JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
156

Eeva-Riikka Vehniäinen

Boreal Fishes and Ultraviolet Radiation

Actions of UVR at Molecular and Individual Levels

JYVÄSKYLÄN YLIOPISTO

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 156

Eeva-Riikka Vehniäinen

Boreal Fishes and Ultraviolet Radiation

Actions of UVR at Molecular and Individual Levels

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Villa Ranan Blomstedt-salissa
elokuun 26. päivänä 2005 kello 12.

Academic dissertation to be publicly discussed, by permission of
the the Faculty of Mathematics and Science of the University of Jyväskylä,
in the Building Villa Rana, Blomstedt Hall, on August 26, 2005 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2005

Boreal Fishes and Ultraviolet Radiation

Actions of UVR at Molecular and Individual Levels

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 156

Eeva-Riikka Vehniäinen

Boreal Fishes and Ultraviolet Radiation

Actions of UVR at Molecular and Individual Levels

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2005

Editors
Jukka Särkkä
Department of Biological and Environmental Science, University of Jyväskylä
Pekka Olsbo, Marja-Leena Tynkkynen
Publishing Unit, University Library of Jyväskylä

Cover picture by Eeva-Riikka Vehniäinen

URN:ISBN:9513921891
ISBN 951-39-2189-1 (PDF)

ISBN 951-39-2149-2 (nid.)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2005

ABSTRACT

Vehniäinen, Eeva-Riikka
Boreal fishes and ultraviolet radiation – Actions of UVR at molecular and individual levels
Jyväskylä: University of Jyväskylä, 2005, 52 p.
(Jyväskylä Studies in Biological and Environmental Science,
ISSN 1456-9701; 156)
ISBN 951-39-2189-1
Yhteenveto: Pohjoisen kalat ja ultraviolettisäteily – UV-säteilyn vaikutukset molekyyli- ja yksilötasolla
Diss.

Anthropogenically-caused stratospheric ozone depletion has increased the amount of UVB on Earth, and the predictions in Fennoscandia for the future suggest a further 20–60 % increase in the coming decades. This work explores the effects of UVB on larval boreal freshwater fishes at both the cellular and molecular level, and connects them to impacts at the individual level (mortality and growth retardation). Larval coregonids were very tolerant against UVR, but simultaneous exposure to UVB and retene, a polynuclear aromatic hydrocarbon, resulted in high mortality. On the contrary, in larval northern pike no photoinduced toxicity of retene was detected, but irradiation with currently occurring fluence rates and doses of UVB alone resulted in high mortality. Sublethal effects of UVB on pike included severe behavioural disorder followed by delayed mortality, growth attenuation, DNA damage, and changes in mRNA and protein expression. DNA damage occurred in the skin of pike larvae irradiated with current fluence rates of UVB. The higher the fluence rate the deeper in tissues the DNA damage was found. The DNA damage in the brain of pike larvae was followed by changes in protein expression, such as HSP/HSC70 decline. DNA damage and changes in protein expression in brain coincided with the behavioural disorder, suggesting the disorder was of neural origin. UVB caused less DNA damage in whitefish, the more tolerant and more pigmented species, than in pike. Also the UVB-induced transcriptomic changes differed between species and age groups. The results suggest connections between DNA damage and other molecular changes at the cellular level and the abnormal behaviour in pike.

Key words: Boreal fishes; DNA damage; larvae; phototoxicity; UVB.

E.-R. Vehniäinen, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland

Author's address Eeva-Riikka Vehniäinen
University of Jyväskylä
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä, Finland
E-mail: eevehnia@cc.jyu.fi

Supervisor Professor Aimo Oikari
University of Jyväskylä
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä, Finland
E-mail: aoikari@bytl.jyu.fi

Reviewers Professor Chris D. Metcalfe
Environmental & Resource Studies Program
Trent University
1600 West Bank Drive
Peterborough, Ontario K9J 7B8
Canada
E-mail: cmetcalfe@trentu.ca

Associate professor Terrence J. Piva
School of Medical Sciences
Science Engineering and Technology Portfolio
RMIT University
P.O. Box 71
Bundoora, VIC 3083
Australia
E-mail: terry.piva@rmit.edu.au

Opponent Professor Helmut Segner
University of Bern
Centre for Fish and Wildlife Health
Laenggass-Str. 122
CH-3012 Bern
Switzerland
E-mail: helmut.segner@itpa.unibe.ch

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, referred to in the text by their Roman numerals I–VI.

I Vehniäinen, E.-R. Protecting fish embryos from ultraviolet radiation: The role of chorion transmittance and methanol-extractable substances in boreal species. Manuscript submitted to Boreal Environmental Research

II Vehniäinen, E.-R., Häkkinen, J. & Oikari, A.O.J. 2003. Photoinduced lethal and sublethal toxicity of retene, a polycyclic aromatic hydrocarbon derived from resin acid, to coregonid larvae. Environmental Toxicology and Chemistry 22: 66–71.

III Häkkinen, J., Vehniäinen, E. & Oikari, A. 2004. High sensitivity of northern pike larvae to UVB but no UV-photoinduced toxicity of retene. Aquatic Toxicology 66: 393–404.

IV Vehniäinen, E.-R., Häkkinen, J. M. & Oikari, A. O. J. Fluence rate or cumulative dose? Vulnerability of larval northern pike (*Esox lucius*) to ultraviolet radiation. Manuscript submitted to Photochemistry and Photobiology

V Vehniäinen, E.-R., Vähäkangas, K. & Oikari, A. Ultraviolet-B exposure causes DNA damage and changes in protein expression in northern pike (*Esox lucius*) larvae. Manuscript

VI Vehniäinen, E.-R., Krasnov, A. & Oikari, A. Comparison of ultraviolet radiation-induced changes in larvae of two fish species with different susceptibility: DNA damage and transcriptomic responses. Manuscript

In addition, some unpublished data is also presented in this thesis.

ABBREVIATIONS

A_{500}	Absorbance at 500 nm
CIE	Commission Internationale de l'Eclairage; International Commission on Illumination) i.e., the action spectrum specific for human erythema
CPD	cyclobutane pyrimidine dimer
CYP1A	cytochrome P450 1A
DMSO	dimethylsulfoxide
DOC	dissolved organic carbon
DOM	dissolved organic matter
HSC70	heat shock cognate 70
HSP70	heat shock protein 70
LC50	lethal concentration causing 50 % lethality
MAA	mycosporine-like amino acid
PAH	polycyclic aromatic hydrocarbon
PAR	photosynthetically active radiation
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SOD	superoxide dismutase
UV	ultraviolet
UVA	ultraviolet A (315–400 nm)
UVB	ultraviolet B (280–315 nm)
UVC	ultraviolet C (200–280 nm)
UVR	ultraviolet radiation

1 INTRODUCTION

The solar spectrum consists of a wide range of wavelengths with different effects on life on Earth. The shorter the wavelength, the more energetic the radiation is, and the greater the potential for harm to living organisms. Ultraviolet (UV) radiation, i.e. radiation between 200 and 400 nm (UVR), has always been there, even in very dramatic quantities and qualities in the very prehistoric times when molecular oxygen was absent from the atmosphere (Cockell 1998).

Nowadays, the most energetic part of the solar spectrum, UVC (200–280 nm), does not reach the surface of the Earth, because the ozone layer and molecular oxygen prevent it from passing through the atmosphere (Frederic et al. 1989, Madronich et al. 1998). Also the shortest wavelengths of UVB (280–315 nm) are greatly attenuated by the ozone layer in the atmosphere (Madronich et al. 1995, 1998). UVA (315–400 nm), which is less energetic than the other two, passes through the atmosphere almost unattenuated (Madronich et al. 1995, 1998). Visible light (400–700 nm) is less energetic still, and crucial for the life on Earth, because its energy is absorbed by plants via photosynthesis.

1.1 Factors affecting the quantity of UVB on earth and in natural waters

The amount of UVB reaching the surface of the earth depends on the thickness of the ozone layer and the atmosphere the radiation has to get through. This varies due to solar zenith–the distance through the ozone layer is shortest and thus the attenuation of UVB lowest when the solar angle is 90° (Gerstl et al. 1986). The angle of solar radiation changes with latitude, season and time of day, so that the highest fluence rates of UVB radiation occur at the equatorial region in midsummer at midday (Gerstl et al. 1986). However, the relative ratio of UVR to photosynthetically active radiation (PAR) is greatest during

crepuscular periods (dawn and dusk) because at those times the light field is mostly composed of skylight (Leech & Johnsen 2003). Also the altitude plays a role: the higher the place the greater the amount of UVB (Blumthaler et al. 1997). Clouds usually attenuate UVR, but UVR can also be reflected and thus become spatially enhanced by clouds (Mims & Frederick 1994).

The ozone hole over Antarctica was reported in 1985 (Farman et al. 1985) and thinning of the ozone layer has also been observed in the Northern Hemisphere (Stolarski et al. 1992, Gleason et al. 1993), causing an increase in the UVB fluxes (Kerr & McElroy 1993). Predictions for the future show that due to ozone depletion UVB levels in boreal region are likely to increase 10–60 % in April in years 2010–2020 (Taalas et al. 2000).

1.1.1 Attenuation by water

The UVB fluence rates differ greatly between aquatic habitats. The concentration of dissolved organic matter (DOM) or dissolved organic carbon (DOC) and habitat depth are the main factors contributing to the differences. DOM/DOC attenuates UVR effectively, but also suspended particulate material, vegetation coverage and hydrological characteristics (such as turbulence) play a role in attenuating UVR (Scully & Lean 1994, Morris et al. 1995, Bukaveckas & Robbins-Forbes 2000).

In Finnish lakes 99 % attenuation depths of UVB vary from < 10 cm (DOC 16.0 mg/l) to ~50 cm (DOC 4.9 mg/l) (Huovinen et al. 2000, 2003). In lakes with lower concentrations of DOC, UVB is likely to penetrate deeper still. UVR affects its own penetration by degrading DOC. This phenomenon is called photobleaching, and it has a remarkable role especially in systems with very low DOC (Morris & Hargreaves 1997, Zagarese et al. 2001). In the process of photobleaching DOC is made available to the food chain (usually via the microbial loop), and also phototoxic compounds may be formed (Morris & Hargreaves 1997, Zagarese et al. 2001). The ongoing climate change may further affect DOC concentrations via changes in precipitation, and acidification and changes in land use may regionally change the DOC concentrations and thus the water transparency (Schindler et al. 1996, Yan et al. 1996). In addition, the climate change may cause extreme weather conditions such as long-lasting sunny periods, thus increasing UVR exposure. The warming temperature may also increase the duration of UVR exposure in aquatic environments due to decrease in ice-cover time.

1.2 Detrimental effects of UVR on fish

In all living organisms UVR directly affects the molecules absorbing in the UV-region. These include molecules containing aromatic or conjugated aliphatic chains, such as bases in nucleic acids, aromatic amino acids (tyrosine,

tryptophan, phenylalanine) and lipids (Cockell 1998, Schwarz 1998). Indirectly UVR produces oxidative stress (Jurkiewicz & Buettner 1994). In an individual, these effects may be manifested in many ways. In fishes, these effects range from minor changes in epidermis to death, and there is great variation between species in vulnerability to UVR.

Eggs and larval stages of fish are more vulnerable to UVR than juveniles or adults. Ultraviolet radiation causes mortality of embryonal and larval stages of both marine and freshwater fish, including northern anchovy (*Engraulis mordax*), Pacific mackerel (*Scomber japonicus*) (Hunter et al. 1979), Atlantic cod (*Gadus morhua*) (Béland et al. 1999, Kouwenberg et al. 1999), plaice (*Pleuronectes platessa*) (Steeger et al. 1999), *Galaxias maculates* (Battini et al. 2000), sockeye salmon (*Oncorhynchus nerka*) (Novales Flamarique & Harrower 1999), and bluegill sunfish (*Lepomis macrochirus*)(Gutiérrez-Rodríguez & Williamson 1999). Brain and eye lesions have been detected after UVR exposure in larval northern anchovy, Pacific mackerel (Hunter et al. 1979), dab (*Limanda limanda*) and plaice (Dethlefsen et al. 2001). UVR attenuates the growth of larval northern anchovy, Pacific mackerel (Hunter et al. 1979) and plaice (Steeger et al. 1999). Metabolic depression indicated by decreased heart rate has been detected in larval plaice exposed to UVR (Steeger et al. 1999), while a very high flux of UVB results in the loss of buoyancy in dab (*Limanda limanda*) and plaice eggs (Dethlefsen et al. 2001).

Although adult fish are more tolerant than embryos and larvae, UVR may have effects on them as well. For example, both UVA and UVB potentially suppress the immune response in adult roach (*Rutilus rutilus*) (Salo et al. 1998, Jokinen et al. 2001), a most characteristic benthic species in Fennoscandia.

1.3 Protective mechanisms in fish

Fishes, like other organisms, have multiple ways to protect themselves from harmful effects of UVR. For some species and life stages avoidance appears to be the most important way of protection. For example many salmon and trout lay their eggs inside gravel (Koli 1998), where they are physically protected from UVR. Northern pike spawns amongst vegetation, and pike, European perch and yellow perch spawn the deeper the higher the UVR intensity is (Gillet & Dubois 1995, Williamson et al. 1997, Koli 1998). Many fishes, such as rainbow trout, have UV photoreceptors enabling them to see in the UVR region, and many species are negatively phototactic to UV light (Browman & Hawryshyn 1992, Thorpe et al. 1993, Leech & Johnsen 2003).

Many fishes produce, or acquire in their diet, UVR screening compounds. Melanin is a brownish pigment that absorbs in a wide range of the UV spectrum (Cockell & Knowland 1999). It is found in skin and eyes of many fishes and its production typically begins a few days before hatching (Ahmed & Setlow 1993, Lowe & Goodman-Lowe 1996, Speekmann et al. 2000, Häkkinen et

al. 2002). UVR induces melanin synthesis in some fishes–that means they tan like people after exposure to UVR (Lowe & Goodman-Lowe 1996, Häkkinen et al. 2000). There is some controversy about the protective role of melanin, however, because in addition to acting as a radical scavenger it can also produce reactive oxygen species (Korytowski et al. 1987, Bustamante et al. 1993).

Another group of UVR-screening compounds typically found in fishes are mycosporine-like amino acids (MAAs). The group of MAAs includes some 20 compounds that absorb in a wide range of UVR wavelengths (Shick & Dunlap 2002). Fishes lack the shikimate pathway by which MAAs are synthesized in algae and cyanobacteria. Thus they most likely acquire MAAs from their food, as observed in krill, sea hare, copepods and medaka (Carefoot et al. 1998, Mason et al. 1998, Moeller et al. 2005). MAAs are found in fish eyes and skin, and also in developing eggs, where the parents have sequestered them (Dunlap et al. 1989, Thorpe et al. 1993, Fabacher & Little 1995, Mason et al. 1998, Zamzow & Losey 2002). In addition to UV-screening, MAAs may function as antioxidants (Yakovleva et al. 2004).

