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Effects of ultraviolet-B radiation and pH on early development of the moor frog *Rana arvalis*

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Summary

1. Although the potential negative effects of increased ultraviolet-B (UV-B) radiation on early life stages of aquatic organisms are widely recognized, possible synergistic effects with other stressors have seldom been studied outside the laboratory. We investigated the effects of UV-B radiation and pH on hatchability and early development of moor frog *Rana arvalis* eggs in the field and in laboratory experiments conducted during April 1998 and April 2000 in central Sweden.

2. In the field experiments, no evidence was found for reduced hatchability or increased frequency of developmental anomalies of embryos exposed to ambient levels of UV-B compared with embryos shielded from UV-B radiation.

3. Hatchlings shielded from ambient UV-B radiation did not grow larger than their exposed full-sibs, giving no support to the hypotheses that (i) the repair of cellular UV-B damage might be energetically costly nor (ii) that UV-B-induced photoproducts directly reduce growth.

4. Although low pH (5·0) reduced hatchability, increased frequency of developmental anomalies and reduced early embryonic growth in *R. arvalis*, there was no evidence for synergistic effects of pH and UV-B on any of these traits.

5. The lack of UV-B radiation effects on the development of *R. arvalis* embryos cannot be ascribed to relatively low effective daily doses of radiation (*c.* 0·43 kJ m^{-2}) during the field experiments, as in the laboratory even higher doses at UV-B 1·25 kJ m^{-2} and 1·58 kJ m^{-2} (all DNA weighed) had no negative effects.

6. These results suggest that current levels of UV-B radiation in northern Europe are not likely to reduce fitness in natural populations of the moor frog, even in areas already stressed by acidity.

Key-words: acidification, amphibians, growth, synergism, UV-B.

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Introduction

Increased ultraviolet-B (UV-B) radiation at the earth's surface as a result of ozone depletion (Kerr & McElroy 1993; Madronich *et al*. 1998) has been widely discussed as a potential cause of world-wide amphibian decline (Wake 1991; Blaustein *et al*. 1995; Houlahan *et al*. 2000), prompting several laboratory (Blaustein *et al*. 1994; Ankley *et al*. 1998; Bruggeman, Bantle & Goad 1998) and field investigations (Anzalone, Kats & Gordon 1998; Blaustein *et al*. 1998; Lizana & Pedraza 1998; Cummins, Greenslade & McLeod 1999; Merilä, Laurila & Pahkala 2000; Pahkala, Laurila & Merilä 2000) into the effects of UV-B on amphibians. Several field studies have found evidence for higher mortality and incidence of developmental anomalies among amphibian eggs exposed to ambient levels of UV-B radiation, than among those sheltered from UV-B (reviewed in Blaustein *et al*. 1998). However, other studies have not detected effects on egg development at realistic, or even considerably increased (Cummins *et al*. 1999), doses of UV-B radiation (Blaustein *et al*. 1998; Corn 1998; Blaustein *et al*. 1999). Hence, the emerging view

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is that not all species are equally sensitive to UV-B radiation (Blaustein *et al*. 1994), with those living in habitats naturally exposed to high doses of UV-B being more tolerant than those living in less UV-B-exposed habitats (Blaustein *et al*. 1994, 1999).

Many amphibian populations are also subjected to other stressors, such as low pH (Pierce 1985; Böhmer & Rahman 1990) which increases both egg mortality and the time needed for embryonic development (Andrén *et al*. 1988; Beattie & Tyler-Jones 1992; Grant & Licht 1993). Although there is a growing concern that such stressors could act synergistically with UV-B radiation as agents in amphibian declines (Kiesecker & Blaustein 1995; Long, Saylor & Soulé 1995; Ankley *et al*. 1998; Hatch & Burton 1998), there are only two studies that have examined the synergistic effects of low pH and UV-B radiation on amphibian development (Long *et al*. 1995; Pahkala *et al*. 2000). Moreover, most studies on the effects of UV-B radiation on amphibians have involved North American or Australian species (Blaustein *et al*. 1998, 1999; Corn 1998), with little known about the responses of palaearctic species (but see Nagl & Hofer 1997; Lizana & Pedraza 1998; Cummins *et al*. 1999; Langhelle, Lindell & Nyström 1999).

