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Identification of influential events concerning the Antarctic ozone hole over southern Brazil and the biological effects induced by UVB and UVA radiation in an endemic treefrog species

André Passaglia Schuch [a,b], Mauricio Beux dos Santos [a], Victor Mendes Lipinski [a], Lucas Vaz Peres [a,b], Caroline Peripolli dos Santos [a], Sonia Zanini Cechin [a], Nelson Jorge Schuch [b], Damaris Kirsh Pinheiro [a,b], Elgion Lúcio da Silva Loreto [a,*]

[a] *Federal University of Santa Maria, RS, Brazil*
[b] *Southern Regional Space Research Center, CRS/INPE-MCTI, Santa Maria, RS, Brazil*

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ABSTRACT

The increased incidence of solar ultraviolet radiation (UV) due to ozone depletion has been affecting both terrestrial and aquatic ecosystems and it may help to explain the enigmatic decline of amphibian populations in specific localities. In this work, influential events concerning the Antarctic ozone hole were identified in a dataset containing 35 years of ozone measurements over southern Brazil. The effects of environmental doses of UVB and UVA radiation were addressed on the morphology and development of *Hypsiboas pulchellus* tadpole (Anura: Hylidae), as well as on the induction of malformation after the conclusion of metamorphosis. These analyzes were complemented by the detection of micronucleus formation in blood cells. 72 ozone depletion events were identified from 1979 to 2013. Surprisingly, their yearly frequency increased three-fold during the last 17 years. The results clearly show that *H. pulchellus* tadpole are much more sensitive to UVB than UVA light, which reduces their survival and developmental rates. Additionally, the rates of micronucleus formation by UVB were considerably higher compared to UVA even after the activation of photolyases enzymes by a further photoreactivation treatment. Consequently, a higher occurrence of malformation was observed in UVB-irradiated individuals. These results demonstrate the severe genotoxic impact of UVB radiation on this treefrog species and its importance for further studies aimed to assess the impact of the increased levels of solar UVB radiation on declining species of the Hylidae family.

1. Introduction

Recently, amphibian populations have suffered a widespread decline resulting in the extirpation of many species (Mendelson et al., 2006; Stuart et al., 2004). Ever since amphibian declines were recognized as a global problem, several causes for the phenomenon have been suggested (Alford, 2011). Numerous stressors contribute to these losses, such as habitat fragmentation and destruction (Becker et al., 2007; Hof et al., 2011), exposure to chemicals (Chang et al., 2009; Wolkowicz et al., 2013) or pesticides (Brühl et al., 2013; Rohr et al., 2008), the impact of climate changes (Foden et al., 2013), and the pathogenic fungus *Batrachochytrium dendobatidis* that causes the disease chytridiomycosis (Berger et al., 1998; Lips et al., 2006) the occurrence of which is influenced by global warming and climate changes (Pounds et al., 2006; Rohr and Raffel, 2010).

However, the occurrence of rapid ozone depletion and ensuing global amphibian declines has led to intense investigation of the effects of increased solar UVB radiation (280–315 nm) on amphibians. Indeed, UVB radiation has a major role in the occurrence of malformations and mortality, mainly due to its genotoxic action during the embryonic and larval stages (Blaustein et al., 1997; Hader et al., 2007; Kiesecker et al., 2004; Roy, 2002). The main cellular consequences induced by UVB light are mutagenesis and cytotoxicity and these are directly associated to the induction of large distortion in DNA double helix, known as cyclobutane

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; TOMS, Total Ozone Mapping Spectrometer; OMI, Ozone Monitoring Instrument; DS, Direct to the Sun; HYSPLIT, HYbrid Single-Particle Lagrangian Integrated Trajectory; NOAA, National Oceanic and Atmospheric Administration

* Correspondence to: Av. Roraima, 1000, Building 16-A, Room 3211, Camobi, Santa Maria, RS 97105-900, Brazil. Fax: +55 55 3220 8020.
E-mail address: elgion@base.ufsm.br (E.L. da Silva Loreto).

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pyrimidine dimers (CPDs) and pyrimidine (6–4) pyrimidone photoproducts (6–4PPs) (see review Schuch et al., 2013). Furthermore, exposure to UVB radiation can cause spinal curvature in tadpole, reduced growth and development rates, delayed metamorphosis, reduced locomotive ability, behavioral changes, and susceptibility to disease and predation (Alton et al., 2010, 2011, 2012; Ankley et al., 2000; Belden and Blaustein, 2002; Croteau et al., 2008a, 2008b; Fite et al., 1998; Flamarique et al., 2000; Hays et al., 1996; Kiesecker et al., 2004; Kiesecker and Blaustein, 1995).

Thus, the discovery of the Antarctic ozone hole in the 1980s has caused grave concern worldwide. Although the hole is located over the polar region, it can also influence the ozone content over southern South America during spring season increasing UVB incidence at the surface of the mid-latitudes (de Laat et al., 2010; Kirchhoff et al., 1997; Pazmiño et al., 2005). It has been already demonstrated that polar air masses with low ozone content can disturb the stratospheric ozone concentration in the South of Brazil (Kirchhoff et al., 1996), where a 1% depletion in ozone content results in an average increase of 1.2% in UVB incidence (Guarnieri et al., 2004). Additionally, this same atmospheric phenomenon has already been observed over other mid latitude regions, such as New Zealand (Brinksma et al., 1998) and South Africa (Semane et al., 2006).

