Photochemistry and Photobiology, 2010, 86: 909–919

Effects of UV Radiation and Diet on Polyunsaturated Fatty Acids in the Skin, Ocular Tissue and Dorsal Muscle of Atlantic Salmon (*Salmo salar*) Held in Outdoor Rearing Tanks

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Received 16 September 2009, accepted 8 February 2010, DOI: 10.1111/j.1751-1097.2010.00733.x

ABSTRACT

The effect of UV radiation (UVR) on juvenile Atlantic salmon (*Salmo salar*) was assessed by measuring the fatty acid (FA) profiles of muscle, dorsal and ventral skin, and ocular tissues following 4-month long exposures to four different UVR treatments in outdoor rearing tanks. Fish were fed two different diets (Anchovy- and Herring-oil based) that differed in polyunsaturated fatty acid (PUFA) concentrations. Anchovy-fed salmon had higher concentrations of ALA (alpha-linoleic acid; 18:3n-3), EPA (eicosapentaenoic acid; 20:5n-3) and DPA (docosapentaenoic acid, 22:5n-3) in their muscle tissues than fish fed the Herring feed. Fish subjected to enhanced UVB levels had higher concentrations of LIN (linolenic acid, 18:2n-6) and ALA, total omega-6 FA and SAFA (saturated fatty acids) in their tissues compared with fish in reduced UV treatments. Concentrations of ALA, LIN, GLA (gamma-linolenic acid; 18:3n-6), EPA, PUFA and total FA were higher in ventral skin of fish exposed to enhanced UVB compared with fish in reduced UV treatments. Salmon exposed to reduced UV weighed more per-unit-length than fish exposed to ambient sunlight. The FA profiles suggest that fish exposed to UV radiation were more quiescent than fish in the reduced UV treatments resulting in a buildup of catabolic substrates.

INTRODUCTION

The degree to which UV radiation (UVR; 280–400 nm) penetrates water is mainly affected by depth and the inherent optical properties of the water mass (1–3). Ozone depletion increases levels of UVB radiation (280–320 nm) reaching the earth's surface and, therefore, the dose that penetrates into natural waters. Although there is some uncertainty with respect to model predictions, ozone levels are expected to remain lower than in the 1970s with a return to pre-1980 concentrations expected only by the mid-21st century (4).

UVR has many harmful effects on aquatic organisms (5,6), especially in shallow, clear-water habitats where organisms cannot readily escape into deeper water and/or in situations where riparian canopy cover has been removed through natural (*e.g.* fire) or anthropogenic (*e.g.* clear cutting) causes. For example, eggs of landlocked *Galaxias maculates* from Patagonia exhibited increased mortality as a function of UV dose (7) and juvenile rainbow trout (*Oncorhynchus mykiss*) increased their swimming activity, restless behavior and oxygen consumption when they were exposed to UVR (8). In general, the early life stages of fishes are more sensitive to the effects of UVR than are older fish (9).

In natural situations, juvenile salmon limit their exposure to ambient UVR by hiding between or under overhanging rocks or under the shadow of the riparian canopy. In contrast, in some aquaculture situations (*e.g.* uncovered, relatively shallow, outdoor rearing tanks filled with clear water), or in situations where cover has been removed through natural or anthropogenic activity, juvenile salmon may be exposed to UVR. Because salmon farming is expanding rapidly (10) we wanted to evaluate whether exposure to UVR had the potential to have negative consequences on the health and growth of juvenile Atlantic salmon. We assessed this by quantifying the fatty acid (FA) signatures in four different tissues of salmon fed two different feeds and subjected to four different UV treatments. The length and weight of salmon was recorded in each treatment at the beginning and end of the experiment. Two artificial feeds were designed to differ in concentrations of two essential omega-3 FA: eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (a complete list of lipid abbreviations is provided in Table 1). These two FAs were emphasized because EPA is a precursor to anti-inflammatory eicosanoids (11) whereas DHA plays a crucial role in vision (see below) and in membrane competency in general (11).

METHODS

Salmon culture. Juvenile Atlantic salmon (*Salmo salar*; Norsk LakseAvl strain) from the Matre Aquaculture Research Station, Institute of Marine Research (IMR), Matre, Norway were used for these experiments. Fish were transported to the IMR's Austevoll Research Station (60°5′42″N, 5°13′8″E) where the experiment was conducted. A sample of 50 fish was randomly selected from the batch that was delivered to the experimental facility in May 2002. The mean (±SE) length and weight for this sample of 50 fish was 5.69 cm (±0.13) and 1.85 g (±0.12). At the beginning of the experiment salmon were placed into tanks (3 m wide × 1 m deep and filled with ~6000 L water) in which net cages (50 × 60 × 60 cm; $L \times W \times H$)

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Table 1. Common lipid abbreviations.

Compound or class of lipid	Abbreviation	Formula or definition
Alpha-linoleic acid	ALA	18:3n-3*
Linolenic acid	LIN	18:2n-6
Gamma-linolenic acid	GLA	18:3n-6
Arachidonic acid	ARA	20:4n-6
Eicosapentaenoic acid	EPA	20:5n-3
Omega-3 docosapentaenoic acid	DPA	22:5n-3
Docosahexaenoic acid	DHA	22:6n-3
Fatty acid	FA	
Fatty acid methyl ester	FAME	Fatty acid esterified to a terminal methyl group
Saturated fatty acid	SAFA	Fatty acid with no double bonds
Monounsaturated fatty acid	MUFA	Fatty acid with one double bond
Polyunsaturated fatty acid	PUFA	Fatty acid with ≥2 double bonds
Highly unsaturated fatty acid	HUFA	Fatty acid with ≥20 carbons and ≥3 double bonds
Omega-3 fatty acid	Omega-3 FA	FA with the first double bond at the third carbon from methyl end of molecule
Omega-6 fatty acid	Omega-6 FA	FA with the first double bond at the fourth carbon from methyl end of molecule

*"m:pn-x" denotes a fatty acid (FA) with "m" carbon atoms, "p" ethylenic bonds (methylene-interrupted) if more than 1, and "x" carbon atoms from and including the terminal group to and including the carbon atom nearest the first ethylenic bond.

were immersed 30 cm into the water (*i.e.* the distance from the water surface to the bottom of the net cages was 30 cm). Each net cage was stocked with 100 juvenile Atlantic salmon at the beginning of the experiment. At the end of the experiment all fish were euthanized using tricaine methanesulfonate (MS-222). There were eight tanks (experimental units) corresponding to the eight possible feed × UV treatment combinations. Additional details of the experimental setup and conditions can be found elsewhere (12).

Salmon diets. Two feed types were manufactured by Nofima (http://www.nofima.com/; formerly SSF, Norway), Norway. The two feeds were designed so as to have different concentrations of key omega-3 FA (Fig. 1, Table 2). This was accomplished by adding Atlantic herring (*Clupea harengus*) oil to one of the feeds and Peruvian anchovy (*Engraulis ringens*) oil to the other. For brevity these feeds are hereafter referred to as the Herring feed and the Anchovy feed. The detailed ingredients, gross chemical composition and energy content of the feeds are provided in Table 2. The batch-produced feeds were processed into 1.0, 1.5 and 2.0 mm diameter pellets so that salmon could be fed appropriately sized particles as they grew.

