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COMPARING DIET, SEED SIZE AND RICHNESS IN FRUIT INGESTED BY RED HOWLER MONKEYS (*ALOUATTA JUARA*) IN TWO HABITAT TYPES IN CENTRAL AMAZONIA

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Abstract

Primates in the genus *Alouatta* (howler monkeys) inhabit a wide range of environments due to their flexible folivorous-frugivorous diet. However, the feeding ecology of *Alouatta* species that inhabit continuous and difficult-to-access forest environments, such as the Amazon, is poorly known. In this study, we compared diet composition, seed size, and richness of fruits ingested by Juruá red howler monkeys (*Alouatta juara*) in white-water flooded forest and upland forest. Our analysis was based on 47 stomachs donated by subsistence hunters of central Amazonia in Brazil. We found no difference in the proportion of structural parts, reproductive parts, and animal matter (arthropods) ingested between these environments. However, we found a positive relationship between the richness of ingested seeds and the water level for the white-water flooded forest. This correlation was not observed for upland forest, where other environmental factors such as precipitation may influence tree phenology. The stomach analysis revealed a high diversity of seeds and a substantial percentage of whole seeds (73%) in the red-howler monkeys' stomachs. This suggests that they have the potential to be effective seed dispersers for at least 40 taxa in the white-water flooded forest and 14 in the upland forest. The population decline or extinction of this species could have an impact on seed dispersal services, particularly for plant species with seeds larger than 20 mm of diameter, as the number of dispersing agents tends to be limited. Further studies are needed to investigate the environmental dynamics involved in the feeding ecology of red howler monkeys. These studies would help to understand the ecological impacts of local subsistence hunting and wild meat trading on seed dispersal, as well as the economic implications for human populations.

Keywords: *Alouatta juara*, Amazon, feeding ecology, seed dispersal, subsistence hunting, upland forest, white-water flooded forest

Resumo

Primatas do gênero *Alouatta* (guaribas) habitam uma ampla variedade de ambientes florestais devido à sua dieta folívoro-frugívora flexível. No entanto, a ecologia alimentar de espécies de *Alouatta* que habitam ambientes florestais contínuos e de difícil acesso, como a Amazônia, ainda é pouco conhecida. Nesse estudo, comparamos a composição da dieta, o tamanho e a riqueza de sementes ingeridas por guaribas-vermelhos (*Alouatta juara*) em florestas de várzea e de terra-firme. Nossa análise foi baseada em 47 estômagos doados por caçadores de subsistência na Amazônia central brasileira. Não encontramos diferença na proporção de partes estruturais, partes reprodutivas e matéria animal (artrópodes) ingeridas entre esses ambientes. No entanto, encontramos uma relação positiva entre a riqueza de sementes ingeridas e o nível d'água na várzea. Essa correlação não foi observada para terra firme, onde outros fatores ambientais, como a precipitação, devem influenciar a fenologia das árvores. A análise dos estômagos revelou uma alta diversidade de sementes e uma porcentagem substancial de sementes inteiras (73%) nos estômagos dos guaribas-vermelhos. Isso sugere que eles têm o potencial de serem dispersores eficazes de sementes para pelo menos 40 taxa de sementes para várzea e 14 para terra firme. Declínios populacionais ou extinções locais pode ter um impacto nos serviços de dispersão de sementes, especialmente para espécies com sementes >20 mm de diâmetro, para as quais o número de agentes dispersores tende a ser limitado nessas áreas. Mais estudos são necessários para investigar a dinâmica ambiental envolvida na ecologia alimentar de guaribas-vermelhos. Esses estudos ajudariam a compreender os impactos ecológicos da caça de subsistência local e do comércio de carne selvagem na dispersão de sementes, bem como as implicações econômicas para as populações humanas.

Palavras-chave: *Alouatta juara*, Amazônia, caça de subsistência, dispersão de sementes, ecologia alimentar, floresta de *terra firme*, floresta de várzea

Introduction

The *Alouatta* genus (Primates, Atelidae), composed of howler monkeys, has a flexible folivorous-frugivorous diet (Crockett et al., 1998). In general, howler monkeys consume large amounts of leaves and fruits (representing 50% and 34% of their feeding records, Norconk et al., 2009), with variation according to the food availability in the environment (Chapman, 1990; Hawes and Peres, 2014). This ecological flexibility allows their survival in highly diversified habitats, ranging from primary forests to small fragments (Bicca-Marques, 2003), spanning from southern Mexico to northern Argentina and southern Brazil (Hirsch et al., 1991; Gregorin, 2006). Howler monkeys play crucial ecological roles in the forests they inhabit (Julliot, 1996, 2001; Chaves et al., 2018), particularly as seed dispersers of trees with seeds larger than 12 mm diameter, which are rarely dispersed by smaller species (Julliot, 1996; Bufalo et al., 2016).

