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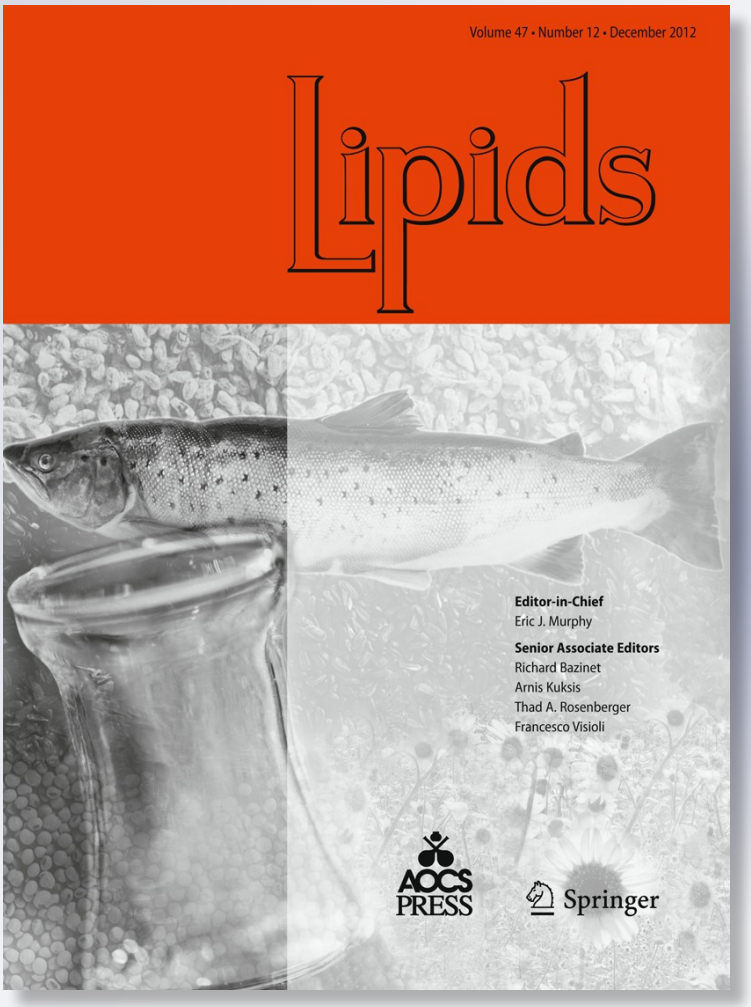

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ORIGINAL ARTICLE

UVB Radiation Variably Affects n-3 Fatty Acids but Elevated Temperature Reduces n-3 Fatty Acids in Juvenile Atlantic Salmon (*Salmo salar*)

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Abstract Temperature and ultraviolet B radiation (UVB 290–320 nm) are inextricably linked to global climate change. These two variables may act separately, additively, or synergistically on specific aspects of fish biochemistry. We raised Atlantic Salmon (*Salmo salar*) parr for 54 days in outdoor tanks held at 12 and 19 °C and, at each temperature, we exposed them to three spectral treatments differing in UV radiation intensity. We quantified individual fatty acid (FA) mass fractions in four tissues (dorsal muscle, dorsal and ventral skin, and ocular tissue) at each temperature × UV combination. FA composition of dorsal muscle and dorsal and ventral skin was not affected by UV exposure. Mass fractions of 16:0, 18:0, and saturated fatty acids (SFA) were greater in dorsal muscle of warm-reared fish whereas 18:3n-3, 20:2, 20:4n-6, 22:5n-3, 22:6n-3, n-3, n-6, polyunsaturated fatty acids (PUFA), and total FA were significantly higher in cold-reared fish. Mass fractions of most of the FA were greater in the dorsal and ventral skin of warm-reared fish. Cold-reared salmon exposed to enhanced UVB had higher ocular tissue mass fractions of 20:2, 20:4n-6, 22:6n-3, n-3, n-6, and PUFA compared to fish in which UV had been removed. These observations forecast a host of ensuing physiological and ecological responses of juvenile Atlantic Salmon to increasing temperatures and UVB levels in native streams and rivers where they mature before smolting and returning to the sea.

Keywords Atlantic Salmon · Fatty acids · Temperature · UV radiation · Climate change · Aquaculture

Abbreviations

DHA	Docosahexaenoic acid
EFA	Essential fatty acid(s)
FA	Fatty acid(s)
FAME	Fatty acid methyl ester(s)
LC-PUFA	Long-chain polyunsaturated fatty acid(s) (carbon chain length ≥C20 and typically with ≥3 double bonds)
MUFA	Monounsaturated fatty acid(s)
PUFA	Polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)
UVA	Ultraviolet A radiation (320–400 nm)
UVB	Ultraviolet B radiation (290–320 nm)
UVR	Ultraviolet radiation (both UVA and UVB)

Introduction

The proximate physical environment experienced by animals results from complex higher-order interactions among

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local topography, wind, cloud cover, aerosols, ozone, green house gases, variable solar radiation fluxes and season [1]. The latest Intergovernmental Panel on Climate Change [2] report predicts, for non-mitigation global warming scenarios, increases in average surface air temperature of between 0.64 and 0.69 °C for 2011–2030 and, depending upon the modeling scenario, increases of as much as 1.8 °C by mid-century (2046–2065). In addition, the IPCC report predicts an increase in the number of extreme heat days for northern temperate ecosystems [2]. Sustained and extreme increases in surface air temperatures have an immediate effect on shallow systems (i.e. streams, ponds) as their water temperatures are directly influenced by air temperatures [3, 4]. Global warming is also contributing to polar stratospheric ozone loss, delaying the recovery of the ozone layer [5] which helps to protect life on the surface of the planet from damaging UVB radiation. Thus, temperature and UVB radiation are inextricably linked to global climate change where they function as key drivers of the proximate physical environment experienced by animals.

Temperature induces strong effects on the physiology of poikilotherms, including fishes. At the cellular level, temperature can be regarded as a stressor to which fish must respond in order to establish a new equilibrium between their environment and the physico-chemical properties of their membranous structures; a phenomena called "homeoviscous adaptation" by Sinensky [6]. Thus, biochemical and physiological adaptations in response to temperature, at the membrane level, provide the most sustained and specific response to fluctuating temperatures experienced by fish. These biochemical responses are principally mediated by changes in the composition and concentrations of individual fatty acids (FA) and sterols in cell membranes [7].

