

Natural history notes and micro-habitat use by *Bokermannohyla luctuosa* (Pombal & Haddad, 1993) (Anura: Hylidae)

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ABSTRACT

This study investigates the activity, habitat use, and ecological patterns of the arboreal treefrog *Bokermannohyla luctuosa*, based on long-term monitoring from April 2020 to August 2022 in São Francisco Xavier, São Paulo state, southeastern Brazil. Over 126 field trips, we recorded this frog 203 times across 17 sites, with significant seasonal variation in activity and habitat use. During the dry season (April to September), the animals frequently occupied waterfall spray zones, while wetter months saw decreased reliance on these habitats. Reproductive activity, including vocalizing males, gravid females, and tadpoles, was observed across multiple months, which indicates an extended reproductive period. Notable trophic interactions included predation by the larger treefrog *Boana faber* and the consumption of a harvestman by *B. luctuosa*. These findings highlight the importance of long-term monitoring to address knowledge gaps, particularly as climate change threatens amphibian habitats. Understanding seasonal microhabitat preferences can guide conservation strategies and improve fieldwork outcomes.

Key Words: Amphibian Ecology; Atlantic Forest; Climate Change Impacts; Predation; Seasonal Habitat Use.

Studies on diversity depend on the success of field trips to underexplored areas. Tropical forests are biodiversity hotspots, and the Atlantic Forest in Brazil is home to thousands of species, many of them endemic (Meyers *et al.*, 2000; Jenkins *et al.*, 2015). Expeditions focusing on Atlantic Forest amphibians are frequent, as this ecoregion hosts more than 600 species of anurans (Rossa-Feres *et al.*, 2017). As in most of the Neotropical ecoregions with seasonal climate regime, the reproductive season for most anurans occurs in the warmer and wetter months, from October to February (Duellman and Trueb,

1986; Donnelly and Crump, 1998; Haddad and Prado, 2005; Prado *et al.*, 2005; Gomez-Mestre *et al.* 2012; Verdade *et al.*, 2019), despite the consistently high humidity levels throughout the year and the diversity of reproductive modes observed among Atlantic Forest anurans (Nunes-de-Almeida *et al.*, 2021).

Focusing herpetological campaigns during the wetter months creates a major knowledge gap in relation to behaviours displayed during the dry season, such as microhabitat and shelters use, movement patterns, and activity levels. Global climate change

is threatening delimitation and predictability of seasons, altering temperature and rainfall patterns, therefore impacting biodiversity, particularly of frogs (Luedtke *et al.*, 2023). There is an urgent need for natural history studies, as long-term monitoring plays a crucial role in providing insights into animal behaviour and strategies to overcome environment challenges (see Prado *et al.*, 2005; Verdade *et al.*, 2012; Muscat *et al.*, 2020; Moroti *et al.*, 2022; Botelho *et al.*, 2023).

Bokermannohyla luctuosa (Pombal and Haddad, 1993) is a large hyloid treefrog (males SVL 55 mm, females 70 mm) from the *B. circumdata* group (Faivovich *et al.*, 2005; Carvalho *et al.*, 2012). This frog has arboreal and nocturnal habits and is commonly found in forested areas, forest edges, and wetlands. It is endemic to the mountainous regions of the Atlantic Forest (Haddad *et al.*, 2013). Some aspects of the defensive (Pombal and Haddad, 1993; Toledo and Haddad, 2009; Toledo *et al.*, 2011; Moroti *et al.*, 2018) and reproductive (Zornosa-Torres and Toledo, 2019) behaviours of *B. luctuosa* during the wet season are formally described: males release advertisement calls perched on arboreal vegetation;

females lay eggs in temporary pools, streams, or on the edge of lentic streams; and tadpoles initially develop in mud depressions, rock crevices or under branches near streams, from where heavy rains carry them to larger bodies of water to complete their cycle. (Pombal Jr. and Haddad, 1993; Malagoli *et al.* 2021). Nothing is known about the species' habitat use during drier times of the year.

