Lethal and Sublethal Effects of UV-B/pH Synergism on Common Frog Embryos

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Abstract: *Although the negative effects of ultraviolet-B (UV-B) radiation on the development of many amphibian species have been demonstrated, some species—such as the common frog (*Rana temporaria*)—seem to be tolerant of UV-B radiation. The amount of UV-B radiation received is likely to vary among populations of the same species, but little is known about geographic variation in UV-B tolerance. Similarly, although UV-B radiation can have synergistic effects with other stressors, no studies have focused on geographic variation of these effects on amphibians. We investigated the synergistic effects of UV-B radiation and low pH on hatchability and early development of* R. temporaria *embryos in a factorial laboratory experiment with animals originating from southern and northern Sweden. Newly fertilized eggs were exposed to three different UV-B treatments (no UV-B [control], 1.254 k/J/m^2 [normal] and 1.584 k/J/m^2 [26% enhanced]) and two pH treatments (4.5 [low] and 7.6 [neutral]). Ultraviolet-B radiation in combination with low pH lead to markedly (approximately 50%) reduced survival rates and increased (approximately 30%) frequency of developmental anomalies in the northern but not in the southern population. The UV-B-exposed embryos hatched at smaller size in the southern population, whereas low pH reduced hatchling size in both populations. In both populations and pH treatments, embryos in the normal UV-B treatment developed significantly faster than embryos in the enhanced or control UV-B treatments. No interaction between pH and UV-B on developmental rates or hatchling size was detected. The results demonstrate—contrary to earlier belief—that* R. temporaria *embryos are not insensitive to increased levels of UV-B radiation. The lethal effects of UV-B radiation may, however, become manifested only in combination with other stressors, such as low pH, and the effects of this synergism may differ among different populations of the same species.*

Efectos Letales y Subletales del Sinergismo B-UV/pH en Embriones de Rana Común

Resumen: *Aunque se han demostrado los efectos negativos de la radiación B-ultravioleta (B-UV) sobre el desarrollo de muchas especies de anfibios, algunas especies—tal como la rana común (*Rana temporaria*)—parecen ser tolerantes a la radiación B-UV. La cantidad de radiación B-UV recibida varia entre poblaciones de la misma especie, pero poco se sabe sobre la variación geográfica en cuanto a la tolerancia a B-UV. De manera similar, aunque la radiación B-UV puede tener efectos sinérgicos con otros factores estresantes, ningún estudio se ha centrado en la variación geográfica de estos efectos en anfibios. Investigamos los efectos sinérgicos de la radiación B-UV y pH bajo sobre la eclosión y el desarrollo temprano de embriones de* R. temporaria *en un experimento de laboratorio factorial con animales provenientes del sur y del norte de Suecia. Se expusieron huevos recién fertilizados a tres tratamientos diferentes de UV-B (sin B-UV [control], 1.254 k/J/m^2 [normal] y 1.584 k/J/m^2 [26% más de lo normal]) y dos tratamientos de pH (4.5 [bajo] y 7.6 [neutral]). La radiación B-UV combinada con pH bajo condujo a tasas de supervivencia significativamente bajas (aproximadamente 50%) e incrementó (aproximadamente 30%) la frecuencia de anormalidades en el desarrollo en la*

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población del norte, pero no en la del sur. Los embriones expuestos a B-UV eclosionaron con menor tamaño en la población sureña, mientras que el pH bajo redujo el tamaño al eclosionar en las dos poblaciones. En ambas poblaciones y tratamientos de pH los embriones en tratamiento de B-UV normal se desarrollaron significativamente más rápido que los embriones en los tratamientos control y por arriba de lo normal. No se detectó ninguna interacción entre pH y B-UV y las tasas de desarrollo o tamaño al eclosionar. Los resultados demuestran—contrariamente a lo que se pensaba—que los embriones de R. temporaria *no son insensibles al incremento de niveles de radiación B-UV. Sin embargo, los efectos letales de la radiación B-UV pueden manifestarse solo en combinación con otros estresantes, tal como pH bajo, y los efectos de este sinergismo pueden variar entre diferentes poblaciones de la misma especie.*

Introduction

Recent reports indicate that many amphibian species have exhibited population declines and range reductions (Pechmann et al. 1991; Wake 1991; Pechmann & Wilbur 1994; Alford & Richards 1999; Houlahan et al. 2000). Several anthropogenic factors are thought to contribute to these declines, including habitat destruction, climate changes, chemical pollutants, introduced exotic species, and increased levels of ultraviolet-B (UV-B, 280–315 nm) radiation (Alford & Richards 1999). As for the last possibility, several researchers have found higher mortality rates among amphibian eggs exposed to ambient levels of UV-B radiation than among those sheltered from UV-B (e.g., Ankley et al. 1998; Broomhall et al. 2000; Kiesecker et al. 2001; for a review of earlier literature see Blaustein et al. 1998). However, amphibian species differ in their sensitivity to UV-B radiation (Blaustein et al. 1994, 1999; Ovaska et al. 1997; Anzalone et al. 1998; Lizana & Pedraza 1999; Langhelle et al. 1999; Broomhall et al. 2000), and some species, such as the common frog (*Rana temporaria*), are considered tolerant of even high levels of UV-B radiation (Cummins et al. 1999; Langhelle et al. 1999; Hofer & Mokri 2000; Merilä et al. 2000*a*; Pahkala et al. 2000; but see Pahkala et al. 2001).

