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Eveliina Markkula

Ultraviolet B Radiation Induced Alterations in Immune Function of Fish

In Relation to Habitat Preference and Disease Resistance

JYVÄSKYLÄN YLIOPISTO

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ABSTRACT

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Solar ultraviolet B (UVB) radiation is an environmental stressor known to have harmful impacts on aquatic organisms. While the immunomodulatory effects of UVB on fish have been demonstrated in short-term exposures, the present work focused on the long-term effects of low-dose UVB on fish species adapted to live at high or low levels of natural solar radiation. In general, the immunomodulatory effects of the long-term exposures with low UVB doses were less profound than those induced by short-term exposures. Suppressed functioning of head kidney phagocytes was detected after different exposure regimes ranging from a single UVB dose to an irradiation of 6 weeks, and was the most sensitive of the studied cellular parameters. Natural cytotoxic cells and lymphocytes were more inconsistently affected by radiation treatments, as were the hematological and humoral parameters of the blood. Cortisol-mediated stress reaction was common after short-term exposures. A comparison of the fish species revealed that benthic carp (*Cyprinus carpio*) is more sensitive to UVB than rainbow trout (*Oncorhynchus mykiss*), a representative species of clear water habitats. In outdoor experiments, the exposure simulating 20 % stratospheric ozone loss for 8 weeks negatively affected the growth, condition, and plasma IgM concentration of the juvenile Atlantic salmon (*Salmo salar*) indicating potentially compromised immune defense. The exposure simulating 8 % ozone loss for 20 weeks, however, caused no detectable change in fish when compared to the natural solar radiation. The relationship between the UVB exposure and disease resistance was demonstrated in rainbow trout irradiated over 1-2 weeks. Resistance of exposed fish to parasite *Diplostomum spathaeum* was significantly decreased, and clearance of bacteria *Yersinia ruckeri* from the tissues of infected fish was affected as well. The results suggest that increased UVB radiation levels can potentially harm fish health in long-term exposures, and that there is an association between UVB exposure and disease resistance.

Keywords: Disease resistance; immune function; fish; ultraviolet B radiation.

Eveliina Markkula, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland

Author's address Eveliina Markkula
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland
eveliina.s.markkula@jyu.fi

Supervisors Docent Ilmari Jokinen
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Professor Aimo Oikari
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Reviewers Professor Johan Garssen
Utrecht University
Department of Pharmaceutical Sciences
Sorbonnelaan 16
3508 TB Utrecht
The Netherlands

Docent Jarmo Laihia
Department of Biological and Environmental Sciences
P.O. Box 56
FI-00014 University of Helsinki
Finland

Opponent Professor Dieter Steinhagen
School of Veterinary Medicine
Centre for Infection Medicine
Buenteweg 17
D-30559 Hannover
Germany

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which will be referred to in the text by their Roman numerals I-V.

Responsibilities of Eveliina Markkula in the articles of this thesis: In I-III, the experiments were planned with Dr Ilmari Jokinen and Dr Harri Salo. Sampling and laboratory analysis were performed together with the co-authors. I was responsible for analyzing the data and writing the articles. In IV, the study was planned by Dr Howard Browman, Dr Ilmari Jokinen, and Dr Michael Arts. The exposures of fish and measurement of ambient radiation were performed by Dr Howard Browman. I corresponded to the sampling, laboratory analysis, and data analysis with Ilmari Jokinen and Harri Salo. Ilmari Jokinen wrote the article. In V, the study was planned, and experiments performed together with the co- authors. Sampling and laboratory analysis were also performed together. I was responsible for analyzing the data and writing the article.

I Markkula, S. E., Salo, H. M., Immonen, A. K. & Jokinen, E. I. 2005. Effects of short- and long-term ultraviolet B irradiation on the immune system of the common carp (*Cyprinus carpio*). Photochemistry & Photobiology 81: 595-602.

II Markkula, S. E., Salo, H. M., Rikalainen, A.-K. & Jokinen, E. I. 2006. Different sensitivity of carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) to the immunomodulatory effects of UVB irradiation. Fish & Shellfish Immunology 21: 70-79.

III Markkula, E., Salo, H. M., Rikalainen, K. & Jokinen, I. 2009. Long-term UVB irradiation affects the immune functions of carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*). Photochemistry & Photobiology 85: 347-352.

IV Jokinen, I. E., Markkula, E. S., Salo, H. M., Kuhn, P., Nikoskelainen, S., Arts, M. T. & Browman, H. I. 2008. Exposure to increased ambient ultraviolet B radiation has negative effects on growth, condition and immune function of juvenile Atlantic salmon (*Salmo salar*). Photochemistry & Photobiology 84: 1265-1271.

V Markkula, S. E., Karvonen, A., Salo, H. M., Valtonen, E. T. & Jokinen, E. I. 2007. Ultraviolet B irradiation affects resistance of rainbow trout (*Oncorhynchus mykiss*) against bacterium *Yersinia ruckeri* and trematode *Diplostomum spathaceum.* Photochemistry & Photobiology 83: 1263-1269.

ABBREVIATIONS

BSA	bovine serum albumin
CFC	chlorofluorocarbon
CFU	colony forming unit
CHS	contact hypersensitivity
CL	chemiluminescense
CPD	cyclobutane pyrimidine dimer
CPS	counts per second
CPM	counts per minute
CYP1A	cytochrome P450 1A
DOC	dissolved organic carbon
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
EMR	salmonid enteric redmouth disease
GADD45	growth arrest and DNA damage gene 45
HSP70	heat-shock protein 70
IgM	immunoglobulin M
ISC	immunoglobulin-secreting cells
K	condition factor
K_d	diffuse attenuation coefficient
LD_{50}	lethal dose 50 %
LPS	lipopolysaccharide
Mb	megabase
NCC	natural cytotoxic cells
NER	nucleotide excision repair
NK	natural killer
PBS	phosphate buffered saline
PAH	polycyclic aromatic hydrocarbon
PER	photoenzymatic repair
PHA	phytohemagglutinin
PMA	phorbol 12-myristate 13-acetate
RB	respiratory burst
SI	stimulation index
SOD	superoxide dismutase
SSR	solar-simulated radiation
UVA	radiation in wavelength band 315-400 nm
UVAI	radiation in wavelength band 340-400 nm
UVAII	radiation in wavelength band 315-340 nm
UVB	radiation in wavelength band 280-315 nm
UVC	radiation in wavelength band 100-280 nm
UVR	ultraviolet radiation

1 INTRODUCTION

1.1 Ultraviolet B radiation

1.1.1 Ambient ultraviolet radiation

Solar UV radiation consists of ultraviolet C (UVC, 100-280 nm), ultraviolet B (UVB, 280-315 nm), and ultraviolet A (UVA, 315-400 nm) radiation. As the most energetic UVC wavelengths are absorbed by stratospheric ozone in the upper atmosphere, UVB is the highest energy-level radiation able to reach the surface of the earth. UVA radiation travels through the atmosphere without significant attenuation, and it is the least harmful of the UV wavelengths to living organisms.

A significant amount of the solar UVB radiation is absorbed by the stratospheric ozone layer. Changes in the ozone layer have, therefore, a major impact on the amount of tropospheric UVB. The ozone hole over the Antarctica was detected in 1985, and the total column of ozone declined also at mid-latitudes in 1980s and 1990s, leading to increased UVB levels on Earth. In 1987 the use of major ozone depleting substances, chlorofluorocarbons (CFCs), was limited by an international treaty, the Montreal Protocol, to prevent further ozone loss.

Measured annual cumulative terrestrial UV doses (erythemally weighted and altitude corrected) during 1974-1998 varied from 304 (Poland) to 968 (Southern California) kJ m^{-2} (Godar 2005). The highest readings, 1675 kJ m^{-2} per year, have been obtained in Darwin, Australia. Both the latitude and altitude affect the UV irradiance, but at any given site the solar zenith angle has a profound effect on the terrestrial readings. Cloud cover usually reduces the UVB radiation by 15-30 %, and air pollution may cause a 20-50 % decrease in urban sites. Surface reflections vary, for example, while water reflects about 5 % of the UV radiation, 60-80 % is reflected from snow. Differences in stratospheric ozone changed the terrestrial UVB radiation in New Zealand by 10 % at most (Godar 2005).

At present, concentrations of the ozone-depleting substances in the atmosphere are decreasing and, according to recent models, the stratospheric ozone is recovering (Reinsel et al. 2005, McKenzie et al. 2007). The ozone is expected to reach the pre-1980 levels by the mid 21st century, but in polar regions the recovery will probably take longer. There are interactions between the ozone depletion and other aspects of climate change, but it is not clear whether the overall effect delays or accelerates ozone recovery. Because of the high natural variability in the levels of stratospheric ozone, and the unknown rates of future increases in greenhouse gases, the actual development remains uncertain (McKenzie et al. 2007).

1.1.2 UVB in aquatic environment

The optical qualities of the water have a major impact on the attenuation of radiation. In open oceanic waters 10 % irradiance depths for UVB may be over 15 m, and in coastal waters up to 5 m (Tedetti & Sempere 2006). While in the clearest alpine lakes 1 % attenuation depths up to 13 m have been detected (Laurion et al. 2000), UVB penetrates down to several meters also in the most transparent lakes in Northern America (Williamson et al. 1996). The penetration depth of UVB can be just a few centimeters in the most colored humic lakes. In Finnish lakes with different optical qualities 10 % of the UVB measured just below the surface penetrated to a depth of 5-19 cm (Huovinen et al. 2000). Another study demonstrated 1 % of the UVB radiation to penetrate to 57 cm water column in the clearest of the lakes studied, and to 12 cm in a small humic lake (Huovinen et al. 2003). The UVB environment of small forested streams also varies considerably. The highest and lowest measured UVB fluxes at 5 cm depth corresponded to the transmission of about 10–20 % and 0.5 %, respectively, of above-canopy incident UVB radiation. This variation in transmission was linked to the tree canopy cover and dissolved organic carbon (DOC) concentration of the water (Frost et al. 2006). The DOC concentration was also seen to have a high impact on the attenuation of UV radiation in temperate lakes, and the effects of particulate organic carbon or chlorophyll a were less prominent (Scully & Lean 1994, Williamson et al. 1996).

UVB radiation at current levels is harmful to aquatic organisms and can reduce the productivity of marine ecosystems, which is considered a significant alteration (Bancroft et al. 2007, Häder et al. 2007). It has also been suggested that increased UVB changes the food web structure and function by the differential UV sensitivities of the phytoplankton species, the major marine biomass producers. At the cellular level, exposure to UVR impairs the survival of the bacterioplankton and DNA damage has been detected at depths down to 5 m in tropical coastal waters. UVB affects the motility, protein biosynthesis, nitrogen fixation, and survival of cyanobacteria, as well as the photosynthesis of flowering aquatic plants. UVB constitutes a significant stressor for macroalgae even without the ozone depletion, affecting photosynthesis, morphology, and growth rates (Häder et al. 2007).