Among the environments inhabited by howler monkeys, the Amazon rainforest is characterized by a high diversity of habitats resulting from different levels of soil drainage and nutrient availability (Wittmann et al., 2006). One of these habitats is the white-water, periodically flooded forests, which are found in areas that are regularly inundated by nutrient-rich white-water rivers during the annual flood season (Junk, 1993; Ramalho et al., 2009). These forests have higher nutrient availability due to the regular flooding and sediment deposition, resulting in fertile soils that support the growth of flood-adapted plant species (Junk, 1993). However, these forests have a lower tree species diversity compared to upland forests, which are typically located in elevated areas with well-drained soil. Upland forests are characterized by diverse tree species composition and high species richness (Haugaasen and Peres, 2005a). These habitat differences may lead to distinct patterns of fruiting and fruit availability (Haugaasen and Peres, 2005a, 2005b; Hawes and Peres, 2016) and may require different strategies of primate species for obtaining sufficient nutrients. However, our understanding of how howler monkeys deal with these differences in terms of diet and ecological role in Amazonian regions is limited.

These gaps in research can be partially explained by the high costs associated with conventional methodologies to assess feeding ecology, particularly in challenging environments, such as the Amazon Forest. These habitats have high canopies that are commonly used by arboreal primates for foraging and locomotion but are difficult for observers to identify and accurately quantify food consumption, leading to observation bias towards places with greater visibility (Milton and Nessimian, 1984; Nielsen et al., 2017). Additionally, the presence of wild animal hunting by humans in these environments impairs the habituation and continuous monitoring of animals in their natural habitats, both for direct observation of feeding behavior (Altmann, 1974; Dew, 2003) or for non-invasive fecal sample collection (Moreno-Black, 1978; Nielsen et al., 2017). In such scenarios, the use of participatory methods that rely on the collaboration of local people, such as the voluntary donation of biological material by subsistence hunters, has proven to be a valuable alternative for studying ecological aspects of wild animals (Mayor et al., 2017; Jesus et al., 2022a), including diet studies of arboreal primates that are difficult to observe and monitor (e.g., Jesus et al., 2022b; Torres et al., 2022).

Studies on primate diets in different environments provide valuable information regarding various aspects of feeding behavior and directly contribute to conservation efforts aimed at protecting primate populations and their food resources. In this study, we aimed to compare the feeding ecology of red howler monkeys and assess their potential role in seed dispersal in terms of size and richness of ingested seeds in white-water and upland forests in central Amazonia. We achieved this by analyzing the contents of howler monkey stomachs that otherwise would be discarded by hunters. Specifically, we aimed to (i) identify the dietary composition of red howler monkeys, (ii) quantify the richness and number of seeds per diameter class and (iii) assess variations in seed richness in relation to seasonal environmental changes in white-water flooded and upland forests. Finally, we discuss the potential impact of red-howler monkey hunting on their ecological role as a seed disperser.

Methods

Study areas

We conducted the study in two types of environments, white-water flooded forest and upland forest, which are located in two protected areas in central Amazonia, in the state of Amazonas in northern Brazil (Figure 1). The Mamirauá Sustainable Development Reserve (hereafter Mamirauá Reserve) is situated between the middle Solimões River and the lower Japurá River (2°S, 65°W) and covers an area of 1,124,000 ha, entirely consisting of white-water flooded forests that are seasonally flooded by white-water rivers. The Amanã Sustainable Development Reserve (hereafter Amanã Reserve) covers an area of 2,350,000 ha between the Negro and Japurá rivers (01°S, 64°W). The Amanã Reserve comprises various environments, with about 73% being upland forests, 17%

