liver and gill from Atlantic salmon juveniles fed diets containing containing either fish oil (FO; ●), rapeseed oil (RO; ▲), linseed oil (LO; ■) or linseed oil supplemented with 20:4n-6 (LOA; □) up to seawater transfer. Each point represents the mean ± SD for three replicate samples. The dotted line represents the time of transfer from freshwater to seawater.

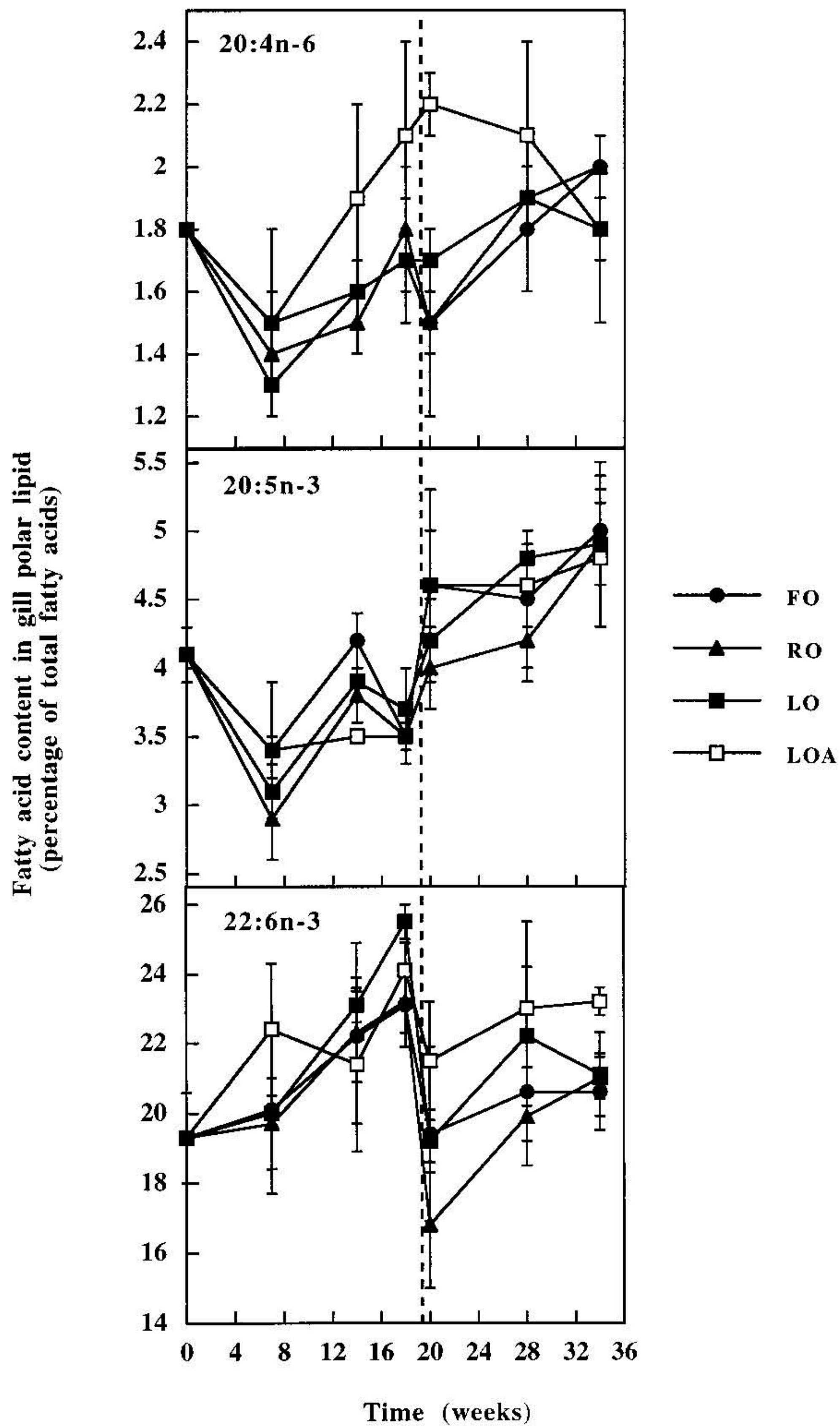
FIG. 4. Levels of 18:2n-6, 18:3n-3, and n-3 highly unsaturated fatty acids (n-3HUFA) in total lipid of muscle (flesh) from Atlantic salmon juveniles fed diets containing either fish oil (FO; □), rapeseed oil (RO; □), linseed oil (LO; □) or linseed oil supplemented with 20:4n-6 (LOA; ■) up to seawater transfer. Values are % weight of total fatty acids and are means ± SD for three replicate samples. Transfer from freshwater to seawater occurred in week 19. Mean contents for a specific fatty acid type (18:2n-6, 18:3n-3 or n-3HUFA) with a different superscript letter are significantly different ($P < 0.05$) as determined by one-way analysis of variance and Tukey's test as described in the Materials and Methods section.

