Host specificity and activity synchronization drive frog-biting midge incidence on torrent frogs (Hylodidae) in southeastern Brazil

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Abstract

Frog-bitting midges (Corethrellidae) are widespread micropredators that feed on the blood of frogs. Furthermore, frog-biting midges carry pathogens such as *Batrachochytrium dendrobatidis* (Bd), an important cause of worldwide amphibian declines. Female midges usually target calling male frogs by using acoustic cues. However, how midges target frogs that use conspicuous visual cues, especially diurnal species, is still poorly understood. We explored micropredation by corethrellid midges on two syntopic diurnal frogs, *Hylodes asper* and *H. phyllodes*, in Brazil's Atlantic Forest. We tested host selection (species and sex) as well as their spatial and temporal patterns. Midges were recorded exclusively on *H. asper*, indicating host preference, potentially linked to acoustic parameterss preferred by midges. Males were more frequently targeted, particularly those calling near the main current of the streams. Yet, midge abundance per individual did not differ between sexes, with unusual high abundance in females. Midges concentrated on the nostrils and gular region, likely due to higher vascularization. Midge activity was strictly diurnal, despite frogs being available at night while resting exposed on vegetation. Micropredation levels were strongly influenced by precipitation, especially under high maximum temperatures. These patterns suggest that host traits, as well as environmental conditions, shape micropredation dynamics for frogs that use visual cues. Considering that midges can carry pathogens, their micropredation could influence infection risk at the population level. This may be particularly critical for *H. asper*, a species with rapid annual turnover and documented susceptibility to Bd, which highlights the importance of integrating behavior and environmental factors into disease risk assessments.

Keywords: abundance, Atlantic Forest, diel activity, hematophagous, micropredation, rainforest, seasonal, stream-dwelling frog.

Introduction

Frog-biting midges (Diptera: Corethrellidae) are a specialized group of micropredators with wide distribution, typically occurring below 1500 m (Borkent 2008). They are closely associated with sites where frogs aggregate, such as streams, swamps, and bromeliads (Borkent 2008), since eavesdropping females obtain their blood meals from calling male frogs (Ambrozio-Assis et al. 2019). Calling males are especially targeted due to their conspicuous acoustic displays and exposed positions on or around waterbodies (e.g., Bernal et al. 2006, 2007). The attraction of frog-biting midges is largely driven by species-specific acoustic traits such as call rate, frequency, and pulse structure (Bernal et al. 2007; Meuche et al., 2016, Virgo et al., 2019, but see Virgo et al., 2009), reflecting a long history of coevolution between midges and their anuran hosts that dates back to the Early Cretaceous (Borkent 2008). Yet, knowledge about host specificity, preferred biting regions of the body, and the ecological consequences of this interaction remains limited, especially for most tropical systems.

Midges typically target thin and vascularized regions of the skin and can remove substantial amounts of blood, up to 10% of host blood volume in a single night (McMahon et al. 2017), potentially impacting males that invest heavily in calling. Moreover, since *Corethrella* midges are hematophagous and interact directly with skin surfaces, they facilitate the transmission of pathogens such as trypanosomes (Sigl et al. 2025) and *Batrachochytrium dendrobatidis* (Bd; Toledo et al. 2021a) — a major driver of amphibian declines worldwide (Carvalho et al. 2017; Olson et al. 2021; Carvalho et al. 2024). Environmental temperature is linked to infection dynamics (Murray et al., 2011) and to frog activity. Thus understanding how these interactions vary across time, due to diel variation in frog calling activity and seasonal shifts in frog abundance and climate, is therefore crucial for assessing both the ecological impact of micropredation and its potential role in amphibian disease ecology.