A sample of each of the three feed pellet sizes was collected at the beginning of the experiment and analyzed for fatty acid methyl esters (FAME) following the procedures described below. The bags of feed were stored throughout the experiment, in the dark, in a −50°C chest freezer. At the end of the experiment an additional sample of each of the three feed pellet sizes was collected and analyzed for FAME to determine if any FA degradation had occurred during storage. Salmon were hand fed *ad libitum*.

Light measurements and irradiance treatments. The purpose of the net cages was to keep salmon: (1) at approximately the same depth in all treatments so that the UV dose that they received could be more accurately estimated and (2) centered under the shielding materials and/or lamps. The underwater irradiance measurements (Optronic Laboratories OL754 scanning spectrophotometer), were made by placing the aperture of the sensor at the bottom of the net cages (*i.e.* 30 cm depth). Salmon were exposed to four irradiance treatments: (1) natural sunlight filtered through Rohm Plexiglas® GS-231 (hereafter referred to as −UVR) which has a sharp cutoff below 400 nm (*i.e.* no UVB or UVA radiation), (2) natural sunlight filtered through Mylar-D® (hereafter, −UVB) which removes wavelengths below 320 nm (50% transmittance at 318 nm), (3) ambient sunlight (hereafter, Sun) and (4) natural sunlight enhanced with additional UVB radiation (hereafter, +UVB). The +UVB treatment received its extra UVR *via* supplementation by one 40 W fluorescent lamp (Philips TL40/12RS) located 1 m above the water and switched on for 1 h day^{-1} for a total of 130 days. The lamps were wrapped in cellulose triacetate film (CTA, 95 μm; Clarifoil Co., UK) to remove any UVC radiation emitted by the lamp (< 1% in air before screening with CTA). The CTA film was changed after a maximum of 18 h of use. The Plexiglas and Mylar-D filters were the width of the tank (3 m) and were ~2 m long. They were attached to a wooden frame that overhung the edges of the tanks at their ends. The filters were oriented on a ~20° angle to shed rain and were washed regularly to prevent the accumulation of dust and other debris. The distance from the top of the water to the filters ranged from 10 to 40 cm. This arrangement ensured that all the sunlight always passed through each of the filter treatments. Exposures ran from 30 April to 26 September 2002. Specific exposure regime details can be found elsewhere (12).

Ambient radiation data, covering the full duration of the experiments, were obtained from a multichannel radiometer (305, 313, 320, 340, 380, 395 and 400–700 nm; GUV-541, Biospherical Instruments, CA) situated in Bergen (60°22′43″N, 5°20′33″E, University of Bergen), 22 km north of the location at which the experiment took place. The total ambient dose (kJ m^{-2} nm^{-1}) was calculated by summing daily totals over the exposure period (Table 3). The doses received under the −UVB and −UVR treatments (Table 3) were calculated by multiplying the spectral transmission (measured with the OL754) of the materials by the ambient total dose measured by the GUV-541 (which was interpolated to 1 nm resolution, 299–367 nm). The irradiance output of the supplemental UV lamp was multiplied by total exposure time (130 h) and added to the ambient total dose to give total exposure for the +UVB treatment (Table 3). To further characterize the exposures, spectral scans were acquired just above the water surface on an overcast day for each of the treatments (Table 3). Effective erythemal irradiance (W m^{-2}) was calculated using the CIE reference action spectrum (13). In Table 3, the results are presented as the time (minutes) needed to be exposed to one standard erythema dose (SED), defined as 100 J m^{-2} of erythemal radiant exposure (ISO/CIE Standard ISO 17166:1999E/CIE S 007/E-199830).

Tissue collections and processing. At the end of the experiment (26 September 2002) four different tissues were collected from salmon: dorsal and ventral skin, dorsal muscle (skin removed and landmarked to either side of the dorsal fin) and eyes. The dorsal skin was sampled adjacent to the dorsal fin. Ventral skin was obtained from the region between the pectoral fins and anus. We could not efficiently separate retina from surrounding tissues, therefore, the entire posterior portion of the eye was sampled. Thus, these tissues were comprised of retina and supporting tissue and are hereafter referred to as ocular tissues.

We chose the dorsal muscle because it is the largest tissue in fish and because we hypothesized that if we observed major changes in FA profiles in this tissue then the effects of UVR must be systemic in nature and the dorsal skin because this tissue should be the major direct target site for UVR damage (14,15). The ventral skin was selected because it is relatively less pigmented (protected) than what we anticipated to be the main target tissue (*i.e.* dorsal skin). Ocular tissue was chosen because: (1) dietary supply of DHA is known to affect retinal DHA concentrations in fish (16,17), (2) DHA is known to be crucial to visual acuity in vertebrates (18–20) and (3) UVR negatively affects several aspects of vision in vertebrates (*e.g.* major cytoskeletal structures such as microtubules and actin) leading to cataract formation (21,22).

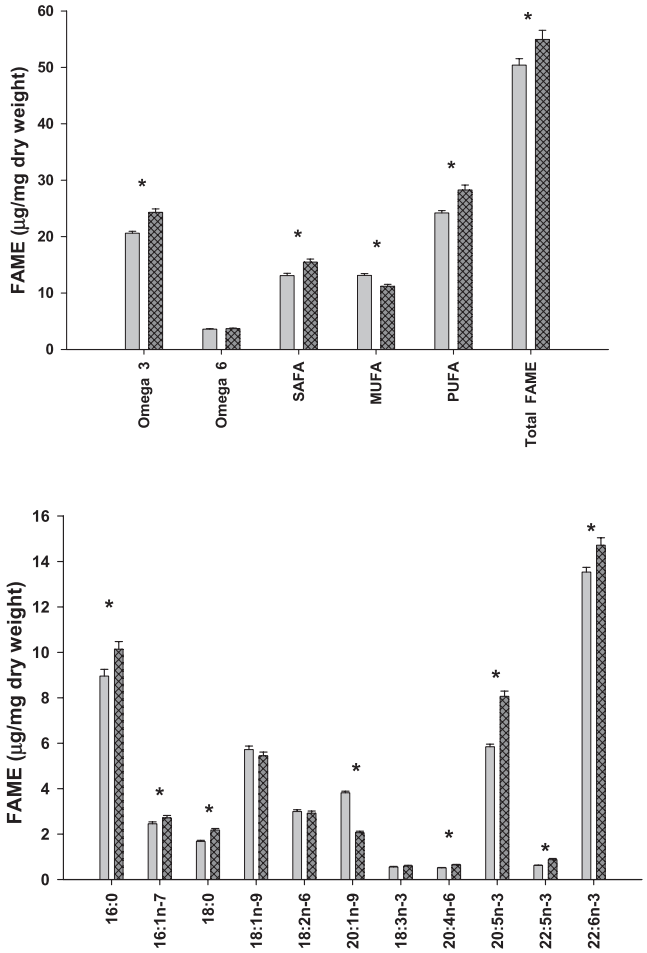

Figure 1. Gross (upper panel) and detailed (lower panel) principal fatty acid (FA) composition of the two experimental feeds. Light gray fill and dark gray fill (with cross hatch pattern) correspond to Atlantic herring (*Clupea harengus*) and Peruvian anchovy (*Engraulis ringens*) oil-based feeds, respectively. With the exception of the physiologically important essential FAs (alpha-linoleic acid and arachidonic acid), only FAs with a concentration >1 μg mg^{-1} dry weight are shown. FAME = fatty acid methyl esters; SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. Error bars are standard errors. An asterisk above a pair of FA indicates a statistically significant difference at $P < 0.05$ (Mann–Whitney rank sum test) between the two feeds.