Herein we present year-round monitoring and natural history data of *B. luctuosa*. We highlight occasional trophic interactions and demonstrate how the occurrence sites of *B. luctuosa* are driven by temperature and humidity variations. This allows for the prediction of where these frogs can be found during different moisture conditions that vary seasonally in its occurrence area.

We monitored the activity of *B. luctuosa* from April 2020 to August 2022 in the Projeto Dacnis private reserve in São Francisco Xavier, São Paulo state (22°53'S 45°56'W, 842 m above sea level) (Fig. 1). Projeto Dacnis is a non-governmental organization dedicated to Atlantic Forest conservation through scientific research and environmental education. The climate is subtropical highland climate (Cwb)

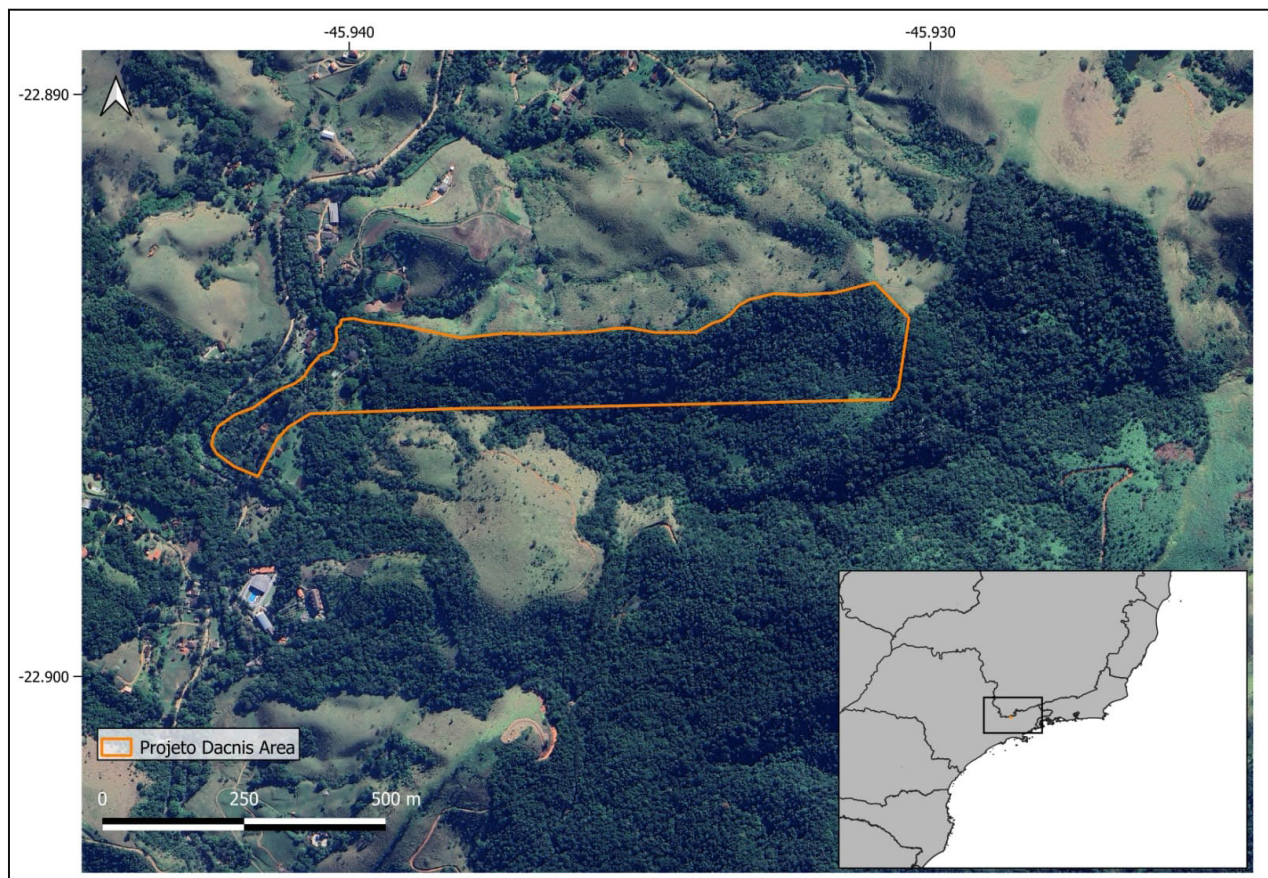


Figure 1. The Projeto Dacnis study area in which the survey took place. São Francisco Xavier, São José dos Campos, São Paulo, Brazil.

with substantial rainfall, according to the Köppen classification (Alvares *et al.* 2013). The annual mean rainfall exceeds 1,800 mm.

Sampling was carried out during routine trails once a week, covering 3 km alongside streams and waterfalls, and lasting 3 h per night. Two researchers conducted the field study, resulting in a total sampling effort of 756 person/hours across 126 field trips. For each individual of *B. luctuosa* found on the trail, we recorded the observation site and individual activity, and the site's air temperature (°C) and relative air humidity (%), measured with a digital thermo-hygrometer (Metrins INS-1383). Rainfall data during monitoring were obtained from a weather station at the Projeto Dacnis area. Average temperature and rainfall for the area during our monitoring is in a climogram (Fig. 2).