UV radiation also exerts strong effects on the physiology of fishes and other aquatic organisms [8]. In an earlier study, we demonstrated that juvenile Atlantic Salmon (*Salmo salar*, Linnaeus, 1758) exposed to enhanced levels of UVB radiation had higher mass fractions of 18:2n-6 and 18:3n-3, total n-6 FA and saturated fatty acids (SFA) in their tissues compared with fish in reduced UV treatments, suggesting that salmon exposed to UV radiation were more quiescent than fish in the reduced UV treatments resulting in a buildup of catabolic substrates [9]. This finding is broadly consistent with observations of depressed feeding behavior and reduced agonistic interactions in juvenile salmon exposed to UVR in artificial flumes [10]. In addition, exposure to enhanced UVB radiation retarded growth, decreased hematocrit value and plasma protein concentration, in Atlantic Salmon parr [11]. Such effects may, in general, decrease resistance to pathogens in salmonids [12]. Finally, exposure to UVB, regardless of water temperature, had a negative effect on immune function parameters, growth and physiological condition of Atlantic Salmon parr, although interactive effects between temperature and UVB were also observed [13].

It is important to consider the effects of both UVB and temperature because these two physical forcing variables may act additively or synergistically on specific aspects of fish biochemistry, physiology, and behavior. Thus, we exposed Atlantic Salmon parr to three environmentally realistic [9, 11, 13] spectral treatments differing in the intensity of UVB radiation at two different temperatures (12 and 19 °C). We assayed FA mass fractions in Atlantic Salmon, held in outdoor rearing tanks subjected to different UV and temperature treatments, because lipids are key molecules involved in many cellular and physiological processes and because they are known to be sensitive to stress, including UVR. We tested the null hypotheses that juvenile (parr) Atlantic Salmon exposed to different temperature or UVB regimens demonstrated no direct and/or interactive effects on FA profiles in their dorsal skin, ventral skin, dorsal muscle, or ocular tissue.

Materials and Methods

Experimental Setup

The experiment was conducted at the Institute of Marine Research's (IMR) Austevoll Research Station (60°5′42″N, 5°13′8″E). At the beginning of the experiment 100 juvenile Atlantic Salmon (*Salmo salar*; Norsk LakseAvl strain; mean weight at start = 8.3 g) were placed in tanks (3 m wide × 1 m deep) which were filled with ~4,500 L of sand-filtered freshwater from a nearby lake. Incoming water was routinely aerated, the turnover rate was high, and the fish biomass to water volume ratio was very low, so oxygen was considered to be saturated. The fish were held in nylon net cages (50 × 60 × 60 cm; L × W × H) which were immersed in the water so that the distance from the surface to the bottom of the net cages was 30 cm.

The fish were randomly divided into the cages to achieve an even size distribution, and were fed with a specially prepared salmon feed (see below). The duration of the experiment was 54 days (July 17–September 8, 2003). At the end of the experiment all fish were euthanized using tricaine methanesulfonate (MS-222, Sigma Chemical Co). There were 6 tanks (experimental units) corresponding to the six possible spectral × temperature combinations (see below). Additional details of the experimental setup and conditions can be found elsewhere [9, 11, 13]. Fish were handled, and sacrificed, following standard procedures accepted by the Institutional Animal Care Committee of the Institute of Marine Research, Austevoll Aquaculture Research Station, Storebø, Norway.

Spectral Treatments

The three spectral treatments were: (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) UVB-depleted solar radiation (hereafter, −UVB) i.e. sunlight screened though polyester plastic film (0.2 mm thick Mylar-D®, DuPont Teijin Films, Delaware, USA, 50 % transmittance at 318 nm), and (3) solar radiation supplemented with UVB radiation (hereafter + UVB) from an overhead fluorescent tube lamp (TL40/12 RS, Philips Lighting, Rosendal, NL, emission maximum at 315 nm) placed 100 cm above the water surface. To remove UVC radiation the lamp was wrapped in cellulose triacetate film (95 mm, Clarifoil Co., UK), and the film was changed every 18 h. The lamp was turned on at noon for 4 h. For the spectral output of the TL40/12 RS lamp, see [14]. For details concerning the average daily irradiances in the +UVB and −UVB treatments see [13] Table 1).

These three spectral treatments were carried out simultaneously at two temperatures; at the normal rearing temperature of salmon in the area of the experimental site (∼12 °C) and at ∼7 °C above the normal rearing temperature (increased to ∼19 °C by a thermostatic heater) to explore potential additive or synergistic effects of different combinations of the two environmental stressors. The temperature of the warm water treatment was consistently higher than the cold water treatment (repeated measures ANOVA on ranks, Tukey multiple comparison p value ≤ 0.0001; Fig. 1).

Salmon Diet

The feed was manufactured by Nofima (http://www.nofima.com/; formerly SSF, Norway), in spring 2002 and was utilized in another experiment conducted in 2002 [9].

Table 1 Degrees of freedom (d.f.) for three-way and two-way ANOVA tests conducted to assess the effects of temperature and exposure to ultraviolet radiation on fatty acids of Atlantic Salmon parr

Source of variation	Three-way ANOVA d.f.	Two-way ANOVA d.f.
Total	143	35
Temperature	1	1
UV exposure	2	2
Temperature × UV exposure	2	2
Tissue	3	
Tissue × temperature	3	
Tissue × UV exposure	6	
Tissue × temperature × UV exposure	6	
Error	120	30

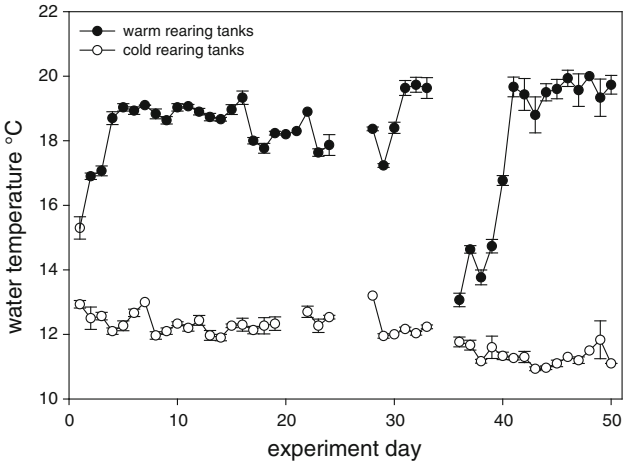

Fig. 1 Mean (±SD) water temperature of the experimental tanks used to test for the effects of temperature and UV exposure on juvenile Atlantic Salmon tissues ($n = 3$). The average water temperatures of the warm and cold-rearing tanks were 19 and 12 °C, respectively. Note: the average temperature of the cold rearing tanks was incorrectly reported as 14 °C in Jokinen et al. [13]

