









## ABSTRACT

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Effects of enhancing UV-B irradiance on the behaviour, survival and metabolism of coregonid larvae

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Yhteenvedo: Lisääntyvän UV-B säteilyn vaikutukset siikakalojen poikasten käyttäytymiseen, kuolleisuuteen ja metaboliaan

Diss.

Anthropogenically-caused stratospheric ozone depletion has increased UV-B irradiance in the Earth's atmosphere and under water. The latest results suggest that a further 20-50 % increase in erythemal UV doses can be expected in northern latitudes over coming decades. This work evaluates the effects of enhanced UV-B irradiance on coregonid larvae. The study species, vendace and whitefish, have a clear, positive phototactic response after hatching that makes them potentially susceptible to UV exposure. Thus, the behaviour of larvae was studied to find out whether larvae were able to avoid high UV-B exposure despite their tendency to stay in the most illuminated depth zone. The results suggest that UV radiation plays an important role in the regulation of vertical distribution of vendace larvae in Finnish lakes. The most important factor in UV avoidance behaviour is suggested to be cumulative UV-B dose. The survival, growth and food consumption of coregonid larvae were not affected by present or predicted erythemal UV-B doses, but high UV-B exposure for two weeks produced low survival of whitefish larvae with high individual variation. High UV-B exposure also decreased the maximum metabolic rate and the metabolic scope of coregonid larvae and disturbed oxygen consumption of the digestive system in whitefish larvae, but the mechanism remains unclear. In the field, direct effects of UV-B radiation on larval coregonids will be negligible. However, some indirect effects, such as increased predation risk and reduced growth rate, can occasionally be expected.

Key words: Avoidance behaviour; coregonid larvae; metabolism; survival; UV-B radiation.

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## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which are referred to in the text by Roman numerals I-V.

- I Ylönen, O., Huuskonen, H. & Karjalainen, J. 2004. UV avoidance of coregonid larvae. Proceedings of the VIII International Symposium on the Biology and Management of Coregonid Fishes, Rovaniemi, Finland. Ann. Zool. Fennici 41: 89-98.
- II Ylönen, O., Huuskonen, H. & Karjalainen, J. 2004. Effects of solar radiation on the vertical distribution of vendace (*Coregonus albula* (L.)) larvae in Finnish lakes. Submitted to Ecol. Freshwat. Fish
- III 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. Env. Biol. Fish. 64: 451-459.
- IV Ylönen, O. & Karjalainen, J. 2004. Growth and survival of European whitefish (*Coregonus lavaretus* L. s. l.) larvae under enhanced UV-B irradiance. Submitted to J. Fish Biol.
- V Ylönen, O., Heikkilä, J. & Karjalainen, J. 2004. Metabolic depression in UV-B exposed larval coregonids. Ann. Zool. Fennici (in press)



# 1 INTRODUCTION

## 1.1 UV radiation in the Earth' s atmosphere

Solar radiation is essential for life on Earth. However, the shortest wavelengths of solar radiation, the ultraviolet (UV) radiation, is known to have harmful effects on living organisms. UV radiation is divided into UV-C (200-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm) according to wavebands. The biologically most harmful UV-C is completely absorbed by atmospheric ozone and oxygen (Frederic et al. 1989, Madronich et al. 1998). The stratospheric ozone layer absorbs UV-B partially and UV-A only weakly (Madronich et al. 1995, 1998). Thus, any changes in the ozone layer affect mainly the amount of UV-B radiation in the Earth' s atmosphere.

UV-B levels have increased and are still increasing due to anthropogenically-caused stratospheric ozone depletion (Stolarski et al. 1992, Kerr & McElroy 1993, Austin et al. 1999, Taalas et al. 2002), but long-term prediction of future UV levels is difficult and uncertain. Taalas et al. (1996, 2000) suggested that, relative to 1979-1992 conditions, the 2010-2020 conditions will have a springtime enhancement of erythemal (CIE) weighted UV doses of up to 90 % in the 60-90 °N region. The erythemal weighting means that the action spectrum is specific for human erythema (McKinlay & Diffey 1987) and both UV-B and UV-A radiation are included but weighting diminishes towards longer wavelengths. The corresponding maximum increases in the annual Northern Hemispheric UV doses are estimated to be 14 % in 2010-2020, and 2 % in 2040-2050. However, the latest results actually suggest a 20-50 % increase of erythemal UV doses in northern latitudes over coming decades (Taalas et al. 2002).

Besides stratospheric ozone depletion, several factors affect the intensity of UV radiation reaching the Earth' s surface. These include clouds, aerosols, tropospheric ozone and other gaseous pollutants, surface albedo and elevation (Madronich et al. 1995). Clouds, aerosols and tropospheric ozone and other gaseous pollutants usually decrease the intensity of UV radiation, at least in the

polluted regions, whereas surface albedo and increasing surface elevation above sea level increase the intensity. The increase caused by snow and ice may be 75-100 % and that by surface elevation over 10 % per 1000 m (Madronich et al. 1995, Blumthaler et al. 1997).

Several authors have shown that thick cloud cover considerably reduces UV irradiance. In the UV-B waveband the reduction is 40-72 % (Németh et al. 1996, Seckmeyer et al. 1996) and in the UV-A waveband about 50 % (Seckmeyer et al. 1996). Under medium cloud cover (25 %), on the other hand, scattering from the sides of cumulus clouds can increase UV-B irradiance by 15-20 % (Mims & Frederick 1994, Németh et al. 1996).

In the worst-case scenario, short-term increase in UV irradiance may occur due to ozone minihole events or due to a large volcanic eruption that increases the amount of stratospheric sulfate aerosols (Vogelmann et al. 1992, Madronich et al. 1995, Reuder et al. 2001). The latter can produce a marked increase, up to 20 %, in erythemal UV doses in several months. During ozone minihole events, with maximum occurrence in spring, UV irradiance is typically increased for a few days. During these events, erythemal UV doses may be 40 % higher than average (Reuder et al. 2001).

## 1.2 UV radiation under water

In many water bodies, UV radiation can penetrate to ecologically significant depths (Smith & Baker 1979, Smith et al. 1992, Kirk 1994). Absorption of UV by water itself is negligible, and most of the UV is absorbed by the dissolved and particulate organic matter (Kirk 1994), the most important being dissolved organic carbon DOC (Scully & Lean 1994, Morris et al. 1995, Williamson et al. 1996, Bukaveckas & Robbins-Forbes 2000, Huovinen 2000). In the clearest ocean waters, 10 % of UV-B (310 nm) irradiance just beneath the surface can penetrate to 20 metres (Kirk 1994). In freshwater lakes, due to higher humic content, the 1-10 % attenuation depths can vary from just a few centimetres in waters having high DOC concentration to over 10 m in low DOC lakes (Kirk 1994, Morris et al. 1995, Bukaveckas & Robbins-Forbes 2000, Huovinen et al. 2003). Attenuation depths for UV-A radiation are approximately 2.5 times as deep as those for UV-B (Williamson et al. 1996). In the clearest lakes in Finland, UV-B radiation can penetrate deeper than 1 metre (Huovinen et al. 2000). In the future, decreases in dissolved organic carbon caused by climate warming and acidification may cause increased penetration of UV radiation in freshwaters (Schindler et al. 1996, Yan et al. 1996, Schindler 2001).

During winter in northern boreal latitudes, aquatic organisms are well protected from UV exposure as lakes are covered by ice for several months. It has been shown that only a few per cent of UV-B irradiance penetrates through ice and snow (Belzile et al. 2000, 2001, Schubert et al. 2001). Immediately after the ice-melt, in Finland usually in April-May, primary production is scarce and therefore the water is clearer than one to two weeks later allowing deeper

penetration of UV-B down the water column. As that is also both the time of spawning or hatching of most boreal fishes and of maximum ozone depletion (Taalas et al. 2002), the time just after ice-melt is the most critical when evaluating possible effects of increasing UV-B irradiance on boreal freshwater ecosystems and fish stocks.