Other ecological factors may also be important drivers of frog-biting midge incidence. Since midges use mainly acoustic cues, ambient noise may impact their ability to locate calling frog hosts. However, this has been seldom tested. Torrent frogs of the genus *Hylodes* provide a particularly interesting context to explore this. Unlike most nocturnal anurans targeted by *Corethrella* (Bernal et al. 2007; Meuche et al., 2016), *Hylodes* males are diurnal and call near waterfalls and fast-flowing streams (Haddad & Giaretta 1999). To communicate in these noisy stream environments

Hylodes rely on not only acoustic but also visual signals, such as conspicuous foot-flagging (Haddad & Giaretta 1999; Augusto-Alves et al. 2021). Due to these ecological and behavioral factors, it is thus possible that incidence of micropredation differs from nocturnal amphibian species that provide only acoustic cues.

We investigate micropredation by *Corethrella* spp. midges in two syntopic frogs of the genus *Hylodes* (*H. asper and H. phyllodes*) in the Atlantic Forest of Brazil by exploring the effects of i) frog species, ii) sex, iii) distance from the main stream flow, iv) body region, v) time of day, and vi) seasonal climate on the abundance of midges on frogs. We predict that i) midges are attracted by species-specific frog calls such that there are differences in midge occurrence between *H. asper* and *H. phyllodes*; ii) if midges are attracted by frog calls, adult males will show midges more frequently than adult females and juveniles; iii) if midges are associated to calling males, the probability of finding midges on frogs decreases with distance to the main stream flow, as males tend to call near or on the main stream flow; iv) if midges are attracted by frog calls, resting frogs at night will present less or no midges when compared to frogs found during the day; v) since midges are hematophagous, there will be a higher abundance of midges in vascularized regions of the skin; and vi) given that frogs show seasonal patterns of abundance throughout the year (Ruggeri et al., 2015), temperature and/or rainfall will affect the abundance of midges on frogs. By integrating host traits, behavior, and environmental conditions, this study aims to clarify the ecological drivers of micropredation and assess their potential implications for disease dynamics in Neotropical amphibians.

Material and Methods

Frog species

Species of the genus *Hylodes* are endemic to the Atlantic Forest in Brazil (Haddad & Pombal Jr. 1995). *Hylodes* is the most diverse genus within the family Hylodidae, with 26 recognized species (Frost 2024). *Hylodes phyllodes* and *H. asper* exhibit sexual dimorphism, where males are smaller than females (Heyer et al. 1990). *Hylodes asper* males have an snout-vent length (SVL) = 39–43 mm (mean SVL = 41 mm) and females have an SVL 43–51 mm (mean of SVL = 46 mm) (Heyer et al. 1990), while for *H. phyllodes* the males have SVL = 27–32 mm (mean SVL = 29 mm) and females have SVL = 29–36 mm (mean SVL = 32 mm) (Heyer et al. 1990), respectively. Besides size differences, *Hylodes asper* are also more conspicuous than the cryptically coloured *H. phyllodes*. Both species have diurnal habits and are predominantly found active on rocks and resting on low vegetation at night in small to large streams in the forest (Heyer et al. 1990). Adult males and females, as well as juveniles of *H. asper* are restricted to streams, whereas males and females of *H. phyllodes* grow on the forest floor apart from streams after hatching and move back to the streams only to breed; in both species, eggs and tadpoles are restricted to streams (Toledo et al. 2021b; Augusto-Alves & Toledo, 2022). Both species produce pulsed calls that are adapted to environments with high ambient water noise (Haddad & Giaretta, 1999; Augusto-Alves et al., 2021). Furthermore, both species (especially *H. asper*) use stereotyped visual signals for male territoriality and agonistic encounters which include foot and toe flagging (Hartmann et al., 2005).