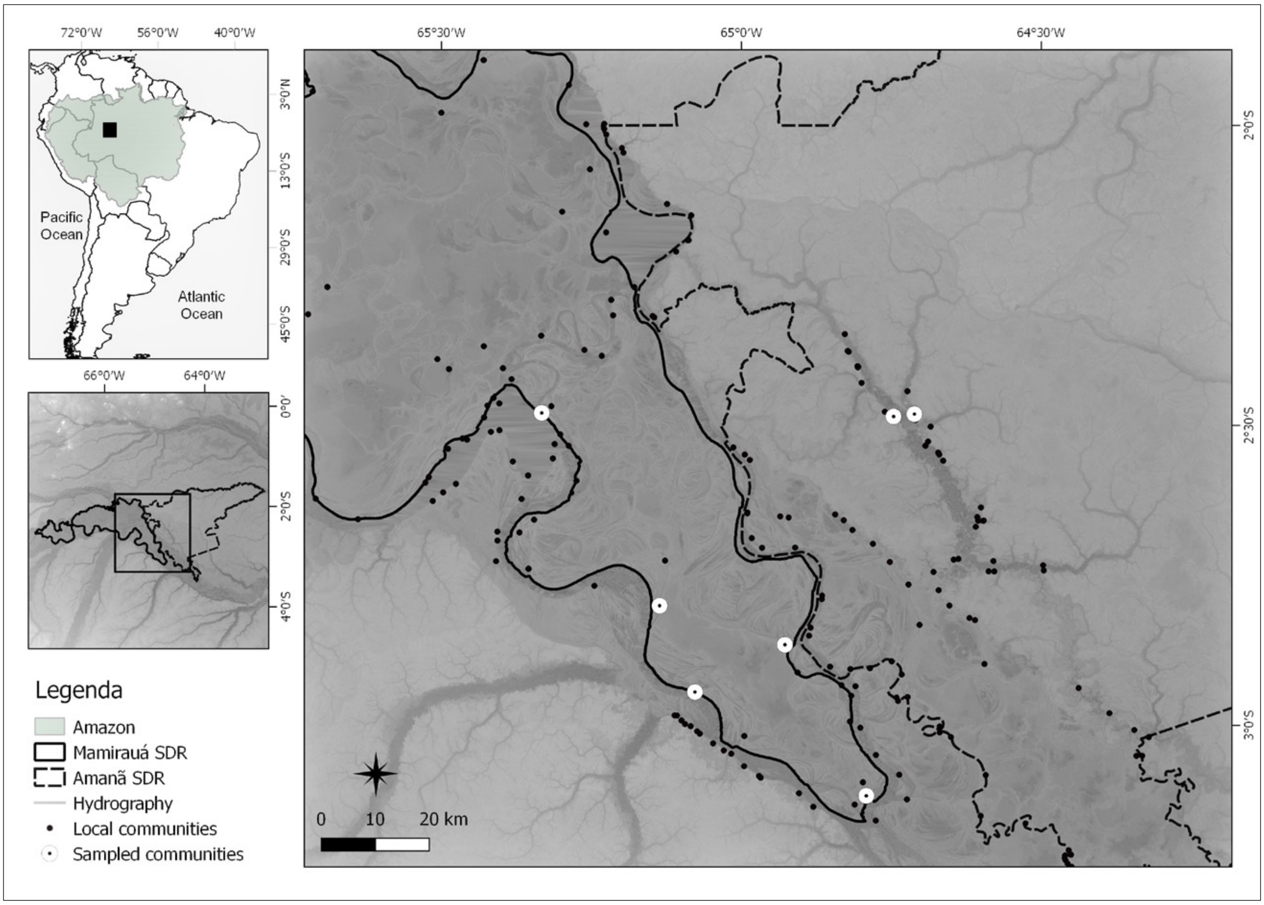


Figure 1. Location of study areas and communities with sample collection. The white-water flooded forest is represented in dark grey and the upland forest in paleovarzea soil in intermediate grey. The lighter grey area represents the upland forest *sensu strictu* not sampled in this study.

white-water flooded forests and 10% igapó forests that are flooded by blackwater rivers (SEMA, 2020). Annually, the floods of the Solimões and Japurá rivers influence the water level of the rivers adjacent to the sampling sites, raising the water column by an average of 10.6 m, particularly between May and July (Ramalho et al., 2009).

The protected areas have been assigned the conservation category Type VI (according to IUCN classification), which permits local rural, indigenous and non-indigenous peoples to use natural resources in accordance with management plans (SNUC, 2000). Hunting is a common activity in the area, mainly practiced for subsistence purposes and playing a vital role in the food security of rural traditional or indigenous populations (Torres et al., 2018). Game species include ungulates, caviomorph rodents and primates (Torres et al., 2018), particularly targeting large-bodied primates (Peres, 2000). Although primate hunting in the reserves is considered sustainable due to the constant size of hunting areas over 15 years of participatory monitoring, Juruá red howler monkeys (*Alouatta juara*) (hereafter, red howler monkeys) are among the primates with the largest body size and are frequently targeted by local hunters (Pereira et al., 2017). They represent one of the species with the highest number of samples received in the Mastozoological Collection of Instituto de Desenvolvimento Sustentável Mamirauá.

Sample collection

The collection of viscera was made possible through voluntary donations of 47 red howler monkey stomachs, between 2002–2017. We obtained stomachs harvested by subsistence hunters from white-water flooded (n=29 from Mamirauá Reserve) and upland forests (n=18 from Amanã Reserve). These donations were part of long-term projects aimed at promoting sustainable fauna management and food sovereignty for the local human villages. The research data were collected through participatory monitoring of hunting activities, wherein the community voluntarily provided information about hunting trips (such as the date and type of environment) and donated thoracic and abdominal organs of hunted animals (Valsecchi et al., 2014). No animals were killed specifically for this study; all specimens were hunted as part of the usual livelihood activities of local hunters. The donation of biological samples encompassed all animals that are usually hunted, not just focusing on howler monkeys. However, for this study, we used only the sample set of red howler monkeys.

The organs were preserved in vats with 4% (v/v) formaldehyde buffered solution and assigned an individual code indicating the sample and the date of the individual's hunting (following Mayor et al., 2017). They were then transported to the scientific collection of the Mamirauá Institute for Sustainable Development in Tefé, Amazonas, where they were individually stored in glass bottles containing 70% alcohol (v/v). The research protocol was approved by the Chico Mendes Institute for Biodiversity Conservation in Brazil (SISBIO License n° 29092-1) and the Ethics Committee in the Use of Animals of the Mamirauá Institute for Sustainable Development (004/2017).