### **1.3 Effects of UV-B radiation on aquatic life**

Solar UV-B radiation has been shown to cause damage to early developmental stages of fish (Hunter et al. 1981, Häder et al. 1995, Beland et al. 1999, Browman et al. 2000), shrimp (Wuebben 2000), crab (Hovel & Morgan 1999), amphibians (Merilä et al. 2000, Kiesecker et al. 2001), and other aquatic animals (Adams & Shick 2001). In fish, exposure to UV-B radiation causes reduced larval and embryonic survival and growth (Hunter et al. 1979, 1981, Williamson et al. 1997). It also induces sunburn in several species (Berghahn et al. 1993, Little & Fabacher 1994, Blazer et al. 1997) and causes lesions in the brain and eye (Hunter et al. 1979). However, UV-B sensitivity varies widely between species and life history stages (Blazer et al. 1997, Kouwenberg et al. 1999, Steeger et al. 1999, Häkkinen 2003). Studies with adult roach have shown that UV radiation is also an immunosuppressive agent (Salo et al. 1998, 2000a, 2000b, Jokinen et al. 2001).

### **1.4 Mechanisms protecting fish from UV-B**

There are at least three ways that fish larvae can cope with enhanced UV-B levels: DNA photorepair mechanisms, skin melanin pigmentation and avoidance behaviour. The distribution of fish larvae in the presence of UV radiation has been little studied, although movements of other planktonic organisms in response to UV radiation have been well documented (e.g. Leech & Williamson 2001, Rautio 2001, Rhode et al. 2001). However, the results of Speckmann et al. (2000) and Kelly & Bothwell (2002) suggest that fish larvae and juveniles are able to actively avoid high UV irradiance and, thus, decrease the harmful UV exposure.

Enhanced melanin pigmentation under UV exposure is well documented in cladocerans and in some fish species, and it seems to act as a photoprotective mechanism (e.g. Ahmed & Setlow 1993, Lowe & Goodman-Lowe 1996, Rhode et al. 2001, Rautio & Korhola 2002, Häkkinen et al. 2003). In addition to enhanced melanin pigmentation, the induction of photorepair mechanisms (e.g. Shima & Setlow 1984, Ahmed & Setlow 1993) may serve to protect fish against enhanced UV irradiance. Although UV-A radiation has been found to have some negative effects (Winckler & Fidhiany 1996a, b, 1999, Fidhiany & Winckler 1999) more often it has been shown to induce DNA repair. For example,

Applegate & Ley (1988) exposed fish embryos to photoreactivating UV-A light, and the photorepair of pyrimidine dimers was accompanied by a substantial increase in survival.

## 1.5 Study species

Whitefish (*Coregonus lavaretus* L. s. l.) and vendace (*Coregonus albula* (L.)) are widely distributed freshwater species in the Northern Hemisphere and they have great commercial and recreational value in Scandinavian inland waters. These fish are most UV-sensitive as recently hatched larvae. In Finnish lakes, spawning takes place in October-November, egg incubation under UV blocking ice-cover, and hatching after ice-melt in May (Karjalainen & Viljanen 1994). After hatching, coregonid larvae have a clear, positive phototactic response and aggregate in shallow littoral areas or in surface waters in the pelagic zone (Karjalainen et al. 1998) where they may be exposed to high UV-B levels.

Although the two study species are from the same genus, their life histories clearly differ from each other. Whitefish is a relatively long-lived species. Sexual maturity is generally attained at the age of three years and the main part of the whitefish catch from Finnish lakes consists of fish at the age of 4-10 years (Turunen et al. 1991, Heikinheimo-Schmid 1992).

Vendace, on the other hand, is a short-lived species. In the vendace catch from Finnish lakes, 2-3 year-old fish dominate and 5-6 year-old fish are rare (Turunen et al. 1991). Female fish normally spawn in the second autumn and occasionally even in the first autumn (Viljanen 1986, Sarvala et al. 1992). Vendace stocks may have regular two- to four-year cycles, depending on the lake (Marjomäki 2003). The reasons for this cyclicity have been studied intensively for decades and external environmental factors (e.g. Auvinen 1988, Helminen & Sarvala 1994), intraspecific competition (e.g. Hamrin & Persson 1986, Auvinen et al. 2000), and fishing (e.g. Auvinen 1994, Valtonen & Marjomäki 1988, Helminen et al. 2002) have all been suggested as possible causal factors.

## 2 OBJECTIVES

The first aim of this thesis was to study the behaviour of coregonid larvae under UV radiation both in the laboratory and in the field. Although the behaviour of an animal has a key role when laboratory results are evaluated under field conditions, it is not always studied. Especially, the UV avoidance behaviour of fish has been studied very little. The second aim of the thesis was to study possible effects of enhanced UV-B irradiance on growth, survival and metabolism of coregonid larvae. Several series of experimental and field studies were carried out

- 1) to determine whether vendace and whitefish larvae are capable of detecting and avoiding UV radiation under laboratory conditions (I);
- 2) to analyse the possible UV avoidance behaviour of the larvae in the field (I, II);
- 3) to study possible effects of enhanced UV-B irradiance on the survival and growth of coregonid larvae (III, IV); and
- 4) to measure how metabolism and energy allocation of coregonid larvae is affected under UV exposure (V).

## 3 MATERIALS AND METHODS

### 3.1 Avoidance experiments

UV avoidance of coregonid larvae was studied in three experimental series in the laboratory (I, unpublished data) and by analysing field data gathered in 1995-2001 from eight Finnish lakes with different optical properties (I, II). In addition, avoidance of visible light (II) was studied in acrylic tubes placed vertically in a lake.

#### 3.1.1 Laboratory exposures to UV radiation

Fertilised vendace (Lake Vanajavesi stock in 2000 and Lake Pyhäselkä stock in 2001) and whitefish (Lake Pyhäselkä stock) eggs were incubated in glass jars until the larvae hatched in April 2000 and 2001. In the first two experimental series three size groups of vendace larvae were used each year and similarly three size groups of whitefish larvae were tested under laboratory conditions in 2001. Two or four glass aquaria were used in each experiment. They were placed side-by-side in one or two glass fibre pools, two aquaria in each pool. The water circulating in the pool served as a water bath which regulated the temperatures of the aquaria. A water layer of 5 cm was used in the aquaria to minimise the vertical movement of the larvae. There was no oxygenation in the aquaria. Grey tape or white plastic was used to increase the visibility of the aquarium wall for the larvae.

*In experimental series one*, a visible light fluorescent tube (Philips TLD 36 W/950) and a UV-B fluorescent tube (Q-Panel UVB-313) were attached above both aquaria. Two different and similarly sized light zones were created in the aquaria with cellulose diacetate and Mylar-D filters. One included UV-B radiation whereas the other one did not. 50 vendace larvae were used at a time. One experiment included three phases: 1) control phase without UV radiation, 2) UV exposure phase, and 3) recovery phase without UV radiation. The control and recovery phases each lasted for one hour and the exposure phase for two

hours. The position of the larvae was observed by naked eye every ten minutes. The number of larvae below the Mylar-D and cellulose diacetate filter in both aquaria was counted through a small window in the curtain.

*In experimental series two*, there were two test and two control aquaria. Above the test aquaria the same lamps were used as in series one, and above the control aquaria only a visible light fluorescent tube was attached. Again, half of an aquarium was covered with Mylar-D filter and the other half with cellulose diacetate. Two hours before the experiment started, 20 vendace or whitefish larvae were taken from the rearing aquarium and transferred into the test (UV exposure) and control aquaria. UV exposure lasted for two hours. The experiments were recorded with four digital video cameras and a digital video recorder was used to save the data. The position of the larvae was observed both from the videofilm and by naked eye through a small window in the curtain every ten minutes. The number of larvae below the cellulose diacetate filter (UV-B exposure in test aquaria) in each aquarium was counted. In both experimental series the mean percentages of larvae during the different phases in an experiment were used in the analysis.

Before experimental series one and two, UV irradiance was measured at 21 points (10 cm intervals) with a Hamamatsu Photonic Multichannel Spectral analyser. Both UV-B and UV-A irradiance diminished markedly in the middle of the aquarium. A more detailed description of the experimental setup and measurements is in paper I.

*In experimental series three*, eight acrylic tubes with 10 cm depth zones marked on them were used. Four tubes served as controls and above them only visible light fluorescent tube was attached. Above four exposure tubes a visible light fluorescent tube and a UV-B or UV-A fluorescent tube (Q-Panel UVA-340) were attached. In addition, Mylar-D or cellulose diacetate filters were used (II). Three different treatments were created : a high UV-B treatment (erythemal UV dose rate  $780 \text{ mW m}^{-2}$ , two-hour UV dose  $5.62 \text{ kJ m}^{-2}$ ), a low UV-B treatment (erythemal UV dose rate  $50 \text{ mW m}^{-2}$ , two-hour UV dose  $0.36 \text{ kJ m}^{-2}$ ) and a UV-A treatment (erythemal UV dose rate  $3 \text{ mW m}^{-2}$ ; two-hour UV dose  $0.024 \text{ kJ m}^{-2}$ ).

