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Seasonal and Sexual Variation of the Intermediate Metabolism and Body Condition Indexes in the Lizard *Tropidurus catalanensis* (Gudynas and Skuk, 1983) (Squamata: Tropiduridae)

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Abstract. Throughout the life cycle of organisms, nutrient intake is necessary for the maintenance of the functions of daily life and in preparation for a variety of biological events, such as molting, migration, hibernation, and reproduction. One of the most energetically costly events is reproduction. Present study sought to elucidate the seasonal variations in the markers of intermediate metabolism (total proteins, uric acid, glucose, total lipids, triglycerides, total cholesterol, VLDL cholesterol, and glycogen) and body condition indices (gonadosomatic, hepatosomatic, and abdominal fat index) in *Tropidurus catalanensis*, in order to know the physiological costs of reproduction in this species, also allowing these parameters to be used as markers in studies of environmental alterations. During the study period, 38 males and 35 females were collected. *Tropidurus catalanensis* is a lizard that inhabits the Pampa grasslands, a biome that stretches across Argentina, Brazil, and Uruguay. Results obtained reveal that this lizard exhibits a clear pattern of seasonal variation in its metabolism, as reflected mainly in levels of plasma total protein, liver glycogen, total lipids, and triglycerides in males and females; and in blood glucose and lipid, and liver protein levels in females. It bears stressing that all body condition indices analyzed varied over the course of the year. The parameters studied can be used to evaluate the impact of changes in environmental conditions on these animals. Winter months appear to trigger a reduction in overall metabolism, most intensely in males. Reproductive period seems to be the biological event that demands greater energy demand, leading to the mobilization of energy stocks mainly in females, where the search for food may be reduced in the months of reproductive peak (spring). The response pattern observed during the reproductive period suggests that this time represents a critical point in terms of the animal's adaptation to its life cycle.

Keywords. Biochemical composition; Life cycle; Lizards; Reproduction; Seasonality.

Resumo. Ao longo do ciclo de vida dos organismos, a entrada de nutrientes é necessária para a manutenção e antecipação de eventos biológicos. Um dos eventos com maior custo energético é a reprodução. Este estudo procurou elucidar as variações sazonais em marcadores do metabolismo intermediário (proteína total, ácido úrico, glicose, lipídios totais, triglicerídeos, colesterol total, colesterol VLDL e glicogênio) e índices de condição corporal (gonadaossomático, hepatossomático e de gordura abdominal) em *Tropidurus catalanensis*, a fim de conhecer os custos fisiológicos da reprodução, permitindo também que esses parâmetros sejam utilizados, no futuro, como marcadores em estudos de alterações ambientais. Durante o período de estudo, 38 machos e 35 fêmeas foram coletados. *Tropidurus catalanensis* é um lagarto que habita as pastagens de Pampa, um bioma que se estende por toda a Argentina, Brasil e Uruguai. Os resultados obtidos revelam que esse lagarto apresenta um claro padrão de variação sazonal em seu metabolismo, o que foi constatado para os níveis plasmáticos de proteínas totais e na concentração de glicogênio, lipídios totais e triglicerídeos no fígado de machos e fêmeas, e para os níveis plasmáticos de glicose e lipídios totais e proteínas totais no fígado de fêmeas. É importante ressaltar que todos os índices de condição corporal também variaram ao longo do ano, confirmando que os parâmetros estudados podem ser utilizados para avaliar o impacto de alterações das condições ambientais sobre estes animais. Os meses de inverno parecem levar a um estado de hipometabolismo, principalmente em machos. O período reprodutivo parece ser o evento biológico que exige maior demanda energética, levando à mobilização dos estoques de energia principalmente nas fêmeas, cuja busca por alimento pode estar reduzida nos meses de pico reprodutivo (primavera). O padrão de resposta observado durante a reprodução sugere que esse período representa um ponto crítico em termos de adaptação do animal ao seu ciclo de vida.

INTRODUCTION

Throughout the life cycle of organisms, nutrient intake is necessary for the maintenance of the functions

of daily life and in preparation for a variety of biological events, such as molting, migration, hibernation, and reproduction (McEwen and Wingfield, 2003). Normally, organisms store extra resources (glycogen, triglycerides and

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proteins) in specific tissues (such as liver, adipocytes, and muscle) to be used in these processes. Growth rate and reproductive effort are largely the result of the amount of resources that organisms can allocate from energy reserves and/or nutrients after meeting the basic maintenance needs of the body. When organic and environmental conditions are favorable, reproduction is triggered, and part of the energy reserves are directed to this process (Hirshfield and Tinkle, 1975; Shine, 1980; Tytler and Calow, 1985; Harshman and Zera, 2006; Van Dyke and Beaupre, 2011). According to Speakman (2008), the physiological costs of reproduction may be direct or indirect. Direct costs include the energy and nutrient demands of the reproductive event and the morphological changes needed to facilitate the realization of these demands. Indirect costs can be optional compensatory costs, so animals choose to reduce their investment in some other aspect of their physiology to maximize the inflow of resources into reproduction.

Seasonal fluctuations in biotic and abiotic factors have a major influence on energy metabolism in reptiles (Bennett and Dawson, 1976). As ectothermic animals, reptiles undergo profound alterations in physiological processes and patterns of activity, particularly regarding the reproductive cycle, as a result of temperature changes. Factors such as food availability and photoperiod also contribute to differences in metabolism (Southwood et al., 2006; Williard and Harden, 2011). In temperate-zone reptiles, reproduction appears to occur seasonally, with peaks of reproductive activity interleaved with periods of inactivity (Brown and Shine, 2006). Temperature appears to be the single greatest regulator of the reproductive cycle in reptiles. Nevertheless, other factors, such as photoperiod, rainfall pattern, and availability of food, may also influence this biological event (Callard and Kleis, 1987).