The consumers of the aquatic ecosystems are also influenced by UVB and negative effects on the embryonic development of amphibians, fish, and

molluscs have been detected. Zooplankton may be affected at the cellular level like the primary producers, as well as by possible alterations in the quality of the food. Corals may be affected by UVB directly or through symbiotic algae, and *in situ* UVR induces apoptosis and developmental delay in sea urchin embryos. Furthermore, sea anemones have been noted to express different UV absorbance and acclimatization capacities (Häder et al. 2007).

1.2 Effects of excess UVB radiation on fish

1.2.1 Alterations in skin and eyes

The harmful effects of the excess solar radiation on fish were first recognized in fish farmed outdoors, and sunburn was reported in several species (Dunbar 1959, Bullock et al. 1983, Lowe & Goodman-Lowe 1996). According to a retrospective study, Atlantic salmon suffering from summer lesion syndrome typically had lesions in the skin behind the pectoral fins, rapidly deepening to involve the muscle layers. The changes in the skin included the appearance of the sunburn cells, irregularity of the skin surface, epidermal oedema and necrosis, and even separation of the basal layer of the epidermis from the basement membrane. The syndrome was associated with significant losses of fish. The summer lesion syndrome did not occur in farms that shaded the fish cages from direct sunlight (Rodger 1991). Later, skin alterations were also detected in the natural fish populations inhabiting environments with high radiation levels, such as residual waters on the tidal flats (Berghahn et al. 1993) and an oligotrophic high mountain lake, that did not significantly absorb UVR (Kaweewat & Hofer 1997). It has also been discussed whether the decline in salmonid populations since the mid-1980s is related to solar radiation (Walters & Ward 1998).

The skin of fish is particularly vulnerable to the intense radiation because it generally lacks a keratinized outer layer and has dividing cells in all layers of the epidermis (Bullock 1982b). Mucus is an important factor maintaining the protective function of the skin, and artificial UVB has been seen to decrease the numbers of mucus-secreting goblet cells in exposed fish (Kaweewat & Hofer 1997, Noceda et al. 1997). The size of mucus-secreting cells was significantly reduced following the 6 d exposure of flatfish larvae with UVB doses corresponding to those predicted for the sea surface around the UK with an estimated 15 % ozone depletion (McFadzen et al. 2000). Other histological studies have revealed alterations in the epidermal structure, sloughing off the cells, and secondary infections in the dorsal skin of exposed fish (Bullock 1982a, Fabacher & Little 1996, Blazer et al. 1997, Ewing et al. 1999).

UVB irradiation affects the mechanism of wound repair in the skin of Atlantic salmon, and the wound contracture and resolution of the epidermal structure fail to occur after normal, unaffected epidermal migration (Bullock & Roberts 1992). Sunlight and UVB wavelengths also induce tumors in hybrid *Xiphophorus*, the melanoma-sensitive platyfish and swordtail, which are

commonly used as animal models for tumorigenesis in humans (Setlow et al. 1993, Nairn et al. 1996).

The outer surfaces of the eye are also damaged by UVB, which was demonstrated in an ultrastructural study of irradiated ayu, *Plecoglossus altivelis* (Sharma et al. 2005). With higher doses, UVB can produce corneal damage and persistent cataractous changes in the lens (Doughty et al. 1997). Solar radiation-induced lesions have also been detected in the eye and brain of larval Northern anchovy (*Engraulis mordax*) (Hunter et al. 1982).

1.2.2 Mortality in early life stages

Fish eggs and larvae may be comprised of only a few cell layers, a structure suggested to make them vulnerable to radiation. The harmful impact of artificial and solar UVB radiation on the early life stages of fish has been demonstrated with several marine (Hunter et al. 1981, Beland et al. 1999, Kouwenberg et al. 1999) and freshwater species (Williamson et al. 1997, Gutiérrez-Rodríguez & Williamson 1999, Battini et al. 2000, Häkkinen et al. 2004, Ylönen & Karjalainen 2004, Vehniäinen et al. 2007a). Furthermore, the UVB-induced mortality has been shown to be related to the increasing dose (Hunter et al. 1981, Kouwenberg et al. 1999, Browman et al. 2000) or fluence rate (Vehniäinen et al. 2007b).

In freshwater lakes, solar radiation has been demonstrated to significantly decrease the survival of bluegill sunfish (*Lepomis macrochirus*) embryos with increasing irradiation dose (Gutiérrez-Rodríguez & Williamson 1999). The eggs of yellow perch (*Perca flavescens*) also perish, when incubated in quartz tubes in a low-DOC lake (Williamson et al. 1997). Almost complete mortality of landlocked *Galaxias maculatus* eggs exposed to *in situ* UVR in northwestern Patagonia, Argentina, was recorded near the surface, and a mortality of 22 % at a depth of 43 cm in a lake with a diffuse attenuation coefficient of 3.08 m-1 at 320 nm (K_d320). In a more transparent lake (K_d320 = 0.438 m-1) high mortality still occurred at 2.5 m (Battini et al. 2000).

The impact of UV on the egg populations of Atlantic cod (*Gadus morhua*), and a copepod *Calanus finmarchicus* in the Gulf of St. Lawrence, Canada, has been estimated with a mathematical model including the biological weighing functions (BWFs), vertical mixing of eggs, meteorological and hydrographic conditions, and 50 % stratospheric ozone depletion. According to this model, the average daily survival of cod eggs was about 99 % over several alternative conditions, but that of *C. finmarchicus* eggs could decrease even 32.5 % by UV (Browman et al. 2000, Kuhn et al. 2000).

1.2.3 DNA damage

Studies with several fish species have led to the suggestion that the UVB-induced mortality of the early life stages results from the DNA damage (Applegate & Ley 1988, Kouwenberg et al. 1999, Browman et al. 2003). Two common types of DNA damage are the cyclobutane pyrimidine dimers (CPDs), and the pyrimidine (6-4) pyrimidone photoproducts. The increased load of 10

CPD/Mb of DNA has been estimated to result in 10 % mortality in the eggs of cod and *Calanus finmarchicus* (Browman et al. 2003).

Significant DNA damage, an average of 31-35 CPDs/Mb, has been detected in the eggs and larvae of icefish (*Chaenocephalus aceratus*) exposed to the solar radiation under the ozone hole in Antarctica. However, the fish larvae (*Notothenia larseni*) of similar in size and coloring, but collected during the lower UVB flux had no detectable DNA damage (Malloy et al. 1997). The CPD concentration at the time of sampling appeared to be a good estimate of the dose rate, but did not correlate to the cumulative dose in Northern anchovy, *Engraulis mordax*. It was suggested that the accumulation of CPDs during the time was prevented by the active DNA repair (Vetter et al. 1999).

1.2.4 Physiological stress and metabolic changes

Alterations related to the physiological stress response, increased plasma cortisol concentration followed by blood lymphopenia, and granulocytosis, were detected in roach (*Rutilus rutilus*) exposed to a moderate level UVB dose inducing no visible signs of edema, sunburn, or signs of infections in the skin of fish (Salo et al. 1998, 2000a, 2000b, Jokinen et al. 2001). UVB has also been shown to have stress effects on rainbow trout, manifested as increased oxygen consumption (Alemanni et al. 2003), and the increased ventilation rate and impaired respiratory control have been demonstrated with UVB-exposed plaice (Freitag et al. 1998, Steeger et al. 2001).

The effect of UVB radiation on proteins related to the oxidative stress, or stress in general, heat-shock protein 70 (HSP70), cytochrome P450 1A (CYP1A), and superoxide dismutase (SOD) have been studied in the larvae of boreal fish. Inductions of HSP70 and CYP1A were seen in whitefish (*Coregonus lavaretus*) exposed to 2.8 kJ m^{-2} UVB daily for 2 d, but not in vendace (*Coregonus albula*). The UVB doses were measured at the water surface and corresponded to slightly subambient radiation (Vehniäinen et al. 2003). These species are considered rather tolerant to UVB radiation (Häkkinen et al. 2002). Northern pike (*Esox lucius*), on the other hand, is much more sensitive, and significantly decreased concentration of the HSP70 was observed in the UVB-exposed larvae. It was suggested that cellular damage, and inactivation or destruction of the HSP70 by UVB were possible explanations for this contradictory finding. CYP1A and SOD were not affected, although the treatment induced a severe neurobehavioral disorder and increased the mortality in the larvae (Häkkinen et al. 2004). The expression of tumor suppressor p53, a transcription factor with a central role in cellular stress responses, was up-regulated in UVB-irradiated hepatocytes isolated from zebrafish (Sandrini et al. 2009).

Reduced growth, an ecologically severe outcome of exposure to UVB, has been recorded in larval Northern anchovy (Hunter et al. 1981), but newly hatched whitefish (*C. lavaretus*) larvae tolerated higher UV doses than usually encountered in Finland in May without decreased growth or survival (Ylönen & Karjalainen 2004). In another study, however, UVB was shown to affect the energy allocation in whitefish larvae (Ylönen et al. 2004b). When compared to the control fish, the digestion costs of the larvae under UV radiation decreased

more than those of activity and maintenance. These changes are likely to lead to the reduced growth of fish (Karjalainen et al. 2003, Ylönen et al. 2004b).

1.2.5 Immunomodulation and disease resistance

The effects of UVB on the fish immune system have been studied using roach (*Rutilus rutilus*) as a model fish. This species often inhabits humic or turbid lakes with low environmental radiation levels. Significant UVB-induced changes in the innate and acquired immune system were noted, demonstrating the harmful sublethal effects of UV radiation on adult fish. Chemotactic migration and the respiratory burst activity of phagocytes, and the activity of natural cytotoxic cells of the head kidney were suppressed in roach exposed to a single UVB dose of 430 mJ cm^{-2} in 72 min (Salo et al. 1998, 2000b). The dose corresponds to 120 mJ cm^{-2} erythemally weighted UVB, which can be received outdoors in 4 h in Finnish latitudes (Salo et al. 2000a, 2000b). These parameters showed enhanced activity in the blood, leading to the increased defense potential after UVB treatment (Salo et al. 2000a, 2000b). Proliferation of lymphocytes was also suppressed after a single UVB irradiation, but the antibody production following immunization and the plasma total IgM concentration were not affected (Salo et al. 2000b, Jokinen et al. 2001). The effects of exposure extended over a relatively long period and in some cases the functioning of leucocytes remained altered for 2 weeks (Salo et al. 1998, 2000a, 2000b, Jokinen et al. 2001).