Analysis of stomach contents

We opened the stomachs at their greatest curvature, and the contents were washed on granulometric sieves with different mesh sizes (4.0, 0.6 and 0.075 mm) for an initial sorting of the contents. We dried the contents in an oven at 60°C for up to 6h (following Jesus et al., 2022b and Torres et al., 2022). After drying, we separated and classified the types of food items according to Kurland and Gaulin (1987) into structural vegetative parts (S), primarily composed of leaves, fibers and woody parts; reproductive parts (R), including flowers, fruits and seeds; and animal matter (A), mainly represented by arthropods. We used a stereoscopic microscope with magnification ranging from 10x to 40x to examine portions of the content containing small particles that could not be separated macroscopically. After a thorough screening of the contents of each stomach, the food items were weighed using an analytical balance with an accuracy of 0.0001 g. The total biomass (in dry matter) of the stomach contents for each individual was calculated as the sum of all items.

We carried out the taxonomic identification of arthropods at the Order level with the assistance of an experienced entomologist (see Acknowledgments). For the majority of seeds, we identified them at the genus level with the help of an experienced botanist (see Acknowledgments) and seed identification guides (Loayza and Paine, 2014; Mozombite, 2018; Vásquez-Arévalo et al., 2021). We classified unidentified seeds into morphotypes based on their morphological characteristics and considered each morphotype as a distinct species. For each consumed plant taxon, we counted the total number of intact and damaged seeds, measured the largest diameter using a precision caliper with an accuracy of 0.1 mm, and classified them into six biometric categories based on the mean diameter: <2 mm; 2.0-5.5 mm; 5.6-10 mm; 10.1-15 mm; 15.1-20 mm; and >20 mm.

Environmental variables

We used information on river water levels (in meters above sea level, m.a.s.l.) as a seasonal environmental variable corresponding to the hunting date of each individual. We obtained these data through the fluviometric monitoring system (see Ramalho et al., 2009; IDSM, 2020).

Data analysis

We described the diet composition of the red howler monkeys in each environment, focusing on the proportions of consumed items and seed richness. Prior to analysis, we checked the normality of the data using the Shapiro-Wilk test and applied non-parametric tests. We compared the proportions and richness of consumed taxa between environments using the Mann-Whitney test (U) for unpaired data. Additionally, we presented the average percentage of seeds within each size category in terms of quantity and biomass. Finally, we conducted a Spearman correlation analysis for each forest type to assess the relationship between the variation in fruit richness consumed and the seasonal variation in river water levels. We performed all data analyses using the Past 4.03 software (Hammer et al., 2001).

Results

The diet composition of red howlers consisted of structural parts, reproductive parts and animal matter. We found no difference in the diet of red howler monkeys between the two environments (Table 1). Arthropods, although ingested in small proportions, were present in 95.5% of the samples (white-water flooded forest=97%; upland forest=94%), belonging to four Orders of the Class Insecta (Coleoptera, Diptera, Hymenoptera, Lepidoptera) and one of Arachnida (Araneae).

We found a total of 10,856 seeds, of which about 73% (white-water flooded forest = 74.5%; upland forest = 65.1%) were whole. The maximum seed diameter found was 25.4 mm for white-water flooded forests and 34.1 mm for upland forest (Table 2). Seeds with a diameter less than 5.5 mm constituted the majority, accounting for 80.8% and 69.4% of seeds ingested by red howler monkeys in white-water flooded and upland forests, respectively (Figure 2A). However, seeds with diameter less than 5.5 mm represented only 2.8% and 5.1% of the seed biomass ingested in the white-water flooded forest and upland forest, respectively (Figure 2B). In the white-water flooded forest, the largest number of ingested seeds fell within the 2.1 mm and 5.5 mm diameter category, whereas the largest biomass was represented by seeds with a diameter greater than 20.1 mm (Figure 2). In upland forest, seeds with a diameter between 10.1 and 15 mm constituted the majority both in terms of the number of seeds and in biomass (Figure 2).

Seed richness in the stomachs ranged from 1 to 7 taxa. The average seed richness per stomach was higher in white-water flooded forest compared to upland forest (Table 1). We identified a total of 40 seed taxa in the white-water flooded forest samples, with quantities ranging from one to approximately a thousand seeds per stomach, constituting between 0.3% and 56.2% of the total biomass consumed by the individuals. In the upland forest, we identified 14 taxa of ingested seeds, contributing from 0.2% to

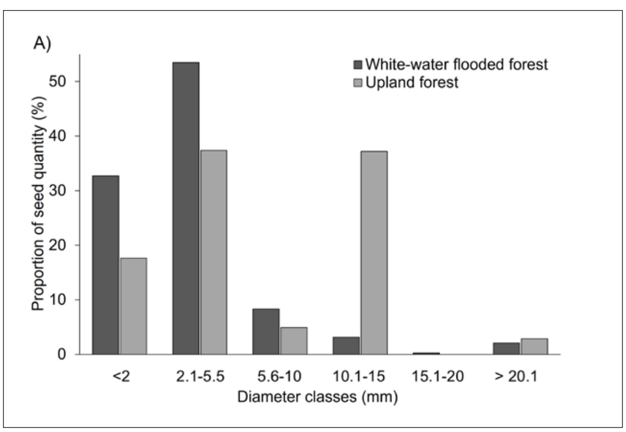
Table 1. Proportion of dry weight of stomach content (%, mean ± standard deviation) of food items for 47 red howler monkeys (*Alouatta juara*) from white-water flooded and upland forests in central Amazonia. Richness indicates number of taxa.