Peruvian Anchovy (*Engraulis ringens*) oil was added to the base feed formulation to provide the salmon with key dietary essential n-3 FA. This feed is hereafter referred to as the anchovy feed. The detailed ingredients, gross chemical composition, and energy content of the anchovy feed is provided elsewhere [9]. The feed was processed into 1.0, 1.5 and 2.0 mm diameter pellets so that juvenile salmon could be fed appropriately-sized particles as they grew. The feed was stored in the dark in a −50 °C chest freezer from the time it was manufactured until the end of the 2003 experiment. Salmon were hand-fed ad libitum.

Tissue Collections and Processing

At the end of the experiment, four different tissues were collected from six randomly selected Atlantic Salmon juveniles from each of the spectral and temperature combinations. The tissues sampled included dorsal and ventral skin, dorsal muscle) and ocular tissue. Dorsal skin, adjacent to the dorsal fin, was sampled by gripping it with a pair of surgical pliers and peeling it off the underlying muscle tissue. A surgical razor blade was then used to trim excess fat and/or muscle that remained attached to the skin. Ventral skin was obtained from the region between the pectoral fins and anus. Because we could not efficiently separate the retina from surrounding tissues we here refer to samples consisting of the entire posterior portion of the inner regions of the eye as ocular tissues. Ocular tissue was chosen because dietary supply of docosahexaenoic acid (DHA; 22:6n-3) is known to affect retinal DHA concentrations in fish [17, 18] and visual acuity in vertebrates [19–21]. Finally, UVR negatively affects several aspects of

vision in vertebrates (e.g. major cytoskeletal structures such as microtubules and actin) leading to cataract formation [22, 23]. We chose skinless dorsal muscle (landmarked to either side of the dorsal fin) because it is the largest tissue in these fish and because of its economic importance (i.e. filets). Dorsal skin was chosen because it should be a major target site for UVR damage [15, 16]. Ventral skin is relatively less pigmented (protected) than dorsal skin and, therefore, should be more vulnerable to the effects of UV radiation when/if exposed.

Fatty Acid Analyses

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 for 48 h prior to analyses. A total of 144 individual tissue samples were analyzed for FA (3 light treatments × 2 temperatures × 4 tissues × 6 fish per treatment cell). Lipids and FAME of the freeze-dried salmon feed and salmon tissues were obtained in a three-step process: extraction [24], derivatization using the boron trifluoride method [25] and quantification on a HP6890 gas chromatograph (as in [26]). Each freeze-dried sample was weighed to the nearest microgram (Sartorious ME5 microbalance), and homogenized to extract the lipids in 2 mL of 2:1 (v/v) chloroform:methanol (modified from Folch et al. [24] in that dry tissues were extracted). This was repeated three times. The resulting supernatants (after centrifugation to remove non-lipid containing material) were combined in a 15-mL centrifuge tube. The lipid extract was then accurately adjusted to 8 mL with 2:1 (v:v) chloroform:methanol, and 1.6 mL of a 0.9 % NaCl in water solution was added. The two phases were then thoroughly mixed and centrifuged (2,000 rpm at 4 °C). The upper aqueous layer was removed and discarded. The FA were dissolved in 2 mL hexane prior to derivatization. Two milliliters of BF3-methanol (10 % w/w) was added and vials were heated (70 °C) for 2 h after which 1 mL of water was added. The FAME-containing hexane-layer was carefully removed and put into a 2-mL Kuderna-Danish receiving vial (Sigma #6-4689U). One milliliter of hexane was then added to the original Shimadzu vial to extract the remaining FAME. This step was repeated once more to get the best extraction efficiency (90–95 %). The FAME-hexane solution was evaporated to 2.0 mL using nitrogen gas and transferred to a 2 mL glass GC vial and stored in a −80 °C cryogenic freezer prior to GC analysis. FAME were quantified using a Hewlett Packard 6890 GC (splitless injection; column = Supelco SP-2560, 100 m × 0.25 mm ID × 0.20 μm thick film) by comparing peak retention times and areas between the samples and standard curves created using a 37-component FAME standard (Supelco #47885-U). FA results are reported as mass fractions (i.e. μg FAME/mg dry weight tissue).

Statistical Analyses

The effect of water temperature and UV exposure on the FA composition of juvenile Atlantic Salmon tissues was examined using a series of analysis of variance (ANOVA) tests. FA mass fractions were log-transformed prior to analysis to satisfy normality and homogeneity of variance assumptions of parametric analysis. Three-way ANOVAs were used to compare FA composition among the four tissue types (dorsal muscle, dorsal skin, ventral skin and ocular) at two water temperatures (cold and warm) and three UV exposures (+UVB, −UVB and −UVR). Since multiple 3-way ANOVA testing was employed, the false discovery rate correction, which controls the proportion of errors among the rejected hypotheses, was applied to reduce the risk of Type I errors [27]. Two-way ANOVA and Tukey multiple comparison tests were used to compare FA responses to water temperature and UV exposure within each tissue type. Tukey multiple comparison tests control the Type I error rate for multiple pairwise comparisons [28]. Results are presented as untransformed means ± SD. Differences were considered significant at $p < 0.05$. Statistical analyses were performed using SigmaStat for Windows software (version 3.11; Systat Software Inc., San Jose, CA, USA).

Results

Fatty Acids Profiles of Salmon Tissues

Juvenile Atlantic Salmon tissues had high mass fractions of 22:6n-3, 16:0 and 18:1n-9 (Fig. 2). The mean mass fraction of all other FA was <5 μg/mg dry tissue. Mass fractions of total FA across all tissue types averaged 43.3 ± 15.1 μg/mg dry tissue. FA composition differed among tissue type (three-way ANOVA; Tables 1, 2). In general, mass fractions of FA were greater in ocular tissue as compared to dorsal muscle and dorsal and ventral skin (Fig. 2). Differences among tissues were temperature dependent (Table 2). Temperature also interacted with UV exposure although UV exposure main effects were not significant.

