BOREAL ENVIRONMENT RESEARCH 12: 673–680
ISSN 1239-6095 (print) ISSN 1797-2469 (online)

Helsinki 18 December 2007

Responses to ultraviolet radiation in larval pike, *Esox lucius,* of two origins and ages

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Received 30 Nov. 2006, accepted 7 Aug. 2007 (Editor in charge of this article: Outi Heikinheimo)

Vehniäinen, E.-R., Häkkinen, J. M. & Oikari, A. O. J. 2007: Responses to ultraviolet radiation in larval pike, *Esox lucius,* of two origins and ages. *Boreal Env. Res.* 12: 673–680.

Ultraviolet radiation (UVR)-induced mortality and behavioural disorder were studied in larval northern pike of two origins and ages. Newly hatched larvae of two differently coloured populations and six-day-old larvae of one population were exposed to four fluence rates of UVR, resulting in total doses from 11.5 to 63 kJ m^{-2}, and monitored for mortality and behaviour. The rate of mortality and the severity of behavioural disorder differed by origin and age of the animals, but the effect was fluence-rate dependent. Total melanin concentration of newly hatched larvae was measured to assess if sensitivity to UVR correlated with pigmentation, but no differences in melanin concentration between larvae from different origins were found.

Introduction

Ultraviolet B (UV-B; 280–320 nm) radiation has been shown to be detrimental to fishes, especially to embryo and larval stages (Hunter *et al*. 1979, Hunter *et al*. 1981, Williamson *et al*. 1997, Béland *et al*. 1999, Kouwenberg *et al*. 1999, Novales Flamarique *et al*. 1999, Steeger *et al*. 1999, Battini *et al*. 2000, Browman *et al*. 2000, Häkkinen *et al*. 2002). However, there are extremely large differences between species. It was recently shown that larval pike (*Esox lucius*) are highly sensitive even to current levels of UV-B encountered in early summer (Häkkinen *et al*. 2004, Vehniäinen *et al*. 2007). The larvae displayed abnormal behaviour named spiral swimming, which means that they were unable to swim straightforward and orientate, and the affected larvae spin uncontrollably (Häkkinen *et al*. 2004, Vehniäinen *et al*. 2007). This condition was usually followed by mortality (Häkkinen *et al*. 2004, Vehniäinen *et al*. 2007).

In Fennoscandia, pike spawns in April–May in shallow waters (depth < 1 m) with vegetation as spawning base. Fertilized eggs swell and adhere to plants, which prevents them from sinking down to the bottom. Newly hatched larvae attached to plants remain nonmotile for the first few days of life. One-week-old freely swimming larvae are positively phototactic and often swim very near the water surface, and they may thus become severely exposed to UV-B radiation (Urho *et al*. 1989, Zigler and Dewey 1995).

Melanin is a brownish pigment that absorbs a wide range of the UV spectrum (Cockell and Knowland 1999). It is found in skin and eyes of many fishes and its production typically begins a few days before hatching (Ahmed and Setlow 1993, Lowe and Goodman-Lowe 1996, Speekmann *et al*. 2000, Häkkinen *et al*. 2002).

UVR induces melanin synthesis in some fishes — they tan like people after exposure to UVR (Lowe and Goodman-Lowe 1996, Häkkinen *et al*. 2002). Regarding to the protective role of melanin, however, there is some controversy about whether it actually protects from UVR, because in addition to acting as a radical scavenger it can also produce reactive oxygen species (Korytowski *et al*. 1987, Bustamante *et al*. 1993, Agar and Young 2005, Wood *et al*. 2005). Some studies have suggested that melanin has no protective influence at all, or that melanin may actually increase the UV-induced damage (Blazer *et al*. 1997, Armstrong *et al*. 2002, Setlow *et al*. 1993).