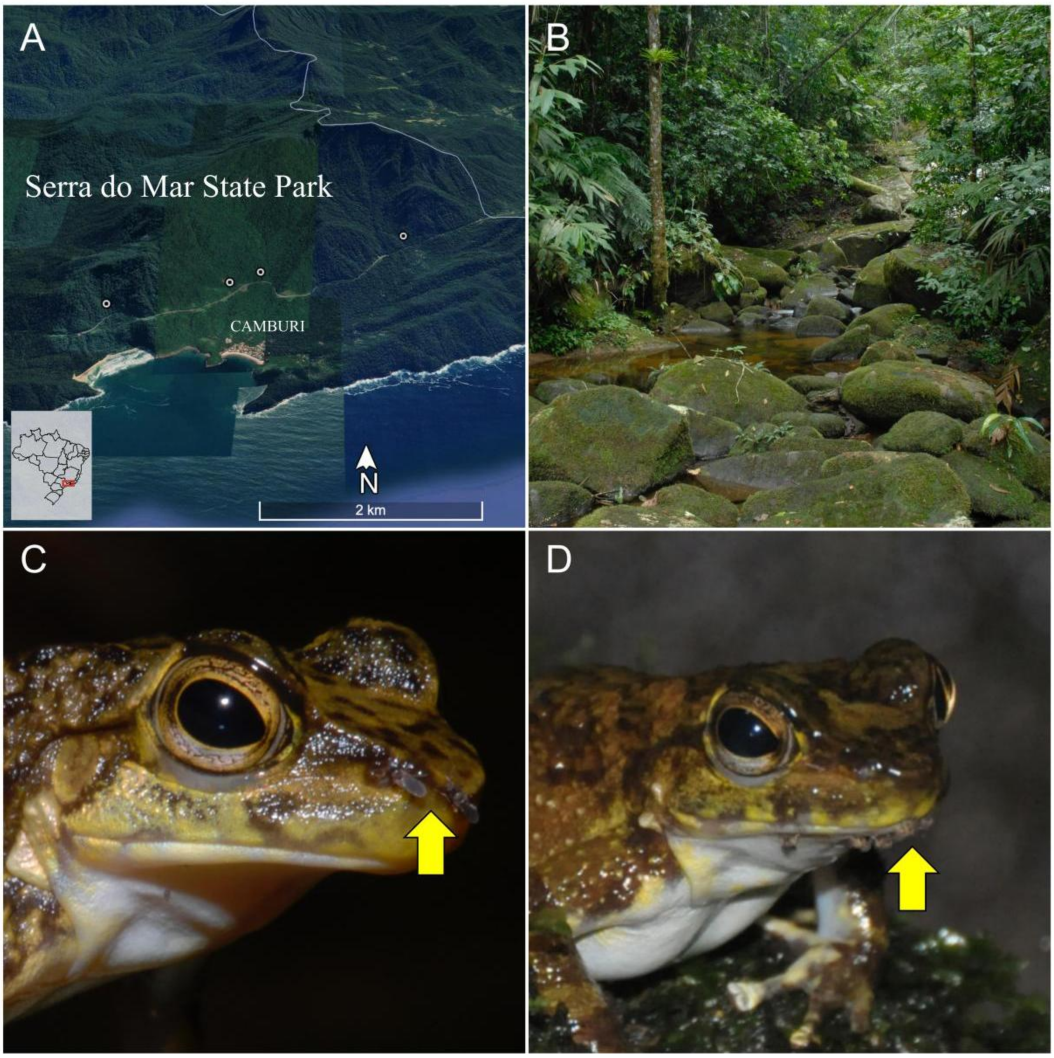
Study site

We conducted standardized transect surveys in streams at Parque Estadual da Serra do Mar (PESM), Núcleo Picinguaba, in the municipality of Ubatuba, on the coast of the state of São Paulo, Brazil (Fig. 1A). The study site is characterized by typical Atlantic Forest vegetation, with dense rainforest (Fig. 1B). The climate in this region is seasonally wet with mean annual rainfall of 2519 mm, a warmer season from October to April (monthly rainfall 215–376 mm; mean temperatures from 21–26 °C), and a drier, colder season from May to September (monthly rainfall 11–166 mm; mean temperatures from 18–21 °C; EMBRAPA, 2014). The high rainfall, even during the winter, is due to orographic effects due to the proximity of the Serra do Mar to the coast (Pinheiro & Geise, 2008).

