Evolutionary ecology of ultraviolet-B radiation stress tolerance in amphibians

BY

MAARIT PAHKALA
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Abstract


During the last decades many amphibian species and populations have experienced declines and extinctions in different parts of the world. Anthropogenic activities are believed to account for these declines, and one of the hypothesized causes has been the increased level of ultraviolet-B (UV-B) radiation due to depletion of the stratospheric ozone layer.

Although negative effects of UV-B radiation on development of many amphibian species have been demonstrated, a number of potentially critical issues around assessment of amphibian UV-B radiation tolerance have remained unexplored. For instance, next to nothing is known about geographic variation in UV-B tolerance and about possible carry-over effects of early UV-B exposure to later life-stages. Likewise, synergistic effects with other stressors, as well as sublethal effects on growth have received little attention.

The results from field and laboratory experiments show that *R. temporaria* and *R. arvalis* are relatively tolerant to even high levels of UV-B in terms of embryonic survival. However, it was found that even normal levels of UV-B can reduce early embryonic growth. In addition, the effects of early exposure to UV-B became manifested mostly or only after a considerable time-lag (i.e. at metamorphosis). Furthermore, it was found that the sublethal effects of UV-B may become manifested only in combination with other stressors, such as low pH, and this synergism may differ among different populations. No evidence for genetic differentiation in UV-B tolerance was found.

These findings suggest that even a relatively tolerant species, such as *R. temporaria*, may be sensitive to increased levels of UV-B radiation, but that this sensitivity may be highly population, environment and trait dependent. The observed carry-over effects over life-stages emphasise the importance of the early life environment on later life fitness.

Key words: amphibians, anomalies, embryonic development, geographic variation, growth, *Rana arvalis*, *Rana temporaria*, survival, UV-B radiation

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To my parents
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The thesis is based on the following articles which are referred to by their Roman numerals in the text:


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Introduction ..........................................................................................7
Aims of this study ..................................................................................9

Material & Methods ...............................................................................10
  The study species .................................................................................10
  Experimental procedures .....................................................................10
    Field exposures to solar UV radiation ...............................................10
    Laboratory exposures to UV-B radiation ..........................................12
    UV-B treatments ..............................................................................14
  Response variables ...........................................................................14

Results & Discussion ..........................................................................14
  Field and laboratory studies in R. temporaria .....................................14
  Field and laboratory studies in R. arvalis .........................................15
  Synergistic effects of UV-B and pH ....................................................16
  Population differences .......................................................................17
  Carry-over effects .............................................................................18

Conclusions .........................................................................................19

Acknowledgements .............................................................................19

Yhteenveto (Résumé in Finnish) .............................................................21
  Ultraviolettii-B säteilyn sietokyvyn evolutiivinen ekologia sammakkoeläimillä.........21

References..........................................................................................22
During the last decades, the distribution range of many amphibian species has dramatically reduced and species extinctions have occurred in various parts of the world (e.g. Alford & Richards 1999; Houlahan et al. 2000). Most dramatic losses have been detected in Australia, South and Central America, and high-altitude regions of the western United States (Drost & Fellers 1996; Laurance et al. 1996; Pounds et al. 1997; Berger et al. 1998; Lips 1998). Also, in Europe species declines have occurred (Berglund 1976, 1998; Terhivuo 1981, 1993; Ahlén & Tjernberg 1992; Gasc et al. 1997; Corn 2000).

Several anthropogenic factors have been suggested to contribute these declines. The most evident threat to amphibians is the loss and fragmentation of forest and wetland habitats, but other hypothesized causes for the declines include acidification, introduced exotic species, diseases, fungi, parasites, chemical pollutants, climatic changes and interactions between these different factors (Blaustein et al. 1994a; Kiesecker & Blaustein 1995; Long et al. 1995; Ankley et al. 1998; Berger et al. 1998; Hatch & Burton 1998; Lips 1998; Walker et al. 1998; Zaga et al. 1998; Carey et al. 1999; Green 1999; Johnson et al. 1999; Kaiser 1999; Lawler et al. 1999; Monson et al. 1999; Morell 1999; Pounds et al. 1999; Hatch & Blaustein 2000; Kiesecker et al. 2001). However, the causes for amphibian population declines have been difficult to establish, because often only some species are declining, whereas some other species show no sign of decline (e.g. Blaustein et al. 1998; Alford & Richards 1999). Furthermore, as population declines appear in undisturbed areas, speculation about a global phenomenon has been raised (Wake 1991, 1998; Pechmann et al. 1991; Pechmann & Wilbur 1994; Blaustein & Wake 1995; Alford & Richards 1999; Houlahan et al. 2000). Increased levels of ultraviolet-B (UV-B, 280-315 nm) radiation due to depletion of stratospheric ozone layer have been suggested as one factor causing global declines of amphibians (Barinaga 1990; Blaustein & Wake 1990, 1995; Alford & Richards 1999; Houlahan et al. 2000).