Due to the possible harm UVR may evoke, the aim of this study was to compare the UVR-dependent mortality and behavioural disorder of pike larvae from two lakes (Lentua and Päijänne). The lakes differ in their location and water characteristics. The larvae from lake Lentua seemed darker by eye than the larvae from lake Päijänne and therefore the melanin content of the larvae was measured. Because at the age of about one week behaviour of the larval pike changes from stationary to positively phototactic free-swimming (making the larvae more likely to be exposed to UVR) the UVR-dependent mortalities of newly hatched and six-day-old larvae were compared. Similar UVR fluence rates were used as in a previous experiment, ranging from subambient with no effects on mortality to unrealistically high with 100% mortality in 5 days (Vehniäinen *et al*. 2007). The irradiation time was 3 hours on two consecutive days (instead of 6 hours in the previous experiment) to better mimic the natural UVR (high dose at noon).

Table 1. The experimental design showing the UVR fluence rates and total doses used in the experiment (mean ± variance). Newly hatched pike larvae were exposed to UVR in Pyrex glass bowls, 40 larvae in each bowl. Each treatment was replicated three times ($N = 3 \times 40$).

Fluence rate (mW m^{-2})	Dose in 6 h (kJ m^{-2})
550 ± 45	11.5 ± 0.8
1480 ± 150	30.8 ± 3.2
2200 ± 130	45.6 ± 2.8
3040 ± 210	63.0 ± 4.4

Materials and methods

Animals and water characteristics

The fertilized eggs of pike were obtained from the municipality of Kuhmo (Kuhmon Kala hatchery, lake Lentua (64°22´N, 29°75´E), Finland) and the municipality of Jämsä (Timo Paajoki, lake Päijänne (61°24´N, 25°24´E), Finland). Once transferred to the wet laboratory of the University of Jyväskylä, the eggs were incubated and hatched in flow-through hatchery cones at 10 °C. The conductivity of the water was 24.6 mS m^{-1}, alkalinity 0.79 mmol l^{-1}, pH 7.6, and concentrations of Mg 7.9 mg l^{-1} and Ca 19 mg l^{-1}. Newly hatched (< 24 h) larvae from lakes Lentua and Päijänne and six-day-old larvae from lake Päijänne were used in the experiments.

Exposure system and sampling

UV radiation was provided in the laboratory by a fluorescent lamp (UVB-313, Q-Panel, USA). Ultraviolet C (UV-C; under 280 nm), not reaching the surface of the earth, was blocked with a cellulose diacetate filter (Clarifoil, UK), replaced after each six-hour UV irradiation. Control treatments without UVR received visible light (TLD 36W/950 daylight, Philips, the Netherlands). The photoperiod was 12h:12h.

UVR was quantified using Hamamatsu Photonic Multichannel Spectral analyser (model PMA-11), measuring the wavelength area 280–380 nm. The UVR intensities were measured at the water surface at the beginning and the end of each experiment at various spots to ensure equal doses for all replicates in each treatment. The daily doses were calculated as unweighted J m^{-2}. The UVR fluence rates and cumulative doses are shown in Table 1.

Larvae were carefully transferred to one-litre Pyrex glass bowls filled with water from the hatchery. The water depth was 5 cm. Each treatment was replicated three times with 40 larvae

in each bowl. Larvae were exposed to UVR radiation at four dose rates for 3 hours on two consecutive days (i.e. 2 × 3 h). After the UVR irradiation episodes the larvae received visible light only (TLD 36W/950 daylight).

Mortality and behaviour of the larvae were monitored twice a day. Larvae were gently disturbed by blowing air next to them with a glass pipette, and the ability of the animals to respond by their movements was monitored. Non-moving larvae were designated as dead, and those unable to swim straight were designated as abnormal. The experiment was finished and animals sampled for analysis when all larvae in the treatment with lowest UVR intensity showed behavioural symptoms (newly hatched larvae from lake Päijänne) or when the larvae had used up more than 3/4 of their yolk sac (six-day-old larvae and larvae from lake Lentua).

Melanin analysis

Total melanin was analyzed spectrophotometrically using a method developed for mammalian hair (Ozeki *et al*. 1995, 1996, Häkkinen *et al*. 2002). Heads of newly hatched larvae were trimmed off before homogenization to remove the eyes with high melanin content. Homogenized samples (20 mg tissue ml^{-1} distilled water) were placed in 10-ml capped glass test tubes, to which 1.8 ml Soluene-350 (Packard Instruments, USA) 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 into total melanin by referring it to the A_{500} value of solubilized sepia melanin standard (Sigma-Aldrich, Germany).