However, the responses of developing amphibians to UV-B radiation can depend on other abiotic or biotic stressors that are present. Polycyclic aromatic hydrocarbons (Hatch & Burton 1998; Walker et al. 1998; Monson et al. 1999), other chemical contaminants (Ankley et al. 1998; Zaga et al. 1998), pathogens (Kiesecker & Blaustein 1995; Kiesecker et al. 2001), and low pH (Long et al. 1995; Hatch & Blaustein 2000) amplify the negative effects of UV-B radiation exposure to amphibian embryos. For instance, Long et al. (1995) found that the negative effects of UV-B on survival of *Rana pipiens* eggs were expressed only under low pH conditions. The synergistic effects of UV-B and low pH could be a particular concern in Scandinavia and northern America, where large areas have been exposed to acid rain (Brodin 1993; Arctic Monitoring Assessment Program 1998). Environmental acidification reduces the amount of dissolved organic carbon (DOC), thus enhancing the penetration of UV-B into the water column and increasing UV-B radiation stress (Schindler et al. 1996; Lean 1998). Consequently, studies of the synergistic effects of low pH and UV-B radiation on the development of species considered UV-B tolerant can be illuminating.

Because the amount of UV-B radiation received differs in different populations of the same species (e.g., Belden et al. 2000; Merilä et al. 2000*b*), it is possible that populations inhabiting areas differing in their UV-B regimes also have diverged genetically in their resistance to UV-B radiation (Blaustein et al. 1994; Williamson et al. 1997, Corn 1998; Belden et al. 2000). Furthermore, if the synergistic effects of UV-B radiation and other abiotic stress factors (e.g., low pH, temperature) are important determinants of UV-B stress tolerance, then populations subject to different environmental conditions may also differ in their tolerance of UV-B radiation. Several researchers have compared UV-B radiation tolerance among amphibian populations in field experiments conducted in different localities (Blaustein et al. 1994, 1999; Kiesecker & Blaustein 1995; Corn 1998). These studies are useful for comparing UV-B radiation tolerance within the environments where the investigations were conducted (Blaustein et al. 1998), but they may tell little about the possible intrinsic differences among the populations because the comparisons were not conducted under uniform environmental conditions. Few studies have compared several amphibian populations under uniform conditions. Recently, Belden et al. (2000) found in a laboratory experiment that two populations of long-toed salamander (*Ambystoma macrodactylum*) differed in larval sensitivity to UV-B radiation. To know whether spatial variability in intrinsic tolerance to UV-B radiation occurs in amphibians, more studies are needed that compare the tolerance to UV-B radiation of multiple populations under common environmental conditions.

Apart from increasing mortality rates, UV-B radiation may have sublethal effects on amphibians in terms of altered behavior (Zaga et al. 1998; Belden et al. 2000; Blaustein et al. 2000; Hatch & Blaustein 2000; Kats et al. 2000), developmental anomalies (Worrest & Kimeldorf, 1975, 1976; Grant & Licht 1995; Kiesecker & Blaustein 1995; Langhelle et al. 1999; Merilä et al. 2000*a*; Pahkala

et al. 2000), and larval growth and development (Grant & Licht 1995; Bruggeman et al. 1998; Belden et al. 2000; Smith et al. 2000; Pahkala et al. 2001). Ultraviolet-B radiation may also have a negative effect on embryonic growth and development (size and age at hatching) of amphibians (Hatch & Burton 1998; Merilä et al. 2000*a*; Pahkala et al. 2000), which may have consequences for an individual's later performance. Small size at hatching may have negative effects on survival and growth, because smaller larvae are more vulnerable to predators (Caldwell et al. 1980; Wilbur et al. 1983), are less efficient in exploiting resources (Steinwascher 1979; Travis 1984), and may be smaller at metamorphosis (reviewed by Kaplan 1998). Small size at metamorphosis can lead to delayed breeding, reduced fecundity, and lower mating success (Kaplan & Salthe 1979; Howard 1980; Smith 1987; Semlitsch et al. 1988; Berven 1990). Consequently, investigations of sublethal effects of UV-B radiation on amphibian embryonic stages may reveal important mechanisms affecting later performance.