We aimed to determine whether ambient UV-B radiation, alone or in combination with low pH, had negative effects on embryonic development of central Swedish moor frogs *Rana arvalis* (Nilsson), as reflected in levels of embryonic mortality, developmental anomalies and early growth performance in the field. Because the conclusions from field experiments may be sensitive to the specific UV-B conditions during the experiments (cf. Cummins *et al*. 1999), we also conducted laboratory experiments in which embryos were subjected to both normal and enhanced UV-B radiation regimes.

Methods

THE STUDY SPECIES

The moor frog has a euro-asiatic distribution (Gasc *et al*. 1997) and it occurs typically in semi-temporary or permanent ponds, but also in bogs and marshes (Gislén & Kauri 1956). In Sweden, the moor frog is more acid tolerant than other frog species (Andrén *et al*. 1988) and occurs frequently at pH < 5. Globular egg masses are usually laid at a depth of 5–10 cm, with extensive exposure to the sun (A. Laurila & J. Merilä, personal observations). The eggs usually hatch within 2 weeks after fertilization.

FIELD EXPERIMENT

The effects of UV-B and pH on survivorship and growth performance of moor frog embryos were investigated in a field experiment in Uppsala (59°50′N, 17°50′E; 50 m a.s.l.), Sweden, between 22 April and 17 May 1998, coinciding with the natural reproductive period (A. Laurila & J. Merilä, personal observations). Ambient UV-B radiation and freshly (< 2 h) laid egg masses (n = 22) were used. The eggs were obtained from laboratory matings where the moor frog pairs, collected from two different localities in the vicinity of Uppsala (pH 7·6–7·9), were allowed to spawn at 14 °C in plastic buckets filled with dechlorinated tap water. This procedure ensured that the age of different egg masses was similar in all experimental units, and that the eggs had no prior exposure to low pH.

The effects of ambient UV-B radiation and pH on hatchability and early growth of the embryos were investigated in a 3 × 2 factorial experiment (Fig. 1). Each clutch was divided into six different treatments (*c.* 40 eggs in each), which consisted of all possible combinations of two values of pH (low = pH 5·0; neutral = pH 7·6) and three radiation treatments (1, unfiltered sunlight; 2, sunlight filtered to remove UV-B and shorter wavelength components; 3, sunlight filtered to remove shorter wavelength components than UV-B). The second treatment was attained with the aid of Mylar filters (0·10 mm; Erik S. Ekman, Stockholm, Sweden), which were placed over the eggs. The third treatment was created with a preburned cellulose diacetate filter (0·13 mm; Courtaulds, Derby, UK), which was included to control for filter effects, such as enhanced thermal environment created by the filter

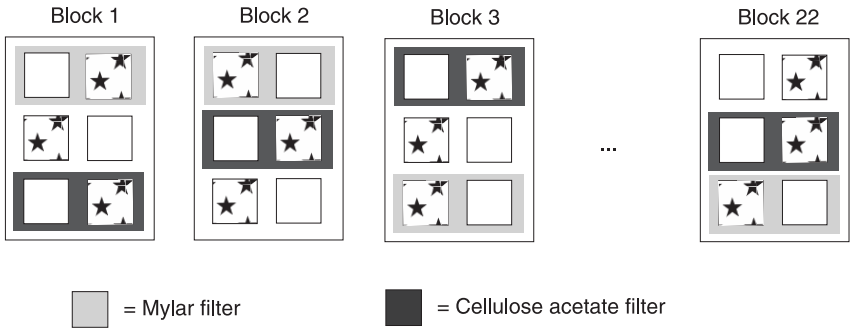

Fig. 1. Schematic presentation of a field experiment assessing the effects of pH and UV-B treatments on moor frogs. All eggs in each of the blocks (n = 22) were derived from the same mating, and exposed to six different combinations of UV-B (Mylar filter, cellulose acetate filter, open) and pH (5·0, 7·6) treatments. Smaller star-filled and empty boxes depict low and neutral pH, respectively. The order of pH and UV-B treatments within each block was randomized.

