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Effects of ambient UV-B radiation on early development of the common frog (*Rana temporaria*) embryos in the subarctic

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Several recent field experiments have demonstrated that ambient ultraviolet-B radiation (UV-B; 280–315 nm) may cause severe mortality of amphibian embryos. We investigated the effects of ambient UV-B on early embryonic performance of the common frog (*Rana temporaria*) in subarctic Finland (ca. 69°03´N, 20°50´E), where levels of UV-B radiation during the egg laying period are roughly twice as high as those in southern Fennoscandia. We found no evidence for decreased embryonic mortality, decreased frequency of developmental anomalies or improved growth performance of embryos shielded from UV-B as compared with embryos exposed to ambient levels of UV-B. Our results concur with the results of earlier studies with this species conducted in more southern populations, and suggest that current UV-B levels alone are not likely to pose significant threat for the well being of subarctic common frog populations.

1. Introduction

In recent years, many amphibian populations have undergone declines and range reductions (e.g. Semb-Johansson 1989, Wake 1991, Pounds & Crump 1994, Drost & Fellers 1996, Fisher & Shaffer 1996, Laurance *et al.* 1996, Lips 1998). Hypothesised causes for these declines include habitat destruction, introduced exotic species, pollution, acidification and increased ultraviolet-B radiation (Wake 1991, Blaustein & Wake 1995, Licht 1996). The fact that the declines have taken place in diverse locations, including undisturbed habitats, has prompted a concern (e.g. Blaustein

et al. 1998) that the declines might owe to increased solar ultraviolet-B (UV-B; $\lambda = 280 – 315$ nm) irradiance associated with the depletion of the stratospheric ozone column (Kerr & McElroy 1993, Madronich *et al*. 1995, 1998).

Several field experiments have found evidence for higher mortality among amphibian eggs exposed to ambient levels of UV-B radiation as compared to UV-B shaded controls (reviewed in Blaustein *et al.* 1998). These studies provide evidence that even current levels of UV-B may have a negative impact on individual fitness in many, but not in all amphibian populations. Hence, these findings are most alarming in the face of the fact that there has been a downward trend in the amount of ozone in mid-latitudes, and an even stronger negative trend in springtime ozone levels in higher latitudes (e.g. Kerr & McElroy 1993, Mardonich *et al.* 1998). However, within the European continent, the taxonomic and geographic coverage of studies on impact of UV-B radiation of amphibian development is still limited (Nagl & Hoffer 1997, Lizana & Pedraza 1998, Cummins *et al*. 1999, Laughelle *et al*. 1999, Pahkala *et al.* 2000).

The aim of this study was to test whether ambient levels of UV-B radiation have negative impacts on development of the common frog (*Rana temporaria*) embryos in subarctic Finland. Because of their later initiation of spawning, embryos of the common frog at high latitudes (> 65°N) are exposed to much higher UV-B radiation doses (Josefsson 1996) than those of their more southern (≈ 55°N) conspecifics. Consequently, unless adaptation to high doses of UV-B radiation has occurred after recolonisation of northern Fennoscandia after the last ice age, one could expect that the negative effects of ambient UV-B radiation on embryonic development could be more easily detected at higher latitudes.

2. Material and methods

The common frog (*Rana temporaria*) is a widespread Palearctic frog that breeds in various kinds of small waters from the northern Spain to the most northern parts of Fennoscandia (Gasc *et al.* 1997). We studied the UV-B radiation tolerance of the common frog embryos at Kilpisjärvi located in northernmost part of Finland (ca. 69°03´N, 20°50´E), where eggs are usually deposited in shallow water devoid of UV-absorbing organic materials and shading vegetation. The snow in valleys melts in late May–early June, and the mean air temperature in June is + 8 °C (Järvinen 1987). The study pond used for the experiment is situated 485 m a.s.l. In 1999, the first egg masses appeared on 1 June, and the egg laying continued until about 15 June. In 1998, egg laying was initiated on 5 June.