Methods

Study Species and Populations

Rana temporaria is found throughout most of the western Palearctic up to 71°N (Gasc et al. 1997). It breeds in various kinds of water bodies in early spring and tends to avoid waters with low pH (Strijbosch 1979; Beebee 1983; Laurila 1998). Eggs are usually laid in shallow water with extensive surface contact, which allows for faster development but also exposes the eggs to UV-B radiation. Depending on water temperature, the eggs usually hatch within 2 weeks after fertilization (J. M. & A. L., personal observation).

We studied the effects of UV-B radiation and pH on *R. temporaria* embryos in a laboratory experiment in April–June 1999. The experiment was conducted with two populations, one originating from southern Sweden (Lund: lat. 55°40′N, long. 13°30′E; elevation 19 m; pH ≈ 7) and one from northern Sweden (Umeå; lat. 63°50′N, long. 20°25′E; elevation 64 m; pH ≈ 7).

We collected 10 adult males and females from the southern population and 4 adult males and females from the northern population and brought them to a laboratory in Uppsala. Each male was then artificially mated with one female following the procedure of Berger et al. 1994, resulting in 10 and 4 families (clutches) per population, respectively. Artificial mating ensured that all offspring in a given clutch were full siblings (multiple paternity occurs in nature; Laurila & Seppä 1998) and that the eggs had no prior exposure to low pH or UV-B radiation. Apparently damaged eggs were discarded prior to experimentation. After fertilization, the eggs (<2 hours old) were divided into batches of 30–50 and placed in experimental vessels (0.28 L; polystyrene; 5.5 × 10 cm) about 4.5 cm below the water surface.

Experimental Design

We conducted the experiment in a constant-temperature room (15° C, corresponding to the typical spring temperature in Scandinavia) in three aquarium systems, each of which consisted of two experimental aquaria (120 × 120 × 25 cm; about 320 L) positioned one on top of the other with a reservoir tank (90 × 90 × 35 cm; about 280 L) below them. Each aquarium system was filled with reconstituted soft water, which was continuously circulated through a cooling unit (flow rate: 3 L/minute) to reduce temperature fluctuations caused by the greenhouse lamps.

The experiment consisted of the fully factorial combination of three UV-B and two pH treatments, with each family replicated three times within each treatment combination. Replicates were randomly placed in the aquaria so that within each aquarium the total number of vessels per treatment was equal. To control for possible variation of irradiance in the aquarium, the placement of vessels within them was changed randomly each day.

The experimental vessels containing the eggs were filled with reconstituted soft water and placed on top of a plastic netting situated 5 cm below the water surface. There was no exchange of water between the experimental vessels and the surrounding water circulating in the aquarium systems. As a direct consequence of the radiation from the greenhouse lamps, there were regular daily temperature variations (10–17° C) in the aquaria. The average daytime (0800–1700 hours) water temperature during the experiments was 15.2 ± 0.10° C for the southern and 15.6 ± 0.01° C for the northern population. These temperatures are within the range encountered in both populations in the wild (A.T. Laugen & J.M., unpublished data). The average temperatures in different UV-B treatments differed slightly (15.9 ± 0.11° C, 15.1 ± 0.11° C, and 15.1 ± 0.09° C for control, normal, and high UV-B treatments, respectively) but significantly ($F_{2,101} = 34.9, p < 0.001$). Therefore, mean temperature in each experimental unit was included in the analyses as a covariate to control for possible temperature effects.

UV-B Treatments

We divided the UV-B treatments into six blocks, two for each UV-B treatment, over the three aquarium systems; thus, each system contained two blocks. The daily photoperiod was 17 hours light and 7 hours dark, and the UV-B exposure periods occurred around noon (1100–1400), mimicking the situation in nature (Josefsson & Karlsson 1997). We used a computer model (Björn & Murphy 1985; Björn & Teramura 1993) to calculate the

daily irradiance of UV-B in Uppsala on 24 April (the normal breeding time of *R. temporaria*) and the daily increase in UV-B radiation that would follow from 15% ozone depletion under clear-sky conditions resulting in 26% enhanced UV-B levels. The DNA-weighted daily UV-B exposures were 1.254 and 1.584 kJ/m^2 for normal and enhanced UV-B, respectively. The UV-B exposures from Uppsala were used as an intermediate reference point because Uppsala is situated in the middle of Sweden halfway between the two localities. We adjusted the levels of UV-B by regulating daily irradiation regimes in the following way: (1) normal UV-B (irradiation time, 2 hours 17 minutes/day); (2) high UV-B (2 hours 53 minutes/day); and (3) control (2 hours 17 minutes/day), where UV-B and UV-C were blocked with a Mylar filter (0.10 mm, Erik S. Ekman, Stockholm). The UV-B radiation for each aquarium was provided by four fluorescent tubes (120 cm, 40W, Q-PANEL, UV-B 313, Cleveland, Ohio) preburned for 100 hours to give a stable output. In each aquarium, the four fluorescent tubes were placed 50 cm above water level and uniformly parallel (40 cm between each lamp) to one another. The midsection (approximately 40 cm) of the two central tubes was covered with aluminum foil to obtain an even radiation distribution within the aquarium.