Four hours before the experiment started, 15 newly hatched vendace larvae were introduced to each tube and two hours before the experiment water circulation was stopped and the lights were switched on. After the habituation period, the positions of the larvae in each tube were observed with naked eye three times at half hour intervals. The number of larvae in each depth zone was converted to a percentage of the total number introduced ( $n = 15$ ).  $\chi^2$ -test was used to test whether the frequency distributions of larvae in control and exposure tubes were different.

### 3.1.2 Vertical distribution in the field

The differences in the vertical distribution of coregonid larvae between sunny and cloudy periods were analysed from the field data gathered from eight Finnish lakes. The lakes were Lake Puruvesi, Paasivesi, Onkamo, Höytiäinen,

Puula, Konnevesi, Oulujärvi and SW Pyhäjärvi. The samples were collected by Bongo nets (Karjalainen et al. 1998, 2002) from both littoral and pelagic zones.

Newly hatched vendace and whitefish larvae were collected immediately after ice-melt, which occurred between mid-April (SW Pyhäjärvi) and mid-May (Paasivesi, Onkamo). A jet-powered boat or the research vessel R/V Muikku with two Bongo nets attached to the front of the boat was used. The depth of the water in the randomly selected sampling areas was more than 1 m.

During surface vertical sampling of all the study lakes, one Bongo net gathered larvae from the vertical depth layer of 0-0.4 m and another net from the depth layer of 0.4-0.8 m. The analysis of these data from the lakes Puruvesi, Paasivesi, Onkamo and SW Pyhäjärvi was performed for two time periods, 1996-2000 (I) and 1999-2001 (II). During deep vertical sampling performed in the pelagic zone of the lakes Paasivesi and Puruvesi, one Bongo net gathered larvae from the vertical depth layer of 0-1 m and another net from the depth layer of 1-2 m. In the laboratory, the number of vendace and whitefish larvae in each tow was counted and the relative number of larvae caught by the upper net was calculated. The surface percentages (SP = catch in the upper net/total catch in a tow) of vendace and whitefish larvae were used to compare the vertical distribution of larvae in sunny and cloudy periods.

Cloud cover was estimated visually during each tow and classified into nine (0-8) classes. These original cloud cover values were recoded into three cloud cover classes: CCC 1, no cloud cover (0-2); CCC 2, medium cloud cover (3-5); and CCC 3, full cloud cover (6-8). The cloud cover was used as an indirect estimate of the amount of UV irradiance. To make sure there really were differences in UV irradiance during sampling periods, only CCC 1 and CCC 3 were compared in the analysis.

### 3.1.3 Avoidance of visible light

Ten acrylic tubes, the same as in the laboratory experiments, were used in the field experiments. The framework to which the tubes were attached was anchored on the bottom of Lake Konnevesi near the Konnevesi research station, Central Finland. Five of the tubes were filled with unfiltered lake water and water for the other five tubes was filtered using a plankton net (48 µm mesh size) to remove zooplankton. A Mylar-D filter was attached to the top of every tube to remove UV radiation coming from above.

During the experiments visible light was measured above the surface with a Delta-Ohm lux-meter (model HD-9221). The attenuation of visible light (400 – 800 nm) in Lake Konnevesi was measured with a MACAM spectroradiometer (model SR9910, double monochromator) outside the tubes. At 1 m deep the irradiance was 35 % of the irradiance just below the surface.

Newly hatched vendace larvae were observed during two sunny and two cloudy days by two scuba-divers in May 2002. One hour before the first observation 30 seine netted vendace larvae from Lake Konnevesi were introduced to each tube. On one day counting was repeated three times with 30 minute breaks producing six observations from each tube on sunny and cloudy



days. The number of larvae in each depth zone was converted to a percentage of the total number introduced.

### 3.2 Rearing experiments

Three rearing experiments with four different treatments in each experiment were performed (III, IV). In experiment one, both newly hatched vendace and whitefish larvae and in experiments two and three, only whitefish larvae were reared in glass aquaria.

Fertilised vendace and/or whitefish eggs were incubated in glass jars until hatching in May and newly hatched larvae were then transferred to aerated flow-through aquaria with 200 (100 vendace and 100 whitefish) or 150 larvae per aquarium. Before the start of experiment, samples of 50 or 30 larvae were taken for assessment of the total length (mm) and both fresh and dry masses.

In all three experiments there were four aquaria for both the control (without UV-B radiation) and for the three UV-B treatments. Fish in all treatments were exposed to UV-A radiation and visible light. In the control treatment UV-B radiation was blocked by a Mylar-D filter, which was replaced once during the experiment. Adjustable UV-B lamps produced the UV-B radiation in the different UV-B treatments. The UV-C radiation (under 280 nm) produced by the UV-B lamps was blocked by a cellulose diacetate filter which was replaced after each three-hour exposure period.

In experiments one and two (III, IV), coregonid larvae were reared for two weeks. Larvae were fed *ad libitum* on *Artemia* nauplii during the experiments. The erythemal daily UV doses measured just above the water surface in experiments one and two varied from 0.93 kJ m<sup>-2</sup> to 6.15 kJ m<sup>-2</sup>. For comparison, according to the Finnish Meteorological Institute, the average erythemal daily UV dose was 1.66 kJ m<sup>-2</sup> measured at Jokioinen (60.82N 23.50E) in the beginning of May (between 2-8 day), 1998-2000. However, the highest erythemal daily UV doses in the middle of May 2000 were ~3 kJ m<sup>-2</sup>.

The amount of food given was adjusted according to the mortality in each treatment. Growth assessments were made after one- and two-week exposures in experiment one, and every fifth day in experiment two. In experiment one, 24 larvae (six from each aquarium) and in experiment two, 20 larvae (five from each aquarium) from each treatment were randomly taken and total length (mm) and both fresh and dry masses were measured. The total survival ( $S_t$ ) during the experiments was calculated from the equation:

$$S_t = (e^{-Z}) * 100,$$

where  $Z$  is the total instantaneous mortality rate during the experiment and this is the sum of the instantaneous mortality rates of the larvae during the periods between two sampling occasions. For example in experiment two,  $Z$  is the sum of  $Z_{1-5}$ ,  $Z_{6-10}$  and  $Z_{11-15}$  which are the instantaneous total mortality rates for the

periods days 1-5, days 6-10 and days 11-15, respectively. The numbers of fish sampled after the periods were subtracted before the instantaneous mortality rates were calculated.

In experiment three, whitefish larvae were reared for nine days and fed in excess on *Artemia* nauplii. On five of the nine days, food consumption of larvae was estimated in each aquarium. At the end of a six-hour feeding period, the aquaria were emptied, *Artemia* were sieved from the water and the number recovered subtracted from that originally given. The fish were gently collected and transferred to new aquaria, and were stressed by keeping them in the sieve in the air for five seconds. The same procedure was used by Lappivaara (2001) to stress juvenile whitefish. The erythematous daily UV doses varied from 0.93 kJ m<sup>-2</sup> to 2.33 kJ m<sup>-2</sup>. At the end of the experiment, 20 larvae from each treatment (five from each aquarium) were randomly taken and total length (mm) and fresh mass were measured.

### 3.2.1 Melanin pigmentation

At the end of rearing experiment one, samples were taken for determination of the body total melanin concentrations of whitefish (10 samples per treatment, two larvae pooled in one sample) and vendace larvae (in control and highest dose levels 14 samples, otherwise seven samples per treatment, three larvae pooled in one sample). Spectrophotometric determination of total melanin (eu- and pheomelanin) in larvae was made, with slight modifications, by the method developed to assay melanin concentrations in mammalian hair (Ozeki et al. 1995, 1996). A detailed description of the method is given in paper III.

## 3.3 Oxygen consumption measurements

### 3.3.1 Routine rate experiments

Oxygen consumption of larval vendace was measured in a series of experiments using an intermittent-flow respirometer (Forstner 1983, Forstner et al. 1983) equipped with a polarographic oxygen sensor (POS, YSI 5750) and three parallel acrylic swimming chambers. Before the experiments, larvae were collected randomly from the rearing aquaria and transferred to the exposure aquaria. In every experiment, there was a control treatment without UV-B radiation and a UV-B exposure treatment. The erythematous UV dose measured just above the water surface in the UV-B exposure treatment was 3.58 kJ m<sup>-2</sup>.