Plasma metabolites are an important tool for understanding, monitoring, analyzing the biological and health conditions of free-living animals, and providing information on the management and conservation of these animals. These markers include intermediary metabolism products such as glucose, proteins, uric acid, urea, and different types of lipids (triglycerides, cholesterol, and beta-hydroxy-butyrate) that reflect the balance between anabolic/catabolic pathways, absorption capacity through the gastrointestinal tract and excretion through the excretory system. Nutrients and metabolites carried through blood have proven value as direct and effective markers for estimating nutritional status in birds, fish, mammals, and other animals under a variety of feeding conditions (Jenni-Eiermann and Jenni, 1998; Artacho et al., 2007; Cooke et al., 2013). Considering metabolic aspects, another important organ to be studied is the liver, since this organ performs numerous functions, although it is also a discrete organ with many of its functions relating to each

other, having a high metabolic rate, synthesizing and processing multiple substances that will be transported to other areas of the body. Almost all lipid synthesis from carbohydrates and proteins occurs in the liver. In addition, the detoxification of several exogenous and endogenous substances, for example, several hormones secreted by the endocrine glands that are chemically altered or excreted by the liver.

Biomarkers such as those linked to the biochemical blood profile of different animal species have been widely used in ecotoxicological studies, which are early warning indicators of the exposure of these animals to toxic substances, and are often used to compare reference areas with other contaminated ones. However, very few studies have been conducted on lizards and mainly focusing on the range of natural variability of these biomarkers (Norris and Jones, 2012), which is critical to understanding the differences detected and whether these are actually determined by any adverse effects due to pollution or only linked to the life cycle of the species. The use of energy stocks (glycogen, triglycerides and proteins) during the reproductive period can reduce survival rates, hampering growth, immune functions and energy storage to cope with periods of potential environmental stress, as well as environmental changes that have been imposed by man on wildlife.

The reproductive period of *Tropidurus catalanensis* (Gudynas and Skuk, 1983) is September through January when females produce single clutches of six eggs on average; males, in turn, produce sperm continuously, and maintain aggressive, territorial behaviors (Arruda, 2009; Kunz and Borges-Martins, 2013), as we believe that at least females have a high degree of allocation of their energy reserves to support the reproductive period. The present study sought to elucidate the seasonal variations in the markers of intermediate metabolism and body condition indices in *T. catalanensis* in order to know the physiological costs of reproduction in this species, also allowing these parameters to be used as markers in studies of environmental alterations.

MATERIALS AND METHODS

Tropidurus catalanensis occurs on rocky outcrops in the Pampas grasslands (Argentina, Brazil, and Uruguay) and is a mid-sized saurian lizard with a short body and tail, imbricate, keeled dorsal scales, dark coloration, and marked sexual dimorphism (Rodrigues, 1987; Lema, 2002). Arruda (2009) reports for this same lizard population that the smallest reproductive female had a snout-vent length (SVL) of 74.8 mm, and the smallest male one 65.3 mm. It has a seasonal reproductive cycle, as is typical for species from temperate regions (Wiederhecker et al., 2003). Another study using the same lizards collected in

Table 1. Abiotic parameters observed during the collection period for the region of Alegrete, RS, Brazil. Results are expressed as the mean \pm SE. Different letters represent a difference for a $P < 0.05$.

	Spring	Summer	Autumn	Winter	Annual average
Temperature (°C) (F= 11.38; P = 0.002)	20.69 \pm 0.99 ^{ab}	26.38 \pm 0.57 ^a	18.83 \pm 0.59 ^b	14.7 \pm 0.76 ^b	19.66 \pm 0.66
Relative air humidity (%) (F= 3.53; P = 0.061)	72.36 \pm 2.67 ^a	68.48 \pm 1.75 ^a	78.41 \pm 1.19 ^a	79.09 \pm 1.32 ^a	74.41 \pm 1.68
Precipitation(mm) (F= 6.111; P = 0.0292)	138 \pm 10.65 ^{ab}	135.66 \pm 3.75 ^{ab}	156.66 \pm 5.81 ^b	116 \pm 5.68 ^a	136.67 \pm 6.47
Wind (M/S) (F= 1.392; P = 0.307)	2.57 \pm 0.18 ^a	2.11 \pm 0.16 ^a	2.16 \pm 0.11 ^a	2.03 \pm 0.13 ^a	2.25 \pm 0.15
Radiation (KJ/M²) (F= 7.073; P = 0.009)	1,264.3 \pm 72.18 ^{ab}	1,814.9 \pm 64.53 ^a	1,229.4 \pm 55.09 ^b	978.12 \pm 45.08 ^b	1,321.68 \pm 59.22

the present study found that this lizard consumes vegetal fiber in a reduced amount, having a primarily carnivorous diet (arthropods), with little variation and preference for hymenoptera (Rosa, 2015).

Tropidurus catalanensis specimens were captured monthly from September 2013–September 2014. Animals were hand-caught between 10:00 and 18:00 h, during active searches of seven rocky outcrops located within a rural property in the municipality of Alegrete, state of Rio Grande do Sul, Brazil (55°41'79"W, 29°97'10"S). Data on temperature, relative air humidity, wind velocity, and solar radiation throughout study period were obtained for the municipality from the meteorological station of Alegrete, together with the National Institute of Meteorology of Brazil (Table 1). Specimens were sexed, weighed (Pesola® dynamometer) and the mean SVL was measured with digital calipers (resolution 0.01 mm) to confirm if the subjects were adults. Sexual maturity was determined according to criteria established by Arruda (2009); only adult animals were captured.

Blood samples were obtained by cardiac puncture in heparinized syringes; they were conditioned in ice bath inside a thermal box. Animals were euthanized with ketamine hydrochloride and were conditioned in ice bath inside thermal box. After arrival at the field base, the plasma was collected with individual Pasteur pipettes and frozen along with the animals in a freezer (-20°C). After, the plasma and animals were transported in ice bath to the Laboratory of Conservation Physiology. At the laboratory, livers were extracted, weighed on an analytical balance (Bel Engineering, precision: 0.001 g), and immediately flash-frozen in liquid nitrogen; gonads and abdominal fat were also weighed for subsequent calculation of body condition indices. All samples were stored at -20°C until the time of analysis.

Body condition indices

The hepatosomatic index, gonadosomatic index, and abdominal fat index were calculated for each of the captured specimens with the following mathematical formula: (tissue mass (g) / animal mass (g)) \times times 100. Results are expressed as percentages (%).