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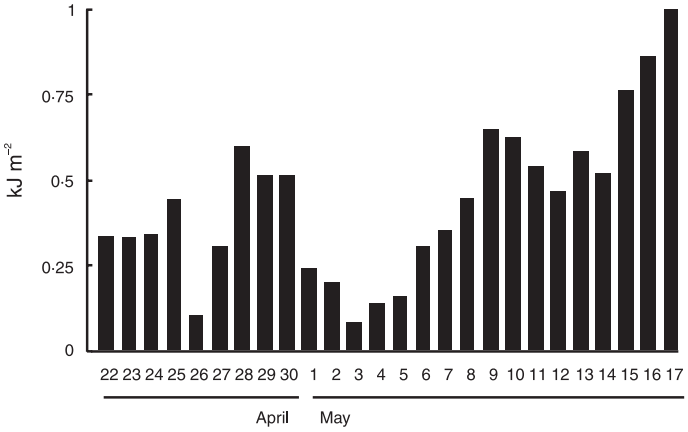

Fig. 2. Estimated effective daily doses of UV-B radiation (DNA-weighed, cloud corrected) during the field experiments (see the Methods).

coverage. The filters were tested with an Optronics 754 spectroradiometer (Optronics, Orlando, FL) calibrated using an OL 752-10 plug-in standard lamp (200 W tungsten lamp with a quartz envelope), which in turn was calibrated at the National Institute of Standards and Technology (Orlando, Florida, USA). The filters were placed 3 cm above the vessels to allow air circulation, and they were exchanged once a week to ensure that their properties remained homogeneous during the experiments. The two different pH levels corresponded to naturally low (5·0) and normal (7·6) pH in the typical breeding localities of this species. The experiment was conducted in 22 blocks in which all of the six different treatment combinations were applied to eggs from the same clutch (i.e. all embryos in each block were full-sibs; Fig. 1). Each block consisted of green 112-litre plastic boxes ($80 \times 40 \times 35$ cm) filled with water to stabilize temperature fluctuations. The six treatment vessels within each of the blocks were 16×20 cm wide and 5 cm high propane vessels filled with 1·6 litres of water. The treatment vessels were mounted on a wooden frame covered with wire mesh. There was no water exchange between the large box and treatment vessels.

Dechlorinated tap water, mixed with deionized water at a 1 : 1 ratio, was used in the experiments. Water was exchanged every third day, and always immediately after heavy rain. Water for both pH treatments was prepared in 80-litre storage tanks, adjusted with 1 or 0·1 M H_2SO_4 (or 1 M NaOH) and stabilized over two 48-h periods before use. pH levels were monitored daily using a Hanna HI 9025 pH-meter (Hanna Instruments Inc., Woonsocket, RI) equipped with a Ross (Orion Ross sure-flow 8172; Orion Research Inc., Beverly, MA) electrode. Because the developmental rate was likely to be influenced by temperature, we recorded temperatures in each of the three UV-B treatments in each block three times a day (06:00, 12:00 and 18:00 h) during the experiments. The temperatures differed significantly among blocks ($F_{21,42} = 15{\cdot}69$, $P < 0{\cdot}001$) and under different filter treatments ($F_{2,42} = 5{\cdot}25$, $P < 0{\cdot}01$). The open controls had the lowest temperatures [$x = 12{\cdot}90 \pm 0{\cdot}08$ (SE)], whereas the temperatures under Mylar ($x = 13{\cdot}65 \pm 0{\cdot}09$) and cellulose acetate filters ($x = 13{\cdot}70 \pm 0{\cdot}09$) did not differ (Tukey's test, $P > 0{\cdot}05$). The amount of UV-B irradiance during the experiments was monitored with an Optronics 752 spectroradiometer every hour between 06:00 and 18:00. However, because measurements were not taken continuously, and the data were missing for some occasions due to rain or other logistic reasons, the daily UV-B exposure was estimated using (i) the algorithm of Björn & Murphy (1985), (ii) ozone column values and (iii) a correction for cloudiness based on a comparison between measured and calculated irradiance values and the cloudiness at the time of measurement. Ozone values from satellite (total ozone mapping spectroradiometer; TOMS) measurements were used, except for one day when such values were not available. On this occasion a value measured (by Weine Josefsson at the Swedish Meteorological and Hydrological Institute, SMHI) from the ground in Norrköping (60 km south-east of Uppsala) was used. Cloudiness was measured at hourly intervals [using the octa-scale (0–8) recommended by the World Meteorological Organization (WMO; http://www.wmo.ch/)] or measurements obtained via SMHI from observations at Arlanda airport situated 25 km south-east of the study area. For 3 days cloudiness data were not available from any of these sources, and the cloudiness was estimated from satellite pictures obtained from the satellite NOAA-7 via the Dundee Satellite Receiving Station.