UVB exposure makes fish more susceptible to pathogens, and radiation-induced lesions in the skin are often accompanied by secondary fungal and myxobacterial infections (McArdle & Bullock 1987, Fabacher et al. 1994, Little & Fabacher 1994, Blazer et al. 1997). UVB and skin infections probably have an overall additive effect on fish, since plaice (*Pleuronectes platessa*) infested with the ectoparasite *Ichtyobodo necator* were noted to be more susceptible to UVB than those with healthy skin (Bullock 1985). The effects of radiation on the infections occurring outside the actual site of exposure are, however, so far unknown.

1.3 Photoprotective mechanisms in fish

1.3.1 Avoidance of UV radiation

Fish may actively regulate the amount of UV exposure by changing the location. Juvenile coho salmon (*Oncorhynchus kisutch*) have been demonstrated to strongly prefer shade to the unattenuated full-spectrum sunlight, and also to selectively avoid UVR (Kelly & Bothwell 2002). Even larval coregonids, normally showing a distinct positive phototaxis after hatching, avoided UV exposure both in the laboratory and field experiments (Ylönen et al. 2004a, 2005). It has been suggested that the UV photosensitivity in several species of

juvenile or small fish provides protection against the excessive exposure by the awareness of elevated irradiance levels (Losey et al. 1999).

Yellow perch (*Perca flavescens*) have been detected to spawn deeper in high- than low-UVR lakes, suggesting avoidance of the high UVR environment (Williamson et al. 1997). Similarly, bluegill sunfish (*Lepomis macrochirus*) constructed their nests deeper in the lake with a high underwater UVR environment than they did with less UVR, also suggesting behavioral response to radiation (Gutiérrez-Rodríguez & Williamson 1999). However, the bluegill in the high UVR environments did not always build their nests at depths protecting from the harmful levels of radiation. Temperature is an important factor in bluegill sunfish nesting, and it was suggested that a trade-off between the optimal temperature and the UVR levels took place in nesting (Gutiérrez-Rodríguez & Williamson 1999). Different environmental factors affect the exposure of fish to UVB, and opportunities to avoid high radiation levels may be limited in some cases.

1.3.2 UV-absorbing compounds

At the cellular level, the radiation-caused injuries may be prevented by UV-absorbing compounds. A sunlight-induced increase in the integumental melanin, leading to the darkening of the skin, resembling tanning in humans, has been demonstrated in hammerhead shark (Lowe & Goodman-Lowe 1996). An increase in the melanin concentration of vendace *(Coregonus albula*) and whitefish (*Coregonus lavaretus*) larvae was detected after exposure to the artificial UVB levels (Häkkinen et al. 2002), but the ambient UVB levels were insufficient to modify the degree of melanin pigmentation of coregonid fish larvae in a field study (Häkkinen et al. 2003a). The melanin concentration of larvae collected from five Finnish lakes with different optical properties correlated positively to the color of the water, but negatively to the UVB attenuation depth (Häkkinen et al. 2003a), suggesting that the main purpose of pigmentation is adaptation to the background color. UVB did not affect the melanin concentration in pike larvae (Vehniäinen et al. 2007a), also the UV tolerances of pigmented (*Oryzias latipes*) and albino medaka were similar, suggesting that photoprotection is not connected to the amount of melanin (Fabacher et al. 1999). Overall, the role of melanin in the UV protection of fish is controversial, and in some studies melanin has been related more to harmful UV effects than the protection of fish (Armstrong et al. 2002).

Photoprotective pigments, identified as mycosporin-like amino acids, have been detected within fish eye lenses (Truscott et al. 1992, Thorpe et al. 1993), and eggs (Grant et al. 1980). The tryptophan derivative 3-hydroxykynurenine and other UV-absorbing compounds have also been demonstrated in the lens and cornea of cuttlefish (Shashar et al. 1998). A colorless substance absorbing the UV wavelengths was isolated from the skin of rainbow trout (*Oncorhynchus mykiss*), Apache trout (*O. Apache*), Lahontan cutthroat trout (*O. clarki henshawi*), and razorback suckers (*Xyrauchen texanus*) (Fabacher & Little 1995, 1996, Blazer et al. 1997), but not in fingerling channel catfish *Ictalurus punctatus* (Ewing et al. 1999), and was later identified as a

gadusol-like compound (Fabacher & Little 1998). The amount of this substance correlated to the UV sensitivity of the fish, and it was suggested that it acts as a photoprotective agent. Recently, mucus from 137 species of the coral reef fish were screened, and 90 % were found to have strong absorbance peaks in the UV wavelengths. A tropical wrasse, *Thalassoma duperrey*, was demonstrated to be able to adapt to the UV environment by altering the absorbance of its epithelial mucus (Zamzow & Losey 2002).

1.3.3 DNA repair

The UVR-induced DNA damage is repaired mainly by two mechanisms. Photoreactivation is a single enzyme system that needs UVA and blue light (450-495 nm) energy in repair (photoenzymatic repair; PER), whereas the light-independent nucleotide excision repair (NER) requires a series of DNA replication enzymes. PER is faster, and only 10 % of UVR-induced CPDs remained after 1 h incubation in fathead minnow (*Pimephales promelas*) embryos. A similar clearance of CPDs was obtained with NER after 24 h (Applegate & Ley 1988). Recently, larvae of the five species of temperate freshwater fish (bluegill; *Lepomis macrochirus*, brook trout; *Salvelinus fontinalis*, Northern pike; *Esox lucius*, rainbow trout; *Oncorhynchus nerka*, yellow perch; *Perca flavescens*) were studied for the active PER and NER. All species had NER at some level, but PER was demonstrated only in bluegill and yellow perch. Although PER was recognized as a faster process, it was suggested that NER repairs more damage (Olson & Mitchell 2006). In Antarctic ichtyoplankton, the most effective PER was detected in organisms exposed to the high radiation levels during early development (Malloy et al. 1997). Four differently pigmented strains of larval Japanese medaka (*Oryzias latipes*) showed different levels of photorepair capability, the fish lacking white melanophore having more effective repair than the wild-type medaka (Armstrong et al. 2002).

1.4 UVB susceptibility of fish

Fish species have adapted to certain levels of UVR and exhibit different ranges of tolerance to radiation. UVB induces less sunburn in the skin of razorback suckers than in medaka, rainbow trout, or cutthroat trout (Little & Fabacher 2003). Heavily pigmented turbot (*Scophthalmus maximus*) larvae are more tolerant to UVB than sole (*Solea solea*) with less pigmentation (McFadzen et al. 2000). Different sensitivities to UVB radiation were also demonstrated within the same taxonomic family, in rainbow trout, apache trout, and Lahontan cutthroat trout, when skin pigmentation, sunburn, and fungal infections were monitored (Little & Fabacher 1994). It has been suggested that fish have individual differences in UV susceptibility as well, since the amount of radiation-induced harmful effects varies between individuals exposed to the same UVB doses (Fabacher & Little 1996).

Age affects the UV susceptibility, and some life stages are more vulnerable to radiation than others. The 1-2 d old yolk-sac Northern anchovy larvae were seen to be more sensitive to UV than the eggs, or the 4-5 d old larvae (Hunter et al. 1982). The 6 d old pike larvae were also more tolerant than the newly hatched ones in terms of UVB-induced neurobehavioral disorders, and mortality (Vehniäinen et al. 2007a). Generally, adult fish are believed to tolerate radiation better than the early life stages.

The effect of different ambient radiation environments on UV sensitivity is seen among juvenile Antarctic fish, whose DNA repair rates appear to correlate with the life histories of their embryonic stages. Species whose eggs and larvae remain in the water during spring and summer have higher DNA repair capacities than species whose early development takes place during seasons of low solar illumination (Malloy et al. 1997). The same phenomenon is also noted within species. Recently spawned eggs of yellow perch collected from the high-UVR lake tolerated the UVR treatment better than the eggs collected from the low-UVR lake (Williamson et al. 1997). The pike larvae from the more colored Lake Lentua were also more tolerant to UVB than those from Lake Päijänne. However, this difference in tolerance was not directly related to UVR, but it was suggested that it was the side effect of pigmentation, probably formed to protect the fish from predation (Vehniäinen et al. 2007a).

2 AIMS OF STUDY

Solar ultraviolet B (UVB) radiation is an environmental stressor affecting both wild and farmed fish. So far it has become well established that UVB exposure induces pronounced immunomodulation in cyprinids. The effects of a single, moderate-level UVB dose on roach (*Rutilus rutilus*) have been thoroughly examined, and markedly changed immune functions were demonstrated (Salo et al. 1998, 2000a, 2000b, Jokinen et al. 2001).

The goal of this thesis is to establish whether long-term exposure to low-level UVB doses has an impact on the immune status of fish. It was also studied whether UVB-induced changes are associated with habitat preferences of fish in relation to light, and if the modulation of immune function eventually leads to an altered defense against pathogens.

The specific aims of this study were to determine:

1) the characteristic effects of short- and long-term UVB exposure on fish immune function (I-III)

2) the UVB sensitivity of the immune system in fish adapted to live at high or low levels of natural solar UVB (II, III)

3) the immunomodulatory potential of long-term, low-level UVB exposure under laboratory (III) and outdoor conditions (IV)

4) the ability of fish to resist pathogens after exposure to UVB radiation (V)

3 SUMMARY OF MATERIALS AND METHODS

3.1 Fish used in experiments

Juvenile carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) for laboratory experiments at the University of Jyväskylä were obtained from commercial fish farms in Central Finland. The fish were maintained in 300 l flow-through tanks filled with dechlorinated, aerated tap (I) or ground water (II, III, V) at 13-20 ± 1 °C over the acclimation period. Fish were fed commercial dry food daily, and were allowed to adapt to laboratory conditions for at least 1 week before the experiment. Exposure to UVB was conducted in shaded 60 l flow-through tanks, where the water depth was 40 cm.

Atlantic salmon (*Salmo salar*) juveniles for the outdoor experiments at the Institute of Marine Research (IMR, Norway), Research Stations of Matre and Austevoll, were obtained from IMR Matre stock. Salmon were kept in open 50 x 60 cm cages, with the 30-46 cm watercolumn (between the water surface and the bottom of the cage), in 500 l flow-trough tanks. The water was sand-filtered river water at 14 ± 2 °C, and the fish were fed commercial salmon feed.

3.2 UVB irradiation and measuring dose rates

In the laboratory (I-III, V), fish were exposed from above to UVB by two unfiltered Philips TL40/12RS lamps. To obtain homogenous exposure, the vertical movement of the fish was restricted to a lower part of the aquaria with a UV-penetrating plastic sheet during the exposure. The underwater spectra were measured with Hamamatsu PMA-11 model C5965 spectrograph with an integrating sphere Oriol-70451 as the diffuser. The UVR penetrating into the water was measured with a UVX Digital Radiometer equipped with a waterproof sensor, UVX-31 (peak sensitivity at 310 nm). Both, the spectrograph and radiometer were calibrated with an Optronic-750 spectroradiometer by Radiation and Nuclear Safety Authority, Helsinki.