Effect of Temperature and UV Exposure on Individual Tissues

The FA composition of dorsal muscle and dorsal and ventral skin tissues were not significantly affected by UV exposure (two-way ANOVA, UV exposure main effect $F_{2,30} \leq 3.1$, p values ≥ 0.06), but were often significantly

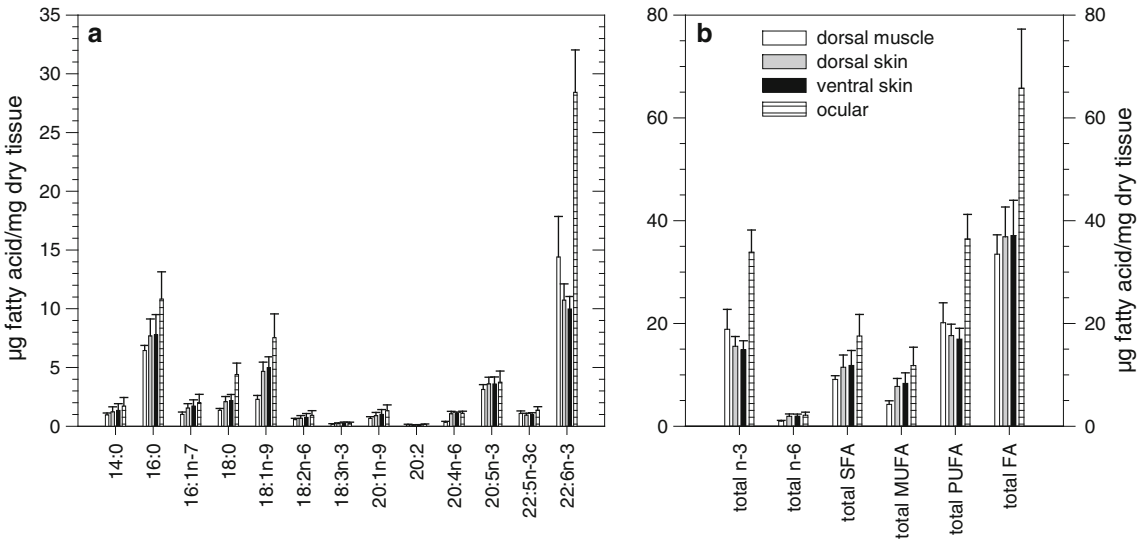

Fig. 2 Mean (±SD) mass fractions of individual (**a**) and summary (**b**) fatty acids in juvenile Atlantic Salmon tissues ($n = 36$ per tissue)

Table 2 Three-way analysis of variance F values summarizing the response of juvenile Atlantic Salmon tissues to water temperature and UV exposure ($n = 6$ per treatment combination)

Fatty acid	Main effects			Two-way interactions			Three-way interaction
	Tissue	Temperature	UV exposure	Tissue × temperature	Tissue × UV exposure	Temperature × UV exposure	Tissue × temperature × UV exposure
14:0	28.71*	164.47*	0.57	15.48*	0.55	3.46*[a]	1.53
16:0	138.90*	214.15*	0.12	17.01*	0.67	7.70*	1.52
16:1n-7	61.11*	73.35*	0.73	10.47*	0.50	3.27*[a]	1.68
18:0	650.60*	271.33*	0.96	10.91*	0.53	8.71*	0.72
18:1n-9	411.96*	79.76*	1.59	14.16*	0.32	3.80*[a]	1.33
18:2n-6	16.91*	109.64*	1.06	16.10*	0.37	2.75	1.67
18:3n-3	11.53*	22.50*	1.40	12.26*	0.57	1.76	1.68
20:1n-9	40.90*	93.35*	1.23	12.63*	0.52	2.60	1.45
20:2	33.04*	18.64*	1.30	55.70*	0.78	3.18*[a]	2.52*[a]
20:4n-6	621.22*	3.15	1.54	25.25*	0.90	6.19*	1.66
20:5n-3	12.43*	95.05*	1.95	14.91*	0.84	3.18*[a]	2.28*[a]
22:5n-3	46.09*	4.08*[a]	0.67	27.72*	1.54	2.95	1.48
22:6n-3	512.22*	19.30*	1.81	19.49*	1.04	9.78*	1.90
Total n-3	357.57*	2.08	1.79	21.32*	1.06	8.30*	2.02
Total n-6	206.97*	91.53*	0.23	24.41*	0.12	4.10*	2.79*
Total SFA	185.27*	239.61*	0.18	17.04*	0.65	7.09*	1.44
Total MUFA	218.93*	96.18*	1.23	13.01*	0.36	3.37*[a]	1.49
Total PUFA	323.64*	0.00	1.71	22.39*	1.00	8.30*	2.16
Total FA	265.73*	52.51*	0.86	25.58*	0.71	8.23*	2.18*[a]

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *FA* fatty acids

*F value was significant at $p < 0.05$

[a] F value was not significant after false discovery rate correction for multiple inferences

related to the water temperature at which fish were reared (temperature main effect p values <0.05). For dorsal muscle tissue, mean mass fractions of the individual FA 16:0 and 18:0, as well as total SFA, were greater in fish raised at the warm water temperature (Table 3). Conversely, 18:3n-3, 20:2, 20:4n-6, 22:5n-3, 22:6n-3, total n-3,

Table 3 Fatty acid mass fractions (μg FA/mg dry tissue) of tissue in juvenile Atlantic Salmon reared at different water temperatures ($n = 18$)