The estimated effective UV-B doses during the field experiments averaged 0·435 kJ m^{-2} (Fig. 2), which is lower than the value from Langhelle *et al.* (1999) in southern Sweden (0·864 kJ m^{-2}). However, they measured UV-B levels much later in the season when the solar angle is higher, and also the weather conditions and ozone levels may have been different.

Survival rates were recorded when the majority of larvae in a given vial had reached stage 25 (absorption of external gills; Gosner 1960; hereafter G25) and were defined as the proportion of eggs that survived from the beginning to the end of the experiment. Larvae with visible developmental anomalies (tail flexure or swollen

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body) were excluded from the survival estimates because their future survival was unlikely. Anomaly frequency was defined as the proportion of larvae with visible developmental anomalies. Hatchling size was determined from formalin-preserved samples for eight larvae from each of the six treatments in each block by measuring their total (from nose tip to tip of tail), body and tail length under a stereomicroscope (to the nearest 0·13 mm). All measurements were taken blind in respect of the experimental treatments, and the repeatability of all size measurements was high, as assessed from two repeated measures of the same individuals (repeatability > 0·90, $F_{24,25} > 500{\cdot}0$, $P < 0{\cdot}001$). Sample sizes for the analysis of hatchling size were lower than those for survival and anomaly analyses because all or part of the larvae in some blocks died before reaching stage G25, or because samples for the measurements were not available. The experiments ended when the majority of the larvae in a given vial had reached stage G25.

LABORATORY EXPERIMENT

In April 2000, adult male and female frogs were collected from one population in the vicinity of Uppsala and brought to the laboratory. Each male was then artificially mated (following the procedure of Berger, Rybacki & Hotz 1994) with one female, resulting in four full-sib families. Artificial mating ensured that all offspring from a given family were full-sibs, and that the eggs had no prior exposure to UV-B radiation. Any eggs that appeared abnormal were discarded prior to experimentation. After fertilization, the eggs (< 2 hours old) were divided into batches of *c.* 30 and placed into experimental vessels (0·25 litre; polypropene; 5 cm × 5·5 cm).

Experimental design

The experiment was conducted in a constant temperature room (+15 °C) in three aquarium systems, each of which consisted of two experimental aquaria (120 × 120 × 25 cm; about 320 litres) situated on the top of each other and a reservoir tank (90 × 90 × 35 cm; about 280 litres) below them. Each aquarium system was filled with reconstituted soft water (RSW, APHA 1985), which was circulated continuously (flow rate 3 l min^{-1}) to reduce temperature fluctuations. Each aquarium system was equipped with a water-cooling unit.

The experiment consisted of the fully factorial combination of three UV-B treatments, with each family being replicated four times within each treatment combination. Because there were two experimental aquaria (blocks) for each of the UV-B treatments, two replicate vessels were randomly placed in each aquarium. Placement of the vessels within the aquaria was changed randomly each day to ensure uniform irradiance. The experimental vessels containing the eggs were placed on top of a sheet of plastic netting situated 5 cm below the water surface. The vessels had direct contact with the surrounding water circulating in the aquarium systems. As a direct consequence of the radiation from the greenhouse lamps (see below), there was regular daily temperature variation in the aquaria. The average daytime (08:00–17:00) water temperature during the experiments was 16·6 ± 0·07 °C (min = 13·3 °C, max = 19·7 °C).