In the rearing aquaria, larvae were fed *Artemia* nauplii *ad libitum*. Before the measurements, larvae were starved for 18 hours. Oxygen consumption of 10-60 vendace larvae (depending on the total mass, in every size group approximately 150 mg) was recorded for 15 minutes in each chamber. The average rate during this period was extrapolated to an hourly value. One experiment lasted for 24 hours yielding 24 hourly observations. The maximum

( $R_{\max}$ ) and minimum ( $R_{\min}$ ) oxygen consumption rates in these experiments were, respectively, the means of the three highest and three lowest values obtained during the 24 hour period. The routine rate was the mean of all values obtained during the 24 hour period, excluding the values used for the maximum and minimum rates. The metabolic scope was the difference between maximum and minimum rates.

### 3.3.2 Maximum rate experiments

Maximum oxygen consumption rates ( $R_{\max}$ ) of larval vendace and whitefish exposed to different UV doses was measured in a series of swimming respirometer experiments. The same respirometer system was used as in routine rate experiments. However, the three parallel acrylic swimming chambers in the swimming respirometer had a lower diameter than in the intermittent-flow respirometer. Thus, water flow and swimming speed of fish in the swimming respirometer were higher than in the intermittent-flow respirometer. The maximum oxygen consumption rates are, however, comparable between the different respirometer configurations (V).

In every experiment there was a UV-B exposure treatment and a control treatment without UV radiation. Before the exposure, larvae were fed *Artemia* nauplii *ad libitum* to ensure that UV irradiation did not affect the quantity of food consumed. The erythematous UV doses varied from 1.81 kJ m<sup>-2</sup> to 3.65 kJ m<sup>-2</sup> for vendace larvae, and from 1.37 kJ m<sup>-2</sup> to 5.58 kJ m<sup>-2</sup> for whitefish larvae.

During the measurement period, the school of five fish swam against a current and maximum oxygen consumption rates were recorded. In both the maximum rate and energy allocation experiments, oxygen consumption in each chamber was recorded for seven minutes at a time, and the average rate during this period was extrapolated to an hourly value.

### 3.3.3 Energy allocation experiments

Energy allocation by larval whitefish for activity and digestion was examined in two swimming respirometer experiments. Before both experiments, larvae were starved for 15 hours. After that, half of the fish were fed on *Artemia* nauplii for two to three hours and the other half remained without food before the oxygen consumption measurements. In experiment 1, only a high ration (*ad libitum*, ratio 2) was offered, but in experiment 2 both low (10 % of FM, ratio 1) and high (*ad libitum*, ratio 2) rations were offered. One group of both fed and unfed fish was exposed to UV-B radiation for three and two hours in experiment one and two, respectively. The erythematous UV doses were 5.58 kJ m<sup>-2</sup> and 5.35 kJ m<sup>-2</sup> in experiment one and two, respectively. After the exposure, oxygen consumption of 6-24 schools (five larvae per school) was measured in the respirometer. The costs of digestion ( $R_{\text{sda}}$  = the post-prandial rise in the metabolic rate, SDA as in Jobling (1983)) were calculated by subtracting the mean  $R_{\max}$  of unfed larvae from the mean  $R_{\max}$  of fed larvae.

## **4 RESULTS AND DISCUSSION**

### **4.1 UV avoidance in the laboratory**

#### **4.1.1 Horizontal avoidance**

Both in experimental series one and two all three size groups of vendace and whitefish larvae clearly avoided high UV-B exposure horizontally by staying under the Mylar filter during UV exposure (Table 1). In both series the difference between control and exposure phases or treatments was highest in the smallest vendace larvae. This suggests that the smallest vendace larvae are probably the most sensitive to UV radiation of the species and size groups studied. It also seems that whitefish larvae are less sensitive to changes in UV irradiance than vendace larvae.

Although the mean percentages of two-hour exposure periods were used larvae did not change their behaviour immediately after the UV-lamps were switched on. It took approximately half an hour before larvae clearly started to avoid high UV-B exposure. This suggests that avoidance behaviour is not exclusively visual because in that case larvae would have started to avoid UV-B exposure immediately.

TABLE 1 Mean percentages ( $\pm$  S. D.) of vendace and whitefish larvae under Mylar-D foil (UV-B cover) and lengths (mean  $\pm$  S. D.) of size groups in experimental series one and two. In series one there was consecutive control and exposure phases and in series two separate control and exposure treatments (see paper I).

Series one			
Size group	Length (mm)	Control phase	Exposure phase
Vendace 1	12.7 $\pm$ 0.9	45.3 $\pm$ 7.7	
Vendace 2	18.8 $\pm$ 2.0	42.2 $\pm$ 12.2	
Vendace 3	24.2 $\pm$ 2.7	61.6 $\pm$ 12.2	
Series two			
Size group		Control treatment	Exposure treatment
Vendace 1	9.4 $\pm$ 0.6	55.1 $\pm$ 10.3	80.3 $\pm$ 5.8
Vendace 2	11.2 $\pm$ 0.6	63.1 $\pm$ 4.6	70.5 $\pm$ 5.4
Vendace 3	15.6 $\pm$ 1.1	56.2 $\pm$ 6.3	71.5 $\pm$ 6.0
Whitefish 1	12.6 $\pm$ 0.7	62.8 $\pm$ 3.4	70.8 $\pm$ 12.2
Whitefish 2	13.7 $\pm$ 0.9	63.8 $\pm$ 3.9	80.1 $\pm$ 3.3
Whitefish 3	18.3 $\pm$ 1.3	57.5 $\pm$ 11.7	68.9 $\pm$ 4.6

#### 4.1.2 Vertical avoidance

In the vertical tube experiments under laboratory conditions, without UV exposure (control) most vendace larvae stayed in the top 10 cm depth zone (Fig. 1). In the high UV-B treatment, the frequency distributions were different between control and UV exposure treatments and the mean percentage of vendace larvae in the top 10 cm depth zone was markedly lower in UV exposure than in the control treatment without UV radiation. In the low UV-B treatment the frequency distributions were different between control and UV exposure treatments, but in the top 10 cm there were more larvae in UV exposure than in control treatment. UV-A exposure had no effect on the frequency distributions (Fig. 1). Thus, vendace larvae clearly avoided high UV-B exposure but low UV-B or UV-A exposure did not have the same effect.

The transparency of the water used in the experiments was high (colour of water 2.0 mg Pt l<sup>-1</sup>; DOC concentration 2.0 mg l<sup>-1</sup>), making for low attenuation of UV. Although as much as 64 % of the UV-B irradiance (310 nm) just below the water surface penetrated to a depth of 10 cm in the tubes, at a depth of 20 cm the irradiance was already less than 50 % of the irradiance just below the surface. This seemed to be a sufficiently low UV-B level for larvae to tolerate, at least for short periods.

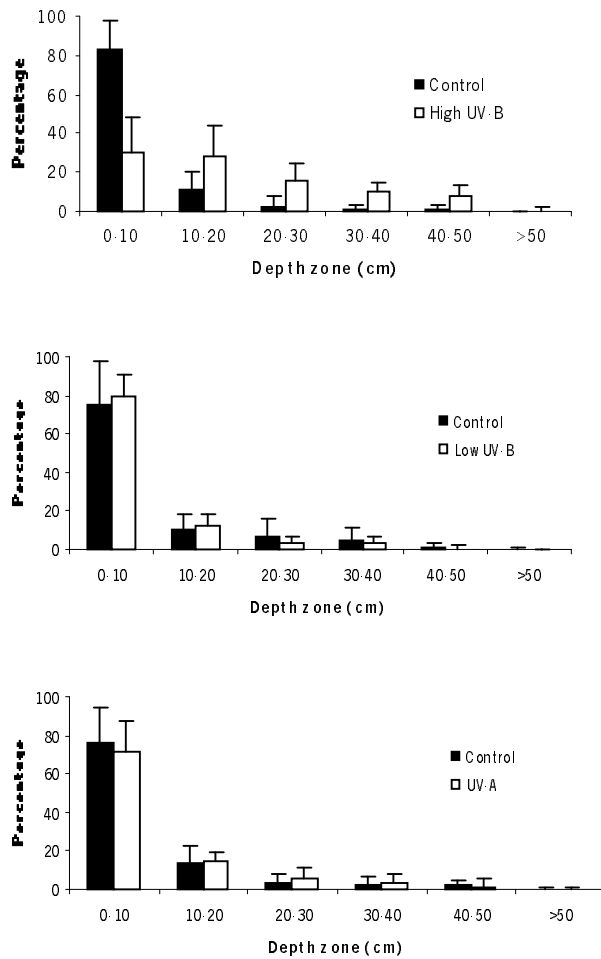


FIGURE 1 Mean percentages ( $\pm$  S. D.) of vendace larvae in different UV treatments (shown in the figure legends) in the five depth zones in the vertical tube experiments under laboratory conditions. Depth zones 0-10, 10-20 cm etc. are from the surface.

