

Exposure to Increased Ambient Ultraviolet B Radiation has Negative Effects on Growth, Condition and Immune Function of Juvenile Atlantic Salmon (*Salmo salar*)

Ilmari E. Jokinen^{*1}, Eveliina S. Markkula¹, Harri M. Salo¹, Penny Kuhn², Sami Nikoskelainen³, Michael T. Arts⁴ and Howard I. Browman²

¹Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland

²Institute of Marine Research, Austevoll Research Station, Storebø, Norway

³Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland

⁴National Water Research Institute, Burlington, ON, Canada

Received 21 November 2007, accepted 3 March 2008, DOI: 10.1111/j.1751-1097.2008.00358.x

ABSTRACT

Atlantic salmon (*Salmo salar*) parr were exposed in two outdoor experiments, ranging in duration from 52 to 137 days, to spectral treatments: (1) natural sunlight (= present ambient UVB level), (2) solar radiation supplemented with enhanced UVB radiation from lamps simulating 20% or 8% stratospheric ozone loss or (3) UVB-depleted sunlight achieved by screening with Mylar-D film. The growth, condition and immune function of the salmon were quantified after treatments. Exposure to enhanced UVB radiation retarded growth, and decreased hematocrit value and plasma protein concentration. Further, enhanced UVB radiation affected plasma immunoglobulin concentration. The results demonstrate that juvenile Atlantic salmon are not able to fully adapt to increased ambient UVB levels in long-term exposures, and the interference with immune system function suggests a negative effect of UVB on disease resistance in Atlantic salmon.

INTRODUCTION

Due to ozone layer depletion, levels of UVB radiation (wavelength 280–315 nm) incident on the earth's surface have increased significantly at polar regions and at mid-latitude areas of the northern and southern hemispheres (1–3). According to recent models, atmospheric ozone remains depleted and increases in doses of UV radiation are expected in northern latitudes over coming decades (4,5). UVB radiation also penetrates the water column to greater depths than had previously been anticipated (6,7). In clear ocean waters UVB can penetrate down to 20 m (8) and in the clearest lakes of Finland to depths of >1 m (9). In shallow rivers and streams with low dissolved organic carbon (DOC) concentrations, UVB can penetrate right down to the bottom. Several studies indicate that UVB radiation, at current levels, is harmful to aquatic organisms, and can reduce the productivity of marine ecosystems (10–13). Studies on eggs and larvae of fish indicate that exposure to UVB can increase mortality (14–19). Sublethal effects, such as reduced growth (20),

changes in ventilation rate and respiratory control (21,22) and skin injuries (23–25), have been reported (for a summary of UV effects in fish, see also Zagarese and Williamson [26]). Exposure to UVB affects also immune system and the resistance against diseases (27). Overexposure to solar UVB can cause skin lesions and these are often accompanied by microbial infections (28), but the susceptibility to bacterial and parasite infections can increase even after moderate doses of UVB (29). Exposing roach (*Rutilus rutilus* L.), carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) to UVB radiation in the laboratory has resulted in disturbed immunologic functions—suppressed function of phagocytes and natural cytotoxic cells (30), alterations in blood leukocytes (31) and decreased lymphocyte responses to mitogenic activators, suggesting deviant regulation of lymphocyte-dependent immune functions (32).

Most studies have concentrated on embryos and larvae, and have not examined the effects of long-term, low-level increased UVB radiation on the physiology of postlarval stage of fish in experimental designs relevant to natural conditions. Here, juvenile Atlantic salmon (*Salmo salar*) were exposed to natural sunlight, increased UVB radiation levels and UVB-depleted solar radiation in outdoor tanks for several weeks. The growth, condition of fish and the immune function were determined to investigate the impact of UV radiation on juvenile fish.