If avoidance and screening compounds fail and damage by UVR takes place, repair mechanisms come into play. All cells have non-specific base excision repair mechanisms for repairing the DNA damage, and at least some fish species possess also a specific UVR-induced repair mechanism called DNA photolyase for repairing the UVR-induced damage (Shima & Setlow 1984, Applegate & Ley 1988, Ahmed & Setlow 1993, Thoma 1999). Fishes have chaperoning systems for repairing proteins and getting rid of too damaged proteins via ubiquitinylation and degradation, and antioxidants and antioxidative enzymes for quenching the UVR-produced active oxygen forms and for reducing oxidative stress (Dyer et al. 1991, Winston 1991, Sanders 1993, Duffy et al. 1999, Lewis et al. 1999, Charron et al. 2000).

1.4 Photoinduced toxicity

In addition to the direct harmful effects of UVR, indirect effects may be a risk to aquatic organisms. One of these is the photoinduced or photoenhanced toxicity of chemicals, which means that chemicals absorbing UVR become more toxic. Chemicals can either be photomodified to more toxic daughter compounds, or photosensitization reactions may take place. In photosensitization, the chemical is transformed from the low-energy ground state to a higher energy state, and this energy is then transferred to other molecules, creating reactive species and radicals that are potentially damaging (Larson & Berenbaum 1988). These processes can also occur simultaneously for the same compound; for example photomodification products of anthracene are more toxic than the parent compound, and these products can be further photosensitized or photomodified to yield even more toxic compounds and harmful intermediates

(Mallakin et al. 1999). Compounds that are known to be photoinduced include various polynuclear aromatic hydrocarbons (PAHs) such as pyrene, anthracene, fluoranthene and benzo(a)pyrene; other ring-containing structures such as azaarenes and humic compounds; and metals such as arsene, copper, cadmium and manganese (Oris & Giesy 1985, 1987, Larson & Berenbaum 1988, Arfsten et al. 1994, 1996, Krylov et al. 1997).

Retene (7-isopropyl-1-methylphenantrene) is a PAH formed from resin acids via action of anaerobic microbes or during incomplete combustion of resinous softwood e.g. in forest fires (Ramdahl 1983, Tavendale et al. 1997, Gabos et al. 2001). In natural waters, retene is mainly formed anaerobically from resin acids, and low background levels (ng/g dry weight) occur in sediments (Bouloubassi et al. 1997, Judd et al. 1998). In lake areas contaminated by treated pulp and paper mill effluents it is found in sedimenting particles (highest observed concentration 54 μg/g dw) as well as in sediment surface (500–1600 μg/g) (Leppänen & Oikari 1999, Leppänen et al. 2000, Lahdelma & Oikari 2005). Dissolution from sediments also occurs, and retene is bioavailable to fish (Oikari et al. 2001, Oikari et al. 2002a). Retene is teratogenic to fish, and it induces their mixed function oxygenase (MFO) system (Fragoso et al. 1998, Billiard et al. 1999, 2000). Retene is phototoxic to *Daphnia magna* at concentrations where no toxicity is observed in the absence of UVB (Huovinen et al. 2001).

1.5 Ecological relevance–hatching and phototaxis

Spring is the time of reproduction for many boreal aquatic organisms. Also most boreal freshwater fishes hatch in spring, right after the breaking up of ice. The coregonids used in this study, whitefish and vendace, both spawn in autumn, but hatch in spring (Koli 1998). Pike both spawns and hatches in spring (Koli 1998). All the species studied are positively phototactic in the larval stage, and thus likely to be exposed to UVR (Shkorbatov 1966, Urho et al. 1989, Viljanen et al. 1995, Zigler and Dewey 1995, Karjalainen et al. 1998).

2 OBJECTIVES

The purpose of this thesis was to evaluate the risk of UVB radiation to boreal freshwater fish larvae, and to explore the cellular and molecular targets and modes of action of UVB in these animals. The aim was to connect the effects at the cellular and molecular level to effects at the individual level.

More specifically, the following matters were examined:
1) UVR protection of fish egg shells, and UVR screening compounds in the embryo (I)
2) UVB-induced phototoxicity of a model PAH, retene (II, III)
3) Investigating whether reciprocity occurs between mortality and total UV dose (IV)
4) DNA damage as a primary insult of UVB, and its amount and location in whitefish and pike (V, VI)
5) Differences between species in UVB-induced changes at mRNA and protein levels, in mechanisms of damage, and in protective mechanisms (II–VI)

3 MATERIALS AND METHODS

The materials and methods are described in detail in the original articles (I–VI).

3.1 Animal models

Developing, unhatched eggs of rainbow trout (*Oncorhynchus mykiss* W.), whitefish (*Coregonus lavaretus* Svärdson *s.l.*), vendace (*Coregonus albula* L.), lake (brown) trout (*Salmo trutta* (L.) *m. lacustris*), sea trout (*Salmo trutta* (L.) *m. trutta*), Atlantic salmon (*Salmo salar* L.), Northern pike (*Esox lucius* L.) and European perch (*Perca fluviatilis* L.) were used (I). Three species were studied in larval stages: vendace (*C. albula*), whitefish (*C. lavaretus*) and Northern pike (*E. lucius*) (II–VI).

3.2 Egg shell transmittance measurements

Developing eggs were pushed through a metal sieve to obtain egg shells free of embryos, and the egg shells were washed in water. Egg shells were placed in a quartz cuvette in a uniform layer and their transmittances at 290–800 nm were recorded with Beckman DU-640 spectrophotometer (I).

3.3 Determination of UVR protective pigments

3.3.1 Determination of methanol-extractable substances

Eggs and egg shells were frozen in liquid nitrogen and preserved in –80 °C until analyzed. They were thawed quickly, weighed, homogenized and methanol

extracted (Fabacher & Little 1995, 1998). Briefly, 400 mg of eggs, egg shells, larvae or commercial fish food were homogenized with 1 ml 100 % methanol in 4 °C. The extract was centrifuged for 15 min, 3000 rpm in +4 °C, and the supernatant used for absorbance analysis between 250–400 nm on a Beckman DU-640 spectrophotometer (I).

3.3.2 Determination of melanin

Melanin concentrations in whole body of pike larvae were examined from animals from two lakes, Lake Lentua and Lake Päijänne, differing in their latitude and water characteristics (IV). Melanin concentrations were determined also from animals from both lakes that had been exposed to UVB. Total melanin was analyzed spectrophotometrically by a method developed for mammalian hair (Ozeki et al. 1995, 1996, Häkkinen et al. 2002). Heads of larvae were removed before homogenization to get rid of the eyes with high melanin content. Homogenized samples (20 mg tissue / ml distilled water) were placed in 10 ml capped glass test tubes, to which 1.8 ml Soluene-350 was added. The tubes were sonicated for 5 min, vortexed and placed in a boiling water bath for 30 min. After cooling and revortexing, the tubes were placed in the boiling water bath for 15 min. Cooled samples were analyzed for absorbances at 500 nm (A_{500}). The A_{500} value was converted to total melanin by referring it to the A_{500} value of solubilized sepia melanin standard.

3.4 Experimental UVB exposures

The UVB was provided in the laboratory using Q-Panel UVB-313 fluorescent lamps (II–VI). UVC was blocked using a cellulose diacetate filter (Clarifoil), which was replaced after each 3-hour UVB radiation. Control treatments without UVB received visible light (Philips TLD 36 W/950 daylight).

UV was quantified using Hamamatsu Photonic Multichannel Spectral analyser (model PMA-11), measuring the wavelength area 280–380 nm. The device was calibrated with a NIST-traceable lamp at the Finnish Meteorological Institute, and the fluence rates were calculated using the calibration curve, yielding fluence rates in mW/m^2. The CIE-weighted (Commission Internationale de l'Eclairage; International Commission on Illumination) doses, i.e., the action spectrum specific for human erythema, were calculated using a weighting function (McKinlay & Diffey 1987).

Fluence rates, irradiation times and daily doses in experiments are presented in Table 1. All UVB exposures were acute, lasting maximally 6 hours a day or three hours on two consecutive days (II–VI). The experiment was finished and animals taken as samples either immediately after irradiation (II, III), after monitoring the behaviour and mortality for up to 11 days (IV, V) or as a time series after irradiation (VI).

TABLE 1 Summary of UVB fluence rates, doses and irradiation times of laboratory experiments conducted with three Finnish freshwater fish species. All fluence rates and doses are human erythema weighted (CIE). Sample calculation relating fluence rate to dose: 260 mW/m² x 3 hours = 260 mW/m² x 10800 s = 2 808 000 mJ/m²= 2 808 J/m² ~2.8 k J/m².

Species	UVB fluence rate (mW/m²)	Fluence rate compared to mean fluence rate in May (132 mW/m²)	Daily UVB dose (kJ/m²)	Irradiation time	Daily dose compared to mean (1.66 kJ/m²) daily dose in May	Daily dose compared to maximum (3.0 kJ/m²) daily dose in May	Reference
Whitefish	260	200 %	2.8	2 x 3 h	170 %	90 %	II
(*Coregonus*	500	380 %	5.9		340 %	190 %	
lavaretus)	80	60 %	0.8	3 h	50 %	25 %	VI
	195	140 %	2.0		120 %	70 %	
Pike	92	70 %	1.0	2 x 3 h	60 %	30 %	III
(*Esox lucius*)	167	130 %	1.8		110 %	60 %	
	250	190%	2.7		160 %	90 %	
	80	60 %	0.4	1.5 h	25 %	10 %	IV, V
			0.8	3 h	50 %	25 %	
			1.7	6 h	100 %	50 %	
	190	140 %	1.0	1.5 h	60 %	30 %	
			2.0	3 h	120 %	70 %	
			4.1	6 h	250 %	140 %	
	300	230 %	1.6	1.5 h	100 %	55 %	
			3.3	3 h	200 %	110 %	
			6.5	6 h	400 %	220 %	
	540	410 %	2.9	1.5 h	170 %	95 %	
			5.9	3 h	340 %	190 %	
			11.7	6 h	680 %	380 %	
	80	60 %	0.8	3 h	50 %	25 %	VI
	185	140 %	2.0		120 %	70 %	
Vendace							
(*Coregonus albula*)	500	380 %	5.9	2 x 3 h	340 %	190 %	II

3.5 Acute phototoxicity experiments

In order to explore the possible photoinduced toxicity of polyaromatic hydrocarbons (PAHs) to vendace, whitefish and pike, retene was used as a model compound (II, III). Newly hatched larvae were pre-exposed for 24 h to several concentrations of retene (nominal concentrations 3.2–100 µg retene/l for all species, measured concentrations (III) 3–82 µg retene / l for pike) in Pyrex glass bowls. After the accumulation period, larvae were further exposed to retene. At the same time, they were irradiated with either UVB or visible light for 3 h a day on two consecutive days. The solutions were renewed daily before irradiation by replacing 60 % of each solution. Dead larvae were removed and counted twice daily, and the behaviour of larvae was monitored four times a day. Larvae were sampled after 72 h from the start by anesthetizing the larvae in MS222 (50 mg /l) and freezing in liquid nitrogen.

3.6 Mortality, behaviour, and growth measurements

Mortality and behaviour of larvae were monitored twice daily (II–V). At the end of the experiment (IV), the larvae were anesthesized (MS222, 50 mg/l), and the total length of viable larvae and the size of fry sac determined with the help of stereomicroscope. At the same context, the behavior of larvae was examined and the animals from each replicate were sorted into two groups, normally and abnormally behaving (= unable to swim straightforward) (IV).

3.7 Determination of UVB induced DNA damage

UVB-induced DNA damage, manifested as cyclobutane pyrimidine dimers (CPDs), was determined as whole body analysis (V, VI). Larvae frozen in liquid nitrogen were homogenized quickly with plastic homogenizers in eppendorf tubes, 500 µl extraction buffer (50 mM Tris, pH 8.0; 5 mM EDTA; 1% SDS; 0.25 M NaCl; 20 µg / ml proteinase-K) was added, and the tubes were placed on 55 ^{o}C heat block for a minimum of 3 hours. After this, DNA was purified using MOBIO Microbial DNA purification kit (MOBIO). The DNA was eluted in TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The concentration of DNA was analyzed fluorometrically using SYBR Gold dye (Molecular Probes) with lambda DNA as standard.

The CPD standards were prepared as described in Vetter et al. (1999). Briefly, larval whitefish and pike genomic DNA were irradiated with UVC lamp emitting at 254 nm for 12 s to obtain a dose of 12 J / m^2. UVC lamp was

used because the absolute number of CPDs/kb DNA produced by UVC is known from earlier studies (Bohr et al. 1985). Molecular weight of 640 000 per kb of DNA was assumed, and the absolute number of CPDs/kb DNA was determined based on studies by Bohr and coworkers (Bohr et al. 1985). So the absolute number of CPDs per kilobase DNA was not measured but derived from published measurements of other DNA, as was also done for northern anchovy (Bohr et al. 1985, Stapleton et al. 1993, Vetter et al. 1999).

Prior to blotting, extracted DNA was denatured by adding 0.1 volume 3 M NaOH and heating at 60 °C for 1 h. Ammonium acetate was added to obtain 1 M final concentration, and samples were vacuum blotted onto 0.45 μm cellulose acetate membranes (Protran™, Schleicher & Schuell, Germany) using a 72 well slot blotter (Minifold II™, Schleicher & Schuell, Germany). The membrane was baked for 1 h in 80 °C to fix the DNA onto the membrane.

The membranes were blocked with 9% (w/v) non-fat dry milk in TBS-T (tris-buffered saline–Tween 20). They were then washed with TBS-T and incubated in 1: 800 dilution of antibody against CPDs (TDM-2, provided by prof. Osamu Nikaido, Mori et al. 1991) for 1 h. After wash with TBS-T the membranes were incubated in 1: 3000 peroxidase labelled anti-mouse IgG (A9044, Sigma) for 30 min. The membranes were then washed and the immunodetection was performed via enhanced chemiluminescence. Scion Image 4.0.2 was used for quantification of immunoreactive bands.

3.8 Transcriptome analysis

The design of the microarray is described in detail elsewhere (Krasnov et al. 2005). In brief, the platform included 1300 genes printed in 6 replicates each (VI). The salmonid nucleotide sequences were compared with the known vertebrate proteins using blastx (Altschul et al. 1997) and 984 gene were identified; the functional annotations were transferred from the putative homologs. Random clones from common and subtracted cDNA libraries were supplemented with 300 genes selected by the categories of Gene Ontology. Overall, each microarray was enriched in a number of functional classes, such as stress and defense response (145 and 105 genes, respectively), cell cycle (62 genes), signal transduction (114 genes), chaperone activity (41 genes), and apoptosis (79 genes).

Larvae frozen and preserved in liquid nitrogen were homogenized quickly using glass homogenizers, and total RNA was extracted using Tri Reagent (Sigma). Labeling with Cy3- and Cy5-dCTP (Amersham Pharmacia) was made using SuperScript III (Invitrogen) and oligo(dT) primer; cDNA was purified with Microcon YM30 (Millipore). Each sample was hybridized to two microarrays. For the first slide, test and control cDNA were labeled with Cy5 and Cy3 respectively, and for the second array dye assignments were reversed. The slides were pretreated with 1% BSA, fraction V, 5 x SSC, 0.1% SDS (30 min

at 50°C) and washed with 2 x SSC (3 min) and 0.2 x SSC (3 min) and hybridized overnight in a cocktail containing 1.3 x Denhardt's, 3 x SSC 0.3% SDS, 0.67 µg/µl polyadenylate and 1.4 µg/µl yeast tRNA. All chemicals were from Sigma-Aldrich. Scanning was performed with ScanArray 5000 and images were processed with TIGR Spotfinder.