Table 2. Composition (in kg), gross proximate composition (% of wet weight) and energy content (MJ kg^{-1}) of the two experimental Atlantic salmon feeds.

Ingredient	Detailed composition (kg)
Fish meal 52/02*	76.58
Fish meal 358/01†	32.88
Atlantic herring oil	15.75 in Feed 1; 0.00 in Feed 2
Peruvian anchovy oil	0.00 in Feed 1; 15.75 in Feed 2
Soya lecithin	0.75
Suprex® Corn	21.00
Vitamin mix	2.25
Mineral mix	0.60
Inositol	0.05
Betafin® (betaine)	0.15
Carophyll® Pink (astaxanthin)	0.06
Component (both feeds)	**Gross composition**
Protein	53.9
Lipid	17.7
Carbohydrate	11.5
Ash	10.3
Water	6.4
Sum	99.8
Energy (MJ kg^{-1}) in both feeds	150

*62% blue whiting (*Micromesistius poutassou*) and 38% capelin (*Mallotus villosus*). †70% sand eel (*Ammodytes marinus*) and 30% Atlantic herring (*Clupea harengus*).

Table 3. Total dose (over duration of experiment; 30 April to 26 September 2002), in air, for each exposure treatment group. The time for one standard erythemal dose (SED; in minutes), in air, is provided for the treatments in which UV radiation was present.

	Dose (kJ m^{-2})			
Waveband (nm)	−UVR	−UVB	Sun	+UVB
299–320	0	84	4995	5197
321–367	0	30 451	63 095	63 247
Time for 1 SED (min)	N/A	1212.1	119.9	20.6

N/A = not applicable.

The tissue samples were placed in cryovials, frozen in liquid nitrogen and immediately transferred to a cryogenic freezer (−85°C) where they remained until FA analyses. All tissues were freeze-dried prior to analyses. Tissues from a total of six haphazardly selected fish were collected from each possible combination of UV treatment × feed. Thus, a total of 192 individual tissue samples were analyzed for FA (4 treatments × 2 diets × 4 tissues × 6 fish per treatment cell). The remaining fish were photographed and their length and weight was determined. In all, we measured lengths and weights of 130, 114, 118, and 122 fish in the Herring-fed group and 121, 119, 114, and 108 fish in the Anchovy-fed group in the −UVR, −UVB, Sun and +UVB groups, respectively (for both feed types).

Lipids and fatty acids. FAMEs of freeze-dried salmon feeds and salmon tissues were obtained in a three-step process: extraction (23), derivatization using the boron trifluoride method (24) and quantification on a HP6890 gas chromatograph (25). FAMEs were identified and quantified using Supelco's 37 component FAME standard (#47885-U) by comparing peak retention times between samples and standards. FA results are reported as μg FAME mg^{-1} dry mass of tissue. The commonly accepted abbreviations used here include: ALA (alpha-linoleic acid;18:3n-3), LIN (linolenic acid, 18:2n-6), GLA (gamma-linolenic acid; 18:3n-6), EPA (20:5n-3), ARA (arachidonic acid; 20:4n-6), DPA (docosapentaenoic acid, 22:5n-3), DHA (22:6n-3), SAFA (saturated fatty acid), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acid) and FAME. A complete list of lipid abbreviations is provided in Table 1.

Statistical analyses. Kruskal–Wallis one-way ANOVA on ranks tests revealed that there was no systematic difference in concentrations of EPA and DHA amongst the three feed pellet sizes ($P > 0.05$). Two-way ANOVA was used to test if there was significant degradation in EPA or DHA concentrations within each of the two feeds over the course of the experiment by comparing concentrations of these two labile omega-3 FAs in triplicate feed samples collected in June and September. Because no significant differences in concentrations of labile EPA or DHA over time in either feed were observed ($F(1,8) = 2.4$, $P = 0.16$; $F(1,8) = 0.91$, $P = 0.38$, respectively), the triplicate samples collected in June were pooled with triplicate samples in September in order to provide data for testing if there were any differences in individual FA between the two feeds. This larger dataset was found to be non-normal for some FAs. Therefore, all differences between feeds, with respect to individual FA, were assessed using Mann–Whitney ranks sum tests. Concentrations of three long-chain omega-3 fatty acids (EPA, DPA

Table 4. Summary of ANOVA degrees of freedom. Unreplicated three-way ANOVAs were used to detect UV, diet and tissue effects on the fatty acid content of juvenile Atlantic salmon while two-way ANOVAs were used to detect tissue-specific effects of UV and diet.

	Three-way ANOVA d.f.	Two-way ANOVA d.f.
Total	31	7
Model	22	5
UV	3	2
Diet	1	1
Tissue	3	
UV × Diet	3	2
UV × Tissue	9	
Diet × Tissue	3	
Error	9	2

and DHA) in skinless dorsal muscle were compared (*t*-tests) at the end of the experiment in the −UVR treatment in order to assess whether or not the test feeds were able to produce the intended enhancement in physiologically important omega-3 FA concentrations in the fish.

The effect of UV and diet on the FA content of Atlantic salmon was examined using a series of unreplicated, three-way univariate, ANOVAs with UV (+UVB, Sun, −UVB and −UVR), diet (Herring and Anchovy oil) and tissue type (dorsal muscle, ocular tissue, and dorsal and ventral skin) included as fixed factors (Table 4). The FA content was characterized using metrics known to have important physiological properties. These metrics included the concentration (μg mg^{-1} DW) of key individual FAs (LIN, GLA, ALA, ARA, EPA, DPA and DHA), and summary indices (total omega-3 FA, total omega-6 FA, SAFA, MUFA, PUFA and total FA). Total lipid content (%) was also examined. Response values were mean FA concentrations (for the various metrics described above), as well as lipid content per tissue type for the six fish subsampled from each experimental tank. The three-way ANOVA interaction term was assumed negligible and used as the error term (26). This assumption of effect sparsity was confirmed by examination of normal probability plots (27) and Lenth exact tests (28,29) using a modified design where each 4-level factor (UV and tissue type) was replaced with two 2-level factors for a total of five 2-level factors (30). Stepwise regression on the original three factor design also indicated the three-way interaction was not significant ($P > 0.05$). ANOVA *P*-values were adjusted for multiple inferences using the false discovery rate (31) and significant UV and diet effects were further examined using Tukey multiple comparison procedures. Analysis of ANOVA residuals indicated variances for percent lipid were irremediably unequal and that therefore results for this individual metric should be interpreted with caution. All other FA metrics met parametric analysis requirements. Tissue-specific responses to UV and diet were examined using a series of two-way ANOVAs (Table 4) followed by Tukey or Tukey–Kramer multiple comparison tests depending on whether the interaction term was significant.