MATERIALS AND METHODS

Experimental setup. The study consisted of two experiments carried out during summer at two locations in Norway. Experiment 1 (Exp. 1) was carried out in 2001 at the Institute of Marine Research's (IMR) Matre Research Station (60°52'29"N, 5°34'40"E) and Exp. 2 in 2002 at the IMR's Austevoll Research Station (60°5'42"N, 5°13'8"E), 89 km southwest of Matre (Table 1). The Atlantic salmon juveniles used in both experiments were from the IMR, Matre stock. The experimental fish were kept outdoors in three round 4500 L, flow-through tanks. For each spectral treatment, three cages made of nylon fabric with a 10 mm mesh size, 50 × 60 cm in size, were placed in a row in one tank. The fish in the outermost two cages were used for the assessment of growth, condition and immune function, and the fish in the middle cage were used for other purposes. Each cage was stocked with 50 (mean weight 5.2 g, Exp. 1) or 100 juvenile Atlantic salmon (mean weight 1.8 g, Exp. 2). The fish were divided into cages randomly to

*Corresponding author email: eijokine@cc.jyu.fi (Ilmari E. Jokinen)

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Table 1. Average daily irradiance in two wavebands, UVB and UVA, in the spectral treatments—natural sunlight, sunlight supplemented with enhanced UVB radiation and UVB-depleted sunlight.

Treatment	Average daily irradiance (kJ m ⁻²)			
	Waveband UVB (305–320 nm)		Waveband UVA (321–367 nm)	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Sunlight				
Ambient	28.3	38.0	331	448
At depth 1 cm	28.0	32.0	318	394
At bottom of cage	2.40	0.13	54.0	10.5
Sunlight supplemented with UVB				
Ambient	33.0	39.6	335	447
At depth 1 cm	31.0	33.0	322	393
At bottom of cage	3.50	0.14	55.0	10.0
Simulated O ₃ loss (%)	20	8		
UVB-depleted sunlight				
Ambient	0.48	0.65	161	217
At depth 1 cm	0.45	0.50	155	192
At bottom of cage	0.00	0.00	28.0	5.60

achieve even size distribution, and were fed commercial salmon feed. The depth of the water column above the bottom of the net cages was 46 cm (Exp. 1) or 30 cm (Exp. 2). The water in the tanks was sand-filtered fresh water from a local river (Exp. 1) or a lake (Exp. 2), and the temperature of water in both experiments was a constant 14°C. Oxygen was monitored continuously and was near full saturation.

Spectral treatments. There were three spectral exposure treatments: (1) natural sunlight, (2) natural sunlight enhanced with UVB from an overhead lamp to simulate increased level of UVB radiation resulting from depletion of stratospheric ozone and (3) UVB-depleted solar radiation—sunlight was screened with polyester plastic film Mylar-D® (0.2 mm; DuPont Teijin Films, DE) which effectively removes wavelengths below 320 nm (50% transmittance at 318 nm) (33). To increase the proportion of UVB wavelengths two fluorescent tube lamps (TL40/12 RS; Philips Lighting, Roosendaal, The Netherlands), with emission maximum at 315 nm, were placed 1 m above the water surface. 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 lamps were kept turned on at noon for 2 h (Exp. 1) or 1 h (Exp. 2) for the spectral output of the TL40/12 RS lamp, see Salo *et al.* [34]. The duration of treatments was 52 days (July–August) and 137 days (April–September) in Exp. 1 and Exp. 2, respectively. Daily irradiances in spectral treatments are presented in Table 1.

Radiometry. Ambient radiation data, covering the full duration of the experiments, was obtained from multi-channel 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) 66 km southwest of Matre and 22 km north of Austevoll. To obtain comparative data, ambient radiation was monitored in Exp. 2 using a multichannel rotating shadow band radiometer (299, 305, 310, 317, 324, 332, 367 nm; UVMFR-7, Yankee Environmental Systems, MA) placed at IMR, Austevoll. The range of exposure experienced by the fish was determined by calculating irradiance at depths of 1 and 46 cm (Exp. 1), or 1 and 30 cm (Exp. 2), using the diffuse attenuation coefficient (K_d) (6,35) for the water in which the fish were housed. K_d was measured using a spectroradiometer (OL-754; Optronic Laboratories, FL) equipped with an underwater detector (OL-86T-WP; Optronic Laboratories) submerged at different depths in the tanks. The irradiance output of the UV supplemental lamp was added to the ambient levels to calculate mean daily irradiance of enhanced UVB treatment. Simulated ozone loss was calculated using the delta-Eddington approximation algorithm (36,37) in conjunction with ambient radiation data collected during the

exposure period, and the irradiance output of the supplemental lamps. The irradiance received in the UVB-depleted treatment was calculated by multiplying the percent spectral transmission of the Mylar-D® material (measured with OL-754 spectroradiometer) by the ambient irradiance.