In the air, TL40/12RS lamps (emission maximum at 315 nm) emitted UVC less than 2 %, 53 % UVB and 45 % UVA. No UVC radiation was detected in underwater measurements in the tank. The irradiation consisted of 37 % UVB and 63 % UVA in tap water, and 46 % UVB and 54 % UVA in the ground water. For spectral characteristics of the irradiation, see Fig. 1 in I and II. When erythemally weighted, the underwater dose was composed of more than 98 % UVB and less than 2 % UVA. UVB was given at a mean irradiance of 74 μW cm^{-2}. In control exposures, UV wavelengths were filtered out with a screen made of glass and polyacrylic sheets, and unexposed fish were kept in the shaded aquaria. To minimize the effects of factors other than spectral treatment, both exposed and unexposed fish were subjected to similar handling during the experiment.

In the outdoor experiments (IV), fish were exposed to an additional UVB from TL40/12 RS lamps daily, for 1 h at noon. All UVC wavelengths were removed by filtering the radiation from lamps with cellulose triacetate film. UVB-depleted solar radiation was produced by screening the experimental tanks with Mylar-D foil. Ambient radiation data was obtained with GUV-541 multi-channel radiometer situated in Bergen (66 km Southwest from Matre and 22 km North from Austevoll, and local radiation levels were confirmed with UVMFR-7 a multi-channel rotating shadow band radiometer at the site of the experiments in Austevoll. The range of exposure experienced by the fish was calculated using the diffuse attenuation coefficient (K_d) for the water. K_d was measured using an OL-745 spectroradiometer equipped with an underwater detector OL-86T-WP.

The UVB doses and irradiation regimes in the experiments are summarized in Table 1.

TABLE 1 Summary of the average UVB doses (mJ cm^{-2}, unweighted) and irradiation regimes (duration of exposure, number of irradiations, and accumulated UVB dose) in experiments with different fish species.

Species	UVB dose	Exp.	No.	Accumulated dose	Ref.
Carp	50, 250, 500	1 dose	1	50, 250, 500	II
(*Cyprinus carpio*)	60, 120, 240	1 wk	3	180, 360, 720	I
	60, 120, 240	4 wk	12	720, 1440, 2880	I
	7, 20, 60	6 wk	17	120, 340, 1020	III
Rainbow trout	50, 250, 500, 1000	1 dose	1	50, 250, 500, 1000	II
(*Oncorhynchus mykiss*)	150	1 wk	6	900	V
	150	1 wk	4	600	V
	150	2 wk	7	1050	V
	7, 20, 60	6 wk	17	120, 340, 1020	III
Atlantic salmon	350	8 wk	52	18 200	IV
(*Salmo salar*)	14	20 wk	137	1 918	IV

3.3 Sampling

Before sampling, the fish were anesthetized with 0.01 % tricaine methanesulfonate, MS-222. The length (mm), and weight (g) of the fish were measured, and the condition factor (K) was calculated using the equation

$$K = 100\ w\ l^{-3}$$

where w=weight (g) and l=length (cm) (Fulton 1902). A blood sample was collected from the caudal vessels of each fish using a heparinized 1 ml syringe with a 25 G needle (I), or blood was allowed to flow directly into a heparinized capillary tube after cutting the tail (III). Fresh blood was used for the hematological and immunological assays, plasma samples were stored frozen (-70 ˚C) until used. The fish were decapitated, and cell isolation of the head kidney (apart from the trunk kidney) and blood were carried out using Percoll density gradients after rupturing the head kidney against nylon net (I). The cell culture media in all the experiments were modified for carp and rainbow trout (II). The cells were counted by trypan blue exclusion (viability > 95 %) and adjusted to the desired concentration.

3.4 Hematology and blood chemistry

3.4.1 Hematocrit and differential blood cell counts

Hematocrits were measured in heparinized 75 mm hematocrit tubes. Blood leucocytes and erythrocytes were counted under a microscope after staining according to Shaw's method (Shaw 1930). Thin blood smears were prepared on objective glass from fresh heparinized blood. The smears were air dried and stained by a Diff-Quick hematological staining. At least 200 leucocytes were counted and classified as lymphocytes, thrombocytes, granulocytes, monocytes, or unidentified cells based on morphology and staining properties under a light microscope using a 63x objective with oil immersion (I). A percentage of the leukocyte types were calculated.

3.4.2 Plasma total protein, cortisol, lysozyme, and IgM

The plasma protein concentration was determined by the Bradford method, using a BioRad Protein Assay Kit with bovine serum albumin (BSA) as a standard. The cortisol concentration was measured with a GammaCoat™ Cortisol radioimmunoassay kit (I). Plasma lysozyme activity was determined with a turbidometric assay, by adding plasma and *Micrococcus lysodeicticus* suspension in phosphate buffer (pH 6.2) to microtiter plate. After a brief mixing the decrease in the optical density of bacterial suspension was monitored with a microplate reader at 450 nm for 30 min (II).

The plasma total IgM concentration was measured with a modification of ELISA (enzyme-linked immunosorbent assay) method by Aaltonen et al. (1994).

The trapping and detection agent was the monoclonal mouse anti-roach IgM antibody (R1a4, made in our laboratory) for carp (I), polyclonal goat anti-trout Ig antibody (Kirkegaard & Perry Laboratories Inc.) for rainbow trout (II), and goat anti-salmon IgM antibody CLF002 (Cedarlane Inc.) for Atlantic salmon (IV). The flat-bottomed 96-well microtiter plates were coated with trapping antibody. After masking the plate with BSA, diluted plasma samples were incubated in the wells. The trapped IgM was detected with a biotin-conjugated detecting antibody. Next, alkaline phosphatase-conjugated avidin was added. The wells were washed between each step with phosphate buffered saline-Tween 20, pH 7.4 (PBS-Tween). P-nitrophenylphosphate was used as a coloring substrate and the optical density read with a plate reader at 405 nm. The assay was standardized with a pooled carp or rainbow trout serum collected from 5-30 fish (I, II). The concentration of IgM in the pooled serum was given 1000 artificial units per ml (U ml^{-1}). Atlantic salmon IgM -ELISA was standardized with known concentrations of a chromatographically purified salmon IgM (V).

3.5 *In vitro* immune function assays

3.5.1 Respiratory burst by phagocytes

Phorbol 12-myristate 13-acetate (PMA) -stimulated respiratory burst (RB) by the whole blood, and isolated blood or head kidney leucocytes was determined by the luminol-enhanced chemiluminescence (CL) method (I, II). The CL was monitored with a microplate luminometer at 25 °C, and the peak value of CL in counts per second (cps) and the peak time (min) were determined. Blood CL in Paper I was measured using a luminometer for sample vials, and the peak value in millivolts (mV) was recorded. One to two peaks were determined for each CL response curve, and the higher peak value was considered the actual peak of the respiratory burst (I).

3.5.2 Spontaneous cytotoxicity

The natural cytotoxic cell (NCC) activity of the whole blood and isolated blood or head kidney leucocytes against K562 target cells was determined with a ^{51}chromium release assay. Sodium ^{51}chromate-labeled K562 cells and fish effector cells were cultured in microtiter plates. Optimized culture conditions were used for carp (I) and rainbow trout (II) cells. Supernatant from each well was harvested to measure the radioactivity, and the percent cytotoxicity of effector cells was calculated. Spontaneous-release values were obtained from targets incubated without effectors, and the maximal release values from lysed target cells.

3.5.3 Lymphocyte proliferation

Phytohaemagglutinin (PHA) and lipopolysaccharide (LPS) stimulated, as well as non-stimulated, proliferation of blood and head kidney lymphocytes were assayed (I, III). The lymphocytes in the culture medium were added to round-bottomed 96-well plates in triplicates, and the cultures supplemented with 2 % autologous plasma. The cells were activated with PHA or LPS (from *Salmonella typhosa*). After 66 h of culture at 26 °C (*methyl*-^{3}H)-thymidine was added to the wells. The plates were incubated for an additional 18 h and the lymphocytes harvested with deionized water onto glass fiber filters. Radioactivity (cpm) was recorded with a scintillation counter, and the mean cpm of triplicate cultures was used as a value representing the proliferation of the cells. The stimulation index (SI) was calculated for each fish using the equation

SI = (cpm of mitogen-stimulated culture) (cpm of non-stimulated culture)$^{-1}$.

3.5.4 Enumeration of immunoglobulin secreting lymphocytes (ISC)

A modified enzyme-linked immunospot (ELISPOT) assay (Aaltonen et al. 1994) was used for the enumeration of the total immunoglobulin-secreting cells (ISC) in the suspension of isolated carp head kidney leucocytes (I). The monoclonal mouse anti-roach IgM antibody (R1a4) was used as the trapping and detection agent after cross-reactivity with carp IgM was established. The cells suspended in a carp culture medium were pipetted into antibody-coated and albumin-saturated flat-bottomed 96-wells, and allowed to secrete immunoglobulin. The trapped IgM was detected with a biotin-conjugated R1a4 antibody followed by alkaline phosphatase-conjugated avidin. The substrate, bromo-chloro-indolyl phosphate, was mixed with warm agarose and added to the wells. Finally, blue spots were counted using a stereomicroscope.

3.6 Disease resistance models

3.6.1 Infection with bacterium *Yersinia ruckeri*

Bacteria for the infection (V) were isolated from the liver of rainbow trout, after once passaged through fish, then sub-cultured on tryptone soy agar and identified as *Y. ruckeri* by determining the biochemical properties (API 20 E). The stock suspension was prepared by culturing the isolate as a suspension for 18 h, small aliquots in PBS were stored at -24 °C until use. The concentration of viable *Y. ruckeri* in a thawed stock suspension was 2.4 x 10^{10} CFU ml^{-1}, the number of cells was approximately 7 x 10^{10} ml^{-1}. The fish were infected by injecting the bacterial suspension (10^{7} CFU) into the peritoneal cavity.

The spleen was carefully removed from each fish to determine the *Y. ruckeri* infection, and the surface of the organ disinfected and rinsed with sterile saline. The peritoneal membrane lining the body cavity of the fish was also disinfected and rinsed and the tissue sample taken aseptically from the

posterior kidney. Tissue samples were weighed and stored frozen until determining the *Y. ruckeri* infection. The thawed samples of the spleen and head kidney were aseptically homogenized, and the series of dilutions of tissue homogenates were cultured on SW bacterial agar dishes (Waltman & Shotts 1984), then the *Y. ruckeri* colonies on the agar were counted.

3.6.2 Infection with trematode *Diplostomum spathaceum*

Parasite cercariae for the infection (V) were obtained from naturally infected snails (*Lymnaea stagnalis*) collected earlier from earth ponds of a commercial fish farm. Newly produced cercarial suspension was combined from the snails, and the number of cercariae in the suspension estimated by counting their numbers under a microscope. The infection dose of 500 cercariae, less than 4 h old, was then introduced into each fish placed individually in aerated 1 l containers. After 30 min of exposure, the fish were returned to experimental aquaria and maintained there until dissection. From day 1 post-challenge both eye lenses from each fish were dissected and studied under a microscope to determine the number of metacercariae.