Field sampling

We surveyed four low elevation streams (150–330 m asl) on the escarpments of the Serra do Mar, located along 4 km of the Rio-Santos highway (see Ruggeri et al., 2015). We conducted monthly surveys from January to December 2008. In each stream, we sampled a 100–120 m-long transect, both during the day and at night. Approximately 40 species of anurans occur in Núcleo Picinguaba (Toledo et al., 2014), but we chose species of the genus *Hylodes* due to their conspicuousness and high local abundance. We searched for adults of both focal species (*H. asper* and *H. phyllodes*) by walking upstream once during daytime (approximately from 07:00 to

16:30) and once at night (approximately from 18:30 to 22:00) for 30–90 minutes in each transect. Upon finding each frog, we recorded the individual frog stage, sex, species, distance to the main flow of the stream, and the presence, number, and position of midges on each individual based on photographs made using close-up lenses. We identified midges to the generic level (i.e., genus *Corethrella*) based on pictures sent to experts. Fieldwork was approved by ICMBio (sampling permit #16593). Approval by an Animal Ethics Committee was not required for this study as no animal was captured or collected.



Analyses

To assess the difference in the abundance of midges between *Hylodes asper* and *H. phyllodes* and between males and females we used a zero-inflated Poisson regression model, which accounts for overdispersion (Desmarais & Harden, 2013).

Since the detection of midges depends on the time of the observation, this allows for the estimation of true zeros (i.e., midges were not present) separate from count zeros (conditional, i.e., midges could be present but were not observed at that moment). We then tested the relationship between the presence of midges and distance to stream with a generalized linear model due to the binomial nature of the model and including month as a random effect, as in the main reproductive months, frogs may be closer to water to call and perform visual display (see Haddad & Giaretta, 1999; Augusto-Alves & Toledo, 2022).

To assess the relationship between the abundance of midges on frogs and time of day, we fitted a zero-inflated Poisson regression model to examine the relationship between the number of midges and the hour of the day, modeled as daily cyclical sine and cosine terms, adding the log-transformed number of *H. asper* as an offset. We tested for differences in the abundance of midges among body regions with a Kruskal-Wallis test followed by a post-hoc pairwise comparison using the Wilcoxon rank sum test with continuity correction. Body regions were categorized as anterior limbs, posterior limbs, dorsum, nostrils, gular region, snout and mouth.

We collected data on climatic variables (minimum and maximum daily temperatures; T_{Min} and T_{Max} in °C, respectively) and precipitation (in mm) from a local weather station of INMET, the Brazilian National Institute of Meteorology (INMET, 2024). Lastly, to assess if temperature and/or rainfall influence the abundance of midges throughout the year, we fitted a zero-inflated Poisson regression model to examine the relationship between the number of midges and three bioclimatic variables (daily precipitation, daily minimum temperature, and daily maximum temperature), including yearly cyclical sine and cosine terms. Furthermore, we added the log-transformed number of *H. asper* as offset.

Results

In total, we recorded 377 frog encounters (75 *Hylodes phyllodes* and 302 *H. asper*; Figure 1). Since no midges were found on individuals of *H. phyllodes*, consistent with our first hypothesis, all subsequent analyses were conducted only for *H. asper*. We further excluded *H. asper* juveniles (n = 9, none with midges) and undetermined sex individuals (n = 10) due to their low representation in the dataset.

Male *H. asper* (0.53 ± 0.96 midges per male; range 0 – 6; n = 178) had, on average, more midges than females (0.29 ± 0.59 midges per female; range 0 – 2; n = 111) (Fig. 2A). Regarding the abundance of midges, males were estimated to host about 1.8 times more flies than females, although this difference was not significant (Table 1). Therefore, sex did not influence the probability of micropredation or the abundance of midges present on an individual.

We found a negative, marginally non-significant relationship between the presence of midges on *H. asper* males and distance to water (p = 0.059). Males were also more likely to be targeted than females (p = 0.010), although the abundance of midges per individual did not differ between sexes. The probability of a male frog directly on the water having midges was ~45%, which quickly decreases to 0% for frogs more than 10 cm away from the water (Fig. 2B). At very close distances to water, males were more likely to have midges than females. For females, distance to water did not affect the probability of midge presence, while for males, we found a marginally significant trend suggesting that midge presence decreased with as distance from the stream increased (Fig. 2B).