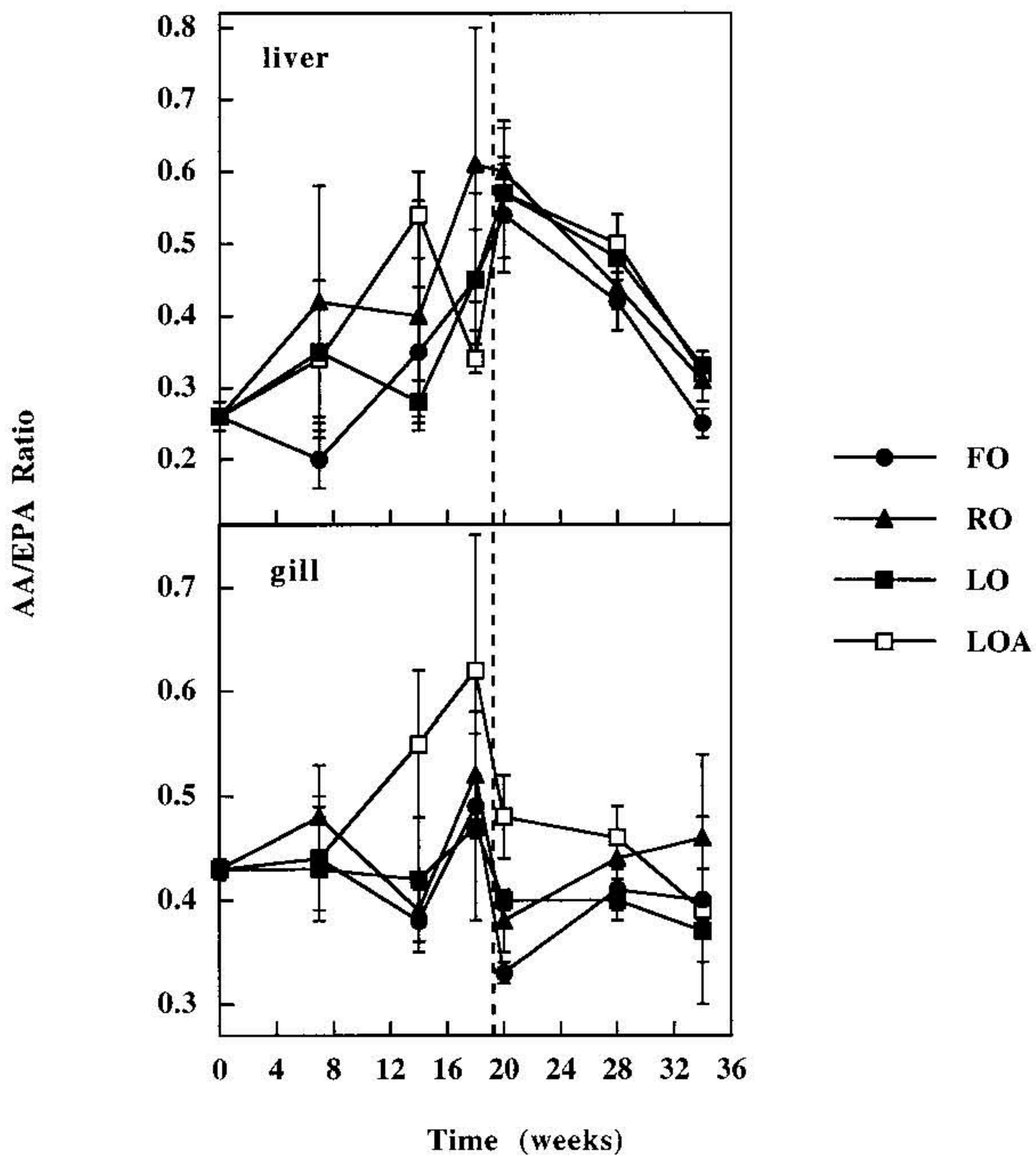
FIG. 5. Total amount of [1-^{14}C]18:3n-3 desaturated and elongated/hour per mg protein by isolated hepatocytes from Atlantic salmon juveniles fed diets containing either fish oil (FO; ●), rapeseed oil (RO; ▲), linseed oil (LO; ■) or linseed oil supplemented with 20:4n-6 (LOA; □) up to seawater transfer. Each point represents the mean ± SD for three replicate samples. The dotted line represents the time of transfer from freshwater to seawater. Points at the same sampling time with a different assigned letter are significantly different between dietary treatments ($P < 0.05$) as determined by one-way analysis of variance and Tukey's test as described in the Materials and Methods section.

