



## Relationships between food shortages, endoparasite loads and health status of golden-headed lion tamarins (*Leontopithecus chrysomelas*)

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**Abstract:** Both anthropogenic actions and abiotic parameters, such as rainfall, temperature and photoperiod, can affect fruit and flower availability for animals, which consequently affects nutritional status and thus animals' health. Herein, we investigated whether abiotic factors are related to changes in fruit availability that can lead to changes in feeding behavior and, consequently, in endoparasite load and general health status in two groups of golden-headed lion tamarins (*Leontopithecus chrysomelas*) living in degraded fragments of Atlantic forest in Southern Bahia, Brazil. We detected that there was a high variation in availability of ripe fruits throughout the year, with lower availability occurring at the end of spring and beginning of summer. Despite this, there was no difference in tamarins' general health status, body mass and blood counts between seasons. This is probably because during native fruit scarcity, the tamarins eat cultivated species, such as banana (*Musa* spp.) and jackfruit (*Artocarpus heterophyllus*). Temperature and daylength were negatively correlated with golden-headed lion tamarin endoparasite loads. Contrary to our expectations, endoparasite loads are not linked to fruit scarcity and consequent changes in feeding behavior. Nevertheless, we found higher parasite diversity in the group of golden-headed lion tamarins that occupied the smallest home range. The smaller the area available, the greater the contact with parasites the animal will have, as they are forced to travel constantly along the same routes in the forest, increasing infection risk and re-infection rates. Our results highlight how animals' health is associated with environmental health as well as the need for constant monitoring to ensure the effective conservation of endangered species, such as the golden-headed lion tamarin.

**Keywords:** biodiversity loss; conservation; fruit availability; one health; parasites; primates.

## Relações entre escassez alimentar, carga endoparasitária e estado de saúde do mico-leão-de-cara-dourada (*Leontopithecus chrysomelas*)

**Resumo:** Parâmetros abióticos, como precipitação, temperatura e fotoperíodo, podem afetar a disponibilidade de frutos e flores para os animais, o que consequentemente afeta o estado nutricional e a saúde dos animais. Neste estudo, investigamos se fatores abióticos estão relacionados com alterações na disponibilidade de frutos, o que pode levar a mudanças no comportamento alimentar e, consequentemente, na carga de endoparasitas e estado de saúde geral em dois grupos de mico-leão-de-cara-dourada (*Leontopithecus chrysomelas*) que vivem em fragmentos degradados de Floresta Atlântica no sul da Bahia, Brasil. Detectamos que houve grande variação na disponibilidade de frutos maduros ao longo do ano, com menor disponibilidade no final da primavera e início do verão. Apesar disto, não houve diferenças no estado geral de saúde, na massa corporal ou nas contagens de células sanguíneas dos animais entre as estações do ano. Isto provavelmente ocorreu porque durante a escassez de frutos nativos, os micos comem espécies cultivadas, tais como a banana (*Musa* spp.) e jaca (*Artocarpus heterophyllus*). A temperatura e a duração do dia foram negativamente correlacionadas com a carga de endoparasitas de mico-leão-de-cara-dourada. Contrário ao previsto, a carga de endoparasitas não está ligada à escassez sazonal de frutos e consequentes mudanças no comportamento alimentar. Entretanto, encontramos maior diversidade de endoparasitas

no grupo de mico-leão-de-cara-dourada que usou uma área de vida menor. Quanto menor a área disponível, maior o contato com parasitas, porque os micos são forçados a se deslocar constantemente pelas mesmas rotas na floresta, aumentando o risco de infecção e as taxas de reinfeção. Nossos resultados destacam como a saúde dos animais está associada à saúde ambiental, bem como a necessidade de monitoramento constante para a conservação eficaz das espécies ameaçadas de extinção, como o mico-leão-de-cara-dourada.

**Palavras-chave:** perda de biodiversidade; conservação; disponibilidade de frutos; saúde única; parasitas; primatas.

## Introduction

Abiotic factors, such as rainfall (Opler et al. 1976), temperature (Williams-Linera 1997), photoperiod (Rivera et al. 2002) and soil moisture (Lobo et al. 2003), influence phenological processes in plants, causing fluctuations in fruit and flower availability for animals (Mendoza et al. 2017). Anthropogenic actions, which cause habitat fragmentation, also impact abiotic parameters and fruit availability (Mendoza et al. 2017). Some non-human primate species can change their feeding behavior as a result of food shortage periods caused by both abiotic factors and anthropogenic impact. Howler monkeys (*Alouatta palliata mexicana*), for example, change their diet, including items with lower nutritional and energy value, increasing the intake of leaves as a strategy to counterbalance the decreased availability of ripe fruits (Asensio et al. 2007). On the other hand, black howler monkeys (*Alouatta pigra*) increase the time spent foraging on the ground rather than in trees, due to food scarcity in small forest fragments due to human action, exposing themselves to terrestrial predators (Pozo-Montuy & Serio-Silva 2007).

The nutritional alternatives may lead to immunosuppression, resulting in increased susceptibility to parasites (Chapman et al. 2006). When western gorillas (*Gorilla gorilla*) experience a scarcity of food during the dry season, they usually show higher parasitic load compared to the rainy season and more food availability (Masi et al. 2012). Changes in environmental temperature and rainfall may intensify parasite infection due to increased host biomass or increased exposure to parasite infection stages (Behie et al. 2013). Moreover, environmental changes can influence the ecological balance between vector, parasite and host as well (Daszak et al. 2000, Masi et al. 2012). Chimpanzees (*Pan troglodytes schweinfertii*) living in degraded forest fragments show increased infection by *Oesophagostomum* sp., which was associated with intensification of rainfall and decrease in temperature (McLennan et al. 2017). In this regard, efforts should be made to ensure the interpretation of 'animal health' within the One Health concept (Thompson 2013) for wild animals, monitoring deleterious effects of food shortages and investigating the effects on endoparasite load.