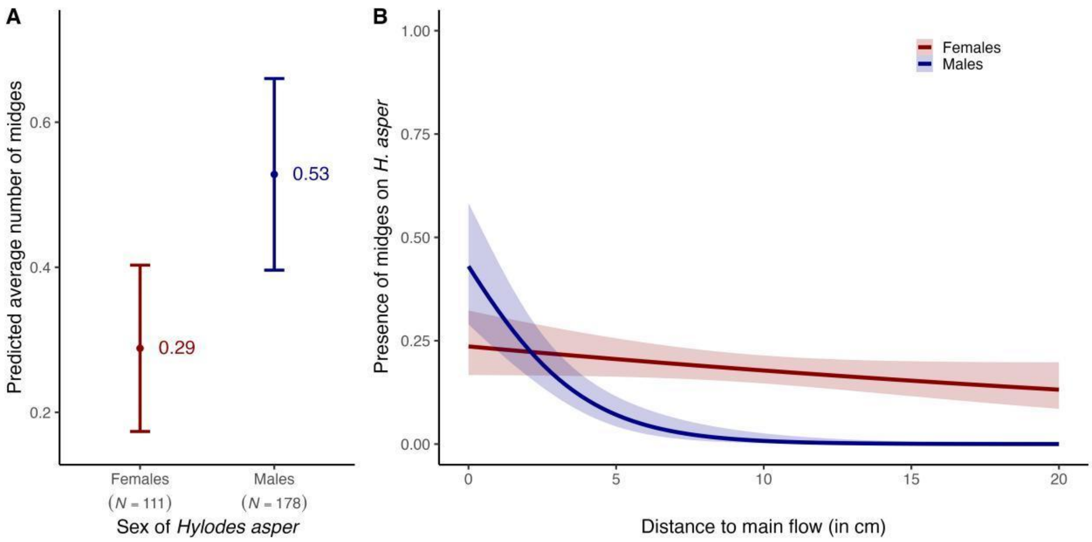


Figure 2. A) Predicted average abundance of midges for females (blue) and males (red) of *Hylodes asper* in Picinguaba, São Paulo, Brazil. Vertical bars represent the 95% confidence interval for each predicted average. B) Probability of midge presence on *H. asper* in relation to distance from the mainstream flow, for females (blue) and males (red).

Table 1. Estimates, standard errors (SE), and Z values for the abundance of frog-biting midges on males and females of *Hylodes asper* in Picinguaba, São Paulo, Brazil. None of the variables were significant.

	Estimate	Std. Error	Z value
Poisson predictors			
Female	-0.501	0.339	-1.480
Male	0.558	0.372	1.496
Binomial predictors			
Female	0.097	0.583	0.166
Male	-0.093	0.646	-0.144

No midges were found on limbs or dorsum thus we only compared head regions (mouth, nostrils, gular regions and snout). The Kruskal-Wallis test showed that there were differences between regions where midges landed on frogs' heads ($\chi^2 = 107.73$, $p < 0.001$). The gular region ($n = 53$) and nostrils ($n = 48$) had more midges than all the other regions (Table 2) albeit with no differences among them ($p = 0.99$).

Table 2. Pairwise Wilcoxon test for differences in the abundance of midges on different body regions of frogs. An asterisk indicates significant differences.

	Mouth	Nostrils	Gular	Snout
Nostrils	<0.001*	-	-	-
Gular	<0.001*	0.84	-	-
Snout	1	<0.001*	<0.001*	-
Head	0.22	<0.001*	<0.001*	0.22

Individuals of *H. asper* were found all day long (active by day and resting at night), being especially abundant during the morning. However, midges were only found during the day, with no midge being found after 18:00h despite frogs being present (Fig. 3A). Our results show that there is a daily cyclical effect on the expected abundance of midges on frogs, with time of day (sine = 0.652, p = 0.059 and cosine = -2.308, p = 0.010), being statistically significant or marginally non-significant (Table 3). The predicted abundance of midges peaked at noon (Fig. 3B).