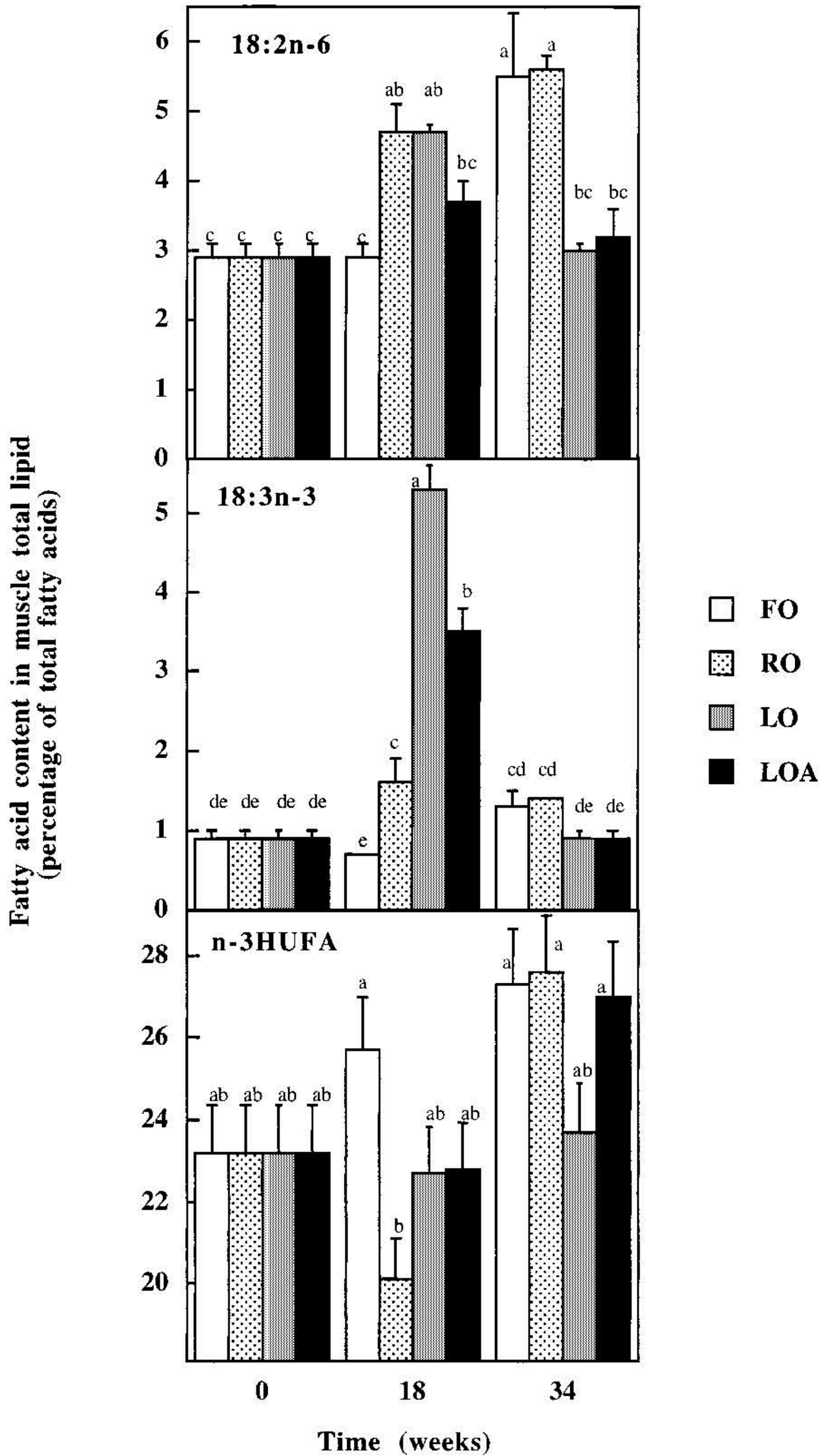
FIG. 6. Amount of [1-^{14}C]18:3n-3 desaturated and elongated to tetraene (18:4n-3 and 20:4n-3) products, pentaene (20:5n-3 and 22:5n-3) products and 22:6n-3 by isolated hepatocytes from Atlantic salmon juveniles fed diets containing either fish oil (FO; ●), rapeseed oil (RO; ▲), linseed oil (LO; ■) or linseed oil supplemented with 20:4n-6 (LOA; □) up to seawater transfer. Results are experessed as pmol produced/hour/mg hepatocyte protein and are means ± SD for three replicate samples. The dotted line represents the time of transfer from freshwater to seawater.

