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Evaluation of the seasonal variation of parameters of oxidative status of *Tropidurus catalanensis* Gudynas and Skuk, 1983

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Abstract. Markers of oxidative status may become important tools in conservation biology as a means of evaluating survival and reproductive expectations in organisms, as well as to elucidate the effects of anthropogenic impacts on populations living in their natural environments. The objective of the present study was to evaluate seasonal variations in oxidative status in a population of *Tropidurus catalanensis* through the analysis of lipid peroxidation levels and quantification of activity of the antioxidant enzymes superoxide dismutase, catalase, and glutathione S-transferase in liver, kidneys, and tail muscle. This lizard inhabits southern Brazil, in the Pampas grasslands. A total of 73 animals were collected over a 1-year period through active search and hand capture, separated into males and females, euthanized with ketamine hydrochloride, and frozen. A detailed approach on the variation of climatic variables over the seasons is also provided in order to subsidize a better comprehension of seasonal variation in oxidative stress biomarkers of *T. catalanensis.* Results obtained reveal that this lizard exhibits a clear pattern of seasonal variation in redox balance markers across different tissues. Antioxidant system of these animals is highly efficient, being able to maintain constant levels of lipid peroxidation in liver and muscle in males and in all tissues studied in females. In the renal tissue, an increase in lipid peroxidation was found only in males in spring. The enzymatic antioxidant defense system studied herein exhibited tissue and sex-specific responsiveness, and was possibly modulated by factors both abiotic (e.g., photoperiod, temperature, radiation, precipitation) and biotic (e.g., reproductive cycle, nutritional status, type of food consumed).

Keywords. Lizard; Kidney; Liver; Lipid peroxidation; Natural environment; Oxidative stress; Tail muscle.

Resumo. Marcadores do *status* oxidativo podem se tornar ferramentas importantes na biologia da conservação, tanto como um meio para avaliar a sobrevivência e as expectativas reprodutivas nos organismos como para elucidar os efeitos dos impactos antropogênicos sobre as populações em seus ambientes naturais. O objetivo do presente estudo foi avaliar as variações sazonais no *status* oxidativo de uma população de *Tropidurus catalanensis* por meio da análise dos níveis de peroxidação lipídica e quantificação da atividade das enzimas antioxidantes superóxido dismutase, catalase e glutationa S-transferase no fígado, rins e músculo caudal. Este lagarto habita o sul do Brasil, nas pastagens dos Pampas. Para tal, foram coletados 73 animais, durante um período de um ano, por meio de busca ativa e captura manual; eles foram separados por sexo, eutanasiados com cloridrato de cetamina e congelados. Uma abordagem detalhada sobre a variação das variáveis climáticas ao longo das estações do ano também é fornecida, a fim de subsidiar uma melhor compreensão da variação sazonal em biomarcadores de estresse oxidativo de *T. catalanensis.* Os resultados obtidos revelam que este lagarto exibe um padrão claro de variação sazonal nos marcadores do balanço oxidativo tanto para os diferentes tecidos como para os sexos. O sistema antioxidante desses animais é altamente eficiente, sendo capaz de manter níveis constantes de peroxidação lipídica no fígado e no músculo em machos e em todos os tecidos estudados em fêmeas. No tecido renal, um aumento na peroxidação lipídica foi encontrado apenas nos machos na primavera. Estas respostas foram, possivelmente, moduladas por fatores abióticos (por exemplo, fotoperíodo, temperatura, precipitação, radiação) e bióticos (por exemplo, ciclo reprodutivo, estado nutricional, tipo de alimento consumido).

INTRODUCTION

It is well known that oxidative status can have significant impacts on biological performance. Oxidative status might not only reflect the environmental conditions to which an animal is exposed, but also influence its future odds of reproduction and survival in its natural habitat (Beaulieu and Costantini, 2014). However, the survival of various species has been drastically threatened by several other disturbances in addition to natural variations in the life cycle, including habitat loss and fragmentation, overexploitation, invasive species, disease, chemical compounds introduced into the environment, and climate change (Carey, 2005; McNab, 2006; Brook et al., 2008; Beaulieu and Costantini, 2014). These disturbances lead to altered homeostasis, thus triggering different types of stress, including oxidative stress.

The imbalance in redox status often caused by exposure to contaminants can lead to oxidative stress, cell apoptosis, mutagenesis, and carcinogenesis, among other changes, including different pathological alterations (Sies et al., 1992; Jones, 2006). Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and host antioxidant defenses (Jones, 2006). This imbalance is known to cause damage to all types of biomolecules, including proteins, lipids, and nucleic acids

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(Halliwell and Gutteridge, 2008). Chronic stress can cause tissue damage and metabolic dysfunction, thus leading to cell death and/or to death of the entire organism (Costantini et al., 2009). To deal with ROS, the cells of aerobic organisms rely on a system of antioxidant defenses, which include enzymes such as superoxide dismutase, catalase, peroxiredoxins, glutathione reductase, and glutathione peroxidase. These enzymes are able to neutralize excess ROS production and prevent damage to the cell structure (Costa et al., 2008; Dowling and Simmons, 2009; Righetti et al., 2014). Glutathione S-transferase exerts a secondary antioxidant role of clearing toxic byproducts of lipid peroxidation, and it is involved in the endobiotic and xenobiotic metabolism (Hermes-Lima and Storey, 1993).