For the normal and high UV-B treatments the radiation passed through a cellulose diacetate filter (0.13 mm, Courtaulds, Derby, United Kingdom) to cut off ultraviolet-C radiation (UV-C $<$ 280 nm). Details of filter properties follow those of Pahkala et al. (2000). Filters were placed 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 homogenous 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, 350–700 nm) were fitted over each of the six aquaria. The amount of radiated light (white light, including UV-A) was measured with a LI-COR light meter (Li-Cor, Lincoln, Nebraska) that had a quantum sensor, giving an irradiance of 320 μmol/m^2/second.

Water Preparation and pH Treatments

The two pH levels corresponded to naturally low (4.5) and neutral (7.6) pH in the typical breeding localities of this species (Leuven et al. 1986; Aston et al. 1987). Throughout the experiment we used reconstituted soft water consisting of $NaHCO_3$ (48 mg/L), $CaSO_4 \times 2H_2O$ (30 mg/L), $MgSO_4 \times 7H_2O$ (61.37 mg/L), and KCl (2 mg/L; American Public Health Association 1985). Water for both pH treatments was prepared in 80-L storage tanks, adjusted with 1 or 0.1 M H_2SO_4, and stabilized for 48 hours before use. The pH levels were monitored with a portable Hanna HI 9025 pH meter equipped with an Orion Ross sure-flow 8172BN electrode. We changed the water in each vessel daily and measured pH randomly from a few vessels. The pH remained stable between water changes.

Response Variables

The response variables we measured were survival, frequency of developmental anomalies, development time, and hatchling size. The experiment was terminated when 10 larvae in a given vessel had reached stage 25 (Gosner 1960), at which point survival (recorded as the proportion of eggs surviving until the end of the experiment) and the number of seemingly abnormal individuals (flexure of the tail or edema) were recorded. Larvae with visible developmental anomalies were excluded from the survival estimates because their future survival was highly unlikely (Beattie et al. 1992). The post-hatch larvae were not fed because the experiment was termi-

Table 1. Mixed-model nested analyses of covariance of survival rate in response to UV-B and pH treatments in two *Rana temporaria* populations.*

	Southern population				*Northern population*			
Effects	*variance*	*SE*	z	p	*variance*	*SE*	z	p
Random								
family	0.0055	0.0048	1.13	0.1285	0.0072	0.0080	0.90	0.1829
residual	0.0647	0.0079	8.17	0.0001	0.0377	0.0076	4.95	0.0001
	ndf	ddf	*F*	*p*	ndf	ddf	*F*	*p*
Fixed								
UV-B	2	135	0.84	0.4333	2	49.6	9.71	0.0003
pH	1	134	8.0	0.0054	1	49.4	34.42	0.0001
UV-B × pH	2	135	1.92	0.1512	2	49.6	10.04	0.0002
temperature	1	134	0.54	0.4645	1	49.3	0.35	0.5553

**Tests were performed with arcsine-square-root-transformed data. Abbreviations: ndf, numerator degrees of freedom; ddf, denominator degrees of freedom.*

