

# Environmental and biological drivers of prevalence and number of eggs and oocysts of intestinal parasites in red howler monkeys from Central Amazonia

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**Abstract** – Host-parasite relationships can be directly affected by host's biological aspects and environmental factors, which influence both the survival of infective forms and the incidence of parasites. However, logistical difficulties in accessing biological samples for parasitological studies makes the Amazon Forest into a poorly known region in relation to the dynamic of parasites of wild animals. Here, using 34 red howler monkeys' biological samples donated by local subsistence hunters from two Amazon habitat types (white-water flooded forest and upland forest) as an opportune alternative, we detected four intestinal parasite taxa infecting this species (two nematodes – *Trypanoxyuris* sp. and *Strongyloides* sp., one protozoan – *Entamoeba* sp. –, and one not-identified trematode, the last just found for white-water flooded forest). *Trypanoxyuris* was the most prevalent intestinal parasite (56.5% at flooded forest and 54.5% at upland forest). There was no difference between habitat types or individual sex regarding the prevalence for any parasite taxa. On the other hand, we found a strong influence of seasonality, with increasing prevalence of all parasite taxa as the river water level increased. In terms of egg and cyst counts, we found a difference between sexes (females > males,  $p = 0.002$ ) and habitat types (upland forest > white-water flooded forest,  $p = 0.02$ ), and a positive relationship with river water level ( $p = 0.002$ ). Although some of these parasite taxa can be shared between humans and howlers, further investigations are necessary to study the parasites taxonomy thoroughly and to assess the potential zoonotic cross-transmission of these pathogens to local people living in the Amazon. In this study, we unveiled a seasonal effect for howler monkeys' intestinal parasites, that also might occur in other non-human primates of the Amazon. In addition, our results on periods of high risk of intestinal parasite infection are useful to estimate future impacts of climate change on host-parasite dynamics.

**Keywords** – *Alouatta juara*, nematodes, protozoans, seasonality, subsistence hunting.

## Introduction

Parasites are intrinsic functional components of natural environments and can be used as important indicators of the health and stress levels of hosts and ecosystems (MacKenzie, 1999; Gómez and Nichols, 2013). In anthropogenically disturbed ecosystems, such as fragmented forests or those subjected to intensive hunting, the network of parasite-host natural interactions can be compromised (Gillespie and Chapman, 2006; Kowalewski and Gillespie 2009; Klain et al., 2021), benefiting opportunistic and pathogenic parasites (Hudson et al., 2006). In a healthy ecosystem, host populations should present natural parasites that use hosts as a microhabitat that protect them, favouring the maintenance of their physiological functions and propitiating their reproduction at levels that do not cause disease to the host (Hudson et al., 2006). Therefore, biological aspects of the host and environmental factors can directly affect host-parasite relationships (Klein, 2004; Altizer et al., 2006).

Most parasites are dependent on host density, with hosts occurring at higher density and/or living in larger social groups presenting higher levels of parasitic infections because these are more exposed to the infective forms of parasites (Maldonado-López et al., 2014; Helenbrook et al., 2015). However, environmental factors have been regarded as better predictors of parasitic infections than host density (Gillespie, 2006; Martínez-Mota et al., 2015). Environmental conditions such as humidity, temperature, and seasonal events can influence the survival of infective forms and affect the incidence of parasites (Altizer et al., 2006; Kowalewski and Gillespie, 2009). Furthermore, parasites can benefit from reduced efficiency of the host's immune response against parasitic infections during periods of stress occurring repeatedly or for a prolonged period (Romeo et al., 2020), i.e., increased energy expenditure and metabolic demands during pregnancy and breastfeeding periods (Maldonado-López et al., 2014; Chaves et al., 2019), defensive behaviors (Martinez-Mota et al., 2016) or reduced fruit availability (Chaves et al., 2019).

Determining the underlying biological and environmental effects and the life cycles of different parasites are crucial to assess periods of high infection risk to hosts due to increased susceptibility to parasite proliferation. Identifying these periods is important to support more accurate predictions about the future impact of climate change on host-parasite dynamics (Laffert and Kuris, 2005). Changes in parasite patterns are particularly concerning in the case of non-human primates due to the greater risks of cross-transmission of parasites with humans because of the phylogenetic proximity between these groups (Wolfe et al., 1998).

Among Platyrrhini primates, the howler monkey (*Alouatta* spp.) is the taxon with the largest number of parasites reported (at least 276 endo and ectoparasites taxa), including species of helminths and protozoa (Solórzano-García and de León, 2018). Like many non-human primate species, howler monkeys live in close social groups, which can facilitate parasite transmission and make these species particularly vulnerable to parasitic infections (Stoner, 1996). Furthermore, their wide geographic range encompassing forest environments from southern Mexico to northern Argentina and southern Brazil (Hirsch et al., 1991; Gregorin, 2006) may contribute to the high diversity of parasites associated with this genus. However, many locations are still poorly sampled in terms of parasitological studies. In the Amazon, very few parasitological studies have been carried out so far especially due to logistical difficulties of accessing appropriate biological samples, particularly faecal samples that are often used to develop coprological studies (Moreno-Black, 1978; Hopkins and Nunn, 2007).