Fatty acid	Dorsal muscle		Dorsal skin		Ventral skin	
	Cold water temperature	Warm water temperature	Cold water temperature	Warm water temperature	Cold water temperature	Warm water temperature
14:0	0.94 ± 0.13[a]	1.00 ± 0.19[a]	0.91 ± 0.24[a]	1.56 ± 0.29[b]	0.90 ± 0.23[a]	1.80 ± 0.43[b]
16:0	6.26 ± 0.25[a]	6.63 ± 0.52[b]	6.65 ± 0.74[a]	8.74 ± 1.17[b]	6.26 ± 0.76[a]	9.31 ± 0.78[b]
16:1n-7	1.02 ± 0.16[a]	1.01 ± 0.22[a]	1.32 ± 0.27[a]	1.80 ± 0.28[b]	1.35 ± 0.28[a]	2.08 ± 0.48[b]
18:0	1.27 ± 0.09[a]	1.46 ± 0.13[b]	1.77 ± 0.22[a]	2.42 ± 0.32[b]	1.74 ± 0.25[a]	2.64 ± 0.19[b]
18:1n-9	2.32 ± 0.28[a]	2.27 ± 0.37[a]	4.19 ± 0.52[a]	5.16 ± 0.70[b]	4.29 ± 0.60[a]	5.67 ± 0.64[b]
18:2n-6	0.57 ± 0.08[a]	0.55 ± 0.13[a]	0.51 ± 0.14[a]	0.85 ± 0.16[b]	0.52 ± 0.14[a]	0.99 ± 0.28[b]
18:3n-3	0.21 ± 0.02[a]	0.17 ± 0.03[b]	0.19 ± 0.04[a]	0.26 ± 0.05[b]	0.20 ± 0.04[a]	0.31 ± 0.10[b]
20:1n-9	0.68 ± 0.10[a]	0.67 ± 0.16[a]	0.71 ± 0.18[a]	1.09 ± 0.19[b]	0.71 ± 0.20[a]	1.30 ± 0.35[b]
20:2	0.17 ± 0.02[a]	0.08 ± 0.01[b]	0.10 ± 0.02[a]	0.11 ± 0.02[b]	0.10 ± 0.01[a]	0.12 ± 0.02[b]
20:4n-6	0.39 ± 0.03[a]	0.30 ± 0.06[b]	0.99 ± 0.10[a]	1.14 ± 0.24[b]	0.95 ± 0.11[a]	1.15 ± 0.10[b]
20:5n-3	3.14 ± 0.35[a]	3.12 ± 0.48[a]	3.26 ± 0.27[a]	3.97 ± 0.54[b]	3.13 ± 0.33[a]	4.08 ± 0.36[b]
22:5n-3	1.26 ± 0.11[a]	0.92 ± 0.12[b]	0.91 ± 0.17[a]	1.00 ± 0.11[b]	0.89 ± 0.14[a]	1.06 ± 0.17[b]
22:6n-3	16.89 ± 2.61[a]	11.93 ± 2.13[b]	10.98 ± 1.24[a,B]	10.49 ± 1.50[a]	9.57 ± 1.21[a]	10.38 ± 0.74[b]
Total n-3	21.54 ± 2.92[a]	16.19 ± 2.69[b]	15.37 ± 1.66[a]	15.77 ± 2.09[a]	13.82 ± 1.66[a]	15.89 ± 1.17[b]
Total n-6	1.05 ± 0.09[a]	0.94 ± 0.14[b]	1.65 ± 0.15[a]	2.24 ± 0.45[b]	1.61 ± 0.16[a]	2.30 ± 0.24[b]
Total SFA	8.76 ± 0.45[a]	9.45 ± 0.78[b]	9.71 ± 1.22[a]	13.27 ± 1.81[b]	9.28 ± 1.23[a]	14.39 ± 1.42[b]
Total MUFA	4.24 ± 0.56[a]	4.24 ± 0.82[a]	6.68 ± 1.07[a]	8.80 ± 1.24[b]	6.82 ± 1.14[a]	9.87 ± 1.53[b]
Total PUFA	22.76 ± 2.96[a]	17.51 ± 2.74[b]	17.12 ± 1.75[a]	18.15 ± 2.55[a]	15.53 ± 1.78[a]	18.34 ± 1.36[b]
Total FA	35.77 ± 3.04[a,A]	31.20 ± 2.93[b]	33.51 ± 3.86[a]	40.22 ± 5.49[b]	31.64 ± 3.99[a]	42.59 ± 4.10[b]

Values are expressed as means ± SD. Values in the same row with different letters indicate significant differences between water temperature treatments within each tissue type (two-way analysis of variance temperature effect $p < 0.05$). Interaction terms were not significant ($p \geq 0.05$) with two exceptions

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *FA* fatty acids

[A] Total FA was greater in the +UVB treatment as compared to the −UVB treatment at cold water temperature

[B] 22:6n-3 was greater at cold water temperature as compared to warm water temperature within the −UVB treatment

total n-6, total polyunsaturated fatty acids (PUFA), and total FA were greater in fish reared at cold water temperature (Table 3). For dorsal and ventral skin tissues, mean mass fractions of the individual FA were greater in fish reared at the warm water temperature with the exception of 22:6n-3 in dorsal skin. The mean mass fractions of the summary FA indices were also greater in fish reared at warm water temperatures although differences in total n-3 and total PUFA were not significant in dorsal skin tissue (Table 3).

For ocular tissue, the mean mass fraction of most FA was greater in fish reared at warm water temperature (two-way ANOVA, temperature main effect p values <0.05; Table 4). The effect of water temperature on FA composition in ocular tissue differed among UV exposure level (interaction effect $F_{2,30} \geq 3.3$, p values <0.05; Table 4). At the colder temperature, juvenile salmon exposed to +UVB had higher mass fractions of 20:2, 20:4n-6, 22:6n-3, total n-3, total n-6, total PUFA and total FA in their ocular tissues compared to fish exposed to −UVR (Tukey multiple comparisons, p values <0.05). At warm water temperature, fish exposed to −UVR had higher mass fractions of 14:0, 16:0, 16:1n-7, 20:5n-3, 22:5n-3, total n-3, total n-6, total SFA, total PUFA and total FA in their ocular tissues compared to fish exposed to +UVB (Table 4). At both cold and warm water temperatures, FA mass fractions in ocular tissues of fish exposed to −UVB were intermediate to mass fractions in fish exposed to +UVB and −UVR (Table 4).