The effect of UV and diet on the length–weight relationship of Atlantic salmon was assessed using ANCOVA followed by Tukey–Kramer multiple comparison tests. Prior to ANCOVA, length and weight measures were log transformed to linearize relationships and the assumption of regression slope homogeneity was confirmed ($F_{0.05,7,929} = 0.7$, $P > 0.05$). Analysis of regression residuals identified a single influential outlier in the Anchovy fed, −UVB treatment group; this individual was removed from analysis. All UV and diet effects were analyzed using SAS Institute, Inc. (version 9.1) statistical software.

RESULTS

Feed analyses

The bulk composition of the two feeds was identical, with the exception of the type of oil added: Atlantic herring or Peruvian anchovy (Table 2). Because there were no significant relationships (Kruskal–Wallis ANOVA) in either EPA or DHA concentrations among the three pellet sizes for either Feed 1 or for Feed 2 we pooled the FA data from the different pellet sizes. Further, there was no significant difference between the June and September sampling periods with respect to concentrations of labile EPA and DHA in the feed confirming that freezing the feed at −50°C sufficed to preserve the feed for the duration of the experiment. There was no significant interaction between sampling time and feed type for either EPA or DHA ($F(1,8) = 0.05$, $P = 0.84$; $F(1,8) = 0.51$, $P = 0.497$, respectively).

Although the bulk composition of feeds was identical, there were, as expected, significant differences in the overall FA profiles between the two feeds (Fig. 1). Omega-3 FA, SAFA, PUFA and total FAME concentrations were all higher in the Anchovy feed; however, MUFA concentrations were higher in the Herring feed principally because concentrations of 20:1n-9 (a FA biomarker typically associated with the consumption of copepods such as *Calanus* spp. and, thus, consistent with the diet of Atlantic herring) were higher in this feed (Fig. 1). In keeping with the objective of creating two distinct feed types differing in concentrations of physiologically important omega-3 highly unsaturated fatty acids (HUFA), the Anchovy feed had higher concentrations of both EPA and DHA, consistent with what we would expect given the origin of Peruvian anchovies (*i.e.* diatom-rich waters off the coasts of Peru and Chile) (Fig. 1). The Anchovy feed also had higher concentrations of omega-3 docosapentaenoic acid (DPA; 22:5n-3) as well as higher concentrations of palmitic acid (16:0), palmitoleic acid (16:1n-7; a diatom marker and thus broadly consistent with the diatom-rich diets of Peruvian anchovies) and ARA.

Effect of diet (Herring *vs* Anchovy) on FA content of muscle tissue

In order to assess whether or not the feed manipulation translated into the intended effects on FA profiles of Atlantic salmon we compared FA profiles of skinless dorsal muscle of six fish (from each feed type) from the −UVR treatment collected at the end of the experiment. Although omega-3 FA, PUFA and total FAME concentrations were higher in the dorsal muscle tissues of Anchovy-fed fish than in Herring-fed fish, these differences were not significant (*t*-tests; $P = 0.09$, 0.18 and 0.39 for omega-3, PUFA and total FAME, respectively) due to variability in these measures amongst the relatively small number of fish examined. Despite this, we were able to measure significantly higher concentrations of ALA, EPA and DPA (but not DHA; again due to high variability among fish) in dorsal muscle tissues of fish fed the Anchovy diet (Fig. 2). These results confirm that our dietary manipulation had the desired effect of altering key omega-3 FA concentrations in the largest tissue of salmon.

Fatty acid profiles of the four different tissues

Concentrations of physiologically important omega-3 and omega-6 FAs were similar among dorsal muscle, dorsal and ventral skin; however, as expected, DHA concentrations were much higher in ocular tissues compared to the three other tissues (Fig. 3). The high levels of EPA and DHA are typical of

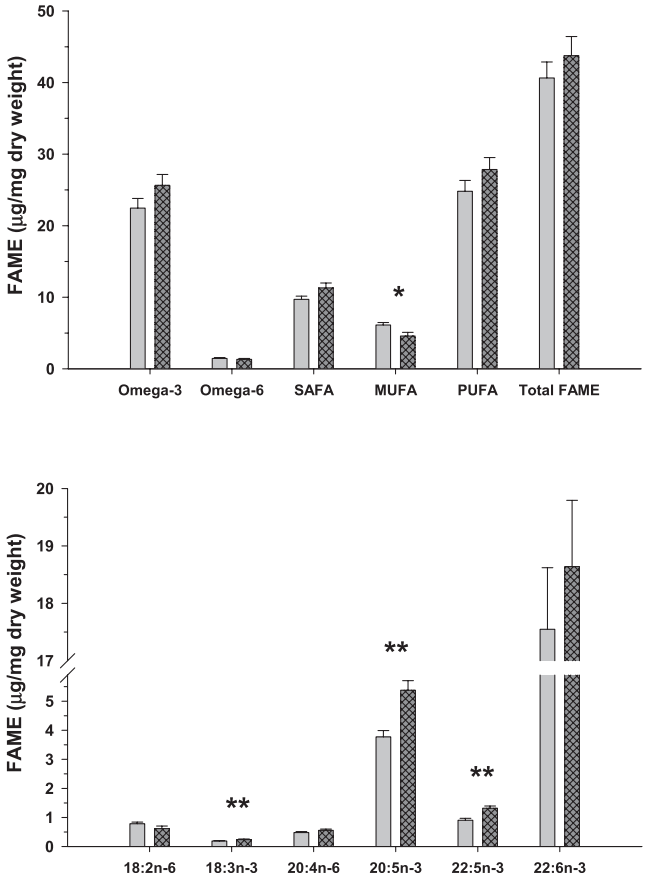

Figure 2. Detailed (upper panel) and gross (lower panel) fatty acid (FA) composition of the dorsal muscle tissue (skin removed) of Atlantic salmon (*Salmo salar*) in the −UVR treatment at the end of the experimental period. Fills and abbreviations as in Fig. 1. Probabilities associated with differences between pairs of FAs are provided when the differences were significant (*t*-tests; $*P < 0.05$; $**P = 0.002$).

Atlantic salmon and account for the high concentrations of total omega-3 FAs (compared to total omega-6 FAs) in this species (Fig. 4). Total lipid concentrations (simple gravimetric analyses) were ranked (highest to lowest) in the following order: ventral skin, ocular tissue, dorsal skin, dorsal muscle (Fig. 4).