Statistics

Statistics were performed with SPSS 12.0.1. Effects of age and population on mortality and behaviour were tested with repeated measures ANOVA (fixed effects model).

Results

Mortality

Both origin and age affected the UVR-induced mortality, but the magnitude varied with the fluence rate. Newly hatched larvae from lake Lentua were more tolerant to UVR than newly hatched ones from lake Päijänne (repeated measures ANOVA: df = 1, $F = 25.851$, $p < 0.01$). This difference in mortality was seen at the three highest fluence rates (1480 ± 150–3040 ± 210 mW m^{-2}) (Fig 1). At the lowest fluence rate, however, the larvae from both origins were equally tolerant, and the mortality of this treatment did not differ from that of controls (repeated measures ANOVA: $p > 0.05$).

The six-day-old larvae were more tolerant to UVR than the newly hatched ones (Fig. 1; repeated measures ANOVA: df = 1, $F = 4.873$, $p = 0.033$). This difference could be seen especially clearly at the intermediate doses (Fig. 1).

Behavioural abnormality

The syndrome of spiral swimming was seen in all irradiation groups (Fig. 2). This syndrome was typically followed by mortality in all UVR treatments except in the treatment with the lowest UVR dose (11.5 kJ m^{-2}) (Fig. 2). The only animals that showed distinct recovery were the larvae from lake Lentua irradiated with the lowest UVR dose (Fig. 2a).

Melanin concentration

The melanin concentration was 0.80 ± 0.24 μg mg^{-1} dry weight (mean ± SD) and 0.81 ± 0.29 μg mg^{-1} dry weight for lakes Lentua and Päijänne larvae, respectively. There was no difference between populations in their melanin content.

Discussion

Large differences between species in tolerance to UVR have been documented for many organisms