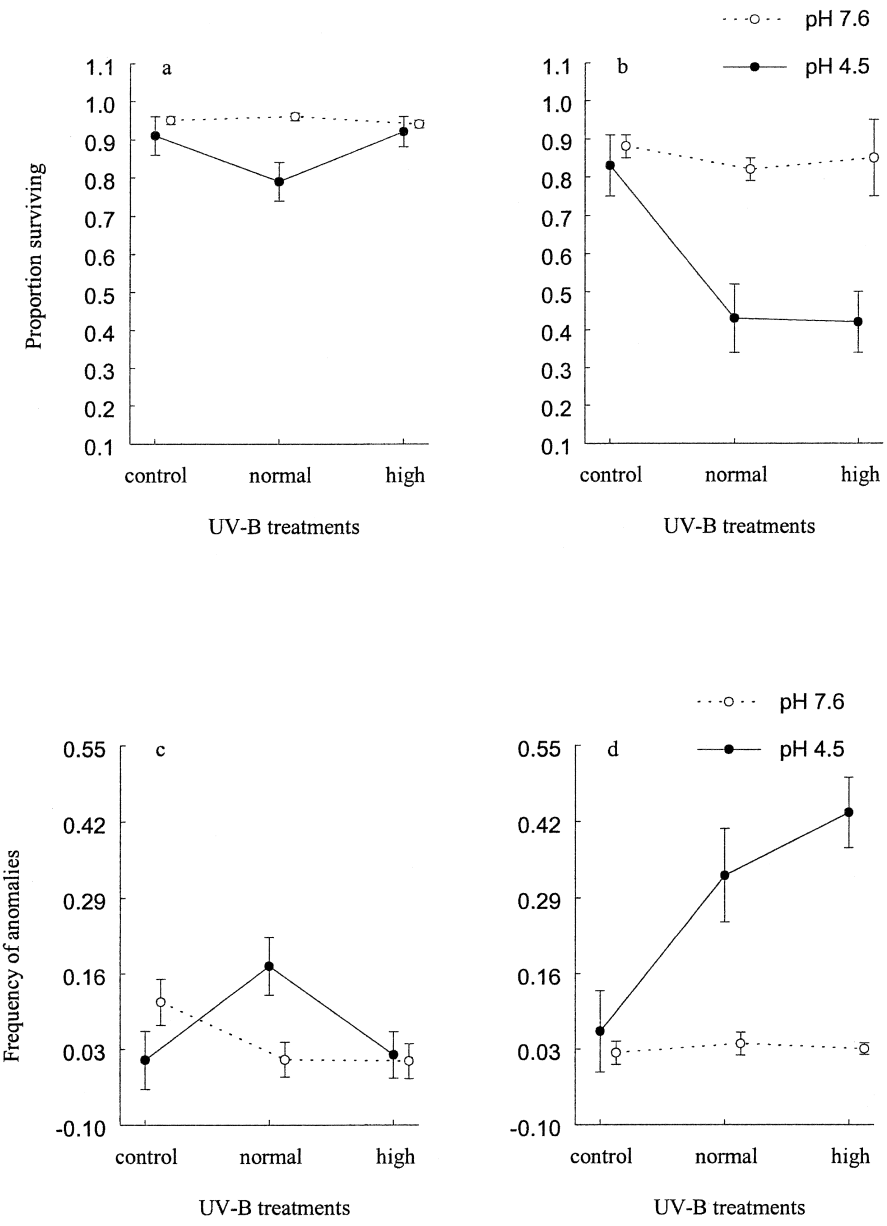

Figure 1. Survival and frequency of developmental anomalies of Rana temporaria *embryos in different UV-B and pH treatments: (a) survival of southern and (b) northern embryos and frequency of developmental anomalies among (c) southern and (d) northern embryos. Values are least square means (±SE) from models presented in Tables 1 and 2.*

nated before the feeding stage (cf. Gosner 1960). Development time was determined as the number of days from the start of the experiment until the 10 larvae reached stage 25. The larvae from each replicate were preserved in 10% formalin for later measurement. Hatchling size was determined from five preserved larvae from each replicate as total length of the larvae from tip of the nose to tip of the tail under a stereomicroscope (to the nearest 0.1 mm). All measurements were taken blind with respect to the experimental treatments.

Statistical Analyses

We used mixed-model analysis of covariance (ANCOVA) to investigate the effects of UV-B and pH treatments on the measured variables, as implemented in PROC MIXED in the SAS statistical package (SAS Institute 1996). Because our experimental design made predictions about the effects of population and pH and UV-B treatments, these factors were treated as fixed effects in the models, whereas family (nested under population) was considered a random effect (Zar 1996). Although family effects are given in the tables, they are not commented on in the results because this term was included solely to control for nonindependence of data points within a family. Family interactions (family × pH and family × UV-B) were excluded from further analyses because none was significant. Temperature was included as a covariate to control for temperature variation. To keep the interpretation simple, separate ANCOVAs were first run for both

Table 2. Mixed-model nested analyses of covariance of the frequency of developmental anomalies in response to UV-B and pH treatments in two *Rana temporaria* populations.*

	Southern				*Northern*			
Effects	*variance*	*SE*	z	p	*variance*	*SE*	z	p
Random								
family	0.0000	.	.	.	0.0002	0.0038	0.06	0.4743
residual	0.0646	0.0076	8.46	0.0001	0.0499	0.0102	4.89	0.0001
	ndf	ddf	*F*	*p*	ndf	ddf	*F*	*p*
Fixed								
UV-B	2	143	3.16	0.0454	2	49.9	7.00	0.0021
pH	1	143	4.46	0.0363	1	49.3	41.00	0.0001
UV-B × pH	2	143	3.27	0.0408	2	49.6	7.51	0.0014
temperature	1	143	2.15	0.1440	1	48.7	2.18	0.1459

**Tests were performed with arcsine-square-root-transformed data. Abbreviations: ndf, numerator degrees of freedom; ddf, denominator degrees of freedom.*

populations. This procedure was followed by a common ANCOVA for both populations to extract the between-population effects. To obtain correct degrees of freedom for the effects in the models, we used the Satterthwaite procedure, as implemented in PROC MIXED of the SAS statistical package (Littell et al. 1996). For analyses of hatchling size, we used the mean value of the hatchlings in each replicate as a unit. Before statistical testing, both survival and anomaly estimates were arcsine-square-root transformed to normalize their distributions. All statistical analyses were performed with 6.12 version of the SAS statistical package (SAS Institute 1996).

