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An UV-sensitive anuran species as an indicator of environmental quality of the Southern Atlantic Rainforest

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A B S T R A C T

The Southern Atlantic rainforest is continuously suffering from wood extraction activity, which results in the increase of clearings within the forest. Although the direct impacts of deforestation on landscape are already well described, there is an absence of studies focused on the evaluation of its indirect effects, such as the increase of solar UV radiation levels inside forest environment and its consequences for forest specialist anuran species. The results presented in this work clearly show that the threatened tree frog species *Hypsiboas curupi* presents severe traits of sensitivity to UV wavelengths of sunlight, making it a vulnerable species to this environmental stressor, as well as a biological indicator of the quality of forest canopy coverage. In addition, the measurement of solar UVB and UVA radiation incidence upon *H. curupi* breeding site and the analyses of a 20-year dataset of satellite images regarding the management of canopy coverage indicate that the photoprotection provided by trees of the Southern Atlantic rainforest is critical for the conservation of this forest specialist anuran species. Therefore, this work demonstrates that the deforestation process enhances the exposure of *H. curupi* embryos to solar UVB and UVA radiation, negatively affecting their embryonic development, inducing mortality and population decline.

1. Introduction

Since the first reports of the global phenomenon of amphibian decline, many species have become extinct or have faced reduction in their natural distribution ranges [1,2]. Among several factors related to this process, forest canopy is historically under severe anthropogenic pressure [3,4]. Canopy removal has been shown to have long-term negative effects on amphibian abundance, with some populations taking two to seven decades post-harvesting to reach pre-disturbance levels [5–8]. In addition, it has been also demonstrated that forest specialist amphibian species decline in abundance after forest disturbance while most habitat generalist species are not affected by canopy removal, thereby indicating a shift in the relative abundance towards habitat generalists [9].

Similar observations were also obtained at the Brazilian Atlantic Rainforest. This biome has lost 88% of its original area [10], and it is suggested that approximately 30% of endemic anuran species are facing decline due the suppression/change of the native forest by anthropogenic activities [11,12]. More specifically, the southernmost region of this forest has been facing a gradual deforestation since 1910, which resulted in the increase of open areas within the forest [13]. Consequently, in 1947 the government of the Rio Grande do Sul state created the Turvo State Park (TSP), which is a conservation unit that covers >17,000 ha of the Southern Atlantic rainforest [14]. However, the management plan of the park was incipient until the beginning of the year 2000, which prompted the state government to apply new conservation measures that began to be strictly implemented in this area [14].

An important consequence of canopy removal in Brazilian territory is the incidence of very high levels of solar UV radiation inside this environment. Indeed, UVB radiation has a major role in the occurrence of amphibian population decline due to its genotoxic impact during the embryonic and larval stages [15–18]. On the other hand, amphibian photolyases play the main role in the repair of DNA damage induced by sunlight, such as the cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine-pyrimidone photoproducts (6-4PPs) [19–22]. However, it has been recently shown that tree frog tadpoles present low efficacy in repairing both CPDs and 6-4PPs induced by UVB and UVA radiation, which resulted in the generation of serious biological effects on the development of these organisms (i.e., changes on body mass and total length, as well as induction of malformation after metamorphosis and mortality) [23]. Furthermore, it is predicted that anuran species whose eggs are naturally highly exposed to sunlight have higher enzymatic activity than species whose eggs are naturally protected [24].

Despite the wide knowledge in the field regarding the effects of forest harvesting on the landscape, there is an absence of studies showing

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its impact on the increase of solar UV radiation incidence inside forest environment, as well as its biological consequences to forest specialist anuran species. In the present work, we measured the influence of deforestation on the solar UVB (280–315 nm) and UVA (315–400 nm) radiation incidence upon breeding sites of the tree frog species *Hypsiboas curupi*. This species was chosen as a model because it is currently restricted to well conserved (highly forested) portions of streams inside the Southern Atlantic rainforest [25]. Despite *H. curupi* is listed as least concern at IUCN list, both national and state lists consider it as an 'endangered' species because of its very fragmented distribution on portions of Southern Atlantic rainforest with anthropogenic pressure, which are generally located outside conservation units [26,27]. Therefore, this species was recently included as a target-species on the National Action Plan for the Conservation of Southern Brazil's Herpetofauna, thus requiring more scientific studies focused on its biology and ecology [27].

The detrimental effects of solar-simulated UV doses were addressed in terms of hatching success, developmental rates, mortality, and malformation induction in embryos and newly hatched tadpoles. Additionally, the management of the canopy coverage over the TSP's area was also evaluated through the analyses of 20 years of satellite images. By comparing the biological data with UV light incidence and satellite images, we demonstrate the importance of conservation politics focused on maintaining this biome in order to avoid the severe biological effects of sunlight upon UV-sensitive populations of this endangered amphibian species.