The golden-headed lion tamarin (*Leontopithecus chrysomelas*) is endemic to the Atlantic Forest of Southern Bahia, Brazil, and it is classified as an endangered (EN category) species, mainly due to deforestation (Kierulff et al. 2008). Most populations of *L. chrysomelas* are found in degraded areas or in cocoa agroforestry systems, known as *cabruca* (Raboy et al. 2004, Oliveira et al. 2011, Catenacci et al. 2016a). Costa et al. (2020) reported endoparasite infection in groups of golden-headed lion tamarins living in degraded areas, named Degraded Forest fragments embedded within an Agricultural Matrix of pastures (DFAM), but not in populations living in *cabruca*. These authors associate these findings with individual behavioral traits and endoparasite infection, possibly caused both by the intake of infected

insects during food scarcity and by social grooming. Nevertheless, the intake of insects may be associated with abiotic factors, which alter food availability and consequently parasitological cycles, causing increased infection of animals by parasites (Masi et al. 2012). Thus, we will test the hypothesis that reduced fruit availability caused by abiotic factors affects the general health status of golden-headed lion tamarin. Therefore, herein, we aimed to investigate whether abiotic factors (rainfall, temperature and daylength) and fruit availability were related to changes in feeding behavior (exploratory and food intake), general health status and, specifically, endoparasite loads in golden-headed lion tamarins living in degraded areas of Atlantic forest in Southern Bahia, Brazil. If abiotic factors indeed influence fruit availability, we predict greater availability of ripe fruits in the months with higher rainfall, lower temperatures and shorter daylength, as observed in the southeast of Bahia (Pessoa 2008, Pessoa et al. 2012, Catenacci et al. 2016a). Moreover, if fruit availability indirectly influences endoparasite load, as suggested by Costa et al. (2020), we expect that during lower availability of ripe fruits, tamarins will show higher endoparasite infection, because they increase their substrate exploration to supplement feeding with insects, as observed by Guidorizzi (2008). Furthermore, if our previous predictions are confirmed, and if higher endoparasite load results in detrimental effects on general health status of tamarins, as verified by Monteiro et al. (2010), we also expect differences in body mass, clinical measures and blood cell counts between seasons.

## Material and Methods

### 1. Animals and study area

We studied 12 free-living golden-headed lion tamarins (*L. chrysomelas*) (10 adults and 2 subadults), belonging to two groups – named RIB (N = 6) and MRO (N = 6) – inhabiting fragments of Atlantic Forest (details in Table 1). The number of individuals in each group varied throughout the study period due to events such as births, predation, disappearance and migration to other groups. For instance, the dominant male of the RIB group (82M, Table 1) was preyed upon on December 13<sup>th</sup>, 2016. Our suspicion that this male underwent predation is based on hearing an alarm vocalization in the forest, after which we did not see the animal again. Sometime later, field assistants found its radio collar on the ground. The day after the predation, another adult male (126M, Table 1) joined the group. In turn, in the MRO group, the number of individuals varied from six to eight in November 2016 with the birth of twins. The data collected on the health and behavior of these infants, however, did not enter into the analyses presented here. At the beginning of the study, we classified as adults those individuals older than 18 months and/or weighing above 550g, following Dietz & Barker (1993); while sub-adults were those between 12 and 18 months, following Miller et al. (2003).

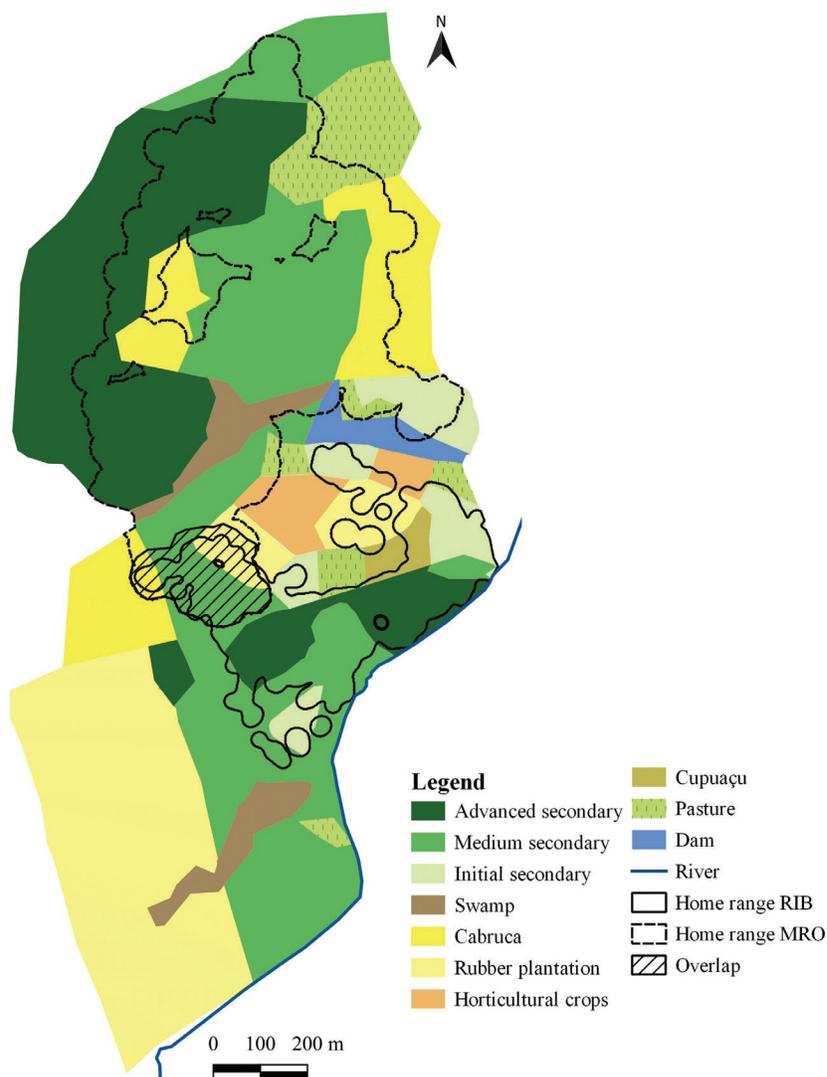
**Table 1.** Characterization of individual golden-headed lion tamarins in each group.