UV treatments

The Mylar-D removed 98.3% of solar UVB irradiance in air, and no UVB was detected at the bottom of the cages. The UV lamp treatment delivered only a slight increase in total UV dose (although a much higher dose rate for the 1 h exposure). The supplementation of UVB radiation with Philips lamps increased UVA irradiation by <1.5% (321–367 nm waveband). The lamps increased the average daily UVB irradiance in air by 4.2%. Based on measurements in the 305–310 nm waveband, the increased total dose delivered simulated a stratospheric ozone loss of 8%.

Overall effect of light regime, feed and tissue type on fatty acid profiles

Fish subjected to enhanced UVB (the +UVB treatment) had higher levels of the individual FAs LIN (three-way ANOVA UV effect, $F_{3,9} = 6$, $P = 0.016$) and ALA ($F_{3,9} = 10.7$, $P = 0.003$), as well as the summary FA indices total omega-6 ($F_{3,9} = 5.9$, $P = 0.016$) and SAFA ($F_{3,9} = 6$, $P = 0.016$), in their tissues compared with fish in the reduced UV treatments (Tukey $P < 0.05$; Table 5). The FA content in fish from the Sun treatment did not differ from that of fish from the other UV treatments ($P > 0.05$). Diet also affected FA content as ARA (three-way ANOVA diet effect, $F_{1,9} = 31.4$), EPA ($F_{1,9} = 61.2$), DPA ($F_{1,9} = 105.8$), and total omega-3 ($F_{1,9} = 18.5$) and PUFA ($F_{1,9} = 15.1$) were elevated in tissues from fish fed the Anchovy feed diet while LIN ($F_{1,9} = 25.9$) and total MUFA ($F_{1,9} = 76.7$) were higher in fish fed the Herring feed diet (Tukey $P < 0.05$). Differences in FA content due to UV and diet were not interrelated (interaction effect $P > 0.05$). The FA and lipid content (with the exception of EPA) differed greatly between tissues (three-way ANOVA tissue effect, $F_{3,9} \geq 31.5$, $P < 0.05$); differences were unrelated to UV or diet treatment (interaction effect $P > 0.05$).

The FA content in fish subjected to the −UVB and −UVR treatments did not differ (see Table 5) and these treatments were, therefore, combined as a single, low UV, treatment (−UV) in subsequent tissue-specific analysis of UV and diet effects on FA profiles. Tissue-specific responses to diet and UV (+UVB, Sun and −UV) are detailed below with reported significance set at $P \leq 0.05$ (two-way ANOVA and multiple comparison procedures).

Effect of UV and diet on FA profiles of dorsal muscle and dorsal skin tissues

FA and lipid content in dorsal muscle tissue was not affected by UV treatment. Diet, however, influenced FA content with Anchovy-fed fish containing a higher concentration of DPA (diet effect, $F_{1,2} = 27.3$) and lower concentrations of LIN ($F_{1,2} = 19.2$) and total MUFA ($F_{1,2} = 16.9$) compared with Herring-fed fish. Total MUFA in dorsal skin tissue was also lower in Anchovy-fed fish irrespective of UV treatment ($F_{1,2} = 149.9$). LIN was similarly lower in Anchovy-fed fish ($F_{1,2} = 11.2$) but only for fish in the Sun treatment.

Effect of UV and diet on FA profiles of ventral skin tissue

Concentrations of ALA, LIN, GLA, EPA, PUFA and total FA were higher in ventral skin tissue of fish exposed to enhanced UVB compared with fish in reduced UV treatments (UV effect, $F_{2,2} \geq 18.6$). Diet also influenced FA content of ventral skin tissue with Anchovy-fed fish exhibiting higher levels of GLA, ARA, EPA, DPA, PUFA and total omega-3, but lower levels of MUFA (diet effect, $F_{1,2} \geq 21.1$). UV and diet treatments had an interactive effect on total omega-6 FAs ($F_{2,2} = 64.6$) although omega-6 levels were consistently higher in +UVB (Herring and Anchovy diets) and Sun treatments (Herring diets) compared to the reduced UV treatments (Herring and Anchovy diets).

Effect of UV and diet on FA profiles of ocular tissue

Ocular tissue FA concentration was primarily affected by diet and the interaction of diet with UV. Fish fed the Anchovy diet had reduced LIN concentration and increased concentrations