Results

Survival

Low pH reduced embryonic survival rates in the southern population, but there was no evidence of UV-B treatment or synergistic effect of pH and UV-B on survival rates (Table 1; Fig. 1a). In contrast, both UV-B and pH had a significant effect on embryonic survival rates in the northern population. A significant interaction between UV-B and pH in the northern population (Table 1; Fig. 1b) showed that the negative effects of normal and high UV-B treatments on survival were seen only in the low pH treatment (Table 1; Fig. 1b), indicating that the two stressors acted in a synergistic fashion. A combined analysis of the two populations confirmed that the population differences seen in the separate analyses were significant (population × UV-B × pH: $F_{2,186} = 5.54$, $p = 0.0046$; population × UV-B: $F_{2,186} = 7.22$, $p = 0.001$; population × pH: $F_{1,185} = 5.74$, $p = 0.0175$). Furthermore, survival rate was significantly lower for the northern than for the southern embryos (Fig. 1a & 1b; population: $F_{1,13.1} = 25.59$, $p = 0.0002$), but this was due largely to the strong effects of UV-B radiation and interactions between UV-B and pH the northern population.

Developmental Anomalies

Analyses of the frequency of developmental anomalies generally concurred with the results of the survival analyses: significant main effects of UV-B and pH were found in both populations, but these were much stronger in the northern than in the southern population (Table 2; Fig. 1c & 1d). Again, synergistic effects of UV-B and pH in the northern population were manifested by a significant UV-B × pH interaction (Table 2), which was due to the proportionally higher increase in the frequency of anomalies in the normal and high UV-B treatments in low-pH conditions than with the control treatment (Fig. 1d). Nevertheless, there was also a significant interaction between UV-B and pH in the southern population owing to an increased frequency of anomalies in the normal UV-B treatment under low-pH conditions (Fig. 1c). A combined analysis of the two populations confirmed, however, that their responses to UV-B and pH treatments were significantly different (population × UV-B × pH: $F_{2,196} = 4.97$, $p = 0.0078$), suggesting higher sensitivity of northern embryos to combined effects of UV-B and pH on embryonic development (Fig. 1c and 1d).

Development Time

Effects of UV-B and pH treatments on development time were similar in both populations (Fig. 2a & 2b). Low pH prolonged the development time significantly in both populations, but the normal UV-B treatment had the opposite effect (Table 3; Fig. 2a & 2b). Embryos experiencing a normal UV-B regime developed faster than those experiencing high or no UV-B radiation (Table 3; Fig. 2a & 2b). No significant interaction between UV-B and pH was detected in either of the populations (Table 3), and a combined analysis of both populations confirmed the similarity of the treatment effects in both populations (population × pH × UV-B: $F_{2,208} = 1.40$, $p = 0.25$). How-