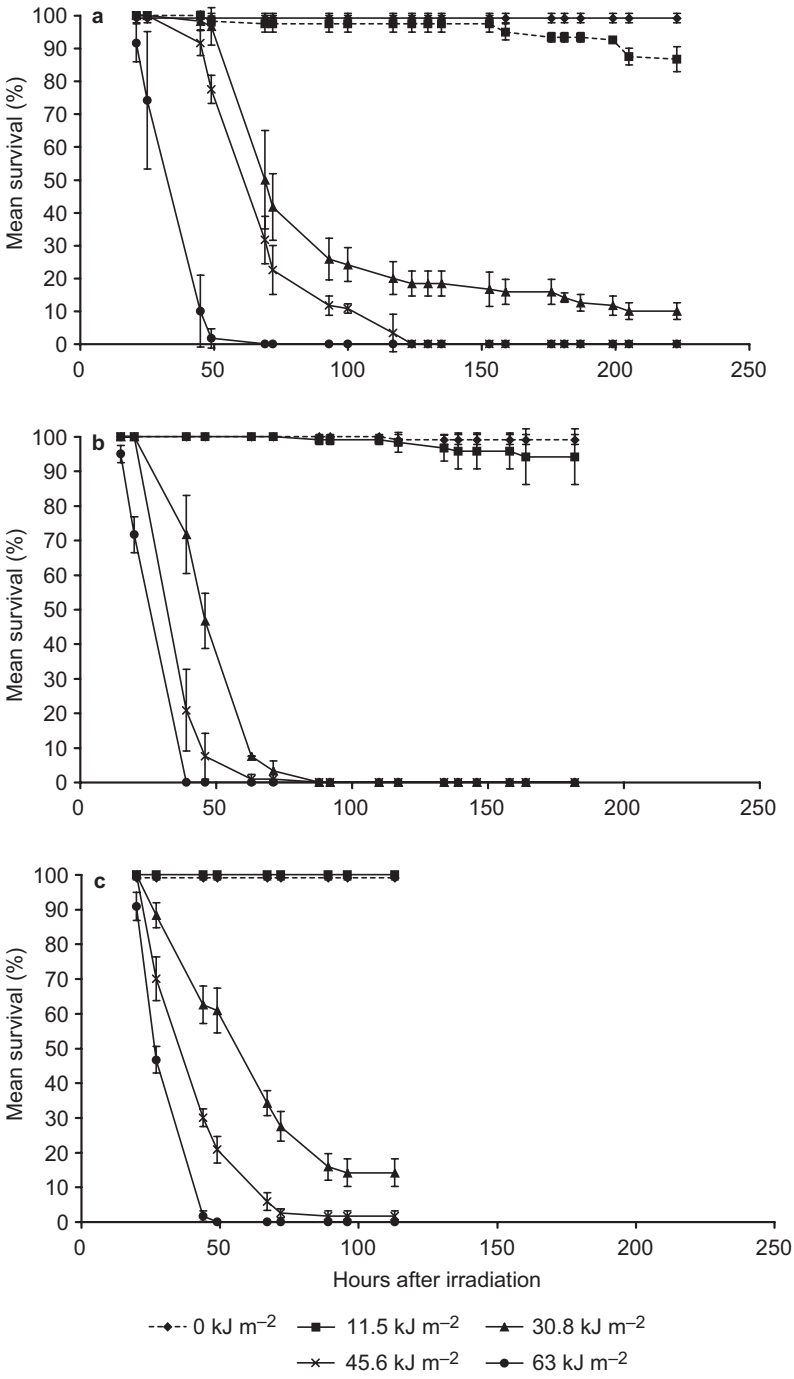

Fig. 1. Mean survival of pike larvae as a function of time after irradiation. Larvae were irradiated for 3 h on two consecutive days (2 × 3 h). — **a**: larvae from lake Lentua, — **b**: newly hatched larvae from lake Päijänne, — **c**: six-day-old larvae larvae from lake Päijänne.

such as bacteria, plants, zooplankton, and fishes (Sommaruga 2001, Häkkinen *et al*. 2002, 2003b, Kakani *et al*. 2003, Yanqun *et al*. 2003, Ylönen and Karjalainen 2004). In contrast, intraspecific differences in sensitivity to UVR have not been studied as vastly, the only exception being (crop) plants. Differences in sensitivity to UVR between strains have been observed in freshwater fishes Japanese medaka (*Oryzias latipes*) and *Xiphophorus* (Ahmed and Setlow 1993, Armstrong *et al*. 2002). In the present study the darker larvae from lake Lentua were more tolerant to UVR than