A non-enzymatic defense system also exists, comprising a wide range of small-molecule compounds such as glutathione, ascorbic acid, tocopherol, various selenium compounds, ubiquinones (coenzyme Q), uric acid, α-lipoic acid, zinc, taurines, hypotaurines, β-carotene, and carotene. The effects of this system are twofold: to remove noxious agents before they can cause harm or to repair injury after it has occurred (Andrade et al., 2010; Zanette et al., 2015).

Studies of oxidative stress have focused mostly on mammals and birds, with comparatively less attention given to reptiles, although the sensitivity of the latter to environmental degradation factors is well established (Campbell and Campbell, 2002; Costantini et al., 2009). Studies have reported that certain natural events might induce production of ROS and trigger oxidative stress over the course of the life cycle of different reptile species, such as in *Caiman yacare* (Daudin, 1802) individuals at different stages of the life cycle (mature adult males, young adult males, ten pups, and five embryos); during diving in *Chelonia mydas agassizii* (Linnaeus, 1758) (Valdivia et al., 2007); during anoxia and freezing in *Thamnophis sirtalis parietalis* (Say, 1823) and *Lacerta vivipara* (Laurenti, 1768); and during hibernation in *Salvator merianae* (Duméril and Bibron, 1839) (Hermes-Lima and Storey, 1993; Voituron et al., 2006; Moreira et al., 2018).

The species used in the present study, *Tropidurus catalanensis* (Gudynas and Skuk, 1983), is a lizard of the family Tropiduridae Bell, 1843. It is found in open continental areas and its populations are large across its range (Kunz and Borges-Martins, 2013). They are particularly present on rocky outcrops in the Pampas grasslands biome; according to Carvalho et al. (2013), the incidence of the species in this environment might be explained by its dispersal ability and ecological plasticity. Adults of this lizard consume moderate amounts of plant fiber and follow a little-varied, invertebrate-based carnivorous diet composed primarily of arthropods, showing preference for Hymenoptera (Carvalho et al., 2007; Rosa, 2015). They are diurnal, territorial, and exhibit sit-and-wait predation behavior (Carvalho et al., 2007; Arruda, 2009; Vieira et al., 2011). The reproductive period of the species begins in September and ends in January (Arruda, 2009).

Results obtained by Oliveira et al. (2018) show that males and females of *Tropidurus catalanensis* exhibit a clear pattern of seasonal variation in their metabolism, as reflected mainly in levels of total protein, liver glycogen, total lipids, and triglycerides, blood glucose and lipid, and liver protein levels in females. It should be noted that the body condition indices (hepatosomatic, gonadosomatic, and abdominal fat index) varied throughout the year. The same authors state that the winter months seem to trigger a reduction in overall metabolism, most intensely in males. The reproductive period seems to be the biological event that demands greater energy, leading to the mobilization of energy stocks mainly in females.

External environmental factors such as temperature, photoperiod, and type and availability of food have a profound impact on the metabolism of reptiles that is possibly reflected in the antioxidant system. Not much information is available on free radical metabolism in reptiles in general (Chainy et al., 2016). It is, therefore, imperative to differentiate variations in biomarkers of oxidative stress due to natural events of the life cycle from those due to environmental disruptions. The present study sought to elucidate seasonal variations in oxidative status in a *Tropidurus catalanensis* population by analyzing markers of lipid peroxidation (LPO) and quantifying the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) in the liver, kidneys, and tail muscle of males and females. To facilitate comprehension of seasonal variation in oxidative stress biomarkers, a detailed consideration of the variation of climatic variables over the seasons is also provided.

MATERIALS AND METHODS

Capture and study area

Specimens of *Tropidurus catalanensis* were collected monthly from September 2013 to September 2014 for a total of 13 collections. The data from each monthly collection were grouped by season (spring: September, October, and November; summer: December, January, and February; autumn: March, April, and May; winter: June, July, and August). Animals were hand-caught during active searches (10:00–18:00) of seven rocky outcrops located within a rural property in Alegrete municipality, state of Rio Grande do Sul, southern Brazil (55°41'79"W, 29°97'10"S). This region is located in the subtropical zone of the Pampas biome, which presents four well-defined seasons and features natural grasslands and riparian forests vegetation (Boldrini, 1997).

After the collection of each animal, it was euthanized with *S*(+)-ketamine hydrochloride, sexed, weighed (Pesola® dynamometer), and measured (digital calipers, resolution 0.01 mm) in the field, and then conditioned in an ice bath. After the field collection, the animals were transported to the field base, where they were frozen in a freezer (−20°C). The field base is about 30 minutes from the animal collection site, and all animals throughout the collection period were submitted to the same sequence of procedures in the field.

Animals were kept in a cooler on ice and at the end of each expedition, transported to the PUCRS Conservation Physiology lab, where liver, kidney, and tail muscle samples were collected, weighed on an analytical balance (Bel Engineering, 0.001-g resolution), and immediately flash-frozen in liquid nitrogen. All samples were stored at –20°C until analysis.