To gain a detailed understanding of the phenology of *B. luctuosa*, we employed circular statistics and the Rayleigh test (Agostinelli and Lund, 2024). We performed a bootstrap analysis (1000 replicates; Canty and Ripley, 2024) to estimate the average rainfall at which individuals were found associated to the waterfall in the area. Additionally, we used a logistic regression to test whether the occurrence of

B. luctuosa in the waterfall was related to average rainfall during the monitoring period (Fig. 3). Monthly mean rainfall values were transformed using their natural logarithm. We conducted all analyses in the R environment (R Core Team 2020).

During the 126 field trips, we found *Bokermannohyla luctuosa* 203 times. In most encounters, there was more than one individual, usually perched on vegetation, not interacting, totalling 447 encounters in 29 months of monitoring. Throughout these observations, our records on activity, if any, life stage, calling, presence of females with eggs, and occurrence of tadpoles, provide detailed insights of the reproductive and ecological patterns of this treefrog in the area (see Table 1 and Figs. 2-5).

We found individuals of *B. luctuosa* in 17 different sites within the study area. Records for this frog were strongly seasonal (Rayleigh Test of Uniformity = 0.55, p-value < 0.01), predominantly concentrated during the drier months (n=398), from April to September (Figs. 2 and 3A), when it was the single frog species recorded in our monitoring. There were 181 sightings at waterfalls over the whole study period, of which 173 happened during the dry season. Waterfalls accounted for 43.5% of all