Animal	Group	Sex	Weight (kg)	Length (mm)	Observations
82M	RIB	M	0.65	610	Breeder
92F	RIB	F	0.69	590	Breeder
93F	RIB	F	0.65	605	Breeder
118M	RIB	M	0.61	607	
119F	RIB	F	†	†	
126M	RIB	M	0.58	615	
102M	MRO	M	0.58	690	Breeder
115M	MRO	M	0.63	636	
120F	MRO	F	0.48	565	Subadult
121F	MRO	F	0.43	586	Subadult
125F	MRO	F	0.61	630	
1F	MRO	F	†	†	Breeder

Symbol codes: RIB: Ribeiro; MRO: Manoel Rosa; Sex: M: male; F: female.

† No data available.

The groups live in a degraded area, named Degraded Forest fragments embedded within an Agricultural Matrix of pastures (DFAM) (Costa et al. 2020) (Figure 1). Besides the cultivation of cocoa (*Theobroma cacao*), through the traditional system of growing cocoa under the native canopy, which is locally known as ‘cabruca’, this area is characterized by forest fragments (classified as advanced secondary, medium secondary and initial secondary forest fragments, Figure 1) interspersed with different types of agricultural activities such as horticultural crops (vegetables, medicinal, aromatic, and ornamental plants), pasture, rubber plantation (*Hevea brasiliensis*) and cupuaçu (*Theobroma grandiflorum*) (Figure 1). This area belongs to two neighboring private properties (Santo Antônio and Manoel Rosa farms) located in the municipality of Una, Bahia state, Brazil (15°15’52” S, 39°8’46” W). During the study period (Aug/2016 to Aug/2017), the RIB group that lived at Santo Antônio farm occupied 17.7 ha of home range, while the MRO group that lived at Manoel Rosa farm occupied 48.1 ha of home range. Both groups share a common home range area – home



**Figure 1.** Characterization of the study area, with all the forest types (classified as advanced secondary, medium secondary and initial secondary forest fragments); sites with anthropogenic activities (‘cabruca’: cocoa (*Theobroma cacao*) under the native canopy, horticultural crops, pasture, rubber plantation (*Hevea brasiliensis*) and cupuaçu (*Theobroma grandiflorum*)); and the home range of the two groups of golden-headed lion tamarins (*L. chrysomelas*) (group Ribeiro: RIB and group Manoel Rosa: MRO) living in Una, Bahia, Brazil.

range overlap – of 3.0 ha (Coutinho 2018). Throughout the observations, we recorded some occasional contacts between the groups. At the time of contact, there were many agonistic vocalizations by all individuals in the groups and chases between males from different groups that, in some cases, ended in fights and bites. On other occasions, in contrast, we also saw copulation between males and females from different groups.

Individuals were captured prior to the start of the study in March 2016 (late summer) and recaptured in September 2016 (late winter) using tomahawk traps (0.48 m length × 0.15 m width × 0.15 m height), baited with bananas, following Dietz et al.'s (1996) procedures. Following capture, we took the animals to a field laboratory where they were anesthetized (10 mg/kg Ketamine and 0.3 mg/kg Midazolam, IM) (see Catenacci et al. 2016a) to receive a unique tattoo number, made on the interior part of their right thigh for permanent identification, and a dye mark (Nyanzol Dye®) for field observations. Thereafter, we determined the tamarins' sex, estimated their age and, besides taking blood samples, performed a clinical evaluation. We collected biometric data [body mass (kg), total and tail length (cm)]; and collected other clinical measures during sedation [heart rate (bpm); respiratory frequency (mpm); and body temperature (°C)]. One must take into account that physical restraint and sedation may have affected such measures. Later, we calculated the body mass index, which was determined by the relationship between the tamarin's body mass and size (the body weight in grams divided by the square of the head and body length in cm (excluding the tail) following Soto-Calderón et al. (2016).

We collected blood samples by puncture of the femoral vein, at the arteriovenous plexus in the inguinal region, in a maximum collection volume of 3.0 mL. These samples were stored in sterile 4.0 mL tubes containing EDTA K<sub>3</sub> and cooled for hematological analyses, which took place the day after collection. The hematocrit, leukocytes and the hemoglobin concentration were performed using an automated cell counter (ABX Vetcounter, Horiba™, Montpellier, France). The leukocyte differential count, aiming to determine the percentage of basophils, eosinophils, neutrophil rods, segmented neutrophils, lymphocytes and monocytes, was obtained from the examination of blood smears stained with Panoptic. The reference values used for the evaluation of hematological parameters were based on the intervals established for *Leontopithecus* sp. (Verona & Pissinati 2014).