Whether UV-B or UV-A radiation is the major directive factor in UV avoidance of coregonid larvae is unclear. Microspectrophotometric analysis of the spectral sensitivity of larval retinas would give important information about the ability of larval vendace and whitefish to sense UV radiation (e.g. Beaudet et al. 1993). However, the peak absorption of visual pigments of many fish species is in the UV-A waveband at around 360 nm (e.g. Beaudet et al. 1993, Browman & Hawryshyn 1994, Losey et al. 1999) suggesting that neither coregonid larvae are able to detect UV-B radiation. In this case, UV-A radiation would be the directive factor. However, it seems obvious that not only UV perception but the length of UV exposure have some role in UV avoidance. The results of Kouwenberg et al. (1999) support this suggestion as UV-B induced mortality of cod embryos was strongly influenced by cumulative UV-B dose and not by dose-rate. Thus, after prolonged high UV-B exposure coregonid larvae start avoiding the exposure because otherwise they would suffer from sunburn or other UV-B induced lesions. This time-dependent avoidance explains the rather

high relative frequencies of larvae in the high UV-B exposed zones (horizontal and vertical) in all the experiments described in this thesis.

## 4.2 Avoidance of solar radiation

From the deep vertical sampling (0–2 m) of the lakes Paasivesi and Puruvesi, most vendace larvae were in the 0–1 m depth zone during both sunny and cloudy periods (II). However, in Lake Puruvesi where UV attenuation was low (Table 2) the surface percentage (SP) was higher during cloudy than during sunny periods whereas in humic Lake Paasivesi the opposite was the case.

Also, from the surface vertical sampling of Lake Puruvesi and other lakes with colour of water 20 or lower, SP (0–0.4 m) of vendace larvae was higher during cloudy than during sunny periods (Fig. 2, II). In Lakes Höytiäinen, Paasivesi and Oulujärvi with the higher water colour (Table 2) SP was higher during sunny than during cloudy periods (Fig. 2, II) and SP during sunny periods increased by the water colour (II). It has to be recognised, however, that SP was at least 40 % even in the clearest Lake Puruvesi during sunny periods (II). Thus, it is obvious that even during high UV levels larvae can swim just under the surface for short periods if they spend most of the time at lower depths.

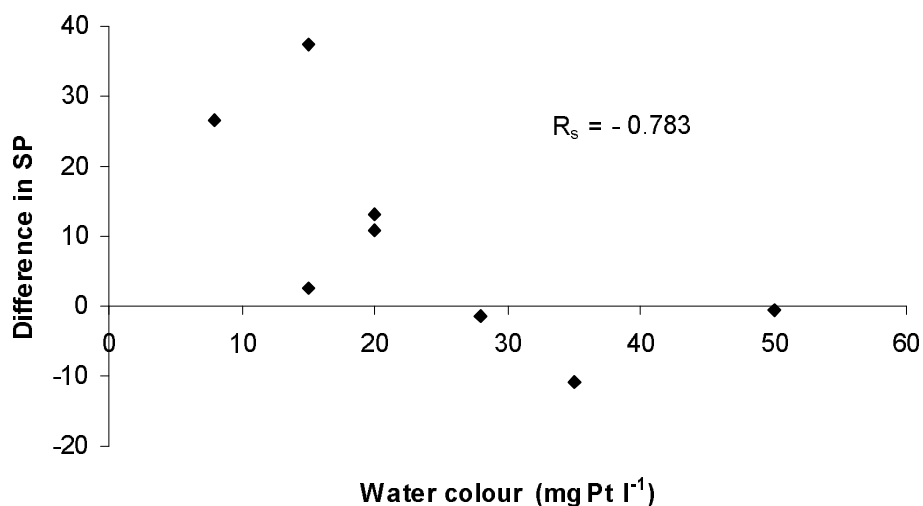


FIGURE 2 Differences in surface percentages (SP, see methods) of vendace larvae between sunny and cloudy periods. SPs are from the surface vertical sampling (0–0.8 m water column) of the study lakes in 1999–2001 (II). Spearman's correlation ( $R_s$ ) is shown in the figure ( $p < 0.05$ ).

The results from the lakes Puruvesi, Onkamo, SW Pyhäjärvi and Paasivesi were almost the same for 1996–2000 and 1999–2001. The only difference was that avoidance of high solar irradiance of vendace larvae in Lake SW Pyhäjärvi was

less clear in 1999-2001 (I) than in 1996-2000 (II). I suggest this may be due to the length of the sunny period before sampling. If the sun has shone continuously for several days before sampling the avoidance behaviour is likely to be more pronounced than if there have not been sunny days before sampling.

The results suggest that UV radiation plays an important role in the regulation of vertical distribution of vendace larvae in Finnish lakes. In the surface layer (0-0.8 m) of lakes with low UV attenuation and water colour, avoidance of high UV irradiances was clear. On the other hand, in those lakes with high water colour and UV attenuation, avoidance behaviour was not observed.

UV avoidance was less clear in whitefish larvae than in vendace larvae (I). Because whitefish larvae are more often found in the littoral zone of lakes than vendace larvae (Karjalainen et al. 2002) whitefish have developed better repairing mechanisms against UV radiation, such as heat shock protein 70 (HSP70, Häkkinen 2003). As there are whitefish larvae in the littoral zone of Finnish lakes where water depth is less than 0.5 m they cannot usually avoid high UV exposure by vertical adjustments. When water colour is high UV avoidance is possible even in very shallow water.

If we could have used Bongo nets designed for different lakes according to their UV-A and UV-B attenuation depths it might have been possible to detect UV avoidance even in the more humic lakes. The Bongo nets used were best suited for the lakes Onkamo and SW Pyhäjärvi, because in those lakes the upper net caught larvae exposed to both UV-B and UV-A radiation while the lower net caught larvae exposed only to UV-A radiation. In Lake Onkamo the avoidance behaviour was very clear but in Lake SW Pyhäjärvi it was not so clear. The reason for the difference between these two lakes with similar water colour may be that Lake SW Pyhäjärvi had lower Secchi depth due to higher biomass of algae (chl-a  $16.0 \text{ mg m}^{-3}$ ) than in Lake Onkamo (chl-a  $6.4 \text{ mg m}^{-3}$ ). Although in inland waters the contribution of phytoplankton to absorption of UV-B is small compared to the contribution of DOC (Kirk 1994), in Lake SW Pyhäjärvi algae may have some effect on the attenuation of UV-B radiation. However, this does not fully explain the difference between the lakes Onkamo and SW Pyhäjärvi.

Other factors that could affect the vertical distribution of newly hatched vendace larvae in the lakes include, water temperature, wind-induced turbulence, food distribution and predator avoidance. In fact, water temperature in Finnish lakes in spring when coregonid larvae hatch is low and the water column is not yet stratified. Also, Ventling-Schwank & Meng (1995) suggested that water temperature does not affect the vertical distribution of newly hatched whitefish larvae. The effect of wind-induced turbulence on the larvae is difficult to estimate, but the mean wind velocities on the lakes during larval sampling were not very high, only from 1 to 5  $\text{m s}^{-1}$ .



TABLE 2

Secchi depth, water colour, surface area and UV-B (310 nm) and UV-A (360 nm) attenuation depths of the lakes studied (II). Attenuation depths are the values where the irradiance is 1 % of the value measured just below the surface. The data for the water analyses are from the databases of the Finnish Regional Environmental Centres. Hyphen denotes that data are not available and asterisk (\*) own analysis or calculations.

