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Research Article

Basking Behaviour and Ultraviolet B Radiation Exposure in a Wild Population of *Pelophylax lessonae* in Northern Italy

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ABSTRACT - Amphibians are facing catastrophic global declines under pressure from a variety of threats. Many of these are so acute and poorly understood that ex-situ conservation has been employed where in-situ efforts may be unable to act quickly enough to save species in the wild. However, our knowledge of the captive requirements of most amphibians is unknown or poorly understood and this knowledge gap jeopardises the success of ex-situ programs. A lack of data from the habitat of wild populations underpins many husbandry failures in captivity, as without it husbandry is based on best guess. Ultraviolet B radiation has been shown to be critical in the care of many reptile species, but its importance to amphibians is not well understood. We present the first data on UV-B exposure and basking behaviour in wild amphibians, using *Pelophylax lessonae* as a model species. We show that wild frogs inhabit a UV-B microclimate defined by physical features of their habitat and by the basking behaviour of the animals themselves. This data may encourage the future gathering of such wild environmental data, which could be fed directly into ex-situ programs.

INTRODUCTION

With growing threats to amphibians worldwide and more than a third of known species threatened by extinction (IUCN, Conservation International and NatureServe, 2008) ex-situ conservation is becoming more important in the long term survival of amphibian species (Gascon, 2007). As such, a number of species have been 'evacuated' from the wild and installed in captive breeding programs, with the eventual aim of reintroduction. However, knowledge of the captive requirements of many species is poor and this deficit of knowledge has the potential to undermine captive conservation efforts (Gascon, 2007; Gagliardo et al., 2008). Without a good understanding of captive requirements, evacuated populations may be no more likely to survive than their wild counterparts and ex-situ conservation efforts may fail.

One approach to addressing this lack of knowledge is to study amphibians and their environment in the wild, with the aim of replicating wild parameters in captivity. By aligning the captive environment more closely to wild conditions, the success of captive husbandry may be improved. Furthermore, replicating wild conditions in the terrarium may help to address the issue of adaptation to captivity. Where captive conditions differ from those to which organisms have evolved (i.e. those in the wild), this may either relax selection pressures that existed in the wild (e.g. predation pressures), alter the direction of selection pressures (e.g. fear of humans, foraging behaviour) or exert entirely new ones (e.g. dealing with crowding or novel pathogens) (Woodworth et al., 2002; Frankham, 2008). This phenomenon has been identified in captive colonies of *Alytes muletensis*, which, after 9-12 generations in captivity, showed little or no response to predator cues as tadpoles (Kraaijeveld-Smit et al., 2006). Reproducing wild conditions more faithfully in the captive environment can help to address this problem to some extent (Frankham, 2008; Williams & Hoffman, 2009).

Ultraviolet B (UV-B) radiation is a form of solar radiation that many organisms, including at least some amphibians, use in the synthesis of vitamin D3, which is vital in calcium uptake, as well as for other biological functions (Antwis & Browne, 2009). Although the understanding of UV-B requirements in captive lizards and chelonians has revolutionised the way these animals are maintained in captivity and has resolved many previously common pathologies, including metabolic bone disease (e.g. Ferguson et al., 1996; Divers, 1996; McArthur & Barrows, 2008), the importance of UV-B radiation for captive amphibians is very poorly understood (Antwis & Browne, 2009).

Anecdotal, and some experimental (e.g. Verschooren et al., 2011), evidence suggests that UV-B may be important for some amphibians (Antwis & Browne, 2009), particularly those that are known to expose themselves to solar radiation by basking (e.g. *Trachycephalus resinifictrix*; Verschooren et al., 2011). Conversely, UV-B radiation has been suggested as possible driver of amphibian extinctions (e.g. Kiesecker et al., 2001) and some studies have found impacts of UV-B radiation on mortality in captive amphibians (Blaustein et al., 2005).

However, neither set of captive studies (either those finding a benefit or those finding detrimental effect) used data from the field and, more specifically, from the microclimate of these amphibians to inform UV-B exposure levels in the laboratory, so leaving their results open to interpretation. Nor did either study refer to data on basking behaviour in the wild. For example, (Blaustein et al., 2005) found mortality associated with 'ambient' levels of UV-B exposure in *Anaxyrus* (*Bufo*) *boreas*. UV-B levels were measured at the site where study animals were collected and replicated in the lab. However, UV-B levels were not measured within the microclimate where the toads live and so the exposure in captivity may not reflect levels of UV-B to which these toads are normally exposed. Furthermore, basking behaviour was not recorded and so any effect of behaviour on actual UV-B exposure in the field could not be taken into account. While this study certainly shows that UV-B radiation can have negative effects of amphibians, it should not be used to justify a lack of exposure in captivity. This study also did not make use of earlier work (Lillywhite et al., 1973), which defined the thermal optima of *A. boreas*, dependent on nutritional status. The UV-B-associated mortality may, therefore, have been influenced by laboratory temperatures being up to 10°C lower than the thermal optimum for this species.

