Ecotoxicology and Environmental Safety 143 (2017) 12–18

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Impacts of UVB radiation on food consumption of forest specialist tadpoles

James Eduardo Lago Londero[a,b], Caroline Peripolli dos Santos[a,b], Ana Lúcia Anversa Segatto[a], André Passaglia Schuch[a,b,*]

[a] Department of Biochemistry and Molecular Biology, Federal University of Santa Maria, RS, Brazil
[b] Southern Regional Space Research Center, CRS/INPE-MCTIC, Santa Maria, RS, Brazil

ARTICLE INFO

*Keywords:*
Sunlight
DNA damage
DNA repair
Treefrog
Weight loss
Amphibian decline

ABSTRACT

Solar ultraviolet radiation B (UVB) is an important environmental stressor for amphibian populations due to its genotoxicity, especially in early developmental stages. Nonetheless, there is an absence of works focused on the UVB effects on tadpoles' food consumption efficiency. In this work, we investigated the effects of the exposure to a low environmental-simulated dose of UVB radiation on food consumption of tadpoles of the forest specialist *Hypsiboas curupi* [Hylidae, Anura] species. After UVB treatment tadpoles were divided and exposed to a visible light source or kept in the dark, in order to indirectly evaluate the efficiency of DNA repair performed by photolyases and nucleotide excision repair (NER), respectively. The body mass and the amount of food in tadpoles' guts were verified in both conditions and these data were complemented by the micronuclei frequency in blood cells. Furthermore, the keratinized labial tooth rows were analyzed in order to check for possible UVB-induced damage in this structure. Our results clearly show that the body weight decrease induced by UVB radiation occurs due to the reduction of tadpoles' food consumption. This behavior is directly correlated with the genotoxic impact of UVB light, since the micronuclei frequency significantly increased after treatments. Surprisingly, the results indicate that photoreactivation treatment was ineffective to restore the food consumption activity and body weight values, suggesting a low efficiency of photolyases enzymes in this species. In addition, UVB treatments induced a higher number of breaks in the keratinized labial tooth rows, which could be also associated with the decrease of food consumption. This work contributes to better understand the process of weight loss observed in tadpoles exposed to UVB radiation and emphasizes the susceptibility of forest specialist amphibian species to sunlight-induced genotoxicity.

## 1. Introduction

Amphibians are ectotherms characterized by having permeable exposed skin and eggs that may readily absorb substances from the environment (Blaustein and Belden, 2003). Moreover, many species have complex life cycles that can potentially expose them to both aquatic and terrestrial environmental changes (Blaustein and Belden, 2003). In addition, amphibian populations around the world have been suffering a general decline that culminated in the extinction of several species (Stuart et al., 2004). Several factors may be contributing to this phenomenon, such as climate changes (Foden et al., 2013), the pathogenic fungus *Batrachochytrium dendobatidis* (Carvalho et al., 2017), habitat fragmentation and destruction (Cushman, 2006), release of chemicals (Rissoli et al., 2016), and the introduction of exotic species (Kats and Ferrer, 2003). Besides the agents mentioned above, the increased incidence of ultraviolet (UV) radiation due to stratospheric ozone depletion (Kerr and McElroy, 1993) has been proposed as an important factor for amphibian decline due to its genotoxicity on embryonic and larval stages (Alton et al., 2012; Belden and Blaustein, 2002; Blaustein and Belden, 2003; Blaustein et al., 1994; Schuch et al., 2015a, 2015b).

The UV component of sunlight, which corresponds to ultraviolet B (UVB, 280–315 nm) and ultraviolet A (UVA, 315–400 nm) wavelengths, can induce cell death and mutagenesis as a consequence of DNA lesions induction, known as pyrimidine dimers (Pfeifer et al., 2005; Schuch and Menck, 2010; Schuch et al., 2015b). The most frequent DNA lesions induced by UVB radiation are the cyclobutane pyrimidine dimers (CPDs), which constitute ~80–90% of the photoproducts, and pyrimidine-pyrimidone (6-4) photoproducts (6,4PPs), which account for the 10–20% of the UVB lesions (Sancar, 2008). The genotoxicity induced by UVB radiation has been shown to be very detrimental to amphibian species (Schuch et al., 2015a, 2015b).