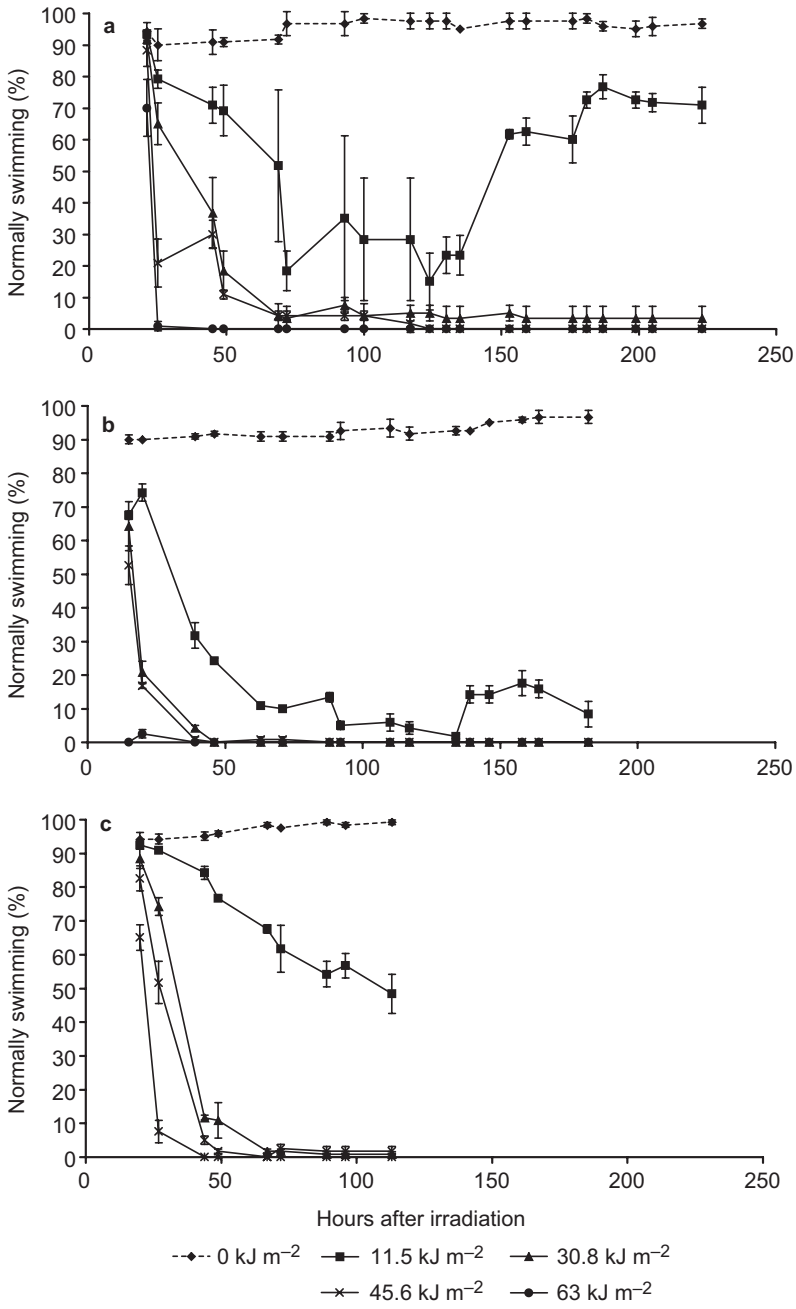

Fig. 2. Behaviour of pike larvae as a function of time after irradiation: proportion of normally swimming individuals as a percentage of the original number of larvae (40). Larvae were irradiated for 3 h on two consecutive days (2 × 3 h). — **a**: larvae from lake Lentua, — **b**: newly hatched larvae from lake Päijänne, — **c**: six-day-old larvae larvae from lake Päijänne.

the lighter ones from lake Päijänne. Larvae from lake Lentua showed lower mortality than those from lake Päijänne in all treatments except that with the lowest UVR dose, and at the lowest dose the larvae from lake Lentua were the only ones to show 60% recovery from spiral swimming.

The difference in tolerance between larvae from different origin most likely does not reflect direct adaptation to different UVR regimes, because the water of lake Lentua is darker than that of lake Päijänne (Table 2). Actual transparencies to UVR are not known for the given lakes, but as the UVR attenuation depth correlates negatively with the colour of water (Häkkinen *et al.* 2003a), it can be assumed that UVR is attenuated more quickly in lake Lentua than in lake Päi-

jänne. Thus the difference in tolerance to UVR may be merely a side-effect of different pigmentation. Positive correlation between water colour and larval pigmentation has been observed in vendace (*Coregonus albula*) and whitefish (*C. lavaretus*) (Häkkinen *et al*. 2003a). The reason for the correlation in coregonids was assumed to be phenotypic adaptation to the colour of the background water (Häkkinen *et al*. 2003a).

Although the larval pike from lake Lentua seemed darker by eye than larvae from lake Päijänne, there was no difference in the amount of melanin. However, the amount of melanin was so small in all pike (0.38–1.33 μg mg^{-1} dry weight, average 0.8 μg mg^{-1} dry weight) as compared with that in e.g. whitefish (*Coregonus lavaretus*) larvae (4–9 μg mg^{-1} dry weight, average 5.5 μg mg^{-1} dry weight; Häkkinen *et al*. 2002) that the method may not be able to discern any differences at such a low level. The minute amount of melanin in pike larvae as compared with that in whitefish larvae raises the question whether this lack of protective pigmentation is the reason for the extreme sensitivity of pike to UV-B (Häkkinen *et al*. 2003b, Vehniäinen *et al*. 2007). Furthermore, it may not be only the amount but also the location of melanin that plays a role in protecting fishes from UVR (Hunter *et al*. 1979, Kaweewat and Hofer 2001).