As such, until thorough research reveals the true extent of amphibian dependence on UV-B radiation, or any detrimental effects thereof, the safest course of action may be to provide captive amphibians with levels of UV-B radiation similar to those that they are exposed to in nature, within a spectrum of microclimates allowing for downwards regulation of exposure.

Meteorological data, including UV-B Indices, are readily available for many regions and are sometimes used as a guide for designing captive conditions. However, amphibians live in and use microclimates within the larger environment (e.g. McClanahan et al., 1978; Kluber et al., 2009; Heath, 1975; Seebacher & Alford, 1999; 2002), so meteorological data for the wild range of a species is unlikely to be representative of conditions within the microclimate. Therefore data taken from the wild microclimate must be gathered, rather than using anthropocentric data as a proxy.

The actual amount of UV-B radiation to which wild amphibians are exposed will be dependent on many aspects of species biology. The maximum levels of UV-B exposure that a species can be exposed to will be limited by the micro-climate in which the animals live. Amphibians are not simply passive objects within the environment, however, and UV-B exposure can also be regulated through basking behaviour. This has been demonstrated in reptiles (e.g. Karsten et al., 2009) and there is no reason to suspect that it is not the case in other taxa. Many amphibians are known to bask to thermoregulate (e.g. McClanahan et al., 1978; Wells, 2007) and this behaviour will inevitably also regulate exposure to UV-B radiation, the environmental gradient of which mirrors that of sunlight and solar heat.

Water, or green, frogs (*Pelophylax* spp.) are well-known for their basking behaviour. They exist throughout Europe and Asia and inhabit still or slow-moving bodies of water from which, in contrast to the brown frogs of the genus *Rana*, they do not stray far (pers. obs.). Several species of this genus are threatened in the wild, according to the IUCN Red List (IUCN, 2012; Uzzell & Isailovic, 2009). The common pool frog, *Pelophylax lessonae* (the focal species of this study) represents a common and readily accessible water frog species, which is itself held within several institutional collections (e.g. Horniman Museum and Gardens and Slimbridge Wildfowl and Wetlands Trust, both in the UK), as well as being used in laboratory research and the culinary industry, for both of which they are collected from the wild (although they are often advertised as 'farmed') (pers. comm. J. Bentley).

We present data on basking behaviour and exposure to UV-B radiation in the wild, basking amphibian *P. lessonae*. Although the measures and observations presented are conceptually and practically simple, they represent the first such study in amphibians and provide a baseline for comparison with future studies. We identify the levels of UV-B radiation to which this frog was actually exposed during one day in the breeding activity season (late May) and demonstrate that this species inhabits a UV-B microclimate while basking. Our data, although limited to a single day of sampling, has implications for the captive husbandry of water frogs (*Pelophylax* spp.) and for the way that environmental data should be collected for wild amphibian populations.

METHODS

Study site

The study site, shown in Fig. 1, is a small, mature, artificial pond in a private residential/agricultural setting in Savorgnano, San Vito al Taglimento, Pordenone, Friuli-Venzia Giulia, NE Italy (N 045°53' 848" E 012°51'180"). It is a rough oval in shape, measuring approximately 2 m in maximum length and 1.5 m in maximum width, with a maximum depth of around 1 m. The pond is located in full sun, with a fringe of long grass. Adult pool frogs (*P. lessonae*) are resident in the pond and were observed to bask in four major positions (marked with 'x's in Fig. 1) on the bank near a fringe of long grass running round the perimeter of the pool; henceforth referred to as 'basking sites'. All

Figure 1. Study site, a small pond inhabited by *P. lessonae* in northern Italy.

readings were taken on the 20/5/2012. During sampling, at least seven adult frogs were present in the pool. All of the following readings were taken on a day of full sun with high visibility and a maximum ambient temperature of 23°C. Frogs were active, and males called during the evening and early morning. Spawning took place 2 weeks after sampling.