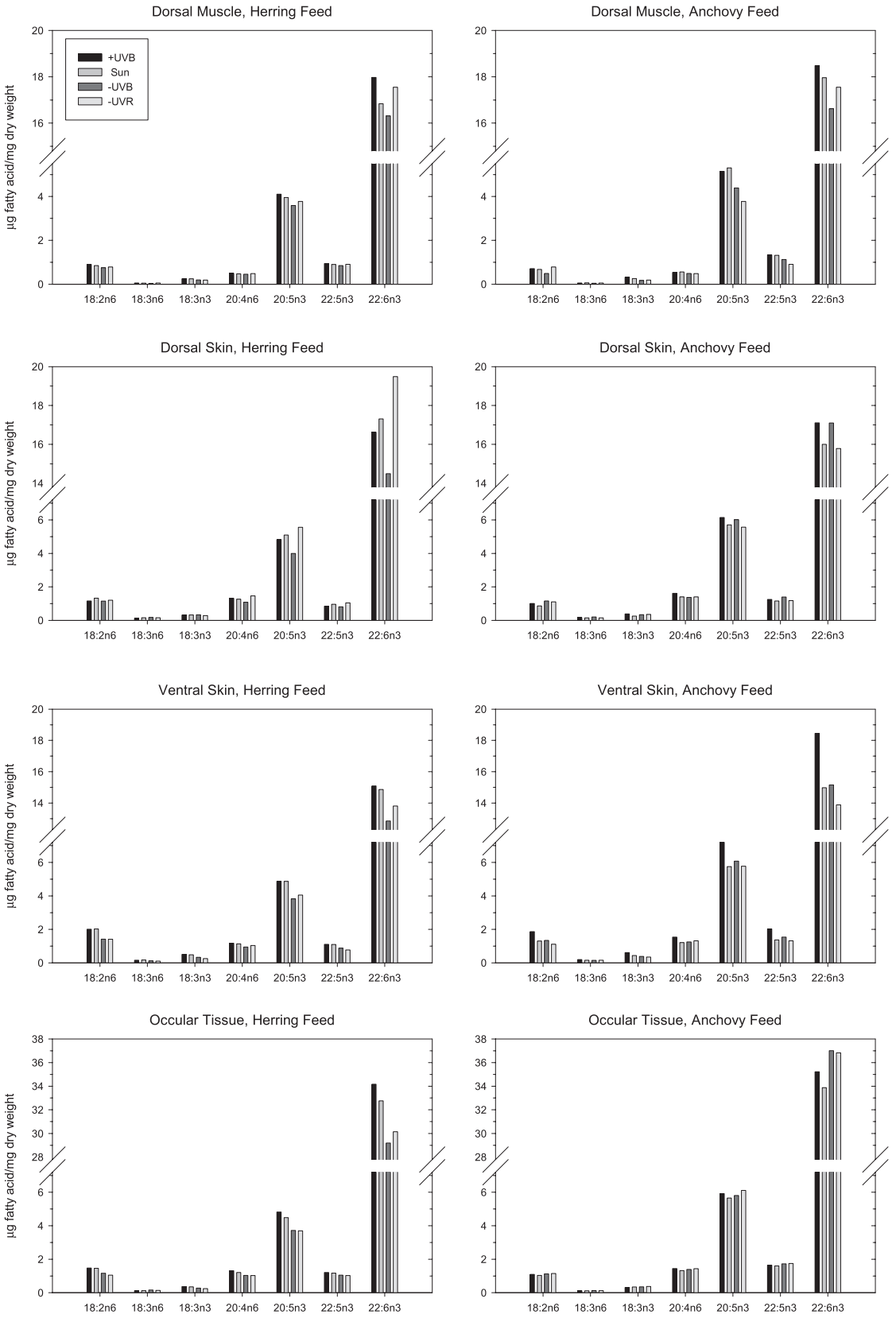

Figure 3. Mean concentrations of physiologically important individual omega-3 and omega-6 fatty acids measured in four tissues of Atlantic salmon (*Salmo salar*) fed two different feeds (Atlantic herring or Peruvian anchovy oil) and subjected to four different UV treatments (+UVB, Sun, −UVB or −UVR). Each mean measurement comes from six fish (pseudo-replicates) collected from the same experimental treatment unit (tank). See Table 1 for a complete list of lipid abbreviations.

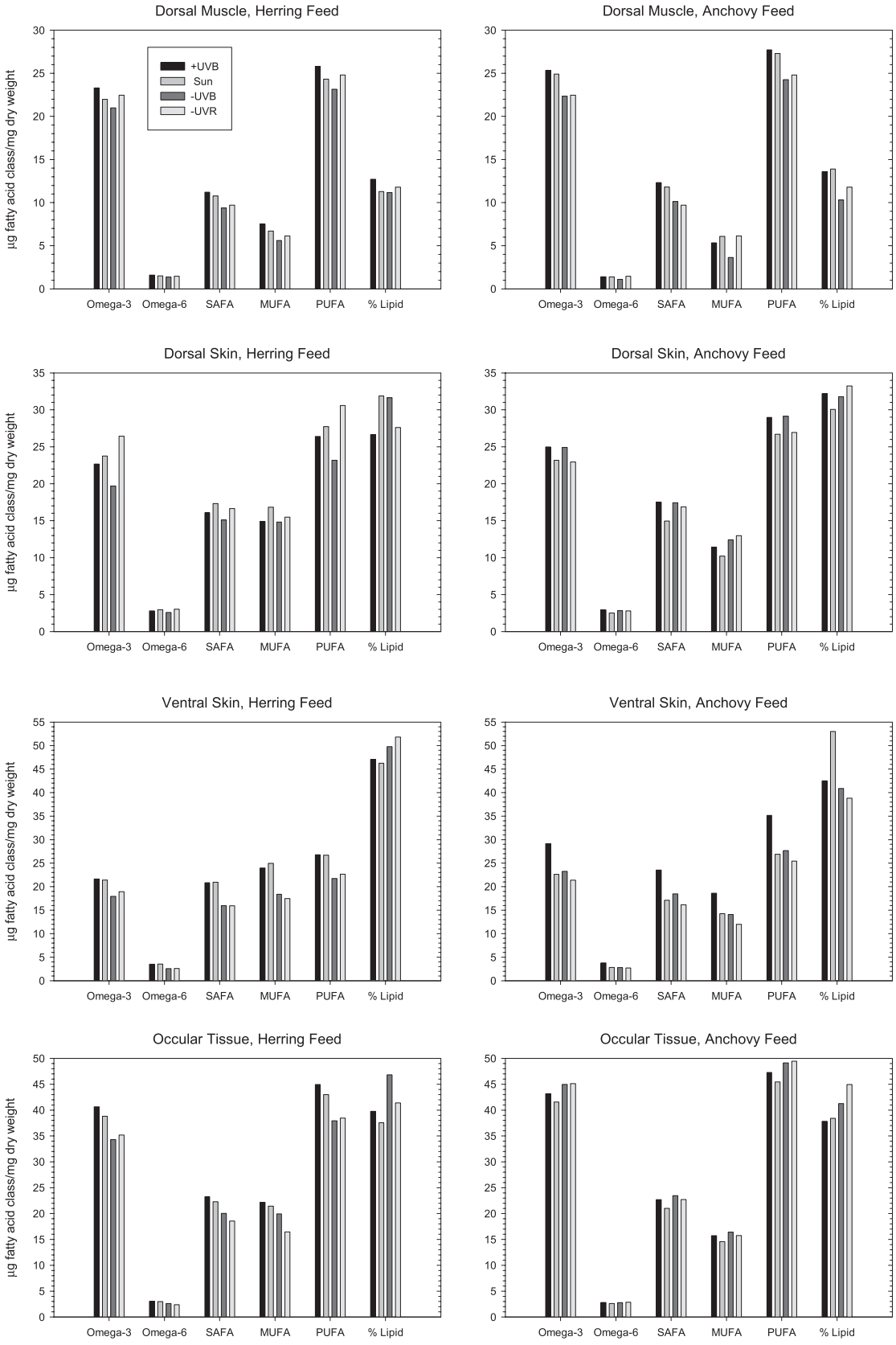

Figure 4. Mean concentrations of different fatty acid classes as well as total lipid concentrations (determined gravimetrically as % of dry weight of extracted tissue) measured in four tissues of Atlantic salmon (*Salmo salar*) fed two different feeds (Atlantic herring or Peruvian anchovy oil) and subjected to four different UV treatments (+UVB, Sun, −UVB or −UVR). Each mean measurement comes from six fish (pseudo-replicates) collected from the same experimental treatment unit (tank). See Table 1 for a complete list of lipid abbreviations.

Table 5. Summary *P*-values from three-way ANOVAs testing the effects of UV treatment (+UVB, Sun, −UVB and −UVR), diet (Herring and Anchovy feed) and tissue type (dorsal skin, dorsal muscle, ventral skin and ocular tissues) on key individual fatty acids (FA) and FA summaries in juvenile Atlantic salmon. Significant UV and diet effects were further examined using Tukey multiple comparison procedures. Multiple comparisons for (1) Sun *vs* −UVB, (2) Sun *vs* −UVR and (3) −UVB *vs* −UVR were not significant and are therefore not shown.