Plasma

Measurements of metabolites in plasma and liver were carried out in duplicate by spectrophotometry and compared to a curve constructed with a series of diluted standard solutions for each quantified metabolite. The plasma total protein levels were expressed as mg/mL and all other metabolites were expressed as mg/dL. In liver, all metabolites were expressed as mg/g.

Total protein concentration was determined using a commercially available kit (Labtest). This assay is based on the biuret method, whereby a reaction with the peptide bonds in serum proteins yields a purple product. Uric acid levels were quantitated using a commercially available kit (Analisa), which uses an enzymatic colorimetric method based on oxidation of uric acid to allantoin, CO₂, and H₂O₂ by uricase. Glucose levels were quantitated using a commercially available kit (Labtest) based on the glucose oxidase method, which catalyzes the oxidation of glucose to form a red product.

Total lipids were quantitated by the sulfo-phospho-vanillin method (Frings and Dunn, 1970), which consists of oxidizing cell lipids into small fragments after chemical digestion with hot concentrated sulfuric acid. Upon addition of a solution of vanillin, a red complex is formed. Triglycerides (TGL) were quantitated using a commercially available kit (Labtest). Briefly, lipoprotein lipase catalyzes the hydrolysis of triglycerides to yield glycerol, which is converted by glycerol kinase to glycerol 3-phosphate. This is oxidized to dihydroxyacetone and hydrogen peroxide by glycerol phosphate oxidase. A peroxidase-catalyzed coupling reaction of hydrogen peroxide with 4-aminoantipyrine and 4-chlorophenol then occurs, yielding a quinone imine. Very low-density lipoprotein cholesterol (VLDL) levels were derived from triglyceride measurements (VLDL = TGL/5). Total cholesterol levels were quantitated using a commercially available kit (Labtest). Briefly, cholesterol esters are hydrolyzed by cholesterol esterase to free cholesterol and fatty acids. Free cholesterol is oxidized by cholesterol oxidase to form cholest-4-en-3-one and hydrogen peroxide; phenol and 4-aminoantipyrine are oxidized to form an antipyrine quinone imine.

Liver

Glycogen was extracted using the method described by Van Handel (1965) and quantitated as glucose after acid hydrolysis (HCl) and neutralization (Na₂CO₃), using a commercially available kit (Labtest). Total protein levels were quantitated in an aliquot of the glycogen extraction medium, using a commercially available assay kit (Labtest).

Total lipids and triglycerides were extracted via the 2:1 (v/v) chloroform/methanol method described by Folch et al. (1957). Lipids were quantitated by the sulfo-phospho-vanillin method (Frings and Dunn, 1970), and triglycerides were measured using a commercially available kit (Labtest).

Statistical analysis

Results are expressed as mean \pm SE. 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. For nonparametric data, we employed the Kruskal-Wallis test with post-hoc Dunn's test. Results obtained for different sexes were compared through two-way ANOVA (fixed parameters the sex and the seasons of the year). Significance level was set at 5%, and all analyses were performed in the software Statistical Package for the Social Sciences for Windows (17.0) (IBM Corporation, 2008) and BioEstat 5.3 (Ayres et al. 2007).

Table 2. Summary of the statistical analysis of body indexes and biochemical parameters determined over a seasonal cycle in male and female of the lizard *Tropidurus catalanensis*.

Male Body condition indices	Test	Number of animals	df	H or F	P
Gonadosomatic index	Kruska-Wallis + Dunn	38	3	27.21	0.0001
Hepatosomatic index	Kruska-Wallis + Dunn	38	3	8.51	0.01
Body Fat index	Kruska-Wallis + Dunn	38	3	23.08	0.0001
Plasma					
Total proteins	ANOVA + Bonferroni	38	3	4.298	0.014
Uric acid	ANOVA + Bonferroni	38	3	0.646	0.594
Glucose	Kruska-Wallis + Dunn	38	3	2.816	0.061
Total lipids	Kruska-Wallis + Dunn	38	3	1.373	0.712
Triglycerides	Kruska-Wallis + Dunn	38	3	8.044	0.054
Total cholesterol	Kruska-Wallis + Dunn	38	3	3.289	0.349
VLDL	Kruska-Wallis + Dunn	38	3	1.296	0.730
Liver					
Total proteins	Kruska-Wallis + Dunn	38	3	8.4	0.384
Total lipids	Kruska-Wallis + Dunn	38	3	8.04	0.024
Glycogen	Kruska-Wallis + Dunn	38	3	12.221	0.007
Triglycerides	Kruska-Wallis + Dunn	38	3	8.043	0.045
Female Body condition indices					
Gonadosomatic index	Kruska-Wallis + Dunn	35	3	16.52	0.0009
Hepatosomatic index	Kruska-Wallis + Dunn	35	3	18.8	0.0003
Body Fat index	Kruska-Wallis + Dunn	35	3	17.69	0.0005
Plasma					
Total proteins	ANOVA + Bonferroni	35	3	3.268	0.035
Uric acid	Kruska-Wallis + Dunn	35	3	1.928	0.587
Glucose	Kruska-Wallis + Dunn	35	3	2.855	0.045
Total lipids	Kruska-Wallis + Dunn	35	3	13.199	0.0042
Triglycerides	Kruska-Wallis + Dunn	35	3	4.629	0.201
Total cholesterol	Kruska-Wallis + Dunn	35	3	6.55	0.087
VLDL	Kruska-Wallis + Dunn	35	3	3.355	0.340
Liver					
Total proteins	Kruska-Wallis + Dunn	35	3	3.86	0.019
Total lipids	Kruska-Wallis + Dunn	35	3	3.35	0.0004
Glycogen	Kruska-Wallis + Dunn	35	3	2.426	0.488
Triglycerides	Kruska-Wallis + Dunn	35	3	0.31	0.818

RESULTS

Temperatures and solar radiation were highest in the summer months ($26.38 \pm 0.57^\circ\text{C}$ and $1814.9 \pm 64.53 \text{ KJ/m}^2$, respectively) and lower in the winter months ($14.7 \pm 0.76^\circ\text{C}$ and $978.12 \pm 45.08 \text{ KJ/m}^2$, respectively). Precipitation was higher in the autumn and lower in winter (156.66 ± 5.81 and $116 \pm 5.68 \text{ mm}$, respectively). For the relative humidity of the air ($74.41 \pm 1.68\%$) and speed of the winds ($2.25 \pm 0.15 \text{ m/s}$) were observed constant values throughout the year (Table 1).