The measurements in spots were filtered by criterion (I-B)/(SI+SB)>1, where I and B are the mean signal and background intensities and SI, SB are the standard deviations. After subtraction of mean background, Lowess normalization (Cleveland et al. 1992) was performed separately with each slide. Using the dye swap design of hybridization, each gene was analyzed in 12 replicates. Data for the 2 slides were consolidated and differential expression was assessed by the difference of expression ratios at the reverse labeling (Student's t-test). In order to correct for multiple testing, we determined the false discovery rate (FDR) using Q-value (Storey and Tibshirani, 2003).

3.9 Determinations of heat shock protein 70, cytochrome P450 1A and p53 protein

Three inducible biomarker proteins, HSP70, CYP1A and p53 were measured in whole body tissue samples (II, III, V, VI). Specimens frozen in liquid nitrogen and preserved in -80 °C were homogenized with potassium gluconate buffer (pH 7.8), centrifuged, and the supernatant used for analysis. Total protein concentrations of the supernatants were analyzed by modified Lowry method (BioRad DC) (Lowry et al. 1951).

The HSP70, CYP1A and p53 determination was made by Western blot after sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) separation (20, 100 and 100 µg protein / lane, respectively); (Laemmli 1970, Towbin et al. 1979). The positive controls were heat-shocked vendace and whitefish larvae (20 µg protein / lane, II) or HSP70 from bovine brain (Sigma H9976, 50 ng protein / lane or slot, III–VI) for the HSP70, the liver of β-naphthoflavone injected juvenile whitefish (3.15 µg protein / lane, II–III) for CYP1A, and nuclear extract of MCF-7 cells exposed to benzo(a)pyrene (1 µg protein / lane, Turpeinen et al. 2002, V–VI). Proteins were transferred to a nitrocellulose membrane (Protran™, Schleicher & Schuell, Germany), which was blocked with 9% non-fat dry milk and probed with 1: 5000 anti-HSP70 (MA3-006, Affinity BioReagents, Inc.), 3 µg/ml anti-CYP1A (Mab 1-12-3, kindly provided by Dr. John Stegeman) or 1: 500 anti-p53 (NCL-p53-CM1, Novocastra Laboratories). After washing, the blot was probed with secondary antibody, 1: 3000 peroxidase labelled anti-mouse IgG (A9044, Sigma) for HSP70 and CYP1A or 1: 10 000 peroxidase labelled anti-rabbit IgG (81-6120, Zymed Laboratories) for p53. The immunodetection was performed via enhanced chemiluminescence, and Scion Image 4.0.2 was used for quantification of

immunoreactive bands. Blots were made comparable to each other by calibration with positive controls, fixing each positive control at a value of 1.

3.10 SOD analysis

Pike larvae were homogenized in 0.3 M phosphate buffer (pH 7.8), and the samples centrifuged at 600 x *g* for 10 min. Superoxide dismutase activity was measured from the supernatant with SOD-assay kit (Dodindo Molecular Technologies Inc.) (III). Protein concentration was measured from the supernatants by Bio Rad DC Protein Assay Kit.

3.11 Immunohistochemistry

The animals were dehydrated through a graded series of ethanol solutions up to 100 %, followed by xylene before embedding in paraffin (V). Animals were sectioned longitudinally along the vertical axis at 5 μm using a Leica microtome. The sections were placed on Superfrost Plus slides to avoid floating of the sections in later incubations. Fresh sections (< 48 h) were always used for the analysis.

Slides were deparaffinized with xylene followed by rehydration with a graded series of ethanol. In order to unmask the antigens, the slides were cooked in 10 mM sodium citrate, pH 6.0, in waterbath in microwave oven (Shi et al. 1993). Slides were then washed three times in water, and after that indigenous peroxidase was quenched with 3 % hydrogen peroxide, 1 % sodium azide in phosphate salt buffer. After washing, the slides were incubated with primary antibody (1: 1000 TDM-2 against cyclobutane pyrimidine dimers, 1: 1000 NCL-p53-CM1 against p53 or 1: 1000 MA3-006 against HSP-70) in 4 ^{o}C overnight. The next day the slides were washed with PBS and incubated with secondary antibody (1: 500 anti-rabbit-HRP for p53 slides and 1: 400 anti-mouse-HRP for others) for 30 min in room temperature. After washing the slides were stained with 3,3'-diaminobenzidine tetrahydrochloride (DAB), counterstained with Mayer's hematoxylin, dehydrated with a graded series of ethanol solutions up to 100 %, followed by xylene and mounted in DePex. Negative controls consisted of sections in which all the reagents were added with the exception of the primary antibody.

4 RESULTS AND DISCUSSION

4.1 Egg shell transmittance and UV-protective substances

The results show that fish egg chorion appears to have a role in protecting the developing embryo from UVR (I). The unhatched embryos of northern anchovy (*Engraulis mordax*) and Atlantic cod (*Gadus morhua*) are partly shielded by the egg shell from UVR, because less pyrimidine dimers are formed in them than in hatched larvae (Vetter et al. 1999, Browman et al. 2003).

4.1.1 Methanol-extractable UV-absorbing substances

The chorions of various boreal fish species and populations differed in both their visible light and UVR transmittance (I). The same was noticed for the copepod, *Nucella emarginata*: the spectral properties of capsule walls differed significantly between populations of *N. emarginata*, and among capsules laid by different species of *Nucella* (Rawlings 1996). However, the chorions of fish eggs contained no methanol-extractable substances absorbing in the UVB, UVA or visible light region of the spectrum, and thus the mechanism of UVR protection is not mycosporine-like amino acids (MAAs) (I). The same result was also observed in *N. emarginata* (Rawlings 1996).

The methanol-extractable substances found in the developing embryos and larvae possessed absorption peaks at 270, and 320–330 nm, and could be MAAs, working as sunscreens (Shick & Dunlap 2002). These compounds are synthetized by cyanobacteria and algae, and are acquired in the diet by adult fishes, especially females, which then transfer them into eggs and developing larvae (Mason et al. 1998, Shick and Dunlap 2002).

Though the species studied were fed with same commercial fish food, the amounts of ultraviolet radiation-absorbing methanol-extractable substances differed between species. Compared to the other fishes, the eggs of rainbow trout, sea trout and Atlantic salmon had a comparatively high absorption peak at 324 nm. These results show that species differ either in their ability to acquire

ultraviolet-screening substances from their diet, or in transformation of these substances e.g. by gut microflora (Shick & Dunlap 2002). This may be one reason for differences in sensitivity to UVR between species at embryonal stages.

4.1.2 Melanin

Though the larval pike from Lake Lentua seemed darker by eye than larvae from Lake Päijänne, there was no difference in the amount of melanin with the used analysis method. In addition, there was no difference in the amount of melanin between UVB-irradiated and unirradiated larvae. However, the amount of melanin was so small in all pike (0.38–1.33 μg/mg dry weight, average 0.8 μg/mg dry weight) compared e.g. to whitefish larvae (4–9 μg/mg dry weight, average 5.5 μg/mg dry weight; Häkkinen et al. 2002) that our method may not be able to discern any differences at such a low level.

The minute amount of melanin in pike larvae compared to coregonid larvae raises the question whether this lack of protective pigmentation is the reason for the extreme sensitivity of pike for UVB.

4.2 UV-induced changes in mortality, behaviour and growth

4.2.1 Mortality

Ultraviolet radiation caused no mortality in whitefish and vendace larvae (II, VI). In pike, however, increase in mortality was seen even with rather low fluence rates and doses (III, IV). The lowest fluence rate (80 mW/m^2 CIE) used in the experiments did not cause mortality except negligible late mortality with the longest irradiation time in youngest larvae (IV). The highest fluence rate (540 mW/m^2 CIE), on the other hand, led to 100% mortality in 100 h even with the shortest irradiation time (1.5 h, total cumulative dose 2.9 kJ/m^2 CIE) (IV).

When the two populations of pike were compared for the part of continuous irradiation for 6 h, Lake Lentua population was more tolerant when the dose was 4.1 or 6.5 kJ/m^2. In the reverse, with the lowest dose (1.7 kJ/m^2), the Lake Päijänne population was more tolerant. With the highest dose the mortality was similar in both populations. Of the animals irradiated for 2 x 3 h, the larvae from Lake Lentua were more tolerant against UVB than those from Lake Päijänne, except those irradiated with the lowest fluence rate (80 mW/m^2), where the mortality was similar (IV).

The six-day-old pike larvae were more tolerant than the 1-day-old ones with all doses except the lowest one. The time to 50% mortality was reached 1 to 10 hours earlier by the younger animals (IV).

When the mortality of the larvae that had been exposed to the same fluence rates and total doses, either continuously for 6 hours or 2 x 3 hours,

were compared, it was seen that in both populations the recovery time had an influence on the time to 50% mortality. However, the direct effect of the difference was difficult to compare by statistics as the same total dose was reached 21 hours later in the experiment based on 2 x 3 h irradiation periods, i.e. animals were that much more developed.

4.2.2 Behavioural disorder

No changes in behaviour were noticed in coregonids (II). In pike, all UVB fluence rates caused spiral swimming, i.e. the inability to swim straightforward (III, IV). Although there was almost complete recovery at the lowest total doses (0.4 and 0.8 kJ/ m^2 CIE) given at the lowest fluence rate (80 mW/ m^2 CIE), as well as marked recovery in the group of Lake Lentua larvae irradiated with lowest fluence rate for 2 x 3 hours (cumulative total dose 1.7 kJ/m^2 CIE), all other doses resulted in complete mortality.

4.2.3 Growth

When considering only the pike designated as normal at the end of the experiment, the control larvae were longer than irradiated ones, but there was no difference in the size of yolk sacs (IV). Larvae designated as abnormal for behaviour in the end of the experiment were shorter and possessed more yolk than larvae deemed to be normal. The larvae that had been exposed to UVB were shorter and possessed more yolk than controls (IV).

As the yolk represents predetermined amount of energy that can be used either for tissue growth or respiration, increased metabolism will result in a decrease of growth (Kamler 1992). Growth can be retarded due to DNA damage either due to the energy costs of repair or due to cell cycle arrest before complete repair takes place.

Part of the growth attenuation was due to the larvae's inability to absorb yolk (IV). This can be a result of defects in vascular development, also proposed as a reason for decrease in oxygen consumption allocated for digestion caused by high doses of UVB in whitefish larvae (Ylönen et al. 2004).

4.3 Acute phototoxicity of retene

Retene revealed no acute lethality to any of the species. However, in combination with UVB it was highly toxic to coregonids, with UVB doses that by themselves had no effect (II, Fig. 1a; Häkkinen et al. 2002, Ylönen & Karjalainen 2004). On the contrary, no photoinduced toxicity of retene was seen in pike (III, Fig. 1b). This could not be explained only by the fact that pike was much more sensitive to UVB alone than coregonids, as the same combination of

retene + UVB caused 100% mortality in coregonids but not in pike (II, III, Fig. 1a, b).

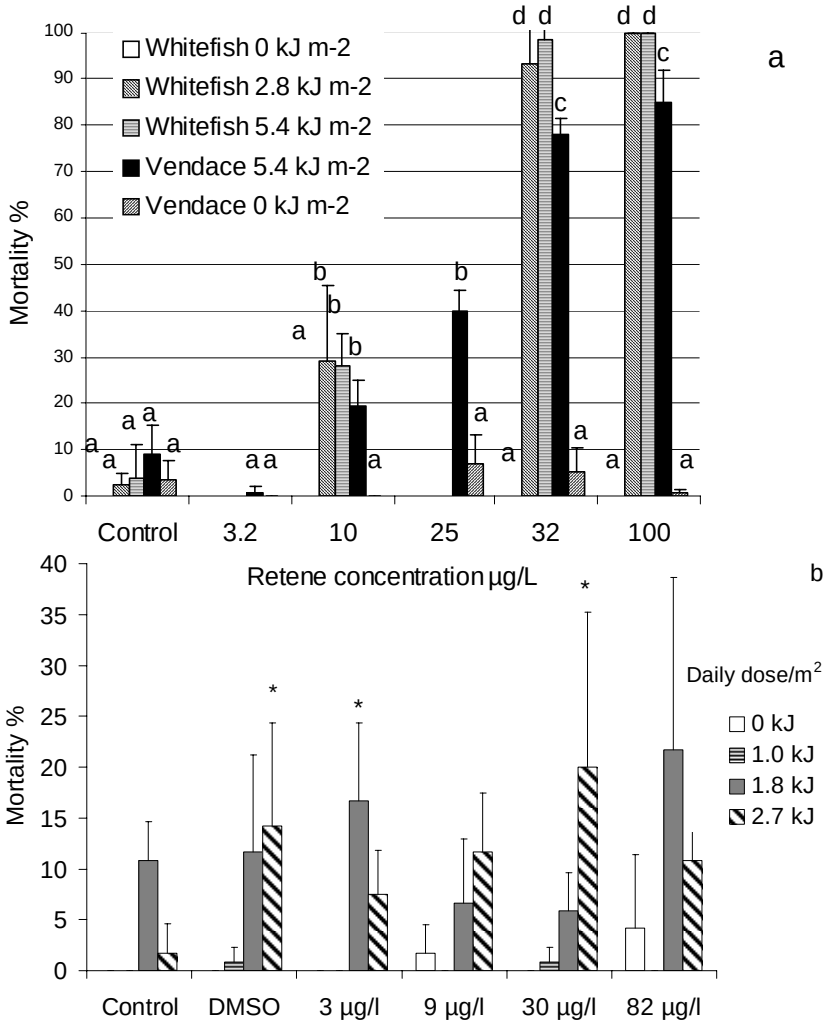

FIGURE 1 Mean mortality (± SD) caused by UVB irradiation and retene in fish larvae. a) Coregonids. Groups denoted by the same letter do not differ from each other. ($P > 0.05$). b) Pike. * indicates statistically significant ($P < 0.05$) difference from the control.

The reason for difference in vulnerability remains unclear, but it can rise from a multiple of reasons: 1. Pike need less oxygen than coregonids and therefore the damage to skin, a functional respiratory organ in this stage of development, is not as crucial to pike. 2. The damage to skin is due to photosensitization of internally transformed retene the amount of which depends on CYP1A activity, which is lower in pike (see below). 3. For some reason there is less damage to skin in pike than in coregonids.

The mechanism of retene phototoxicity in coregonids is not yet fully understood. Though photoproducts are formed when retene is irradiated (III), no toxicity can be seen by these products without further UV radiation in *Daphnia magna* nor primary cell culture of liver cells (Huovinen et al. 2001,

Vehniäinen et al. 2004). Simultaneous retene and UVB exposure causes severe damage to the skin of larval whitefish, suggesting that photosensitization of either retene or its photoproducts is the primary mode of action (Häkkinen et al. 2003). However, simultaneous exposure to retene and UVB causes also liver damage in larval whitefish, suggesting that photomodification products may be of importance (Häkkinen et al. 2003).

4.4 Reciprocity

The experiment undertaken testing reciprocity (IV)–i.e. if biological response to UVR depends solely on the total dose, independent of fluence rate and irradiation time–showed that fluence rate is of crucial importance when assessing the effects of UVR. Reciprocity was tested with larval pike by estimating the time to reach 50% mortality (ttd50%) values from the mortality curves, or, if 50% mortality was not reached, calculating it with Probit analysis (2 kJ/m^2 CIE), and testing if the doses and ttd50% values were in good linear relationship.