In the Amazon, subsistence hunting is an important source of protein for rural human populations (Torres et al., 2018), and may provide relevant biological information on the parasites of game species (e.g., Conga et al., 2019; Gomez-Puerta et al., 2020). In this study, we used biological samples donated by local subsistence hunters in Central Amazonia as an opportunity to analyze the richness, prevalence and periods of higher transmission risk

of intestinal parasites in the red howler monkey (*Alouatta juara*). We also assess the factors underlying parasitic dynamics, including biological (sex and body mass of individuals) and environmental (habitat type and river water level) factors.

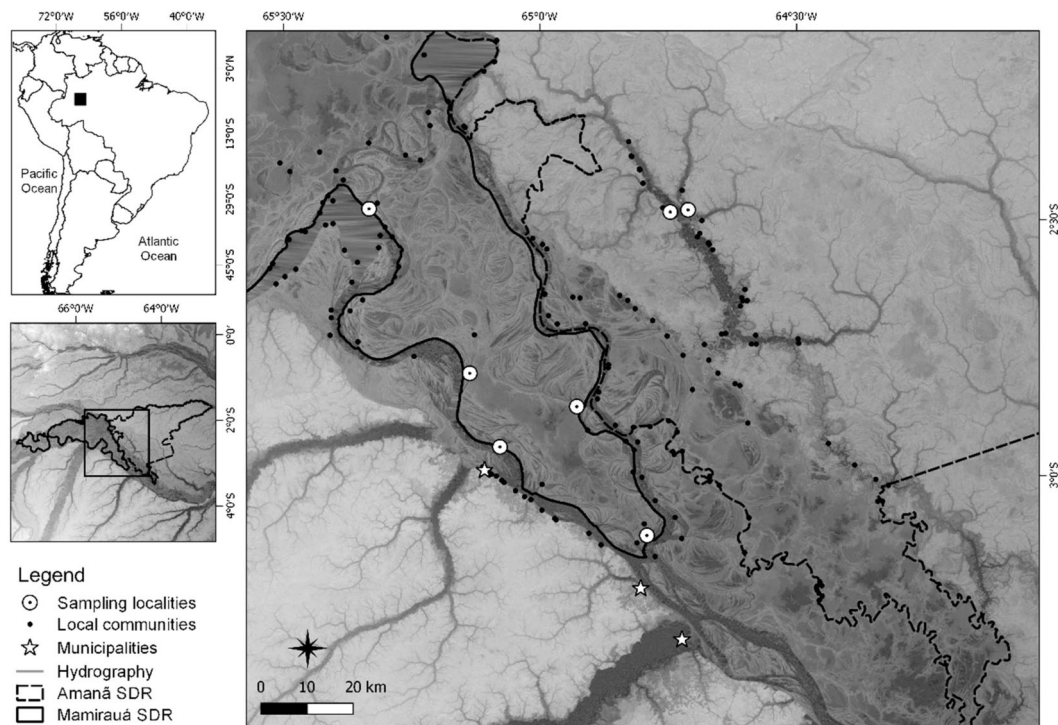
## Material and methods

### STUDY AREAS

This study was conducted in two protected areas with continuous forest in Central Amazonia, located in the state of Amazonas in northern Brazil (fig. 1). The Mamirauá Sustainable Development Reserve (MSDR) covers 1,124.00 ha of exclusively white-water flooded forests between the middle Solimões River and the lower Japurá River (2°S, 65°W). The population of residents and users of the MSDR is 11,304 people (SIMDE, 2019). The Amanã Sustainable Development Reserve (ASDR) (01°S, 64°W) covers an area of 2,350,000 ha located between

the Negro and Japurá rivers and is distributed among upland forests (covering about 73% of the reserve extension), white-water flooded forests (covering ca. 17%) and black-water flooded forests (covering ca. 10%) (SEMA, 2020). The population of residents and users of the ASDR is 5,458 people (SIMDE, 2018).

The periodic flooding of the Solimões River influences the level of rivers adjacent to the sampling locations, raising the water level by an average of 10.6 m each year, with a peak of river water level between May and July (Ramalho et al., 2009). This flood pulse results in a high deposition of sediments in the soils of white-water flooded forests, contributing to high primary productivity (Junk, 1993). This high productivity is ultimately related to the high density of red howler monkeys (26.6 ind/km<sup>2</sup>, Paim et al., 2012), and the high primate richness in this environment, which houses 11 species of primates (Pereira et al., 2017): *Atotus*



**Figure 1.** Sampling localities in the white-water flooded forest (dark gray) of the MSDR and in upland forest in paleovarzea soil (intermediate gray) in the ASDR. The lighter gray area represents upland forest *strictu sensu* not sampled in this study.

*vociferans* (Cebidae: Aotidae), *Cebuella pygmaea* (Cebidae: Callitrichinae), *Sapajus macrocephalus*, *Saimiri macrodon*, *S. cassiquiarensis* and *S. vanzolinii* (Cebidae: Cebinae); *Alouatta juara* (Atelidae: Alouattinae) and *Ateles chamek* (Atelidae: Atelinae); *Cacajao calvus calvus*, *Pithecia cazuzai* *C. c. rubicundus* and (Pitheciidae: Pitheciinae). On the other hand, the ASDR's upland environment has a very low density of howler monkeys (1.7 ind/km<sup>2</sup>, Gonçalves et al., 2014), and houses eight species of primates (Pereira et al., 2017): *Aotus vociferans* (Cebidae: Aotinae), *Saguinus inustus* (Cebidae: Callitrichinae), *Cebus albifrons*, *Sapajus macrocephalus* and *Saimiri cassiquiarensis* (Cebidae: Cebinae); *Alouatta juara* (Atelidae: Alouattinae); *Cacajao melanocephalus* (Pitheciidae: Pitheciinae) and *Cheracebus torquatus* (Pitheciidae: Callicebinae). The high primate richness and density of red howler monkeys in MSDR reflects the higher hunting pressure on howler monkeys compared to ASDR (Pereira et al., 2017).