To see whether ambient levels of UV-B radiation had an impact on development, growth and/or survival of the common frog embryos, we collected 19 freshly laid (< 3 hours old; determined by checking the pond for fresh egg masses every third hour) egg masses and brought them into the laboratory on 2 June 1999. From each of the egg masses, we clipped three egg samples containing ca. 50 eggs ($\bar{x} = 50.1$, SD = 11.4, $n = 57$), and maintained them in a +4 °C walk-in cold room in pond water until the morning of 3 June, when the experiments started.

The experiments were carried out in ten 112 l green PVC-boxes (= blocks), filled with pond water, and placed outdoors in an open non-shaded space on ground. Within each of the ten large PVC-boxes, we placed six smaller opaque plastic vessels (16 × 20 × 5 cm; Fig. 1). These smaller vessels had four 35 mm holes, covered with fine insect mesh, in each of the bottom corners to allow water exchange between them and the large box. The three different UV-B treatment conditions were (1) unfiltered sunlight (2) sunlight filtered to remove UV-B and shorter wavelengths, and (3) sunlight filtered to remove wavelengths shorter than UV-B (*see* Pahkala *et al.* 2000 for details of filter properties). The second radiation treatment was attained with the aid of Mylar filters (0.1 mm thickness; Erik. S. Ekman, Stockholm, Sweden), and the third treatment was created with pre-burned cellulose diacetate filters (0.13 mm, Courtaulds, Derby, UK). The cellulose diacetate filters were included to control for filter effects, such as enhanced thermal environment created by filter coverage. The filters were placed on wooden rigs about 4 cm above the water level to allow air circulation beneath them. The filters were changed twice during the 14 day long

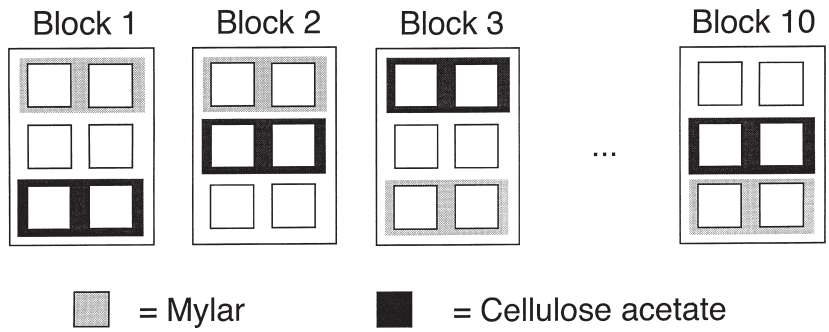

Fig. 1. Schematic presentation of the experimental set up. Large rectangles (= blocks; $n = 10$) represent 112 l boxes, and the small ones ($n = 60$) the vessels in which the eggs were placed. The two filter treatments and open control were assigned in random order within each of the blocks. Each block (except one) contained eggs from two families (i.e. a total of 19 families).

experiment to ensure that their transmission properties did not change due to accumulation of dust or pollen. The water depth in vessels was adjusted to about 2 cm. All experiments were carried out in pond water (pH ≈ 6.5) taken from the pond from which the eggs originated.

Temperatures within the egg masses under different filters were measured with a digital thermometer in each of ten blocks 9–10 times during the experiment between 1000 and 2300 hrs. The temperatures varied widely between days ($F_{6,273} = 964.85$, $P = 0.001$; note that this also includes variations due to time of the day) and slightly between different blocks ($F_{9,273} = 2.29$, $P = 0.038$). There were also slight differences in temperature under different filter treatments (open: $\bar{x} = 13.49 \pm 0.11$ °C [SE]; mylar: $\bar{x} = 14.04 \pm 0.11$ °C; cellulose acetate: $\bar{x} = 14.16 \pm 0.11$ °C; $F_{2,273} = 12.27$, $P < 0.001$), but only the ca. 0.5 °C difference between open and the two filter treatments was significant (Tukey's test, $P < 0.05$). Consequently, any differences in developmental performance between mylar and cellulose acetate treatments cannot be ascribed to temperature effects, whereas this is a possibility between filter (mylar + cellulose) and open treatments. The temperatures measured in experimental vessels correspond well with the measurements taken from egg masses in nature during the experimental period (J. Merilä, unpubl.).