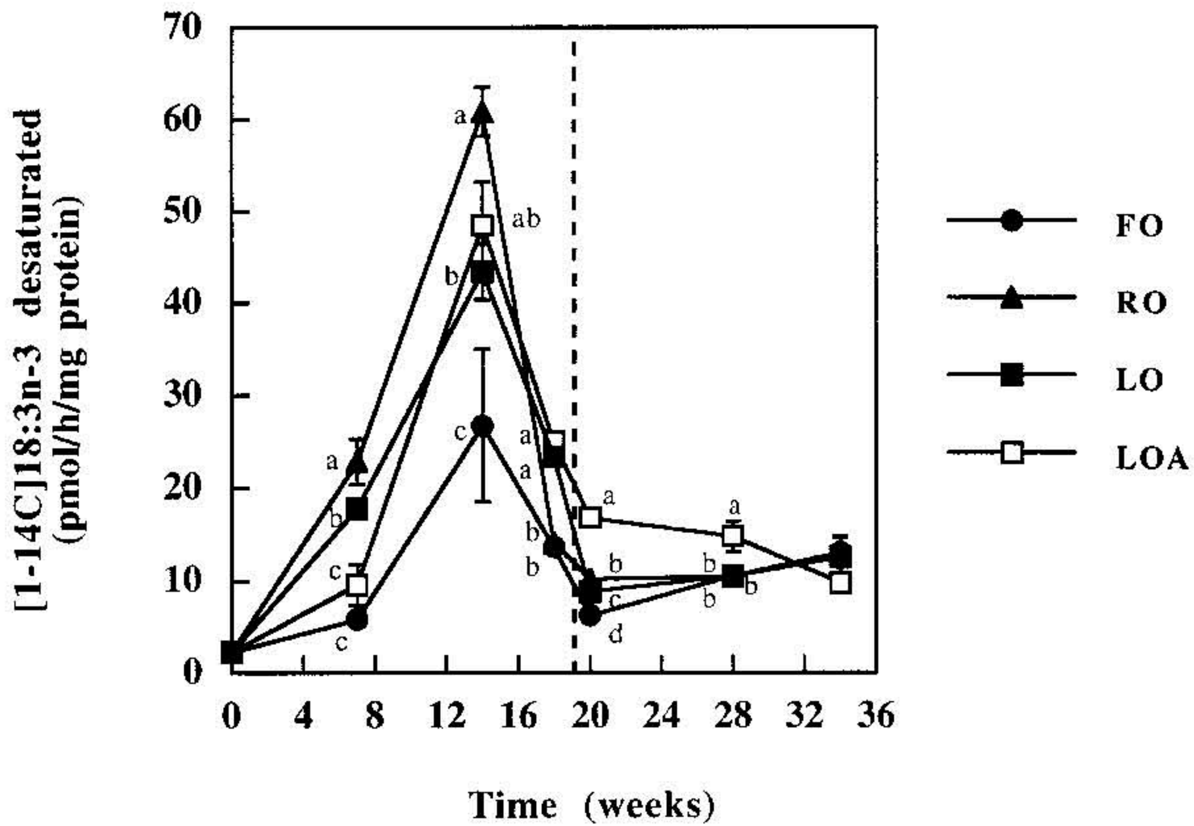
FIG. 7. Production of total prostaglandin (PG) F by isolated gill cells, stimulated with the calcium ionophore A23187, from Atlantic salmon juveniles fed diets containing either fish oil (FO; ●), rapeseed oil (RO; ▲), linseed oil (LO; ■) or linseed oil supplemented with 20:4n-6 (LOA; □) up to seawater transfer. Results are expressed as pmol/mg protein and are means ± SD for three replicate samples. The dotted line represents the time of transfer from freshwater to seawater. Points at the same sampling time having a different assigned letter are significantly different between dietary treatments ($P < 0.05$) as determined by one-way analysis of variance and Tukey's test as described in the Materials and Methods section.