Climatic variables

Data on climatic variables (temperature [°C], precipitation [mm], relative air humidity [%], wind velocity [m/s], and solar radiation [kJ/m²]) throughout the study period were derived from the Meteorological Station of Alegrete municipality and National Institute of Meteorology of Brazil (INMET) and taken from Oliveira et al. (2018), which studied the intermediated metabolism of the same population of *Tropidurus catalanensis.* According to a study developed in a natural habitat by Vieira (2009), this species is active throughout the year from 8:00–18:00, with higher activity rates in the warmer months (spring and summer) and lower rates in the colder months (autumn and winter); the standard of activity of this animal is bimodal in hot seasons and unimodal in cold seasons. Considering this activity pattern, and mainly the activity interval of 8:00–18:00, we chose to use the average of 24 h. Thus, the daily values of each variable were grouped by months of sampling and were calculated as mean by season of the year.

Tissue homogenization

All chemical reagents used in the present study were obtained from Merck and Sigma-Aldrich. For analysis of lipid peroxidation and antioxidant enzyme levels, tissues were homogenized using the following protocol: in a mixture of 1 g tissue and 5 mL of a phosphate buffer solution (20 mM, pH 7.4) containing 140 mM of potassium chloride and 100 mM phenylmethanesulfonyl fluoride (PMSF; protease inhibitor) at a ratio of 10 µL PMSF: 1 mL solution, tissues were homogenized in an ice bath with the aid of an Ultra-Turrax disperser (IKA-WERK). The homogenate was then centrifuged in a refrigerated centrifuge at 3,000 rpm for 10 minutes at 4°C. The supernatant was collected and aliquoted into four plastic tubes, which were stored at –20°C for later quantitation of preset parameters (Llesuy et al., 1985).

Total protein content

Lipid peroxidation and enzymatic activity are expressed in relation to the soluble protein content, which was quantified in supernatants of the homogenate with a commercially available total protein content quantitation kit (Labtest). This assay is based on the biuret method, which when in contact with the sample, yields a purple liquid with peak absorbance at 545 nm.

Lipid peroxidation

Lipid peroxidation levels were measured through the quantitative thiobarbituric acid reactive substances (TBARS) method. This method consists of heating the sample in the presence of thiobarbituric acid under acidic conditions (Buege and Aust, 1978) to yield a colored product. The following compounds were added to a test tube, in sequence: 300 µL trichloroacetic acid (TCA) 10%, 200 µL thiobarbituric acid 0.67%, 100 µL distilled water, and 100 µL of sample, to a final volume of 700 µL. Tubes were shaken and heated to 100°C for 15 minutes and subsequently cooled in ice for 10 minutes. Then, 600 µL n-butanol was added to extract the colored product from the aqueous solution. Tubes were shaken again and centrifuged at 3,000 rpm for 10 minutes. The supernatant was quantified by spectrophotometry at 535 nm. The TBARS concentration was expressed as nmol TBARS mg protein^{-1}.

Antioxidant enzymes

Antioxidant enzyme activity was quantified by spectrophotometry performed in triplicate on liver, kidney, and tail muscle samples using the methods described below.

Superoxide dismutase

The method used for the determination of SOD activity is based on inhibiting the reaction between the superoxide radical and adrenaline, in which the enzyme competes with the detection system for the superoxide radical. As the actual concentration of the enzyme cannot be determined, nor can its activity in terms of substrate consumed over time (minutes), it is quantified in relative units. One unit (U) of SOD activity is defined as the amount of enzyme that inhibits the rate of reduction of the detector (adrenaline) by 50%. The oxidation of adrenaline yields a colored product, adrenochrome, which is detected at 480 nm. The reaction medium employed consists of glycine-NaOH (50 mM, pH 10.5) and adrenaline (1 mM; Boveris, 1984). The SOD concentration was expressed as U SOD mg protein^{-1}.

Catalase

Catalase activity is quantified by measuring hydrogen peroxide consumption. Phosphate buffer (50 mM) was pipetted into a quartz cuvette previously placed in the spectrophotometer, to which a 5-µL aliquot of homogenate was added, followed by 17.5 µL hydrogen peroxide (H_2O_2). Enzyme kinetics behavior is determined by the downslope (decline in absorbance) at 240 nm over 84 s, with readings obtained every 7 s (Boveris and Chance, 1973). Catalase activity was expressed as nmol H_2O_2 consumed min^{-1} mg protein^{-1}.

Glutathione S-transferase

To quantify enzyme activity, we employed the method described by Boyland and Chasseaud (1969), which

consists of measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH). Activity is measured by assessing the increase in absorbance at 340 nm. Enzyme activity is proportional to the rate of production of the conjugate compound (Habig and Jakoby, 1981). The background rate, obtained by reading the reaction between assay GSH and CDNB in the absence of the sample, is subtracted from this activity. The enzyme assay was run for 120 s, with readings obtained every 10 s in potassium phosphate (KPi) buffer (100 mM potassium phosphate, 1 mM EDTA, pH 7.0, containing 1 mM GSH and 1 mM CDNB), and background absorbance was subtracted from the reaction rate, as noted above. A 10 or 25 µL aliquot of homogenate was used to initiate the reaction. Enzyme activity is expressed as nmol CDNB conjugate/min^{-1}/mg protein^{-1}.