One adult male of the MRO and two adult males of the RIB group were equipped with a radio collar (model RI-2D, Holohil Ltd., Ontario, Canada) for subsequent monitoring and field observation using radio-telemetry. As a rule, we established that the tamarin needed to weigh over 550 g to receive the radio-collar. Therefore, the radio-collars were placed on the dominant individuals, who were usually the heaviest. Besides being heavier, the chances that the dominant ones would leave the group and thus take the radio-collar were smaller. Tamarins were kept in the field laboratory overnight to ensure complete recovery from anesthesia and were released in the early morning in the same site where they were captured. All procedures were carried out with the assistance of a primate-specialist veterinarian.

## 2. The abiotic data collection and evaluation of temporal availability of ripe fruits

Daily temperature, total rainfall and daylength data were obtained from the INMET website ([www.inmet.gov.br](http://www.inmet.gov.br), accessed 18 June 2018)

for Una-BA Automatic Station (A437). Minimum and maximum temperatures of each day were determined and mean monthly temperature was calculated. To determine the total monthly rainfall, all daily precipitation data were summed. Finally, the mean monthly daylength was calculated based on the time the sun rose and set each day.

We used linear transects to evaluate temporal change in availability of ripe fruits from August 2016 through August 2017. Six transects were made within the home range of the studied groups. Transect size varied between 45 and 225 meters (mean = 155 meters), depending on home range size and format. We marked trees that are part of golden-headed lion tamarins' diet, following Catenacci et al. (2016a) procedures. Six to 10 trees of each species (native or not) were tagged on or near the transects, considering only trees in sites up to five meters on each side of transect, totalizing 129 marked trees, belonging to 14 species. For phenological evaluation, only trees with diameter at breast height (DBH) ≥ 5 cm were considered. In order to quantify fruiting phenophase, we considered a scale from zero to four (0 = no fruiting, 1 = between 1% and 25% of tree crown with mature fruits, 2 = between 26% and 50% of tree crown with mature fruits, 3 = between 51% and 75% of tree crown with mature fruits, 4 = between 76% and 100% of tree crown with mature fruits) (Fournier 1974). To correct the differences of crown volume between trees of the same and different species, the intensity index of mature fruits of each tree was multiplied by its diameter at breast height (DBH), producing values without unity for monthly availability of fruits of each tree (Catenacci et al. 2016a). Based on these individual scores, overall fruit availability per month was calculated for all trees monitored along transects.

## 3. Behavioral data collection and parasitological analysis of fecal sample collection

We followed the groups for 12 months in two periods (from August 2016 to January 2017 and March to August 2017) for behavioral observation. Each group was followed for two consecutive days per month, approximately 11 hours per day, resulting in 528 hours of data collection. Data collection started in the morning (approximately 0530 am), using radio-telemetry to localize the tamarins before they woke up in their sleeping site (tree hollows). Thus, groups were followed from the moment animals left the sleeping site until they returned to the same sleeping site or a different one in the late afternoon (approximately 0500 pm). The paint marks of animals disappeared in February 2017 and observations were suspended and resumed in March 2017, following the capture and remarking of the animals.

We collected behavioral data using the focal sampling method (Altmann 1974); each focal lasted 10 min/animal. Collecting 10 minutes of focal animal from the tamarins in the field was very difficult, because sometimes the animal would hide and we could not find it again. Sometimes they walked a lot, making it difficult to follow the focal animal, which led to a pause (time-off) in observation. Some days, we managed to collect two or three rounds for each animal, but on other days it was impossible to collect more than one round per individual. Thus, for the analyses, we standardized one round with all individuals of the group for the analyses (10 min/animal per day), which we had in common for all animals (from both groups) on all collection days. As we observed each group for two days each month, this totaled 20 minutes/animal/month. At the beginning of each observation day, we randomized the order in which animals were observed. If the animal

disappeared from sight, the observation was interrupted and resumed when the animal was visible again. The behaviors of animals were voice-recorded (RR-US450 Panasonic, Ontario, Canada). Later, we calculated the proportion of observation time that each animal spent on specific behavioral patterns (exploration, eating insects and eating fruits) (Table 2).

During behavioral data collection, we also collected feces samples immediately after defecation and stored these on ice-styrofoam for further qualitative and quantitative endoparasite evaluation. Sometimes the feces fell on leaves in the trees, sometimes on the forest floor. When they fell on the forest floor, we collected only the inner portion of the sample to decrease the chances of contamination. At the end of each observation day we weighed the collected feces samples and stored them in 4% formaldehyde for further parasitological analysis (see Monteiro et al. 2007) one week later. The amount of each feces sample ranged from 0.5 to 1.5 g. If more than one feces sample from the same individual was collected at different times of the day, a pool was made with these samples, which were stored in formaldehyde in the same container. The identification of parasite and parasite load (EPG: number of eggs/g of feces) was performed following the modified Ritchie technique, adopted by Monteiro et al. (2003) and Monteiro et al. (2007) for golden lion tamarin (*Leontopithecus rosalia*).

**Table 2.** Behavioral patterns recorded by direct observation of individual golden-headed lion tamarins.

Behavioral patterns	Description
Exploration <sup>†</sup>	Individual manipulates substrates such as tree trunks, bromeliads, foliage and vines, with hands, in search of food. The exploration can be initially visual (visual scanning), following manipulation of substrates.
Eating insects <sup>†</sup>	Individual grabs, chews, and intakes insects.
Eating fruits <sup>†</sup>	Individual grabs, chews, and intakes fruits.