Sampling. The total number of fish sampled from each spectral treatment were $n = 70$ and $n = 84$ in Exp. 1 and Exp. 2, respectively. Fish were first anesthetized with 0.01% tricaine (MS-222; Sigma), and weight (W) and length (L) were measured. Fulton's condition factor (K) was calculated as $K = 10^2 \times W(g) \times L^{-3}(cm)$ (38). Blood samples were drawn from the caudal vein using a heparinized 1 mL syringe and 25-G needle (Exp. 1), or taken directly into heparinized capillaries (75 µL; Hirschmann, Germany) after cutting off the tail (Exp. 2). Capillaries were centrifuged at 10 500 g for 5 min to determine hematocrit; plasma was then separated from packed cells and frozen (–70°C) until analyses.

Plasma protein concentration and lysozyme activity. The plasma total protein concentration was measured with a Bio-Rad Protein Assay Kit (Bio-Rad, Inc.) using bovine serum albumin (BSA) as the standard. The lysozyme enzyme activity in plasma was determined with a turbidometric microplate assay (39,40) using a *Micrococcus lysodeicticus* (Sigma) suspension (1 mg mL⁻¹ phosphate buffer, pH 6.2) as the substrate. The optical density of bacterial suspension in wells was measured with a plate reader (Victor2 1420 Multilabel Counter; Wallac Co., Finland) at 450 nm for 30 min with 1 min intervals.

Quantification of plasma IgM. The concentration of IgM in plasma was determined with a micro-plate modification of a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (41). Flat-bottomed, 96-well microplates (Nunc MaxiSorp, Nunc, Denmark) were coated with antisalmon IgM-specific antibody (5 µg mL⁻¹, CLF002; Cedarlane, Canada) in carbonate buffer (50 mmol L⁻¹, pH 9.6). After washing, the plates were saturated with 1% BSA in PBS (pH 7.4). After washing the plate, diluted plasma samples and standards were added in duplicates into the wells. The assay was calibrated with known concentrations of chromatographically purified salmon IgM. After washing the plates the trapped IgM was detected with biotin-conjugated CLF002 antibody (42). After washing the plate, alkaline phosphatase-conjugated avidin (Sigma) was incubated in the wells. The substrate (*p*-nitrophenylphosphate, 1 mg mL⁻¹; Sigma) in 1 M diethanolamine buffer (pH 9.8) was added after washing the plate, and the optical density was read with a Titertek Multiskan plate reader (Flow Laboratories) at 405 nm.

Statistical analysis. The variables were grouped into three specified sets of responses for multivariate analysis (43), including growth (weight and length), condition (blood hematocrit and plasma protein concentration) and immune function (plasma IgM concentration and lysozyme activity). The data were analyzed for effects in response sets using nested multivariate analysis of variance (MANOVA) with treatment as the fixed factor, and cage as a random factor hierarchically nested inside treatment. Subsequent to multivariate analyses, protected univariate ANOVA, with treatment as the fixed factor and cage as the random factor nested inside treatment, was used to analyze variables in response sets. Pair-wise differences between treatments in each variable were identified with ANOVA (factors as above, significance level $P < 0.05$). SPSS Statistical software ver. 13 (SPSS, Inc.) was used for all statistical analyses.

RESULTS

Spectral treatments

In Exp. 2, ambient irradiance was simultaneously measured at the experimental site in Austevoll and at the Bergen observatory. The total irradiance measured over the experimental period with the UVMFR in Austevoll was 4995 kJ m⁻² (299–320 nm) and 63095 kJ m⁻² (321–367 nm), and with the GUV in Bergen 4952 kJ m⁻² (305–320 nm) and 57938 kJ m⁻² (321–367 nm). The Austevoll-based UVMFR data were 0.8% higher at wavelengths below 320 nm, and 8.9% higher in the 321–367 nm waveband compared with the data from Bergen-based GUV. The ambient radiation data collected with the GUV

radiometer were used throughout the study because the on-site UVMFR spectrometer was damaged by lightning in Exp. 1.