*Abbreviations:* UV, ultraviolet radiation; UVA, ultraviolet radiation A; UVB, ultraviolet radiation B; UVC, ultraviolet radiation C; CPDs, cyclobutane pyrimidine dimers; 6-4PPs, pyrimidine (6-4) pyrimidone photoproducts; TSP, Turvo State Park; NER, nucleotide excision repair

* Corresponding author at: Federal University of Santa Maria, Av. Roraima, 1000, P.O. Box 5021, room 3010, Camobi, Santa Maria, RS 97110-970, Brazil.
*E-mail address:* schuchap@gmail.com (A. Passaglia Schuch).

http://dx.doi.org/10.1016/j.ecoenv.2017.05.002
Received 19 January 2017; Received in revised form 28 April 2017; Accepted 2 May 2017
Available online 06 May 2017
0147-6513/ 

According to UV-sensitivity hypothesis, the declining amphibian species have lower resistance to UV radiation due to the lower capacity to repair the UV-induced DNA damage (Blaustein et al., 1999, 1994). The DNA damage induced by UV radiation can be repaired by enzymes known as photolyases that use visible/UVA light as an energy source to reverse the DNA damage in an error free process called photoreactivation (Blaustein et al., 1994; Friedberg, 2003). In addition to photolyases, the nucleotide excision repair (NER) pathway can restore the UV-induced DNA damage in a process that needs several proteins and ATP consumption (Sancar and Tang, 1993). However, the role of DNA repair pathways in amphibian decline is still a matter of discussion (Blaustein et al., 1999; Smith et al., 2000; Thurman et al., 2014; Schuch et al., 2015b).

Previous published studies regarding the amphibian foraging behavior were focused on the impact of predation risk, quantity and quality of food (Babbitt, 2001; Eklov and Halvarsson, 2000; Kupferberg, 1997), importance of well-maintained labial tooth row for the feeding knematics (Venesky et al., 2013, 2010b), as well as on the impacts of *Batrachochytrium dendrobatidis* infection on tadpole oral disc (Smith and Weldon, 2007) and foraging efficiency (Venesky et al., 2009). Despite the well documented effects of UVB light on tadpoles' weight (Belden and Blaustein, 2002; Lipinski et al., 2016; Schuch et al., 2015a), there is an absence of works focused on the UVB effects on foraging efficiency, which can have an important role in the tadpoles' weight loss process. Therefore, considering that the UVB radiation reduces tadpoles' normal activity (Alton et al., 2012), and the reduced foraging behavior causes tadpoles to consume less food (Anholt et al., 1996; Skelly, 1994), here we hypothesized that UVB exposure leads to weight loss due to the decrease of food consumption. Furthermore, we also evaluated if the food consumption decrease is a consequence of the UVB-induced genomic instability. Additionally, considering the fact that UVB radiation can alter keratin structures (Biniek et al., 2012), it becomes necessary to evaluate if UVB exposure can disturb tadpoles' food consumption activity due to its impact on the keratinized mouthparts (Venesky et al., 2010b).

In this work we chose the treefrog *Hypsiboas curupi* [Hylidae, Anura] as a model species to evaluate the effects of UVB radiation on tadpoles' food consumption and body weight. This treefrog species is restricted to highly forested areas in the Southern Atlantic Rainforest (Iop et al., 2011), which is part of the Brazilian Atlantic Rainforest biodiversity hotspot (Myers et al., 2000). However, this environment has being severely fragmented during the last century (ICMbio, 2012; SEMA, 2014), and many species, including *H. curupi*, are currently present in both national and state list of endangered species (ICMbio, 2012; SEMA, 2014). After exposures of *H. curupi* tadpoles to a low solar-simulated UVB radiation dose, the food consumption and the total body mass were evaluated. Furthermore, the genotoxic effect of the UVB treatment was assessed through the quantification of micronucleus formation in collected blood samples. In addition, the impact of UVB exposure on tadpoles' keratinized labial tooth rows was evaluated. In all the experiments the results obtained with tadpoles kept in the dark after UVB treatment or with tadpoles exposed to a photoreactivation treatment were compared to evaluate the efficiency of DNA repair pathways to restore UVB-induced DNA damage. The obtained results clearly demonstrate the severe impact of UVB treatment on this endangered treefrog species, as well as the importance of future studies aiming to assess the impact of increased levels of solar UVB radiation on declining forest specialist species of the Hylidae family.