UV-B treatments

The UV-B treatments were divided into six blocks (two for each UV-B treatment) over the three aquarium systems, each system thus containing two blocks. The daily photoperiod was 17 light : 7 dark and the UV-B exposure periods occurred around noon (between 11:00 and 14:00). A computer model (Björn & Murphy 1985; Björn & Teramura 1993) was used to calculate the daily irradiance of UV-B in Uppsala on 24 April (the normal breeding time of *R. arvalis*) as well as the daily increase in UV-B radiation that would follow from 15% ozone depletion under clear sky conditions, resulting in 26% enhanced UV-B above normal levels. This calculation was based on spectrally weighting the radiation with Caldwell's plant action spectrum as parameterized by Thimijan, Carns & Campbell (1978). However, to facilitate comparisons with experiments on frogs by other groups, we expressed the radiation in DNA-weighted units. The DNA-weighted daily UV-B exposures were 1·254 and 1·584 kJ m^{-2} for 'normal' and 'enhanced' UV-B, respectively. The levels of UV-B were adjusted by regulating daily irradiation regimes in the following way: (i) normal UV-B (irradiation time 2 h 17 min day^{-1}); (ii) high UV-B (irradiation time 2 h 53 min day^{-1}); (iii) control (irradiation time 2 h 17 min day^{-1}), where UV-B and UV-C were blocked with a Mylar filter. UV-B radiation for each aquarium was provided by four fluorescent tubes (UV-B 313, 40 W, 120 cm; Q-PANEL, Cleveland, OH) preburned for 100 h to give a stable output. In each aquarium, the four fluorescent tubes were placed 50 cm above water level, uniformly parallel (40 cm between each lamp) to each other. The mid-section (*c.* 40 cm) of the two central tubes was covered with aluminium foil to obtain an even radiation distribution into the aquarium.

In the normal and high UV-B treatments the radiation passed through a cellulose diacetate filter (see above) to cut off UV-C (< 280 nm) radiation. Filters were placed on wooden frames about 25 cm above the water level to allow air circulation beneath them, and were changed every second week to ensure that their properties remained homogeneous during the experiment. To ensure sufficient background light for normal functioning of light-dependent DNA damage repair mechanisms (Zhao & Mu 1998), two 400-W greenhouse lamps (Powerstar HQI-BT 400 W/D, OSRAM, UBA; Växthus, Malmö, Sweden) were fitted over each of the six aquaria. The amount of radiated light was measured using a LI-COR Light Meter (Li-Cor, Lincoln, NE) with a quantum sensor, giving an irradiance of 320 μmol m^{-2} s^{-1}.

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STATISTICAL ANALYSES

The effects of UV-B and pH treatments on survival, frequency of anomalies and size at hatching in the field experiment were investigated with mixed model ANOVAS as implemented in the PROC MIXED routine in SAS (SAS Institute Inc. 1996). In these models, pH and UV-B treatments were treated as fixed effects, whereas block was considered to be a random effect. When analysing the hatchling size traits, we used the mean value of hatchlings in each of the vessels as a unit of analysis because individual values cannot be considered as independent observations. Likewise, to avoid unbalance in the design matrix caused by the death of all hatchlings in some of the vessels, we included only those blocks in the analyses of morphological traits in which hatchlings from all of the six vessels survived. However, the results are insensitive to the inclusion of all available measurements into the analyses. Before statistical testing, both survival and anomaly estimates were arcsine-square root transformed to normalize their distributions. The normality of all response variables was tested with Wilk's statistic applied on the residuals of the models; no deviations from normality were detected (in all cases $P > 0{\cdot}05$). Due to the non-normal distribution of survival rates and the frequency of developmental anomalies in the laboratory experiment, a generalized linear model using logit link function and binomial error structure was applied to these data as implemented in PROC GENMOD procedure in SAS (Allison 1995). All statistical analyses were performed with version 6.12 of the SAS statistical package (SAS Institute Inc. 1996).

Results

FIELD EXPERIMENT

Survival and anomalies

Low pH reduced the survival probability as well as increased the frequency of developmental anomalies (Fig. 3 and Table 1). No effect of UV-B treatment nor UV-B × pH interaction was detected, suggesting that neither ambient levels of UV-B nor low pH together with UV-B reduced the survival of the moor frog embryos (Table 1). The block effects were large (Table 2), suggesting that factors other than ambient UV-B radiation were more important to successful development of moor frog embryos.