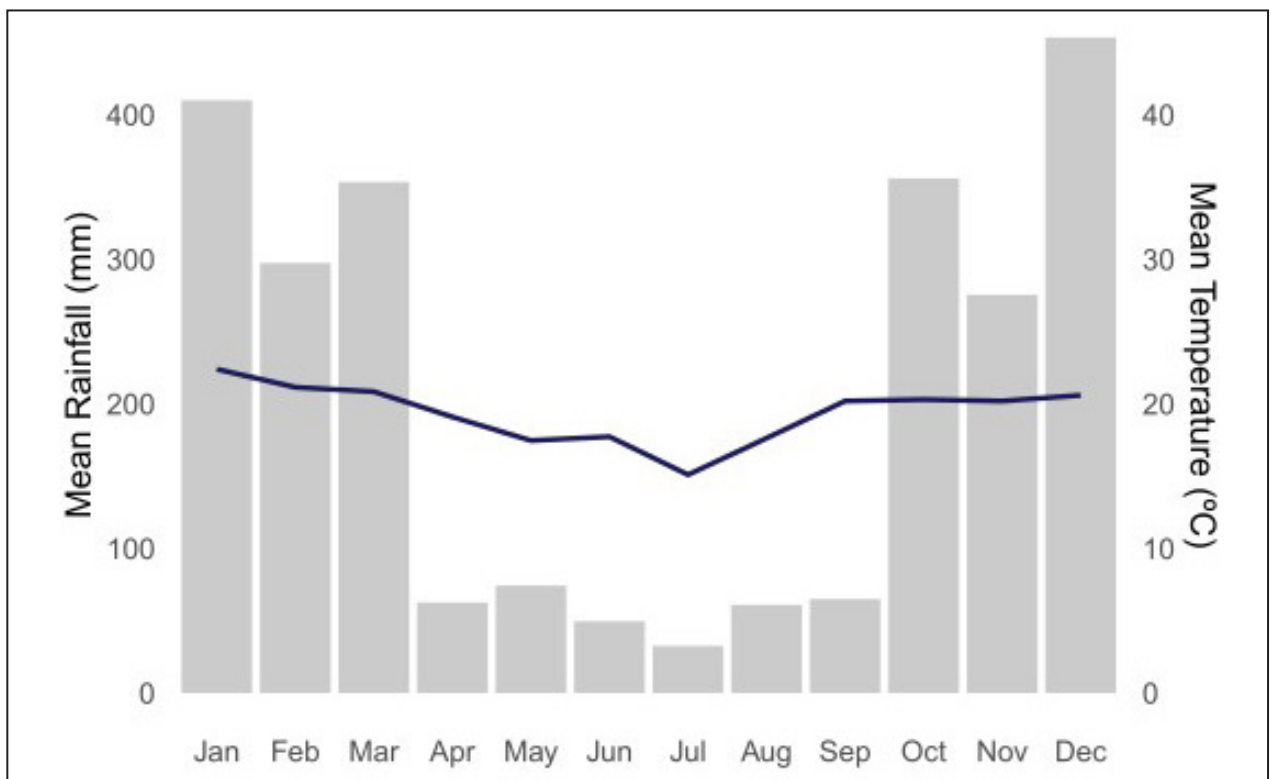


Figure 2. Monthly average temperature and rainfall from April 2020 to August 2022 in São Francisco Xavier, São José dos Campos, São Paulo, Brazil.