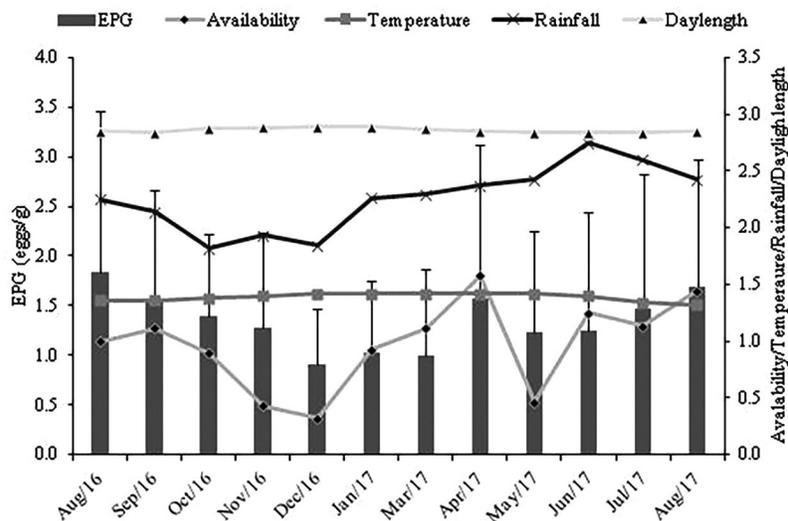
<sup>†</sup>Adapted from (Raboy & Dietz 2004).

#### 4. Statistical analysis

As the data vary in different scales we log-transformed them and, after applying Ryan-Jones tests, we verified that all data showed normal distribution. Following that, to test our predictions of associations between abiotic factors and fruit availability and between fruit availability and endoparasite infection, we applied a Pearson correlation matrix including the abiotic data (ambient temperature, rainfall and daylength), availability of ripe fruits and parasite loads. We included in this correlation matrix the mean monthly proportion of observation time that the tamarins spent on specific behavioral patterns (exploration, eating insects and eating fruits) as well. Subsequently, we performed linear regression tests to verify the relationships between ambient temperature, daylength and endoparasite loads. We used the student's t-test to compare endoparasite load in the RIB and MRO groups. We also used the student's t-test to compare the tamarins' body mass; body mass index; heart rate (bpm); respiratory frequency (mpm); and body temperature (°C) data of the five tamarins (three females and two males), which we captured in both seasons (late summer vs late winter). Blood counts [hematocrit (%); erythrocytes ( $\times 10^6/\mu\text{L}$ ); hemoglobin (g/dL); leucocytes (%); segmented neutrophils (%); and lymphocytes (%)] between seasons (late summer vs late winter) of three tamarins (two males and one female) captured in both seasons were also compared through t-tests. We used Minitab v. 19.1 software (Minitab Inc., State College, PA) for all analyses, considering  $\alpha < 0.05$ .

#### Results

Total rainfall during the study period was 1338 mm, with rainfall peak in June and July 2017, while October and December 2016 were the driest period of study (Figure 2). The mean ambient temperature did not vary much during the study period (Figure 1), with an annual average of 24.4°C (standard error – SE = 0.5). The mean minimum and maximum monthly temperatures were 21.9°C (SE = 0.1) and 26.5°C (SE = 0.5), respectively. Daylength was higher from October to March (late spring and summer) and lower from May to July (late autumn and winter) (Figure 2).



**Figure 2.** Mean (+SE) eggs per gram (EPG, left y-axis) in the RIB and MRO groups of golden-headed lion tamarins (*L. chrysomelas*) living in Una, Bahia, Brazil, mean monthly ambient temperature (°C), total monthly rainfall (mm, right y-axis), mean monthly ripe fruit availability (right y-axis) and mean monthly daylength (right y-axis) over the data collection period. All data were log-transformed.

**Table 3.** Plant species observed with mature fruits (X) and scores of ripe fruit availability (between brackets)\* during phenological survey from August 2016 to August 2017.

Family	Species	2016					2017							
		Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Anacardiaceae	<i>Anacardium occidentale</i>									X (42.9)				
Melastomataceae	<i>Henriettea succosa</i>	X (12.2)	X (13)	X (13)	X (37.8)					X (37.8)	X (37)	X (67)	X (106)	X (112)
	<i>Miconia hypoleuca</i>	X (98.9)	X (89.5)	X (53.1)	X (20.9)			X (15.8)				X (19.5)	X (6.1)	X (21.2)
	<i>Miconia mirabilis</i>	X (48.7)	X (36.3)		X (9.6)	X (19.2)	X (21.9)			X (15.8)	X (81.3)	X (164)	X (169)	X (130.1)
Mimosaceae	<i>Inga thibaudiana</i>									X (62.9)	X (9.1)	X (148)	X (116.1)	
	<i>Inga edulis</i>												X (21.3)	
Moraceae	<i>Pouroma velutina</i>							X (58.7)	X (33.9)	X (34.6)	X (22.7)	X (64.5)		
	<i>Helicostylis tomentosa</i>	X (17)			X (17)								X (21.4)	
Myrtaceae	†									X (12.8)				
Sapotaceae	<i>Chrysophyllum splendens</i>					X (50.5)					X (71.6)	X (113.8)		
	<i>Pouteria grandiflora</i>									X (53.8)	X (53.8)			

\*The fruit availability scores of each tree species were determined by multiplying the intensity index of mature fruits of each tree by its diameter at breast height (DBH). Symbol code: † No data available.

**Table 4.** Endoparasites detected in golden-headed lion tamarin groups in Ribeiro (RIB) and Manoel Rosa (MRO) groups from August 2016 to August 2017.