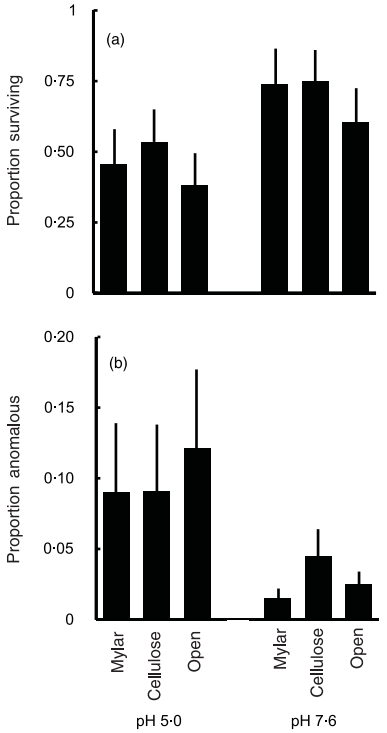

Fig. 3. (a) Survival and (b) frequency of developmental anomalies among moor frogs in different UV-B and pH treatments in the field experiment. Each mean (± SE) is based on 22 independent replicates. For statistical tests, see Table 1.

Hatchling size

UV-B treatment had significant effects on hatchling total, body and tail lengths (Table 2 and Fig. 4). However, the significant contrasts between open and filter (Mylar and cellulose) treatments revealed that the filter itself had a positive effect on early growth performance,

Table 1. Mixed-model ANOVAS assessing the effects of low pH and UV-B on (a) survival and (b) anomaly frequency of *Rana arvalis* larvae until hatching. Tests were performed on arcsine-square root transformed data. *ndf* = numerator degrees of freedom; *ddf* = denominator degrees of freedom

Source	*ndf*	*ddf*	*F*	*P*
(a) Survival				
Block	*var* = 0·023 ± 0·009, *z* = 2·36, *P* = 0·019			
pH	1	105	5·85	< 0·05
UV-B	2	105	0·77	0·46
pH × UV-B	2	105	0·25	0·78
(b) Anomalies				
Block	*var* = 0·240 ± 0·081, *z* = 2·98, *P* < 0·005			
pH	1	105	4·55	< 0·05
UV-B	2	105	1·52	0·22
pH × UV-B	2	105	0·17	0·84

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Table 2. Mixed-model ANOVAs assessing the effects of pH and UV-B on (a) total, (b) body and (c) tail length of *Rana arvalis* hatchlings. *ndf* = numerator degrees of freedom; *ddf* = denominator degrees of freedom

Source	*ndf*	*ddf*	*F*	*P*
(a) Total length				
Block	*var* = 0·112 ± 0·089, *z* = 1·38, *P* < 0·10			
UV-B	2	35	4·99	< 0·05
pH	1	35	35·32	< 0·001
pH × UV-B	2	35	0·05	0·95
Contrasts				
Mylar vs. cellulose	1	35	0·02	0·89
Open vs. filter	1	35	9·95	< 0·001
(b) Body length				
Block	*var* = 0·013 ± 0·014, *z* = 1·38, *P* < 0·10			
UV-B	2	35	3·38	< 0·05
pH	1	35	21·12	< 0·001
pH × UV-B	2	35	0·68	0·51
Contrasts				
Mylar vs. cellulose	1	35	0·86	0·36
Open vs. filter	1	35	5·90	< 0·05
(c) Tail length				
Block	*var* = 0·036 ± 0·034, *z* = 1·07, *P* = 0·14			
UV-B	2	35	4·33	< 0·05
pH	1	35	7·02	< 0·001
pH × UV-B	2	35	0·02	0·98
Contrasts				
Mylar vs. cellulose	1	35	0·09	0·77
Open vs. filter	1	35	8·58	< 0·01

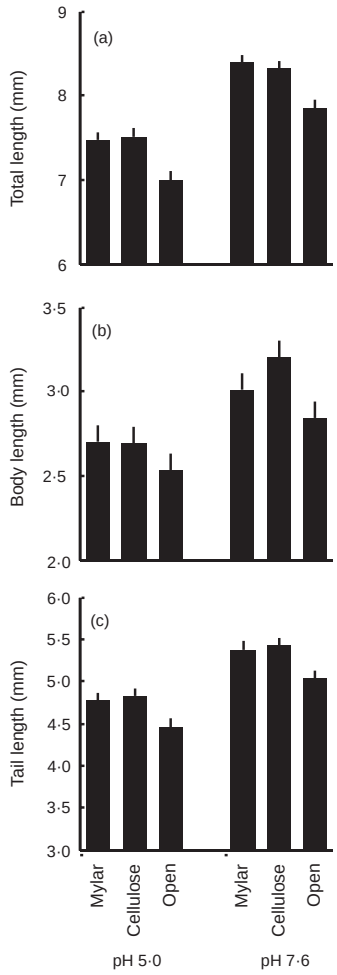

Fig. 4. Effects of UV-B and pH treatment on mean (± SE) hatchling size in the field experiment. (a) Total length, (b) body length and (c) tail length. For statistical tests, see Table 2.