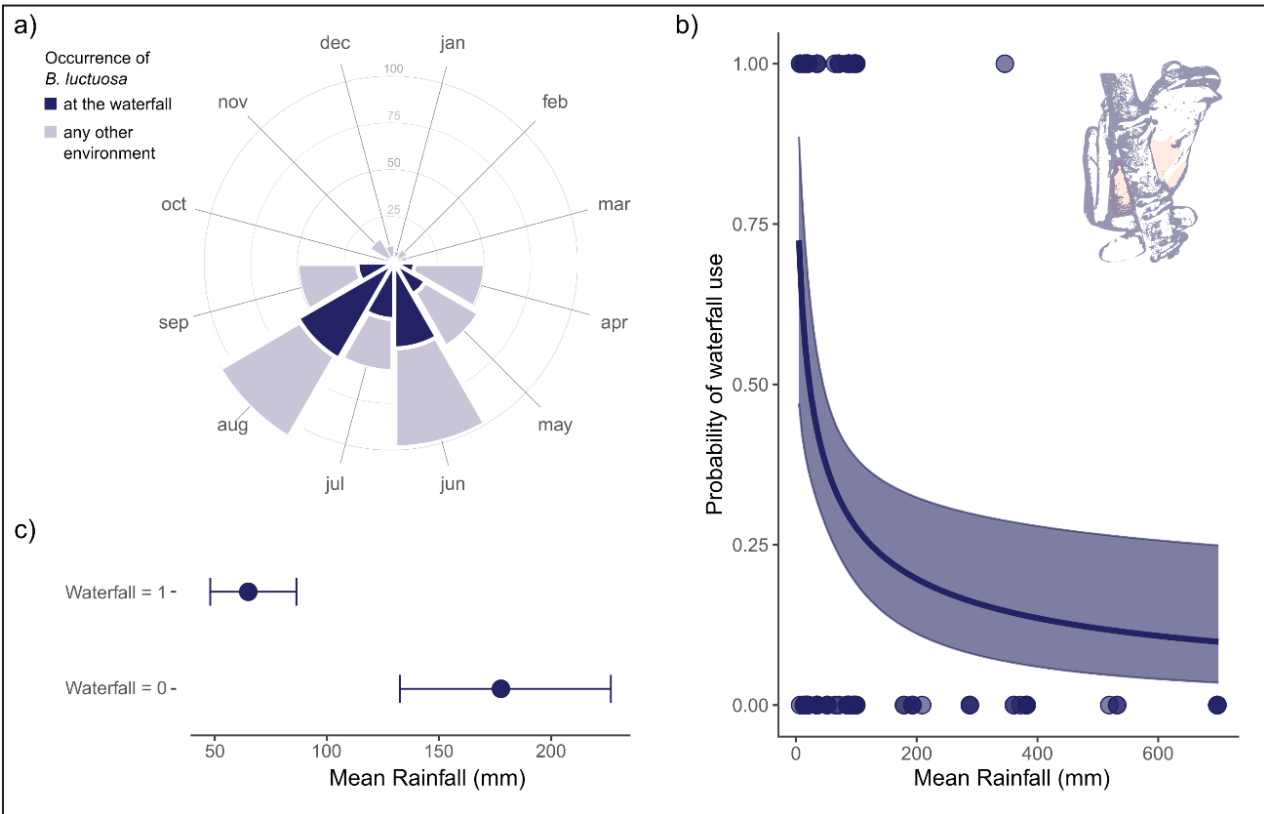


Figure 3. Probability of waterfall use by *Bokermannohyla luctuosa* in the Projeto Dacnis reserve, São Francisco Xavier, São José dos Campos, São Paulo, Brazil. (a) The occurrence of this frog was highly seasonal during the sampling period, with most individuals found during the dry season (April to September). (b) Individuals were commonly observed in the waterfall spray zone when mean rainfall was below 100 mm/month. (c) The frequency of waterfall use increased as rainfall decreased.

B. luctuosa found during this period, highlighting their importance as a water resource when there was reduced rainfall (Fig. 3A). We found that the occurrence of *B. luctuosa* in waterfall zones was associated with a mean rainfall of less than 100 mm/month (Fig. 3B), while the dependence of this moist habitat decreased with increasing rainfall in warmer months (Fig. 3C, Table 1).

We recorded *B. luctuosa* calling activity in April, June, and August, and once in November. We observed a female with eggs far from the waterfall in June. We found tadpoles in May, August, September and October, in different water bodies inside the forest, swimming at a depth of 30 to 50 cm, once observed in water as cold as 16°C. Tadpoles in early

developmental stages were found in shallow, lentic-like streams with muddy or sandy bottoms, and their maximum numbers were around 30 individuals. Juvenile and adult frogs were commonly found perched between 30 and 200 cm high on vegetation within the forest. All observations above occurred during night time surveys, when temperatures ranged from 9.8°C to 25.9°C and humidity ranged from 51 to 100%. During the dry season, we recorded the frogs in retreats in the axils of banana plants (*Musa* sp.) and bromeliads (Fig. 4).

Additionally, we also documented predatory interactions involving *B. luctuosa*. On November 12, 2019, before formal monitoring began, we recorded a *B. luctuosa* adult on vegetation, preying on a gon-

Table 1. Influence of rainfall on the use of waterfall zones by *Bokermannohyla luctuosa*, as inferred from a logistic regression between the natural logarithm of monthly mean rainfall and the species' occurrence.