The conservation category of these protected areas (IUCN Type VI) allows traditional human populations to reside inside them and use their natural resources according to management plans (SNUC, 2000). The communities living within these reserves are isolated from urban centres and carry out subsistence activities such as extraction of timber and non-timber forest resources, fishing, hunting, and small-scale agriculture. The sustained use zone in the MSDR covers about 1,870 km<sup>2</sup> (IDSM, 1995), whereas in the ASDR the intensive use zone is 862.87 km<sup>2</sup> and the extensive use (managed) zone is 9,215.22 km<sup>2</sup> (SEMA, 2020). The average distance covered by hunters to harvest primates during the flooded periods is 6.57 km in white-water flooded forests and 11.62 km in the upland forest. In the dry period, when the routes are made on foot, these distances decrease to 0.48 km and 2.06 km, respectively (Pereira et al., 2017). This region has a typical equatorial climate, annual temperatures varying between 22 and 36°C, relative humidity between 80% and 100% and annual rainfall of 1,500 to 3,000 mm (see El Bizri et al., 2018).

## SAMPLING COLLECTION

We used biological samples stored in the scientific collection of the Mamirauá Sustainable Development Institute, in Tefé, Amazonas state. These samples were obtained in the two study areas through a long-term participatory collection with the participation of local people. From 2002 to 2017, local subsistence hunters living in five white-water flooded forest communities in the MSDR and two upland forest communities in the ASDR collected and donated the thoracic and abdominal organs of 34 howler monkeys, 23 from white-water flooded forests (13 males, 10 females) and 11 from upland forests (eight males, three females), and preserved them in buffered 4% formaldehyde solution (v/v). No animals were killed other than those harvested as part of local hunter's usual activities. Hunters recorded the species, body mass, date, and individual code for each sample. The organs were transported to the scientific collection of the Mamirauá Sustainable Development Institute, and individually stored in glass flasks containing 70° gl alcohol.

## COPROPARASITOLOGICAL ANALYSIS

We extracted 2 g of faeces directly from the colon and rectum of the digestive system of the sampled individuals. To determine the richness of intestinal parasites, their prevalence, and egg and oocyst counts in the hosts, we conducted the spontaneous sedimentation technique (also known as Lutz or Hoffman, Pons and Janer technique; De Carli, 2001a). The spontaneous sedimentation technique is efficient to verify the prevalence and production of parasite dispersant forms due to its effectiveness in simultaneously isolating helminth eggs and protozoan oocysts from samples fixed in formaldehyde (De Carli, 2001a; Tello et al., 2012).

To increase the efficiency of spontaneous sedimentation, we prepared three slides (as suggested by Raso et al., 2019), depositing one drop (0.05 ml) of sample after sedimentation and one drop of Lugol's iodine solution under a coverslip. Each slide was examined under a light microscope. Parasites were identified based on the size, shape, and internal structure of eggs (in

the case of helminths) and oocysts (for protozoa) and compared with images from previous studies on *Alouatta*'s intestinal parasites (e.g., Eckert et al., 2006; Stoner and Di Pierro, 2006; Trejo-Macías et al., 2007; Solórzano-García and de León, 2017; Solórzano-García et al., 2020). Identification was done up to genus level whenever possible. Samples were scored as either positive or negative for each parasite genus or family found.

We systematically scanned the slide and counted the number of eggs and oocysts present in each slide with the aid of a manual counter. The overall egg and oocyst count per digestive sample was measured as the average number of eggs or oocysts found in the three slides analyzed and extrapolated to the equivalent in 1 g of faeces diluted in 10 ml (adapted from De Carli, 2001b).

We reported on the parasitic richness (number of parasite taxa), parasitic prevalence (percentage of positive animals over the total number of animals sampled) and mean and median egg and oocyst counts (mean number of eggs/oocysts per gram of faeces) for all samples and stratified for each taxon, sex and habitat type. We used the egg/oocyst counts as a proxy of the shedding of dispersal stages.

#### BIOLOGICAL AND ECOLOGICAL VARIABLES

Biological variables included the sex and body mass of each primate specimen. As environmental variables, we used information on samples' origin in terms of habitat type (white-water flooded forest or upland forest) and on river water level (in meters above sea level, m.a.s.l.) on the date of hunting of the specimen obtained through the fluviometric monitoring system (see Ramalho et al., 2009; IDSM, 2020).