Discussion

Water temperatures in native streams and rivers inhabited by Atlantic Salmon can increase in response to the removal of riparian cover and/or because of climate warming. Removal of riparian cover (e.g., due to logging operations) can increase water temperature in streams by as much as 4 to 6 °C [29–31]. Climate model scenarios predict an

Table 4 Fatty acid mass fractions (μg FA/mg dry tissue) of ocular tissue in juvenile Atlantic Salmon reared at different water temperatures and UV exposure levels ($n = 6$)

Fatty acid	Temperature main effect F value	Cold water temperature			Warm water temperature		
		+UVB	−UVB	−UVR	+UVB	−UVB	−UVR
14:0	63.79*	1.37 ± 0.39^{a}	1.10 ± 0.29^{a}	1.03 ± 0.25^{a}	1.91 ± 0.55^{a}	2.25 ± 0.55^{ab}	2.65 ± 0.36^{b}
16:0	54.99*	9.91 ± 1.67^{a}	8.91 ± 1.19^{a}	8.57 ± 0.59^{a}	11.52 ± 1.72^{a}	12.22 ± 1.81^{ab}	13.84 ± 1.11^{b}
16:1n-7	39.76*	1.79 ± 0.48^{a}	1.44 ± 0.34^{a}	1.35 ± 0.28^{a}	2.09 ± 0.59^{a}	2.53 ± 0.61^{ab}	2.87 ± 0.36^{b}
18:0	70.82*	3.86 ± 0.69^{a}	3.65 ± 0.59^{a}	3.37 ± 0.18^{a}	4.84 ± 0.60^{a}	5.10 ± 0.66^{a}	5.58 ± 0.44^{a}
18:1n-9	50.59*	6.59 ± 1.39^{a}	6.06 ± 0.89^{a}	5.53 ± 0.47^{a}	8.03 ± 1.40^{a}	9.43 ± 1.70^{a}	9.60 ± 1.61^{a}
18:2n-6	49.14*	0.77 ± 0.25^{a}	0.59 ± 0.18^{a}	0.54 ± 0.15^{a}	1.01 ± 0.33^{a}	1.25 ± 0.34^{a}	1.40 ± 0.19^{a}
18:3n-3	9.04*	0.28 ± 0.08^{a}	0.21 ± 0.06^{a}	0.19 ± 0.05^{a}	0.25 ± 0.06^{a}	0.29 ± 0.09^{a}	0.34 ± 0.05^{a}
20:1n-9	40.85*	1.16 ± 0.28^{a}	0.95 ± 0.26^{a}	0.85 ± 0.19^{a}	1.40 ± 0.38^{a}	1.69 ± 0.47^{a}	1.91 ± 0.25^{a}
20:2	0.51	0.19 ± 0.05^{a}	0.15 ± 0.04^{ab}	0.14 ± 0.03^{b}	0.14 ± 0.02^{a}	0.14 ± 0.03^{a}	0.17 ± 0.02^{a}
20:4n-6	12.72*	1.15 ± 0.14^{a}	0.99 ± 0.15^{ab}	0.90 ± 0.05^{b}	1.09 ± 0.13^{a}	1.15 ± 0.16^{a}	1.30 ± 0.19^{a}
20:5n-3	58.61*	3.42 ± 0.59^{a}	2.91 ± 0.41^{a}	2.82 ± 0.26^{a}	4.03 ± 0.78^{a}	4.37 ± 0.76^{ab}	4.97 ± 0.41^{b}
22:5n-3	21.28*	1.31 ± 0.18^{a}	1.17 ± 0.16^{a}	1.14 ± 0.13^{a}	1.37 ± 0.26^{a}	1.53 ± 0.32^{ab}	1.72 ± 0.18^{b}
22:6n-3	2.35	32.23 ± 3.06^{a}	29.26 ± 3.26^{ab}	26.02 ± 0.90^{b}	26.23 ± 3.11^{a}	26.74 ± 4.09^{a}	30.06 ± 2.54^{a}
Total n-3	0.07	37.30 ± 3.59^{a}	33.61 ± 3.84^{ab}	30.22 ± 1.12^{b}	31.99 ± 4.04^{a}	33.05 ± 5.08^{ab}	37.16 ± 2.98^{b}
Total n-6	52.34*	2.04 ± 0.36^{a}	1.66 ± 0.31^{ab}	1.52 ± 0.19^{b}	2.26 ± 0.39^{a}	2.57 ± 0.52^{ab}	2.90 ± 0.22^{b}
Total SFA	68.83*	15.58 ± 2.74^{a}	14.03 ± 2.01^{a}	13.35 ± 1.03^{a}	18.98 ± 2.96^{a}	20.40 ± 3.14^{ab}	22.99 ± 1.95^{b}
Total MUFA	55.10*	10.20 ± 2.16^{a}	9.01 ± 1.57^{a}	8.23 ± 1.00^{a}	12.59 ± 2.57^{a}	15.04 ± 3.09^{a}	15.72 ± 2.53^{a}
Total PUFA	1.36	39.56 ± 3.90^{a}	35.43 ± 4.18^{ab}	31.88 ± 1.28^{b}	34.71 ± 4.55^{a}	36.21 ± 5.67^{ab}	40.75 ± 3.25^{b}
Total FA	24.35*	65.34 ± 7.98^{a}	58.46 ± 7.56^{ab}	53.46 ± 3.16^{b}	66.28 ± 9.87^{a}	71.65 ± 11.34^{ab}	79.46 ± 7.39^{b}

Values are expressed as means ± SD. Values in the same row with different letters indicate significant differences between UV treatments within each water temperature treatment (Tukey multiple comparison test $p < 0.05$)

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *FA* fatty acids

* F value was significant at $p < 0.05$

increase in the number of extreme heat days as well as globally-averaged increases in surface air temperatures of between +0.64 and +0.69 °C for 2011–2030 and as much as +1.8 °C by mid-century (2046–2065) [2]. Past climate warming (e.g. the period between 1985 and 2009) has already increased mean night-time surface water temperatures of inland water bodies by an average rate of 0.045 ± 0.011 °C year^{-1} (and as high as 0.10 ± 0.01 °C year^{-1}) with the greatest warming in the mid- and high latitude ranges of the northern hemisphere [32] inhabited by Atlantic Salmon.