No direct measurements of UV-B radiation were made, but we obtained data on cloudiness during the experiments from the Karesuvando meteorological station of the Swedish Meteorological Institute situated ca 120 km SE of the study locality. The mean (± SD) cloudiness during the experimental period (3–16 June 1999; 06:00–18:00 hrs) was 71% ± 20% which is not significantly different from the 11 year mean for the same period ($\bar{x} = 72\%$, min. = 38%, max. = 95%, one-sample sign-test, $P = 0.99$). This suggests that although the levels of UV-B in this area are probably reduced by cloud cover rather frequently, the year 1999 was not exceptional in this sense.

The experiments started in the morning of 3 June, and went on until the evening of 15 June when the majority of larvae had hatched (attained stage 25; Gosner 1960). We counted the eggs in each vessel at the beginning of the experiment, and when the embryos reached the stage 16, the number of dead (undeveloped) eggs were counted. Towards the attainment of the stage 25 (start of independent feeding; Gosner 1960), the development in each vessel was monitored twice a day (09:00 and 21:00 hrs) to determine the exact timing of reaching stage 25. The ten first hatched larvae were then stored in 70% alcohol, and their size was later determined in laboratory by measuring the body and tail length under stereomicroscope (to nearest 0.1 mm). The total length was defined as the sum of body and tail length. The remaining larvae, irrespectively of their developmental stage, were preserved in alcohol. They were later examined for their developmental stage and presence of developmental anomalies.

The response variables in our analyses were the proportion of eggs surviving to (1) day 8 post-

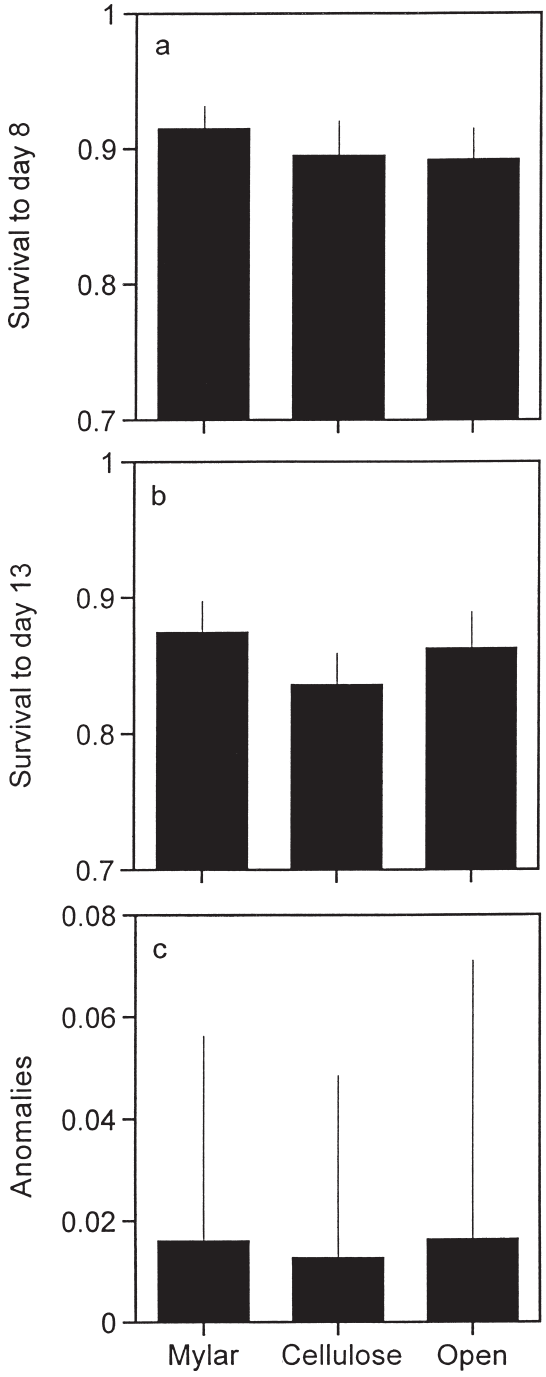

Fig. 2. Survival and frequency of developmental anomalies among the common frog eggs/larvae exposed to different UV-B radiation treatments. Proportion (± SE) of surviving eggs to (a) day 8 and (b) day 13 post-fertilisation, (c) proportion (± SE) of larvae with visible anomalies at the end of the experiment.