However, despite growing knowledge in the field regarding the UVB-induced biological effects in amphibians, there are only few published works concerning its genotoxicty (Blaustein et al., 1994, 1999; Pandelova et al., 2006; Smith et al., 2000; Thurman et al., 2014). Consequently, although the UV-sensitivity hypothesis postulates that the lower resistance presented by declining amphibian species is linked to DNA repair capacity (Blaustein et al., 1994, 1999), the role of DNA repair pathways, specially photolyases activity, in amphibian decline is still a matter for discussion because other studies did not find any difference in photolyase activity among the studied species (Blaustein et al., 1999; Smith et al., 2000; Thurman et al., 2014). Thus, much work is still required in order to show the role of DNA repair activity on the decline of amphibian species, as well as to assess the effects of environmental genotoxic agents on species-specific population decreases (Pandelova et al., 2006). Furthermore, it is important to emphasize that there is an absence of studies showing the genotoxic impact of UVA radiation on these animals, as well as their efficacy in repairing UVA-induced DNA damage and preventing chromosomal breaks.

Considering that the absorption of DNA is rather weak in the less energetic, long-wavelength UV range, it is evident that UVA radiation is far less efficient in producing direct photolesions than UVB (Yagura et al., 2011). Therefore, UVA radiation can indirectly damage DNA after the absorption of its photons by other chromophores, thereby generating reactive oxygen species (ROS) and inducing oxidized bases, such as 8-oxo-7,8-dehydroguanine (8-oxoGua, which is considered a marker of oxidative DNA damage) (Cadet et al., 2005; Kuluncsics et al., 1999; Piette et al., 1986; Schuch and Menck, 2010). In human cells, it is well known that the oxidative stress induced by UVA radiation can also damage other cellular structures and molecules, such as lipids (through lipid peroxidation that results in membrane destabilization) and proteins (the oxidized proteins can induce cross-links with DNA bases blocking the replication and transcription processes) (Blair, 2008; Girard et al., 2008; Hoerter et al., 2008; Moysan et al., 1993). Despite oxidatively generated DNA damage being more effectively induced by UVA than by UVB, studies indicate that UVA radiation also induces CPDs directly on DNA more readily than oxidized bases, suggesting that UVA may be more mutagenic than previously assumed (Jiang et al., 2009; Schuch et al., 2009). Additionally, although UVA absorption by DNA is weak, the presence of 6–4PPs in purified DNA samples after irradiation with UVA light has been demonstrated (Cortat et al., 2013; Schuch et al., 2009). The DNA repair of UVA-induced DNA damage occurs through three main mechanisms: (1) Photoreactivation, which involves the direct monomerization of CPDs and 6–4PPs by the action of photolyases enzymes in the presence of UVA/visible light; (2) Nucleotide Excision Repair (NER) which is critically important in the repair of UV-induced DNA lesion and is one of the most versatile and flexible repair systems found in most organisms; and (3) Base Excision Repair (BER) which acts on small DNA lesions such as oxidized bases, abasic sites and DNA single-strand breaks (Cadet et al., 2005; Rastogi et al., 2010).

Several experiments have been conducting to assess UV-induced genotoxicity in aquatic and terrestrial organisms. Among them, the micronucleus test (MN) has been widely used in several models, as well as in human patients (Calo and Marabini, 2014; Groff et al., 2010; Najafzadeh et al., 2012; Reus et al., 2012). However, amphibian models have been usually employed to evaluate the ecotoxicity of chemicals presented in the environment, such as those used in agriculture (Bosch et al., 2011; Cabagna et al., 2006; Feng et al., 2004; Vera et al., 2010). Micronuclei are chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division in cells of any actively dividing tissue (Fenech, 2007). Thus, considering that the increasing frequency of MN is a biomarker of the genotoxic effect at the cellular level and a response of chromosomal damage, the investigation of MN frequency in peripheral blood of anuran tadpole is of paramount importance for the investigation of genotoxic effects induced by solar UVB and UVA radiation.

In this work, we first evaluated the occurrence of influential events concerning the Antarctic ozone hole over southern Brazil (29°43′S) during a 35-year period (1979–2013) with the purpose to characterize the occurrence of this stratospheric phenomenon over the studied locality. Subsequently, the biological impact of solar UVB and UVA radiation on the endemic treefrog species *Hypsiboas pulchellus* was demonstrated through the investigation of the effects induced by these UV wavelengths on morphology and development concerning the activation of photolyases enzymes by an additional photoreactivation treatment. These approaches were complemented with the quantification of micronucleus formation in collected blood samples. The choice of this species is justified because they are relatively abundant in southern Brazil, tadpole are easy to identify, and they are relatively easy to maintain in the laboratory. Additionally, there is an absence of published studies focused on the impact of solar UV radiation on this anuran species, which belongs to the Hylidae family that is one of the four principal families of rapidly declining species worldwide (Stuart et al., 2004).

By comparing these physical and biological data, here we demonstrate the importance of the photorepair system for the maintenance of genomic integrity, as well as the vulnerability of this amphibian species to deal with the hazards of exposure to increased doses of solar UVB radiation.

2. Materials and methods

2.1. Identification of influential events regarding the Antarctic ozone hole over southern Brazil from 1979 to 2013

The measurement of ozone concentration over southern Brazil was conducted by obtaining daily average data of total ozone content with a Brewer Spectrophotometer (Kipp & Zonen, The Netherlands) (model MKIV#081 from 1992–2000, model MKII#056 from 2000–2002, and model MKIII #167 from 2002 to date), installed at the Southern Space Observatory (SSO/CRS/INPE-MCTI, 29.4°S, 53.8°W). Complementing the analyzes performed by