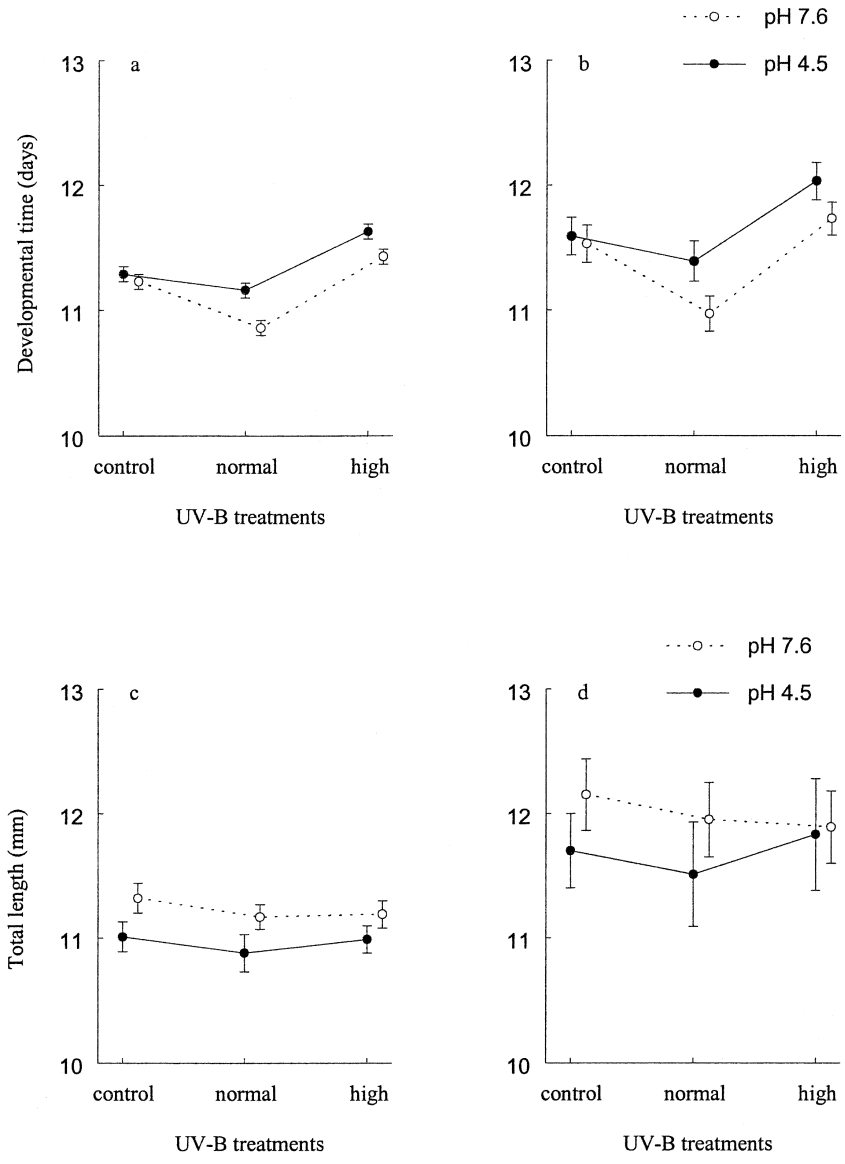

Figure 2. Developmental time and hatchling size in Rana temporaria *in different UV-B and pH treatments: (a) developmental time among southern and (b) northern embryos and hatchling size of (c) southern and (d) northern larvae. Values are least square means (±SE) from models presented in Tables 3 and 4.*

ever, a significant population × pH interaction ($F_{1,208}$ = 9.63, p = 0.0022) revealed that the effect of low pH was more pronounced in the northern population (Fig. 2a & 2b).

Hatchling Size

As in the case of development time, the response of average hatchling size to UV-B and pH treatments was similar in both populations: low pH reduced average hatchling size in all UV-B treatments and the embryos from the control UV-B and neutral pH treatment hatched at larger size than those from normal and high treatments (Table 4; Fig. 2c & 2d). The effects of UV-B treatment were significant only in the southern population (Table 4), however, and there was no evidence for synergism between UV-B and pH in either of the populations (Table 4). Combined analysis of the two populations revealed significant overall effects of UV-B ($F_{2,180}$ = 3.56, p = 0.03) and pH ($F_{1,180}$ = 39.98, $p < 0.001$) on average hatchling size, but there was no evidence of population differences in responses to either of the stressors.

Discussion

Our results demonstrate lethal and sublethal effects of UV-B radiation on embryonic performance—survival, frequency of developmental anomalies, size at hatching, and development time—of *R. temporaria.* These findings appear to conflict with previous evidence suggesting that UV-B radiation does not have negative effects on embryonic survival or the frequency of developmental

Table 3. Mixed-model nested analysis of variance of developmental time in response to UV-B and pH treatments in two *Rana temporaria* populations.*

	Southern population				*Northern population*			
Effects	*variance*	*SE*	z	p	*variance*	*SE*	z	p
Random								
family	0.0032	0.0041	0.77	0.2205	0.0092	0.0164	0.56	0.2868
residual	0.0897	0.0104	8.63	0.0001	0.1833	0.0349	5.26	0.0001
	ndf	ddf	*F*	*p*	ndf	ddf	*F*	*p*
Fixed								
UV-B	2	150	41.96	0.0001	2	55.9	14.38	0.0001
pH	1	150	7.21	0.0080	1	55.9	17.13	0.0001
UV-B × pH	2	150	0.09	0.9184	2	56	1.48	0.2372
temperature	1	150	437.65	0.0001	1	55.6	508.09	0.0001

**Abbreviations: ndf, numerator degrees of freedom; ddf, denominator degrees of freedom.*

anomalies in this species (Cummins et al. 1999; Langhelle et al. 1999; Hofer & Mokri 2000; Merilä et al. 2000*a*; Pahkala et al. 2000, 2001). Our results suggest, however, that the negative effects of UV-B radiation may be highly population-specific and confined to particular, stressful environmental conditions. We observed the negative effects of UV-B on survival in only one of the two populations and only at low pH. The same applied to the frequency of developmental anomalies, although low pH, in combination with a normal UV-B regime, also seemed to increase their frequency in the southern population. These findings provide further evidence for the contention that different populations of the same species may differ in their sensitivity to UV-B radiation (Williamson et al. 1997; Corn 1998; Belden et al. 2000) and that the synergism between UV-B radiation and other stressors may magnify the negative effects of UV-B radiation on amphibian development (Kiesecker & Blaustein 1995; Long et al. 1995; Ankley et al. 1998; Hatch & Burton 1998; Walker et al. 1998; Zaga et al. 1998; Monson et al. 1999; Hatch & Blaustein 2000). Consequently, conclusions based on tests of UV-B radiation tolerance using single populations in the absence of other relevant environmental stressors should be viewed with caution.