Light in the UV-B range is particularly damaging to living organisms, because it is directly absorbed by proteins and DNA and can damage these molecules (e.g. Tevini 1993). At the organismal level, UV-B radiation can suppress the immune system (Mayer 1992; Salo 2000) and cause damage to dermal tissue and eyes in vertebrates (Zavanella & Losa 1981; Fite et al. 1998; Wang et al. 1999; Flamarique et al. 2000). UV-B radiation could be particular damaging to some amphibian species, because they lay their eggs in shallow water, thus exposing the eggs to UV-B radiation. Indeed, it has been found that in some amphibian species UV-B radiation increases mortality (e.g. Blaustein et al. 1998; Ankley et al. 1998; Broomhall et al. 2000; Kiesecker et al. 2001), have sublethal effects in terms of altered behaviour (Zaga et al. 1998; Belden et al. 2000; Kats et al. 2000), and increases the frequency of developmental anomalies (Worrest & Kimeldorf 1975, 1976; Grant & Licht 1995; Kiesecker & Blaustein 1995; Blaustein et al. 1994b, 1997; Langhelle et al. 1999). Also, it affects negatively on embryonic and larval growth and development (Grant & Licht 1995; Bruggeman et al. 1998; Hatch & Burton 1998; Belden et al. 2000; Smith et al. 2000; Häkkinen et al. 2001).

Sublethal effects on embryonic and larval growth and development can also have significant ramifications on later life because hatching size is considered an important component of fitness in amphibians and size at hatching is known to be positively correlated with size at metamorphosis (Kaplan 1992, 1998). Also, timing and size at
metamorphosis can have cascading effects on individuals’ later fitness. For example, delayed metamorphosis can increase risks associated with habitat drying or predation (Newman 1992), and small size at metamorphosis can expose individuals to greater risk of desiccation and predation (Goater 1994; Newman & Dunham 1994). Also, smaller size at metamorphosis can lead to delayed maturation, reduce fecundity and lower mating success (Kaplan & Salthe 1979; Howard 1980; Smith 1987; Semlitsch et al. 1988; Berven 1990).

However, a number of studies have not found evidence for negative effects of UV-B radiation on amphibian early embryonic performance (e.g. Cummins et al. 1999; Merilä et al. 2000a). However, as pointed out by Cummins et al. (1999), it is possible, that the negative effects of UV-B radiation may become expressed at later developmental stages (i.e. have carry-over effects). That this issue may be of some concern is also suggested by the increasing number of studies showing that environmental stressors experienced during early life (Rossiter 1996; Desai & Hales 1997) may have permanent negative effects on later fitness (Pechenik et al. 1998; Lindström 1999).

When faced with risks involved in UV-B radiation exposure, amphibians have basically two kinds of options to cope with its negative effects. They can either avoid the UV-B stress by behavioural mechanisms, for example by seeking shelter or avoiding UV-B exposed habitats, or they can acclimate to this stress either physiologically or genetically (evolutionarily) (Roy 2000). As to the first option, data on egg-laying behaviour suggests that certain amphibian species have adapted so as to reduce exposure of their eggs to UV-B radiation. The tendency of some salamanders to hide their eggs or lay them in relatively deep water shields them from UV-B (Blaustein et al. 1994a; Marco et al. 2001). As to the physiological acclimation, at least two different mechanisms could protect tadpoles from UV-induced skin damage: the accumulation of melanosomes close to the skin surface, shielding the nuclei of epidermal cells, and a substance in the skin specifically absorbing UV-B radiation (UVAS, Licht & Grant 1997; Hofer & Mokri 2000).