Habitat type	Diet composition			Average seed richness per stomach
	Structural parts	Reproductive parts	Animal matter	
White-water flooded forest	87.5% (±22.3)	12.5% (±22.3)	0.05% (±0.08)	3.1±1.7
Upland forest	88.1% (±31.6)	16.9% (±31.4)	0.04% (±0.09)	1.6±1.7
U test	185.4	185	185.3	183.3
degrees of freedom (df)	3	45	45	45
P-value	0.465	0.430	0.142	0.012

Table 2. Identification of taxa and average size (mm), biomass per seed (g) and mean number per taxon of seeds ingested by 47 red howler monkeys (*Alouatta juara*) from the white-water flooded and upland forests in central Amazonia in Brazil.

Family	Taxon	Mean seed size (mm)		Seed biomass (g)	Mean number of ingested seeds
		White-water flooded forest	Upland forest		
Arecaceae	*Astrocarium murumuru*	-	20.6	0.86	7
Anacardiaceae	*Spondias mombin*	22.3	-	1.46	7
Annonaceae	*Bocageopsis* sp.	11.5	-	0.69	15
	Unonopsis sp.	15.3	-	0.88	5
	Xylopia sp.	6.4	-	0.07	66
Apocynaceae	*Macoubea* sp.	24.1	-	0.62	85
Capparidaceae	*Crataeva* sp.	12.2	-	0.35	21
Clusiaceae	*Rheedia* sp.	25.4	-	1.00	3
Combretaceae	*Terminalia* sp.	20.0	-	0.45	1
Euphorbiaceae	*Glycydendron* sp.	-	34.1	2.30	1
Fabaceae	*Inga* sp. *1*	15.0	-	0.48	1
	Inga sp. *2*	12.2	-	0.24	16
	Parkia sp.	-	10.0	0.07	2
Hypericaceae	*Vismia* sp.	3.1	-	0.002	5
Lauraceae	*Nectandra* sp.	20.2	-	1.16	16
	Sp. 1	13.5	-	0.19	48
Malpighiaceae	*Byrsonima* sp.	11.5	-	1.20	1
Meliaceae	*Trichilia* sp.	-	5.7	0.001	9
Moraceae	*Ficus* sp. *1*	1.8	-	0.001	800
	Ficus sp. *2*	1.4	1.5	0.0006	267
	Ficus sp. *3*	4.1	-	0.0001	4
	Ficus sp. *4*	1.1	-	0.0002	5
	Ficus sp. *5*	3.0	-	0.004	1000
	Ficus sp. *6*	2.7	-	0.002	61
Myrtaceae	*Eugenia* sp. *1*	10.9	10.7	0.72	9
	Eugenia sp. *2*	12.1	-	1.00	3
Myristicaceae	*Iryanthera* sp.	-	22.9	1.70	2
	Virola surinamensis	-	13.9	1.03	46

Family	Taxon	Mean seed size (mm)		Seed biomass (g)	Mean number of ingested seeds
		White-water flooded forest	Upland forest		
Rubiaceae	*Chomelia* sp.	11.9	-	0.29	9
	Coussarea sp.	8.9	-	0.18	132
	Genipa sp.	4.6	5.7	0.02	122
	Psychotria sp.	3.5	-	0.02	42
Sapotaceae	*Poutheria* sp. *1*	-	34.1	3.79	18
	Poutheria sp. *2*	17.2	-	0.60	3
	Poutheria sp. *3*	-	14.2	0.27	5
Urticaceae	*Cecropia* sp. 1	3.0	-	0.001	35
	Cecropia sp. 2	3.4	3.4	0.004	1000
	Coussapoa sp. 1	3.6	-	0.005	60
	Coussapoa sp. 2	3.9	-	0.005	146
Not identified	Sp. 2	10.9	-	0.33	15
	Sp. 3	23.0	-	0.60	1
	Sp. 4	0.6	0.5	0.005	57
	Sp. 5	21.2	-	0.57	7
	Sp. 6	8.7	-	0.16	98
	Sp. 7	-	14.2	0.30	268
	Sp. 8	11.4	-	0.61	3
	Sp. 9	12.0	-	0.58	10
	Sp. 10	23.3	-	1.87	3
	Sp. 11	14.9	-	0.30	8
	Total richness of taxa	**40**	**14**		



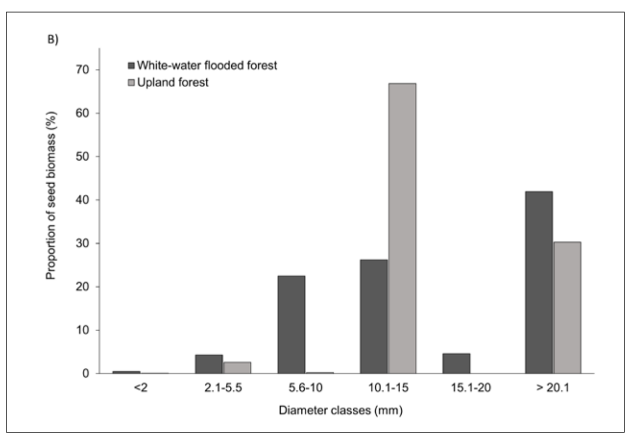


Figure 2. Proportion (%) of quantity (A) and biomass (B) by diameter class of seeds present in the stomach contents of 47 red howler monkeys (*Alouatta juara*) from white-water flooded and upland forests in central Amazonia.