Statistical analysis

Climatic variables

All climatic variables obtained were log-transformed (log10(x+1)) prior to statistical analyses to ensure normality of the data set and equality of variances. The correlation matrix between climatic variables (temperature: °C, precipitation: mm; relative air humidity: %; wind velocity: m/s; and solar radiation: kJ/m²) was constructed using Pearson's coefficient. A Principal Component Analysis (PCA) was used to order the variation of the climatic variables data set and show their seasonal pattern based on the correlation matrix. This analysis is useful to define orthogonal axes that express the greatest variability (linear relations) possible in a dataset allowing the interpretation of the variation and relationships among a large set of variables. All analyses related to climatic variables were performed in the statistical software PAST (Hammer et al., 2001). The significance level assumed was $P \leq 0.05$.

Biomarkers

The data are presented as $\bar{x}$ ± SE for each season of the year (spring: September, October, and November; summer: December, January, and February; autumn: March, April, and May; winter: June, July, and August). The distribution of data was assessed by means of the Shapiro-Wilk test of normality, and homogeneity was assessed with Levene's test. For parametric data, we performed one-way analysis of variance (ANOVA) with Bonferroni correction when $P > 0.05$ or the Games-Howell post-hoc test if $P < 0.05$. All tests were performed in IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., 2011). For nonparametric data, we employed the Kruskal-Wallis test with post-hoc Dunn's test, performed in BioEstat 5.3 (Ayres et al. 2007). Results are expressed as $\bar{x}$ ± standard error. Results obtained for the different sexes were compared through two-way ANOVA. A significance level of 5% was adopted for all statistical analyses.

RESULTS

The number of animals collected was seasonally variable for both males (total = 38, spring = 13, summer = 10, autumn = 11, and winter = 4) and females (total = 35, spring = 7, summer = 10, autumn = 10, and winter = 8). The mean snout–vent length (SVL) was 102.07 ± 2.69 in males and 86.47 ± 1.34 in females.

Climatic variables

Temperature and solar radiation were highest in the summer months (26.38 ± 0.57°C and 1814.9 ± 64.53 kJ/m², respectively) and lower in the winter months (14.7 ± 0.76°C and 978.12 ± 45.08 kJ/m², respectively). Regarding relative humidity of the air (74.41 ± 1.68%), precipitation (136.67 ± 6.47 mm), and speed of the winds (2.25 ± 0.15 m/s), constant values were observed throughout the year (Oliveira et al., 2018).

Temperature was positively correlated with solar radiation ($r = 0.83$; $P = 0.0004$). Radiation was negatively correlated with air humidity ($r = 0.87$; $P = 0.0001$), and temperature and radiation were negatively correlated with air humidity ($r = -0.87$; $P = 0.121$). The other climatic variables were not correlated ($P > 0.05$).

The first three PCA axes explained 94.7% of the total variation in the climatic variables (Table 1). According to the PCA loadings, the climatic variables solar radiation ($r = 0.95$) and air temperature ($r = 0.9$) were positively related with Axis 1, while air humidity was negatively related to this axis ($r = -0.89$). The most important climatic variables related to Axis 2 were precipitation ($r = 0.81$) and wind velocity ($r = 0.6$). Axis 3 was negatively related to wind ($r = -0.73$) and positively to precipitation ($r = 0.52$).

The PCA scatter plot (Fig. 1) summarizing the results of the first two axes only (76% of cumulative explanation) shows that climatic variables were structured and grouped by season. Spring and autumn present similar temperatures, but they differed because of the higher precipitation and mainly the higher humidity in autumn. Summer and winter presented a greater difference, mainly because

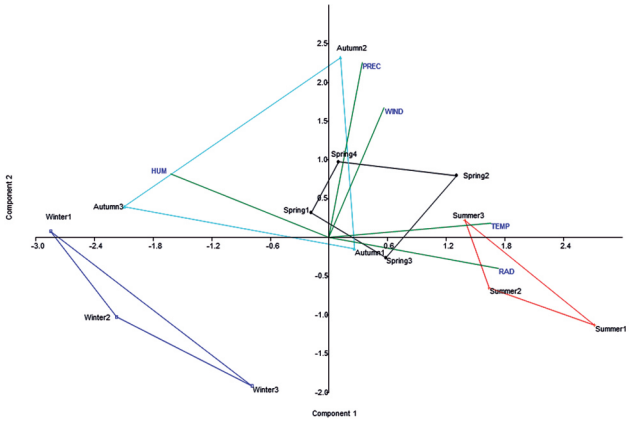

Figure 1. Principal Component Analysis (PCA) ordination biplot describing the variation in climatic variables throughout the seasons (summer, autumn, spring, and winter).

Table 1. Detailed Principal Component Analysis (PCA) results of seasonal variation in climatic variables over the course of the studied period.