#### STATISTICAL ANALYSIS

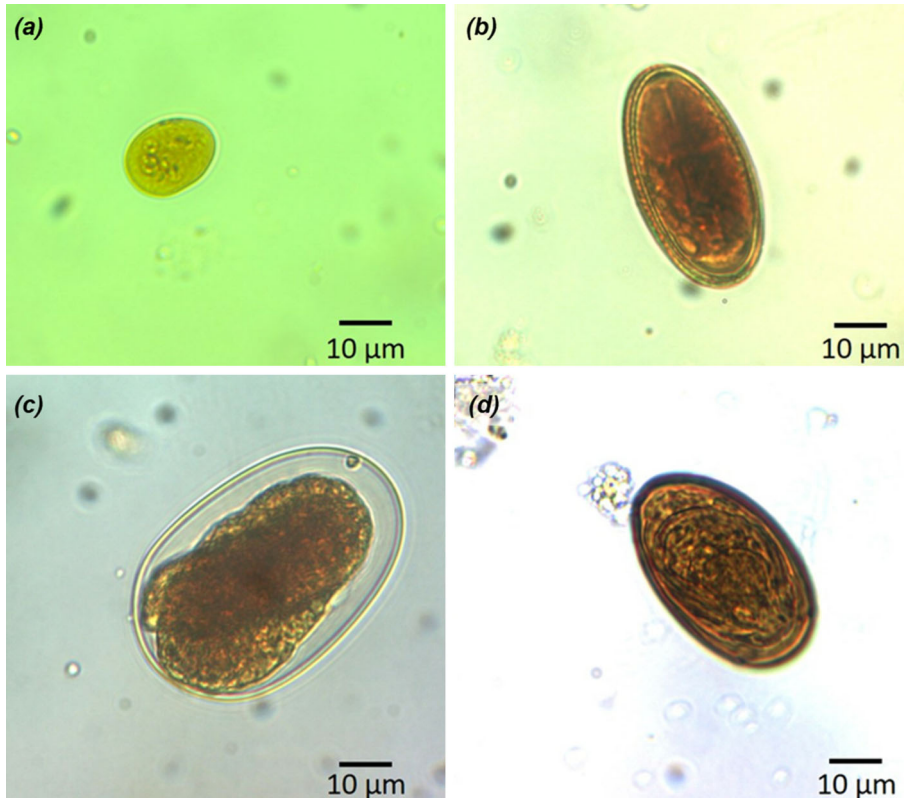
We used generalized linear models (GLMs) to assess the relationship between the parasitic prevalence and egg and oocyst counts (for infected individuals only) with the river water level, habitat type, parasite taxon, body mass

and sex of the individuals. For the parasitic prevalence model, we used the Binomial family of distribution with link function 'logit', while for the egg and oocyst counts model we used the Log-Normal distribution. We used QQ-Plots to assess the fit of the models, both in terms of normal distribution and homoscedasticity of the residuals.

We built a null model (no effect of predictor variables) and models with different combinations of predictor variables, from simplest ones (only one predictor variable) to the more complex one (all variables in the model). As body mass is collinear to sex, these two variables were never included in the same models, but rather tested independently. We also included models with interactions between river water level and parasite taxon to test for possible difference in the effects of river water level among taxa. Final models were compared based on Akaike information criterion (AIC) values, and models with the  $\Delta AIC < 2$  in relation to the model with lowest AIC were considered as with strong support (see Burnham and Anderson, 2004). We considered the best-fitted model the one with lowest number of parameters, that is fewest degrees of freedom. We used R 3.3.3 software for all statistical procedures, and the *gamlss* package (Stasinopoulos et al., 2007) for the conduction of GLMs. For variables effects, a  $p$ -value  $< 0.05$  was considered significant.

#### Results

Of the 34 samples of red howler monkeys analyzed, 21 presented at least one taxon of intestinal parasite; a general prevalence of 62%. We recorded the presence of two nematodes (*Trypanoxyuris* sp. in 55.9%, 19/34; and *Strongyloides* sp. in 26.5%, 9/34) and one protozoan (*Entamoeba* sp. 14.7%, 5/34) in faeces from both habitat types, and an unidentified trematode egg found only in a sample of a male individual from white-water flooded forest (fig. 2, table 1). Overall, 52% (11/21) of parasitized individuals were monoparasitized, and 47% (10/21) were polyparasitized, in which two parasite taxa were observed in 33% (7/21) of the infected individuals and three taxa in 14.3%



**Figure 2.** Oocyst of (a) *Entamoeba* sp.; and endoparasitic eggs found in faeces of howler monkeys (*Alouatta juara*) analysed through the spontaneous sedimentation technique: (b) *Trypanoxyuris* sp., (c) *Strongyloides* sp., and (d) Trematode egg.

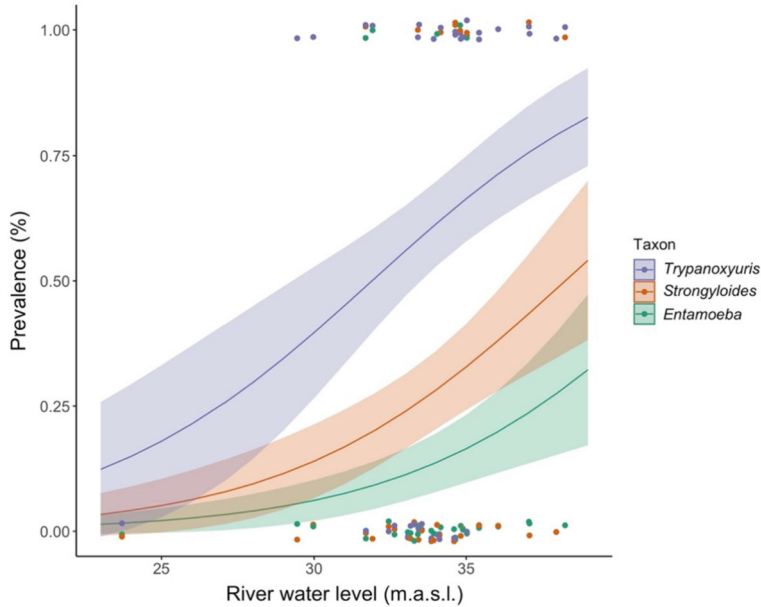
(3/21) (supplementary table S1). The datum on the trematode was not included in the statistical analyses.