There is evidence to indicate that different species (e.g. Blaustein et al. 1994c, 1999; Langhelle et al. 1999; Häkkinen et al. 2001) and even different populations of the same species may respond differentially to UV-B radiation (Corn 1998; Belden et al. 2000; Broomhall et al. 2000). It has been found that differential sensitivity to UV-B could be due to differences in photolyase activity (the genetic adaptation to cope with UV-B radiation, Blaustein et al. 1994c, 1996). Photolyase is believed to be the most important mechanism of removal of UV-B-induced DNA damaging photoproducts from cells (Blaustein et al. 1994c; Hays et al. 1996), and it has been found that species with high photolyase activity are more tolerant to UV-B radiation than species with low levels of photolyase (Blaustein et al. 1994c). Also, thick layers of jelly, which absorb UV-B radiation, protect eggs of many species from harmful effects of UV-B radiation (Higgins & Sheard 1926; Beundt 1930; Gurdon 1960; Grant & Licht 1995; Ovaska et al. 1997). However, next to nothing is known about intra-specific variation in UV-B tolerance as no rigorous among population comparisons have been carried out.

Because mean yearly levels of UV-B radiation are lower at high latitudes than at more lower latitudes, due to both a naturally thicker ozone layer and decreasing solar
inclination, it has generally been assumed that organisms inhabiting high latitudes may be particularly vulnerable to increasing levels of UV-B radiation (Caldwell et al. 1980; Barnes et al. 1987; Gehrke 1998). This generalization is based on assumption that the total effective doses of UV-B received by individuals at different latitudes follow strictly the latitudinal variation in total amounts of UV-B radiation reaching the Earth's surface. However, when the differences in phenology are accounted for, populations occurring at higher latitudes may actually be exposed to higher effective doses of UV-B radiation than those occurring at lower latitudes. This especially in Sweden where the amount of UV-B (during the breeding time of amphibians) in northern Sweden is about twice as much as in southern Sweden (Merilä et al. 2000b). Therefore, if adaptation to local UV-B regime has occurred, there appear to be good reasons to hypothesise that populations from higher latitudes may actually be more resistant to UV-B radiation than populations from lower latitudes.

Aims of this study

Most of the amphibian UV-B radiation studies have been performed with North American species (e.g. Blaustein et al. 1994c, 1995, 1996; Grant & Licht 1995; Long et al. 1995; Hays et al. 1996; Ovaska et al. 1997; Ankley et al. 1998, 2000; Anzalone et al. 1998; Corn et al. 1998), whereas the sensitivity of Palaearctic species has received less attention (Nagl & Hofer 1997; Lizana & Pedraza 1998; Cummins et al. 1999; Langhelle et al. 1999; Hofer & Mokri 2000; Häkkinen et al. 2001) even though reports of thinning of the ozone layer over Europe has also been reported (e.g. Stolarski et al. 1992; Müller et al. 1997, 1999; Rex et al. 1997; Björn et al. 1999; Josefsson 2000). Therefore, I examined how ambient and enhanced levels of UV-B radiation affected Swedish common frog (Rana temporaria) and moor frog (Rana arvalis) embryonic development (i, II).

As synergistic effects of different stressors may be important, I studied the effects of low pH and UV-B radiation on embryonic development of R. arvalis and R. temporaria (I, II, III). Synergistic effects of low pH and UV-B radiation could be of particular concern in Scandinavia where large areas have suffered from acidification (Brodin 1993; AMAP 1998). This in particular because acidification reduces the amount of dissolved organic carbon (DOC), thus enhancing the penetration of UV-B in the water column, and increasing UV-B radiation stress in acidified regions (Schindler et al. 1996; Yan et al. 1996; Lean 1998).