	PCA axis				
	PC 1	**PC 2**	**PC 3**	**PC 4**	**PC 5**
Eigenvalues	2.66064	1.14952	0.873836	0.275533	0.0404675
% variance	53.213	22.99	17.477	5.5107	0.80935
Pearson's correlation / Sampling periods					
Spring1	-0.18455	0.32298	-0.1026	-0.8001	-0.23711
Spring2	1.3013	0.79999	0.011903	-0.95882	0.17952
Spring3	0.57829	-0.2533	-1.7163	0.10089	-0.2955
Spring4	0.095371	0.98085	-0.63242	1.1257	-0.0083169
Summer1	2.7111	-1.1314	0.17812	-0.088226	-0.26909
Summer2	1.6356	-0.65	0.79871	0.41711	0.14339
Summer3	1.3888	0.22108	0.23446	0.36871	0.13191
Autumn1	0.25925	-0.14737	1.2299	0.18917	0.029871
Autumn2	0.11432	2.3181	-0.43886	-0.17638	0.18796
Autunm3	-2.0948	0.39511	1.8801	-0.029444	-0.21339
Winter1	-2.846	0.08202	-0.75121	0.079495	-0.067973
Winter2	-2.1714	-1.023	0.078955	0.046699	0.096753
Winter3	-0.78732	-1.9151	-0.7708	-0.27482	0.32197
Climatic variables					
Precipitation (PREC)	0.1852	0.8181	0.5281	-0.1284	-0.03239
Temperature (TEMP)	0.9084	0.06827	0.04892	0.4053	-0.05927
Wind velocity (WIND)	0.3102	0.606	-0.7309	-0.03473	0.03406
Sola Radiation (RAD)	0.9531	-0.1439	0.2062	-0.0716	0.1523
Relative air humidity (HUM)	**-0.8925**	**0.2962**	**0.1256**	**0.2974**	**0.1075**

of the antagonistic values of temperature and radiation, which were higher in the summer. In the winter, temperature and precipitation were also low.

Biomarkers

Lipid peroxidation

No significant differences in lipid peroxidation levels were found between liver and tail muscle samples of males (liver: $P = 0.177$, $H_{3,38} = 4.93$; and muscle: $P = 0.233$, $H_{3,31} = 2.91$), and in all studied tissues of females (liver: $P = 0.717$, $H_{3,35} = 1.35$; kidney: $P = 0.095$, $H_{3,35} = 6.36$; and muscle: $P = 0.511$, $H_{3,31} = 2.31$; Figs. 2A, 3A, and 4A). The only significant difference was found in male kidney tissue samples, in which lipid peroxidation levels in spring were 78.09% higher than in winter ($P = 0.0156$, $H_{3,37} = 10.376$; Fig. 3A). In male tail muscle ($P = 0.233$, $H_{3,31} = 2.91$), the assay employed was unable to detect TBARS in samples obtained from winter months; for this tissue type and sex, a statistical analysis was performed only for the remaining seasons of the year (spring, autumn, and winter).

Superoxide dismutase

Significant differences in SOD activity were observed in male liver tissue ($P = 0.006$, $F_{3,10} = 12.34$); namely, SOD levels in summer were 60.24% higher than in autumn and 80.38% higher than in winter. In female liver tissue, there was no significant difference ($P = 0.453$, $F_{3,21} = 0.92$) in activity of this enzyme across the seasons (Fig. 2B). In male kidney tissue, significant differences ($P = 0.0418$, $H_{3,12} = 8.21$) were observed in spring and summer, when enzyme activity was lower ($\bar{x} = 2.50$ and $\bar{x} = 2.44$, respectively) as compared with autumn and winter, when enzyme activity was higher ($\bar{x} = 5.16$ and $\bar{x} = 5.17$, respectively). As in the liver tissue, there were no significant differences in SOD activity in female kidney samples ($F_{3,16} = 0.685$, $P = 0.578$; Fig. 3B). Significant sex differences were observed, however; in males, activity was 31.57% higher than in females. The test employed was unable to detect enzymatic activity (SOD) in samples obtained in the summer in male muscle, so the comparison was conducted between the other seasons only. In muscle tissue (Fig. 4B), males exhibited a significant difference ($F_{2,9} = 4.78$, $P = 0.069$) between spring and autumn; namely, activity increased 68.31%

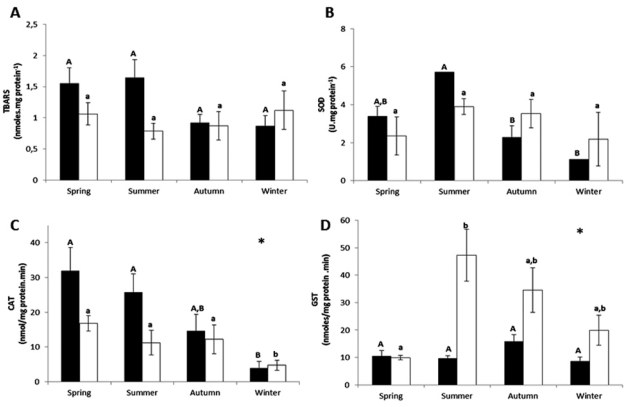

Figure 2. (A) Lipid peroxidation levels (TBARS) and (B) antioxidant enzyme activity (superoxide dismutase = SOD, C: catalase = CAT, and D: glutathione S-transferase = GST) in the liver tissue of Tropidurus catalanensis over the course of a year. Bars represent $\bar{x}$ ± SE. Black bars = males; white bars = females. Different letters represent the differences among the seasons of the year for the sexes, and the asterisk represents the existence of the season and sex interaction ($P < 0.05$). Uppercase letters = males; lowercase letters = females.