3.7 Statistics

The data (I-III, V) were analyzed for statistically significant differences between the means using the Mann-Whitney U-test. The levels of statistical significance were set at $P \leq 0.05$ (*), $P \leq 0.01$ (**) and $P \leq 0.001$ (***). In the case of day-to-day intra-assay variation, the data were modified with an equation
Relative value (%) = value of the fish/mean value of the daily controls x 100
before pooling the replicates and testing the effect of the treatment. Modification was also applied when combining the data from different experiments.

The variables in IV were grouped into sets of responses for multivariate analysis. The data were analyzed for effects in response sets using a nested multivariate analysis of variance (MANOVA). The variables in response sets were analyzed with univariate ANOVA.

4 RESULTS AND DISCUSSION

4.1 UVB-induced immunomodulation in fish

4.1.1 Innate immune system and hematology

The respiratory burst (RB) activity represents the oxygen-dependent killing of microorganisms by phagocytes (Secombes & Fletcher 1992), and it was used as a marker for the functioning of fish macrophages and granulocytes in the present study. In carp blood, the RB activity was markedly different after short- and long-term UVB exposures. The UVB-induced increase in the RB activity was demonstrated after exposing fish to a single dose (II), and after 1-week exposure (I). However, the RB activity in the blood remained at the control level following the long-term exposure of 4 weeks (I), and suppressed activity was observed after 6-week irradiation of carp (III) (Fig. 1).

Phagocytes play an important role in the innate defense system, and one of the first responses to an environmental stressor is the increased plasma cortisol concentration, followed by an increased amount of circulating granulocytes (Wendelaar Bonga 1997). These changes lead to an enhanced defense potential in the blood. It is suggested that this series of events explains the increased RB in the blood of carp exposed to short-term UVB (I, II). Similar findings have also been obtained with roach exposed to a single dose of UVB (Salo et al. 2000a, 2000b). In the present study, the altered differential blood leucocyte counts related more clearly to the UVB treatment, than did the plasma cortisol values. Plasma cortisol levels are, however, easily affected by many, even minimal, environmental stressors that may conceal the effects of treatment. Changes related to the cortisol-mediated stress reaction were temporary (Table 2), that is, the increased RB activity and the percentage of circulating granulocytes recovered at the control level in a few weeks despite the ongoing exposure of the fish (I). The question of an effect of UVB on the RB activity in individual cells remains open, since the possible changes are likely masked by the prominent alterations in differential blood cell counts.

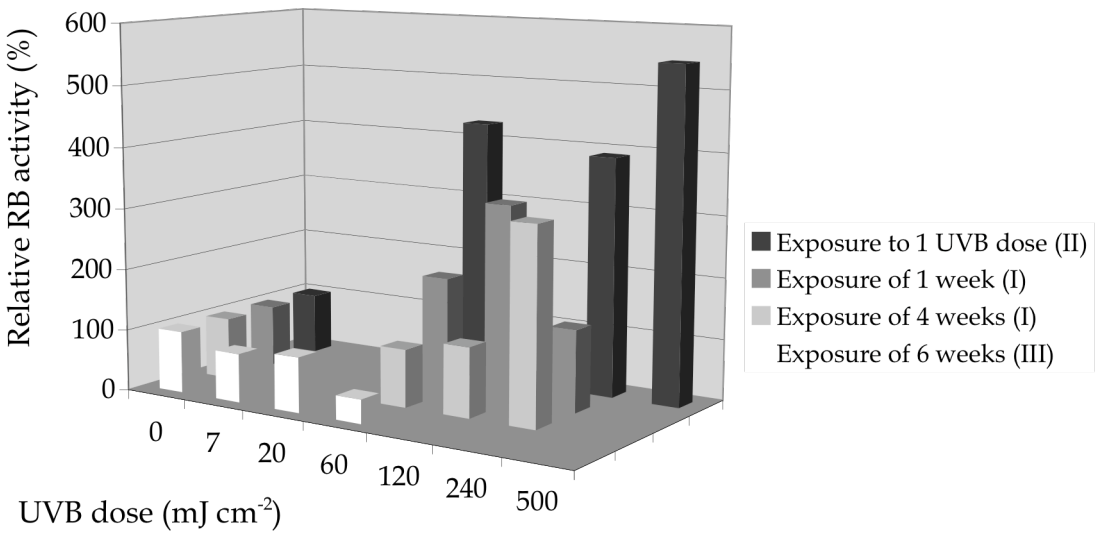

FIGURE 1 The effects of UVB exposure (3 weekly doses) on the RB activity of isolated blood leucocytes (III) or whole blood (I, II) of carp. The data are expressed as relative RB activity (% of the representative controls), and the columns represent the means of the groups (n=10-14). The SEs varied from 8 to 135, but were no more than 30 % of the mean.

The fish with skin lesions (240 mJ cm^{-2} UVB for 4 weeks, I) had highly increased RB activity. The mean RB activity of the fish with healthy skin in this group remained at the control level.

In contrast to short-term irradiations, the long-term exposure with low UVB doses suppressed the RB activity in carp blood, while the percentage of circulating granulocytes and monocytes remained unchanged (III). Some alterations related to the cortisol-mediated stress reaction were also seen after 6-week irradiation (Table 2), indicating the chance of prolonged stress response in the fish, or the response caused more directly by accumulated UVB doses. Chronic stress is recognized as an effective suppressor of immunity and can lead to decreased immune competence in fish (Maule et al. 1989, Fast et al. 2008).

The effect of UVB on the leucocyte functions over time was not monitored in detail, but indications of altered response dynamics were noted in the differential blood cell counts and RB activity in the blood of UVB-exposed carp. The highest UVB dose induced the most significant alterations after a single irradiation (Fig. 1, II), but after a 1-week exposure changes were only seen in the blood of fish exposed to midsized doses (I). The responses induced by the highest dose had probably peaked earlier and already returned to the control level at the time of sampling. Complex events have also been distinguished in the leucocyte functions of roach exposed to UVB (Salo et al. 1998, 2000a, 2000b, Jokinen et al. 2001). A single irradiation of the fish was seen to alter the functioning of the cells measured 1-2 d after the irradiation. The alteration was

sometimes followed by a strong compensatory change in the opposite direction 3-7 d after the irradiation. The leucocytes' final recovery to their normal functional state varied from 3 d to over 2 weeks. In longer exposures, similar alterations probably proceed together with the ongoing exposure of fish, increasing the harmful changes or inducing new ones. The spectral characteristics of radiation, fluence rate, and exposure regime also likely affect the treatment outcome, as well as the different UV susceptibilities of the fish studied.

In contrast to the carp, UVB had no significant effect on the RB activity in the blood of rainbow trout (II, III). This may be partly due to the UVB doses, which in some cases were too low to induce any changes in this rather tolerant fish. However, the lack of blood granulocytosis was also noted after otherwise immunomodulatory UVB treatment (II), indicating that the low response level may be characteristic for this species. The innate immune defense of rainbow trout was also studied by monitoring plasma lysozyme activity in UVB-exposed fish. This enzyme effectively lyses the cell walls of gram-positive bacteria. Lysozyme activity was significantly suppressed after exposing rainbow trout to a single dose of 1000 mJ cm^{-2} (II), but not following the 1-week irradiation with the same accumulated dose (III). Long-term exposures of Atlantic salmon did not affect the plasma lysozyme activity (IV).

In conclusion, UVB exposures altered the innate immune functions in the blood of fish, and in most cases short-term exposures caused more prominent changes than did long-term exposures with lower daily UVB doses. However, the suppressed RB activity of phagocytes in carp indicated that the low-dose, long-term irradiation also has harmful immunosuppressive potential.

TABLE 2 The concentration of plasma cortisol and the percentage of granulocytes and lymphocytes in the blood of juvenile carp after different UVB exposures (I-III). The values represent the mean ± SE of group (n=9-14 individuals). The statistical differences were tested by comparing to controls within the experiments ($P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***)).

	Single dose (II)	1 week (I)	4 weeks (I)	6 weeks (III)
Cortisol (ng ml^{-1})				
Unexposed	299 ± 38	63 ± 9	67 ± 10	237 ± 34
UVB 50-60 mJ cm^{-2}	199 ± 32	185 ± 22 ***	69 ± 10	178 ± 16
UVB 240-250 mJ cm^{-2}	241 ± 24	92 ± 11	66 ± 12	-
Granulocytes (%)				
Unexposed	2 ± 1	3 ± 1	3 ± 1	2 ± 1
UVB 50-60 mJ cm^{-2}	7 ± 2 **	4 ± 1 *	4 ± 1	2 ± 1
UVB 240-250 mJ cm^{-2}	12 ± 2 ***	3 ± 1	7 ± 2	-
Lymphocytes (%)				
Unexposed	48 ± 2	29 ± 4	30 ± 2	35 ± 2
UVB 50-60 mJ cm^{-2}	41 ± 2 *	34 ± 3	30 ± 3	29 ± 2 *
UVB 240-250 mJ cm^{-2}	37 ± 2 **	24 ± 2	14 ± 2 **	-

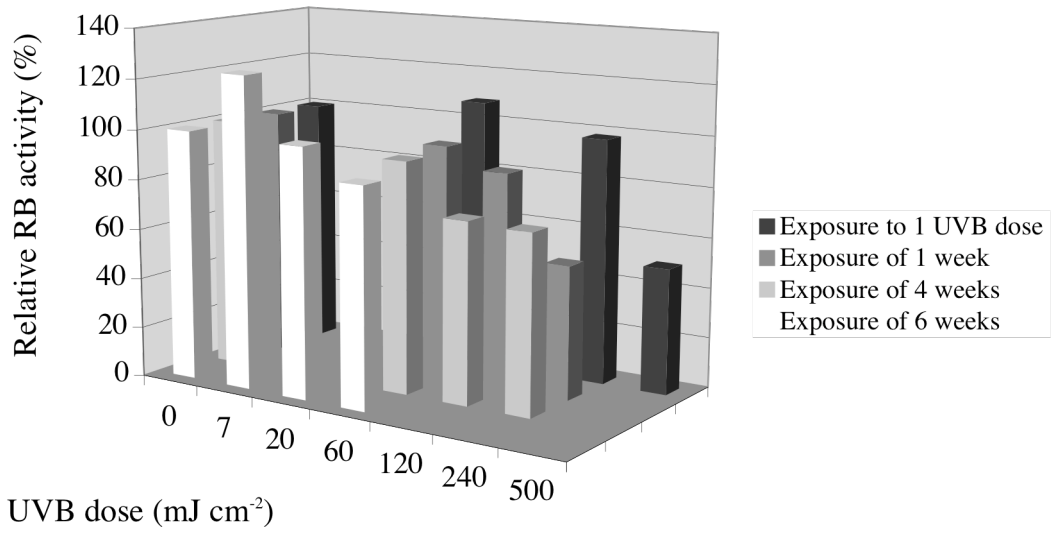

FIGURE 2 The effects of UVB exposure (3 weekly doses) on the RB activity of isolated head kidney granulocytes of carp. The data are expressed as relative RB activity (% of the respective controls), and columns represent the mean of the group (n=10-12). The SE of the mean varied from 7 to 30, and at its highest was 30 % of the mean.