## 2. Materials and methods

### 2.1. Animal collection and maintenance

Four freshly laid egg masses of *H. curupi* were collected in a stream at the Turvo State Park (TSP) (27°07'–27°16'S, 53°48'–54°04'W), which is a remaining fragment of the Southern Atlantic Rainforest (SEMA, 2005). The egg masses were packed in plastic sac half filled with air and water from the stream and transported to the laboratory at Federal University of Santa Maria. The collected egg masses were put together in the same plastic tank and kept aerated until the beginning of hatching. The recently hatched tadpoles were then transferred to individual plastic tanks (one tadpole per plastic tank of 14 cm of diameter, 9 cm of height and 42 cm of circumference) filled with dechlorinated water (19 ± 2 °C) to a minimum depth of 55 mm (between 55 and 70 mm). The total volume of water was changed every two days and tadpoles were fed with boiled spinach ad libitum. Tadpoles were maintained under these conditions for about 20–30 days prior to the beginning of the experiments.

### 2.2. 48-h fasting period, UVB irradiation, photoreactivation treatment and food availability time

Before the beginning of experiments, groups of ten tadpoles (Gosner stage 25–26; Gosner, 1960) were selected based on their similarity in total body length (cm) and mass (g) because we noticed that there was variability regarding these measures inside the same stage. These analyses were made using a stereomicroscope with 40× magnification (Nova Optical Systems, Brazil), an analytical balance (Shimadzu BL3200H, Japan), and a digital 0.01 mm precision caliper rule (Serie 500, Mitutoyo, Brazil). A total of 130 tadpoles were selected for this work (keeping one tadpole per plastic tank). The selected individuals firstly remained in a 48-h fasting period in order to standardize the amount of food in their guts. To prevent tadpoles to use their own feces as a food source, plastic mesh nets were submerged in the water and tadpoles were placed above the nets.

After fasting, tadpoles were divided in two groups: non-irradiated control tadpoles (70 individuals) and UVB-irradiated tadpoles (60 individuals). The UVB dose used in the experiments was 2.0 kJ/m$^2$ and this simulate 15 min of natural sunlight exposure (12:00–12:15) on a clear sky summer day on a stream located in a deforested area at the TSP (Lipinski et al., 2016). All irradiation was performed using the same 15 W UVB lamp (T15M, Vilber Lourmat, France) filtered with a polycarbonate sheet to block UVC wavelengths. The dose rates of the UVB lamp was 5.9 J/m$^2$/s and the exposure time to achieve the desired UVB dose was 5 min and 40 s. The UVB dose applied in this work was established through the use of a portable radiometer (UV Monitor MS-211-1, EKO Instruments, Japan). The amount of UVC contamination for the UVB lamp was below the detection limit of the equipment. The spectral characteristic of the UVB lamp was previously presented by Schuch et al. (2015b).

Immediately after UVB exposures, 30 tadpoles were subjected to 3 h of photoreactivation to activate the photolyase enzymes (group UVB L). Photoreactivation was performed by using two 40 W fluorescent lamps (General Electric, Brazil) at a distance of 40 cm from tadpoles. The spectral characteristic of the fluorescent lamps was previously presented by Schuch et al. (2015b). These tadpoles were then maintained in transparent plastic tanks and exposed to a constant photoperiod (12 h light:12 h dark) until the end of the experiment. In parallel, 30 UVB-exposed tadpoles were kept in the dark (each plastic tank was wrapped with aluminum foil) until the end of the experimental period (group UVB D). The same photoreactivation treatment was performed with 40 tadpoles of the non-irradiated control group (group CTL L), while 30 tadpoles from the non-irradiated group were also kept in the dark (group CTL D). Immediately after UVB irradiation, all tadpoles were fed with boiled spinach ad libitum for 3, 24 or 48 h.

### 2.3. Analysis of the impact of UVB exposure on tadpoles' total body mass and on micronuclei frequency in blood samples

In this experiment, the same tadpole was weighed in three different periods: before fasting (first measurement), after 48 h of fasting (second measurement), and after adding food ad libitum at three different times