	Estimate	Std. error	z-value	p-value
Intercept	2.00	0.87	2.30	0.02
Ln (Mean rainfall)	-0.64	0.21	-3.09	<0.01



Figure 4. Habitat and microhabitats of *Bokermannohyla luctuosa* in the Projeto Dacnis reserve in São Francisco Xavier, São José dos Campos, São Paulo, Brazil. (a) Waterfall where the frog was usually found. (b-c) The frog in day shelters, the axil of a banana plant (*Musa* sp.) and bromeliads, recorded during the night after active search. Photos by Edelcio Muscat.

yleptid opilion, *Gonyleptes* sp. (Fig. 5A). On April 1, 2020, we recorded the larger hylid treefrog *Boana faber* (Wied-Neuwied, 1821) perched on vegetation and preying on *B. luctuosa* (Fig. 5B). At the time we found the two frogs, only the hind legs of *B. luctuosa* were protruding from the mouth of *B. faber*. To be sure of the identity of the preyed frog, we caught *B. faber* and carefully removed the prey from its mouth. Remarkably, the juvenile *B. luctuosa* was still alive. Both frogs were released at the same site and a video documenting this event was deposited in the digital collection of the Jacques Vielliard Neotropical Sound Archive (ZUEC-VID 0001321).

Our data indicate seasonal habitat variation by *B. luctuosa* related to humidity levels. Rainfall below 100mm/month is more common from April to September in the study area, during which the frogs were often found in humid habitats like waterfall splash zones. As rainfall declined, the frogs increasingly depended on these spray zones, where humidity remained high throughout the dry season. Conversely, the number of frogs recorded near waterfalls decreased during wetter months. These findings are in line with our expectations based on the life history traits of anurans in general, and of some Atlantic Forest species (Duellman and Trueb 1994; Moroti *et al.* 2022).