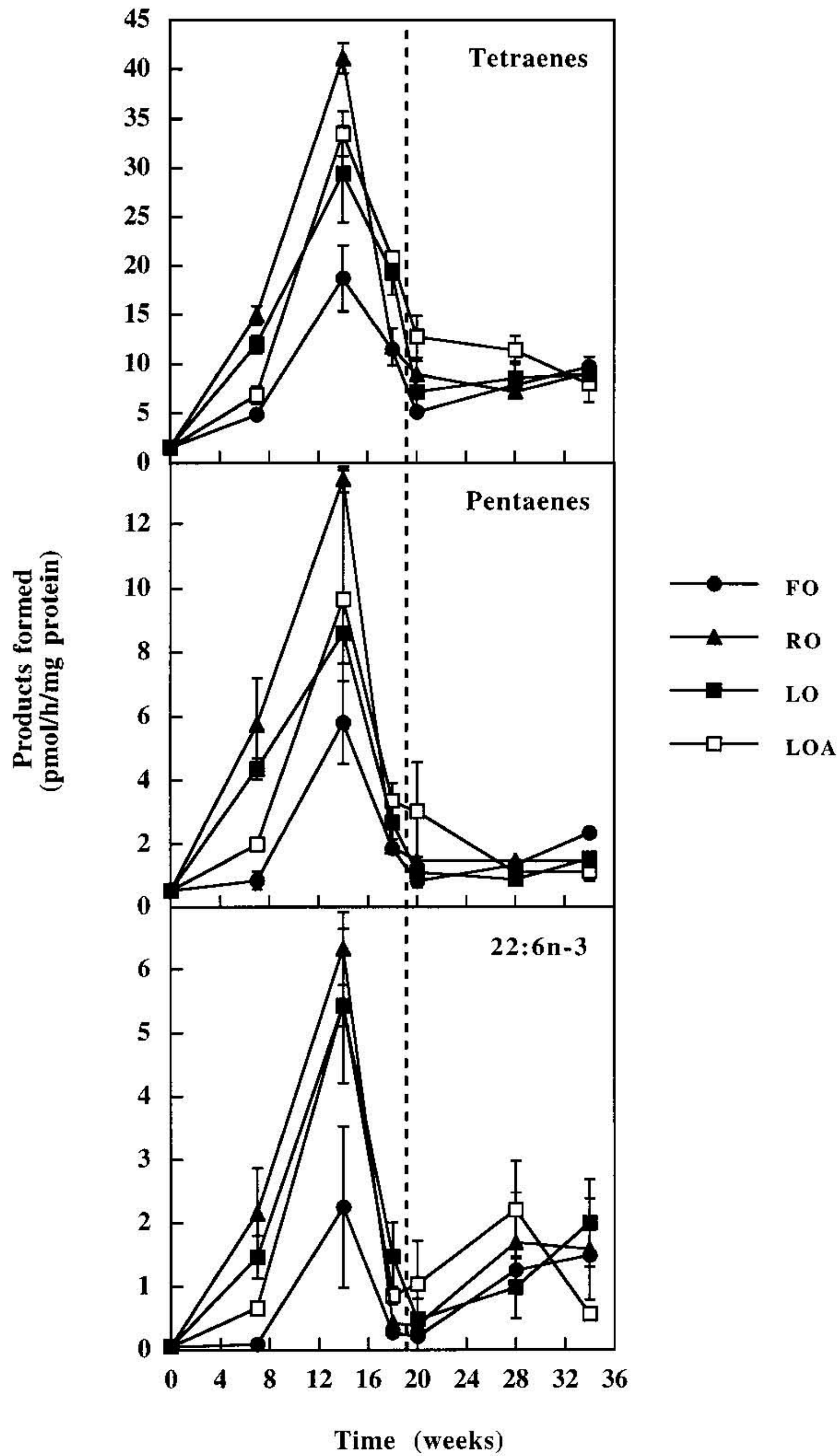

20:4n-6
20:5n-3
22:6n-3
Fatty acid content in liver polar lipid
(percentage of total fatty acids)
Time (weeks)
FO
RO
LO
LOA
3.5
3
2.5
2
1.5
1
9
8
7
6
5
4
45
43
41
39
37
35
33
31
0
4
8
12
16
20
24
28
32
36