Several studies have compared UV-B radiation tolerance among amphibian populations in field experiments conducted in different localities (e.g. Blaustein et al. 1994c, 1999; Kiesecker & Blaustein 1995; Corn 1998). While these studies are useful in comparing the UV-B radiation tolerance within the environments where the investigations were conducted (Blaustein et al. 1998), they may tell little about the possible intrinsic differences among the populations as the comparisons were not conducted under uniform environmental conditions. I examined geographic variation in UV-B radiation stress tolerance under controlled laboratory conditions to find out whether populations differ intrinsically in their tolerance to UV-B radiation (III, IV), and whether there is any evidence for latitudinal adaptation to differing UV-radiation regimes across different populations of R. temporaria. (IV)
If UV-B radiation has negative effects, for example, on embryonic development and growth rates, these effects can be translated to reduced performance at later life-stages (Smith et al. 2000; V). However, most of the studies of amphibian UV-B tolerance have used a protocol where the early embryonic stages have been exposed to UV-B radiation and the experiments have usually been terminated when the embryos have hatched. This kind of study design may only pick-up part of the negative effects of UV-B on embryonic development but it leaves open the question about possible delayed effects of UV-B, which may become expressed at later developmental stages. In my final paper I concentrated on the carry-over effects of UV-B radiation received during embryonic development on larval development (V).

In all works, I paid special attention on sublethal effects of UV-B radiation on individuals’ development, i.e. assessed its impact on growth, development time and size at hatching. These effects have been seldom studied, despite of the fact that they may have far reaching influences on individual fitness.

Material & Methods

The study species

The species selected for this study are the two most common frog species in Fennoscandia, which occur in a variety of habitats ranging from small temporary ponds to lakes. The common frog (Rana temporaria) is the most widespread of the two species, whereas the moor frog (Rana arvalis) has a more restricted distribution, and does not occur commonly at higher latitudes and altitudes (Gasc et al. 1997). Although these species prefer ponds with moderately dense vegetation as breeding sites, R. temporaria occurs frequently also in ponds devoid of vegetation (Laurila 1998). Breeding season in Sweden commences after snowmelt, in late March – early April in the southern Sweden and in mid-June in the northernmost mountains. Globular egg masses are laid in shallow water, thus exposing the eggs to solar UV radiation. After 1.5 – 2 weeks, the larvae hatch and start to feed on algae and detritus. The larvae metamorphose 30 - 90 days after hatching depending on temperature.

Experimental procedures

Field exposures to solar UV radiation

Effects of ambient UV-B radiation and pH on hatchability and early development of common frog and moor frog embryos were studied in field experiments conducted in Uppsala (59°50’ N 17°50’ E, R. temporaria and R. arvalis) and Ammarnäs (65°54’ N 16°18’ E, R. temporaria) (Fig. 1, I, II). For R. temporaria, the freshly laid egg masses collected from field was used. The eggs of R. arvalis were obtained from laboratory matings, where the moor frog pair were allowed to pair in plastic buckets. All the field exposures were performed in outdoor tanks under ambient UV-B radiation.
Fig. 1. A map showing the location of the study populations used in experiments presented in papers I - V.
Fig. 2. Schematic presentation of the experimental set up in papers I and II. All eggs in each block were derived from the same mating, and exposed to six different combinations of UV-B (Mylar filter, Cellulose acetate filter and open) and pH (5.0 [star filled squares] and 7.6 [open squares]) treatments. The order of pH and UV-B treatments within each block was randomised. Each of the six vessels in a given block had initially ca 40 eggs.

The experiment consisted of the fully factorial combination of three UV-B and two pH treatments (Fig. 2). In this design, each of the 22 (R. arvalis) clutches from Uppsala and 14 and 20 clutches (R. temporaria) from Uppsala and Ammarnäs, respectively, were divided into six different treatments, which consisted of all possible combinations of two pH (low: pH = 5.0; neutral: pH = 7.6) and three radiation treatments. The radiation treatments were (1) unfiltered sunlight, (2) sunlight filtered to remove UV-B (Mylar), and (3) sunlight filtered to remove wavelengths shorter than UV-B (Cellulose acetate). Cellulose acetate filters were included to control for possible filter effects, such as enhanced thermal environment created by the filter coverage.

Laboratory exposures to UV-B radiation

Adult R. temporaria and R. arvalis were collected from spawning sites (Fig. 1) at the onset of the breeding season and transported to the laboratory in Uppsala. Eggs used in the experiments (II, III, IV, V) were obtained by using artificial fertilisations following the procedure of Berger et al. (1994). Artificial matings provided homogenous material for population comparisons and ensured that the eggs had no priori exposure to UV-B radiation or low pH. Damaged or unfertilised eggs were not used in the experiments. After fertilisation the eggs (< 2h old) were divided into batches of about 30 - 50, and placed into experimental vessels about 4.5 cm under the water surface.
Fig. 3. One of the three aquaria systems used in experiments described in papers II - V. To even out temperature fluctuations, water is circulated from the reservoir tank on the floor to the two aquaria at constant rate. Water in the reservoir tank is constantly looped through a water-cooling unit (the black box).