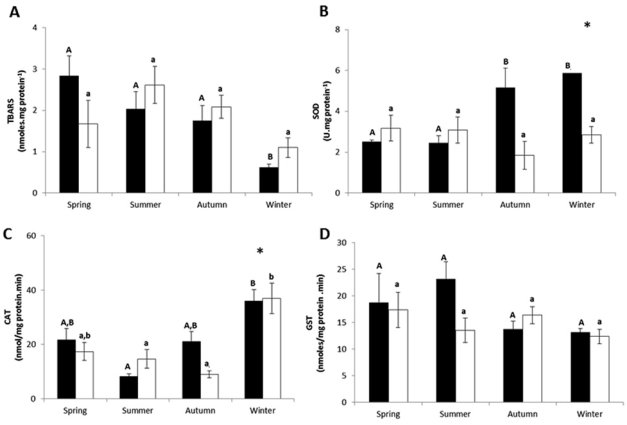

Figure 3. Lipid peroxidation levels (A: TBARS) and antioxidant enzyme activity (B: superoxide dismutase = SOD, C: catalase = CAT, and D: glutathione S-transferase = GST) in the kidney tissue of Tropidurus catalanensis over the course of a year. Bars represent $\bar{x}$ ± SE. Black bars = males; white bars = females. Different letters represent the differences among the seasons of the year for the sexes, and the asterisk represents the existence of the season and sex interaction ($P < 0.05$). Uppercase letters = males; lowercase letters = females.

from springtime to autumn. Although the difference was not significant ($F_{3,11} = 6.58$, $P = 0.19$) for SOD activity in female muscle, activity levels of this enzyme in spring were 3.5 times higher than in the other seasons. Significant ($P < 0.05$) sex-related seasonal differences were observed, whereby enzyme activity in males was 76.96% higher than that in females in autumn and 74.73% higher than that in females in winter. Females, in turn, exhibited muscle SOD activity 62.67% higher than that of males in spring.

Catalase

Significant differences were observed both in male ($H_{3,38} = 11.22$, $P = 0.0106$) and female ($H_{3,35} = 8.57$, $P = 0.035$) mean liver tissue values in spring vs. winter (Fig. 2C), with spring enzyme activity being 93.73% higher in males and 71.04% in females than winter activity. Significant sex differences were also observed ($P = 0.043$), with activity being up to 59.07% higher males than females. Regarding CAT activity in kidney samples (Fig. 3C), males exhibited a significant seasonal difference between summer and winter ($P = 0.0048$, $H_{3,38} = 12.91$), with values in the winter months being 77.01% higher than in summer. In females, a significant difference ($F_{3,35} = 17.22$, $P = 0.0006$) was observed between the winter months and summer and autumn; activity was highest in winter ($\bar{x} = 36.88$) and lowest in autumn ($\bar{x} = 9.02$). A significant seasonal difference ($P = 0.010$) was also observed when comparing values found in males and those found in females; activity was highest in winter for both sexes, and lowest in summer in males ($\bar{x} = 8.25$) and in autumn in females ($\bar{x} = 9.02$). Males exhibited no significant seasonal differences in CAT activity in muscle tissues ($P = 0.281$, $H_{3,37} = 3.82$), whereas females ($P = 0.004$, $H_{3,35} = 13.33$) exhibited differences between winter and summer/autumn (Fig. 4C); in winter, enzyme activity was 79.53% higher than in summer and 80.94% higher than in autumn. A significant difference ($P < 0.05$) between males and females was observed in winter, with activity 60.27% higher in females.

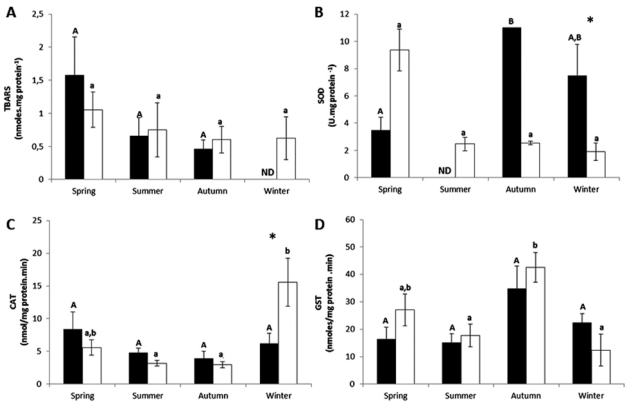

Figure 4. Lipid peroxidation levels (A: TBARS) and antioxidant enzyme activity (B: superoxide dismutase = SOD, C: catalase = CAT, and D: glutathione S-transferase = GST) in the tail muscle tissue of Tropidurus catalanensis over the course of a year. Bars represent $\bar{x}$ ± SE. Black bars = males; white bars = females. Different letters represent the differences among the seasons of the year for the sexes, and the asterisk represents the existence of the season and sex interaction ($P < 0.05$). Uppercase letters = males; lowercase letters = females.