20:4n-6
20:5n-3
22:6n-3
Fatty acid content in gill polar lipid (percentage of total fatty acids)
Time (weeks)
FO
RO
LO
LOA
2.4
2.2
2
1.8
1.6
1.4
1.2
5.5
5
4.5
4
3.5
3
2.5
26
24
22
20
18
16
14
0
4
8
12
16
20
24
28
32
36


liver
gill
AA/EPA Ratio
Time (weeks)
FO
RO
LO
LOA
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0 4 8 12 16 20 24 28 32 36


18:2n-6
18:3n-3
n-3HUFA
Fatty acid content in muscle total lipid
(percentage of total fatty acids)
Time (weeks)
FO
RO
LO
LOA


[1-14C]18:3n-3 desaturated
(pmol/h/mg protein)
Time (weeks)
FO
RO
LO
LOA
0
10
20
30
40
50
60
70
0
4
8
12
16
20
24
28
32
36
a
b
c
c
a
ab
b
c
a
a
b
b
a
b
c
d
b
b
a
b


Tetraenes
Pentaenes
22:6n-3
Products formed (pmol/h/mg protein)
Time (weeks)
FO
RO
LO
LOA
0
5
10
15
20
25
30
35
40
45
0
2
4
6
8
10
12
0
1
2
3
4
5
6
0
4
8
12
16
20
24
28
32
36