The experiments were conducted in a constant temperature room (15 °C) in three aquarium systems, each of which consisted of two experimental aquaria (120 cm x 120 cm x 25 cm; about 320 L) placed on the top of each other and a reservoir tank (90 cm x 90 cm x 35 cm; about 280 L) below them (Fig. 3). The experiments consisted of the fully factorial combination of three UV-B (II, III, IV, V) and two pH (III) treatments. Throughout the experiments reconstituted soft water (RSW, APHA 1985) was used. Water was continuously circulated to reduce temperature fluctuations. To maintain water temperature at the desired level, each aquarium system was equipped with a water-cooling unit (Fig. 3).
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 (Fig. 3). 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. temporaria and R. arvalis) as well as the daily increase in UV-B radiation that would follow from 15% ozone depletion, resulting 26% enhanced UV-B above normal levels. The DNA-weighted daily UV-B exposures were 1.254 and 1.584 kJ m⁻² for normal and enhanced UV-B, respectively. In a control treatment the UV-B and UV-C were blocked with Mylar filter. A detailed description of the experimental system is given in paper (II, III, IV, V).

In paper V, when the majority of the embryos had hatched, randomly selected tadpoles from each experimental unit were reared individually until metamorphosis in the absence of UV-B radiation.

**Response variables**

Four response variables were measured: survival, frequency of developmental anomalies, development time and size at hatching (at stage 25, absorption of the external gills; Gosner 1960) or metamorphosis (at stage 42, the emergence of first foreleg; Gosner 1960). The experiments were terminated when the majority of the larvae in a given vessel reached stage 25 (I, II, III, IV) or when all larvae had metamorphosed (V). At this stage survival rates (i.e. the proportion of eggs or larvae which survived from the beginning of the experiment to the end of the experiment) and the number of abnormal individuals (flexure of the tail or oedema) were recorded. Development time was determined as the number of days from start of the experiment until the majority of the larvae reached the stage 25 (I, II, III, IV, V). Hatchling size was determined as total length (from the tip of the nose to the tip of the tail) of the larvae. Furthermore, in paper V, metamorphosed individuals (stage 42; Gosner 1960) were weighted, and measured for total length. Age at metamorphosis was defined as the number of days elapsed between fertilisation and metamorphosis. The number and type of anomalies was recorded (see results).

**Results & Discussion**

*Field and laboratory studies in R. temporaria*

No strong effects of ambient or enhanced levels of UV-B radiation on survival of the Swedish R. temporaria embryos were detected (I, III, IV, V). This is in accordance with an earlier study on R. temporaria in Sweden, which showed that the embryonic stages of R. temporaria are tolerant to ambient levels of UV-B radiation (Langhelle et al. 1999). The same conclusion is also reinforced by independent studies conducted in different populations of this species (Cummins et al. 1999; Hofer & Mokri 2000; Merilä et al. 2000a; Häkkinen et al. 2001). The good UV-B radiation tolerance may be due to two powerful sunscreen factors possessed by R. temporaria: UV-B absorbing substances and melanin pigmentation (Hofer & Mokri 2000).
There is some evidence for sublethal effects of UV-B on amphibians in terms of anomalies (Worrest & Kimeldorf 1975, 1976; Blaustein et al. 1994b, 1995, 1997; Grant & Licht 1995; Kiesecker & Blaustein 1995; Langhelle et al. 1999), and also in my study the UV-B radiation increased the frequency of developmental anomalies in some of the populations (III, IV, V). Also negative effects on embryonic and larval growth and development have been found (Grant & Licht 1995; Bruggeman et al. 1998; Belden et al. 2000; Smith et al. 2000), but embryonic growth and development have received less attention until very recently (but see Hatch & Burton 1998; Merilä et al. 2000a; Hämäkinen et al. 2001). In general, I found that the hatchling size was biggest in the control treatment compared to normal or enhanced UV-B radiation treatments, but these effects were not entirely consistent in different studies/populations (I, III, IV, V). Hence, these results provide some, albeit equivocal, support for the contention that UV-B radiation may have a negative impact on the growth of amphibian embryos and larvae (Grant & Licht 1995; Bruggeman et al. 1998; Belden et al. 2000; Smith et al. 2000). Similar negative impact of UV-B radiation on growth has been observed also in phytoplankton, plant and fish studies (e.g. Hunter et al. 1979; Sullivan et al. 1992; Johanson et al. 1995; Nielsen et al. 1995).