66.0% to the total stomach contents, with amounts varying from one to 362 seeds per stomach. Only five seed taxa from upland forests matched those found in white-water flooded forest (Table 2). The most prevalent botanical family in the stomach contents was Moraceae (Figure 3), followed by Urticaceae in the white-water flooded forest and Myristicaceae for the upland forest. The main genera within these families were *Ficus* (Moraceae), *Cecropia* (Urticaceae), *Virola* and *Iryanthera* (Myristicaceae).

We found a significant positive relationship between seed richness and water level for white-water flooded forests (rs=5956, p=0.0006), but no significant relationship for upland forests (rs=0.2850, p=0.2516)(Figure 4).

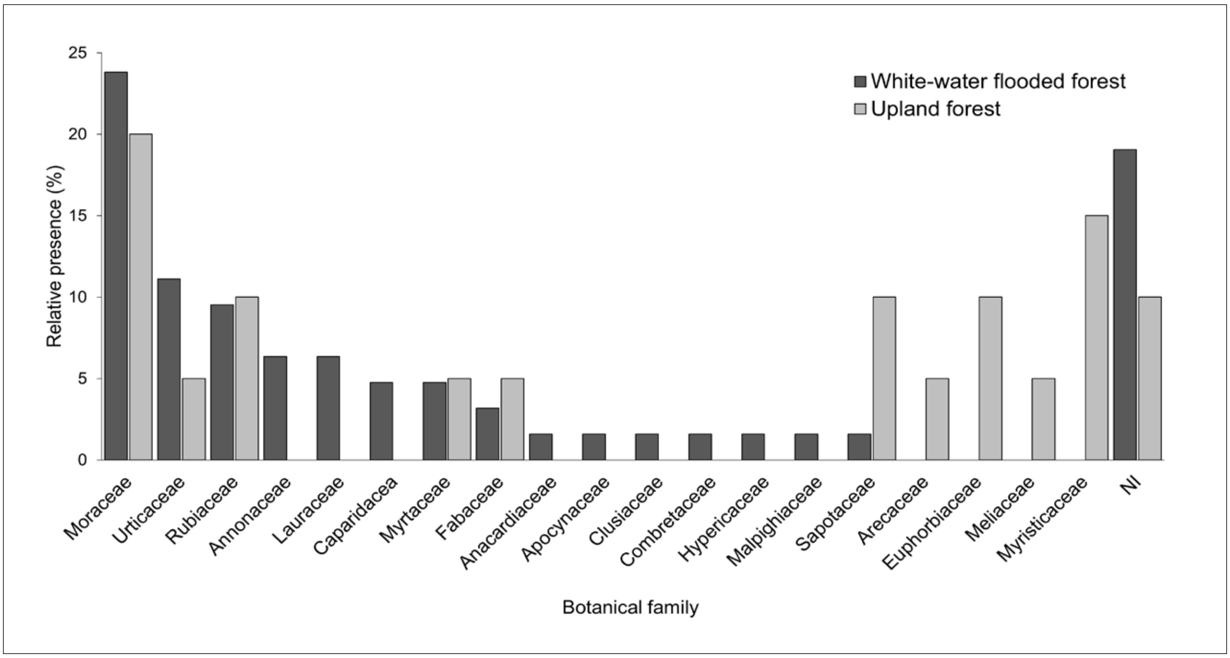


Figure 3. Relative presence (%) of botanical families in the stomach contents of 47 red howler monkeys (*Alouatta juara*) from white-water flooded and upland forests in central Amazonia. NI refers to the category of unidentified seeds.

Discussion

In this study, we report on the diet, seed size, and richness of fruits ingested by red-howler monkeys that were hunted for subsistence in two distinct environments of two Sustainable Development Reserves in central Amazonia. Our findings can contribute to support future studies on the impact of subsistence hunting on the seed dispersal service provided by howler monkeys in each sampled environment. We observed a consistent diet composition across both environments, characterized by the consumption of structural and reproductive parts of plants, as well as arthropods in small proportions. However, the composition of seed taxa differed between the two studied environments, with few taxa being shared among the howlers inhabiting both areas. Furthermore, we found that red howler monkeys in the white-water flooded forest consumed greater mean quantities, biomass, and richness of whole seeds ingested, whereas the seeds ingested by the red-howler monkeys in the upland forest were larger in size. Additionally, we identified a positive relationship between the richness of seeds per stomach and the environmental variations resulting from the increase in the water level in white-water flooded forests.