The results showed that in larval pike reciprocity held with intermediate fluence rates (190 and 300 mW/m^2 CIE). The lowest fluence rate of 80 mW/m^2 CIE did not cause mortality and, on the other hand, the highest fluence rate (540 mW/m^2 CIE) caused total mortality in 5 days no matter what the total dose was. When the fluence rate or the time of irradiation was constant, there was a linear relationship between dose and mortality.

In non-biological systems, reciprocity law failure commonly occurs both with very low and very high fluence rates (Martin et al. 2003). This is exactly what was observed in larval pike: reciprocity failed both with low and high fluence rates. In former experiments with fishes reciprocity has either held or not: with a broad range of doses and fluence rates, and by relatively long exposures (4 to 12 days), reciprocity did not hold in larval northern anchovy (Hunter et al. 1981). However, reciprocity held for cod embryos when exposures were short (1–5 h) and fluence rates high (unweighted fluence rate 1.43–13.7 W/m^2–cf. our highest unweighted fluence rate 1.7 W/m^2); (Kouwenberg et al. 1999). It was hypothesized (Kouwenberg et al. 1999) that reciprocity holds when damage dominates repair, i.e. when repair processes will not significantly compromise reciprocity (short and intense exposures). When repair dominates (low fluence rates, long exposures), reciprocity fails. Grad and coworkers (Grad et al. 2001) have hypothesized and partly proven that photoenzymatic repair may compromise reciprocity in zooplankton. This could explain why our lowest fluence rate resulted in less mortality than expected–the repair mechanisms in pike were possibly able to cope with the damage.

The model of Martin and coworkers (Martin et al. 2003) suggests that the high fluence reciprocity failures commonly seen in non-biological and

biological systems result from a different action mechanism, as described in the band theory. The same discontinuity in the effect of radiation at high fluence rates was seen in our experiments (IV). One explanation to the reciprocity failure is indeed the differential action mechanism, or more specifically the differential target tissue: the UV-specific DNA damage reaches deepest in tissues when irradiated with the highest fluence rate (V; see below).

4.5 DNA damage

The major form of lesions produced by UVB irradiation in DNA is the cyclobutane pyrimidine dimer (CPD) (Ahmed et al. 1993). Also other lesions, such as (6–4) pyrimidine photoproduct, may occur, but CPDs are most frequent (Ahmed et al. 1993, Meador et al. 2000, Yoon et al. 2000).

The cellular responses to DNA damage include alteration in gene expression, cell cycle delay, and apoptosis (Dasika et al. 1999, Kaina 2003). The response depends on the magnitude of DNA damage. Cell cycle delay gives the cells time to repair the damage, but if the damage is too extensive, the cell commits suicide by apoptosis. Prior to these events gene expression may change to the favour of cell cycle delay followed by DNA repair, or apoptosis.

Cells have multiple ways of repairing damage caused by UVB. Some organisms possess specific photoenzymatic repair systems, whereas some repair the damage with a more general nucleotide excision repair system, also called dark repair (Sancar 1994, Sancar 1996, Batty & Wood 2000). In photoenzymatic repair, the DNA damage is reversed with the help of energy from light, typically UVA or blue light (300–500 nm). For example, in many fish species this repair system is much more efficient than the dark repair (Mitchell et al. 1993). The dark repair works by hydrolyzing two phosphodiester bonds, one on either side of the damage, to generate an oligonucleotide carrying the damage. The excised oligonucleotide is then released from the duplex, and the resulting gap is filled in and ligated to complete the repair reaction (Sancar 1996, Wood 1996).

UVB induced CPDs in newly hatched and 7-day-old pike larvae, but the effect on whitefish was not statistically significant (VI, Fig. 2). Pyrimidine dimer loads in controls did not differ between species or age groups, but in UVB-irradiated larvae the following differencies were detected: with the lower fluence rate (80 mW/m^2 CIE) less CPDs were formed in whitefish than in pike of either age, and with the higher fluence rate (195 mW/m^2 CIE) more CPDs were found in newly hatched larval pike than in whitefish or in 7-day old pike. No differences in dimer loads were detected between time points, i.e. no repair of DNA damage was noticed.

Immunohistological examination revealed increasing cyclobutane pyrimidine dimer (CPD) formation with increasing fluence rate (V, Fig. 3a–c). The CPDs were not removed with time, and they were typically found in dorsal

and ventral skin, and with highest fluence rates (300 and 540 mW/m² CIE) also beyond skin in various tissues including eye, brain, heart, blood cells, gills, and layers surrounding yolk sac, the deeper in tissue the greater the fluence rate was (V). Slot blot analysis also confirmed these findings: The amount of CPDs in animals increased with increasing fluence rate (V). In whitefish CPDs were found only in the epidermis even when irradiated with 540 mW/m² CIE for 2 x 3 hours (Fig. 3d).

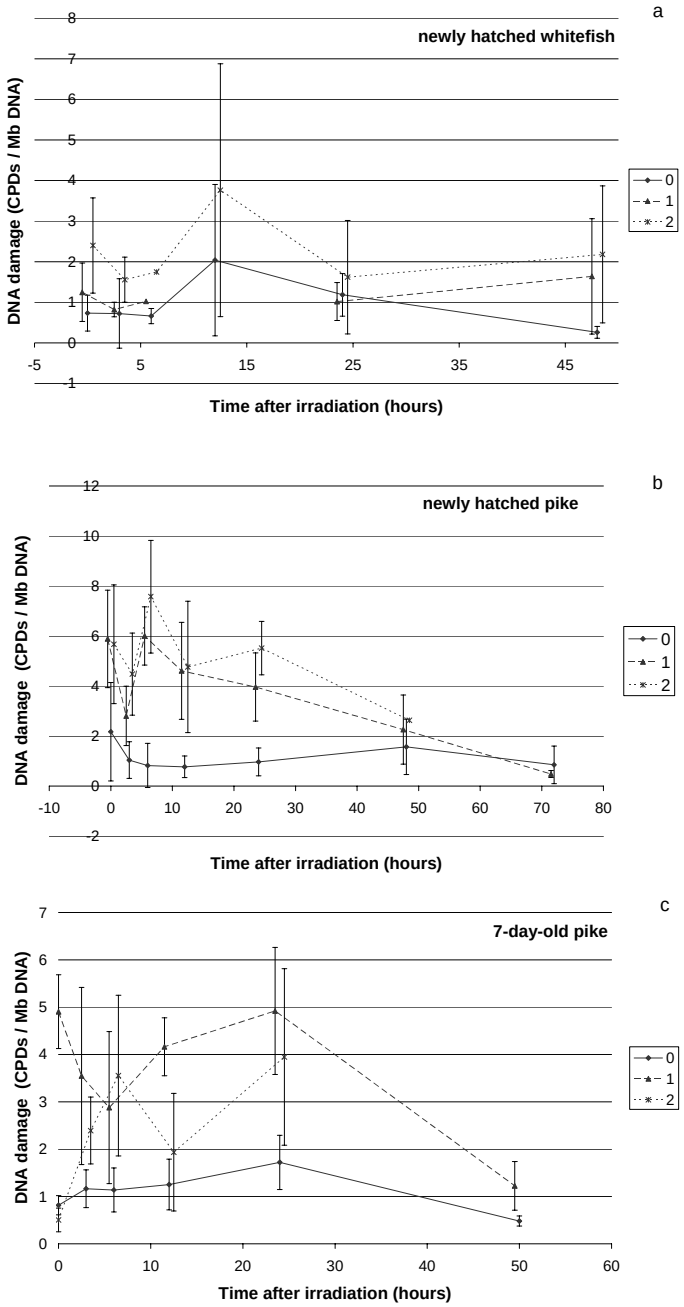

FIGURE 2 Mean (± SE) DNA damage manifested as cyclobutane pyrimidine dimers (CPDs). a) whitefish b) newly hatched pike c) 7-day-old pike. Fluence rates: 0 = control, no UVB; 1 = 80 and 2 = 190 mW/m² CIE.

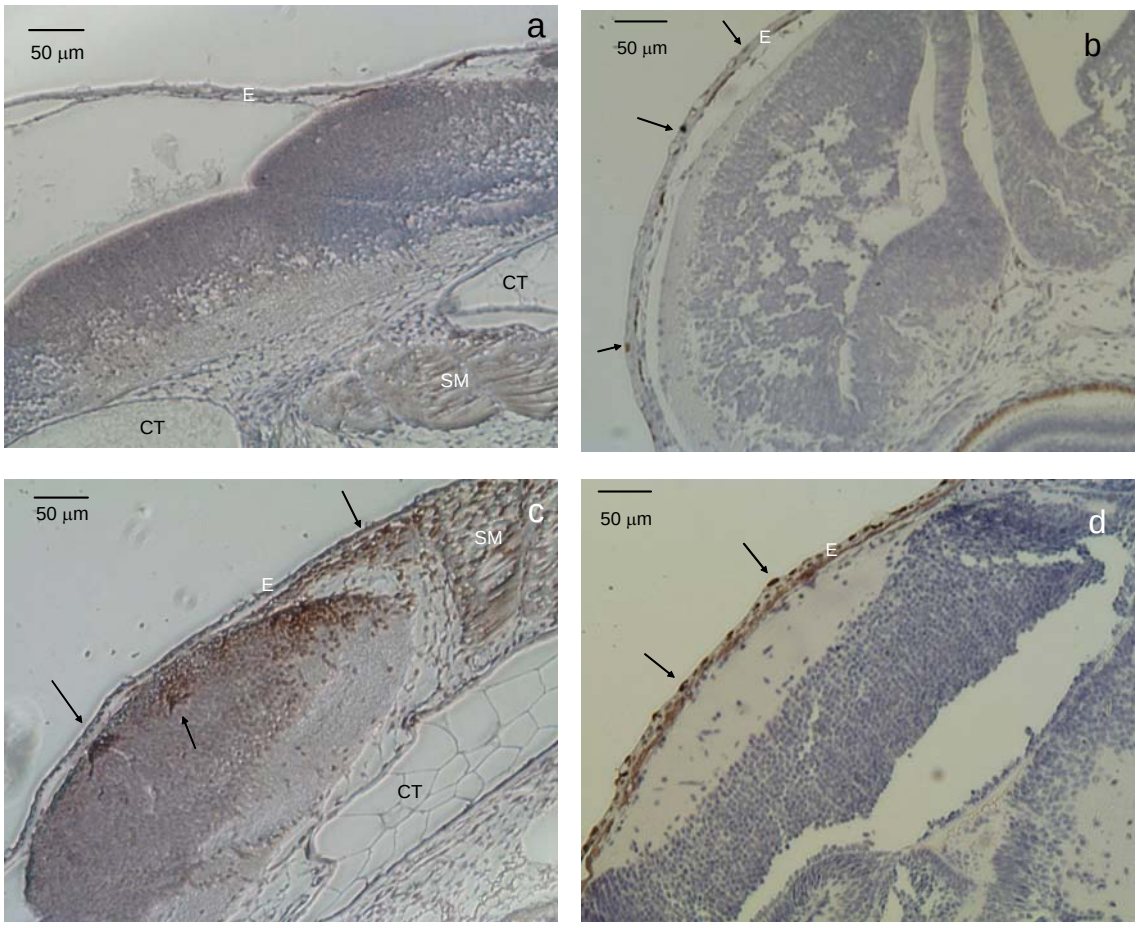

FIGURE 3 Immunohistochemical examination of cyclobutane pyrimidine dimers (CPDs) in the epidermis and brain of a) control newly hatched pike, b) newly hatched pike irradiated for 3 h with 190 mW/m² CIE UVB (2.0 kJ/m² CIE), c) pike irradiated for 3 h with 540 mW/m² CIE UVB (5.9 kJ/m² CIE), d) whitefish irradiated for 2 x 3 h with 540 mW/m² CIE UVB (11.7 kJ/m² CIE). Arrows point to CPD staining in the nuclei. E = epidermis, CT = connective tissue, SM = striated muscle.

4.6 UV-induced changes in transcription

Microarray analysis is a new and powerful technology for exploring transcriptomic responses to external or internal variables that specifically affect the transcriptome. Among other things, it has provided new information about the effects of UVR on mammalian cells and plants (Sesto et al. 2002, Casati and Walbot 2004, Howell et al. 2004, Koch-Paiz et al. 2004, McKay et al. 2004, Boerma et al. 2005, Lee et al. 2005, Molinier et al. 2005).

Ultraviolet radiation induced or repressed around 300 genes in whitefish and pike larvae. The changes observed were both species- and age-specific (VI).

There were altogether 230 genes that responded to UVR in newly hatched larval pike ($P < 0.01$). Of these 92 were down-regulated and 106 up-regulated by lower fluence rate of UVR, and 23 were down-regulated and 68 up-regulated by the higher fluence rate.

Altogether 130 genes responded to UVR in 7-day old larval pike ($P < 0.01$). Of these 36 were down-regulated and 37 up-regulated by the lower fluence rate of UVR and 27 were down-regulated and 44 up-regulated by the higher fluence rate.

In newly hatched whitefish 174 genes responded to UVR ($P < 0.01$): 64 were down-regulated and 67 up-regulated by the lower fluence rate, and 26 down-regulated and 27 up-regulated by the higher fluence rate.

UVB affected most the cytoskeleton category in all species and age groups, but the effect depended on species, age group and fluence rate (V). In whitefish, irradiated with either fluence rate, there were both up- and down-regulated members of this category. In newly hatched pike, UVB irradiation with the fluence rate of 80 mW/m^2 CIE down-regulated transcripts for cytoskeletal proteins, whereas the fluence rate of 195 mW/m^2 CIE up-regulated them. In 7-day-old larval pike both fluence rates down-regulated transcription of genes involved in cytoskeleton, the higher fluence rate even more prominently. Changes in gene expression of cytoskeletal components occur also in human keratinocytes exposed to UVB, either both down- and up-regulation with no clear trend (as seen in whitefish), or an early response of down-regulation (as seen in 7-day old pike and newly hatched pike irradiated with 80 mW/m^2 CIE) (Li et al. 2001, Sesto et al. 2002, Lee et al. 2005). The changes in cytoskeletal proteins in keratinocytes may first soften the cytoskeleton by depolymerization and then harden it again (Li et al. 2001), and this change in hardness of cytoskeleton and in cell shape may happen also in developing larval fishes.

One of the most prominent changes seen in whitefish irradiated with 195 mW/m^2 CIE was the strong down-regulation of transcripts of cytochromes and cytochrome oxidases. This suggests effects on mitochondria, as the translation products of these transcripts occur in the mitochondria. On the contrary, UVB-irradiated human keratinocytes show strong induction of cytochromes, cytochrome oxidases and other mitochondrial proteins, probably due to increased need of energy when protective and repair mechanisms are taking place (Li et al. 2001).

Very few changes in apoptosis-related transcripts were seen in these experiments. However, this lack of apoptosis-related transcripts among the most affected genes is not unique: the same is seen in UV-irradiated human keratinocytes (Li et al. 2001). Moreover, UVB may not only cause cell death by apoptosis but also by necrosis, a phenomenon seen in larval Japanese medaka (Armstrong et al. 2002).