Occurrence period	Group	
	RIB	MRO
Aug/16	<i>Prostenorchis</i> sp.	<i>Prostenorchis</i> sp.
Sep/16	<i>Prostenorchis</i> sp.	<i>Prostenorchis</i> sp.
Oct/16	<i>Prostenorchis</i> sp.	<i>Prostenorchis</i> sp.
Nov/16	<i>Prostenorchis</i> sp., <i>Trypanoxyuris</i> sp.	<i>Prostenorchis</i> sp.
Dec/16	<i>Prostenorchis</i> sp.	<i>Prostenorchis</i> sp.
Jan/17	<i>Prostenorchis</i> sp., <i>Primasubulura</i> sp., Spiruridae	<i>Prostenorchis</i> sp.
Feb/17	†	†
Mar/17	†	<i>Prostenorchis</i> sp.
Apr/17	<i>Prostenorchis</i> sp., Spiruridae	Negative
May/17	<i>Prostenorchis</i> sp., Spiruridae	<i>Prostenorchis</i> sp.
Jun/17	<i>Prostenorchis</i> sp.	Negative
Jul/17	<i>Prostenorchis</i> sp.	<i>Prostenorchis</i> sp.
Aug/17	<i>Prostenorchis</i> sp.	<i>Prostenorchis</i> sp.

†No data available.

Ripe fruit availability varied over the study period (Figure 2, Table 3). The highest scores of ripe fruit availability happened from April to August 2017, while the lowest scores occurred from October 2016 to January 2017 (Figure 2). There were no significant differences in endoparasite loads between the RIB and MRO groups over the year (t-test = 1.48, p = 0.85, Figure 2). We found the acanthocephalus *Prostenorchis* sp. in both groups in all months, except in April and June 2017 in the MRO group (Table 4). The RIB group showed greater endoparasite diversity in comparison to the MRO group (Table 4). In this group, we found nematodes *Trypanoxyuris* sp., *Primasubulura* sp. and Spiruridae together with *Prostenorchis* sp. (Table 4).

Contrary to what we expected, there was no correlation between ripe fruit availability and insect-eating behavioral pattern ( $r_{\text{Pearson}} = 0.08$ , p = 0.809, Table 5). Furthermore, there was no correlation between endoparasite loads and the behavioral pattern of eating insects ( $r_{\text{Pearson}} = 0.28$ , p = 0.382, Table 5). However, there was a negative correlation between mean monthly ambient temperature and endoparasite loads ( $r_{\text{Pearson}} = -0.73$ , p = 0.007, Table 5). Endoparasite loads increased as ambient temperature decreased, according to the equation 1:

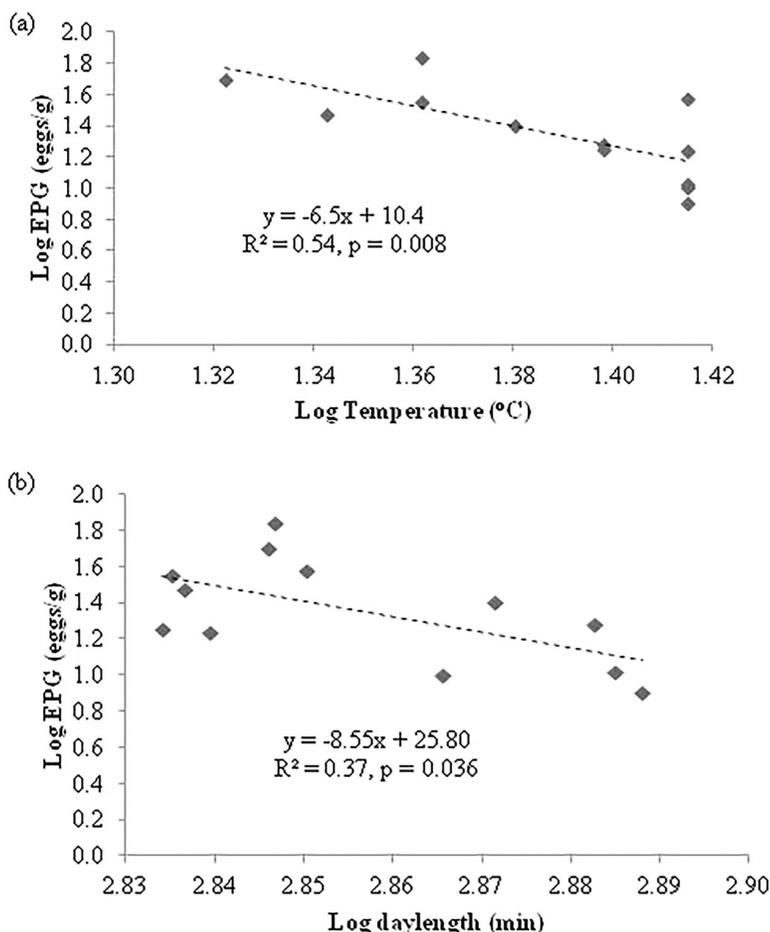
$$\text{Log } y =$$

$$-6.52 \text{Log } x + 10.39 (\text{F1}, 10 = 11.49, R^2 = 0.54, p = 0.007) (\text{Figure 3a})$$

**Table 5.** Pearson correlation matrix (coefficients and p-values) between mean monthly endoparasite loads (EPG: number of eggs/g of feces of tamarins groups), mean monthly environmental temperature (Temp), monthly scores of ripe fruit availability (Availab), mean monthly daylength (Daylength), total monthly rainfall (Rainfall), and mean monthly proportion of observation time that the tamarins spent on specific behavioral patterns (Explor: exploratory behavior; Insects: eating insects; Fruits: eating fruits) of golden-headed lion tamarins living in degraded fragments of Atlantic forest in Southern Bahia.