fertilisation (about at stage 16; Gosner 1960), and (2) to day 13 post-fertilisation (when most of the larvae had attained stage 25), (3) frequency of anomalous larvae (e.g. larvae with coiled tails, asymmetrical bodies, etc), (4) developmental rate (*see* below), (5) and size at hatching in different treatments. Testing of treatment effects on survival rates and frequency of developmental anomalies were performed with generalised linear model as implemented in PROC GENMOD of SAS (SAS Inst., Inc. 1996). Logit link function, binomial error structure and type III sums-of-squares were used (Littell *et al.* 1996). The same type of analysis was used to study the effect of UV-B treatment on development rates: all surviving individuals were coded as having (code = 1) or not having (code = 0) reached stage 25 (Gosner 1960) by the end of the experiment, after which the data was analysed with a logistic regression model exactly as described above. Size measures at stage 25 were analysed with mixed model ANOVAs using PROC MIXED in SAS. Treatment was considered as a fixed effect, whereas family and block effects were considered as random effects. Since two families were reared in each of the blocks (i.e. families and blocks were not crossed), the term family was analysed initially as an effect nested under the block term. However, non-significant block effects were omitted from all models. All statistical analyses were performed with version 6.12 of the SAS statistical package (SAS Inst., Inc. 1996).

3. Results

3.1 Survival

There were no UV-B treatment effects on survival of eggs on day 8 ($\chi^2_2 = 3.18$, $P = 0.20$) or day 13 post-fertilisation ($\chi^2_2 = 5.61$, $P = 0.061$; Fig. 2), but survival varied considerably between the different blocks and families (day 8: block $\chi^2_9 = 96.74$, $P < 0.001$; family within block: $\chi^2_9 = 87.28$, $P < 0.01$; day 13: block $\chi^2_9 = 96.74$, $P < 0.001$; family within block: $\chi^2_9 = 68.44$, $P < 0.001$). At both dates, survival rates were close to 90% in all treatments, and highest among larvae in UV-B

blocked treatment (Fig. 2), although not significantly so, suggesting that UV-B radiation did not directly affect hatching success.

3.2. Anomalies

Anomaly frequencies were low (1%–2% of all eggs), and there was no treatment effect ($\chi^2_2 = 0.18$, $P = 0.90$) on the frequency of visible anomalies (Fig. 2).

3.3. Developmental rate

Developmental rates did not differ between UV-B treatments ($\chi^2_2 = 4.77$, $P = 0.09$) or families ($\chi^2_9 = 13.8$, $P = 0.12$), although there were significant differences between different blocks ($\chi^2_9 = 31.58$, $P = 0.0002$).

3.4. Growth

The UV-B treatment had no significant effect on the body length of the larvae at hatching ($F_{2,274} = 2.49$, $P = 0.08$; Fig. 3). The family effect was large (var = 0.035 ± 0.013 [SE], $z = 2.56$, $P = 0.01$), suggesting that factors other than UV-B were more important determinants of hatchling body size. However, UV-B treatment had a significant effect on tail length of the larvae ($F_{2,270} = 3.23$, $P = 0.041$; Fig. 3), but this was due to fact that larvae in the open treatment attained significantly shorter tails than those under filter treatments (Fig. 3). There was no significant difference in tail length between mylar and cellulose acetate treatments (Linear contrast: $P > 0.10$), and again, family effect was a significant source of variation in tail length (var = 0.15 ± 0.06, $z = 2.50$, $P = 0.012$). The UV-B treatment ($F_{2,270} = 4.23$, $P = 0.015$), but not family ($z = 1.59$, $P = 0.11$), had a significant effect on total body length, and the larvae under cellulose acetate treatment were largest, and the larvae under open treatment smallest (Fig. 3). However, only the difference between open and the two filter treatments was significant (Linear contrast: $P = 0.005$).