Table 3. Mean percentage of dead and abnormal embryos in a laboratory experiment assessing the effects of UV-B on moor frogs. *n* = number of replicates

		Dead	Anomalies
Treatment	*n*	mean ± SD	mean ± SD
Normal	16	1·3 ± 2·5	0·9 ± 2·0
Enhanced	16	1·8 ± 2·9	1·5 ± 2·4
Control	16	2·1 ± 3·0	1·3 ± 2·8

whereas the contrast between Mylar and cellulose treatments was not significant, suggesting that UV-B regime *per se* did not influence growth performance (Table 2). Hatchlings in open treatments grew slower than their full-sibs under cellulose acetate and Mylar filters (Fig. 3), a difference that could be explained by temperature differences between filter and open treatments (see the Methods). Early growth of larvae was strongly influenced by pH treatment (Table 2). In all traits, larvae in low pH treatments were smaller than their full-sibs reared in neutral pH treatments (Table 2 and Fig. 4), whereas block effects were small (Table 2). However, there was no evidence for synergistic effects of low pH and UV-B treatment on hatchling size (Table 2).

LABORATORY EXPERIMENT

There was no evidence for block effects on survival or anomaly frequencies ($\chi^2 \leq 5{\cdot}1$, $P \geq 0{\cdot}16$), and the block effects were pooled into residual variance. Survival was independent of UV-B treatment ($\chi^2_2 \leq 4{\cdot}67$, $P = 0{\cdot}25$) and embryos in all treatments had high and equal survival until hatching (Table 3). Likewise, frequency of

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developmental anomalies was low in all treatments (Table 3) and the UV-B treatment did not explain any variation in frequency of anomalies ($\chi^2_2 = 1{\cdot}67$, $P = 0{\cdot}43$). No family effects on survivorship or frequency of developmental anomalies were detected (in both cases $\chi^2_3 \leq 4{\cdot}26$, $P \geq 0{\cdot}25$).

Discussion

We found no indications that ambient levels of UV-B radiation would have negative effects on the development of moor frog embryos, neither in terms of survival nor in terms of early growth performance. These observations are in agreement with several other amphibian studies that have not found negative effects of UV-B radiation on hatchability (Blaustein *et al*. 1996; Ovaska, Davis & Flamarique 1997; Van de Mortel *et al*. 1998; Blaustein *et al*. 1999; Cummins *et al*. 1999) but contrast with the results of several other studies that have detected lower hatchability of UV-B-exposed compared with UV-B-shielded eggs (Blaustein *et al*. 1998; Anzalone *et al*. 1998; Corn 1998; Lizana & Pedraza 1998). One possible explanation for this dichotomy stems from the observation that activity of the photolyase enzyme involved with removal of UV-B radiation-induced DNA-damaging photoproducts from cells is known to differ between different species (Blaustein *et al*. 1994; Hays *et al*. 1996). Several studies have found that species with high photolyase activity are more resistant to UV-B radiation than species with low photolyase activity (Blaustein *et al*. 1994, 1996, 1999; Van de Mortel *et al*. 1998). The more resistant species include several ranids (Blaustein *et al*. 1996, 1999) and it is possible that *R. arvalis* also belongs to this resistant group of species. This conclusion is also reinforced by the results of the laboratory experiments, which showed that even very high levels of UV-B radiation did not have any negative effects on the development of moor frog embryos. It seems unlikely that more severe effects would have been detected even if the field experiments had been conducted in more extreme weather conditions or under lower levels of atmospheric ozone.