Sunlight average daily ambient irradiances in UV wavelengths were 34% higher in Exp. 2 than in Exp. 1 (Table 1). The Mylar-D removed 98.3% of solar UVB irradiance in air, and no UVB was detected at the bottom of the cages. The lamps increased the average daily UVB irradiance in air by 16.9% in Exp. 1 and by 4.2% in Exp. 2. Based on the measurements in the 305–310 nm waveband, the increased irradiance simulated a stratospheric ozone loss of 20% in Exp. 1 and 8% in Exp. 2. The supplementation of UVB radiation with lamps increased UVA irradiation <1.5% (321–367 nm waveband).

The underwater optics differed markedly between the experimental sites. The diffuse attenuation coefficients at 310 nm were $K_d = 7 \text{ m}^{-1}$ in Exp. 1 and $K_d = 22 \text{ m}^{-1}$ in Exp. 2. Water optical characteristics reduced the UVB dose received by the fish on average by 90% in Exp. 1 and 99.6% in Exp. 2 resulting in markedly different average daily UVB

irradiances at the bottom of cage between the experiments both in exposures to natural sun light and to enhanced UVB (Table 1).

Growth

Exposure to enhanced UVB levels retarded growth of fish in Exp. 1, but not in Exp. 2 (Fig. 1, Table 2). Both the weight and the length of fish in the enhanced UVB treatment were lower than in fish exposed to natural sunlight. The mean of Fulton's condition factor (K) between treatment groups remained unchanged. There was no significant difference in the growth of fish exposed to natural sunlight vs UVB-depleted sunlight.

Condition

No sunburn lesions, overt signs of disease or other abnormalities were noted on the fish in any spectral treatment group. Mortality of fish was low (<2%) and unrelated to spectral treatments. Exposure to enhanced UVB radiation compared to natural sunlight reduced blood hematocrit and plasma total protein concentration of fish in Exp. 1 but not in Exp. 2 (Fig. 2, Table 2).

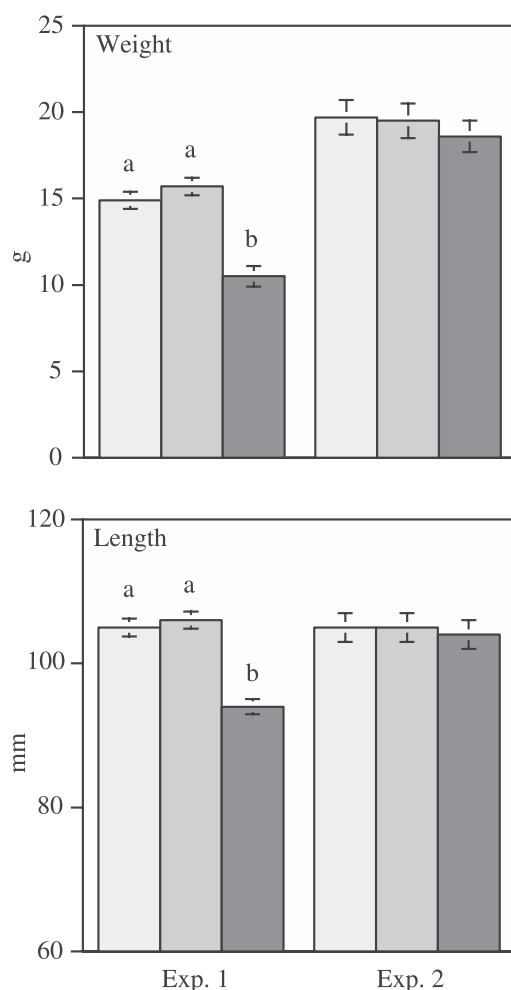


Figure 1. Growth (weight and length) of juvenile Atlantic salmon (*Salmo salar*) after exposure to spectral treatments: UVB-depleted sunlight (open bar), natural sunlight (gray bar) and sunlight supplemented with enhanced UVB radiation (dark bar) in the two experiments (Exp. 1 and Exp. 2). Bars represent mean \pm SE. The total number of fish sampled from each treatment group were $n = 70$ and $n = 84$ in Exp. 1 and Exp. 2, respectively. Identical letters over the bars indicate that the difference between treatments was not statistically significant (ANOVA: treatment, cage nested inside treatment, $P > 0.05$).