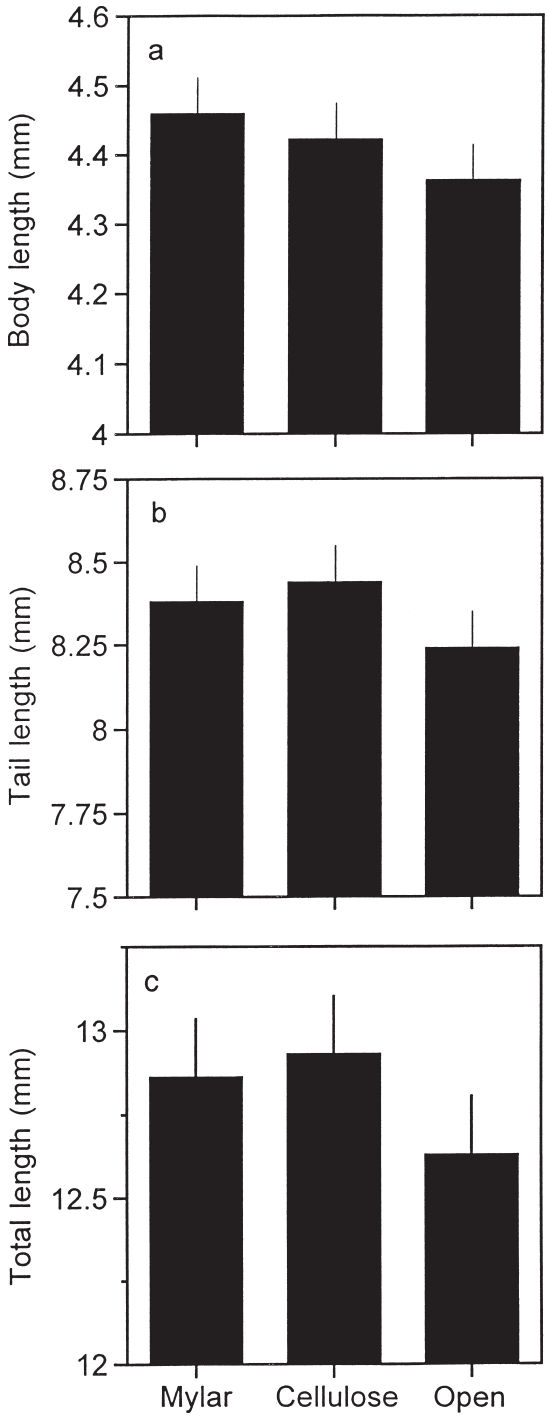

Fig. 3. Size of the hatchling (stage 25; Gosner 1960) common frog larvae exposed to different UV-B radiation treatments. (a) body length, (b) tail length and (c) total length. Plotted values are mean ± SE.

4. Discussion

We found no evidence for reduced mortality or increased frequency of developmental anomalies among the common frog eggs protected from ambient UV-B radiation. This is in agreement with the results of earlier studies with this species performed on more southern latitudes (Cummins *et al*. 1999, Langhelle *et al.* 1999, Pahkala *et al*. 2000), as well as with the results of similar experiments performed with several other amphibian species (Blaustein *et al.* 1996, Ovaska *et al.* 1997, Anzalone *et al*. 1998, Corn 1998, Lizana & Pedraza 1998, van de Mortel *et al*. 1998; Langhalle *et al.* 1999). However, our results contrast with the data from nine other species (reviewed in Blaustein *et al*. 1998) in which field experiments have demonstrated reduced mortality among UV-B shaded eggs as compared with eggs exposed to ambient levels of UV-B. There are several explanations for this apparent inconsistency in effects of UV-B on amphibian development (Licht & Grant 1996, Blaustein *et al*. 1998), but it seems clear that some species are more tolerant to UV-B than the others (e.g. Hays *et al*. 1996). We note, however, that none of the studies performed with European species in realistic conditions have demonstrated any mortality effects of UV-B on embryos (Cummins *et al*. 1999, Langhalle *et al*. 1999). In one study (Lizana & Pedraza 1998), UV-B blocking reduced mortality among the eggs of the common toad (*Bufo bufo*), but since the egg strings of this species are normally submerged in vegetation, it is questionable whether the results of that experiment are representative what might be occurring in natural situations.