In my experiments, UV-B radiation had strong effects on development time. In general, development time was longest in the high UV-B treatment (IV), but again the results were not consistent across different populations (IV). This variability in responses is in accordance with previous studies on R. temporaria: Merilä et al. (2000a) did not find any effect of UV-B treatment on development time, whereas Hämäkinen et al. (2001) found a strong delaying effect. However, it is worth stressing the fact that developmental time is difficult to measure accurately for logistic reasons, and the negative results could, at least partly, owe to bluntness of the assessment protocol. One potential explanation for reduced size and longer development time in hatchlings exposed to UV-B could be the increasing physiological cost associated with repairing cellular damage or producing protective pigments (Epel et al. 1999). Hence, the more energy is allocated to photo protection, the less is available for development and growth (I).

I also found that development time was fastest under normal UV-B treatment (III), and it could be that mild doses of UV-B radiation can have positive effects on growth through, for example stimulated vitamin D (Garman et al. 2000) and pigment synthesis (Stiffler 1993; Cockell & Knowland 1999).

Field and laboratory studies in R. arvalis

In field, no effects of ambient UV-B radiation on embryonic survival or frequency of developmental anomalies in R. arvalis was found (II). This is in contrast with the results of Hämäkinen et al. (2001) who found that the survival of R. arvalis embryos was significantly lower under UV-B treatment. This suggests that there may be variation in UV-B tolerance among different populations of the R. arvalis. However, a caution is needed in here since, the methods were different in the two investigations.

However, ambient UV-B radiation had significant effects on hatching total, body and tail lengths. Nevertheless, the significant contrast between open and filter (Mylar or Cellulose acetate) treatments revealed that the filter itself had a positive effect on
early growth performance, whereas the contrast between Mylar and Cellulose acetate treatments was not significant, suggesting that UV-B regime *per se* did not influence growth performance. Hatchlings in open treatment grew slower than their full-sibs under Cellulose acetate and Mylar filters, a difference that could be explained by temperature differences between filter and open treatments. It is known that temperature may affect hatchling size in amphibians (Kaplan 1992).

In laboratory, no evidence for negative effects of normal or enhanced levels of UV-B radiation on survival or the frequency of developmental anomalies was found. This supports the results of my field experiments, and reinforces the conclusion that *R. arvalis* embryos are indeed tolerant to UV-B radiation. However, the 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. No effects of UV-B radiation on embryonic development or growth in laboratory experiment were recorded.

*Synergistic effects of UV-B and pH*

It has been suggested that the effects of UV-B may only become manifested in combination with other stressors (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; Kiesecker *et al.* 2001). For instance, it has been found that UV-B radiation can act synergistically with low pH in *Rana pipiens* (Long *et al.* 1995), and results from paper III indicate that this may be the case also in some *R. temporaria* populations. I found that low pH magnified the negative effects of UV-B on survival in one (northern population) of the two populations (Fig. 4a, b). The same applied to the frequency of developmental anomalies (Fig. 4c, d).

However, I did not find synergistic effects of UV-B and low pH on *R. arvalis* or *R. temporaria* in studies conducted in field, suggesting that neither ambient levels of UV-B nor low pH together with UV-B reduced the survival of the *R. arvalis* or *R. temporaria* embryos (I, II). This contrasts with my other study, where I found synergistic effects of low pH and UV-B radiation (III). Although broad generalizations about effects of UV-B/pH synergism on amphibians must await further studies, these results suggest that it may not be any general phenomenon, but rather restricted to some populations only.
Fig. 4. Survival and frequency of developmental anomalies of *R. temporaria* embryos in different UV-B and pH treatments. (a) Survival of southern, and (b) northern embryos. (c) Frequency of developmental anomalies among southern and (d) northern embryos. Values are least square means (±S.E.). From III.
**Population differences**