The difference in transcriptome between species probably reflects difference in sensitivity, and that the effects UVB were reaching to dissimilar

tissues in different species and age groups, and when irradiated with different fluence rates. For example the only group that showed significant response to DNA damage by gene ontology was whitefish irradiated with the high UV dose, although more DNA damage was seen in pike. The results fit well with the known difference in tolerance between species: There is less DNA damage in whitefish (the more tolerant species), and moreover its DNA damage response is working while that of pike is not. The cells affected by UVB in pike also may be too damaged for the appropriate induction of DNA damage response. Moreover, the transcriptome even in the same tissue may differ between species and age groups of fast developing larvae. There may also be differences (e.g. due to protective pigmentation) between species and age groups in how deep in tissues the effects of UVB reach. To make the situation still more complicated, higher fluence rates may affect tissues deeper than those lower fluence rates do (V). In mammalian cells, differences are found between cell types in response to UVR (Boerma et al. 2005).

The differences between species, age groups and fluence rates, and also that only small (though statistically significant) differences were found between control and irradiated larvae, can also rise from the fact that the microarray analysis was done with whole body samples. UVB affects most prominently the outermost tissues, especially the epidermis. It would therefore have been ideal to use only the skin of larvae for the analysis, but as the skin at this point is only one or two cell layer thick and eventually impossible to separate from the underlying tissues, and as we even now had to pool several larvae to get enough RNA for the analysis, whole body analysis certainly was the only option. Anyway, the use of whole body samples may have diluted the transcriptomic message.

One of the first effects of UVB on mammalian cells is transcriptional arrest, so the relative changes in gene transcription products may reflect not only the induction or repression of genes by UVB but also ceasing of transcription in tissues affected by UVB (Sesto et al. 2002). Transcriptional arrest may have taken place also in the studied fish, and thus the difference in affected transcripts may again reflect difference in the specific tissue where the effects of UVB are reaching, difference between developmental stages and species in the transcriptome of affected tissues, or difference in half-life of transcripts between species and age groups.

4.7 UV-induced changes in protein expression and activity

4.7.1 HSP70

Heat shock protein 70 (HSP70) is a member of a group of proteins which possess conserved sequences between species and perform multiple tasks inside the cell. There are both constitutive and inducible forms of HSP70, and

their roles include helping other proteins fold correctly, helping them keep their right conformation, transferring proteins into their right compartment in the cell and tagging incorrectly folded proteins into ubiquitylation and degradation (Hartl 1996, Bukau & Horwich 1998). Moreover, HSP70 proteins are part of many multi-protein complexes inside the cell, and they also have a role in endo- and exocytosis and protecting cells from apoptosis (He & Fox 1997, Zinsmaier & Bronk 2001). Inducible forms of HSP70 are upregulated by various stressors including UVR and bleached kraft pulp mill effluent, and they are believed to have a protective role against tissue damage (Suzuki & Watanabe 1992, Sanders 1993, Janz et al. 1997).

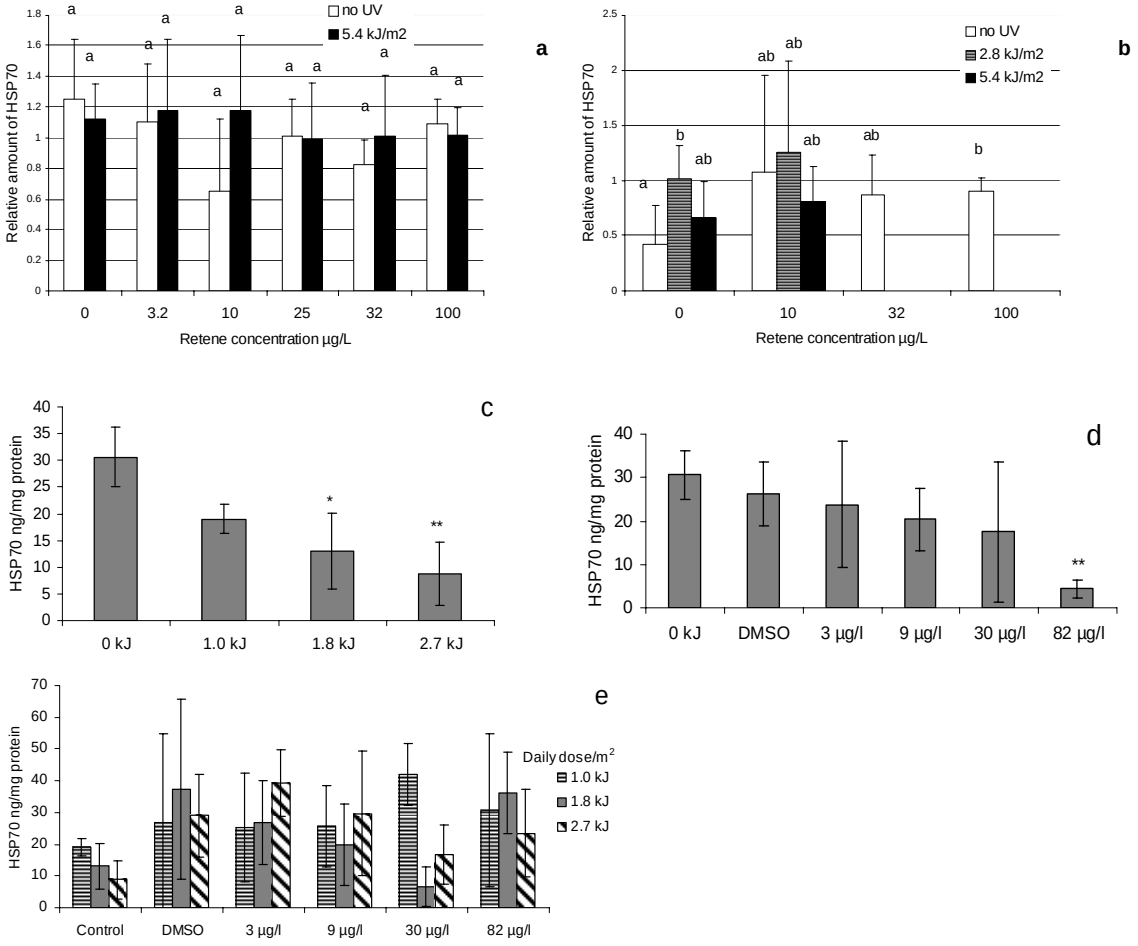

FIGURE 4 The mean amount of HSP70 (± SE) in a) vendace exposed to retene and UVB, b) whitefish exposed to retene and UVB, c) pike exposed to UVB, d) pike exposed to retene, and e) pike exposed to UVB and retene. In a) and b) groups denoted by the same letter do not differ significantly from each other ($P > 0.05$). In c–e significant difference from control is marked with * ($P < 0.05$) and ** ($P < 0.01$).

In vendace, neither UVB nor retene had an effect on HSP70 that was statistically significant (II, Fig. 4a). Also the HSP70 level of the positive control, heat shocked vendace, was similar to control, showing no induction (II). This means that retene, UVB radiation and heat shock were all inefficient in inducing

HSP70 in vendace. The treatments we applied may have been too mild or too short to upregulate HSP70 in this species.

In whitefish, on the other hand, the dose of 2.8 kJ/m^2 CIE caused an induction of HSP70 in retene non-exposed animals (Fig. 4b). Additionally, retene upregulated HSP70 slightly in larvae not exposed to UVB, but only the induction by the highest concentration was statistically significant because of high variability among individuals. The HSP70 level of the positive control, heat-shocked whitefish (transferred from 6 °C to 16 °C for 1 h), was 2.5 times that of the control fish. In whitefish irradiated with low UVB fluence rates no statistically significant effect on HSP70 was seen, even though there was an increasing trend with time (VI).

HSP70 induction is often considered as a protective and acclimatory response, and the inability to upregulate the expression of HSP70 usually correlates with tissue injury (Sanders 1993). This does not directly correlate with our findings where the more tolerant coregonid species, vendace, was unresponsive and the more sensitive species, whitefish, was able to upregulate HSP70. Presumably vendace has other protective mechanisms making it more tolerant than whitefish to UVB radiation.

In pike, HSP70 was down-regulated by retene in a dose-dependent manner and by UVR in a fluence rate-dependent manner, as seen in Western blots (III, V; Fig. 4c–g). Immunohistochemistry showed down-regulation especially in the brain of many individuals irradiated with the two highest fluence rates (V). In controls, high levels of HSP70s were found in the brain. In mammalian brain, both constitutive and inducible HSP70s are continuously expressed (Foster & Brown 1996). HSC70 has a role in regulating neurotransmitter endo- and exocytosis in mammalian nerve cells, and HSP70 helps to protect cells from apoptosis (He & Fox 1997, Zinsmaier & Bronk 2001). The down-regulation of HSP70 is presumably a result of cell damage, and maybe caused by protein degradation or the cessation of protein synthesis. High levels of toxic compounds evoke a similar response in fish: stress protein synthesis slows down or ceases in the mRNA level, and synthesis of house-keeping proteins is induced (Koskinen et al. 2004). In UV-irradiated pike larvae this damage in the brain is manifested as behavioural disorders.

With low ambient fluence rates, however, no statistically significant decrease in HSP70 levels in pike by UVR could be seen, though there was a decreasing trend with time (VI).

4.7.2 CYP1A

Cytochrome P4501A (CYP1A), a dominant heme-containing microsomal monooxygenase, catalyzes the first step in the biotransformation of many xenobiotics.

Subacute exposure of rainbow trout larvae to retene causes CYP1A induction (Billiard et al. 1999). Consistently, acute exposure to retene caused induction of CYP1A both in the presence and absence of UVB radiation in all species studied (II, III). In vendace and pike, the CYP1A induction was

approximately proportional to the concentration of retene, being greatest with the highest nominal concentration (100 μg/l), and UVB radiation alone caused no induction of CYP1A (II, III). In whitefish the induction was greatest with 32 μg/L retene, being slightly submaximal with the highest retene concentration applied (100 μg/l) (II). The inducibility of vendace was slightly higher than that of whitefish, and inducibility of pike was much lower than that of either coregonid (II, III).

The UVB fluence rate of 2.8 kJ/m^2 CIE caused CYP1A induction in whitefish larvae, but not in any other species (II, III). UVB induces CYP1A1 gene expression also in mammalian cells (Goerz et al. 1983). Irradiation of tryptophan with UV causes the formation of formylated indolocarbazoles, which are very potent Ah-receptor agonists (Wei et al. 1999). Thus this was probably the mechanism of induction also in whitefish. In whitefish larvae exposed to 5.4 kJ/m^2 CIE, no induction by UVB was detected, presumably because this high UVB intensity may have caused damage to the skin where the CYP1A induction would have taken place, as observed in our earlier study (Häkkinen et al. 2003).

4.7.3 p53 protein

The tumour suppressor protein p53 plays a major role in regulation of cellular stress response. In stressful conditions, such as hypoxia, hypoglycaemia, and DNA damage, p53 becomes active and in turn transcriptionally activates genes involved in cell cycle control, DNA repair, and apoptosis (Graeber et al. 1994, Ziegler et al. 1994, Amundson et al. 1998, Albrechtsen et al. 1999). Many factors contribute to control the activation of p53, and the downstream response also varies depending on the cellular environment or other modifying factors in the cell (Amundson et al. 1998, Albrechtsen et al. 1999). Protein p53 is also involved in embryonic development: it is typically expressed at high levels in developing tissues with a high number of proliferating and differentiating cells (Choi & Donehower 1999). The activation of p53 usually occurs at the translational level through phosphorylation by multiple kinases (Amundson et al. 1998, Giaccia & Kastan 1998, Albrechtsen et al. 1999, Jimenez et al. 1999).

In larval cod UV-B irradiation increases the amount of p53 (Lesser et al. 2001). In larval pike, however, no clear increase in p53 could be seen by Western blots. Overall, the staining was very faint, and the highest fluence rate (540 mW/m^2 CIE) resulted in a still fainter or no signal (V). In another series of experiments with similar fluence rates (80 and 195 mW/m^2 CIE) and three-hour irradiation, no p53 signal could be seen in Western blots (VI). This may have resulted from absence of p53 induction or too small an induction to be detected in whole-body samples. The antibody may also not have been specific enough for pike p53, as there is no knowledge on its specificity in this species.

Immunohistochemical sections showed very slight overall cytoplasmic staining in all larvae, assumed to be either normal constitutive cytoplasmic level of p53, or background staining (V). In irradiated larvae (fluence rates 190 and

300 mW/m² CIE) sampled right after irradiation an additional nuclear staining in the epidermis of the head region and the uppermost part of the brain could be seen (V). The nuclear form of p53 is the transcriptionally active one, activating specific genes involved in repair of DNA damage, apoptosis, or cell cycle delay; but p53 can trigger apoptosis also via a mitochondrial pathway (O'Brate & Giannakakou 2003). Interestingly, p53 and HSP70 form complexes in the nucleus, and this complex formation leads to p53 stabilization and activation, which may lead to apoptosis (Hinds et al. 1987, Clarke et al. 1988, Halevy et al. 1989). The nuclear p53 and HSP/HSC70 staining seen in some larvae may point to apoptosis, but this remains to be studied in the future.

4.7.4 SOD

As one line of defence against oxidative stress, superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion into hydrogen peroxide and molecular oxygen (Fridovich 1978).

In pike, neither UVB nor retene had an influence on SOD activity that was statistically significant (III). The trend was that SOD activity was slightly induced by both retene and UVB, but the findings were not statistically significant, partly because of high inter-individual variation.

The SOD activity in pike was very low compared to other fish species in different developmental stages: In adult zebrafish the SOD activity was over 200 times higher and in larval turbot ten times higher than in control larval pike (Peters & Livingstone 1996, Charron et al. 2000, III). This can be due to multiple factors: Firstly, both zebrafish and turbot were acclimated to temperatures higher than pike (26–30, 15–18 and 10 °C for zebrafish, turbot and pike, respectively). Both differences in enzyme kinetics in fish adapted to different temperatures (analysis was performed in room temperature for all species), and also presumably faster metabolism due to warmer body temperature (presumably also generating more of active oxygen species) in zebrafish and turbot than pike, can cause the difference. Secondly, yolk sacs of pike larvae may have interfered with the analysis (but so may also yolk sacs of larval turbot). The methods of analysis were also different.

In adult zebrafish irradiated with 0.15 or 1.95 W/m² UVB (unweighted) continuously for 0–24 hours the SOD activity peaked after 6 hours exposure and then fell down to control level (Charron et al. 2000). In pike larvae irradiated with 0.27–0.78 W/m² UVB (unweighted) for 2 x 3 hours there was no induction of SOD activity (III).

4.8 Future directions

Although this thesis gives answers to many important questions it also raises new questions to be answered, problems to be solved and hypotheses to be

tested. One of them is the identification of the methanol-extractable compounds, probably MAAs, in boreal fish eggs.

The ability of different species to absorb MAAs in their food and transfer them to eggs could be tested by feeding experiments in which the identities and amounts of MAAs are known exactly.

To test the real protecting effect of egg shells, the DNA damage in irradiated unhatched eggs of various species should be measured and compared to the differences in UV-transmittance of egg shells and methanol-extractable UV-absorbing compound content of the embryo.

Another interesting open question is the reason for the difference in photoinduced toxicity between species to retene and related PAHs, pike being more tolerant than the two coregonids. Also the actual mode of action of retene phototoxicity still remains to be resolved.

As well, the damage in the brain of pike, and the possible role of apoptosis in it, is one matter to be explored in more detail in the future.