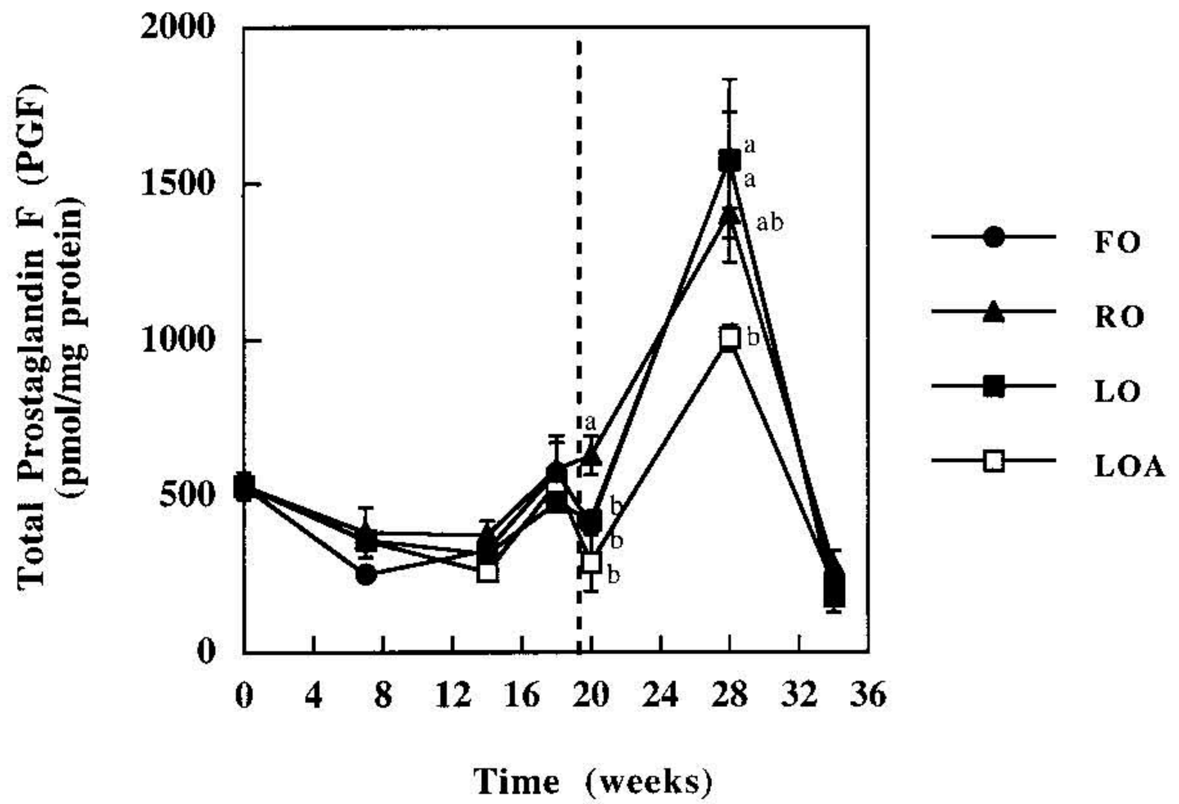

Total Prostaglandin F (PGF)
(pmol/mg protein)
2000
1500
1000
500
0
0
4
8
12
16
20
24
28
32
36
Time (weeks)
a
a
ab
b
a
b
b
b
FO
RO
LO
LOA

Table 1. Feed components (% of dry weight)

	Freshwater diets				Sea water diet
Component	FO	RO	LO	LOA	
Fish meals[1]	30.0	30.0	30.0	30.0	55.3
Plant meals[2]	52.5	52.5	52.5	52.5	31.9
Potato starch	2.0	2.0	2.0	2.0	-
Micronutrients[3]	2.5	2.5	2.5	2.5	2.5
Fish oil[4]	13.0	-	-	-	11.2
Rapeseed oil[5]	-	13.0	-	-	-
Linseed oil[6]	-	-	13.0	12.7	-
ARASCO[7]	-	-	-	0.3	-
<u>Proximate Composition</u>[8]					
Protein	48.9	47.6	48.8	50.8	54.9
Lipid	18.9	19.0	18.1	17.6	19.1
Starch	6.0	5.4	4.9	5.4	10.9
Ash	7.8	6.7	6.4	6.5	9.1
Moisture	6.4	8.0	9.6	9.9	2.4

[1]Hot air-dried Icelandic capelin meal, SR Mjøl, Iceland, Flame-dried Chilean fishmeal, G. Holman, Chile and Fish Protein Concentrates, Soprapeche CPSP G, Boulogne-Sur-Mer, France. [2]Soyameal (HiPro Soya and Supersoy, Unitrition, Selby, U.K. and SoyProtein Concentrate, Loders Croklaan, Netherlands), wheatmeal (Whole wheat, Cargill Swinderby, U.K.) and maize meal (Maize Gluten Meal (60%), Cerestar, Manchester, England). [3]Vitamins, minerals, amino acids and attractant (Finnstim at 0.25%). [4]Danish whole fish oil (stabilised with 250 ppm ethoxyquin), United Fifh Products, Aberdeen. [5]Refined low-erucic acid rapeseed oil, Croda Universal Ltd., Hull, U.K. [6]Refined linseed oil, Croda Universal Ltd., Hull, U.K. [7]Gist-Brocades, Delft, Netherlands.[8]Percentage of weight; determined using the methods described by Woyewoda et al. (1986).

Table 2. Fatty acid compositions (percentage of weight of total fatty acids) of freshwater parr diets and smolt (seawater) diet.

	Freshwater diets				Sea water diet
Fatty acid	FO	RO	LO	LOA	
14:0	5.1	1.4	1.1	1.4	5.5
16:0	14.0	8.4	8.4	9.0	16.0
18:0	2.6	2.3	3.7	3.7	2.2
Total saturates[1]	22.3	12.6	13.4	14.5	24.3
16:1n-7	4.7	1.7	1.3	1.7	5.4
18:1n-9	12.9	38.1	17.5	17.2	16.6
18:1n-7	2.7	3.0	1.3	1.4	2.6
20:1n-9	9.3	3.4	2.2	2.9	6.6
22:1n-11	12.5	3.1	2.1	2.3	5.8
22:1n-9	0.8	0.3	0.1	0.2	0.5
24:1	0.8	0.4	0.3	0.4	0.5
Total monoenes[2]	44.0	49.8	24.7	25.6	38.7
18:2n-6	6.1	19.6	17.3	15.5	6.7
20:2n-6	0.2	tr	0.1	tr	0.3
20:4n-6	0.5	0.2	0.2	0.4	0.5
Total n-6	7.1	19.8	17.6	15.9	7.9
18:3n-3	1.7	11.3	39.2	36.8	1.9
18:4n-3	2.3	0.4	0.3	0.4	2.4
20:4n-3	0.6	0.1	0.1	0.1	0.6
20:5n-3	7.1	2.0	1.8	2.1	7.2
22:5n-3	1.1	0.3	0.2	0.3	1.0
22:6n-3	9.4	3.0	2.8	3.1	10.9
Total n-3[3]	22.8	17.0	44.3	42.7	24.8
Total PUFA[4]	30.6	36.8	61.9	58.5	33.4
n-3/n-6	3.2	0.9	2.6	2.7	3.1