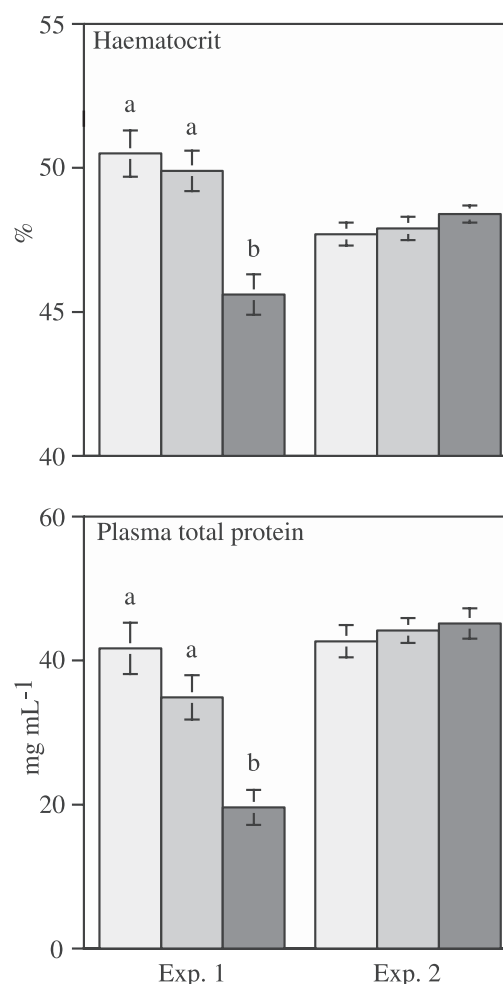


Figure 2. Condition indices of juvenile Atlantic salmon (*Salmo salar*) exposed to different spectral treatments. Bars, treatments and statistical significance are as described in Fig. 1.

Table 2. Effects of spectral exposure (treatment) on growth, condition and immune function as indicated by nested multivariate analysis of variance (MANOVA, design: treatment, cage [in treatment]). The effects of random factor (cage) were not statistically significant, and are not shown.

Response set/variable	Nested MANOVA			Nested ANOVA	
	Wilk's lambda			Univariate	
	Value	Approx. <i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Exp. 1					
Growth	0.0045	13.929	0.013		
Weight				83.060	0.002
Length				66.288	0.003
Condition	0.0140	7.447	0.039		
Hematocrit				39.393	0.006
Protein				78.939	0.002
Immune function	0.0176	6.542	0.048		
IgM				27.581	0.011
Lysozyme				9.000	0.052
Exp. 2					
Growth	0.0482	3.554	0.123		
Condition	0.0484	0.443	0.779		
Immune function	0.3669	0.651	0.656		

Immune function

Immune function was related to spectral treatments in Exp. 1 but not in Exp. 2 (Fig. 3, Table 2). The fish exposed to enhanced UVB radiation had decreased plasma IgM compared either with fish kept under natural sunlight or UVB-depleted solar radiation but there was no statistically significant difference in the activity of plasma lysozyme. No significant differences in IgM levels or lysozyme activity were noted between the fish exposed to natural sunlight and UVB-depleted solar radiation.

DISCUSSION

Because the substances causing the depletion of the ozone layer have a long lifetime in the stratosphere, detectable recovery of the ozone layer is not expected before 2020 and return to pre-1980 levels is predicted between 2040 and 2070 (4,44). In the present study fish were exposed to spectral treatments of varying intensity of UVB to study the impact of an altered spectral environment on the physiology of fish. In exposures to natural sunlight, average daily ambient irradiances in UV wavelengths were higher in Exp. 2 mainly because in 2002 the number of clear-sky days in southern Norway was higher. For enhanced UVB radiation sunlight was supplemented with UVB wavelength delivered by lamps, and this procedure created spectral environments simulating ozone losses of 20% (Exp. 1) and 8% (Exp. 2).