As to the impacts of UV-B treatment on growth and size related traits, the results were inconsistent among different traits and did not give any evidence for the assertion that ambient UV-B levels would impact early development of the larvae. The only significant treatment effect was that observed in tail length, but in this case this was due to the difference between open and cellulose acetate treatments. This suggests that this effect had nothing to do with UV-B *per se*, but with the difference related to the filter cover itself. As explained in Material and methods, the temperature in the open treatments was somewhat lower than that under filter treatments, and temperature is known to affect body-size:tail-size ratio in amphibians (Kaplan 1992).

The results from the kind of experimental design used in this study (i.e. ambient UV-B, no UV-B vs. control) have an unambiguous interpretation when treatment effects are detected (e.g. Blaustein *et al*. 1998). However, as in the case of this study, when no treatment effects are observed the question about the representativeness of radiation conditions during the experiments raises. The radiation conditions (cloudiness) during our experiments were likely to be representative of what frogs in our study locality have been experiencing during the last eleven years (*see* Material and methods), suggesting that the amount of radiation received during the experiments was not unusually low. Therefore, it seems prudent to conclude that there is no reason to believe that results would have been different if the experiments had been performed in some other year.

Since developmental rates, and thereby exposure time to UV-B radiation, are directly related to water temperature, low ambient temperatures will increase the effective cumulative UV-B radiation dose the embryos will experience. In our study, the water temperatures fluctuated widely, and the developmental time to stage 25 was rather short, as compared for example with that in a similar experiment performed elsewhere (ca. 21 days; Pahkala *et al*. 2000). Although water temperatures and UV-B radiation levels in nature are likely to be positively correlated (e.g. fig. 2 in Cummins *et al*. 1999), exceptions are also bound to occur. For example, mean June water temperatures in adjacent study ponds in Kilpisjärvi area can differ by several degrees (J. Merilä & A. Laurila unpubl.), with the consequence that eggs from the colder ponds will be exposed to UV-B for longer periods than those from warmer ponds (up to 5 days; J. Merilä & A. Laurila unpubl.). In this view, future studies should recognise the possible importance of differing water temperatures on UV-B effects on amphibian embryos. Synergistic effects of UV-B and temperature could be important in the same way as documented for example in the case of UV-B and low pH (Long *et al*. 1995): since a low pH slows down the development (Andrén *et al*. 1988), larvae exposed to low pH (or low temperature) will be exposed higher cumulative doses of UV-B as compared to those exposed to

higher pH. However, this has received relatively little attention in the studies carried out so far (but *see*: Licht & Grant 1997, Lizana & Pedraza 1998).

There are several different strategies as to how animals could protect their embryos against UV-B mediated mortality (review in Epel *et al*. 1999). These include: (1) possession of effective DNA damage repair system, (2) minimisation of UV-B exposure by behavioural adjustments, and (3) use of sunscreens (Epel *et al*. 1999). As to the second case, one possible way to avoid UV-B exposure is to spawn at dawn (or deposit eggs to sheltered areas or sink/deposit them in benthos) which will effectively protect the embryos during a UV-sensitive microtubule-dependent movement which occurs shortly after fertilisation (Epel *et al*. 1999). In the case of the common frog, the northern populations have little possibilities to use these strategies because of the continuous daylight (although UV-B levels during the 'nights' are low), and deposition of eggs in shade or on a pond bottom would lead to reduced developmental rates in an already harsh environment. Therefore, it seems likely that if adaptation to local UV-B regime has occurred, this has taken place through the alternatives (1) or (3). We note that there are reports of more heavily pigmented eggs in northern than in more southerly populations of frogs, and that these pigments, could represent adaptation to cope with UV-B radiation (Licht & Grant 1997; *see* also Langhelle *et al*. 1999, Pahkala *et al.* 2000).

In conclusion, our data provides no evidence for significant influence of current levels of ambient UV-B radiation on early development of the common frog eggs in subarctic. Nevertheless, we caution against broad generalisations before more data about possible synergism between UV-B radiation, temperature and other stressors (e.g. low pH owing snow-melting water; e.g. AMAP 1998) becomes available.

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