A total of 73 adult *Tropidurus catalanensis* were collected, with an SVL of $102.07 \pm 2.69 \text{ mm}$ for males and $86.47 \pm 1.34 \text{ mm}$ for females. 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). A summary of the statistical analysis of body indexes and biochemical parameters determined in males and females of this lizard are presented in Table 2.

Body condition indices

The gonadosomatic index (GSI) differed significantly in both males and females when spring was compared to the summer and autumn months, with gonads being proportionally larger in both sexes in spring. Female gonad size was nearly fourfold that of males ($P = 0.001$), with gonads accounting for up to 4% of total body weight; female gonads were approximately 79.76% larger than those of males in the spring and 74.01% larger in the winter, respectively (Fig. 1A).

The hepatosomatic index (HSI) differed significantly in males between the summer and winter. In the summer months, liver tissue accounted for 2.37% of total body weight versus 1.72% in winter. Females also exhibited a difference in this index across seasons; in the spring months, the liver accounted for 8.87% of total body weight (greatest proportion), whereas in the autumn the HSI was only 3.13%, representing the lowest proportion. The HSI in females was up to twofold that calculated in males ($\bar{X} = 4.38\%$ and $\bar{X} = 2.03\%$ respectively), with the greatest difference between sexes observed in spring ($P = 0.001$; Fig. 1B).

In males, the abdominal body fat index (BFI) differed significantly when spring was compared to the summer and autumn months, with the lowest abdominal fat percentage found in spring ($\bar{X} = 0.046\%$). In females, this index differed significantly when spring was compared to the autumn and winter months, with the lowest abdominal fat percentage again found in spring ($\bar{X} = 0.23\%$). A difference between sexes was also found ($P = 0.001$), with the percentage of fat stored in the abdominal cavity being up to 69.88% greater in females ($\bar{X} = 3.12\%$) than in

males ($\bar{X} = 0.94\%$). Females also had a higher abdominal fat percentage than males across all seasons of the year ($P = 0.001$; Fig. 1C).

Plasma

Total protein levels (Fig. 2A) were significantly lower in males than in females (26.27%; $P = 0.043$) throughout the year. Higher levels were found in winter for both males and females ($\bar{X} = 58.97$ and $\bar{X} = 81.96 \text{ mg/mL}$,

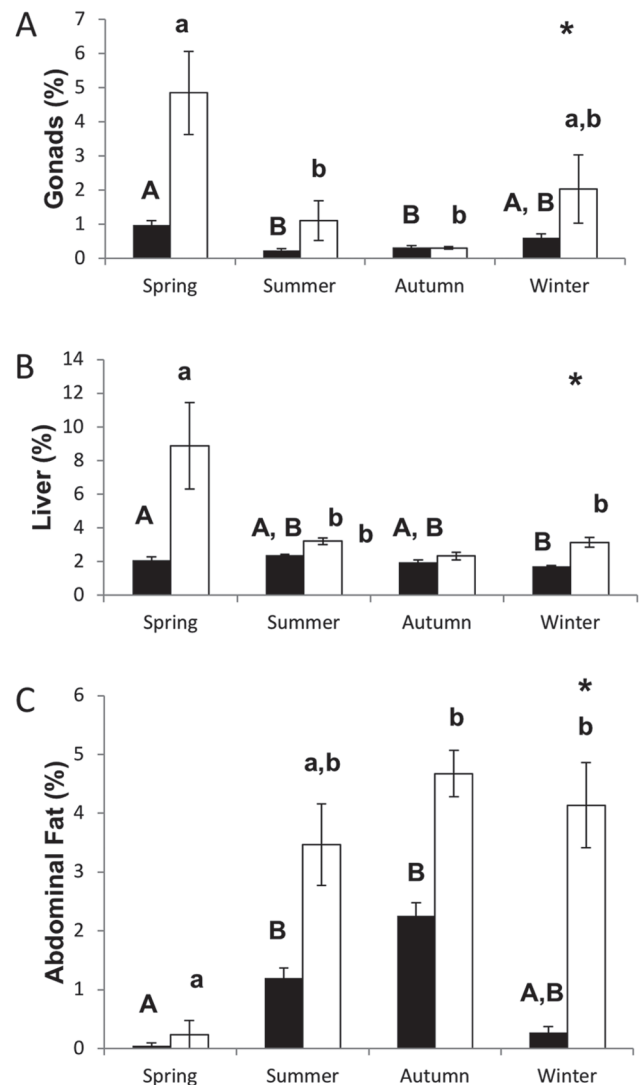


Figure 1. Seasonal gonadosomatic index (A), hepatosomatic index (B), and abdominal fat index (C) over the course of a year in *Tropidurus catalanensis*. Bars represent mean \pm SE. Black bars = males; white bars = females. Different letters denote a significant difference ($P < 0.05$). Upper-case letters = males; lower-case letters = females. An asterisk (*) denotes a difference in the response curve of males and females over the course of the year. The number of animals collected was of 38 males (spring = 13, summer = 10, autumn = 11, and winter = 4), and 35 females (spring = 7, summer = 10, autumn = 10, and winter = 8).

respectively). In males, levels increased 60.34% from spring to winter; a similar pattern was found in females, with a 66.66% increase in total circulating protein levels from spring to winter. There were no significant differences in uric acid between the sexes ($P = 0.711$) or across seasons (Fig. 2B).

Glucose levels (Fig. 2C) did not differ significantly in males, whereas in females, glucose levels increased nearly 2.5-fold in spring compared to winter. There were no significant differences in blood glucose patterns between males and females ($P = 0.577$) over the course of a seasonal cycle or across the seasons of the year.