Atlantic Salmon grow optimally at ~16 °C [33]. Here, we demonstrate that increasing the water temperature by ~7 °C (from 12 to 19 °C) had pronounced effects on mass fractions of individual FA and on summary FA measures for all four tissues of Atlantic Salmon (Tables 3, 4). This suggests that anthropogenically-induced (climate change and/or removal of riparian cover) increases in the water temperature of boreal freshwater streams and rivers could affect the nutritional status of juvenile (parr) Atlantic Salmon. This interpretation must be tempered by the reality that Atlantic Salmon will encounter much larger changes in temperature than the relatively constant conditions to which they were exposed during our experiment. Further, the temperature increase in our experiment should be viewed as a worst-case scenario. Nevertheless, our experimental design isolated the specific effects of temperature on FA profiles, which is difficult to impossible to do in the complex environments of natural streams and rivers. With these caveats in mind, our results can be viewed as indicative of how increasing water temperature in streams and rivers may affect wild juvenile Atlantic Salmon at biochemical and physiological levels during the period prior to and during smoltification. Further, the observed changes in mass fractions of specific FA in dorsal muscle are broadly consistent with what has been observed with respect to membrane adaptations (average membrane lipid order, i.e. fluidity) in response to changing temperature [7]. In agreement with these previous findings we also observed generally higher mass fractions of total and individual SFA (16:0, 18:0) and lower mass fractions of total and individual PUFA (e.g. 18:3n-3, 22:5n-3, 22:6n-3, and 20:4n-6) in dorsal muscle (Table 3) as temperatures increased. Finally, others [34] have commented on the potential of

climate change to affect the health of Atlantic Salmon in native streams/rivers. These authors point out that climate change models for the Maritime Provinces of eastern Canada (where natural reproduction of Atlantic Salmon still occurs) indicate an expected increase in air temperature of between 2 and 6 °C and they highlight how fork lengths (a gross index of fish condition) of Atlantic Salmon are negatively correlated with water temperatures.

Muscle is a good long-term integrator of the effects of changing temperatures and/or diet on a fish's nutritional status [35]. Juvenile salmon remain in freshwater streams and rivers for periods ranging from a year to several years and then transform from parr to smolts during their migration to the ocean. During this period they eat a diet mainly consisting of freshwater invertebrates [36, 37] which contain high levels of C_{18} PUFA, intermediate levels of C_{20} PUFA and generally lower levels of C_{22} PUFA [38–40]. It has been suggested that lipid metabolism in salmon may influence the process of parr-smolt transformation, ultimately affecting successful seawater adaptation [41]. For example, hepatocyte fatty acyl desaturation/elongation activities in juvenile Atlantic Salmon are primarily controlled by environmental factors such as temperature and photoperiod but diet also plays a role [42]. Thus, prior to smoltification the FA composition of parr salmon is influenced by, (1) diet, (2) their ability to modify that dietary FA input via catabolism and by desaturation and elongation pathways and, (3) the influence of water temperature and photoperiod (and possibly also the levels of UV to which they are exposed) on the latter processes. During the period leading up to smoltification, tissue FA composition changes from a typical freshwater pattern, which is relatively low in long-chain polyunsaturated fatty acids (LC-PUFA), to a more typical marine pattern which is relatively rich in LC-PUFA such as 20:5n-3 and 22:6n-3 [42]. Thus, the elevation of n-3 LC-PUFA in salmonids is considered a key pre-adaptive response to seawater entry [43, 44]. Our finding that n-3 LC-PUFA in muscle tissue was reduced at warmer water temperatures—even while diet remained constant—suggests climatic warming and/or removal of riparian cover may affect the physiological competency of Atlantic Salmon with respect to their ability to transition from freshwater to seawater.

The fish feed used in our experiment deviated from the natural, mostly insect-based, food of wild parr salmon in that aquatic insects have relatively higher amounts (both as proportions and mass fractions) of 18:3n-3 and 18:2n-6 and lower amounts of 20:5n-3 and especially 22:6n-3 than the anchovy-based feed. When diets more closely mimic the FA composition of aquatic insects, desaturation of 18:3n-3 and 18:2n-6 to LC-PUFA such as 20:5n-3, 22:6n-3 and 20:4n-6 occurs at a higher rate than when predominantly fish oil diets are fed to Atlantic Salmon parr [41]. Thus, desaturation rates are expected to be more conservative in our experiment. Nevertheless, we observed significantly lower concentrations of 18:3n-3, 20:4n-6, 22:6n-3, total n-3, total n-6 and PUFA in dorsal muscle of fish raised at the warmer temperature (Table 3). This occurred despite the fact that mass fractions of other FA did not change significantly (e.g. 14:0, 16:1n-7, 18:2n-6, 20:1n-9, 20:5n-3) or else increased (16:0). In our experiments, diet quality (anchovy-based feed) and quantity (all fish were fed ad libitum) was the same in all treatments so any changes in the mass fractions of FA in the four tissues examined here, in response to increasing temperature, are not attributable to differences in the underlying diet. It is also important to note that, in natural situations, the essential FA composition of lower trophic level organisms in aquatic food webs (e.g. algae, seston, zooplankton, and aquatic insects) inhabited by, and ultimately consumed by, higher trophic level consumers like salmon may also be negatively affected by water temperature (e.g. [45, 46]). In addition, all of the salmon used in this experiment came from the same broodstock and were randomly allocated to the different treatments, therefore, it is unlikely that there would have been gross systematic differences among the individual fish (for genetic reasons) with respect to their ability to desaturate and elongate shorter-chain FA. Taken together, these results suggest that desaturase and elongase activities were higher in salmon raised in colder water resulting in generally higher tissue PUFA mass fractions, although we cannot rule out the possibility that dietary PUFA were merely retained at a higher rate in the cold water treatment.

We also observed that mean mass fractions of most of the individual FA in dorsal and ventral skin tissues were greater in fish reared at the warm water temperature with the exception of 22:6n-3 in dorsal skin (Table 3). Although a review of the literature did not reveal previous studies relating water temperature to fish skin FA composition, other researchers have provided evidence that subjecting fish to water temperatures that are substantially different than their optimal growth temperature may increase the risk of parasite infection [47, 48]. For example, sea lice (*Lepeophtheirus salmonis*) infections of cultured Atlantic Salmon are highest in summer and/or in shallow bays when temperatures are warmest [49]. The barrier functions of the skin are primarily a consequence of the lipid composition of the stratum corneum [50], therefore, we suggest that the increases in specific individual FA that we observed as a function of increasing temperature may represent a generalized defense response to the increased risk of parasite infection when salmon are raised in water temperatures that are above their optimal growth temperatures.