The consumption of structural and reproductive parts by red howler monkeys aligns with previous direct observation studies conducted in the study areas (see dos Santos, 2006). Although the author's observations indicated a significantly higher frequency of fruit consumption in the white-water flooded forest, representing reproductive parts, this difference can be attributed to the limited availability of fruits during the short study period conducted in the upland forest (dos Santos, 2006). However, the consumption of arthropods was not mentioned in dos Santos' study (dos Santos, 2006). Despite the small proportions of arthropods that we recorded; they were present in a high frequency of stomachs. Although the identified orders (Coleoptera, Diptera, Hymenoptera, and Lepidoptera) may have been accidentally ingested together with the consumption of fruits and leaves, the frequent presence of animal matter in their stomachs raises questions about the intentionality of consumption of arthropods by these primates, especially since the records of consumption of arthropods or other animal matter by howler monkeys in continuous forests are rare (e.g., Queiroz, 1995). Detailed studies focusing on the intake of animal matter, including factors such as seasonality and/or space-time resource availability, are necessary to understand the nutritional implications, whether accidental or intentional, for howler monkeys.

The higher proportion of whole seeds in relation to damaged ones indicates that red howler monkeys are potentially good endozoochoric dispersers for the fruit species they consume (Schupp, 1993; Wrangham et al., 1994; Valenta and Fedigan, 2008). The variation in ingested seed size by the red howler monkeys is within the range presented for their congeneric species, *Alouatta seniculus*, from French Guiana (e.g., 0.01 to 40.0 mm, Julliot, 1996). Comparative studies with other sympatric frugivorous species are necessary to define the role of the species as a disperser relative to other species in terms of pre- and post-dispersal. In a preliminary study that compared the number of seeds ingested by other Amazonian primates in our study areas, only *Sapajus macrocephalus* (Primates, Cebidae) presented seeds larger than 20 mm in their stomachs, but in smaller

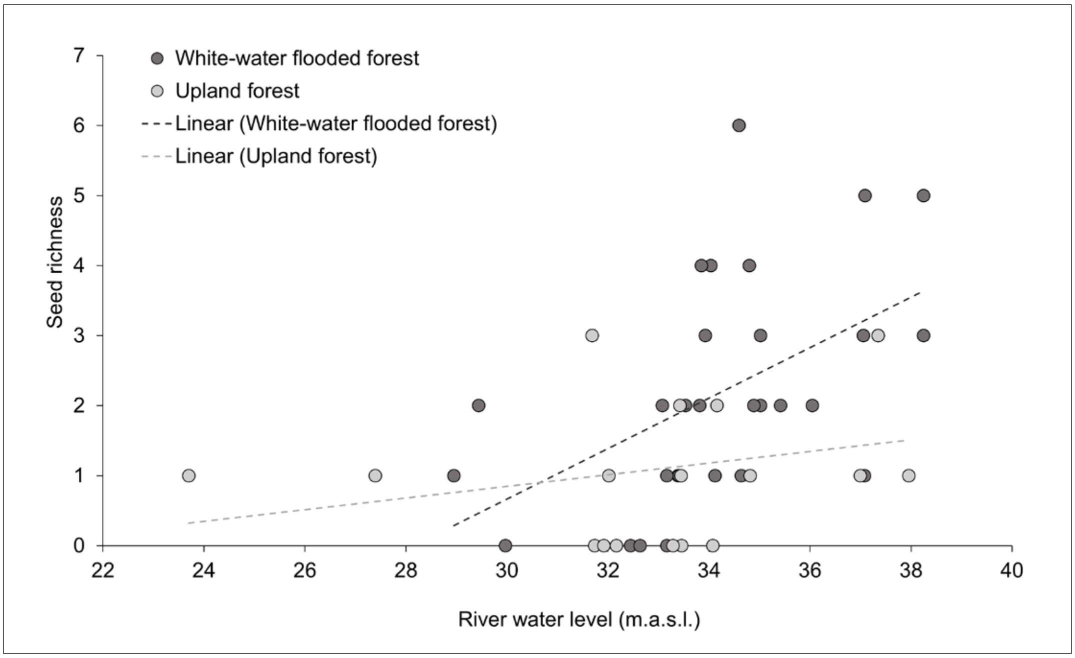


Figure 4. Relationship between the richness of seed taxa found in the stomach contents of the 47 red howler monkeys (*Alouatta juara*) from the white-water flooded and upland forests in central Amazonia and the water level of the rivers (m.a.s.l).

quantities and biomass (Jesus et al., 2021). Seed size seems to be a limiting factor to the number and biomass of seeds ingested (e.g., Julliot, 1997; Stevenson, 2007), and also for dispersal by smaller animals (Peres and van Roosmalen, 2002; Stevenson et al., 2005; Nuñez-Iturri and Howe, 2007). Although *S. macrocephalus* shows some overlap with seed dispersal provided by red howler monkeys in the study areas, it is necessary to verify if this overlap also occurs at the plant taxon level. However, as howler monkeys have the largest body size among the primates present in the study areas (Pereira et al., 2017), it is unlikely they would be surpassed in terms of quantity and biomass of dispersed seeds.