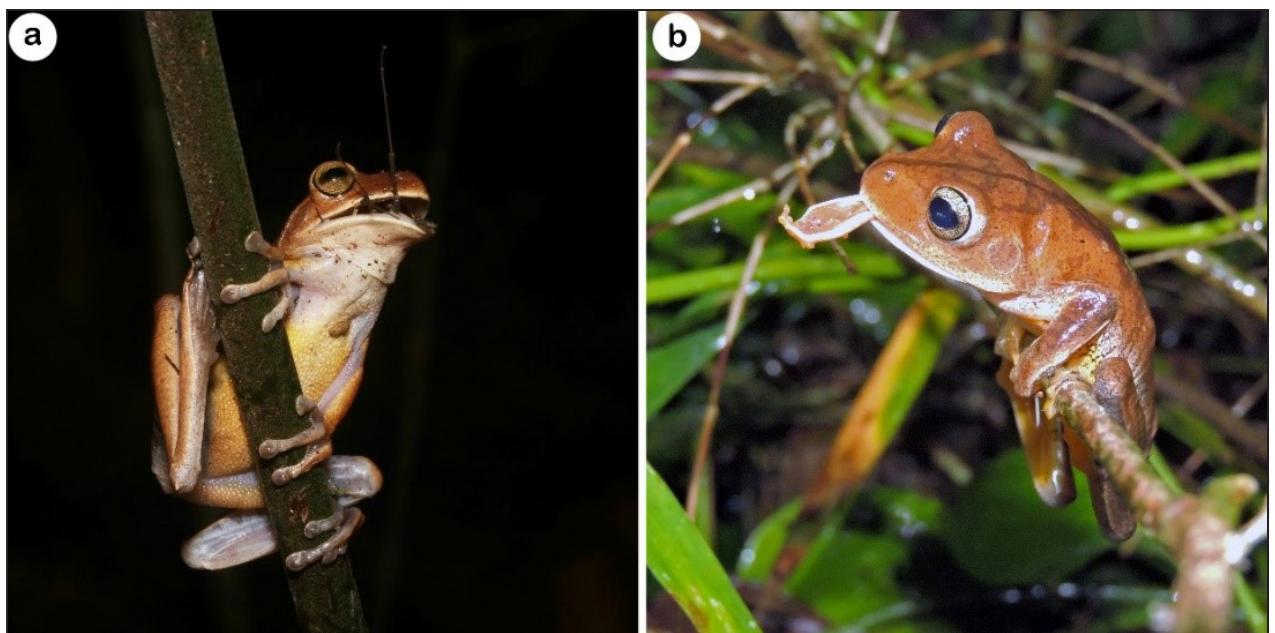


Figure 5. Predation records. (a) *Opilion Gonyleptes* sp. (Gonyleptidae) preyed on by *Bokermannohyla luctuosa* and (b) *B. luctuosa* preyed upon by the larger treefrog *Boana faber* (Hylidae). Photos by Edelcio Muscat.

The high permeability of amphibian skin leads to elevated rates of moisture loss, thereby increasing the risk of dehydration when these animals are in terrestrial environments during dry periods. Water plays a critical role in maintaining the integrity of amphibian skin functions (Feder and Burggren, 1992; McNab, 2002; Junior and Gomes, 2012; Botelho *et al.*, 2023). Evaluations of high-quality habitats for amphibians should consider the availability of water for rehydration during dry periods (Silva *et al.*, 2012), such as splash zones around fast-flowing streams, bromeliads, and water accumulated in axils of large-leaved plants such as *Heliconia* sp. (Botelho *et al.* 2023). Changes in rainfall patterns are potentially harmful if the number of consecutive dry days increases, even if mean precipitation is maintained. Longer dry periods may restrict or eliminate wet microhabitats (Pounds *et al.*, 1999). *Bokermannohyla luctuosa* is the only species of the genus in the study area, and we know climate changes are projected to shrink and cluster occurrence areas for many species, including in the high endemic and diverse areas of south eastern Atlantic forests, affecting differentially species richness, phylogenetic endemism, phylogenetic and functional diversity (e.g. Alves-Ferreira *et al.*, 2025; Bolochio *et al.*, 2025).

During the rainy season between November 2014 and March 2015, Zornosa-Torres and Toledo (2019) conducted their studies and observed that *B. luctuosa* exhibited reproductive activity throughout this interval. Our data indicates a broader period, as males were found vocalizing from April to November, a female with eggs was recorded in June, and tadpoles in May, September and October, supporting the hypothesis that this treefrog has an extended reproductive period. This distribution of tadpole sightings across several months indicates that reproduction may be sustained over time, rather than confined to a single season. Extended reproductive periods are documented for other species in the *B. circumdata* group (Malagoli *et al.*, 2021).

Predation events are poorly documented for *B. luctuosa*. While it is generally known that frog's prey on invertebrates, the records of harvestmen as prey are unusual (see Machado *et al.*, 2005), as these arachnids have chemical defences that may lead to regurgitation by the predator (Machado *et al.*, 2005).

Boana faber is a large treefrog that includes other frogs in its diet (e.g. Craugastoridae, Leite *et al.*, 2008, and Hylidae, Oliveira *et al.*, 2023). Ours is the first record of a treefrog from the genus *Boker-*

mannohyla as part of its diet. The co-occurrence of both species in swamps or flooded areas at the forest edge (Moura *et al.*, 2012; this paper) favours predation events, which may be more common than currently recognised.

Our study adds to the understanding of the natural history of Atlantic Forest frogs, their trophic interactions, and potential future threats resulting from climate changes. The observed seasonality in microhabitat use and its relationship with rainfall can enhance fieldwork success by identifying suitable microhabitats during drier periods. We emphasise the role of long-term monitoring and natural history studies as important tools for understanding fundamental requirements for countless animal species, as well as for management and conservation of frogs and other animals in our quickly-changing environment.

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Declaration of interest

No conflicts of interest.

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