After laboratory exposures, further field exposures would validate to observations and give more environmentally realistic views of risks caused by UVR.

In nature UVA and UVB are present together, and their individual actions are difficult to discern. This thesis has concentrated on the effects of UVB on fish, because the emphasis has been on the effects of ozone depletion. However, also UVA may have detrimental effects such as DNA damage and oxidative stress. In addition, UVA also has beneficial effects: for example, UVA is needed for the activation of DNA photolyase repairing UVB-induced DNA damage. Furthermore, UVA penetrates deeper in the water column. In the future, not only ozone loss, increasing the amount of UVB, but also climate change may change the amount of UVR and the ratio of UVA:UVB in aquatic environments, so the actions of UVA on larval freshwater fish should also be examined.

5 CONCLUSIONS

The aim of this work was to evaluate (a) the risks of current and enhanced UVB radiation to boreal freshwater fish larvae, and (b) to explore the cellular and molecular targets affected by this radiation. The hypothesis was that the cellular and molecular events taking place in UVB-irradiated larvae would work as early-warning biomarkers and help in evaluating the hazards caused by UVB radiation. To achieve this, the biomarkers at the cellular and molecular level had to be connected with endpoints at the individual level.

The most remarkable finding at the individual level was the difference between coregonids and pike in their sensitivity to UVB and to UVB-induced phototoxicity of retene. Compared to pike, the coregonids were very tolerant against UVB itself, but very sensitive to the photoinduced toxicity of retene. Pike may be harmed even by current UVB intensities, and increasing intensities may pose a further risk (Fig. 5). Whitefish and vendace, on the other hand, seem to be well protected from both current and predicted UVB intensities, but photoinduced toxicity of retene and most probably also other PAHs may pose a risk in contaminated waters (Fig. 5).

Growth attenuation of pike larvae was the most sensitive response at the individual level, taking place even with a CIE-weighted dose as low as 0.8 kJ/m^2 CIE (Fig. 5). The attenuation of growth often means also delay in development, prolonging the larval stages at which fish are most vulnerable. Thus it may have indirect effects also on survival of larvae and recruitment of population.

The conclusion from the test of reciprocity was that fluence rate is always of crucial importance when assessing the risk caused by UV radiation. There are differences between species, and maybe also between populations, in tolerable fluence rates and doses received, and therefore likely also in the ranges of fluence rates at which reciprocity holds. This must be taken into account especially when the risk evaluation is based on laboratory experiments.

UVB-tolerance was found to correlate with pigmentation: there was more melanin in whitefish (the more tolerant species) than pike, and the darker (by eye) Lake Lentua population was more tolerant than the fairer (by eye) Lake

Päijänne population. Also one-week-old pike larvae that were darker by eye were more tolerant than the fairer newly hatched ones.

The most remarkable finding at the cellular level was that the amount of DNA damage correlated with susceptibility to UVB and with the lack of pigmentation, growing in the order: whitefish < one-week-old pike < newly hatched pike. Pigmentation also protected the underlying tissues from DNA damage: in whitefish, CPDs were found only in the epidermis, above the melanin layer, whereas in pike CPDs were found also in tissues underneath the epidermis such as brain and muscle.

Another important finding at the cellular and molecular level was that the behavioural changes noticed in UVB-irradiated pike larvae are likely to be of neural origin. There was DNA damage in the brain of larval pike, and the levels of HSP/HSC70 decreased in the brain of irradiated animals with increasing fluence rate. The nuclear HSP/HSC70 and p53 staining seen in some individuals suggests that apoptosis may take place in the epidermis and brain of UVB-irradiated pike.

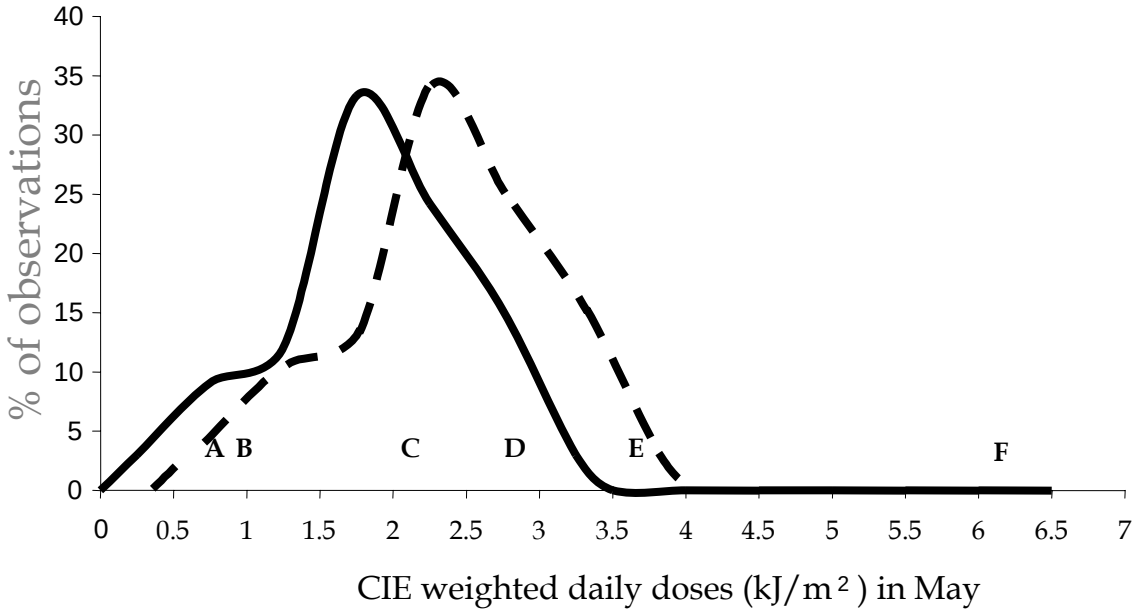

FIGURE 5 The frequency distributions of daily UVB doses in May 1998–2000 (solid line) and predicted for the future (dotted line) based on measurements done in Southern Finland (Jokioinen, Finnish Meteorological Institute) in May 1998–2000, and the scenarios suggesting 50% increase in UVB (Taalas et al. 2000). Letters denote biological endpoints evoked by experimental UVB doses (II–IV): A) Growth attenuation in pike, reversible behavioural disorder. B) Irreversible behavioural disorder in pike followed by mortality. C) Melanin induction in coregonids in two weeks (Häkkinen et al. 2002). D) Mortality by retene + UVB in coregonids in two days. E) 30% mortality of whitefish in two weeks (Ylönen & Karjalainen 2003). F) 60% mortality of whitefish in two weeks (Ylönen & Karjalainen 2003). Picture redrawn and extended from Oikari et al. (2002b).

For practical purposes in ecological risk assessment Figure 5, modified from our earlier version (Oikari et al. 2002b) summarizes the results obtained in this book and earlier studies. The endpoints responding to increasing daily doses are compared with current and predicted daily doses, and they clearly show that from the perspect of fish life, even current daily doses may be risky for the well-being of an individual animal.

Acknowledgements

I want to dedicate my warmest thanks to my supervisor prof. Aimo Oikari for giving me the opportunity to do my PhD thesis on such an interesting subject. I am especially thankful for his invaluable comments on my research plans and manuscripts. He has also been supportive and patient with me, and I recall talks with him that changed my moment of despair to (at least slight) hope.

I am grateful I have had the opportunity to work in such a supportive team. A big thank you to my colleagues Olli Ylönen and Jani Häkkinen for smooth cooperation and all the help. My warmest thanks to laboratory technician Mervi Koistinen whose motherly support has helped all the three of us complete our theses. Mervi has had just the sense of humour we needed in many bad situations, and the optimism we needed in the even worse situations.

My other "roommates" deserve a big thank you, too. I appreciate the ability of being able not only to share the room but also to share the same ambition for science with them. Aarno Karels is one of the most humane people I know. Ilpo Lahdelma solved many of my computer problems, and I learned much from inspiring talks and laboratory work with Maija Pesonen. Lately, I have had a great time with Päivi Meriläinen ("the strange woman") and Leena Sivula (who has e.g. brought my criticism against Microsoft to a completely new level). I have enjoyed the soap opera with you…

I thank prof. Juha Karjalainen for practical work and spirit-raising attitude in the beginning of the project, Jarno Heikkilä for help and a great sense of humour, and Maria Häkkinen, Asta Sarja and Juha Manninen for technical assistance. It has been a pleasure to work with skilful and helpful people such as laboratory technicians Leena Siitonen and Riitta Ryynänen, and technician Juha Ahonen. I also want to thank Marja Tiirola for help with DNA extraction, Ilmari Jokinen for help with computers and measurements, and Arja Mansikkaviita for much appreciated practical help with various issues. It has been a pleasure to work with nice and intelligent people at the Department of Biological and Environmental Science.

Warm thanks for co-work and good comments on my manuscripts to Aleksei Krasnov and prof. Kirsi Vähäkangas from the University of Kuopio.

I want to thank my family and friends for support. Special thanks to Anna-Kaisa Rikalainen who besides of being my sister is also cell biologist and whose work as a laboratory assistant was invaluable for me. It has been good to be able to share both professional and non-professional matters with her.

I am grateful I have the most loving and supportive husband there can be. Eero has been very understanding and patient with me even at the most stressful times, and moreover he has done a great job taking care of our son, Eelis (who in my point of view is the most valuable result acquired during my thesis work).

I want to thank the Academy of Finland (projects 105852 and 212377) and the Maj and Tor Nessling foundation for financial support.

Finally, I want to thank the Creator of life for everything He is and everything He has done in my life. Soli Deo Gloria.

YHTEENVETO (Resumé in Finnish)

Pohjoisen kalat ja ultraviolettisäteily–UV-säteilyn vaikutukset molekyyli- ja yksilötasolla

Ihmisen aiheuttama otsonikerroksen ohentuminen on lisännyt maan pinnalle saapuvan ultravioletti-B-säteilyn (UVB) määrää viime vuosikymmenten aikana, ja lisääntymisen odotetaan jatkuvan vielä noin 2050-luvulle saakka. UVB-säteily on haitallista eliöille, koska se aiheuttaa vaurioita DNA:ssa, proteiineissa ja lipideissä.

Tämän työn tarkoituksena oli arvioida lisääntyvän UVB-säteilyn aiheuttamaa riskiä taloudellisesti ja ekologisesti tärkeille makeanveden kalalajeillemme, siialle, muikulle ja hauelle, niiden herkimmässä elämänvaiheessa välittömästi kuoriutumisen jälkeen. Lisäksi tavoitteena oli selvittää UVB-säteilyn vaikutuksia näissä lajeissa solu- ja molekyylitasolla ja integroida havaittuja vaikutuksia yksilötason vasteisiin.

Tulokset osoittavat, että luonnossa nykyään esiintyvät ja tulevaisuudessa mahdolliset UVB-annokset eivät aiheuta suoraa riskiä siian ja muikun poikasille. Sen sijaan UVB-säteilyn yhdysvaikutukset reteenin ja muiden PAH-yhdisteiden kanssa saattavat aiheuttaa kuolleisuutta näissä lajeissa. Hauenpoikasten havaittiin olevan hyvin herkkiä UVB-säteilylle, ja jo nykyiset annostasot saattavat johtaa kasvun hidastumiseen, epänormaaliin käyttäytymiseen ja kuolemaan.

Pigmentaatioerojen havaittiin selittävän lajienvälistä herkkyyseroa: siiassa ja muikussa oli huomattavasti enemmän melaniinia kuin hauessa. Melaniini suojasi siian ihon alapuolisia kudoksia UVB-säteilyn aiheuttamilta DNA-vaurioilta, kun taas hauessa DNA-vaurioita havaittiin myös ihon alapuolisissa kudoksissa, mm. poikkijuovaisessa lihaskudoksessa ja aivoissa.

UVB-säteily aiheutti hauenpoikasten aivoissa DNA-vaurioita ja muutoksia proteiinien ilmentymisessä: Stressiproteiini HSP70:n määrä väheni säteilyä saaneiden poikasten aivoissa, ja joidenkin poikasten aivoissa havaittiin HSP70:n ja p53-proteiinin siirtymistä tumaan, joka viittaa ohjelmoituun solukuolemaan eli apoptoosiin. Nämä muutokset aivoissa selittävät poikasissa havaittua epänormaalia käytöstä.

REFERENCES

Ahmed, F. E. & Setlow, R. B. 1993: Ultraviolet radiation-induced DNA damage and its photorepair in the skin of the platyfish Xiphophorus. Cancer Res. 53: 2249–2255.

Ahmed, F. E., Setlow, R. B., Grist, E. & Setlow, N. 1993. DNA damage, photorepair, and survival in fish and human cells exposed to UV radiation. Environ. Mol. Mutagen. 22: 18–25.

Albrechtsen, N., Dornreiter, I., Grosse, F., Kim, E., Wiesmüller, L. & Deppert, W. 1999. Maintenance of genomic integrity by p53: complementary roles for activated and non-activated p53. Oncogene 18: 7706–7717.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, Z., Miller, W. & Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389–3402.

Amundson, S. A., Myers, T. G. & Fornace Jr., A. J. 1998. Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. Oncogene 17: 3287–3299.

Applegate, L. A. & Ley, R. D. 1988. Ultraviolet radiation-induced lethality and repair of pyrimidine dimers in fish embryos. Mutation Res. 198: 85–92.

Arfsten, D. P., Davenport, R. & Schaeffer, D. J. 1994. UV-A coexposure enhances the toxicity of aromatic hydrocarbons, munitions, and metals to Photobacterium phosphoreum. Biomed. Environ. Sci. 7: 101–108.

Arfsten, D. P., Schaeffer, D. J. & Mulveny, D. C. 1996. The effects of near ultraviolet radiation on the toxic effects of polycyclic aromatic hydrocarbons in animals and plants: A review. Ecotoxicol. Environ. Saf. 33: 1–24.

Battini, M., Rocco, V., Lozada, M., Tartarotti, B. & Zagarese, H. E. 2000. Effects of ultraviolet radiation on the eggs of landlocked Galaxias maculates (Galaxiidae, Pisces) in northwestern Patagonia. Freshwat. Biol. 44: 547–552.

Batty, D. P. & Wood, R. D. 2000. Damage recognition in nucleotide excision repair of DNA. Gene 241: 193–204.

Béland, F., Browman, H. I., Alonso Rodriguez, C. & St-Pierre, J.-F. 1999. Effect of solar ultraviolet radiation (280–400 nm) on the eggs and larvae of Atlantic cod (Gadus morhua). Can. J. Fish. Aquat. Sci. 56: 1058–1067.

Billiard, S. M., Querbach, K. & Hodson, P. 1999. Toxicity of retene to early life stages of two freshwater fish species. Environ. Toxicol. Chem. 18: 2070–2077.

Billiard, S. M., Hodson, P. V. & Bols, N. C. 2000. Does the potency of polycyclic aromatic hydrocarbons (PAHs) for inducing CYP1A1 in juvenile trout (Onchorhynchus mykiss) predict dioxin-like toxicity in early life stages? Mar. Environ. Res. 50: 308–309.

Blumthaler, M., Ambach, W. & Ellinger, R. 1997. Increase in solar UV radiation with altitude. J. Photochem. Photobiol. B: Biology 39: 130–134.