In carp, isolated head kidney granulocytes showed suppressed RB activity after different irradiation regimes and UVB doses (Fig. 2). Single irradiation of fish induced a prominent change (II) and the exposures of 1 and 4 weeks also suppressed the head kidney RB (I). The effects of 6-week exposure were less profound (III), and this treatment was the only one inducing a reverse alteration. An increased RB of head kidney macrophages was detected in this experiment, while the RB of granulocytes was not affected (III). Earlier, UVB-induced suppression of the RB activity by head kidney macrophages, as well as granulocytes of roach (Salo et al. 1998, 2000b), was demonstrated 1-2 d after the irradiation. Increased RB activity of macrophages was, however, recorded in a 2-week follow-up, on days 3 and 7 after exposing roach to a single UVB dose (Salo et al. 1998). Therefore, we may suggest that the increased RB of carp macrophages after 6-week exposure may also be related to alterations in the dynamics of the response. Overall, the UVB effects on fish tend to have high variability, and both suppression and enhancement of the immune parameters are often seen to result from the exposure. When compared to cyprinids, the studies on rainbow trout phagocytes are fewer and the UVB-induced changes less prominent, but the findings were qualitatively similar.

Natural cytotoxic cells (NCC) also belong to the innate immune system, and are thought to participate in protection against viral, parasitic, and neoplastic diseases in fish (Yoder 2004). Increased NCC activity was detected in the blood of carp after short-term UVB exposure. The alteration was temporary

and related to the amount of circulating granulocytes (I, II). It has been suggested that the neutrophilic granulocytes and macrophages participate in the direct killing of target cells in carp (Yoder 2004). In the head kidney, the NCC activity was suppressed after a single irradiation (II), but remained unchanged in exposures of 1 and 4 weeks (I).

The cytotoxic activity by the head kidney leucocytes of rainbow trout was not affected by 1-week exposure which, however, suppressed RB activity (V). A single UVB dose did not affect NCC, either (II). In rainbow trout, cytotoxic activity has been demonstrated in neutrophils (Sasaki et al. 2002), and in small agranular mononuclear cells (Greenlee et al. 1991). Generally, the NCC of carp and rainbow trout head kidney were less sensitive to the effects of radiation than the RB of phagocytes. The UVB-induced changes were also more significant in the functioning of phagocytes than the NCC in roach (Salo et al. 1998, 2000b).

4.1.2 Acquired immune system

The most straining exposures, long-term irradiations with the high cumulative UVB doses decreased the plasma IgM concentration of fish (Table 3). A study with juvenile Atlantic salmon revealed significantly decreased plasma IgM concentration following an 8-week exposure to sunlight supplemented with UVB simulating 20 % ozone loss (approx. 350 mJ cm^{-2} d^{-1} UVB) (IV). A substantial decrease in plasma IgM concentration was also noted in carp exposed to a UVB dose of 240 mJ cm^{-2} for 4 weeks. Accordingly in carp, UVB exposures for 1 and 4 weeks did not affect the number of immunoglobulin-secreting cells (ISC) in the head kidney (I). Parallel to this, earlier studies have shown that short-term UVB had no negative effect on IgM production or reactivity against the antigen in roach, also a cyprinid fish (Jokinen et al. 2001).

Mitogen-induced proliferation of lymphocytes was used here as another marker for the functioning of the acquired immune system. UVB affected the proliferation of the blood and head kidney lymphocytes of carp, but the changes detected in different experiments were inconsistent. A high variation within and between the groups was a characteristic of this assay. Proliferation of head kidney lymphocytes (cpm) in the presence of a T-lymphocyte activator, PHA, was dose-dependently increased after UVB exposures for 1 and 4 weeks (I), but remained unchanged after a single moderate-level UVB dose (unpublished data). Single irradiation, however, dose-dependently decreased the proliferation (cpm, SI) of carp blood lymphocytes. In contrast to both of these observations, exposure to low doses of UVB for 6 weeks did not affect PHA-stimulated proliferation of the blood or head kidney lymphocytes (III). The suppressed proliferation of carp blood leucocytes may have partly been related to the lower proportion of blood lymphocytes in irradiated fish, because the isolation procedure for blood cells mainly concentrates the leucocytes for the assays. However, in 6-week exposure there was no association between the proliferation and proportion of blood lymphocytes in either carp or rainbow trout (III). Proliferation stimulated with LPS, a B-cell-specific activator, remained unaffected after a single irradiation (unpublished data), like that after

exposures for 1, 4 (I) and 6 weeks (unpublished data), but the spontaneous proliferation was increased by UVB in carp head kidney cells after exposure of 4 weeks (I). Rainbow trout lymphocytes were studied after 6-week irradiation, but UVB did not affect mitogen-induced proliferation (III). Earlier studies have revealed a single UVB exposure to suppress the proliferation of splenic lymphocytes of roach stimulated with a T- or B-cell activator, as well as spontaneous proliferation (Jokinen et al. 2001).

TABLE 3 Plasma immunoglobulin concentration in carp ($x10^{-1}$ U ml^{-1}), rainbow trout ($x10^{-1}$ U ml^{-1}) and Atlantic salmon ($x10^{-2}$ mg ml^{-1}) after different UVB exposures (I-V). The values represent the mean ± SE. The number of fish in groups exposed up to 6 weeks was n=12-30, and in exposures of 8 and 20 weeks n=46-81. The effects of the treatment were tested within the experiments. Statistically significant differences from controls are expressed as $P \leq 0.05$ (*), $P \leq 0.01$ (**).

Exposure	Accumulated dose (mJ cm^{-2})	Carp		Rainbow trout		Atlantic salmon	
		Ctrl	UVB	Ctrl	UVB	Ctrl	UVB
1 dose II	500	22 ± 2	26 ± 2	16 ± 1	18 ± 1	-	-
1 week I; V	720; 900	10 ± 1	8 ± 1	19 ± 1	17 ± 1	-	-
4 weeks I	2880	9 ± 1	3 ± 1 **	-	-	-	-
6 weeks III	1080	20 ± 3	16 ± 2	24 ± 1	21 ± 3	-	-
8 weeks IV	18200	-	-	-	-	43 ± 3	29 ± 3 **
20 weeks IV	1918	-	-	-	-	55 ± 4	62 ± 4

4.2 Condition of UVB-exposed fish

Indications of lowered condition, decreased plasma total protein concentration, and decreased blood hematocrit (Table 4) were seen in fish exposed to UVB from 1-8 weeks (I, III, IV, V). After 20-week exposure with daily 14 mJ cm^{-2} UVB, however, the condition of the salmon was not affected (IV). Plasma total protein concentration reflects the overall metabolism and functional activity of the somatic organs (Rehulka et al. 2005), and a decreased protein level is often accepted as an indicator of poor nutritional status in the long-term. Hematocrit, reflecting the oxygen-carrying capacity and the production of erythrocytes, was used here as another health indicator to assess the condition of exposed fish. The lowered condition was associated with UVB exposures of one week or longer, and was not detected after exposing carp and rainbow trout to a single irradiation dose (II). Single irradiation did not affect these parameters in roach, either (Salo et al. 2000a, 2000b). Generally, long-term exposures affected the immunological parameters less than the condition markers of fish.

The growth of Atlantic salmon fry exposed to UVB for 8 weeks was retarded (IV). Juvenile carp exposed to the highest UVB doses for 4 and 6 weeks

were also smaller than controls (unpublished data), but it cannot be verified whether the finding was caused by the treatment alone because the exact initial weight of the fish, randomly assorted into the experimental groups, was not measured. Earlier, inhibition of growth was demonstrated to be a sensitive endpoint for UV effects in Northern anchovy larvae (Hunter et al. 1981, 1982).

TABLE 4 Concentration of the plasma total protein (Prot, mg ml^{-1}) and blood hematocrit (Hct, %) of carp, rainbow trout, and salmon after different UVB exposures (I-V). The values represent the mean ± SE. The number in fish in groups exposed up to 6 weeks was n=12-30, and in exposures of 8 and 20 weeks n=52-84. The effects of the treatment were tested within the experiments. Statistically significant changes from the controls are expressed as $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***), and $P \leq 0.07$ (°) representing close to significant difference.

Exposure	Accumulated dose (mJ cm^{-2})		Carp		Rainbow trout		Atlantic salmon	
			Control	UVB	Control	UVB	Control	UVB
1 dose II	500; 1000	Prot	40 ± 1	39 ± 1	28 ± 1	30 ± 1	-	-
		Hct	42 ± 1	42 ± 1	46 ± 1	46 ± 1	-	-
1 week I,V	720; 900	Prot	19 ± 2	16 ± 1	29 ± 1	21 ± 1 ***	-	-
		Hct	48 ± 3	37 ± 2 **	52 ± 1	47 ± 1 ***	-	-
4 weeks I	2880	Prot	21 ± 2	21 ± 2	-	-	-	-
		Hct	40 ± 1	37 ± 1 °	-	-	-	-
6 weeks III	1080	Prot	33 ± 1	26 ± 1 ***	23 ± 1	19 ± 2 °	-	-
		Hct	41 ± 1	39 ± 1	47 ± 1	41 ± 1 ***	-	-
8 weeks IV	18200	Prot	-	-	-	-	42 ± 4	20 ± 2 ***
		Hct	-	-	-	-	51 ± 1	46 ± 1 ***
20 weeks IV	1918	Prot	-	-	-	-	43 ± 2	45 ± 2
		Hct	-	-	-	-	48 ± 1	48 ± 1

4.3 Different UVB sensitivity of carp and rainbow trout

In general, rainbow trout was more tolerant to immunomodulatory effects evoked by UVB than carp. Immune functions of these fish were compared after exposure to a single irradiation, and after multiple exposures to UVB for 6 weeks. A single UVB irradiation dose inducing suppressed RB in head kidney leucocytes, and changes in differential blood cell counts of rainbow trout (1000 mJ cm^{-2}) was 200-fold higher than the lowest dose inducing immunomodulation in carp (II). The immune function of roach is markedly affected by a single UVB dose of 430 mJ cm^{-2} (Salo et al. 1998, 2000a, 2000b, Jokinen et al. 2001), suggesting that cyprinid species, carp and roach, express similar sensitivity to radiation. Rainbow trout was more tolerant than carp when also exposed to long-term UVB, but the difference between the species was smaller. The RB activities assayed in the experiments are summarized in Table 5.