In males, there were no significant differences in total lipid levels (Fig. 3A) across seasons, whereas in females

these levels differed significantly in spring compared to the other seasons, as well as between summer and winter. An 82% increase in circulating lipid levels was observed, with the lowest levels found in spring ($\bar{X} = 114.55$ mg/dL) and the highest in winter ($\bar{X} = 623.09$ mg/dL). No significant differences in seasonal response (Fig. 3A) were found between the sexes ($P = 0.141$).

In males, triglyceride levels were 40.36% lower ($P < 0.05$) than in females ($\bar{X} = 158.15$ mg/dL) in the winter months; nevertheless, the behavior of triglyceride levels during the year was similar in both sexes (Fig. 3B). Total cholesterol levels did not differ significantly in either sex across the seasons, or between the sexes over the course of the year (Fig. 3C). VLDL cholesterol levels also did not differ significantly in either sex across the seasons (Fig. 3D). However, a significant difference in the behavior of the VLDL curve over the year was observed between males and females, with females exhibiting levels on average 174.17% higher than males ($P < 0.05$).

Liver

In males, total protein concentrations in the liver did not differ significantly across the seasons of the year (Fig. 4A). In females, a significant difference was found between spring and summer months, with stored protein levels being twice as high as in the spring (spring $\bar{X} = 430.95$ mg/g; summer $\bar{X} = 912.65$ mg/g). On average, females exhibited levels 31.32% higher than those of males ($P < 0.05$). Comparison between males and females revealed significant differences across the seasons of the year ($P = 0.019$), with the highest levels found in summer in females and autumn in males. There was a significant difference between protein levels between males and females throughout the year ($P = 0.019$), with higher levels in females ($\bar{X} = 387.91$ mg/g in males, $\bar{X} = 912.65$ mg/g in females).

In males, total lipids (Fig. 4B) exhibited a significant difference in spring and summer, the seasons with the highest levels in relation to winter. In females, levels were highest in summer ($\bar{X} = 176.99$ mg/g), differing significantly from those measured in spring and winter. Lipid levels measured in females were almost twice as high as those measured in males ($\bar{X} = 112.66$ mg/g vs. $\bar{X} = 60.35$ mg/g, respectively; $P = 0.03$).

Regarding glycogen levels (Fig. 4C), a significant difference was observed for males in winter compared to the spring and autumn levels, with winter levels being 85.54% higher than summer levels and 82.57% higher than autumn levels. There was no significant difference across seasons in females. Comparison between the sexes revealed levels 33.1% higher in males than in females ($P < 0.019$), with winter levels of this polysaccharide reaching 52.21% higher in males than in females.

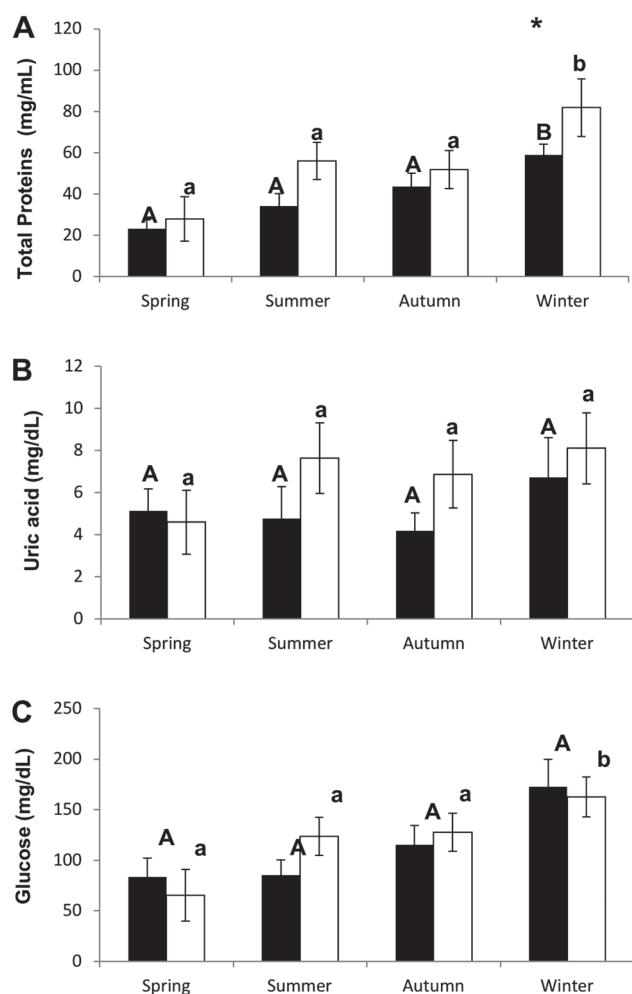


Figure 2. Seasonal plasma levels of total protein (A), uric acid (B), and glucose (C) over the course of 1 year in *Tropidurus catalanensis*. Bars represent mean \pm SE. Black bars = males; white bars = females. Different letters denote a significant difference ($P < 0.05$). Uppercase letters = males; lowercase letters = females. An asterisk (*) denotes a difference in the response curve of males and females over the course of the year. The number of animals collected was of 38 males (spring = 13, summer = 10, autumn = 11, and winter = 4), and 35 females (spring = 7, summer = 10, autumn = 10, and winter = 8).