2. Material and Methods

2.1. Solar UVB and UVA Radiation Measurement

Daily incidence of UVB and UVA radiation was measured during the entire daylight period (6:30 am to 7:00 pm, local time) at the breeding site of the threatened tree frog species *Hypsiboas curupi* (on the edge of a stream inside of a highly conserved forested area), as well as in a converted area (deforested area) of the same stream, both inside the Turvo State Park (TSP) domain, Rio Grande do Sul, Brazil (27°07′–27°16′S, 53°48′–54°04′W), which is the largest remaining fragment of Mesophytic Semideciduous Forest of the state [14]. These measurements were performed at one clear sky summer day (at February 2014 between day 11 and day 26) in each area through the use of a portable radiometer (EKO UV Monitor MS-211-1, Japan).

2.2. Animal Collection and Maintenance

Despite the fact that *H. curupi* is listed as an 'endangered' species on both national and state lists, we also decided to choose this species to link the effects of solar UV radiation with deforestation process because of the fact that the females deposit the egg masses floating on the water surface (above the water surface) at shallow streams, usually attached to rocks or floating branches (this is called Reproductive mode 2 [28–30]). Four freshly laid egg masses of *H. curupi* were collected in the early morning. The egg masses were packed in plastic sacs half filled with air and water from stream to avoid physical shocks during the transportation to the laboratory. Then, the embryos were counted and, immediately, the viable individuals were randomly separated and placed individually into 2.0 ml microtubes. The total amount of microtubes containing one viable embryo formed 9 groups with 90 embryos each in order to perform three independent exposures of 30 individuals to each UVB and UVA dose applied in this work, as well as to compose the non-irradiated control groups.

2.3. UVB and UVA Irradiation Treatments

Each replicate containing 30 embryos were placed together in Petri dishes at a distance about 30 cm from the light sources, and irradiated independently with increasing UVB (0.5, 1.0, 2.0, and 4.0 kJ/m^2) and UVA (25, 50, 100, and 200 kJ/m^2) doses. In parallel to UV irradiation, each replicate of 30 embryos of the non-irradiated control were also placed in a Petri dish filled with dechlorinated water (19° ± 1 °C) and kept under the ambient light until the end of UV treatments (for 36 min). UVB exposures were performed with a Vilber Lourmat T15 M 15 W lamp (Vilber Lourmat, France) filtered with a polycarbonate sheet to block UVC wavelengths. For UVA, tadpoles were irradiated using an Osram Ultramed FDA KY10s 1000 W lamp filtered with Schott BG39 glass filter, 3 mm thickness (Schott Glass, Germany). During irradiation, the water temperature measured under both UV-filters was 20 °C (±1 °C), and the amount of UVC contamination for the UVB lamp as well as UVC and UVB contamination for the UVA lamp was below the detection limit. The highest UV doses applied in this work correspond to 10% of daily solar UVB and UVA doses measured at the converted area, and the exposure times to achieve these energies were 17 min for the UVB and 36 min for the UVA lamps.

2.4. Effects Induced by UV Doses on Hatching, Survival, and Morphology of *H. curupi*

After UV treatments, all embryos (including controls) were again separated and placed individually in 2.0 ml microtubes filled with dechlorinated water (19° ± 1 °C) until the time of hatching. The eggs were analyzed daily to evaluate the success of hatching among non-irradiated controls and UV-exposed embryos. After the period of 96 h, the non-hatched individuals were considered dead. This period was defined a posteriori since all non-irradiated control embryos hatched within 96 h and no UV-treated embryos could hatch after this period. After hatching, the mass of each tadpole was measured with an analytical balance (Radwag WTB 2000, Poland), and all tadpoles from the non-irradiated control group and from each UV treatment were placed together in their respective tank (according to the treatment) containing 1 l of dechlorinated water (19° ± 1 °C). The water volume was replaced every day to maintain the water quality and tadpoles were fed with boiled spinach ad libitum. The newly hatched tadpoles were also monitored for an additional period of ten days (240 h) with the purpose of obtaining the mortality rates (number of dead tadpoles during this period), and the occurrence of malformations due to UVB or UVA exposures (Nova Optical Systems stereomicroscope with 40× magnification, Brazil). To avoid human contact with the eggs and tadpoles, we used an adapted siphon to split and move the eggs to the microtubes, as well as to move the newly hatched tadpoles from the microtubes to the plastic tanks.

2.5. Analyses of a 20 years Dataset of Satellite Images for the Evaluation of Forest Canopy Over the TSP's Area

Satellite images were used to investigate the changes on forest canopy during the period of 1990 to 2010 over the TSP's area. These images were georeferenced for a posterior classification to separate, in distinct colors, the deforested areas (red spots) from the preserved areas (green), in order to calculate the total size of both areas in each decade separately (1990–2000; 2000–2010). The result is presented as the percentage of preserved area in each decade. All images and dataset were obtained from the LANDSAT 5 satellite (TM sensor) from the National Institute of Space Research (INPE, Brazil). The analyses of these images were performed through the use of the ArcGis 10.1 software (Esri, USA).

2.6. Statistical Analysis

For the experiments of success of hatching we used the number of hatched tadpoles (expressed as the average number of hatched tadpole of three independent experiments) as the response variable, and the exposures to UVB or UVA doses, as well as the time of each analysis (24, 48, 72, and 96 h) as predictors.