Lake	Secchi depth (m)	Colour (mgPt l <sup>-1</sup> )	Chl a (mg m <sup>-3</sup> )	Area (ha)	UV-B attenuation depth (m)	UV-A attenuation depth (m)
Puruvesi	7.5	5-10	1.7	31 619	0.65*	1.70*
Onkamo	3.9	15	6.4*	3 227	0.44*	0.68*
SW Pyhäjärvi	3.3	15	16.0	15 400	0.34*	0.71*
Puulavesi	5.4	20	-	19 000	-	-
Konnevesi	4.9	20	5.0	13 000	0.22*	0.55*
Höytiäinen	4.3	28	-	15 392	-	-
Paasivesi	3.5	35	2.9	10 080	0.18*	0.37*
Oulujärvi	2.5	50	-	15 700	0.18*	0.40*

The only experimental evidence for the use of UV vision by fish is connected to planktivory. It has been shown that detection of zooplankton is enhanced by the physical properties of UV light (Browman et al. 1994). Several studies have also shown that cladoceran zooplankton, which are food for the coregonid larvae, react to UV radiation with negative phototaxis (e.g. Storz & Paul 1998, Rhode et al. 2001). However, no clear connection between food availability and vertical distribution of coregonid juveniles or larvae has been shown (Hamrin 1986, Ventling-Schwank & Meng 1995).

One example of predator avoidance by coregonid larvae was presented by Häkkinen et al. (2003). They showed that melanin pigmentation of coregonid larvae correlated positively with water colour, and suggested that larvae adapt to the background colour possibly to avoid visual predation. It could also be possible that vendace larvae avoid visual predators by staying deeper in the water column when the amount of visible light is high. However, because Secchi depths varied from 2.5 to 7.5 m in the study lakes there should be enough light for visual predators deeper than where the avoidance was mainly observed.

#### **4.2.1 The role of visible light**

In the tube experiments, vendace larvae showed a tendency to avoid high solar irradiance even under the conditions where UV radiation was not present. However, without UV radiation the amplitude of avoidance was much smaller than in the lake where UV wavelengths were present (II). Anyhow, larvae may use visible light as an indirect indicator of UV irradiance and avoid it to some extent, but harmful UV-B wavelengths are needed in true avoidance behaviour.

### **4.3 Survival and growth**

In experiments one and two without additional stress (III, IV), survival of both vendace and whitefish larvae was high in subambient and slightly enhanced UV-B treatments, but in the two highest UV-B treatments with erythematous UV doses of 3.65 and 6.15 kJ m<sup>-2</sup>, survival of whitefish larvae decreased markedly (Fig. 3). The survival of whitefish larvae after a two-week UV exposure was 65 % in the 3.65 kJ m<sup>-2</sup> UV dose and only 30 % in the 6.15 kJ m<sup>-2</sup> UV dose. The erythematous daily EC50 UV dose for whitefish larvae was 5.08 kJ m<sup>-2</sup> (probit analysis). It has to be recognised, however, that I did not continue to observe mortality after the end of the experiments which could have slightly decreased the total survival.

Periodic mortality in experiment two was highest in the control and low UV-B treatments during the last five days of the experiment. In the highest UV-B treatment (6.15 kJ m<sup>-2</sup>), mortality was first high but after fifth experimental day mortality decreased. This suggests that some individuals can tolerate very high UV-B doses and there are large differences in UV-B tolerance not only

among species (Little & Fabacher 1994) but within species as well. In addition, although UV-B doses in equatorial regions are several-fold higher than in Finland (Taalas et al. 2002) species living there are well adapted to those high doses (Häder et al. 1998). Thus, it seems evident that in the future boreal coregonid species can adapt to increasing UV-B doses by selection and they have the potential to become more UV-B tolerant.

In experiment two, fresh (FM) and dry body masses (DM) of whitefish larvae were significantly lower in the two high UV-B treatments than in the low UV-B and control treatments after five and ten days of exposure, but at the end of the experiment they were slightly higher in larvae exposed to the high UV-B treatments. It must be that larvae in the two high UV-B treatments had more food than larvae in the low UV-B and control treatment although the amount of food given was adjusted according to mortality in each treatment. Anyhow, it is important to notice that these most UV resistant individuals were able to produce high growth when they had enough food.

In experiment three with additional stress, the survival of whitefish larvae was high (> 95 %) and there were no significant differences between the treatments. However, survival was more variable in the UV-B treatments. The growth of larvae was highest in the control treatment and lowest in the enhanced UV-B treatment. UV-B radiation had no clear effect on food consumption of whitefish larvae (Fig. 4).

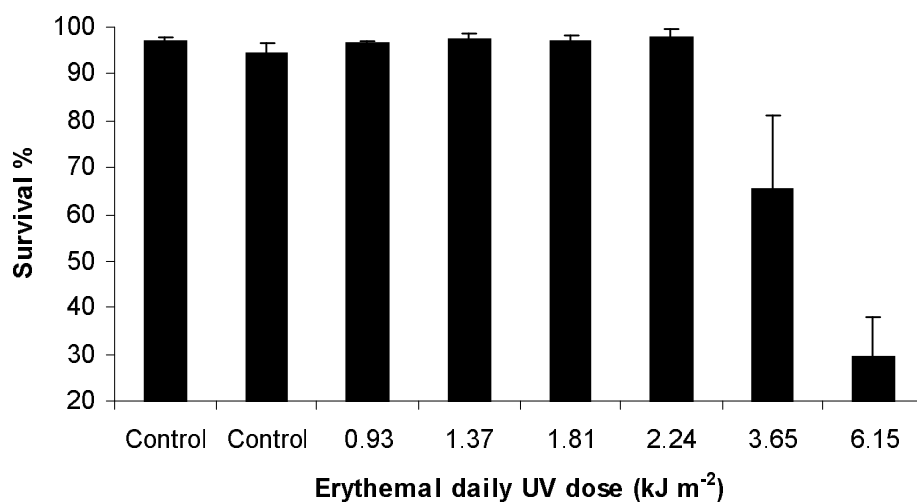


FIGURE 3 Total survival ( $S_t$ , see methods) of whitefish larvae after two weeks of exposure to different above surface UV-B levels (IV). Vertical lines represent standard deviations (S. D.). There are two control treatments because combined results of two separate experiments are shown.

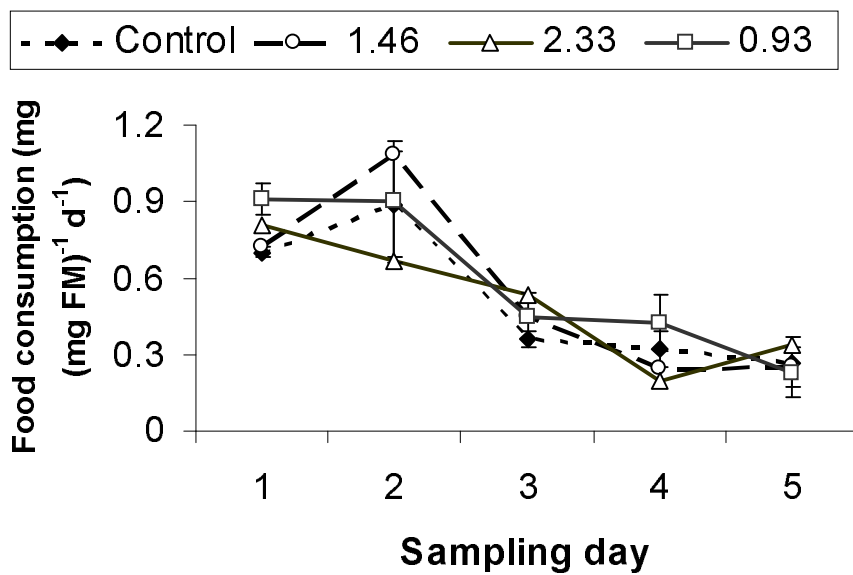


FIGURE 4 Mean food consumption ( $\pm$  S. E.) of whitefish larvae exposed to different erythemal UV doses ( $\text{kJ m}^{-2}$ ) on five sampling days in experiment I.

The durations of the rearing experiments were fairly short, nine days and two weeks (14-15 days). On the other hand, the time whitefish larvae remain close to the water surface after hatching is not much longer. Newly hatched whitefish larvae aggregate to shallow littoral areas or, in the pelagic zone, to water layers near the surface (Karjalainen et al. 1998). After three to four weeks, depending on water temperature, phototaxis is replaced by more complicated behavioural patterns and responses to the environmental factors (Shkorbatov 1966, Karjalainen et al. 2000) and the likelihood of exposure to harmful UV doses is reduced. In addition, skin melanin pigmentation and epidermal thickness increases and coating of scales develop.