	Significant main effects*				Significant multiple comparisons		
Variable	UV	Diet	Tissue	Herring *vs* Anchovy	+UVB *vs* Sun	+UVB *vs* −UVB	+UVB *vs* −UVR
LIN	< 0.05	< 0.05	< 0.05	Herring > Anchovy		+ UVB > −UVB	+ UVB > −UVR
GLA			< 0.05				
ALA	< 0.05	< 0.05†	< 0.05			+ UVB > −UVB	+ UVB > −UVR
ARA	< 0.05†	< 0.05	< 0.05	Herring < Anchovy			
EPA		< 0.05		Herring < Anchovy			
DPA		< 0.05	< 0.05	Herring < Anchovy			
DHA		< 0.05†	< 0.05				
Omega-3		< 0.05	< 0.05	Herring < Anchovy			
Omega-6	< 0.05		< 0.05			+ UVB > −UVB	+ UVB > −UVR
SAFA	< 0.05		< 0.05			+ UVB > −UVB	+ UVB > −UVR
MUFA	< 0.05†	< 0.05	< 0.05	Herring > Anchovy			
PUFA		< 0.05	< 0.05	Herring < Anchovy			
Total FA	< 0.05†		< 0.05				
Lipid			< 0.05				

*Interaction effects were not significant. †Not significant after false discovery rate correction for multiple inferences.

of EPA, DPA and total omega-3 FAs regardless of UV treatment ($F_{1,2} \geq 21.1$). ARA, DHA and PUFA levels were also generally higher in ocular tissues of Anchovy-fed fish but levels were influenced by UV treatment (interaction effect, $F_{2,2} \geq 34.8$) with the lowest concentrations of ARA, DHA and PUFA found in Herring-fed fish subjected to −UV conditions.

Effect of UV and diet on length–weight relationships

Atlantic salmon fed the Herring diet were heavier per unit length compared with fish fed the Anchovy diet (ANCOVA $F_{1,936} = 4.5$, $P = 0.03$; Table 6). Fish weight was also affected by UV ($F_{3,936} = 8.6$, $P < 0.0001$) with fish exposed to reduced UV weighing slightly more than fish exposed to the Sun treatment (Tukey–Kramer $P < 0.05$; Table 7). The length–weight relationship for fish in the +UVB treatment did not differ from that of fish in the −UVB or Sun treatments ($P > 0.05$). The interaction between UV and diet affected fish weight ($F_{3,936} = 2.8$, $P = 0.04$), primarily because the largest difference in weight per unit length was between Herring-fed fish in the −UVR treatment and Herring-fed fish in the Sun treatment. For example, the predicted weight of a Herring-fed, 12 cm long Atlantic salmon exposed to −UVR light was 21.58 g while the same fish in the Sun treatment would weigh 20.93 g, a difference of 3% (Table 7).

Table 6. Summary results from the ANCOVA testing the effects of length (continuous variable), UV treatment (+UVB, Sun, −UVB and −UVR) and diet (herring and anchovy feed) on the weight of juvenile Atlantic salmon.

	d.f.	MS	*F*-value	*P*-value
Model*	8	7.1315	16823.3	< 0.0001
Length	1	57.0356	134548	< 0.0001
UV	3	0.0037	8.61	< 0.0001
Diet	1	0.0019	4.54	0.0334
UV × Diet	3	0.0012	2.76	0.0412
Error	936	0.0004		

*Slope homogeneity tests performed prior to the ANCOVA confirmed slopes were homogeneous and interaction terms with length were not significant.

DISCUSSION

The long-chain omega-3 fatty acids EPA and DHA are produced primarily in aquatic environments and are exported to terrestrial environments by a variety of processes such as insect emergence, fish consumption by terrestrial predators, *etc*. (32). These fatty acids promote optimal physiological health in aquatic and terrestrial animals, including humans (33). Fatty acids are also sensitive and responsive to a broad range of natural and anthropogenic stressors, including UVR (34,35), herbicides (36), persistent organic contaminants (37), temperature (11) and large-scale systemic changes in diet (38). The last issue is of interest to this study because the availability of specific FA in the diet influences the ability of vertebrates, such as fish, to handle stress (11,39,40).

Our specific interest was to quantify the effects of UVR on FA profiles of caged Atlantic salmon juveniles held in outdoor tanks and to see if a change in dietary FA had the potential to alter the final outcome of the UV exposure experiments, both in terms of effects on salmon FA profiles and in terms of effects on their growth (weight per unit length). Our objective, in creating the two high quality fish feeds, was to provide a subtle contrast in concentrations of physiologically important FA without changing overall gross composition (protein, carbohydrate and lipid) of the feeds. We produced a feed (Anchovy oil based) with higher concentrations of omega-3 HUFA such as EPA, ARA and DHA because these FAs are known to have widespread physiological effects in fish (11) and other vertebrates, including humans (33,41). We also wanted to examine whether or not this relatively subtle manipulation would act to offset or otherwise alter the effects of UV radiation, thus potentially shedding light on the complex interactions between diet and this specific form of stress.

Table 7. Comparison of length–weight relationships for juvenile Atlantic salmon cultured in eight UV × Diet treatments. Length–weight relationships from treatments not connected by the same letter are significantly different (Tukey–Kramer multiple comparison tests; $P < 0.05$). The projected weight of a 12 cm long fish from each treatment combination is shown for comparison.

UV	Diet	*N*	Slope	Intercept	Regression R^2	Tukey–Kramer comparisons			Projected weight* (g) of a 12 cm long fish (% difference from greatest weight)
−UVR	Herring	130	3.09	−2.00	0.99	A			21.58
−UVB	Herring	114	3.13	−2.05	0.99	A	B		21.43 (0.69)
−UVB	Anchovy	118	3.16	−2.09	0.99	A	B	C	21.28 (1.39)
−UVR	Anchovy	121	3.13	−2.05	0.99	A	B	C	21.26 (1.48)
+UVB	Herring	122	3.11	−2.02	0.99	A	B	C	21.22 (1.67)
Sun	Anchovy	114	3.15	−2.07	0.99		B	C	21.11 (2.18)
+UVB	Anchovy	108	3.13	−2.06	0.99		B	C	21.08 (2.31)
Sun	Herring	118	3.12	−2.05	0.99			C	20.93 (3.01)

*Back-transformed from log value.

Experiments where food composition is manipulated with the objective of increasing growth rates and/or producing a higher quality food for human consumption are common in the aquaculture literature. In the case of fish feeds, these manipulations are often dramatic, as for example, when fishmeal-based feeds are contrasted with predominantly cereal-based feeds (42). We chose to undertake a comparatively more subtle feed manipulation consisting of the substitution of oil from one oil-rich fish species (Atlantic herring) with a different oil from another oil-rich fish species (Peruvian anchovy) which had slightly higher HUFA concentrations. We did this because we knew that EPA and DHA promote growth, DHA is involved in visual acuity and that ARA has specific, but, as yet not fully understood, stress-modulating effects in fish.