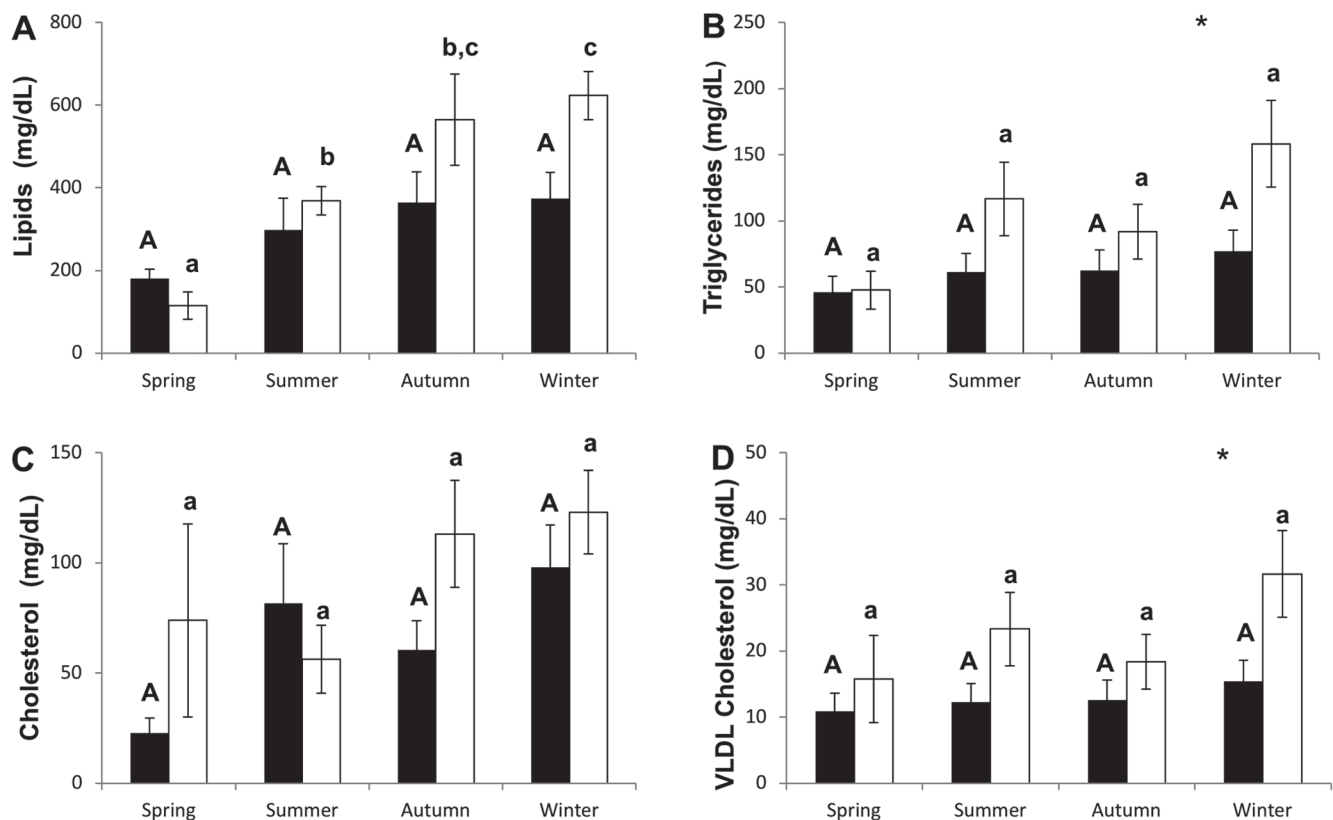


Figure 3. Seasonal total lipids (A), triglycerides (B), total cholesterol (C), and VLDL cholesterol (D) over the course of 1 year in *Tropidurus catalanensis*. Bars represent mean \pm SE. Black bars = males; white bars = females. Different letters denote a significant difference ($P < 0.05$). Uppercase letters = males; lowercase letters = females. An asterisk (*) denotes a difference in the response curve of males and females over the course of the year. The number of animals collected was of 38 males (spring = 13, summer = 10, autumn = 11, and winter = 4), and 35 females (spring = 7, summer = 10, autumn = 10, and winter = 8).

In males, triglyceride levels (Fig. 4D) differed significantly between summer and winter months, with summer levels 90.54% higher than those measured in winter. There was no significant difference across seasons in females, in which liver triglyceride levels were almost twice as high as those measured in males (females: $\bar{X} = 10.25$ mg/g; males: $\bar{X} = 5.85$ mg/g).

DISCUSSION

Results obtained in the present study reveal that *Tropidurus catalanensis* exhibits a clear pattern of seasonal variation in metabolism, as reflected mainly in levels of plasma total protein, liver glycogen, total lipids, and triglycerides in males and females; and in blood glucose, total lipids, and liver protein levels in females. It bears stressing that all body condition indices analyzed (GSI, his, and abdominal fat) varied over the course of the year. The winter months (when the lowest air temperatures solar radiation levels were recorded) appear to trigger a reduction in overall metabolism, most intensely in males,

and the reproductive period appears to be the biological event associated with the greatest energy demand and mobilization of energy stores in females. Vieira et al. (2011) demonstrated the positive association between species activity and variations in temperature for the same population of lizards used in this study.

In spring, which corresponds to the reproductive peak of the species, we observed the lowest circulating protein levels in both sexes. This may be attributable to a reduction in foraging caused by changes in the social behavior of males and females (Arruda, 2009), as well as increased energy requirements to support reproductive events, particularly in females, which allocate nutrients to gamete production, vitellogenesis, and/or offspring (Clutton-Brock, 1991). According to Lourdaís et al. (2004), some species not only mobilize lipid stores for reproduction, but also require essential amino acids—obtained from dietary intake or protein catabolism—for offspring development. In addition, species that tend to exhibit dietary restriction during the reproductive period will also use their lipid and protein stores to maintain energy metabolism (Cherel et al., 1992).

After the reproductive period, males and females exhibited a similar pattern of response to circulating protein levels, whereby levels increased until reaching significantly higher values in the winter months. This pattern might be associated with recovery after the end of the reproductive period. We cannot rule out the hypothesis of reallocation of protein from liver, levels of which declined from autumn onward, reaching significantly lower levels in winter, particularly in females; a similar pattern was observed in males, although the difference was not significant. Throughout the seasonal cycle, circulating protein levels were always higher in females than in males, which might be associated with higher energy demands for reproduction in females, as suggested by Clutton-Brock (1991) and Lourdais et al. (2004).

Uric acid is the main nitrogenous waste product of protein catabolism in reptiles and the end product of degradation of dietary nucleic acids; normal values for blood uric acid are 0–10 mg/dL for most reptiles (Maixner et al., 1987). In this study, we did not detect any significant variation in circulating uric acid levels in either sex, which might suggest a constant protein supply from degradation

of protein from the liver (particularly in winter) and/or of dietary origin (during the other seasons of the year). Intake of these items is consistent with the hypothesis described above.