The doses that fish received were dependent on the underwater optics. DOC is the principal factor controlling UV attenuation in natural waters, and the model based solely on DOC concentration gives a good estimate of K_d (45). Exposure to UVB depends also on the vertical distribution of fish in water column. Avoidance of UV radiation under solar intensities has been reported in salmonids (33), and the most adequate estimate for doses received by fish during exposures is the UVB irradiance near the bottom of the cages. In

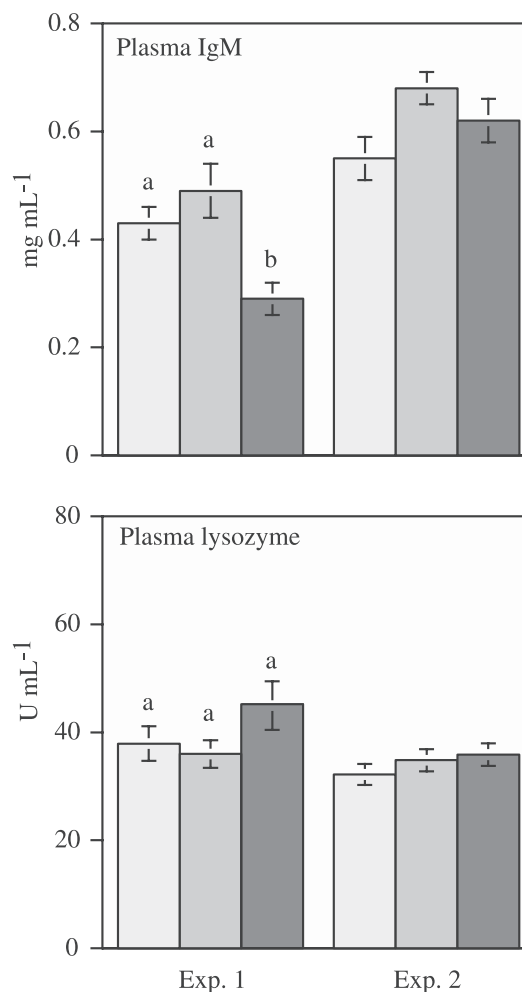


Figure 3. Immune function parameters of juvenile Atlantic salmon (*Salmo salar*) in fish exposed to different spectral treatments. Bars, treatments and statistical significance are as in Fig. 1.

exposure to natural sunlight, the average daily UVB irradiance measured just below the surface was 7% lower in Exp. 1 than in Exp. 2, but near the bottom of cages the value was more than 10-fold in Exp. 1 compared with Exp. 2; even the height of water column was smaller in Exp. 2.

The growth of juvenile Atlantic salmon was reduced in Exp. 1 in fish exposed to enhanced UVB when compared with fish kept under natural or UVB-depleted sunlight. Poor growth resulting from exposure to UVB has been earlier documented in larval anchovy (16) and juvenile rainbow trout (46). The reduced growth observed in the present study most probably reflects the poor nutritional status of fish exposed to increased UVB. UVB radiation has been shown to suppress energy allocation to digestion in European whitefish (*Coregonus lavaretus*) (47) and this could be expected to decrease growth. Reduced growth can also be connected to the costs for repairing the UVB-induced DNA damage as suggested by a study showing that salmonid larvae (brook trout, rainbow trout) rely solely on energetically expensive nucleotide excision repair (48). Taken together, these changes in metabolism could markedly decrease the portion of energy allocated to growth in fish exposed to increased UVB radiation. Chronic elevation of cortisol level is found to change feeding behavior,

carbohydrate metabolism and growth of fish (49,50). Thus the increase in plasma cortisol after exposure to UVB (29,31) might also contribute to the reduced growth observed in Atlantic salmon.