UV Index (UVi) readings

Ultraviolet Index (UVi) readings were taken using a Solarmeter 6.5 UV Index meter (Solartech Inc.) from the four identified basking sites. A Solarmeter is roughly cuboid in shape, being 10.5 x 6 x 2.2 cm in size, with the sensor positioned on one of the small end faces of the device. Readings were taken by placing the Solarmeter upright, with the sensor on top, on the basking sites. By placing the meter flat on the ground, the sensor was orientated in the same direction as the dorsal surface of frogs basking on the same site (i.e. vertically perpendicular to the slope of the bank). This ensured that the UV Index measured by the meter was as similar as possible to the UVi encountered by a basking frog. At each time point, mean UVi was calculated across the four positions. Ambient UVi was recorded from and exposed, elevated (head height) position full sun in the vicinity of the pond, again taking the mean from four readings. Readings were taken hourly from 07:00 (local time) until 18:00 (local time); however, no data were collected at 16:00.

UVi is a unitless measure of the amount of UV-B radiation reaching the surface of the planet, weighted for the erythema (sunburn) action spectrum, which quantifies the differing

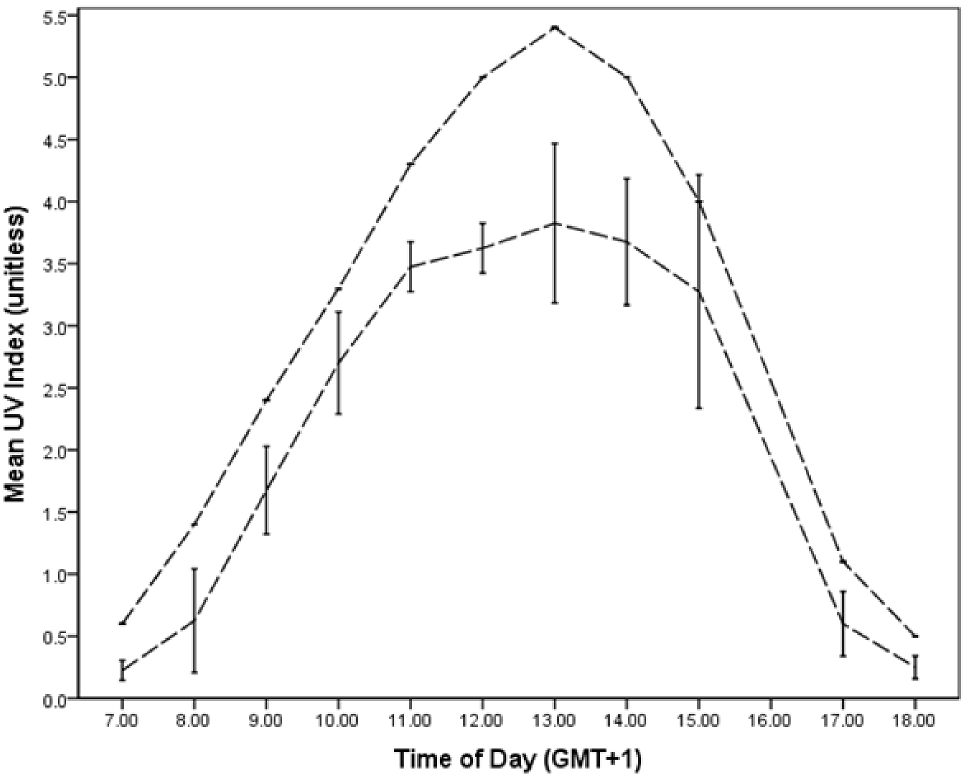

Figure 2. Ambient (broken line) and Basking-site (unbroken line) UV Index readings. Ambient UVi is significantly higher than Basking-site UVi (Paired t-test; t=-6.384, P<0.001). Error bars represent 95% confidence intervals (these are present, but tiny, for ambient data as repeated measurements had the same values).