In paper IV, I tested the proposition (Merilä et al. 2000b) that *R. temporaria* populations along a latitudinal gradient of increasing ambient UV-B radiation levels would have adapted to local levels of UV-B radiation. In other words, I tested whether northern Scandinavian populations, which experience higher doses of UV-B radiation would be better able to cope with high doses of UV-B than the southern Scandinavian populations. However, even though the population differences in tolerance to UV-B radiation were found, there was no evidence for local adaptation (IV). This suggests that there might be little variation in the efficiency of photoprotection mechanisms between different populations, at least in the geographic scale covered in this study. However, the interpretation of these results is complicated by my other findings: it may well be that local adaptation has taken place, but the population differences were not apparent at the time (hatching) the treatment effects were evaluated (V), or in the environment where tests were conducted (III).

**Carry-over effects**

Although I failed to find any severe effects of UV-B on embryonic development, one should interpret the results with caution since not all the potential negative effects of UV-B on development are expressed at hatching stage. In *R. temporaria*, exposure of embryos to enhanced UV-B radiation levels was found to increase the frequency of developmental anomalies at metamorphosis. Most of the hind-limb deformities detected were so severe that the future survival of these individuals would be unlikely (Fig. 5).

![Fig. 5. Examples of hind-limb malformations in *Rana temporaria* observed in experiment described in paper V. The left figure shows a case of ectromelia (part of the limb missing), and the right a case of ectrodactyly (one or more digits missing).](image-url)

Furthermore, I found that UV-B radiation delayed timing and reduced size at metamorphosis. These findings are significant because effects on timing and size at
metamorphosis can have cascading effects on individuals’ later fitness. It has been shown that smaller size and delayed maturation reduces fecundity and lower mating success (Kaplan & Salthe 1979; Howard 1980; Smith 1987; Semlitsch et al. 1988; Berven 1990). Consequently, the negative effects of embryonic exposure to UV-B radiation can have far-reaching consequences from an individual’s and perhaps also from a population dynamic, point of view (Lindström 1999).

Conclusions

In this thesis I have investigated various aspects of UV-B radiation tolerance in two northern European amphibian species, and shown that an apparently tolerant species, such as *R. temporaria*, may be sensitive to increased levels of solar UV-B radiation. However, this sensitivity appears to be highly population and environment specific, so as that different populations may differ in their tolerance to UV-B radiation (III, IV), and that negative effects may become expressed only under presence of other stressors, such as low pH (III). 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 (III). Likewise, even if UV-B radiation does not cause severe mortality in *R. temporaria* embryos, sublethal effects through inhibited growth and developmental anomalies may have effects on future fitness (I, III, IV, V). Consequently, the negative effects of embryonic exposure to UV-B radiation can have far reaching consequences. However, I did not find any evidence for local adaptation for variation in UV-B radiation regime across Sweden (IV). The interpretation of these results was complicated by my other findings: it may well be that local adaptation has taken place, but the population differences were not apparent at time (hatching) the treatment effects were evaluated (cf. V), or in the environment where the tests were conducted (cf. III). The observed carry-over effects of early exposure to UV-B radiation over life-stages emphasise the importance of early life environment on later fitness (V). They also call for further studies in other species now declared to be highly tolerant to UV-B radiation on the basis of experiments terminated at hatchling stage.

In general, population comparisons focusing on delayed and synergistic effects would be necessary to understand the geographic variation in UV-B tolerance. Furthermore, intraspecific comparisons conducted with less photo protected species in a common environment would be crucial in settling the role of local adaptation in UV-B tolerance. Also, because genetic variance is critical in determining the persistence of a species in a changing environment, it would be essential to examine the amount of genetic variability in UV-B tolerance within local populations. This kind of data would help us to evaluate whether amphibian populations have the ability to adapt to predicted increases in levels of UV-B radiation.