Blood glucose and plasma protein levels of these animals exhibited a clear upward trend from the summer months onward (i.e., after the reproductive period), peaking in winter. This is probably attributable to increased food intake after the reproductive period of the species, in the summer and autumn months. A study conducted by Gist (1972) on starved *Anolis carolinensis* (Voigt, 1832) lizards demonstrated a significant increase in glucose concentrations after refeeding. The glucose peak seen in both sexes in the winter months, coupled with a reduction in liver protein levels, suggests increased utilization of amino acids from protein catabolism for glucose synthesis through gluconeogenesis (Lourdais et al., 2004). Studies have shown that hepatic gluconeogenesis plays an essential role in maintaining glycemia during periods of starvation, as may occur during the winter in the species studied herein (Cherel et al., 1992). Further evidence for this hypothesis is provided by the increase in liver

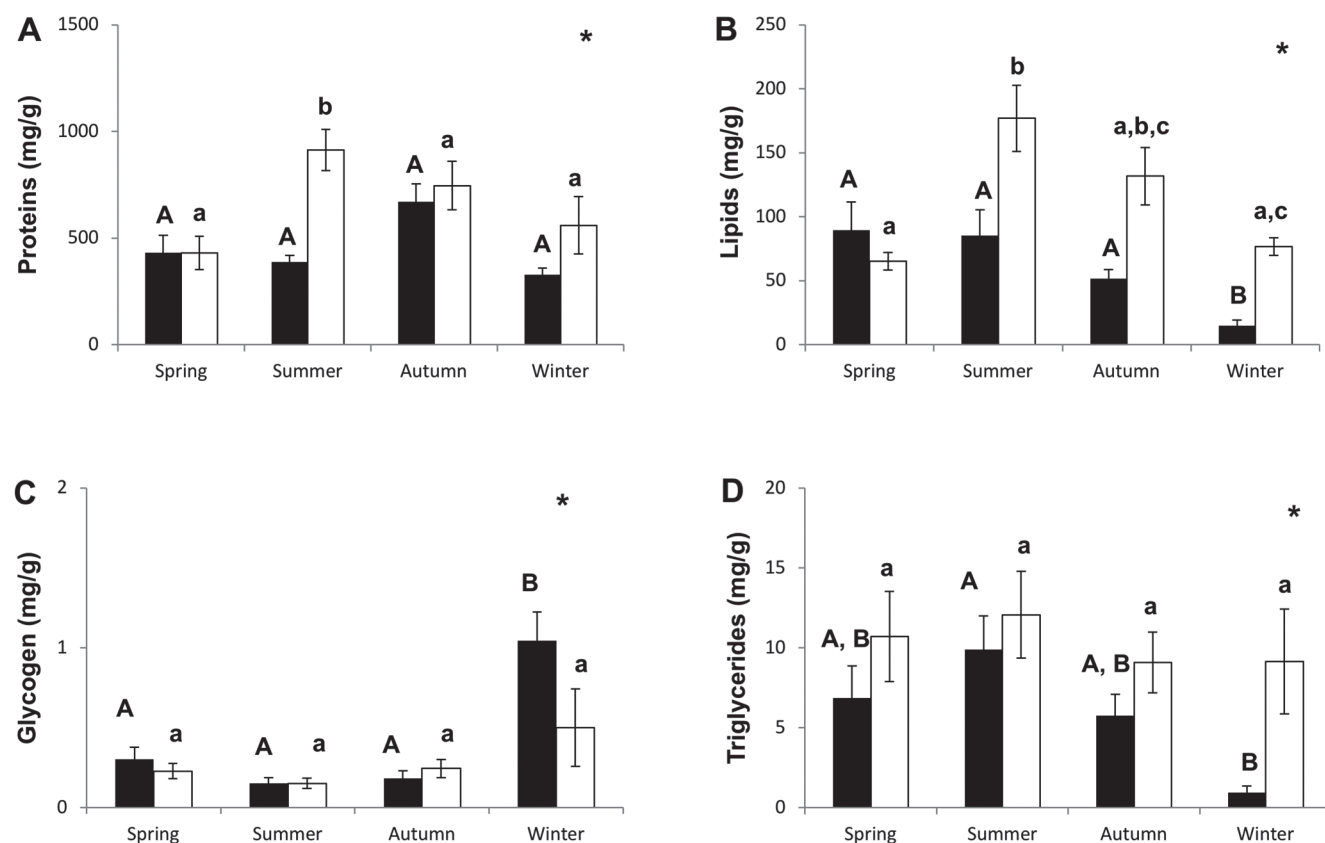


Figure 4. Seasonal hepatic tissue levels of total protein (A), glycogen (B), total lipids (C), and triglycerides (D) over the course of 1 year in *Tropidurus catalanensis*. Bars represent mean \pm SE. Black bars = males; white bars = females. Different letters denote a significant difference ($P < 0.05$). Uppercase letters = males; lowercase letters = females. An asterisk (*) denotes a difference in the response curve of males and females over the course of the year. The number of animals collected was of 38 males (spring = 13, summer = 10, autumn = 11, and winter = 4), and 35 females (spring = 7, summer = 10, autumn = 10, and winter = 8).

glycogen levels and maintenance of these levels, observed in males and females respectively, during the winter. Total hepatic protein levels measured in this study suggest that the reduction observed between autumn and winter is attributable to protein uptake by the liver for subsequent conversion into carbohydrates or lipids, which will then be used for glucose/glycogen synthesis or as a source of energy.

Liver glycogen levels remained constant throughout the year until winter, at which time levels increased in both sexes. The main role of liver glycogen is to maintain circulating glucose levels, particularly during periods of starvation. It is likely that, during the year, glycogen plays an auxiliary role in maintaining blood glucose levels in these animals; however, during the winter, it is possible that synthesis of this polysaccharide from glucose formed via gluconeogenesis occurs, alongside a reduction in metabolic rate due to low ambient temperatures and radiation (Table 1). Unlike in *Tropidurus catalanensis*, Abdel-Raheem et al. (1989) demonstrated in the Kenyan sand boa, *Eryx colubrinus* (Linnaeus, 1758), and Schneider's skink, *Eumeces Schneideri* (Daudin, 1802) that glycogen depletion occurs during hibernation; in both species, this period of reduced activity and energy requirements leads to inactivation or reduction of glucogenesis, as hepatic glycogen stores will be used to maintain blood glucose. However, a study conducted by Haddad (2007) suggests that animals may undergo physiological adjustments during periods of prolonged dormancy and starvation, so that lipids are used as an energy source and the contribution of carbohydrates to the maintenance of homeostasis is reduced.