The prevalence of *Trypanoxyuris* sp. was about three times higher than *Entamoeba* sp. (z-ratio =  $-3.76$ ,  $p = 0.0005$ ) and twice than *Strongyloides* sp. (z-ratio =  $-2.65$ ,  $p = 0.02$ ), but the prevalence between *Strongyloides* and *Entamoeba* sp. was not distinct (z-ratio =  $-1.46$ ,  $p = 0.31$ ). There was a slightly significant association between the rise in river water level and probability of parasite infection ( $p = 0.046$ ), with an increase of around 25% in the odds of being parasitized for each meter of water rise, independently of the parasite taxa (fig. 3, table 2). Habitat type and sex were not retained in the best model, indicating no significant effect of these variables.

The egg and oocyst counts varied according to the taxon. The amount of oocysts of *Entamoeba* sp. was similar to the amount of eggs of *Strongyloides* sp. (z-ratio =  $2.02$ ,  $p = 0.11$ ) and *Trypanoxyuris* sp. (z-ratio =  $-0.73$ ,  $p = 0.74$ ); but egg amount of *Strongyloides* sp. was significantly lower than that of *Trypanoxyuris* sp. (fig. 4c; z-ratio =  $-3.92$ ,  $p = 0.0003$ ). The number of eggs and oocysts for all taxa also increased with the rise in river water level, summarized in fig. 4a (z-ratio =  $3.37$ ,  $p = 0.002$ ) (table 2). Number of eggs and oocysts was also higher in females than males (fig. 4b; z-ratio =  $3.37$ ,  $p = 0.002$ ) and lower in white-water flooded forests compared to upland forests (fig. 4d; z-ratio =  $-2.46$ ,  $p = 0.02$ ), independently of the parasite taxon (table 2).

**Table 1.** Details of the endoparasite taxa present in *Alouatta juara* faeces, with the general prevalence and mean, standard deviation ( $\pm$ SD), median, minimum and maximum load of eggs or oocysts per gram of faeces in upland and white-water flooded forests.

Phylum Class Family	Parasite taxa	Upland forest (n = 11)			White-water flooded forest (n = 23)		
		Prevalence (%)	Mean of eggs or oocysts per gram of faeces ( $\pm$ SD)	Median of eggs or oocysts per gram of faeces (min; max)	Prevalence (%)	Mean of eggs or oocysts per gram of faeces ( $\pm$ SD)	Median of eggs or oocysts per gram of faeces (min; max)
Amoebozoa							
Entamoebidae							
Entamoebidae	<i>Entamoeba</i> sp.	18.2	3,867 ( $\pm$ 4,808.3)	3,866.70 (466.7; 7,266.7)	13	1,689 ( $\pm$ 1,991.5)	1,000 (133.3; 3,933.3)
Nemathelminthes							
Secernentea							
Oxyuridae	<i>Trypanoxyuris</i> sp.	54.5	7,466.70 ( $\pm$ 5688.5)	6,266.70 (2,333.3; 17,066.7)	56.5	9,506.70 ( $\pm$ 19,266.4)	1,733.30 (66.7; 72,800)
Strongyloidiidae	<i>Strongyloides</i> sp.	18.2	66.7 ( $\pm$ 0)	66.7 (66.7; 66.7)	30.4	791.6 ( $\pm$ 1,432.3)	266.7 (66.7; 4,266.7)
Platyhelminthes							
Trematoda		-	-	-	4	333.3 ( $\pm$ 0)	333.3 (333.3; 333.3)



**Figure 3.** Prevalence of intestinal parasites in red howler monkeys (*Alouatta juara*) in relation to the river water level in Central Amazonia. Each point represents one of the 34 faecal samples per parasite taxon, where 0 indicates that the sample was negative for a particular taxon and 1 indicates that it was positive for a particular taxon. Shaded areas represent the 95% confidence interval.

## Discussion

In this study we report on the richness, prevalence and amounts of egg and oocyst of intestinal parasites in red howler monkeys in two habitat types in Central Amazonia. Faecal samples from red howler monkeys in the wild were collected using a participatory sampling technique taking advantage of animals hunted for subsistence purpose as an opportune experimental alternative to the conventional sampling of faeces in the field. Using only one stool concentration method, we recorded the presence and frequency of two nematodes (*Trypanoxyuris* sp. and *Strongyloides* sp.) and a protozoan (*Entamoeba* sp.) in the samples. The prevalence and shedding of eggs and oocysts of these parasite taxa are synchronous with the increase of river water level in both habitats, suggesting a seasonal infectious pattern in red howler monkeys.