The role of melanin in protecting from UVR seems rather controversial. Increased melanin content may be connected with decreased numbers of UV-induced dermal DNA damage in platyfish *Xiphophorus* (Ahmed and Setlow 1993), and pigmented *Clupea pallasi* larvae are more tolerant to UVR than translucent ones (Speekmann *et al*. 2000). On the other hand, the strain with the highest melanin content gets the highest level of UVR-induced tissue damage among differently pigmented Japanese medaka (*Oryzias latipes*) (Armstrong *et al*. 2002), and in one *Xiphophorus* strain the melanin-photosensitized oxidant production seems to be the reason for UV-induced melanoma (Wood *et al*. 2005).

The difference in darkness between larvae from different origin may also be due to some other substance than melanin, as this difference in colour can be seen already in newly fertilized eggs (E.-R. Vehniäinen pers. obs.). In addition to melanin, fish may also contain other substances that absorb UV (Fabacher and Little 1995, 1998, Kaweewat and Hofer 2001). These methanol-extractable, UV-absorbing substances have been found in pike larvae, as well (E.-R. Vehniäinen unpubl. data), but their amounts in larvae from different origins and of different ages remains to be studied. In cyprinids, the amount of UV-absorbing methanol-extractable substances increases with the age of the larvae (Kaweewat and Hofer 2001). This may also explain why the six-day-old pike larvae were more tolerant to UVR than newly hatched ones. Older larvae may also have other protective means that newly hatched animals do not yet possess.

The two highest UVR-fluence rates used in this study are higher than those observed or predicted for the next 50 years in boreal regions, but the two lowest UVR-fluence rates can be referred to as possibly ecologically relevant, because they mimic the natural spectrum for the part of UV-B. Both mortality and behavioural disorder of larval pike occurred at these fluence rates. However, the laboratory spectra never exactly simulate ambient solar radiation and this may lead one to overestimate the effects of UVR, because the unnaturally low levels of UV-A and PAR (photosynthetically active radiation) in the laboratory may attenuate photoenzymatic repair — though according to a recent study, northern pike larvae may not have this mean (Olson and Mitchell 2006). In a field study with similar UV-B fluence rates as the two lowest ones used in this study, larval pike showed behavioural abnormality (Häkkinen and Oikari 2004). This condition most likely leaves the affected larvae vulnerable to predation and makes them unable to forage even if behavioural recovery could occur.

The newly hatched larvae from lake Päijänne showed similar mortality as in the previous

Table 2. Water characteristics in lakes from which the fish eggs were obtained. Data from regional environment centres of Kainuu and central Finland.

	Lake Päijänne	Lake Lentua
Colour (Pt mg l^{-1})	25–40	50–59
pH	6.2–7.3	6.0–7.0
Conductivity (mS m^{-1})	5.8–7.8	2.4–2.8
TOC (mg l^{-1})	6.5–8.1	7.0–9.4

study (Vehniäinen *et al*. 2007), where one single six-hour irradiation was used instead of two three-hour irradiations. There were slight differences in the severity of the behavioural disorder between the studies: Larvae irradiated with the lowest fluence rate showed less behavioural disorder in the present study. Thus some recovery may have taken place between the irradiations.

Although pike larvae from the two locations showed different tolerance to UVR in the laboratory, a question raises whether this difference is ecologically important. Failure to mimic natural light conditions in the laboratory may exaggerate also intraspecific differences in laboratory settings. Small differences in tolerance to UVR in the laboratory may not be directly translated to similar differences under field conditions, and the intraspecific differences should therefore be verified in field studies. Yet the ability of lake Lentua larvae to recover from UVR-induced behavioural disorder may have significance also in the nature.

Acknowledgement: The authors thank Kuhmon Kala and Timo Paajoki for the pike eggs, regional environment centres of Kainuu and Central Finland for providing the data on water characteristics of the lakes, and Mervi Koistinen for excellent technical assistance.

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