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Yhteenveto (Résumé in Finnish)

Ultravioletti-B säteilyn sietokyvyn evolutiivinen ekologia sammakkoeläimillä

Viime vuosikymmeninä sammakkoeläimet ovat taantuneet maailmanlaajuuisesti. Sammakkojen väheneminen johtuu pääasiassa elinympäristöjen häviämisestä, mutta muita syitä ovat esimerkiksi happamoituminen, torjunta-aineet, sienitaudit ja ilmastonmuutokset. Erityisen huolestuttavaa on se, että monet sammakkolajit ovat vähentyneet myöskin luonnonsuojelualueilla, joissa niiden elämän pitäisi olla periaatteessa turvattua. Yhdeksi syyksi on epäilty auringon ultravioletti-B (UV-B) säteilyn, jonka määrä maanpinnalla on lisääntynyt otsonikadon myötä. Viimeikaiset tutkimukset osoittavat, että eri sammakkoeläimet eroavat toisistaan siinä, kuinka herkkia niiden munat kehittyvät UV-B säteilyn aikaansaamalle kuolleisuudelle. Lajit, joiden munat kehittyvät olosuhteissa, joissa luontainen UV-B sääteilyn määrä on korkea, ovat osittautuneet vähemmän herkkiksi kuin lajit, joiden munat kehittyvät ympäristössä, joissa UV-B sääteilyn on vähemmän. Proksimaattiseksi selitykseksi tälle
havainnolle on esitetty sitä, että tärkeimmän UV-B välitteisiä DNA-vaurioita ehkäisevän fotolyaasi entsyymin aktiivisuudet ovat korkeampia lajeilla, jotka elävät ympäristössä, joissa UV-B säteilyn määrä on korkea. Tämä viittaa siihen, että luonnnonvalinta on suosinut tehokkaampia lajeilla, jotka ovat alttiina korkeimilla UV-B sääteilymäärillä. Näin ollen voidaan myös olettaa, että ne laajalle levineen lajin populatiot, jotka ovat alttiina korkeimilla UV-B säteilymäärillä, omaisivat paremmän UV-B resistanssin kuin vähäisemmille sääteilymäärille altistetut populatiot.

Tämän tutkimuksen tarkoitus oli selvittää tavallisensammakon (Rana temporaria) ja viitasammakon (Rana arvalis) muna- ja toukka-asteiden herkkyyttä normaalille sekä kasvavalle UV-B säteilylle sekä selvittää ovatko saman lajin eri populatiot sopeutuneet maantieteellisiin eroihin UV-B sääteilyn määrässä. Lisäksi erityistä huomiota kiinnitettiin siihen, että aiheuttaako UV-B sääteily kuolleisuuden lisäksi myös kehitysvaurioita sekä hidastaako UV-B sääteily kehitystä ja kasvua. Myös yhdysvaikutukset happamoihminen kanssa olivat mielenkiintoinen kohtena, toisin sanoen riippuuko UV-B sääteilyn vaikutus ympäristön happamouudesta. Lisäksi UV-B sääteilyn mahdolliset viivästyneet negatiiviset vaikutukset kehityksen myöhempin vaiheisiin olivat erityisen kiinnostavia.

Tutkimukseni on osoittanut, että erittäin sietokykyiseksi lajiksi epäilty tavallinensammakko, voi olla herkkä kasvavalle UV-B säteilylle. Tämä herkkyyys riippuu kuitenkin sekä populatiosta että elinympäristöstä, ja eri populatioiden välillä on eroja niiden herkkyydessä UV-B säteilylle. Lisäksi erityisesti alueilla, joissa vedet ovat happamoihminen, mukalaiset saattaisivat olla erityisen herkkäiden UV-B sääteilyn ja happamoihminen yhdysvaikutukset. Työni osoittaa myös sen, että UV-B sääteily ei aiheuta suoranaista kuolleisuutta, mutta voi aikaansaada kehitysvauriota, hidastaa kasvunopeutta ja johtaa pieneen ruumiinkokoon - kaikki nämä voivat alentaa yksilön kelpoisuutta. Lisäksi havaitsin, että kehityksen alkuvaiheessa saadun UV-B sääteilyn aiheuttamat vauriot voivat tulla näkyviin vasta kehityksen myöhemmässä vaiheessa. Tämän vuoksi tutkimukset, jotka keskittyvät populatioiden vertailuihin sekä UV-B sääteilyn myöhäis- ja yhdysvaikutuksiin ovat tärkeitä.

References


Rohkea lause – parempi olla sanomatta mielestani.