Variables	EPG	Temp	Availab	Rainfall	Daylength	Explor	Insects
Temp	<b>-0.73</b> (0.007)						
Availab	0.54 (0.069)	-0.40 (0.193)					
Rainfall	0.22 (0.491)	-0.20 (0.533)	<b>0.58</b> (0.048)				
Daylength	<b>-0.61</b> (0.036)	0.48 (0.112)	-0.54 (0.069)	<b>-0.74</b> (0.006)			
Explor	0.23 (0.464)	-0.05 (0.884)	-0.36 (0.247)	-0.33 (0.298)	0.07 (0.841)		
Insects	0.28 (0.382)	-0.23 (0.466)	0.08 (0.809)	-0.52 (0.085)	0.04 (0.898)	0.47 (0.121)	
Fruits	0.21 (0.521)	-0.10 (0.779)	0.39 (0.210)	0.19 (0.558)	-0.22 (0.485)	-0.12 (0.722)	0.21 (0.509)

\*Items in bold are those significant (p < 0.05).



**Figure 3.** Relationship between mean monthly ambient temperature (°C) (a), mean monthly daylength (b), and endoparasite load (EPG: eggs per g) in golden-headed lion tamarins (*L. chrysomelas*). All data were log-transformed.

**Table 6.** Means (standard error) of clinical measures and blood counts of golden-headed lion tamarins living in degraded fragments of Atlantic forest in Southern Bahia according to the season.

Parameters	Data collection		t-value	p-value
	Late summer	Late winter		
Clinical measures (n = 5)				
Body mass	581 (25)	538 (48)	0.80	0.459
Body mass index	0.01 (0.00)	0.01 (0.00)	-0.28	0.786
Heart rate (bpm)	251.2 (16.0)	244.8 (26.0)	0.21	0.840
Respiratory frequency (mpm)	45.6 (7.4)	50.4 (3.2)	-0.59	0.580
Body temperature (°C)	37.7 (0.2)	37.0 (0.2)	2.21	0.062
Blood counts (n = 3)				
Hematocrit (%)	37.2 (1.8)	40.0 (1.9)	-1.05	0.373
Erythrocytes (x10 <sup>6</sup> /μL)	5.6 (0.2)	5.8 (0.3)	-0.64	0.565
Hemoglobin (g/dL)	12.7 (0.7)	12.8 (0.6)	-0.14	0.894
Leukocytes (%)	7.1 (1.5)	7.5 (1.0)	-0.23	0.834
Segmented neutrophil (%)	80.3 (5.5)	77.7 (4.1)	0.39	0.724
Lymphocyte (%)	17.3 (5.0)	19.7 (4.4)	-0.35	0.751

There was also a negative correlation between daylength and endoparasite loads ( $r_{\text{Pearson}} = -0.61$ ,  $p = 0.036$ , Table 5). Endoparasite loads increased as daylength decreased, according to the equation 2:

$$\text{Log } y = -8.55 \text{Log } x + 25.80 (\text{FI}, 10 = 0.84, R^2 = 0.37, p = 0.036) \text{ (Figure 3b)}$$

There was a trend of positive correlation between endoparasite loads and ripe fruit availability ( $r_{\text{Pearson}} = 0.54$ ,  $p = 0.069$ , Table 5), with a tendency of higher endoparasite parasite loads in winter months with higher ripe fruit availability. Despite that, endoparasite loads were not correlated with the behavioral pattern of eating fruits ( $r_{\text{Pearson}} = 0.21$ ,  $p = 0.521$ , Table 5). Moreover, endoparasite loads were neither correlated with rainfall ( $r_{\text{Pearson}} = 0.22$ ,  $p = 0.491$ ) nor with the behavioral pattern of exploration ( $r_{\text{Pearson}} = 0.23$ ,  $p = 0.464$ , Table 5).

All animals appeared in general good health during the clinical examination performed after the captures in late summer and late winter. Both the body mass and body mass index did not differ between seasons (Table 6). There was also no difference between the seasons for the heart rate, respiratory frequency, and body temperature (Table 6); nor were there differences in blood counts between seasons (Table 6).

## Discussion

Although we did not record a great variation in the ambient temperature in southern Bahia, which remained around 24°C throughout the year, we found a negative correlation between temperature and parasite load. The golden-headed lion tamarin showed higher parasite load during the winter and relatively colder months. Additionally, daylength influenced endoparasite infections as well. The precise reasons for these associations, and their causal direction, cannot be determined from the current data. Further research may indicate whether the increase in endoparasite loads during the months of lower temperatures and with shorter days occurs due to a drop in animals' immune system, as reported for other mammals, including humans (Dowell 2001). Furthermore,

physiological changes due to alteration in photoperiod may explain the host's increased susceptibility to pathogens during colder periods (Dowell 2001).

Our study shows that periods of higher ripe fruit availability occurred during the months with higher rainfall. This result follows the general pattern described for tropical forests. In tropical forests, there is usually higher ripe fruit availability during the rainy season (Van Schaick et al. 1993, Mendoza et al. 2017). In agreement with our prediction, ripe fruit availability was higher from the end of fall and during the rainy winter months (from April to August), as previously recorded for this area (Pessoa 2008, Catenacci et al. 2016a). From March, when the days are getting shorter, the availability of ripe fruits begins to increase, and this peaks in June. In this period, the fruits with most availability were from the families Melastomataceae and Mimosaceae. These results may explain the trend of increased ripe fruit availability along with the decrease in daylength.