Boerma, M., van der Wees, C. G. C., Vrieling, H., Svensson, J.P., Wondergem, J., van der Laarse, A., Mullenders, L. H. F. & van Zeeland, A. A. 2005. Microarray analysis of gene expression profiles of cardiac myocytes and fibroblasts after mechanical stress, ionising or ultraviolet radiation. BMC Genomics 6:6 http://www.biomedcentral.com/1471-2164/6/6

Bohr, V. A., Smith, C. A., Okumoto, D. S. & Hanawalt, P. C. 1985. DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. Cell 40: 359–369.

Bouloubassi, I., Lipiatou, E., Saliot, A., Tolosa, I., Bayona, J. M. & Albaigés, J. 1997. Carbon sources and cycle in the western Mediterranean–the use of molecular markers to determine the origin of organic matter. Deep-Sea Res. Part II Top Stud. Oceanogr. 44: 781–799.

Browman, H. I. & Hawryshyn, C. W. 1992. Thyroxine induces a precocial loss of ultraviolet photosensitivity in rainbow trout (Oncorhynchus mykiss, Teleostei). Vision Res. 32: 2303–2312.

Browman, H. I., Vetter, R. D., Alonso Rodriguez, C., Cullen, J. J., Davis, R. F., Lynn, E. & St. Pierre, J.-F. 2003. Ultraviolet (280–400 nm)-induced DNA damage in the eggs and larvae of Calanus finmarchicus G. (Copepoda) and Atlantic cod (Gadus morhua). Photochem. Photobiol. 77: 397–404.

Bukau, B. & Horwich, A. L. 1998. The Hsp70 and Hsp60 chaperone machines. Cell 92: 351–366.

Bukaveckas, P. A. & Robbins-Forbes, M. 2000. Role of dissolved organic carbon in the attenuation of photosyntehtically active and ultraviolet radiation in Adirondak lakes. Freshwat. Biol. 43: 339–354.

Bustamante, J., Bredeston, L., Malanga, G. & Mordoh J. 1993. Role of melanin as a scavenger of reactive oxygen species. Pigment Cell Res. 6: 348–353.

Carefoot, T. H., Harris, M. Taylor, B. E., Donovan, D. and Karenz, D. 1998. Mycosporine-like amino acids: possible UV protection in eggs of the sea hare Aplysia dactylomela. Mar. Biol. 130: 389–396.

Casati, P. and Walbot, V. 2004. Rapid transcriptome responses of maize (Zea mays) to UV-B in irradiated and shielded tissues. Genome Biology 5:R16 http://genomebiology.com/2004/5/3/R16

Choi, J. & Donehower, L. A. 1999. p53 in embryonic development: maintaining a fine balance. Cell. Mol. Life Sci. 55: 38–47.

Cleveland, W. S., Grosse, E. & Shyu, W. M. 1992. Local regression models. In: Chambers, J. M. & Hastie, T. J. (eds), Statistical Models in S: 309–376. Wadsworth & Brooks/Cole, Pacific Grove, California.

Cockell, C. S. 1998. Ultraviolet radiation, evolution and the π-electron system. Biol. J. of the Linnean Soc. 63: 449–457.

Cockell, C. S. & Knowland, J. 1999. Ultraviolet radiation screening compounds. Biol. Rev. 74: 311–345.

Dasika, G. K, Lin, S.-C. J., Zhao, S., Sung, P. Tomkinson, A. & Lee E. Y.-H. P. 1999. DNA damage-induced cell cycle checkpoints and DNA strand break repair in development and tumorigenesis. Oncogene 18: 7883–7899.

Dethlefsen, V., von Westerhagen, H., Tüg, H., Hansen, P. D. & Dizer, H. 2001. Influence of solar ultraviolet-B on pelagic fish embryos: osmolality, mortality and viable hatch. Helgol. Mar. Res. 55: 45–55.

Dunlap, W. C., Williams, D. McB., Chalker, B. E. & Banaszak, A. T. 1989. Biochemical photoadaptation in vision: U.V.-absorbing pigments in fish eye tissues. Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol. 93: 601–607.

Dyer, S. D., Dickson, K. L., Zimmerman, E. G. & Sanders, B. M. 1991. Tissue specific patterns of heat shock protein synthesis and thermal tolerance of the fathead minnow (Pimephales promelas). Can. J. Zool. 69: 2021–2027.

Fabacher, D. L. & Little, E. E. 1995. Skin component may protect fishes from ultraviolet-B radiation. Environ. Sci. Pollut. Res. 2: 30–32.

Farman, J. C., Gardiner, B. G. & Shanklin, J. D. 1985. Large losses in total ozone in Antarctica reveal seasonal ClO_x/NO_x interaction. Nature 315: 207–210.

Foster, J. & Brown, I. 1996. Basal expression of stress-inducible hsp70 mRNA detected in hippocampal and cortical neurons of normal rabbit brain. Brain Res. 724: 73–83.

Fragoso, N. M., Parrott, J. L., Hahn, M. E. & Hodson, P. V. 1998. Chronic retene exposure causes sustained induction of CYP1A activity and protein in rainbow trout (Oncorhynchus mykiss). Environ. Toxicol. Chem. 17:2347–2353.

Frederic, J. E., Snell, H. E. & Haywood, E. K. 1989. Solar ultraviolet radiation at the earth's surface. Photochem. Photobiol. 50: 443–450.

Fridovich, I. 1978. The biology of oxygen radicals. Science 201: 875–880.

Gabos, S, Ikonomou, M. G., Schopflocher, D., Fowler, B. R., White, J., Prepas, E., Prince, D. & Chen, W. 2001. Characteristics of PAHs, PCDD/Fs and PCBs in sediment following forest fires in northern Alberta. Chemosphere 43: 709–719.

Gerstl, S. A. W., Zardecki, A. & Wiser, H. L. 1986. A new UV-B handbook. Vol. I. In: Worrest, R. C. & Caldwell, M. M. (eds), Stratospheric ozone reduction, solar ultraviolet radiation and plant life: 63–74. Springer-Verlag, Berlin, Heidelberg.

Giaccia, A. J. & Kastan, M. B. 1998. The complexity of p53 modulation: emerging patterns from divergent signals. Genes & Dev. 12: 2973–2983.

Gillet, C. & Dubois, J. P. 1995. A survey of the spawning of perch (Perca fluviatilis), pike (Esox lucius), and roach (Rutilus rutilus), using artificial spawning substrates in lakes. Hydrobiologia 300/301: 409–415.

Gleason, J. F., Bhartia, P. K., Herman, J. R., McPeters, R., Newman, P., Stolarski, R. S., Flynn, L., Labow, G., Larko, D., Seftor, C., Wellemeyer, C., Komhyr, W. D., Miller, A. J. & Planet, W. 1993. Record low global ozone in 1992. Science 260: 523–526.

Goerz, G., Merk, H., Bolsen, K., Tsambaos, H. & Berger, H. 1983. Influence of chronic UV-light exposure on hepatic and cutaneous monooxygenases. Experientia 39: 385–353.

Grad, G., Williamson, C. E. & Karapelou, D. M. 2001 Zooplankton survival and reproduction responses to damaging UV radiation: A test of reciprocity and photoenzymatic repair. Limnol. Oceanogr. 46: 584–591.

Graeber, T. G., Peterson, J. F., Tsai, M., Monica, K., Fornace, Jr. A. J. & Giaccia, A. J., 1994. Hypoxia induces accumulation of p53 protein, but activation of a G_1-phase checkpoint by low-oxygen conditions is independent of p53 status. Mol. Cell Biol. 14:6264–6277

Gutiérrez-Rodríguez, C. & Williamson, H. E. 1999. Influence of solar ultraviolet radiation on early life-history stages of the bluegill sunfish, Lepomis macrochirus. Environ. Biol. Fish. 55: 307–319.

Häkkinen, J., Vehniäinen, E., Ylönen, O., Heikkilä, J., Soimasuo, M., Kaurola, J., Oikari, A. & Karjalainen, J. 2002. The effects of increasing UV–B radiation on pigmentation, growth and survival of coregonid embryos and larvae. Environ. Biol. Fish. 64: 451-459.

Häkkinen, J., Vehniäinen, E. & Oikari, A. 2003. Histopathological responses of newly hatched larvae of whitefish (Coregonus lavaretus s.l.) to UVB induced toxicity of retene. Aquat. Toxicol. 63: 159–171.

Hartl, F. U. 1996. Molecular chaperones in cellular protein folding. Nature 381**:** 571–579.

He, L. & Fox, M. H. 1997: Variation of heat shock protein 70 through the cell cycle in HL-60 cells and its relationship to apoptosis. Exp. Cell. Res. 232: 64–71.

Howell, B. G., Wang, B., Freed, I., Mamelak, A. J., Watanabe, H. & Sauder, D. N. 2004. Microarray analysis of UVB-regulated genes in keratinocytes: downregulation of angiogenesis inhibitor thrombospondin-1. J. Dermatol. Sci. 34: 185–194.

Hunter, J. R., Taylor, J. H. & Moser, H. G. 1979. Effect of ultraviolet radiation on eggs and larvae of the Northern anchovy, Engraulis mordax, and the Pacific mackerel, Scomber japonicus, during the embryonic state. Photochem. Photobiol. 29: 325–338.

Hunter, J. R., Kaupp, S. E. & Taylor, J. H. 1981. Effects of solar and artificial ultraviolet-B radiation on larval northern anchovy, Engraulis mordax. Photochem. Photobiol. 34: 477–386.

Huovinen, P. S., Penttilä, H. & Soimasuo, M. R. 2000. Penetration of UV radiation into Finnish lakes with different characteristics. Int. J. Circumpolar Health 59: 15–21.

Huovinen, P. S., Soimasuo, M. R. & Oikari, A. O. J. 2001. Photoinduced toxicity of retene to Daphnia magna under enhanced UV-B radiation. Chemosphere 45: 683–691.

Huovinen, P. S., Penttilä, H. & Soimasuo, M. R. 2003. Spectral attenuation of solar ultraviolet radiation in humic lakes in Central Finland. Chemosphere 51: 205–214.

Janz, D. M., McMaster, M. E., Munkkitrick, K. R. & Van Der Kraak, G. 1997. Elevated ovarian follicular apoptosis and heat shock protein-70 expression

in white sucker exposed to bleached kraft pulp mill effluent. Toxicol. Appl. Pharmacol. 147: 391–398.

Jimenez, G. S., Khan, S. H., Stommel, J. M. & Wahl, G. M. 1999. p53 regulation by post-translational modification and nuclear retention in response to diverse stresses. Oncogene 18: 7656–7665.

Jokinen, I., Salo, H., Markkula, E., Immonen, A. & Aaltonen, T. 2001. Ultraviolet B irradiation modulates the immune system of fish (Rutilus rutilus, Cyprinidae) III: Lymphocytes. Photochem. Photobiol. 73: 505–512.

Judd, M. C., Stuthridge, T. R. & Price, R. W. 1998. Pulp mill sourced organic compounds from New Zealand sediments part 3: Mechanical pulp mills and remote sites. Chemosphere 36: 2311–2320.

Jurkiewicz, B. A. & Buettner, G. R. 1994. Ultraviolet light-induced free radical formation in skin: an electron paramagnetic resonance study. Photochem. Photobiol. 59: 1–4.

Kaina, B. 2003. DNA damage-triggered apoptosis: critical role of DNA repair, double-strand breaks, cell proliferation and signalling. Biochem. Pharmacol. 66: 1547–1554.

Kamler, E. 1992. Early Life History of Fish. An energetics approach. 267 p. Chapman & Hall, London.

Karjalainen, J., Ollikainen, S., & Viljanen, M. 1998. Estimation of the year-class of newly hatched fish larvae in Finnish lakes–how sampling design can influence abundance estimations. Arch. Hydrob. Spec. Issues Adv. Limnol. 50: 73–80.

Koch-Paiz, C. A., Amundson, S. A., Bittner, M. L., Meltzer, P. S. & Fornace Jr., A. J. 2004. Functional genomics of UV radiation responses in human cells. Mutat. Res. 549: 65–78.

Koli, L. 1998. Suomen kalat. 357 p. Werner Söderström Osakeyhtiö, Porvoo, Finland.

Korytowski, W., Pilas, B., Sarna, T. & Kalyaraman, B. 1987. Photoinduced generation of hydrogen peroxide and hydroxyl radicals in melanins. Photochem. Photobiol. 45: 185–190.

Koskinen, H., Pehkonen, P., Vehniäinen, E., Krasnov, A., Rexroad, C., Afanasyev, S., Mölsä, H. & Oikari, A. 2004. Response of rainbow trout transcriptome to model chemical contaminants. Biochem. Biophys. Res. Commun. 320: 745–753.

Kouwenberg, J. H. M., Browman, H. I., Cullen, J. J., Davis, R. F., St-Pierre, J.-F. & Runge, J. A. 1999. Biological weighting of ultraviolet (280–400 nm) induced mortality in marine zooplankton and fish. I. Atlantic cod (Gadus morhua) eggs. Mar. Biol. 134: 269–284.

Krasnov, A., Koskinen, H., Pehkonen, P., Rexroad III, C. E., Afanasyev, S. & Mölsä, H. 2005. Gene expression in the brain and kidney of rainbow trout in response to handling stress. BMC Genomics 6:3 http://www.biomedcentral.com/1471-2164/6/3

Krylov, S. N., Huang, X.-D., Zeiler, L. F., Dixon, D. G. & Greenberg, B. M., 1997. Mechanistic quatitative structure-activity relationship model for the

photoinduced toxicity of polycyclic aromatic hydrocarbons: I. Physical model based on chemical kinetics in a two-compartment system. Environ. Toxicol. Chem. 16: 2283–2295

Larsen, R. A. & Berenbaum, M. R. 1998. Environmental phototoxicity. Solar ultraviolet radiation effects the toxicity of natural and man-made chemicals. Environ. Sci. Technol. 22: 354–360.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T. Nature 227: 680–685.

Lahdelma, I. & Oikari, A. 2005. Resin acids and retene in sediments adjacent to pulp and paper industries. J. Soils & Sediments (in press) DOI: http://dx.doi.org/10.1065/jss2005.05.139

Lee, K. M., Lee, J. G., Seo, E. Y., Lee, W. H., Nam, Y. H., Yang, J. M., Kee, S. H., Seo, Y. J., Park, J. K., Kim, C. D. & Lee, J. H. 2005. Analysis of genes responding to ultraviolet B irradiation of HaCaT keratinocytes using a cDNA microarray. Br. J. Dermatol. 152:52–59.

Leech, D.M. & S. Johnsen. 2003. Avoidance and UV vision. In: Helbling, W. & Zagarese, H. (eds.), UV Effects in Aquatic Organisms and Ecosystems: 455–484. Royal Society of Chemistry, London.

Leppänen, H. & Oikari, A. 1999. Occurrence of retene and resin acids in sediments and fish bile from a lake receiving pulp and paper mill effluents. J. Environ. Toxicol. Chem. 18: 1498–1505.

Leppänen, H., Kukkonen, J. V. K. & Oikari, A. 2000. Concentration of retene and resin acids in sedimenting particles collected from a bleached kraft mill effluent receiving lake. Wat. Res. 34: 1604–1610.

Lewis, S., Handy, R. D., Cordi, B., Billinghurst, Z. & Depledge, M. H. 1999. Stress proteins (HSP's): Methods of detection and their use as an environmental biomarker. Ecotoxiology 8: 351–368.