Our model showed that only precipitation and its non-additive interaction with maximum daily temperature had a positive effect on the abundance of midges found on frogs (Table 3, Fig. 3C), with the interaction of precipitation and minimum temperatures being marginally non-significant. When precipitation is low, the abundance of midges is equally low, independent of daily temperature. However, the abundance of midges found on frogs also increased with precipitation, particularly under higher maximum temperatures. Yearly cyclical sine and cosine were not strong predictors of the abundance of midges on frogs, which suggests that not only time of the year (e.g., spring), but rather specific environmental conditions, may be driving the abundance of frog-biting midges.

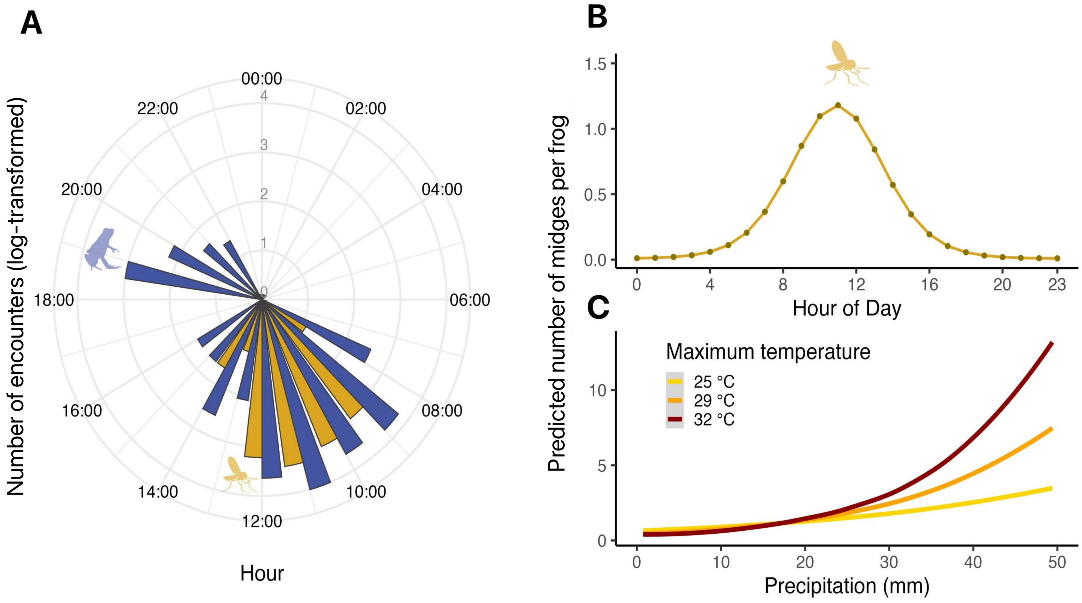


Figure 3. A) Observed number of encounters of midges (gold) and *Hylodes asper* (blue) throughout the day (log-transformed for visualization). Sampling did not occur from 16:00 –18:30 and 22:00 – 07:30. From 18:00 onwards, the number of encounters of frogs is lower due to lack of activity (not calling nor foot-flagging). B) Predicted abundance of midges per frog throughout the day. C) Relationship of predicted abundance of midges per frog and precipitation and maximum daily temperature. Temperature values correspond to the 10% quantile (25 ºC), median (29 ºC) and 90%

quantile (32 ºC), while maximum precipitation (50 mm) corresponds to the 90% quantile.

Table 3. Estimates, standard errors (SE), and Z values for cyclical and scaled bioclimatic predictor variables of midge abundance on *Hylodes asper* in 2008. Asterisks denote significance. #, marginally non-significant, $p \leq 0.06$; *, $p \leq 0.05$; and **, $p \leq 0.01$.