In primates, the exposure to parasitic infections depends on the complexity of the parasite's life cycle. Here, the most prevalent intestinal parasite observed in the red howler monkey was *Trypanoxyuris* sp.. This nematode has

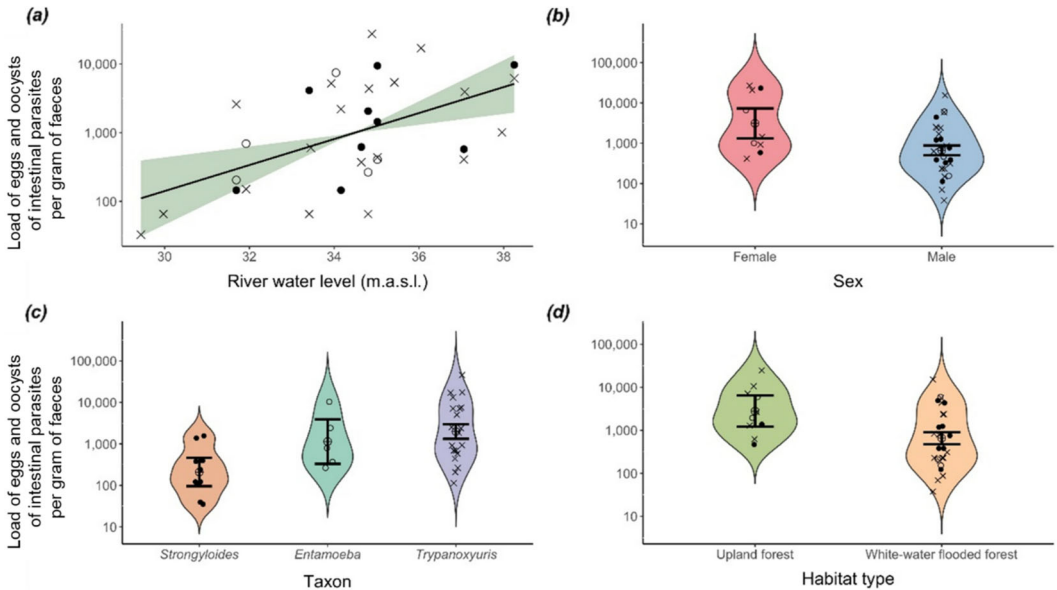
a direct life cycle with an oral-faecal transmission route (Valdespino et al., 2010). Gravid females migrate from the caecum and/or colon to deposit eggs in the animals' perianal region (Adamson, 1989). This movement induces anal itching in the host (Adamson, 1989; Ikeda et al., 2016), which can lead to the behaviour of rubbing the anogenital region (Braza et al., 1981; Hirano et al., 2008), causing reinfections of the host and contaminating the passage and foraging sites of howlers. All these characteristics of the *Trypanoxyuris*' life cycle, allied with the greater rates of eggs shedding found here, may explain its higher prevalence in red howler monkeys.

In addition, *Trypanoxyuris* spp. are one of the most common parasites associated with *Alouatta* in the wild, with records in several species within the genus (e.g., *A. palliata*, *A. seniculus*; Stuart et al., 1998; *A. guariba clamitans*, Amato et al., 2002; *A. belzebul*, Martins et al., 2008; Souza et al., 2010; *A. caraya*, *A. guariba*, *A. pigra*; Solórzano-García and Pérez-Ponce de León, 2018). This may suggest the existence of a specificity in the parasite-host



**Table 2.** Best fitted model for the parasitic prevalence (with *Entamoeba* values retained by the analysis as the intercept) and load of eggs/oocysts in red howler monkeys (*Alouatta juara*) according to AIC selection procedure.

Best-fitted model	Predictor variables	Estimate (SE)	z-value/ t-value	P-value	Family of Distribution	Link functio	AIC (k; df; ΔAIC null)
Parasitic prevalence	(Intercept)	-9.32 (3.75)	-2.42	0.015	BI	Logit	120.78 (4; 4; 16.13)
	River water level	0.22 (0.11)	2	0.046			
	Taxon ( <i>Strongyloides</i> )	0.91 (0.63)	1.46	0.145			
	Taxon ( <i>Trypanoxyuris</i> )	2.30 (0.62)	3.76	0.0002			
Load of eggs/oocysts	(Intercept)	-5.77 (4.27)	-1.35	0.19	LOGNO	Identity	640.46 (4; 7; 16.23)
	River water level	0.44 (0.13)	3.37	0.002			
	Taxon ( <i>Strongyloides</i> )	-1.69 (0.84)	-2.02	0.05			
	Taxon ( <i>Trypanoxyuris</i> )	0.55 (0.75)	0.73	0.47			
	Sex (Male)	-1.54 (0.58)	3.37	0.002			
Habitat type (white-water flooded forest)	-1.45 (0.59)	-2.46	0.02				



**Figure 4.** Load of eggs/oocysts of intestinal parasites in red howler monkeys (*Alouatta juara*) in relation to (a) river water level; (b) individual sex; (c) parasite taxon; and (d) habitat type. Full circle = *Strongyloides*, empty circle = *Entamoeba*, and cross icon = *Trypanoxyuris*. The shaded area in the linear relationship indicates the 95% confidence intervals. In the violin plots, the shaded area indicates the density of the data points, the bar indicates the 95% confidence interval and the cross in a circle indicates the mean.

relationship for these taxa. This specificity may be particularly important as an ecological indicator of environmental health (see Vitazkova and Wade, 2007). For example, in disturbed and fragmented forests, the prevalence of *Trypanoxyuris* spp. in howler monkeys is quite low (e.g., ca. 6.3% to 27%, Vitazkova and Wade, 2006; Maldonado-López et al. 2014; Helenbrook et al., 2015) compared to our results in continuous and preserved environments (ca. 56%).