Glutathione S-transferase

In males, there were no significant differences in GST activity in liver tissues (Fig. 2D; $P = 0.0964$, $F_{3,37} = 0.81$) over the course of a seasonal cycle. In females, a significant difference was found between spring and summer, with a 79.29% higher activity in the latter ($P = 0.0028$, $H_{3,35} = 14.12$). A sex difference was also found ($P < 0.01$), with females exhibiting 60.14% higher activity (male $\bar{x} = 11.12$; female $\bar{x} = 27.9$). The greatest sex-related difference was found in summer, when CAT activity was 79.7% higher in females. GST activity in kidney tissue (Fig. 3D) did not vary significantly over the course of the year in animals of either sex (male: $P = 0.423$, $H_{3,36} = 8.18$ and female: $P = 0.354$, $H_{3,33} = 1.13$). In tail muscle samples (Fig. 4D), there were no significant differences in GST activity in males ($H_{3,35} = 6.62$, $P = 0.084$), whereas in females ($H_{3,35} = 13.85$, $P = 0.0031$), a significant difference was observed between autumn and the winter and summer months; in autumn, activity was 58.41% higher than in summer and 70.95% higher than in winter. There was no significant difference between sexes over the seasons.

DISCUSSION

Our results suggest that a modulation of enzymatic antioxidant defenses (superoxide dismutase, catalase, and GST) occurs during the seasonal cycle of the year; this modulation appears to be tissue and sex-specific (liver, kidneys, and muscle). The antioxidant system of this species was found to be highly efficient, being able to maintain constant lipid peroxidation in liver and tail muscle in males and all studied tissues of females. In the renal tissue, we found the highest levels of lipoperoxidation in spring and a significant reduction in the winter months.

It is well established in the literature that during the reproductive season (September–January), females tend to become resident, decreasing their energy expenditure on the search for food and prioritizing reproduction-related activities instead (Oliveira et al., 2018). This pattern appears to be associated with the maintenance of LPO levels even during reproduction. However, males exhibit a pattern of greater exploratory activity across their habitat, explained by the search for females and by the defense of their territory and harems (Rodrigues, 1987; Arruda, 2009); this suggests a connection between the significant increase in LPO in renal tissue—and the clear trend in increased LPO in the other analyzed tissues (liver and muscle)—and this pattern of activity.

Alabarse et al. (2011) demonstrated that the kidney of male rats presents increased oxidative damage and modifications in the antioxidant defense system during reproduction, being associated with the increase of testosterone and metabolic changes, which corroborates the aforementioned hypothesis. Costantini et al. (2009) stress that sex hormones such as testosterone and estradiol exert antioxidant activity, but, depending on concentration, they also induce ROS production, which can generate oxidative stress. In the target species of the present study, Arruda (2009) observed continuous sperm production during the year, which could be associated with increased testosterone production during the reproductive period, as suggested by Wiederhecker et al. (2002) in *Tropidurus torquatus* (Wied, 1821). Marler and Moore (1988) and Tokarz et al. (1998) demonstrated that high testosterone levels can reduce survival in males. Hence, increased levels of lipid peroxidation in the kidneys of male *T. catalanensis* could be related to an increase in testosterone secretion during the reproductive period (September–January) together with an increase in the activity of these animals and an intense decrease in abdominal fat index and hepatic glycogen levels (Oliveira et al., 2018).

It bears stressing that in males muscle tissue samples we detected lipid peroxidation levels in the spring, summer, and autumn months, but not in winter; this suggests a reduction in exploratory activity during the winter, which would lead to a reduction in energy expenditure (metabolic depression) and, consequently, a decline in ROS formation and lipid peroxidation. Oxidative phosphorylation is known to be one of the main points of synthesis of these molecules (Dowling and Simmons, 2009). In the region where specimens were collected, winter is the coldest season (mean winter temperature 14.7 ± 0.76°C) and is accompanied by a reduction in solar radiation (mean winter radiation 978.12 ± 45.08 kJ/m²) and precipitation (mean winter precipitation 116 ± 5.68 mm; Oliveira et al., 2018). These environmental characteristics might lead the animal to reduce its exploratory activity, leading to hypometabolism. This hypothesis is supported by the difficulty of catching males in winter.

Vieira (2009) observed that the period of activity of this species over the year is 8:00–18:00 h. However, in the autumn and winter months the activity rate is lower and unimodal, contrary to the spring and summer pattern, in which this activity is more intense and bimodal. Oliveira et al. (2018) identified sexual dimorphism in the abdominal fat index, whereby males seem to predominantly use abdominal fat to maintain homeostasis in winter while females maintain their fat reserves intact, prioritizing the allocation of lipid reserves for reproduction during the spring.

Results obtained in the present study for TBARS levels were lower than those reported for other reptiles (Furtado-Filho et al., 2007; Valdivia et al., 2007; Reguera et al., 2014), suggesting that the antioxidant system of this species is highly capable of coping with variations in environmental conditions and within the life cycle itself. Animals that tolerate extreme conditions have an efficient antioxidant defense system against fluctuations in oxygen consumption (Welker, 2009), thus strengthening the aforementioned hypothesis of a yearly hypometabolic period. The adaptations of such a system, already described in other reptiles, include the presence of high concentrations of antioxidant molecules. Further investigation of these aspects in *Tropidurus catalanensis* is needed.