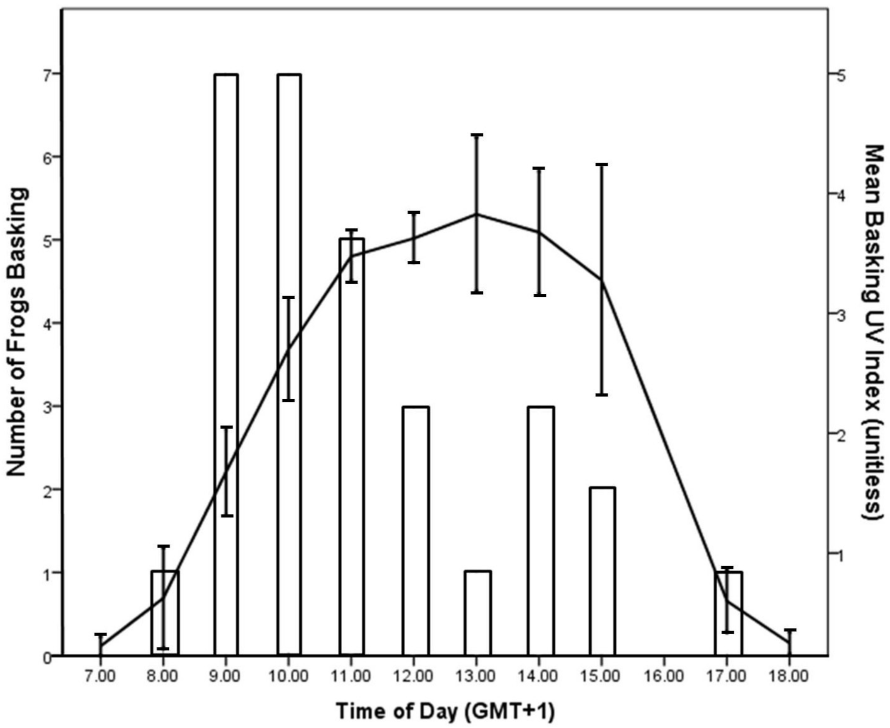

Figure 3. Numbers of basking frogs (bars) and mean UVi (line) at basking sites around the pond. Error bars represent 95% confidence intervals.

biological impact of wavelengths. This value, in W cm-2, is multiplied by an arbitrary value of 40 to give an index value of biologically significant UV irradiance. Although this last transformation is not strictly necessary, it brings values in line with the established UV index scale and makes comparisons more intuitive. This differs from a straightforward measurement of UV-B irradiance, as measured by other devices, which does not correct for biological significance of wavelength. UV indices are widely understood and are thus used to quantify biologically relevant UV irradiance for herptiles by the Reptile and Amphibian Working Group (RAWG) (part of BIAZA) in their UV-Tool, a database designed to compile information relevant to the provision of UV-B radiation to captive reptiles and amphibians. Although this value is used to quantify erythema risk in humans, which is not necessarily relevant to amphibians, it is still a useful representation of the intensity of UV light known to have biological importance in all vertebrates. Furthermore, as well as being linked to the erythema action spectrum, it also describes the action spectrum for the synthesis of vitamin D3 in the skin, with 96% overlap between wavelengths of 290 and 320 nm (F. Baines, pers. comm.), which is of direct relevance to amphibians.

Frog basking counts

When approaching to take UVi readings, the number of frogs basking around the pond was counted. The relatively small size of the pond allowed numbers to be counted easily, with a maximum count of seven frogs.

The pond was left undisturbed between readings so that frogs emerged to bask. Frogs were observed to return to basking positions after 5-10 minutes, so hourly intervals provided sufficient time for normal basking behaviour to be manifested.

Statistical analyses

Data was analysed in SPSS 16.0, specific tests are reported alongside results.

RESULTS

Comparison of basking and ambient UVi; evidence for a UV-B microclimate

Fig. 2 shows mean ambient and mean basking-site UV Indices throughout the day. Error bars are present for ambient readings, but as all four readings were identical, they are very small. Ambient UVi was significantly higher than basking-site UVi (Paired t-test; $t=-6.384$, $P<0.001$). The maximum basking UVi never exceeded the ambient UVi (despite the overlap of the 95% confidence intervals at 15:00).

Frog basking counts and UVi at basking sites

The number of frogs basking on basking sites in relation to UVi is shown in Figure 3. Basking behaviour was bi-phasic; numbers of basking frogs peaked first in the morning, between 09:00 and 10:00 while a second, lower, peak in basking behaviour occurred in mid-afternoon (between 14:00 and 15:00). There were very few basking frogs early in the morning, late in the evening and around the solar zenith (13:00, due to summertime hour change).

DISCUSSION

Many amphibians use environmental microclimates within the broader climate of their geographic range. These microclimates include relatively cool and damp downed wood environments used by plethodontid salamanders (Kluber et al., 2009) and relatively warm microclimates sought out by some juvenile bufonid toads (Lillywhite et al., 1973).