Hatchling size is an important correlate of fitness in amphibians (reviewed by Kaplan 1998). Hatchlings from the UV-B-shielded treatment in the field experiment were larger than their full-sibs from the open treatment that received full ambient UV-B levels. However, there was no difference in hatchling size between the UV-B-shielded Mylar filter treatment and the cellulose acetate treatment receiving full ambient UV-B radiation dose. This indicates that the difference was most probably due to a filter effect, such as increased temperature (see the Methods), which may affect hatchling size in amphibians (Kaplan 1992). Nevertheless, we note that UV-B radiation could potentially have a negative effect on early growth, before larvae reach the free-swimming stage, under two different scenarios. First, there could be a trade-off between DNA-damage repair mechanisms and growth if both are draining energy from a common pool (Pahkala *et al*. 2000). Secondly, UV-B radiation-induced photoproducts could reduce the rate of protein synthesis, and thereby growth, by inhibiting DNA translation and transcription (Zhao & Mu 1998). Although our results give no support for such a scenario, we note that negative effects of UV-B on growth has been documented previously in the common frog *R. temporaria* (Pahkala *et al*. 2000) and in studies of plants and aquatic life (Johanson 1995; Nielsen, Björn & Ekelund 1995; see also Calkins 1982).

The among-block differences in survival rates in the field experiment were large, for which we offer three possible explanations: genetic differences among families, maternal environmental effects induced by egg size (Kaplan 1985, 1998) and among-block environmental heterogeneity. However, because all the UV-B treatments in our design were replicated in each block, these sources of variation do not influence the conclusions regarding the effects of UV-B treatments on different measures of embryonic performance.

The negative effects of low pH on amphibian development are well established (Pierce 1985; Böhmer & Rahman 1990). We found that survival probability and hatchling size were reduced by low pH. This is in agreement with the results of Andrén *et al*. (1988), who found that embryonic mortality in *R. arvalis* increased in acid water. However, in accordance with results on *R. temporaria* (Pahkala *et al*. 2000), we found no evidence for synergistic effects of UV-B radiation and low pH on embryonic development. This contrasts with the results of Long, Saylor & Soulé (1995) who found that UV-B radiation reduced survival of *R. pipiens* eggs at low, but not at neutral, pH. Although broad generalizations about effects of pH/UV-B synergism on amphibians must await further studies, these results suggest that it may not be any general phenomenon.

There has been a recent concern that the lack of effects of UV-B radiation on amphibian larvae in the field experiments could be due to levels of UV-B radiation during experiments being lower than are theoretically possible (Cummins *et al*. 1999), and in the absence of dosimetric data collected during the experiments this possibility cannot be dismissed. In this study, the field data revealed that the UV-B radiation levels during the experiments were low mostly due to bad weather. Hence, without any further laboratory work, the absence of treatment effects would have forced us to conclude that nothing can be said about the sensitivity of moor frog larvae to UV-B radiation. However, our laboratory tests with high doses of UV-B radiation lend support to the results of the field experiments, reinforcing the conclusion that moor frog embryos are indeed tolerant to UV-B radiation. In this context, the radiation during the field experiments, although not reaching theoretical maxima, were not atypical for the locality: the number of sunshine hours (obtained from the SMHI) during the experiments

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(189·3 h) did not differ from the average sunshine hours during the same time period from 1988 to 1997 (mean 205·2, range 111·7–226·6; one-sample *t*-test, $t_9 = 0{\cdot}9$, $P = 0{\cdot}39$). Hence, the irradiance conditions during the experiment were close to the long-term average. Therefore, ecologically relevant doses of UV-B are clearly not the same as the theoretically possible doses.

Our results and conclusions about the effects of UV-B irradiation on *R. arvalis* are limited to embryonic development only, and the possible effects on older larvae remain to be investigated. However, in contrast to the embryonic stage covered by this study, larvae are normally able to seek protection from UV-B irradiation by hiding in vegetation, and moor frogs in Sweden seldom breed in ponds that lack vegetation (J. Merilä & A. Laurila, personal observations). Similarly, turbid water may provide cover from UV-B as it prevents the penetration of radiation through the water column (Nagl & Hofer 1997). Hence, the relevance of UV-B radiation as an environmental factor potentially impacting development is likely to be more important for early embryonic stages than for older larvae (but see Nagl & Hofer 1997). Nevertheless, given the growing evidence that environmental stresses experienced during early development may have an impact on an individual's later performance (Rossiter 1996), the possibility that the negative effects of UV-B radiation experienced at embryonic stages become expressed only at later life stages needs to be studied.

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