Lowe C. & Goodman-Lowe G. 1996. Suntanning in hammerhead sharks. Nature. 383: 677.

Lowry, O. H., Rosebrough, N.J., Farr, A. L. & Randall, R. J. 1951. Protein measurements with Folin phenol reagent. J. Biol. Chem. 193: 265–275.

Madronich, S., McKenzie, R. L., Caldwell, M. M. & Björn, L. O. 1995. Changes in ultraviolet radiation reaching the earth surface. Ambio 24: 143–152.

Madronich, S., McKenzie, R. L., Björn, L. O. & Caldwell, M. M. 1998. Changes in biologically active ultraviolet radiation reaching the Earth's surface. J. Photochem. Photobiol. B: Biology 46: 5–19.

Mallakin, A., McConkey, B. J., Miao, G., McGibben, B., Snieckus, V., Dixon, D. G. & Greenberg, B. M. 1999. Impacts of structural photomodification on the toxicity of environmental contaminants: anthracene photooxidation products. Ecotoxicol. Environ. Saf. 43: 204–212.

Martin, J. W., Chin J. W. & Nguyen, T. 2003. Reciprocity law experiments in polymeric photodegradation: a critical review. Progress in Organic Coatings 47: 292–311.

Mason, D. S., Schafer, F., Shick, J. M. & Dunlap, W. C. 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids (MAAs) are aqcuired

from their diet by medaka fish (Oryzias latipes) but not by SKH-1 hairless mice. Comp. Biochem. Physiol. A 120: 587–598.

McKay, B. C., Stubbert, L. J., Fowler, C. C., Smith, J. M., Cardamore, R. A. & Spronck, J. C. 2004. Regulation of ultraviolet light-induced gene expression by gene size. Proc. Natl. Acad. Sci. USA 101: 6582–6586.

McKinlay, A. F. & Diffey, B. L. 1987. A reference action spectra for ultraviolet induced erythema in human skin. In: Passchier, W. R. and Bosnajakovich, B. M. F. (eds), Human Exposure to Ultraviolet Radiation: Risks and Regulations: 83–87. Elsevier, Amsterdam, Netherlands.

Meador, J. A., Walter, R. B. & Mitchell, D.L. 2000. Induction, distribution and repair of UV photodamage in the platyfish, Xiphophorus signum. Photochem. Photobiol. 72: 260–266.

Mims, F. M. & Frederick, J. E. 1994. Cumulus clouds and UVB. Nature 371: 291.

Mitani, H. & Shima, A. 1995. Induction of cyclobutane pyrimidine dimer photolyase in cultured fish cells by fluorescent light and oxygen stress. Photochem. Photobiol. 61: 373–377.

Mitani, H., Uchida, N. & Shima, A. 1996. Induction of cyclobutane pyrimidine dimer photolyase in cultured fish cells by UVA and blue light. Photochem. Photobiol. 64: 943–948.

Mitchell, D. L., Scoggins, J. T. & Morizot, D. C. 1993. DNA repair in the variable platyfish (Xiphophorus variatus) irradiated in vivo with ultraviolet B light. Photochem. Photobiol. 58: 455–459.

Moeller, R. E., Gilroy, S., Williamson, C. E., Grad, G. & Sommaruga, R. 2005. Dietary acquisition of photoprotective compounds (mycosporine-like amino acids, carotenoids) and acclimation to ultraviolet radiation in a freshwater copepod. Limnol. Oceanogr. 50: 427–439.

Molinier, J., Oakeley, E. J., Niederhauser, O., Kovalchuk, I. & Hohn, B. 2005. Dynamic response of plant genome to ultraviolet radiation and other genotoxic stresses. Mutat. Res. 571: 235–247.

Mori, T., Nakane, M., Hattori, T., Matsunaga, T., Ihara, M. & Nikaido, O. 1991. Simultaneous establishment of monoclonal antibodies specific for either cyclobutane pyrimidine dimers or (6–4) photoproduct from the same mouse immunized with ultraviolet radiated DNA. Photochem. Photobiol. 54: 225–232

Morris, D. P. & Hargreaves, B. P. 1997 The role of photochemical degradation of dissolved organic carbon in regulating the UV transparency of three lakes on the Pocono Plateau. Limnol. Oceanogr. 42: 239–249.

Morris, D. P., Zagarese, H. E., Williamson, C. E., Balseiro, E. G., Hargreaves, B. R., Modenutti, B., Moeller, R. & Queimaliños, C. 1995. The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. Limnol. Oceanogr. 40: 1381–1391.

Novales Flamarique, I. & Harrower, W. L. 1999. Mortality of sockeye salmon raised under light backgrounds of different spectral composition. Environ. Biol. Fish. 55: 279–293.

O'Brate, A. & Giannakakou, P. 2003. The importance of p53 location: nuclear or cytoplasmic zip code? Drug Resistance Updates 6: 313–322.

Oikari, A., Cherr, N. G & Hodson, P. V. 2001. Dissolution, bioavailability and toxicity of retene in industrial sediment. Abstracts, ICT9 Congress in Brisbane, Australia, July 6–12, p 247.

Oikari, A., Fragoso, N., Leppänen, H., Chan, T., Kozin, I & Hodson, P. V. 2002a. Bioavailability to juvenile rainbow trout (Onchorhynchus mykiss) of retene and other mixed function oxygenase-active compounds from sediments. Environ. Toxicol. Chem. 21: 121–128.

Oikari, A., Häkkinen, J., Karjalainen, J., Vehniäinen, E. & Ylönen, O. 2002b. Sensitivity of boreal fish larvae to UV-B radiation: a preliminary risk assessment. In: Käyhkö, J. & Talve, L. (eds), Understanding the global system–the Finnish perspective: 147–152. Finnish Global Change Research Programme FIGARE, University of Turku.

Oris, J. T. & Giesy, J. P. 1985. The photoenhanced toxicity of anthracene to juvenile sunfish (Lepomis spp.). Aquat. Toxicol. 6: 133–146.

Oris, J. T. & Giesy, J. P. 1987. The photo-induced toxicity of polycyclic aromatic hydrocarbons to larvae of the fathead minnow (Pimephales promelas). Chemosphere 16: 1395–1404.

Ozeki, H., Ito, S., Wakamatsu, K. & Hirobe, T. 1995. Chemical characterization of hair melanins in various coat-color mutants of mice. J. Invest. Dermatol. 105: 361–366.

Ozeki, H., Ito, S., Wakamatsu, K. & Thody, A. J. 1996. Spectrophotometric characterization of eumelanin and pheomelanin in hair. Pigment Cell Res. 9: 265–270.

Ramdahl, T. 1983. Retene–a molecular marker of wood combustion in ambient air. Nature 306: 580–582.

Rawlings, T. A. 1996. Shields against ultraviolet radiation: an additional protective role for the egg capsules of benthic marine gastropods. Mar. Ecol. Prog. Ser. 136: 81–95.

Rosette, C. & Karin, M. 1996. Ultraviolet light and osmotic stress: Activation of the JNK cascade through multiple growth factor and cytokine receptors. Science 274: 1194–1197.

Salo, H., Aaltonen, T., Markkula, E. & Jokinen, I. 1998. Ultraviolet B irradiation modulates the immune system of fish (Rutilus rutilus, Cyprinidae) I: Phagosytes. Photochem. Photobiol. 67: 433–437.

Sancar, A. 1994. Structure and function of DNA photolyase. Biochemistry 33: 2–9.

Sancar, A. 1996. DNA excision repair. Annu. Rev. Biochem. 65: 43–81.

Sanders, B. M. 1993. Stress proteins in aquatic organisms: An environmental perspective. Crit. Rev. Toxicol. 23: 49–75.

Schindler, D. W., Curtis, P. J., Parker, B. R. & Stainton, M. P. 1996. Consequences of climate warming and lake acidification for UV-B penetration in North American boreal lakes. Nature 379: 705–708.

Schwarz, T. 1998. UV light affects cell membrane and cytoplasmic targets. J. Photochem. Photobiol. B: Biology 44: 91–96.

Scully, N. M. & Lean, D. R. S. 1994. The attenuation of ultraviolet radiation in temperate lakes. Arch. Hydrobiol. Spec. Issues. Advanc. Limnol. 43: 135–144.

Sesto, A., Navarro, M., Burslem, F. & Jorcano, J. L. 2002. Analysis of the ultraviolet B response in primary human keratinocytes using oligonucleatide microarrays. Proc. Natl. Acad. Sci. USA 99: 2965–2970.

Shi, S. R., Chaiwun, B., Young, L., Cote, R. J. & Taylor, C. R. 1993. Antigen retrieval technique utilizing citrate buffer or urea solution for immunohistochemical demonstration of androgen receptor in formalin-fixed paraffin sections. J. Histochem. Cytochem. 41: 1599–1604.

Shick, J. M. & Dunlap, W. C. 2002. Mycosporine-like amino acids and related gadusols: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu. Rev. Physiol. 64: 223–262.

Shima, A. & Setlow, R. B. 1984. Survival and pyrimidine dimers in cultured fish cells exposed to concurrent sun lamp ultraviolet and photoreactivating radiators. Photochem. Photobiol. 39: 49–56.

Shkorbatov, G. L. 1966. Preferred temperature and phototaxis of Coregonus larvae. Zool. Zh. 14: 1515–1525.

Speekmann, C. L., Bollens, M. S. & Avent, S. R. 2000. The effects of ultraviolet radiation on the vertical distribution and mortality of estuarine zooplankton. J. Plankton Res. 22: 2325–2350.

Stapleton, A. E., Mori, T. & Walbot, V. 1993. A simple and sensitive antibody-based method to measure UV-induced DNA damage in Zea mays. Plant Mol. Biol. Rep. 11: 230–236.

Steeger, H.-U., Wiemer, M., Freitag, J. F. & Paul R. J. 1999. Vitality of plaice embryos (Pleuronectes platessa) at moderate UV-B exposure. J. Sea Res. 42: 27–34.

Stolarski, R., Bojkov, R., Bishop, L., Zerefos, C., Staehelin, J. & Zawodny, J. 1992. Measured trends in stratospheric ozone. Science 256: 342–349.

Storey, J. D., Tibshirani, R. 2003. Statistical significance for genomewide studies. Proc. Natl. Acad. Sci. USA 100: 9440–9445.

Suzuki, K. & Watanabe, M. 1992. Augmented expression of HSP72 protein in normal human fibroblasts irradiated with ultraviolet light. Biochem. Biophys. Res. Commun. 186: 1257–1264.

Taalas, P., Kaurola, J., Kylling, A., Shindell, D., Sausen, R., Dameris, M., Grewe, V., Herman, J., Damski, J. & Steil, B. 2000.The impact of greenhouse gases and halogenated species on future solar UV radiation doses. Geophys. Res. Lett. 27: 1032–1034.

Tavendale, M. H., McFarlane, P. N., Mackie, K. L., Wilkins, A. L. & Langdon, A. G. 1997. The fate of resin acids–1. The biotransformation and degradation of deuterium labelled dehydroabietic acid in anaerobic sediments. Chemosphere 35: 2137–2151.

Thoma, F. 1999. Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair. EMBO J. 18: 6585–6598.

Thorpe, A., Douglas, R. H. & Truscott, R. J. 1993. Spectral transmission and short-wave absorbing pigments in the fish lens--I. Phylogenetic distribution and identity. Vision Res. 33: 289–300.

Towbin, H., Staehelin, T. & Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76: 4350–4354.

Turpeinen, M., Serpi, R., Rahkolin, M. & Vähäkangas, K. 2002. Comparison of anti-p53 antibodies in immunoblotting. Biochem. Biophys. Res. Commun. 293: 850–856.

Urho, L., Laurila, S. & Hildén, M. 1989. Hauen ensimmäinen kesä (The first summer in a pike's life.). Luonnon Tutkija 93: 130–135.

Vehniäinen, E.-R., Häkkinen, J., Pesonen, M. & Oikari, A. 2004. Photoinduced toxicity of retene in larval fish and fish primary cell culture. SETAC Europe 14th Annual Meeting, Prague, Czech Republic. Book of abstracts, p. 106.

Vetter, R. D., Kurtzman, A. & Mori, T. 1999. Diel cycles of DNA damage and repair in eggs and larvae of northern anchovy, Engraulis mordax, exposed to solar ultraviolet radiation. Photochem. Photobiol. 69: 27–33.

Viljanen, M., Karjalainen, J., Helminen, H., Sarvala, J. & Sydänoja, A. 1995. Night day catch ratios of coregonid larvae in three large lakes in Finland. Arch. Hydrobiol. Spec. Issues Advanc. Limnol. 46: 195–201.

Wei, Y.-D., Rannung, U. & Rannung, A. 1999. UV-induced *CYP1A1* gene expression in human cells is mediated by tryptophan. Chem.-Biol. Interact. 118: 127–140.

Williamson, C. E., Metgar, S., Lovera, P. A. & Moeller, R. E. 1997. Solar ultraviolet radiation and the spawning habitat of yellow perch, Perca flavescens. Ecol. Appl. 7: 1017–1023.

Winston, G. W. 1991. Oxidants and antioxidants in aquatic animals. Comp. Biochem. Physiol. C. 100: 173–176.

Wood, R. 1996. DNA repair in eukaryotes. Annu. Rev. Biochem. 65: 135–167.

Yakovleva, I., Bhagooli, R., Takemura, A. & Hidaka, M. 2004. Differential susceptibility to oxidative stress of two scleractinian corals: antioxidant functioning of mycosporine-glycine. Comp. Biochem. Physiol. B 139: 721–730.

Yan, N. D., Keller, W., Scully, N. M., Lean, D. R. S. & Dillon, P. J. 1996. Increased UV-B penetration in a lake owing to drought-induced acidification. Nature 381: 141–143.

Ylönen, O. & Karjalainen, J. 2004. Growth and survival of European whitefish larvae under enhanced UV-B irradiance. J. Fish Biol. 65: 869–875.

Ylönen, O., Heikkilä, J. & Karjalainen, J. 2004. Metabolic depression in UVB exposed larval coregonids. Ann. Zool. Fennici 41: 577–585.

Yoon, J.-H., Lee, C.-S., O'Connor, T. R., Yasui, A. & Pfeifer, G. P. 2000. The DNA damage spectrum produced by simulated sunlight. J. Mol. Biol. 299: 681–693.

Zagarese, H. E., Diaz, M., Pedrozo, F., Ferraro, M., Cravero, W. & Tartarotti, B. 2001. Photodegradation of natural organic matter exposed to fluctuating levels of solar radiation. J. Photochem. Photobiol. B: Biol. 61: 35–45.

Zamzow, J. P. & Losey, G. S. 2002. Ultraviolet radiation absorbance by coral reef fish mucus: photo-protection and visual communication. Environ. Biol. Fish. 63: 41–47.

Ziegler, A., Jonason, A. S., Leffell, D. J., Simon, J. A., Sharma, H. W., Kimmelman, J., Remington, L., Jacks, T. & Brash, D. E. 1994. Sunburn and p53 in the onset of skin cancer. Nature 372: 773–776.

Zigler, S. J. & Dewey, M. R. 1995. Phototaxis of larval and juvenile northern pike. North Am. J. Fish Manag. 15: 651–653.

Zinsmaier, K. E. & Bronk P. 2001: Molecular chaperones and the regulation of neurotransmitter exosytosis. Biochem. Pharmacol. 62: 1–11.