*Entamoeba* species also have a direct life cycle, and the infection may occur by oral-faecal transmission or by the accidental ingestion of water and food contaminated with cysts or trophozoites (Idowu and Rowland, 2006; Aguilar-Días et al., 2011; Berrilli et al., 2011; Dong et al., 2017). The occurrence of *Entamoeba* sp. was previously recorded in four *Alouatta* species (*A. caraya*, *A. palliata*, *A. pigra* and *A. puruensis*; Solórzano-García and de León, 2018), and in some cases it could be an anthroozoonosis (Martins et al., 2008; Trejo-Macías and Estrada, 2012; Rondón et al., 2017). However, studies with improved

techniques (e.g., DNA extraction; Villanueva-García et al., 2017) are necessary to determine the zoonotic potential of this protozoan and whether the species that affects howler monkeys in our study areas are pathogenic and/or resulting from human activities.

In addition to our record for red howler monkeys, *Strongyloides* was reported in at least four other *Alouatta* species (*A. seniculus*; Phillips et al., 2004; *A. caraya*, *A. palliata* and *A. puruensis*; Solórzano-García and de León, 2018). *Strongyloides* infections may also have an anthropic origin (Gillespie et al., 2005; Chapman et al., 2006) and are commonly related to terrestrial primates due to its infection route on the ground (Gilbert, 1994; Campillo et al., 1999; White et al., 2019). *Strongyloides* infections only occur when the infective larvae penetrate the host's skin by contact with contaminated soil, or when larvae are ingested along with water or food contaminated with faeces (Campillo et al., 1999).

Although *Strongyloides* infections are commonly related to an infection route on the

ground, terrestrial locomotion in howler monkeys is rare, occurring mainly in fragmented areas with disconnected canopies or for drinking water (Bicca-Marques, 1992; Bicca-Marques and Calegari-Marques, 1995; Miranda et al., 2005). However, it is possible that other animals, such as sympatric primates like capuchin monkeys, *Sapajus macrocephalus* in both studied environments, and cairara, *Cebus albifrons*, in the upland forest, may narrow this transmission network. The use of ground for foraging is well documented for *Cebus* spp. and *Sapajus* spp. (e.g., Defler, 1979; Milano and Monteiro-Filho, 2009; Bezerra et al., 2014), as well as their association to conflicts with humans in cultivation areas (e.g., mowed; Mikich and Liebsch, 2014). These behaviors can link these primates to the contamination of resources used by howler monkeys. It is likely that the main infection route by *Strongyloides* in *Alouatta* is the accidental larvae consumption with water from tree holes contaminated with faecal material with infectious parasite forms from other howlers or mammal species, such as the abovementioned primate species. The more complex life cycle and lower likelihood of situations leading to infections of this nematode may explain its lower prevalence in our samples.

Despite being widely used as a parasitic intensity parameter, egg counts have been pointed out as an unreliable index of adult parasite load (e.g., Gillespie, 2006). However, the pattern of eggs and oocysts shedding found here is consistent with the results on parasitic prevalence, suggesting a temporal dependence. A similar pattern has recently been identified for the seasonal structure of egg counts in the faeces of vervet monkeys (*Chlorocebus pygerythrus*), meaning that if egg shedding was stochastic, the statistical model used would hardly capture any pattern (Blersch et al., 2021). Our findings show that higher values of both parasitic parameters (prevalence and counts of eggs and oocysts) were associated with the rise in river water level. High humidity in forest microclimates facilitates the viability of parasitic larvae and nematodes' eggs in the environment (Eckert et al., 2006; Cristóbal-Azkarate et al., 2010).

The formation of inactive cysts by protozoa is a strategy to keep viable organisms in the environment for an extended period, until appropriate infection conditions occur (Aguilar-Díaz et al., 2011); therefore, we believe that climatic changes during the humid periods in tropical forests may function as a cue for the activation of *Entamoeba* and other protozoans' cysts and promote the helminthic egg production. Similar association of higher eggs counts in the wet season has been reported for other howler monkeys, such as *A. pigra* and *A. palliatta* (Stuart et al., 1990; Stuart and Strier, 1995; Stoner, 1996; Eckert et al., 2006; Stoner and Di Pierro, 2006; Cristóbal-Azkarate et al., 2010; Trejo-Macías and Estrada, 2012).

The existence of a positive synchronism between the parasitic prevalence and the number of eggs and oocysts and the environmental changes resulting from the process of rising river levels makes the flooded period a season with greater risk of transmission. In flooded forests and areas adjacent to main rivers, the dynamics of rising river levels is a determining factor in several ecological processes, causing a periodic transformation of forests from terrestrial into aquatic environments (Junk, 1993; Ramalho et al., 2009). These environmental changes can result in climate conditions conducive to greater transmission of parasites. This can be especially worrying for potential zoonotic parasites, such as *Entamoeba* sp. and *Strongyloides* sp. (Dong et al., 2017; White et al., 2019). Amoebiasis is the third leading cause of deaths from intestinal parasites in the world, responsible for the death of approximately 100,000 people per year, especially children (Stanley, 2003; Nath et al., 2015). This disease may result in severe diarrhoea events, followed by weight loss and dehydration in humans and non-human primates (Verweij et al., 2003; Montenegro et al., 2010; Genoy-Puerto et al., 2016). On the other hand, in humans, strongyloidiasis, more commonly caused by *S. fuelleborni* and *S. stercoralis* (Oslen et al., 2009), may cause sepsis, bacterial meningitis and gastrointestinal haemorrhages, resulting in a high 80%

mortality rate (White et al., 2019). In immunosuppressed non-human primates, strongyloidiasis can cause weight loss, severe diarrhoea, and secondary respiratory complications, also resulting in lethal cases (Mati et al., 2014).