Comparison of condition markers, that is, plasma total protein concentration and hematocrit, indicated that these responses to UVB irradiation are not very different in carp and rainbow trout. Significant changes were seen in both species after exposures from 1 to 6 weeks, as well as in Atlantic salmon after 8-week exposure (Table 4). The sensitivity of carp and rainbow trout skin to radiation also was not notably different, when the depletion of mucus and appearance of early lesions were monitored in laboratory experiments (I, III, V).

TABLE 5 Respiratory burst (RB) activity of carp and rainbow trout after a single dose (II) and UVB exposures for 6 weeks (III). Whole blood was studied after a single dose, and isolated blood leucocytes after 6 weeks of exposure. The head kidney RB was assayed with isolated granulocytes or unseparated leucocyte suspension. The data are expressed as relative RB activity (% of the representative controls) ± SE, group n=10-32. The statistical differences were tested by comparing to controls within the experiment (* $P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$).

	Single dose		Exposure for 6 weeks	
	Carp	Rainbow trout	Carp	Rainbow trout
Blood				
Unexposed	100 ± 4	100 ± 14	100 ± 14	100 ± 27
50-60 mJ cm^{-2} UVB	185 ± 33 *	139 ± 25	39 ± 8 **	282 ± 118
500 mJ cm^{-2} UVB	424 ± 63 ***	132 ± 20	-	-
Head kidney				
Unexposed	100 ± 12	100 ± 22	100 ± 9	100 ± 7
50-60 mJ cm^{-2} UVB	108 ± 17	171 ± 46	87 ± 11	85 ± 17
500 mJ cm^{-2} UVB	70 ± 10 **	122 ± 29	-	-
1000 mJ cm^{-2} UVB	-	23 ± 4 ***	-	-

4.4 Response of fish immune system to long-term UVB exposure

So far most information on the immunomodulatory effects of long-term UVB exposure concerns mammals. These studies revealed long-term UVB irradiation to have variable effects on immune parameters. For example, exposure to solar-simulated radiation (SSR) from 1 to 4 weeks equally suppressed nickel contact hypersensitivity (CHS) in human subjects (Damian et al. 1999), but in another study the effects of 30 d SSR exposure on CHS and primary allergic reaction were dependent on the accumulated dose (Narbutt et al. 2005). Radiation-induced alterations in the numbers of Langerhans cells and dendritic cells were also most prominent at the end of a 60 d exposure (McLoone et al. 2005), and chronic UV exposure induced a dose-dependent suppression of basal splenic NK activity in nude mice (Toda et al. 1986). UVB-induced suppression of

phagocytes, however, recovered during the 30 d irradiation (McLoone & Norval 2005).

In fish, the short-term UVB doses administered had more immunomodulatory potential than the same doses delivered over a longer period. The functioning of carp head kidney phagocytes and lymphocytes was altered after 1-week irradiation but remained at the control level following the 4-week exposure with the same accumulated UVB dose (720 mJ cm^{-2}) (I). The RB activity of rainbow trout head kidney leucocytes was also studied in exposures varying in length. A single UVB dose of 1000 mJ cm^{-2} induced the most prominent suppression in the RB activity (II), and 1-week daily exposures also caused changes (V). However, the accumulated dose of the 1020 mJ cm^{-2} delivered in 6 weeks had no effect (III). Similar findings have been obtained when studying mortality of Northern anchovy larvae, that is, the UVB dose delivered in 12 d caused less mortality than the same dose delivered over shorter period (Hunter et al. 1981, 1982). Thus, in this thesis UVB-induced modulation of leucocyte functions was more related to the daily irradiation dose than to the accumulated total dose, and an accumulation of effects did not take place. This may still not indicate that long-term exposures would be less harmful than short-term exposures. The negative effects of long-term exposure to low-level UVB doses were seen as a lowered condition (Table 4, I, III-V) and decreased plasma IgM concentration of fish (Table 3, I, IV).

Different effects of UVB at high and low fluence rates have also been demonstrated in the behavior and mortality of larval Northern pike. Within intermediate fluence rates the effects of UVB were determined by the accumulated dose alone (the intensity of radiation, or duration of exposure having no impact). At high fluence rates, the harmful effects were prominent, and at low fluence rates less alterations were detected than would have been expected to result from the accumulated dose (Vehniäinen et al. 2007b). It has been hypothesized that photorepair mechanisms cause the low impact of UVB in long-term exposures with low doses in fish larvae (Kouwenberg et al. 1999, Vehniäinen et al. 2007b).

It has been suggested that photoadaptation protects mammals from the harmful effects of long-term radiation. Long-term exposures to low doses of whole-body SSR produced inability to respond to a local immunosuppressive UV treatment and suppressed UV-induced inflammation in the skin of human subjects (Laihia et al. 2005). Photoadaptation was also demonstrated in the functioning of phagocytes in mice exposed to SSR for 30 d (McLoone & Norval 2005). UV-induced alterations in the numbers of Langerhans cells and dendritic cells of mice were reduced after pre-exposure as well (McLoone et al. 2005). On the other hand, the repeated low doses of UVB protected human subjects only to a limited extent against the effects of an erythemal UVB dose on cytokine expression and DNA damage in the skin, and not on CHS or cyclooxygenase-enzymes (Narbutt et al. 2007). No adaptation developed in mice exposed to SSR up to 90 d and studied for CHS, DNA damage, and proliferative response or cytokine production of skin-draining lymph node cells after immunization (Steerenberg et al. 2006). So far, adaptation of the immune system has not been studied in fish, but a preliminary study with roach showed that a 2-week pre-

exposure to low-level UVB doses enhances the immunosuppressive effect of a single UVB dose of 430 mJ cm^{-2} (Salo, unpublished), suggesting that there is no adaptation to UVB.

4.5 Altered disease resistance after exposure to UVB (III)

4.5.1 Experimental infections with *Y. ruckeri* and *D. spathaceum*

Y. ruckeri bacterium is the causative agent of salmonid enteric redmouth disease (ERM) (Furones et al. 1993). The strain used for experimental infection in this study is an endemic pathogen in Finland and able to infect hosts whose resistance is lowered, for example, because of stress or primary infection (Valtonen et al. 1992, Hietala et al. 1995). As an environmental stressor, UVB exposure was expected to have a negative effect on the resistance to *Y. ruckeri* in fish.

The resistance of rainbow trout against bacterial colonization showed complex alterations associated with time, when bacterial load in the tissues was measured on days 8 and 14 of the experiment (2 and 8 d after the infection, respectively). *Y. ruckeri* were effectively cleared from the kidney and spleen of UVB-exposed fish during the first days after the infection, but at the end of the experiment slightly higher numbers of bacteria remained in UVB-irradiated fish (Fig. 3). The findings clearly suggest altered response dynamics in UVB-exposed fish, but they may also indicate prolonged disease.

The rainbow trout exposed to UVB daily for 6 d were more susceptible to *D. spathaceum* infection and had significantly higher parasite load in the eyes, when compared to the controls (Fig. 3). The genus *Diplostomum* are ubiquitous parasites of wild freshwater fish. The disease, parasitic cataract, is caused by development of larval eye flukes (metacercariae) in the lens (Chappell et al. 1994), and may affect the survival of the infected fish. UV-depleted radiation did not affect the disease resistance of rainbow trout in either of the models described above.

Functioning of the rainbow trout head kidney phagocytes was studied after the 1-week irradiation regime, but only moderate changes in RB activity were detected. UVB treatment, however, was stressful to the fish, and the plasma protein concentration and blood hematocrit were significantly decreased in the irradiated fish (V).

The findings are in accordance with earlier studies demonstrating UVB to lower the resistance against bacterial (Jeevan & Kripke 1989, 1990, Jeevan et al. 1992, Goettsch et al. 1996b), viral (Garssen et al. 2000, Ryan et al. 2000), and parasitic (Goettsch et al. 1994, Goettsch et al. 1996a, Yamamoto et al. 2000) infections in rodents.

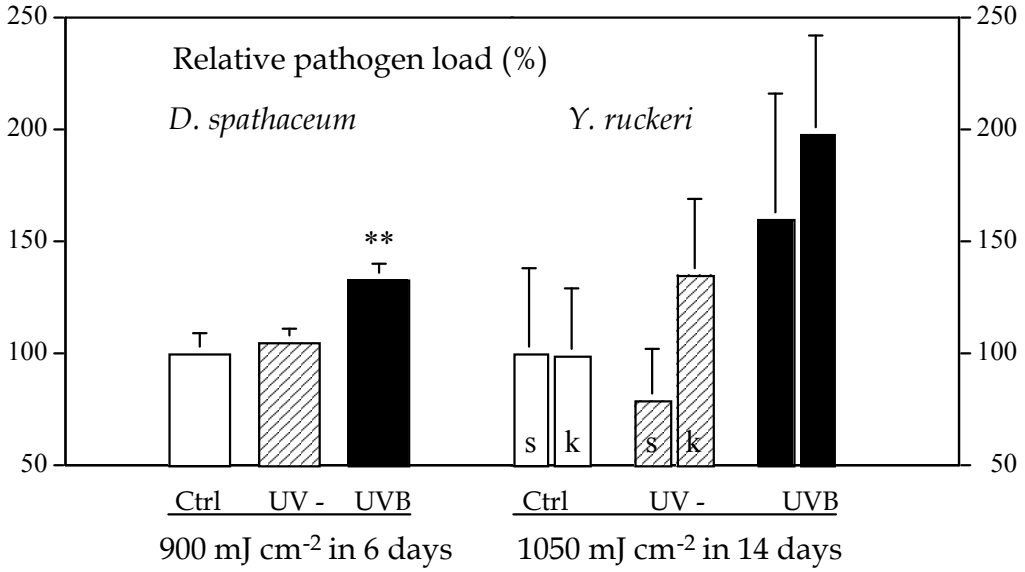

FIGURE 3 The effects of UVB irradiation on the disease resistance of juvenile rainbow trout. The data are expressed as a relative pathogen load (% of the respective controls), and the columns represent the mean of the group (+SE). The pathogen load in the fish was measured as a number of *D. spathaceum* metacercariae in the eye lens (group n=28-45), and the number of *Y. ruckeri* CFU mg^{-1} (group n=28-45) in the spleen (s), and in the kidney (k). The radiation is given as an accumulated dose over the indicated periods.