UV-induced damage including, for example, sunburn [51, 52] and reduced numbers of mucous secreting goblet cells [15] have been observed in some fish species, whereas

other species appear to benefit from protective substances in their skin [53, 54]. Lipids in the outermost layer of skin (*stratum corneum*) are affected by UV exposure in three ways; (1) β scission (fragmentation of the carbon chain), (2) hydrogenation of the double bond of unsaturated compounds and (3) formation of oxygenated entities from unsaturated lipids [50]. In our experiments, the FA composition of dorsal and ventral skin tissues were largely unaffected by UV exposure. Similarly, Arts et al. [9] did not observe significant changes in mass fractions of individual FA of dorsal skin in Atlantic Salmon exposed to enhanced UVB radiation.

Although the main effects of UV exposure on FA profiles were not significant across tissues in our experiment there were, nonetheless, significant interactive effects between temperature and UV exposure for certain FA and FA summary indices (Table 2). These interactive effects were mostly driven by changes in mass fractions of several FA in ocular tissue (Table 4). Enhanced UVB had different effects on individual FA mass fractions when fish were raised in cold versus warm water conditions. Specifically, ocular tissues of fish raised in cold water demonstrated higher mass fractions of 22:6n-3 and 20:4n-6 in response to enhanced UVB stress, whereas this effect was not observed in ocular tissue of fish raised in warm water. We suggest, for the reasons below, that this may be an adaptive metabolic response of ocular tissues to enhanced UVB stress. We further suggest that future studies examine free radical production, vitamin status and histology in skin and ocular tissues as a function of UV exposure and that fatty acid analyses be conducted on specific phospholipid classes (e.g. phosphatidylcholine, phosphatidylethanolamine) so that the overall response to UV radiation can be better understood.

Compromised vision occurs in fishes exposed to UV radiation under controlled experimental conditions. For example, cataract formation increased in Rainbow Trout (*Onchorynchus mykiss*) that received chronic UV exposure [23] while corneal tissue transmission decreased in UV-exposed Hawaiian Saddleback Wrasse (*Thalassoma duperrey*) [54]. Similarly, damage from ambient solar UVR was concluded to be a contributing factor explaining the formation of anterior lens cataracts in cage-reared Atlantic Halibut (*Hippoglossus hippoglossus*) [55]. Finally, wild Atlantic Salmon postsmolts taken from trawl hauls in the Norwegian Sea demonstrated effects ranging from hazy opacity in the anterior part of their lenses to cataracts affecting the whole lens [56]. These authors concluded that the primary cause of these cataracts was defective osmoregulation but they did not rule out oxidative stress caused by exposure to UV radiation as a contributing factor to osmotic cataract development.

Vertebrate retinal tissue is known to contain high concentrations of 22:6n-3 mostly located in membrane phospholipids [57] and retinal 22:6n-3 concentration in fish is known to be responsive to dietary 22:6n-3 manipulations [18]. The 22:6n-3 contained in the vertebrate retina plays a crucial role in visual acuity [21], in part by promoting the survival and differentiation of photoreceptors [20]. Thus, 22:6n-3 is critical for both visual acuity (especially at low light intensities, [17]) and schooling behavior in fish [58].

Desaturases and elongases appear to be up-regulated in Atlantic Salmon raised in cold water compared to warm water, as evidenced by significantly higher concentrations of 22:6n-3 in dorsal muscle (Table 3). Up-regulation of enzymatic activity related to the production of higher amounts of n-3 LC-PUFA is consistent with what other researchers have found when teleosts are exposed to colder water temperatures [59]. Although we did not measure cataract formation or lens opacity, the studies summarized above suggest that compromised vision possibly occurred in the Atlantic Salmon parr exposed to enhanced UVB radiation in our experiment. We further suggest that the increased rate of 22:6n-3 synthesis in cold-water-raised salmon likely provided them with a compensatory mechanism to deal with compromised vision as a result of exposure to enhanced UVB radiation. Thus, we hypothesize that, as visual acuity was compromised following exposure to enhanced UVB radiation, salmon in the cold water treatment were able to compensate for reduced light transmission to the retina by increasing the mass fraction of 22:6n-3 (Table 4). We found a similar pattern when comparing the response of ocular tissue FA between two feeds that differed in mass fraction of LC-PUFA [9]. In that study, ocular tissues of fish in the enhanced UVB treatment had higher 22:6n-3 and 20:4n-6 mass fractions when they were fed a LC-PUFA-rich food source as compared to when they were fed a relatively less rich LC-PUFA food source, again suggesting that when higher amounts of LC-PUFA are available (either through diet or through temperature-induced up-regulation of desaturases and elongases) ocular tissues compensate for reduced function (e.g. cataracts, opacity) by increasing 22:6n-3 concentrations.

Conclusions

Raising the water temperature by $\sim$7 °C had pronounced effects on FA profiles of dorsal muscle, and dorsal and ventral skin of juvenile (parr) Atlantic Salmon raised in outdoor tanks. We observed higher mass fractions of total and individual SFA (16:0, 18:0) at the higher temperature and lower mass fractions of total and individual PUFA (e.g. 18:3n-3, 22:5n-3, 22:6n-3, and 20:4n-6) in dorsal muscle and higher mean mass fractions of most of the individual FA in dorsal and ventral skin tissues in fish reared at the warmer water temperature. Since biochemically-important

essential FA were negatively affected by temperature, these changes probably forecast a host of ensuing physiological and ecological responses of juvenile Atlantic Salmon to increasing temperatures in native streams and rivers where these salmon mature before smolting and returning to the sea [34, 60]. We further suggest that exposure to enhanced UVB radiation as a result of ozone thinning may add another stress factor to juvenile salmon (with respect to vision) especially when protective riparian cover is removed and/or water temperatures increase.

Changes in nutritional status in early life stages can have unforeseen but significant fitness consequences to animals later in life [61, 62]. Thus, we propose that future studies be designed to assess the effects of temperature and/or exposure to UVR early in life on fitness consequences to later life-history stages of Atlantic Salmon. Finally, we hypothesize that, because some of the LC-PUFA produced in aquatic ecosystems is transferred to adjacent terrestrial systems [63], the reduced nutritional quality (from an essential FA perspective) of juvenile Atlantic Salmon maturing in warmer water may also have long-term health consequences for their mammalian and avian fish predators.

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