Journal of Environmental Biology
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January 2008, 29(1) 135-137 (2008)

Short Communication

Influence of ambient ultraviolet radiation on *Bufo calamita* egg development in a semiarid zone (Catalonia, Spain)

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(Received: January 15, 2007 ; Revised received: May 26, 2007 ; Accepted: July 01, 2007)

Abstract: *Several experiments have shown that ambient ultraviolet-B radiation (UV-B) has negative effects on the development of amphibians' embryos. We studied the effects of UV-B radiation on development, survival and frequency of deformity during egg development in the Natterjack toad (Bufo calamita) from a semiarid region of Lleida (Catalonia, Spain). Eggs exposed to ambient levels of UV-B and those protected from UV-B with a filter exhibited similar developmental rate, mortality rate and frequency of developmental anomalies. These experiments show that eggs of Bufo calamita of the studied population are able to develop normally during embryonic period when exposed to current high levels of UV-B observed in Catalonia. These results will be used as reference for future studies on geographic variation in UV-B tolerance in this species.*

Key words: *UV-B radiation, Embryonic development, Bufo calamita, Semiarid zone, Spain*
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Introduction

Several experiments have shown that ultraviolet radiation can alter embryonic development in several amphibian species (Blaustein *et al*., 1994, Blaustein and Bancroft, 2007). The main observed effects are decreases in developmental rate and growth, as well as increased frequencies of mortality and malformation (Blaustein *et al*., 1998; Lizana and Pedraza, 1998; Pahkala *et al*., 2001a; Weyrauch and Crubb, 2006) and they suggest that UV radiation (especially UV-B) can damage the cellular DNA of amphibian embryos and larvae. The energetic cost of the cellular mechanism involved in the repair of DNA damage and the formation of photoproducts that inhibit DNA-transcription were proposed as explanations of the delayed development, mortality and malformation (Blaustein *et al*., 1994; Pahkala *et al*., 2001a). Although some references indicate the influence of current levels of ambient UV-B radiation on the early development of some species, the real effect of this factor is not clear (Licht, 2003; Heyer, 2003). Different methodologies and synergistic effects with other stressors (*e.g.* pH or contaminants: Blaustein and Kiesecker, 2002) can lead to ambiguous results. The amount of UV-B radiation received is likely to vary among populations of the same species and little is known about geographic variation in UV-B tolerance (Pahkala *et al*., 2002a; Pahkala *et al.,* 2002b; Palen *et al*., 2005).

We are currently studying the ecology of amphibians in the plain region of Lleida (Catalonia, Spain) where the most common species is the Natterjack toad *Bufo calamita.* The toads reproduce in temporary water points, an endangered habitat in Mediterranean region (Zacharias *et al*., 2007), where vegetation is absent. In this context, our aim was to estimate the potential effects of ambient UV-B radiation on embryonic development in the Natterjack toad of Catalonia.

Materials and Methods

The study area (province of Balaguer, 41°46'N, 0°46'E) is a semi-arid zone (Conesa *et al*., 1994) of winter cereal fields. The mean annual temperature is about 14.5°C (January = 0.2°C and July = 32.2°C), the mean annual rainfall is 400 mm (meteorological station of Balaguer), and the evaporation-transpiration rate is 805 mm (Thornthwaite and Mather, 1955). The level of UV-B radiation is high (mean global irradiation in 2003 = 15.0 Mj/m^2, Lleida meteorological station, Servei Meterològic de Catalunya : www.smc.es). This climatic context leads to a very particular flora and fauna able to survive in these environmental conditions (Conesa *et al.*, 1994). Behavioural ecology of the Natterjack toad in this area has been described (Miaud *et al*. 2000; Miaud and Sanuy, 2005).

In March 2004, four samples of spawn of *B. calamita* near the city of Balaguer were collected. Spawns were kept in four plastic buckets and transported to the experimental site, on the roof of the University of Lleida (20 km from the collected zone). The experimental design was adapted from Pahkala *et al.* (2000) and the experiment was performed with four blocks. Each block consisted of a 40 litre plastic box (57 x 77 x 20 cm) filled with dechlorinated water to stabilize temperature fluctuation. In each block, we put six plastic floating containers (21 x 14 x 3.5 cm). The containers had four small holes (2 cm in diameter) covered with a fine insect mesh allowing water circulation between the recipient and the block as the temperature above the three types of filters was homogenised. In each plastic recipient, we put a small fragment of a spawn (55 to 70

Table - 1: Survival and malformation rate of Natterjack toad eggs submitted to ambient levels of solar radiation (control), with a filter stopping UV-B (Mylar). The acetate filter did not stop UV-B radiation and was used as a control of the filter effect. n = egg number at the beginning of each experiment. Survival and malfomation expressed in % ± standard deviation