Plasma total protein concentration indicates changes in overall metabolism and functional activity of somatic organs (51). Decreased plasma protein level is often accepted as an indicator of poor nutritional status. Hematocrit, reflecting oxygen carrying capacity and the production of erythrocytes, was used here as another health indicator to assess condition of exposed fish. Plasma protein level and hematocrit were decreased in fish exposed to enhanced UVB radiation in Exp. 1. In an earlier study with carp, and with lamps as the source of UVB radiation, hematocrit values decreased after 6 days, but no decrease in plasma protein was observed (52). The mechanisms for decreased protein and hematocrit values were not studied here but they might be connected, at least in part, to the poor nutritional status of fish.

Immunoglobulin synthesis is a result of a multistep process which includes antigen presentation by antigen-presenting cells, the activation of T- and B-lymphocytes, the operation of cytokines secreted from regulatory T-lymphocytes and finally maturation of B-lymphocytes to plasma cells secreting IgM. The production of IgM thus requires the activity of several cell populations, and serves as an indicator for the overall function of the acquired immune system. IgM levels decreased in Atlantic salmon exposed to enhanced UVB in Exp. 1. Reduced IgM level in carp exposed to UVB for 27 days has been reported (52), but short-term exposures (< 14 days) of roach (32), carp (52) or rainbow trout (53) have not affected IgM concentration, suggesting that the fall in plasma IgM is not an immediate response but requires long-term exposure to UVB to develop.

Along with cellular components, innate immunity is composed of a number of soluble defense factors, such as lysozyme, found in body fluids and blood. Lysozyme is a bacteriolytic enzyme capable of lysing cell walls of gram-positive bacteria. In the present study spectral treatments had no statistically significant effect on lysozyme activity. In line with this, exposure of rainbow trout for 7 days had no effect on lysozyme (29). On the other hand, a single UVB dose can decrease plasma lysozyme (53). It is possible that lysozyme activity, depressed immediately after exposure, can recover (*i.e.* habituation) even if exposure is continued. The effects of short-term exposure on lysozyme activity in Atlantic salmon have not been studied.

Immune modulation after a single dose of UVB, or after a short-term exposure, consists mainly of altered differential blood leukocyte counts and suppressed lymphoid tissue phagocyte functions (30,31,52). Long-term exposure to UVB radiation causes interference with the acquired immune system appearing as reduced plasma IgM level noted in the present study. Exposure to UVB radiation induces stress, as indicated by increased cortisol, leukocytosis, increased proportion of granulocytes and lymphopenia, and the signs of stress have been noted both in short- and long-term exposures (29,31,34,52). Chronic stress is an effective suppressor of immunity, and can lead to decreased immune competence in fish (54). The relevance of altered immune function, in terms of compromised resistance against pathogens, was not tested here. However, a 14-day exposure to UVB decreased resistance

to bacterial and parasitic pathogens in juvenile rainbow trout (29), and demonstrates the potential for UVB to increase susceptibility to diseases also in other fish species.

The dose response of physiologic parameters against the level of UVB was not determined in the present study. Cumulative doses of UVB received during the two experiments were different, as was the fluence rate. In short and intense exposures, DNA damage has been found to dominate and the reciprocity between the dose and the outcome of exposures holds, but in low fluence rates and long exposures it may fail (17,55). Exp. 1 and Exp. 2 were both long-term exposures, but the changes in growth, condition and IgM concentration, however, were significant only in Exp. 1 in which UVB irradiance reaching the fish was higher than in Exp. 2.

The present study was conducted outdoors under near-natural conditions with control over radiation in UVB wavelengths. However, the study did not include factors, such as shelters, underwater foliage and behavioral plasticity of fishes that might modify the UVB dose received by fish. Although estimating the impact of exposure to increased ambient UVB levels on the well-being of fish in nature is complex, the present findings, supported with earlier laboratory studies, suggest that increased ambient UVB has negative impacts on growth and resistance against disease in juvenile fish, especially in clear, transparent waters.

Acknowledgements—We thank 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 for maintaining tanks and taking care of the fish, Johanna Rinne and Outi Väilähö for assistance in sampling and laboratory analysis, and M.Sc. Markku Julkunen for statistical analyses. The project was supported by grants from the Research Council of Norway (140472/130) and the Research Council of Finland (73842).

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