This is the first time, to the authors' knowledge, that a UV-B microclimate to which amphibians are subjected has been identified. Our data suggests that *P. lessonae*, despite being 'sun worshipping' amphibians that appear to bask in full sun, in fact use a UV-B microclimate with significantly lower levels of radiation than ambient exposure. This is perhaps surprising, given the apparently open and un-shaded aspect of their wild habitat. Forest or crop cover reduce UV-B penetration (e.g. Mazza et al., 1999; reviewed by Paul & Gwynn-Jones, 2003), so the reduction in UV-B radiation in basking sites compared with the ambient exposure in this study are likely to be the result of the sparse long-stemmed grasses growing around the pond creating shade.

In this study, while basking, *P. lessonae* are exposed to a UVi of between 1.7 and 3.5 (rounded to 1d.p.; the limit of accuracy for the UV Index meter). Through their bi-phasic basking behaviour, pool frogs are exposed to only a relatively narrow portion of the full UVi range of their habitat, being concealed during the most intense periods of UV-B exposure (at the solar zenith, when only one frog was observed basking) and being generally protected by the low-UV-B microclimate around the pond banks. In reptiles, basking behaviour is often driven by thermoregulatory needs (Huey, 1982) and the pool frogs in this study are likely to be doing the same, with UV-B exposure correlated with, but incidental to, thermoregulatory basking behaviour (pers. comm. F. Baines). However, this cannot be tested with this data.

Despite the detection of a low-UVi microclimate used by basking pool frogs, our findings also highlight the fact that at times, these amphibians are exposed to relatively high levels of UV-B radiation. This reinforces the need for further experimentation into the dependence of amphibians on UV-B radiation. It is to be expected that organisms should exploit a free energy resource to their own metabolic ends and, as with reptiles, UV-B radiation may be important for a number of aspects of amphibian wellbeing, perhaps most importantly vitamin D3 synthesis and calcium metabolism.

Although this species of *Pelophylax* is not a conservation or commercial target, several species of this genus living in very similar habitat are threatened, including *P. shquiperica* (Uzzell & Isailovic, 2009), for which *P. lessonae* may act as an analogue. Other species (e.g. *P. bedriagae*) are part of the frog meat and laboratory animal trade, particularly those imported from Turkey (pers. obs. C. Michaels of laboratory animals imported from Turkey to the University of Manchester via the culinary trade). *P. lessonae* is maintained as part of several educational live collections. This data, collected from a readily accessible and common species, may be used to inform the captive husbandry of other water frog species that require ex-situ conservation breeding or are maintained as part of research, educational or commercial enterprises.

However, any application of this data should be made with the caveat that, given the limited scope in both space and time of sampling, there are likely to be limitations to this dataset. Variation in weather conditions from day to day and solar altitude across the year, as well as variation in the frogs' behaviour across time, will all affect the interaction between the frogs and UV-B radiation. There is also likely to be variation in exposure with the geographic origin of this wide-ranging species. Further investigation to elucidate the nature of this variation, the roles of thermoregulation in determining basking behaviour and the physiological effects of UV-B radiation on this species are all warranted.

The authors suggest that this data is therefore used to provide an indication of the level of exposure within the natural range for this species, which can be used in designing basking microclimates within captive enclosures. If and when further data becomes available, captive conditions can be modified to provide even more natural UV-B exposure.

Our data also draws attention to the issue of detecting microclimates in amphibian habitats when designing captive husbandry protocols, rather than relying on meteorological records. In this case, the maximum ambient recorded UVi would provide excessive (relative to exposure recorded here) levels of UV-B radiation if animals were unable to escape it in the confines of captivity, whereas the assumption that amphibians are not tolerant of UV-B radiation at all could lead to under-exposure. Both over- and under-doses of UV-B radiation can have significant health implications for amphibians (Antwis and Browne, 2009).

CONCLUSIONS

P. lessonae, despite being a basking species of amphibian, appears to use a basking microclimate with significantly lower levels of UV-B exposure than the ambient environment. This, combined with the timing of basking activity, means that this species, as well as in all likelihood other *Pelophylax* frogs, is exposed to relatively lower levels of UV-B radiation in comparison to the maximum available amount of UV-B radiation in its habitat.

These findings may inform the design of captive husbandry protocols for *Pelophylax* species and also demonstrate the importance of collecting microclimate, rather than ambient, data when sampling amphibian habitats, including exposure to UV-B radiation.

Further investigation into temporal and spatial variation in UV-B irradiance and in basking behaviour, as well as other factors that may influence this interaction, are required and encouraged.

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