Although species of howler monkeys usually defecate in groups (e.g., Gilbert, 1997), forming clusters of seeds and, subsequently, seedlings (Julliot, 1997), the consumption of the pulp surrounding the seed reduces the attraction of potential pathogens and predators (Levi and Peres, 2013). Furthermore, in upland forests and in the dry season in white-water flooded forest, the action of secondary dispersers, such as dung beetles, plays a crucial complementary role in the distribution and development of seedlings (Feer, 1999; Andresen, 2003). On the other hand, in the high-water period in the white-water flooded forests, where fruiting follows the increase in river water level (Haugaasen and Peres, 2005; Paim et al., 2017), secondary dispersion carried out by water (Kubitzki and Ziburski, 1994) and/or by fish (Parolin et al., 2013) can help the heterogeneous distribution of seeds in the environment.

The greater number of seeds found in the stomachs of individuals from the white-water flooded forest can be attributed to the prevalence of certain plant families, such as Moraceae and Urticaceae, represented here mainly by *Ficus* and *Cecropia*, respectively. While these genera produce numerous seeds, these plants have a relatively small seeds, with an average diameter of less than 3.9 mm (see Table 2) and can be widely dispersed by smaller animals (e.g., Bufalo et al., 2016). In contrast, howler monkeys from the upland forest ingested a smaller quantity of seeds, biomass and seed richness. However, they likely played a significant role in dispersing larger seeds compared to those in white-water flooded forest. Seeds with diameters between 10.1 mm to 15 mm exhibited both the highest quantity and the largest proportion of biomass among all seeds ingested by howler monkeys from upland forest, and they probably played a significant role as dispersers of larger seeds.

Although no differences in seed biomass and size have been reported between trees from flooded and upland forests (Hawes and Peres, 2014), the differences in seed characteristics consumed by red howler monkeys may also reflect structural differences between the two forest types. This finding supports the idea that the composition of consumed fruit is influenced by the distinct vegetation and plant communities in each environment (Paim et al., 2018). The low overlap in the taxa consumed in each environment further suggests the presence of structural differences between the forests. These findings emphasize the important role of howler monkeys as seed dispersers and suggest a complex interplay between plant species, seed characteristics, and forest ecology. However, due to the difference in sample size between the environments, we cannot confirm whether the generally higher seed richness ingested by howler monkeys from white-water flooded forest is valid or result of sample biases. In this case, we believe that greater participation from local populations would enhance biological sampling and increase the sample size. Further investigations are necessary to improve our understanding of these relationships and their implications for the overall functioning and dynamics of these ecosystems.

The flooded season is the period in which a greater number of trees bear fruit in the white-water flooded forests (Pereira et al., 2010; Paim et al., 2017). This may explain the positive relationship found between the river water level and the richness of seeds ingested per individual in these forests. However, other factors, not analyzed here, seem to be associated with fruit production in upland forests. For example, rainfall showed a strong positive relationship with the proportion of fruit-bearing trees in upland forests in Western Amazonia (El Bizri et al., 2018). Phenological studies and the relationship between phenophases and environmental variables in the upland forests of the Amanã Reserve may clarify the patterns of fruit consumption (and ingestion of their seeds) by red howler monkeys.

Although other factors also need to be evaluated to determine the impact of subsistence hunting on *Alouatta juara*, primate hunting activity seems to occur at sustainable levels in our study areas (see Pereira et al., 2017). However, declining populations of howler monkeys throughout their Amazonian distribution, due to hunting pressures associated with other anthropogenic threats, can have deleterious consequences for forest regeneration and/or tree species composition (e.g., Koné et al., 2008). Predicting the ecological impacts resulting from the population reduction or extinction of seed dispersing species is difficult due to the probability of simultaneous compensatory processes (Zambrano et al., 2008). However, the results of this study indicate that the maintenance of populations of red howler monkeys is important not only for the conservation of the species itself but also for the population dynamics of at least 49 species of plants with seeds found whole in the stomach contents of these primates. In addition, observational records of fruit consumption by red howlers carried out by Queiroz (1995) and dos Santos (2006) in the study areas report at least another 24 species of fruits consumed in the white-water flooded forest and at least another seven fruit species on upland forest.

In addition to the ecological importance of the seed dispersal by red howler monkeys, some taxa found in their stomachs have economic value, and are listed among the timber species of commercial interest in the Brazilian National Forest Information System (SNIF, 2020). This includes species such as *Spondias mombin*, *Virola surinamensis* and species of the genera *Terminalia*, *Parkia* and *Iryanthera*. The economic effects of seed dispersal by primates are still an underexplored field of study (see Lambert, 1998b; Bello et al., 2021); however, our findings indicate that the maintenance of red howler monkey populations is crucial for the sustainable economic utilization of the habitat by human populations. The ecological impacts of local subsistence hunting on the dispersal service provided by these primates and their economic implications for human populations require attention.

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