Vendace larvae had better direct protection from UV-B radiation than whitefish larvae because in the control fish (not exposed to UV-B) there was 53 % more melanin in vendace than in whitefish (III). Nevertheless, newly hatched vendace larvae were more sensitive to UV radiation than whitefish larvae suggesting that the avoidance behaviour was also more developed in vendace. One might expect that whitefish larvae with less UV protecting melanin pigmentation would have better developed avoidance behaviour than vendace larvae. However, in whitefish larvae repairing mechanisms, such as heat shock protein 70 (HSP70, Häkkinen 2003), are more efficient than in vendace larvae.

To conclude, in the laboratory experiments newly hatched whitefish and vendace larvae tolerated higher daily UV doses than those usually encountered in Finland in May without increased mortality or decreased growth. However, additional stress and UV exposure may interact to influence growth and survival of coregonid larvae.

### 4.3.1 Effects in the field

Most of the daily erythemal UV doses above the water surface used in the rearing experiments are environmentally realistic for coming decades. Only the highest UV-B dose that produced 60 % mortality will not be expected, but it was necessary to clarify the upper tolerance limit of coregonid larvae under long-term exposure. The 3.65 kJ m<sup>-2</sup> UV dose may be considered environmentally realistic in the context of ozone depletion, sunny weather and low water turbulence. According to Häkkinen (2003) even daily erythemal UV doses of 4-5 kJ m<sup>2</sup> are realistic above the water surface.

However, there are several factors that may diminish the effects of enhanced UV-B doses in lakes compared to laboratory conditions. Firstly, the irradiance of UV-A in our experiments was lower than the irradiance in the field. As UV-A radiation has been shown to induce DNA repair (e.g. Applegate & Ley 1988) this may improve survival of larvae in the field. Secondly, the attenuation depth of UV radiation in freshwater lakes is much lower than in these rearing experiments. In laboratory experiments 64 % of the UV-B radiation (310 nm) penetrated to 10 cm and the 1 % attenuation depth of UV-B radiation (310 nm) in the eight Finnish study lakes was from 0.18 m to 0.65 m during calm weather (Table 2). During windy conditions water turbulence is higher and as a consequence UV attenuation is further increased. Thirdly, the highest UV doses will presumably not prevail for several consecutive days or weeks at a time as in these experiments. And finally, the UV avoidance behaviour of coregonid larvae shown in this thesis can remarkably reduce UV-B exposure of wild larvae.

## 4.4 Metabolism under UV exposure

UV-B exposure under laboratory conditions had no clear effect on the minimum or routine oxygen consumption rates of vendace larvae, but the metabolic scope of vendace larvae and the maximum oxygen consumption rates of both vendace and whitefish larvae decreased (V). However, the doses needed to decrease the metabolic scope and the maximum oxygen consumption rates were higher than present ambient daily doses in Finland during the hatching of coregonids. In coming decades, the lowest UV dose (3.58 kJ m<sup>-2</sup>) that decreased maximum oxygen consumption rate of coregonid larvae in these experiments will be realistic above the water surface but only for a few days during spring. During these days coregonid larvae may occasionally be exposed to UV levels high enough to decrease their maximum aerobic performance and metabolic scope. But again it has to be recognised that the water used in our laboratory experiments was very clear and in Finnish coregonid lakes the water is generally rather humic producing higher attenuation of UV radiation.

Even short-term depression in maximum aerobic capacity, however, disadvantages larval fish in nature. For example, rapidly growing larvae can

use almost all of their available metabolic power for growth (Hunt von Herbing & White 2002). In this case, decreased metabolic scope under high UV doses decreases growth rate and prolongs time spent in the small, vulnerable larval phase with high risk of predation (Miller et al. 1988). Lowered maximum oxygen uptake rate can also mean that under predator threat and during the postprandial metabolic peak, larvae do not have enough aerobic capacity to escape successfully (Conover & Schultz 1997). Thus, both lower growth rate and maximum aerobic capacity influence the larval survival probability.

#### 4.4.1 Allocation for activity and digestion

Both food ration and UV radiation significantly affected the maximum metabolic rates and digestion costs of whitefish larvae (V). Increasing food ration increased oxygen consumption, whereas UV radiation decreased it. Under UV radiation, the digestion costs decreased much more compared to the control than did the activity and maintenance costs. Also, the SDA decreased more in high than in low ration treatments (Fig. 5).

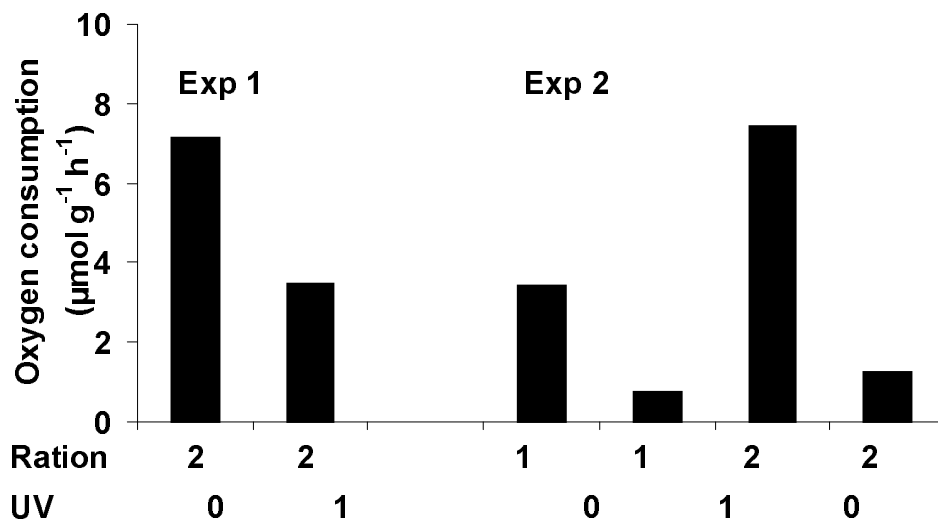


FIGURE 5 The costs of digestion (SDA) in fed (ration 1 = low, and ration 2 = ad libitum) larval whitefish without UV radiation (UV = 0) and under UV radiation (UV = 1) in the two swimming respirometer experiments. The erythemal UV doses were 5.58 kJ m<sup>-2</sup> and 5.35 kJ m<sup>-2</sup> in experiment one and two, respectively.

Karjalainen et al. (2003) suggested that the energy budget of larval whitefish is additive, which means that each component of the budget has its own allocation and this specific capacity cannot be used for other purposes. Compensatory (or competitive) allocation, on the other hand, means that if a fish is swimming at its maximum speed, it cannot digest food simultaneously (Priede 1985).

In these experiments UV radiation mainly decreased the oxygen consumption allocated for digestion in whitefish larvae, which can be caused in

two ways. Firstly, it can be caused by direct disturbance of the digestive system but the exact physiological mechanism for that is unclear. Nevertheless, two possible explanations are proposed: 1) the mitochondrial capacity that supplies energy transfer to the digestive system is decreased, or 2) regulation of blood circulation in the digestive system is disturbed. Secondly, the suppression of digestion can be caused by a compensatory effect on energy allocation. This may be inevitable under unfavourable conditions where metabolic scope is insufficient to cover all metabolic activities running simultaneously. Under field conditions, however, it is probable that the energy budget of larval fish is either additive or competitive, depending on the environmental conditions. Under favourable conditions the energy budget may be mainly additive while under unfavourable conditions, e.g. under high UV exposure, it is compensatory.

#### 4.5 Future study outlooks

As mentioned earlier, the exact physiological mechanism regulating oxygen consumption in the digestive system of larval coregonid whitefish remains unclear. Thus, it would be valuable to perform experiments studying that part of metabolism in larval fish. The results would give important information on digestive mechanisms and hopefully also factors affecting growth in larval fish. One way to investigate the depression of the digestive system capacity would be the method presented by Rønnestad et al. (2001). Their system can provide data for the gut absorption, oxidation and assimilation of nutrients and is based on the release, transfer and entrapment of metabolically produced  $^{14}\text{C-CO}_2$  through manipulation of the water pH.