Values are means of two determinations. [1]Includes 15:0, 17:0, 20:0 and 22:0. [2]Includes 16:1n-9, 20:1n-11, 20:1n-7. [3]Includes 20:3n-3. [4]Includes 18:2n-9 and C_{16} PUFA. tr = trace value < 0.05

Table 3. Results of two-way analysis of variance (ANOVA) on data in Figs.1-7 showing significance of effects and interaction of time and diet.

	Time		Diet		Interaction	
Parameter	F ratio	P value	F ratio	P value	F ratio	P value
Fatty acid composition						
Liver 20:4n-6	43.04	<0.0001	3.26	0.0279	3.78	<0.0001
Liver 20:5n-3	85.58	<0.0001	10.40	<0.0001	1.61	0.0893
Liver 22:6n-3	19.47	<0.0001	1.46	0.2344	1.27	0.2442
Liver 20:4n-6/20:5n-3	22.94	<0.0001	4.06	0.0111	2.26	0.0107
Gill 20:4n-6	1.00	0.4326	0.70	0.5475	0.18	0.9999
Gill 20:5n-3	37.90	<0.0001	2.49	0.0694	1.03	0.4447
Gill 22:6n-3	13.16	<0.0001	5.25	0.0029	1.15	0.3348
Gill 20:4n-6/20:5n-3	8.18	<0.0001	8.26	0.0001	1.96	0.0289
Muscle 18:2n-6	54.26	<0.0001	17.10	<0.0001	25.13	<0.0001
Muscle 18:3n-3	407.92	<0.0001	107.92	<0.0001	153.65	<0.0001
Muscle n-3HUFA	17.15	<0.0001	3.07	0.0470	3.20	0.0187
Fatty acid desaturation						
Total desaturation	456.17	<0.0001	49.16	<0.0001	25.58	<0.0001
Tetraene production	1137.12	<0.0001	135.15	<0.0001	59.75	<0.0001
Pentaene production	120.30	<0.0001	12.46	<0.0001	7.20	<0.0001
22:6n-3 production	109.35	<0.0001	15.56	<0.0001	6.66	<0.0001
Prostaglandin production						
Gill PGF	296.81	<0.0001	11.89	<0.0001	6.23	<0.0001

Table 4. Fish weights[a] at day 0, seawater transfer and termination of the experiment, specific growth rates[b] (SGR) during each phase of the study and overall, and plasma chloride values[a] after a seawater tolerance test prior to seawater transfer.

Parameter/diet	Fish oil (FO)	Rapeseed oil (RO)	Linseed oil (LO)	Linseed/ARASCO (LOA)
Plasma chloride (mmol/l)	155.1 ± 3.2[a]	137.9 ± 2.5[ab]	140.3 ± 2.2[ab]	136.4 ± 1.6[b]
Fish weights (g)				
Week 0 (Initial weights)	30.4 ± 1.1	31.7 ± 1.1	30.1 ± 1.1	30.8 ± 1.1
Week 19 (Prior to seawater transfer)	41.1 ± 1.6	44.0 ± 1.7	40.1 ± 1.4	42.2 ± 1.9
Week 34 (Termination of experiment)	125.4 ± 2.4[a]	126.0 ± 2.2[a]	114.6 ± 1.8[b]	113.3.± 2.0[b]
SGR (Freshwater)	0.23	0.24	0.21	0.24
SGR (Marine)	1.00	0.94	0.94	0.89
SGR (overall)	0.60	0.58	0.56	0.55

[a] Values are mean ± SEM (n=20 for weights and 16 for plasma chlorides). [b] Specific growth rate calculated as % weight gain/day = (e^{Gw} - 1) x 100, where G_w (daily growth rate) = ln W_1 -ln W_0/ T (W_1 = final weight, W_0 = initial weight, T = time in days). Plasma chloride and weight data were subjected to one-way ANOVA as described in the Materials and Methods section. Mean values in the same row having a different superscript letter are significantly different ($P < 0.05$) as determined by Tukey's test.