Differently from our prediction, there was no correlation between ripe fruit availability and endoparasite loads. Additionally, we found no correlation between the intake of insects along with the decrease in the availability of ripe fruits. This last result contradicts the reports of Guidorizzi (2008) on increased searching for insects by the golden-headed lion tamarin due to decreased availability of ripe fruits in semideciduous forest. The relationship between lower ripe fruit availability and intake of other food items, such as the intake of insects, could lead to greater infection by parasites with an indirect life cycle (Gillespie et al. 2005, Kalousova et al. 2014). However, the consumption of these invertebrates occurs throughout the year in ombrophilic forest, as observed by Catenacci et al. (2016a). Therefore, throughout the year animals can become infected with endoparasites, as the consumption of only one infected insect is enough to promote an increase in the parasitic load. Moreover, differences in floristic, climatic and anthropogenic pressures in these two habitats can influence the pattern of consumption of fruits and invertebrates by the golden-headed lion tamarin inhabiting these different forest types (Guidorizzi 2008).

Greater endoparasite diversity was found in the RIB group when compared to the MRO. Some of these endoparasites (Spiruridae,

*Primasubulura* sp. and *Prosthenorchis* sp.) are usually transmitted by the ingestion of infected invertebrates (Stunkard 1953, Melo 2004). Additionally, *Trypanoxyuris* sp., which was also found in tamarins from the RIB group, is transmitted to the host by ingestion of fecal material contaminated with parasite eggs (Stuart et al. 1998). One possible explanation for the greater parasite diversity in the RIB group, and not tested herein, is the smaller home range (17.7 ha) occupied by this group in comparison with the MRO group (48.1 ha) (Coutinho 2018). Previous studies have showed that the smaller the area available, the greater the contact with parasites the animal will have, because they are forced to travel constantly along the same routes in the forest, increasing infection risk and re-infection rates (Gillespie et al. 2005, Gillespie & Chapman 2008, Nunn et al. 2011).

*Prosthenorchis* sp., the most common endoparasite detected in golden-headed lion tamarins, is usually present in primate species (Chandler 1953, Machado-Filho 1950; Stoner et al. 2005) and other mammals, such as carnivores (Pérez et al. 2013). This acanthocephalus parasite is associated with severe intestinal disorders, and may lead to the death of the host (Melo 2004, Pissinatti et al. 2007, Catenacci et al. 2016b). This parasite is mainly found in areas with greater anthropogenic disturbance when compared to more conserved environments (Catenacci et al. 2018), and it could be used as an indicator of environmental health quality. Thus, as previously highlighted by Catenacci et al. (2018), endoparasite evaluation in free-living primates may allow a better understanding of how animals' health is associated with environmental health. Therefore, further study must be done to compare areas with different levels of anthropogenic disturbance to guarantee the causes of this parasite's presence.

Differently from our prediction as well, despite the high variation in availability of ripe fruits throughout the year, there were no differences in body mass, body mass index (which provides a more accurate measure to assess the body condition of primates in the field), clinical measures, and blood counts of tamarins between seasons. The lack of changes in general health status between relatively dry summers and rainy winters can be explained by the behavioral flexibility of tamarins facing anthropogenic changes in their habitat, as previously highlighted by Catenacci et al. (2016a) and Coutinho (2018). In the present and previous studies (Oliveira et al., 2010, Catenacci et al., 2009, Catenacci et al., 2016a, Coutinho 2018), it was possible to observe that golden-headed lion tamarins are usually observed foraging among the hundreds of plant species they use as a food source, which include both native and exotic species. Thus, in times of native fruit scarcity, the tamarins eat cultivated species, such as the banana (*Musa* spp.), jackfruit (*Artocarpus heterophyllus*), and cocoa (*Theobroma cacao*) (Catenacci et al. 2016a, Coutinho 2018). However, we should consider our results as preliminary, because we were able to collect health status data only from a small number of animals. The small sample size is justified because we used a passive trap (tomahawk traps), which allowed us to collect data from those animals that voluntarily entered the traps in both seasons. Additionally, we were not always able to collect blood samples from all captured individuals. Therefore, further study may confirm our comparisons between seasons with greater and lesser availability of ripe native fruits by monitoring a larger number of individuals throughout the year.

Our results showed that abiotic factors, such as rainfall, temperature and daylength, are related to food availability and/or endoparasite infections in golden-headed lion tamarins. Therefore, these results have important implications for *L. chrysomelas* conservation and health and for further epidemiology studies.

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## Author Contributions

Thaise da Silva Oliveira Costa: Contribute to the study's conception and design, was responsible for material preparation, data collection and analysis, contributed substantially to the interpretation of the data and wrote the initial draft of the manuscript.

Sérgio Luiz Gama Nogueira-Filho: Contribute to the study's conception and design, was responsible for data analysis and contributed substantially to the interpretation of the data as well as to the preparation of the manuscript and critical review of the text.

Kristel Myriam De Vleeschouwer: Contribute to the study's conception and design, and commented on previous versions of the manuscript and revised the text critically.

Luciana Aschoff Coutinho: Contribute to the study's conception and design, and commented on previous versions of the manuscript and revised the text critically.

Selene Siqueira da Cunha Nogueira: Principal Investigator, contribute to the study's conception and design, contributed substantially to the data analysis and interpretation of the data as well as to the preparation of the manuscript and critical review of the text.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Ethics

All methods used were approved by the Animal Welfare and Ethical Review Body at the University of Bristol (UB/18/032) in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. Additionally, all methods used were approved by the Committee of Ethics for Animal Use (CEUA) at the Universidade Estadual de Santa Cruz (proc. # 018/2015) and the Brazilian Environmental Agency (ICMBio/SISBIO) (# 23457-6 and # 471783).

## Data Availability

The datasets generated and/or analyzed during the current study are available at:

<https://10.6084/m9.figshare.17111867>

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