Atlantic herring is an abundant marine fish species found on both sides of the Atlantic Ocean. They feed on copepods, krill and small fish, and are themselves eaten by a variety of natural predators, including salmonids. The Peruvian anchovy is another small planktivore that eats mainly phytoplankton, but also small zooplankton and fish larvae. Because they contain high concentrations of omega-3 FA, anchovies are used in the production of high quality fishmeal (in terms of EFA concentrations). This attribute meant that we could use anchovies to enhance the omega-3 HUFA content of fishmeal compared to one based on Atlantic herring oil. The results indicate that we were successful in producing two feeds with the desired characteristics and that these feeds resulted in significantly higher HUFA concentrations in the various tissues of juvenile Atlantic salmon by the end of the experiment.

Overall, juvenile Atlantic salmon subjected to the +UVB treatment had higher concentrations of LIN and ALA as well as higher total concentrations of omega-6 FA and SAFA compared to fish in the reduced UV treatments. However, although diet clearly influenced FA profiles of the various fish tissues sampled, there was no interaction between UV and diet when all treatments and tissues were examined together. LIN and ALA, the precursors of ARA and EPA, respectively, are essential FAs that must be obtained from the diet, *i.e.* fish cannot synthesize these FAs *de novo*. The great majority of ALA (43) and also SAFA and MUFA supply energy for swimming through normal catabolic processes. Thus, unless fish in the +UVB were eating more food (it was supplied *ad libitum* to all fish) the overall increase in LIN and ALA in the +UVB treatment indicates either reduced catabolism of these essential FAs for energy and/or reduced conversion (elongase/desaturase) of LIN and ALA to ARA and EPA, respectively. The former observation is supported by the finding that normal feeding behavior, as well as agonistic interactions among individuals (*i.e.* movement) of juvenile salmon held in artificial flumes, is significantly depressed following exposure to UVR (44). These authors concluded that these effects could have ecological consequences including influencing summer densities, density-dependent growth, and size-dependent winter and early marine survivals. Thus, although we did not specifically measure fish activity in the tanks, our data suggest that fish in the +UVB treatment were more quiescent than fish in the reduced UV treatments resulting in a buildup of normal catabolic substrates (*e.g.* ALA and SAFA). We suggest that, in the reduced UV treatment, normal catabolic substrates (*e.g.* ALA and SAFA) are utilized more to enhance fish growth (see below).

At the individual tissue level, exposure to UV radiation did not produce any detectable effects on FA composition or total lipid of either muscle tissues or dorsal skin. This was surprising, as we expected dorsal skin to be affected as it is an obvious target site for UV radiation. Unexpectedly, FA profiles in ventral skin showed the strongest responses to UV radiation. This was manifested by increases in several physiologically important FAs (LIN, GLA, ALA, and EPA) as well as total PUFA and total FA. Ventral skin tissue in salmon is white and should, therefore, be more susceptible to UV exposure effects than the more darkly pigmented dorsal skin. Although it is possible that ventral skin could have been exposed to UVR, for example if fish swam at an angle to the surface, we consider it unlikely that this tissue received sustained exposure to UVR given: (1) the attenuation of UVR with depth, (2) the normal swimming behavior that was observed and (3) the fact that fish spent most of their time near the bottom of the net cages during the day. We suggest that it is much more likely that the observed increase in these FAs in the +UVB treatment is a result of reduced fish activity. Ventral skin and attached muscle tissues (belly meat) of salmonids are fattier than dorsal tissues; hence, as depot sites for fat, they can

reasonably be expected to show the greatest effects of reduced activity.

Ocular tissue FA profiles were primarily affected by diet. Ocular tissues of juvenile Atlantic salmon raised on the Anchovy-based feed had higher concentrations of EPA, DPA and total omega-3 consistent with the higher concentrations of these compounds in the feed. This result demonstrates the plasticity, in terms of FA profiles, of ocular tissues of fish in response to a dietary manipulation. Diet and UV treatment also interacted such that the lowest concentrations of ARA, DHA and PUFA levels were found in the ocular tissues of fish from the −UV treatments fed the Herring-based diet. We suggested previously that fish in the −UV treatments were more active and that this was manifested by decreased ALA, LIN and SAFA concentrations in other tissues. DHA (and PUFA) dominates FA profiles of ocular tissues; thus, if fish were growing more quickly in the −UV treatments, the requirements for these FAs by other tissues would be expected to compete with the requirements of ocular tissues, especially for fish fed the Herring-based diet which had significantly lower concentrations of DHA than the Anchovy-based diet.

The Herring-based diet produced heavier fish per unit length than the Anchovy diet (Table 6) despite the fact that dorsal muscle tissues of juvenile Atlantic salmon raised on the Anchovy-based diet had higher concentrations of ALA, EPA and DPA (Fig. 2). This suggests that these essential fatty acids were not limiting in either diet and, moreover, that the critically important ratio between omega-3 and omega-6 FA (45) and/or the higher MUFA concentrations in the Herring-based feed (Fig. 1) were more conducive to higher growth rates. Exposing juvenile salmon to UV slightly reduced their weight per unit length (Table 7), suggesting that such exposures are stressful to the fish at some level. This result supports our finding that, overall, juvenile Atlantic salmon subjected to the +UVB treatment likely had reduced activity levels and, thus, higher concentrations of LIN, ALA, total omega-6 FA and SAFA compared with fish in the reduced UV treatments.

CONCLUSIONS

The conclusion that the health of juvenile Atlantic salmon is reduced under mildly increased UV is supported by findings of a companion study (12). In a parallel series of measurements, with another subset of fish exposed to higher UV levels than was the case in this experiment, the authors (12) found decreased weight, hematocrit values and plasma protein concentrations in juvenile Atlantic salmon. There were also effects on plasma immunoglobulin concentrations. They suggested that this interference with immune system functioning could have long-term consequences for disease resistance in UV-exposed Atlantic salmon. It is well established that stress, and especially changes in nutritional status in early life stages, can have unforeseen but significant fitness consequences in fishes, birds and mammals later in life (46,47). The results of this study, a companion study (12) and the work of others (44) all suggest that unprotected juvenile Atlantic salmon may experience negative effects (immune and behavioral responses, changes in fatty acid profiles) following exposure to UVR and that their growth is slightly reduced (this study). Other studies have reported that exposure to UVR can have negative effects on the skin and eyes of various fish species, including salmonids (see above). Future studies should assess the effects of exposure to UVR early in life on fitness consequences to later life history stages of Atlantic salmon.

Acknowledgements—We thank Penny Kuhn for her assistance with the UV measurements and dose estimates and Michelle Palmer (Palmer Processing) for statistical advice. We are grateful to Bjørn Johnson (the Norwegian Radiation Protection Authority), Arne Dahlback (the University of Oslo) and Kåre Edvardsen (the Norwegian Institute for Air Research) for access to the Bergen irradiance data. We also thank Stig Ove Utskot (Institute of Marine Research, Norway) for maintaining tanks and taking care of the fish and Reidun Bjelland for helping with the tissue dissections. Tyler Spencer (Environment Canada) provided expert help with the fatty acid analyses. The project was supported by grants from the Research Council of Norway (140472/130), the Norwegian Institute of Marine Research (Sensory Biology and Behavior Project) and Environment Canada.

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