Although it is probable that coregonid larvae are not able to detect UV-B radiation, it would nevertheless be useful to know exactly which wavelengths larval vendace and whitefish are able to detect. Also, Häkkinen et al. (2003) suggested that larvae adapt to the background colour by melanin pigmentation, possibly to avoid visual predation, and UV radiation may increase the contrast of prey species to the predator (Losey et al. 1999). Thus, we should know how larvae behave under both high UV exposure and predator threat. In addition, the effect of UV exposure time on avoidance behaviour should be studied. All this knowledge together would increase our understanding of the mechanism of UV avoidance in larval fish.

Field rearing experiments under controlled conditions would provide information on how incident UV-A level affects the survival of coregonid larvae compared to laboratory results. In these experiments, UV-B irradiance could be manipulated by UV-B lamps as shown by Häkkinen & Oikari (2004).

## 5 CONCLUSIONS

In this thesis the effects of increasing UV-B irradiance, caused by stratospheric ozone depletion, on coregonid larvae were evaluated. At first, I studied the behaviour of larvae both in the laboratory and in the field to clarify the susceptibility of larvae to confront high UV levels. Then, survival, growth and metabolism of larvae were studied under laboratory conditions to identify possible harmful effects of UV-B exposure also under worst case conditions.

The first important finding of this work was that coregonid larvae were able to avoid high UV exposure both in the laboratory and field. In lakes where UV-B radiation penetrated deeper than 20 cm UV avoidance was clearly seen. As there is a strong possibility that coregonid larvae cannot directly detect UV-B wavelengths, the main directive factor in UV avoidance is perhaps UV-B dose. This means that larvae do not start avoiding high UV exposure immediately but if sunny weather continues for several consecutive days they have to decrease UV dose by staying deeper in the water column.

The second important and novel finding of this work was that high UV-B exposure decreased maximum metabolic rate and metabolic scope of both vendace and whitefish larvae and in whitefish larvae marked suppression in digestion costs was also observed. The exact mechanism regulating the digestive system remains unclear. In the field, however, the direct effect of UV-B radiation on coregonid digestion, and hence on growth will be negligible, but some indirect effects, such as increased predation risk, can occasionally be expected.

UV-B radiation alone did not decrease the survival or growth of coregonid larvae at present UV-B doses or at those predicted for coming decades, but UV-B exposure induced the production of melanin pigmentation that protects transparent larvae from UV-B. However, realistic UV exposure combined to the additional stress caused by predators, wind-induced turbulence etc. may decrease growth rate and survival of coregonid larvae. In the worst case conditions, that is, in the context of ozone depletion, sunny weather, low water turbulence and clear water, some mortality may occur. But it has to be noted



that, at least in the pelagic zone of lakes, wild larvae are able to greatly decrease their UV exposure by avoidance behaviour.

High UV-B exposure that will not be expected in the future did produce high mortality of whitefish larvae. Individual variation in UV-B tolerance was high, however, suggesting that in the future coregonid larvae may become more UV-B tolerant through selection. Hence, although in this thesis life-cycle analysis of vendace and whitefish was not made, the effects of increasing UV-B irradiance on larval coregonids will be so small they hardly have effect on vendace and whitefish stocks in Finnish lakes.

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## YHTEENVETO (Résumé in Finnish)

### Lisääntyvän UV-B säteilyn vaikutukset siikakalojen poikasten käyttäytymiseen, kuolleisuuteen ja metaboliaan

UV-B säteilyn määrä on lisääntynyt viimeisten vuosikymmenten aikana maan pinnalla ja veden alla, ja sen ennustetaan lisääntyvän vielä noin vuoteen 2050 saakka. Syy säteilyn lisääntymiseen on ihmisen toiminnan aiheuttama yläilmakehän otsonikerroksen ohentuminen, koska otsonikerros suodattaa suuren osan ilmakehään tulevasta UV-B säteilystä ja ohuempi kerros päästää lävitseen suuremman säteilymäärän. Eryteema (CIE)-painotetun (painottaa eniten UV-B aallonpituuksia) UV-säteilyn määrän odotetaan lisääntyvän Suomen leveysasteilla vielä noin 20-50 prosentilla. Suurin lisäys on odotettavissa keväällä samoihin aikoihin kuin järvet vapautuvat jäistä.

Tämän työn tarkoituksena oli arvioida, mitä haittavaikutuksia lisääntyvällä UV-B säteilyn määrällä voi olla taloudellisesti tärkeimpien sisävesien kalalajiemme, siian ja muikun, kannalta. Tutkimukset keskittyivät poikasvaiheeseen, koska suojaavan pigmentin puuttuessa tai sen ollessa vasta kehittymässä kalat ovat herkimpää UV-säteilyn vaikutuksille. Lisäksi siikakalojen poikaset uivat kuoriuduttuaan muutaman viikon ajan aivan lähellä veden pintaa ja voivat näin altistua UV-B säteilylle. On kuitenkin huomattava että UV-säteilyn tunkeutuminen veteen riippuu suuresti veden humuspitoisuudesta ja liuenneen orgaanisen aineen määrästä. Kirkkaimmissa Suomen järvissä jotka usein ovat tyypillisiä siika ja muikkujärviä, UV-B säteily voi tunkeutua noin metrin syvyyteen. Toisaalta tummimmissa järvissä UV-B säteily sammuu jo ylimmän 10 sentin matkalla.

Tutkimukset osoittivat selvästi että sekä siian että muikun poikaset pystyvät välttelemään korkeita UV-B säteilyn tasoja. Laboratorio-olosuhteissa ne pysyttelivät UV-B: ltä suojaavan kalvon (Mylar) alla ja järvissä ne oleskelivat pilvettömällä säällä syvemmällä kuin pilvisellä säällä. Paksu pilvipeite voi vähentää UV-B säteilyn määrän noin puoleen pilvettämiin oloihin verrattuna. Järvissä välttelykäyttäytyminen oli kuitenkin havaittavissa vain suhteellisen kirkkaissa järvissä, tummavetisissä järvissä sitä ei havaittu. Tähän voi osaltaan vaikuttaa se että kenttänäytteenotossa käyttämiemme Bongo-haavien korkeus oli kaikissa järvissä sama. Jos käytössä olisi ollut eri korkuisia haaveja, UV-B: n välttely olisi ehkä voitu havaita tummissakin järvissä. Näyttäisi lisäksi siltä, että tärkein tekijä välttelykäyttäytymisessä on kumulatiivisen UV-B annoksen suuruus. Poikaset eivät siis välttämättä heti ui syvemmälle altistuessaan voimakkaalle UV-B säteilylle, mutta jos altistus kestää esim. useita päiviä niin vaurioiden välttämiseksi niiden on pysyteltävä syvemmällä.

Kasvatuskokeissa nykyisillä tai ennustetuilla UV-B tasoilla ei ollut vaikutusta siian ja muikun poikasten kasvuun, kuolleisuuteen tai ravinnonkulutukseen. Tosin UV-altistus yhdistettynä stressikäsittelyyn hidasti hieman kasvua. Korkea UV-B altistus (noin kaksinkertainen nykyiseen

maksimiin verrattuna) tappoi 70 % siianpoikasista ja alensi kasvua kahden viikon aikana. On kuitenkin huomattava, että koeakvaarioissa käyttämämme vesi oli hyvin kirkasta ja UV-A säteilyn, jonka on havaittu korjaavan DNA-vaurioita, taso oli paljon alhaisempi kuin luonnossa. Tästä syystä laboratorionkokeissa saadut tulokset ovat dramaattisempia kuin mitä luonnonolosuhteissa on odotettavissa.

Korkea UV-B altistus alensi siian ja muikun poikasten maksimihapenkulutusta ja hapenkulutuksen vaihteluväliä. Korkea UV-B altistus alensi myös ruuansulatuksen aiheuttamaa hapenkulutusta vähän ennen altistusta ruokituilla siianpoikasilla, mutta tämän ilmiön tarkka fysiologinen mekanismi jäi epäselväksi. Alenema voi johtua lähinnä kahdesta eri tekijästä: 1) ruuansulatuksen energiansaannista huolehtivien mitokondrioiden toimintakyky on alentunut, tai 2) ruuansulatuksen verenkierron säätelymekanismi on häiriintynyt.

Kaiken kaikkiaan lisääntyvän UV-B säteilyn suorat vaikutukset siian ja muikun poikasiin ja sitä kautta myös näiden lajien kantoihin lienevät tulevaisuudessa vähäiset. Joitakin epäsuoria vaikutuksia, kuten lisääntynyttä altistumista saalistukselle sekä kasvunopeuden hidastumista, voi ajoittain olla odotettavissa.

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