The assay used in the present study was unable to detect SOD activity in the muscle of males; in all other samples we were able to detect the activity of this enzyme, but regardless of sex, this activity was very low. In males, SOD activity in liver tissue was higher in the summer, as was the lipid peroxidation level, whereas in females, neither parameter exhibited significant differences. This finding might be associated with increased exploratory activity. According to Olsson et al. (2012), males of the species *Ctenophorus pictus* (Peters, 1866) exhibit greater SOD activity than females during the reproductive cycle; the authors suggested that this pattern is associated with increased physical activity in males, due to long hours patrolling their territory and competing for mating opportunities.

In the kidney, SOD activity exhibited significant seasonal differences ($P < 0.05$) only in males; namely, enzyme activity was greater during the colder seasons (autumn and winter). Females did not exhibit fluctuations in the activity of this enzyme in the renal tissue; this might be related to estrogen production. A study performed on rats concluded that this hormone has a nephroprotective effect, attenuating damage caused by production of the superoxide radical (Ji et al., 2007).

As seen in the kidney tissue, significant differences were found in the muscle tissue in males; again, SOD activity was higher in the colder seasons. A similar result was observed in *Lacerta vivipara* specimens subjected to supercooling and freezing (Voituron et al., 2006). In that study, SOD activity was found to increase in the aforementioned situations, suggesting activation of the antioxidant system as a survival strategy in low-temperature settings; SOD plays an important role in this event (i.e., to protect kidney and muscle tissue from oxidative damage). After thawing, the animals exhibited decreased SOD activity, as observed in males in the present study.

Peak activity of this enzyme in the liver tissue of males and females was found during the reproductive pe-

riod. According to Costa et al. (2008), increased activity of enzymes such as SOD and CAT in hepatic tissue occurs in response to rising ROS levels. As there is a trend toward increased lipid peroxidation levels ($P > 0.05$) during spring and summer, this increase in enzyme activity may be regarded as a response of the liver tissue to reproductive events, thus enabling an increment in metabolic demand by said tissue without incurring oxidative stress. One should also bear in mind that the rise in temperature observed in summer per se might stimulate overall metabolism, thus facilitating a pro-oxidant state that is offset by increased efficacy of the antioxidant system (represented by increased catalase and GST activity), as observed by Manduzio et al. (2004) in the blue mussel *Mytilus edulis* (Linnaeus, 1758) and Nunes et al. (2015) in the European pilchard *Sardina pilchardus* (Walbaum, 1792).

In the kidney, peak CAT activity occurred in the winter months, in males and females alike. We also found high levels of this enzyme in female muscle samples during winter. Increased antioxidant enzyme activity during hypometabolic states appears to be an adaptation of animals resistant to wide fluctuations in oxygen consumption, and it is a tissue-dependent response (Welker, 2009). According to Moreira et al. (2018), survival under stress, such as exposure to hypoxia, anoxia, freezing, dehydration, air exposure of water breathing organisms, and aestivation, is commonly associated with enhanced endogenous antioxidants, a phenomenon coined "preparation for oxidative stress" (POS), and the regulation of free radical metabolism seems to be crucial under these selective pressures, as this response is widespread among animals.

This pattern was observed in *Tropidurus catalanensis,* particularly in males, and might play an important role in preparing these individuals to face the energy demands required to sustain reproduction in subsequent seasons (spring/summer) and prevent oxidative stress. The greater ease of collecting females, even at low temperatures, suggests they continue to forage in the cold season in preparation for the next reproductive period. This behavioral pattern might also influence increased CAT activity in muscle, as physical activity induces a rise in activity of antioxidant enzymes, including catalase (Córdova and Navas, 2000).

In females, the liver exhibited high levels of GST activity throughout the year (summer, autumn, and winter), except the peak reproductive period (spring). This increased GST activity in summer, autumn, and winter, particularly in females, might be associated with recovery from reproduction, preparation for a new reproductive cycle, and/or dietary changes.

Tropidurus catalanensis is a primarily insectivorous species that exhibits active foraging behavior. Carvalho et al. (2007) and Gomides et al. (2013) noted that Formicidae (Hymenoptera) is one of the taxa most commonly found in the stomach of *T. torquatus,* as well as one of the most widely available in the environment. These insects produce formic acid for chemical defense (Hefetz and Blum, 1978). The high GST activity observed in the liver from summer through winter might be related to the metabolism of formic acid, as suggested by Welker (2009) for *Salvator merianae,* in which GST increased after fasting and refeeding.

The results found for GST in the tail muscle show higher levels in the fall months for both sexes; a similar pattern was reported by Aguilera et al. (2012) in *Sceloporus* sp. collected in protected areas. This dataset suggests the presence of a highly efficient antioxidant system in these animals, which maintains lipid peroxidation at low levels in all of the tested tissues. The enzymatic antioxidant defense system studied herein, as represented by superoxide dismutase, catalase, and GST activity, exhibits tissue-specific responsiveness, and is possibly modulated by factors both abiotic (temperature, photoperiod, radiation, among others) and biotic (e.g., reproductive cycle, nutritional status, type of food consumed). The seasonal variation in climatic variables showed that winter months also present, in addition to the lower temperature (often reported in Pampas), a hydric deficit, evidenced by the lower rates of relative humidity and precipitation, which can determine an extreme environmental situation and lower food availability. This profile suggests that these animals are able to maintain homeostasis even in periods that feature adverse environmental condition and/or high energy demands, such as reproduction.

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