4.5.2 Is exposure to increased ambient UVB threat to fish health?

It has been reported that ambient UVR is a potential cause behind the absence of *Galaxias maculatus*, a small freshwater fish widespread in the Southern hemisphere, in highly transparent and shallow lakes. The LD_{50} depths for several lakes within the geographic distribution of *G. maculatus* were calculated, and in some cases the values were greater than the maximum depth of the lakes (Battini et al. 2000). Only a minor increase in ambient UVB levels has been noted to have lethal effects on larvae of a UV-sensitive boreal fish, Northern pike (Häkkinen et al. 2004). On the other hand, UVB doses received by adult, wild fish in their natural environment are likely lower than those received by larvae because of the opportunity for avoidance. Studies with the eggs and larvae of cod and anchovy also suggest that predicted levels of stratospheric ozone depletion will probably not have a major effect on the larval populations of these species. Of the factors studied affecting the UV exposure of fish larvae (e.g. vertical mixing of eggs, meteorological and hydrographic conditions), it was suggested that ozone depletion has the least impact (Hunter et al. 1981, Hunter et al. 1982, Browman et al. 2000, Kuhn et al. 2000). Whitefish and vendace inhabiting Fennoscandian lakes are not expected to be threatened by current or future radiation levels (Häkkinen et al. 2002, Ylönen & Karjalainen 2004). However, a recent study analyzed primary literature of UV-effects on aquatic organisms through meta-analysis, and negative effect on growth and survival was detected despite different habitat types, life history stages, trophic levels and diverse taxonomic groups (Bancroft et al. 2007). UVR may also have an additive effect with other environmental stressors. A high mortality of

whitefish and vendace was noted after simultaneous UVB irradiation and exposure to a PAH compound, retene, although these treatments alone were not lethal to fish (Vehniäinen et al. 2003). Different sensitivities of fish species were also demonstrated as retene had no phototoxic effect on the UV-sensitive Northern pike larvae (Häkkinen et al. 2003b). Alterations related to predicted climate change induced increased temperature, together with increased UV levels are estimated to have harmful effects on farmed fish, and fish populations in Arctic freshwaters (Reist et al. 2006).

Some recent findings indicate that sublethal effects of UV radiation may have a far-reaching impact. UVB exposure for 12 d affected *Daphnia magna* over two generations, that is, the decreased survival and reproduction was also detected in the offspring (Huebner et al. 2008). The mechanisms of UVB effects were not investigated in this study, but it was suggested that the exposure of parents damaged the developing F1, later affecting their reproduction when subsequently exposed to UVB. Such adverse effects of UVB may, thus, be accumulated in successive generations, suggesting that short-term experiments may underestimate the impact on populations. With skin specimens of melanoma patients, the history of sunburn was shown to act on the DNA integrity and altered expression of genes related to small molecule transporters, growth factor and chemokine receptors, transcription factors, and tumor suppressors. Among these were three DNA ligases, the UV excision repair gene RAD23, and the growth arrest and DNA damage gene 45 (GADD45). These findings support the idea that exposure to solar radiation early in life may induce harmful long-term changes at the cellular level (Steinberg et al. 2009).

The UVB doses used in the present work have been of the same order of magnitude, or smaller, as the daily doses (around 200 mJ cm^{-2} erythemally weighted) recorded in Southern Finland during May in 1998-2000 (Oikari et al. 2002). The erythemally weighted daily UVB doses in the laboratory experiments were as low as 2-20 mJ cm^{-2} (III), and approx. 100 and 4 mJ cm^{-2} in outdoor experiments (IV). The lamps also emitted some UVA, but these wavelengths comprise no more than 2 % of the erythemally weighted underwater radiation, while in natural waters fish are exposed to the full solar radiation spectrum including substantially more of the long wavelengths. In mammals, UVA wavelengths are also immunosuppressive, and interactions between UVB and UVA augment each other (Halliday & Rana 2008). UVA radiation has been demonstrated to have harmful effects on catfish (*Clarias gariepinus*) embryos (Mahmoud et al. 2009), as well as on the skin and hematological parameters of adult fish (Sayed et al. 2007). Exposure to the natural full solar spectrum probably affects fish differently than exposure to artificial UVB from lamps, but both types of radiation are expected to be harmful in high doses. UVA and blue light are also known to have a photoprotective potential in fish. Photoreactivating radiation was demonstrated to protect rainbow trout larvae after exposure to low UVB doses, while increasing doses seemed to cause saturation or damage to the repair machinery itself (Mitchell et al. 2009). In a low-radiation environment, photoprotection may further prevent harmful UVB effects on fish.

The present study suggests that UVB radiation can potentially compromise fish health. The detected compromised disease resistance and retarded growth of fish are considered ecologically severe changes. However, the ambient solar radiation during summer months in Norway had no negative effect on juvenile salmon studied after exposure for 4.5 months in shallow cages, not even when supplemented with UVB simulating 8 % ozone loss. It seems, therefore, that only certain species and life stages are exposed to doses high enough to induce a health risk at the current and predicted near-future UVB levels. The potential risk groups may include fish inhabiting clear natural waters in high radiation-level areas, or fish farmed outdoors in shallow cages or ponds.

As it is not known how the global change will proceed and affect future UVB levels, and whether there are additional harmful factors to be expected, the UVB effects on fish should be further examined. Most importantly, field experiments need to be done to verify the effect of long-term UVB on the survival and disease resistance of fish in their normal living environment. The effect of multiple simultaneous environmental stressors on fish should also be examined. To verify the immunomodulatory effects of long-term UVB on fish and to explore the mechanisms behind the present findings, more diverse leucocyte parameters should be monitored; e.g., the functioning of T cells has been shown to be affected by UVB radiation in mammals. It is important to identify suitable early biomarkers for the evaluation of UVB-induced risks on fish health.

5 CONCLUSIONS

In this study, the main goals were a) to establish whether long-term exposure to low level UVB doses has an impact on the immune status of fish, b) to investigate how the habitat preference and mode of life by fish is associated with UVB-induced immunomodulation, and c) to study whether the UVB-induced immunomodulation eventually leads to altered defense against pathogens.

Altered functioning of immune parameters in the blood and head kidney of fish was detected after the UVB exposures of 4 to 6 weeks, but the changes did not accumulate notably with increasing exposure time. Generally, UVB administered in a short exposure regime had more immunomodulatory potential than the same accumulated dose delivered over a long-term experiment. Phagocytes were more sensitive to UVB than NCC or lymphocytes. Solar radiation during summer months in Norway caused no detectable harm to juvenile Atlantic salmon studied after exposures of 2 or 4.5 months in shallow cages, not even when supplemented with UVB simulating 8 % ozone loss. However, salmon exposed to solar radiation supplemented with UVB simulating 20 % ozone loss for 2 months showed significantly decreased growth and condition. The plasma IgM concentration of exposed salmon was also lowered which suggests compromised immune defense.

Rainbow trout, living at higher underwater radiation levels than carp, tolerated UVB better when the functioning of the leucocytes was monitored. However, comparison of condition, that is, plasma total protein concentration and hematocrit, indicated similar responses to UVB irradiation in these species.

The resistance of rainbow trout against bacterial and parasitic pathogens in experimental infections was impaired after exposure to UVB for 1-2 weeks with doses inducing only moderate immunomodulation. The finding suggests an association between UVB exposure and disease resistance in fish, and further emphasizes harmful impact of UVB on fish health.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Ultravioletti B -säteilyn vaikutus kalan taudinvastustuskykyyn ja immunologisen puolustusjärjestelmän toimintaan

Runsas altistuminen auringolle, lähinnä säteilyn ultravioletti B (UVB) -aallonpituuksille, on haitallista ihmisten ja eläinten hyvinvoinnille. Kirkkaisiin järvi- ja merivesiin UVB tunkeutuu annoksina, jotka voivat aiheuttaa haittaa mm. lisäämällä kalojen varhaisten elinvaiheiden epämuodostumia ja kuolleisuutta. Laboratoriokokein on myös osoitettu, että kertaluonteinen altistuminen UVB-säteilylle aiheuttaa muutoksia aikuisen särjen immuunijärjestelmän toiminnassa.

Tässä tutkimuksessa selvitettiin pieninä annoksina annetun pitkäkestoisen UVB-säteilyn vaikutuksia kalojen immunologisen puolustusjärjestelmän toimintaan ja kalojen yleiskuntoon. Karpin ja kirjolohen etumunuaisen fagosytoivien solujen toimintakyvyn havaittiin toistuvasti heikkenevän erilaisten laboratorioaltistusten seurauksena. Luonnollisten tappajasolujen ja lymfosyyttien toimintaan UVB-säteily aiheutti vaihtelevia muutoksia. Merkittävää vaikutusten vahvistumista ei pitkissä altistuksissa kuitenkaan havaittu. Pitkäkestoiset altistukset aiheuttivat yleisesti ottaen vähemmän ja erilaisia immunologisia muutoksia kuin altistuminen vastaavalle UVB-annokselle lyhyessä ajassa. Lyhytkestoisten altistusten seurauksena havaittiin usein veressä kortisolivälitteisenä stressireaktiona granulosyyttien osuuden lisääntyminen, mikä saattaa lyhytaikaisesti tehostaa perifeeristä puolustusta mikrobeja vastaan. Lajien välisistä eroista havaittiin, että paremmin sameisiin vesiin sopeutunut karppi on immunologisten muuttujien osalta kirjolohta herkempi säteilyn vaikutuksille.

Altistuminen luonnolliselle auringonvalolle, johon oli lisätty 20 %:n otsonikatoa vastaava määrä UVB-säteilyä, oli selvästi haitallista nuorille lohille. Kahdeksan viikkoa kestäneen kokeen jälkeen ulkoaltaissa tällaiselle säteilylle altistetut kalat olivat pienempiä kuin vertailuryhmissä. Lisäksi plasman immunoglobuliini-M:n taso sekä kalojen yleiskuntoa kuvaavat plasman proteiinien kokonaispitoisuus ja veren hematokriittiarvot olivat alentuneet. Samankaltaisia muutoksia oli havaittavissa pitkissä laboratorioaltistuksissa myös karpilla ja kirjolohella. Lohen altistuminen 20 viikon ajan 8 %:n otsonikatoa simuloivalle UVB-säteilylle ei sen sijaan vaikuttanut näihin muuttujiin verrattuna luonnollisessa auringonvalossa pidettyihin yksilöihin.

UVB-säteily aiheutti myös muutoksia nuorten kirjolohien kyvyssä torjua *Yersinia ruckeri* -bakteeria ja *Diplostomum spathaceum* -loista kokeellisessa infektiossa. Säteilylle altistetut kalat tuhosivat bakteereja elimistöstään infektion jälkeen aluksi verrokkeja tehokkaammin, mutta myöhemmin kudoksiin jäi enemmän bakteereja. Infektoitaessa kaihia aiheuttavalla imumatoloisella säteilylle altistettujen kalojen silmissä havaittiin merkittävästi vertailuryhmiä suurempi määrä kyseisiä loisia.

Tulosten perusteella pitkäaikaista kohonnutta UVB-säteilytasoa voidaan pitää uhkana kalojen terveydelle. Kokeissa havaittiin myös yhteys UVB-altistuksen ja kalojen tautikestävyyden alentumisen välillä.

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