The dependence on wild meat as a main source of protein by Amazonian societies may facilitate the human exposure to parasites present in faeces and body fluids of hunted animals (Kilonzo et al., 2014; Ellwanger et al., 2020). Although the identification of parasites only at the genus level is one of the limitations of our study, the risks of cross-transmission of pathogens between humans and animals should not be neglected and more careful hygienical practices must be taken by human populations, especially in high river water periods. More accurate parasite diagnoses are needed to properly characterize the zoonotic risks of parasitic infections in order to develop and promote good practice protocols for the safe handling and consumption of game meat, and a healthy and secure use and management of wildlife in Amazonia (e.g., Van Vliet et al., 2017).

We found no differences between males and females in terms of parasitic prevalence, which may indicate that the opportunities for infection for both sexes are similar. However, our results show that female red howlers were more likely to have higher egg/oocyst counts. Other studies have already reported a greater load of parasite eggs in female howler monkeys, suggesting the existence of an immunosuppressive effect resulting from the physiological stress caused by increased energy expenditure and metabolic demands during pregnancy and nursing periods (Stoner and Di Piero, 2006; Maldonado-López et al., 2014; Chaves et al., 2019). Future studies should assess the interaction between the higher load of adult parasites and the eggs in females, sex hormones and reproductive status, in addition to possible effects on offspring, since exposure of infants to parasites may decrease offspring survival (e.g., Hillegass et al., 2010).

This study was carried out using samples obtained through an opportunistic and experimental sampling collection. Although training is offered to local people on how to collect the material to be donated, there was an overall

low adherence for donations. We believe that a greater participation by local populations would improve the biological sampling and increase sample size. Although our sample size was low, it is still above the minimum estimated to determine the prevalence of parasites suggested by Gillespie (2006). Furthermore, as we did not use any smoothing techniques in data analysis, nor used variables with multiple classes, our statistic models may be considered well fitted and with high predictive power. However, as we draw our conclusions based on only 34 samples, we advise caution to readers when considering our results in comparative studies.

It is possible that several other factors, as environmental or social, that we were not able to assess in this study may be behind or influencing further the prevalence and production of eggs and oocysts of the parasites. Future research with greater scope in terms of number of samples and including other predictor variables should be carried out in order to confirm the patterns discovered in this study. These new studies should also include the use of other methods of parasitological analysis (e.g., flotation; see Gillespie, 2006) aimed at parasite groups for which the sedimentation technique is less sensitive, such as helminths with eggs of lower density than the solution. In addition, the use of complementary techniques is necessary for research with the objective of carrying out an exhaustive survey of intestinal parasites, which was not our objective in the present study.

Despite the abovementioned limitations, in contrast to the conventional active collection of faecal samples in the wild by researchers, the use of faecal samples collected directly from the intestine of hunted animals is free from any contamination by parasites present in the environment and without the risk of accidentally collecting repeated samples of the same individual. In addition, we claim that samples donated by hunters have high potential for the development of further parasitological research, i.e., the use of specific techniques for the isolation of adult intestine parasites, refined taxonomic identification (e.g., Conga et al., 2015) and assessment of the intensity of parasitic infections in howler monkeys, which could not be done

hereby through the analysis of faecal material (Gillespie, 2006; Martínez-Mota et al., 2015).

## CONCLUSION

The wild red howler monkey hosts at least four intestinal parasites with different traits in terms of their transmission routes. These parasitic infections are influenced by biological and environmental variables. Females as well as animals from upland forests present higher number of eggs and oocysts of intestinal parasites. We detected a seasonal pattern of intestinal parasite dynamics, with a positive synchrony with variations in the river water level, which is likely to occur in other non-human primates and Amazonian habitats. Additional studies are needed to explain the mechanisms behind the effects of biological and environmental variables on the parasitic parameters found here. The active involvement of local populations in data collection is crucial to the development of alternative methods to fill the knowledge gaps on parasites in Amazonian primates. Additionally, it is necessary to further investigate the potential risk of cross-transmission of parasites between howlers and humans in Amazonia.

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## Statement of ethics

This study is exempted from ethics approval due to the research protocol involving the use of materials belonging to a scientific collection (Animal Use Ethics Committee of the Mamirauá Sustainable Development Institute 004/2017). The research protocol was approved

by the Chico Mendes Institute for Biodiversity Conservation in Brazil (License SISBIO n° 29092-1).

## Conflict of interest statement

The authors have no conflicts of interest to declare.

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## Author contributions

ASJ, MLO-R, JV, HREB and PM designed the study. ASJ and MLO-R collected the data. ASJ and HREB conducted the data analysis. ASJ, MLO-R, HREB and PM interpreted the results and wrote the manuscript. All authors contributed to revise the manuscript and approved the final version of the text.

## Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## Supplementary material

Supplementary material is available online at: <https://doi.org/10.6084/m9.figshare.19076939>

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