Total lipid levels also exhibited a marked upward trend after the end of the reproduction period, particularly in females. Lipids are important sources for energy stores and are used in such distinct processes as development, maintenance of homeostasis, and reproduction (Derickson, 1976). In *Tropidurus catalanensis*, lipid utilization appears to take place largely during the reproductive period, and most intensely in females. Synthesis of sex hormones and gametogenesis (in both sexes), as well as synthesis of the glycolipoprotein vitellogenin in the liver (in females; Gavaud, 1986), is likely the determinant of the greater use of lipid stores by females. This response pattern is corroborated by the marked regression of abdominal fat index (in both sexes) and increase in GSI and HSI (in females) observed in the spring. Several studies of lizards and snakes (Gavaud, 1986; Lacy et al., 2002; Van Dyke and Beaupre, 2011) suggest that energy expended in vitellogenesis is a massive reproductive effort on the part of the female.

Triglycerides are a major source of lipid reserves, and circulating levels of this metabolite were found to remain constant year-round in males and females alike. However, females exhibited a trend toward increased plasma levels in the autumn and winter (i.e., outside the reproductive

period), which suggest the liver might be allocating these energy stores to other tissues, particularly abdominal fat, where greater lipid storage will occur. This allocation is likely related to recovery from the energy expended on vitellogenesis, as well as storage for use during the next reproductive period, as found by Lacy et al. (2002) in the tree lizard *Urosaurus ornatus* (Baird and Girard, 1852). No such trend is observed in males.

Cholesterol is a structural component of cell membranes and plays a major role in the synthesis of several hormones, vitamin D, and bile acids. In the present study, no significant variation in circulating cholesterol levels was observed in either sex; nevertheless, a clear trend toward increased cholesterol levels was seen in males during the spring. This may be associated with production of testosterone, which plays important roles in gametogenesis and in supporting reproductive behaviors, including territory defense, agonistic interactions, and mating (Moore, 1986). Females also exhibited a trend toward increased circulating cholesterol levels, but in the colder seasons; this may suggest fat storage for vitellogenesis, as is observed with triglyceride levels (Gillet and Cruz, 1981).

Lipoproteins are composed of varying amounts of cholesterol and its esters, triglycerides, phospholipids, and apoproteins, being soluble in plasma due to the hydrophilic nature of the protein portion. VLDL is a plasma lipoprotein that promotes the transport of lipids, mainly triglycerides, from the liver and secondarily from the gut to the tissues. Higher levels of this metabolite may indicate peaks in the feeding activity and increased transport of fat to the peripheral tissues, allowing the storage of this metabolite or derivatives for later use. In all seasons, the levels of this lipoprotein were higher in females than in males, which may be related to a higher energy demand for vitellogenesis, as reported by Van Dyke and Beaupre (2011) to snakes.

Liver total lipid profile was inversely proportional to glycogen levels, which corroborates the hypothesis of gluconeogenesis, as lipids may be undergoing allocation to other tissues and/or used in the synthesis of ketone bodies (Williamson, 1981), an important alternative energy substrate in settings of reduced metabolism; hence, this may occur in the winter months. Similar results were obtained in a study of seasonal dormancy in juvenile tegus (Haddad, 2007). In both sexes, liver total lipid levels remain constant throughout the seasons, except in males, which experience a significant decline in winter.

The pattern found for hepatic tissue stores in males and females may suggest starvation during the winter, not due to lack of food (as this is a generalist species) but rather to restricted foraging due to low ambient temperatures. Future studies on the degree of stomach fullness and stomach fullness indices should be conducted to test this hypothesis.

Body condition indices also showed a clear pattern of seasonal variation, strongly correlated with reproduction, in *Tropidurus catalanensis*. Males did not exhibit significant fluctuation in HSI and had lower glycogen and triglyceride stores in liver. Conversely, in females the liver accounts for up to 8% of total body weight in the spring, a proportion that declines markedly to 1% in the summer. Females might use hepatic stores of carbohydrates and lipids in vitellogenesis, which would explain this reduction in the summer. According to Van Dyke and Beaupre (2011), some reptile species exhibit marked liver enlargement due to proliferation of precursors involved in the process of vitellogenesis.

The abdominal fat index revealed sexual dimorphism in this species: males appear to use abdominal fat predominantly to maintain homeostasis in the winter, whereas females keep their fat stores intact, prioritizing allocation of lipid reserves to reproduction during the spring. The same pattern was observed by Lacy et al. (2002) in *Urosaurus ornatus*. In the winter, more females than males were observed in the field (personal observation), which might suggest that males remain inactive during colder periods, consuming their fat stores, while females continue to forage despite unfavorable environmental conditions. Derickson (1976) reported that the sagebrush lizard, *Sceloporus graciosus* (Baird and Girard, 1852), uses its lipid reserves (especially fat bodies) to maintain homeostasis during the colder months, allocating whatever lipids remain to reproduction. The same pattern was observed in male *Tropidurus catalanensis*. GSI values were inversely proportional to the abdominal fat index, especially in females during the reproductive period (spring). Hahn and Tinkle (1965) reported an association between induction of vitellogenesis by estradiol and consequent mobilization of body fat to the gonads in the side-blotched lizard, *Uta stansburiana* (Baird and Girard, 1852).

Results obtained reveal a clear pattern of seasonal variation in markers of intermediary metabolism of males and females; and all body condition indices analyzed. The parameters studied can be used to evaluate the impact of changes in environmental conditions on these animals. The reproductive period, which occurs in spring and early summer, appears to be associated with the greatest energy demand and mobilization of energy stores, mainly in females. The response pattern observed during the reproductive period suggests that this time represents a critical point in terms of the animal's adaptation to its life cycle.

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