Embryos from the northern population were more sensitive to UV-B radiation than those from the south. This is surprising in view of the fact that our UV-B treatments simulating normal and high UV-B radiation levels were based on radiation levels representative of those experienced by Uppsala frogs (approximately 59°N). Because radiation levels during the breeding season of *R. temporaria* increase toward the north (Josefsson & Karlsson 1997; Merilä et al. 2000*b*), the doses experienced by the southern embryos in the normal UV-B treatment are likely to be somewhat higher than they would normally experience, whereas the opposite would be true in the case of the northern embryos. However, one potential explanation for these results resides in the differential sensitivity of the populations to low pH. The environment of southern Sweden has experienced heavy acidification since the early eighteenth century (Bergkvist 1995), and earlier work on amphibians from this area suggests that populations suffering the effects of acidification might have evolved tolerance to low pH

Table 4. Mixed-model nested analysis of variance of the hatchling size in response to UV-B and pH treatments in two *Rana temporaria* populations.*

	Southern population				*Northern population*			
Effects	*variance*	*SE*	z	p	*variance*	*SE*	z	p
Random								
family	0.3530	0.1680	2.10	0.0178	1.6954	1.3942	1.22	0.1120
residual	0.0508	0.0064	7.79	0.0001	0.1651	0.0324	5.10	0.0001
	ndf	ddf	*F*	*p*	ndf	ddf	*F*	*p*
Fixed								
UV-B	2	127	3.90	0.0227	2	52	0.76	0.4722
pH	1	127	44.14	0.0001	1	52	9.35	0.0035
UV-B × pH	2	127	0.65	0.5255	2	52	1.74	0.1848
temperature	1	127	0.29	0.5901	1	52	1.29	0.2606

**Abbreviations: ndf, numerator degrees of freedom; ddf, denominator degrees of freedom.*

(Andrén et al. 1989). Against this background, the better tolerance of the southern embryos over those from north may not be that surprising.

The development times of embryos in both populations and in both pH treatments were consistently shorter under normal than under high and control UV-B radiation treatments. It is known that UV-B irradiation has positive effects in biological systems, such as stimulation of vitamin (Garman et al. 2000) and pigment synthesis (Stiffler 1993; Cockell & Knowland 1999), and there might be an adaptive optimum at intermediate doses of UV-B for these processes to take place. The faster development of embryos under a normal UV-B regime may also represent an adaptive response to avoid prolonged exposure to UV-B radiation. Such a response might not be possible under higher levels of UV-B, however, due, for example, to some trade-off between energy or resources allocated to the development and protection of physiological machinery from UV-B–induced photoproducts (Epel et al. 1999; Pahkala et al. 2000). Further studies of this issue are needed, but the explanations we present are consistent with the contention that rapid development and growth is expected to evolve to minimize the amount of time an organism spends in a vulnerable stage of development (Arendt 1997). However, such responses may be constrained by extreme levels of stress (Hoffmann & Parsons 1997).

In the southern population, hatchling size tended to be larger in control than in normal and high UV-B treatments. This result provides further support for the earlier finding that UV-B radiation may have a negative effect on the growth of amphibian embryos and larvae (Grant & Licht 1995; Bruggeman et al. 1998; Belden et al. 2000; Pahkala et al. 2000; Smith et al. 2000; Pahkala et al. 2001). Similar negative effects of UV-B radiation on growth have been observed in plants (e.g., Sullivan et al. 1992; Johanson et al. 1995; Nielsen et al. 1995). One potential explanation for these observations could be the possible trade-off between the energy allocated to growth and mechanisms protecting organisms from the negative effects of UV-B radiation. The lack of further reduction in hatchling size in high UV-B treatment, compared with normal UV-B treatment, is not in conflict with this explanation because the larvae in the high UV-B treatment had significantly longer development times than those in the normal UV-B treatment. Hence, whatever the proximate causes, the fact that hatchling size is an important fitness correlate in amphibians (Kaplan 1982, 1998; Semlitsch & Schmiedehausen 1994) suggests that UV-B radiation may have negative effects on the later fitness of *R. temporaria* individuals. To this end, it is worth stressing that some of the negative effects of embryonic exposure to UV-B radiation could become expressed only at the later developmental stages (Smith et al. 2000; Pahkala et al. 2001), and further studies are required to assess the occurrence of such delayed effects.

Our results suggest that different populations of the same species may differ in their tolerance to UV-B radiation and, consequently, in their vulnerability to the projected increases in levels of UV-B radiation. Our results further suggest that, especially in regions where acid pollution is a concern, the synergistic effects of UV-B and low pH may have negative effects on amphibian populations. Likewise, even if UV-B radiation does not cause severe mortality in *R. temporaria* embryos, sublethal effects through inhibited growth may have effects on future fitness.

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