Treatments	Control	Mylar	Acetate
Survival	0.994 ± 0.007 (n = 480)	0.983 ± 0.013 (n = 400)	0.983 ± 0.016 (n = 242)
Malformation rate	0.0084 ± 0.008 (n = 477)	0.0084 ± 0.012 (n = 393)	0.0204 ± 0.014 (n = 238)

eggs from one family). Each block was covered with three types of filter in order to test for (treatments): 1) Control: unfiltered sunlight, 2) Mylar: sunlight filtered to remove UV-B and shorter wavelength components (Mylar 0.10 mm) and 3) Acetate: sunlight filtered to remove shorter wavelength components than UV-B (cellulose acetate filter: 0.13 mm). This filter can be considered as a control of the filter effect. The filters were placed 3 cm above the blocks to allow air circulation. This design permitted testing for treatments (3), families (4) and interaction effects. The water was changed every three days. The mean daily water temperature in the blocks was 17.7 ± 0.2°C. The average daily irradiance during the experiment was 23.9 MJ/m^2. At the beginning of the experiment, all the eggs were at stages 0-3 (Gosner, 1960). We observed the egg development twice a day and recorded stages, survival and visible developmental anomalies (*e.g.* oedemas, asymmetric body) at stage 16.

Data were analysed with generalised lineal models implemented in PROC GLM of SAS. Survival rate, malformation rate and body length of embryos were considered as dependent variables. UV exposure condition was considered as fixed factor and block, family and family * condition of UV exposure as random factor. Embryos of all blocks and treatments reached stage 16 at the same time in two days so no statistical analysis were performed on this variable.

Results and Discussion

Reducing UV-B radiation with a filter did not alter development and not increase the survival rate from stage 0 to 16 (F = 2.59, p=0.12, Table 1). Survival rates did not differ significantly between spawns (F = 2.50, p=0.10) and malformation rate were low (1.2 % of all eggs at stage 16, Table 1). The effect of treatment on the occurrence of visible malformation was not significant (F = 0.56, p=0.58) in Natterjack toad eggs of the studied population, as previously observed with this species in the Iberian Peninsula (Sierra de Gredos: Lizana and Pedraza, 1998). Stressing effects of UV were mainly described in North America (*e.g.* Blaustein *et al*., 1998; Palen *et al*., 2005), with large differences in tolerance between species (Kiesecker *et al*., 2001). In Europe, Pahkala *et al.* (2000) studied the effects of UV-B radiation on common frog *Rana temporaria* embryos originating from eight populations spawning along a 1,200 km latitudinal gradient across Sweden. Results indicate that *R. temporaria* embryos are rather tolerant of UV-B radiation, and that there is no clear latitudinal pattern to UV-B tolerance in this species.

The malformation rates differed significantly between spawns (family effect) (F = 3.16, p=0.04). This could due to genetic differences among families and/or among block environmental heterogeneity including maternal effects. However, there was no significant treatment x family interaction.

Differences between species could arise from activity levels of enzyme photolyase (which repairs the DNA damage), which is also higher in populations exposed to high ambient levels of UV-B (Kiesecker *et al*., 2001). The photolyase activity could also be related to the egg-laying behaviour, with species that lay their eggs in shallow open water exhibiting a higher photolyase activity (Blaustein *et al*., 1994; Blaustein and Belden, 2003). Comparing four US Pacific north-western amphibian species, Palen *et al.* (2005), showed that species with high physiological sensitivity to UV-B were those with the lowest field exposure due to oviposition behaviour. Eggs of *B. calamita* are most of the time deposited in shallow temporary waters and are thus directly exposed to solar radiation. Further experiments are needed in *B. calamita* to examine the relation between physiological sensitivity such as photolyase activity and egg resistance to high UV-B doses.

Embryos exposed to UV-B treatment can also manifest deformities, which affect the subsequent survival of larvae (Blaustein *et al.,* 1997). Phenotypic variables were recorded in embryos until stage 16 in this study. However, the deleterious effects of exposure to UV-B can also be delayed in post-embryonary stages. Analysis of larval performance in *R. temporaria* revealed that larvae exposed (as embryos) to increased levels of UV-B radiation suffered a greater frequency of developmental anomalies and metamorphosed later and at a smaller size compared to larvae that had been protected from UV-B radiation as embryos (Pahkala *et al*., 2001b).

The tested *Bufo calamita* population lives in a semiarid region where severe climatic conditions (*e.g.* high temperature, dryness, high UV-B exposure *etc.*) and anthropogenic pressures (high level of fertilizers) are observed. *B. calamita* is widely distributed through western Europe, in a wide variety of habitats. The next step of this study will be to compare embryonic UV-B tolerance in *B. calamita* among populations exposed to decreasing ambient levels of solar radiation.

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