	Estimate	SE	Z value
Poisson predictors			
Intercept	-0.184	0.279	-0.659
TMax	0.034	0.422	0.079
TMin	-0.809	0.458	-1.765
Precipitation*	1.515	0.706	2.145
Cyclical sine	-0.025	0.314	-0.081
Cyclical cosine	0.229	0.352	0.650
TMax:TMin	-0.230	0.208	-1.102
TMin:Precipitation#	-1.990	1.028	-1.936
TMax:Precipitation**	0.328	0.122	2.693
Binomial predictors			
Intercept	-4.203	3.808	-1.104
Precipitation	-7.138	8.919	-0.800
TMin	0.930	1.082	0.860
TMax	-0.624	1.219	-0.512
Cyclical sine	-1.926	1.606	-1.200

Cyclical cosine	-0.626	1.235	-0.507

Discussion

We found a high incidence of midges on torrent frogs, with their occurrence and abundance modulated by both biological and environmental factors. Midges were exclusively recorded on adult individuals of *H. asper,* and mostly on males, especially those calling closer to the main stream flow. Midge activity was restricted to daytime, and abundances increased with precipitation and maximum daily temperatures. Our results suggest that frog-biting midges in the Atlantic Forest streams show a non-random pattern of targetting amphibians. As midges and mosquitoes are potential vectors of amphibian diseases such as trypanosomes (Borkent, 2008), ranavirus (Kinble et al. 2015), and chytrid fungus (Gould et al. 2019; Toledo et al., 2021a; Reinhold et al. 2023), our findings raise important conservation concerns.

We corroborated our first hypothesis that midges exhibit species-specific host preference. Even though *H. asper* and *H. phyllodes* are sympatric and often syntopic, we found no midges on *H. phyllodes*. Similar patterns of selective micropredation have been reported even among congeneric frogs (de Silva et al., 2014; Amaral & Pinho, 2015), although typically expressed as seasonal differences in prevalence rather than complete absence (Legett et al., 2018; Ambrozio-Assis et al., 2019). Host selectivity by frog-biting midges has often been attributed to frog acoustic traits, particularly higher call rates, lower call frequencies (typically 1 – 2.5 kHz), shorter pulse durations, and longer inter-pulse intervals (Bernal et al. 2007; Meuche et al., 2016, Virgo et al., 2019, but see Virgo et al., 2009). Even though the call of both species is pulsed, likely adapted to the high ambient water noise of streams (Haddad & Giaretta, 1999; Augusto-Alves et al., 2021), the calls of *H. asper* have lower dominant frequencies (2 – 4 kHz in H. asper vs. 2.6 – 6 kHz in *H. phyllodes*), shorter pulse duration (2.22 ± 0.37 s vs. 4.65 ± 4.79 s in *H. phyllodes*) and longer inter-pulse intervals (47.9 ± 8.9 s vs. 4.18 ± 1.77 s *in H. phyllodes*) (Haddad & Giaretta, 1999; Hartmann et al., 2006; Augusto-Alves et al., 2021; Augusto-Alves & Toledo, 2022). These acoustic differences likely underlie the strong preference of frog-biting midges for *H. asper*.

Although differences between species were clear, our results suggest that sex was a good predictor of presence but not of the abundance of midges on frogs, despite observing slightly more midges in males than females. Thus, these results align with our second prediction that males are significantly more likely to have midges, despite not in larger numbers. Unlike many hematophagous insects that rely on heat and CO_2 emission as a cue to detect hosts, corethrellids are attracted to frogs by acoustic cues from long distances (Bernal et al. 2006; Amaral & Pinho, 2015; Geisler et al. 2022), often complemented with visual cues at short range (Silva & Breviglieri, 2021). This mechanism may lead them to primarily feeding on male *H. asper*, which call and perform more conspicuous visual displays such as foot-flagging (Haddad & Giaretta, 1999). Female frogs are rarely targeted and if so in low numbers, although occasionally midges can be passed from the male during amplexus (Johnson et al. 1993; Bernal & Silva 2015). In this study, incidence on females was high, which could represent 'collateral damage' due to proximity to calling males, as previously suggested by Silva & Breviglieri (2021). Eavesdropping enemies are known to target non-signaling individuals close to signaling ones (Trillo et al., 2021), albeit most studies concern species-level rather than sex-specific collateral damage (Trillo et al., 2016; Ruether et al., 2022). Although female calls were not heard during this study, females of species from the family Hylodidae have been reported to have courtship calls (Santana et al., 2025), although these short-range calls tend to be rare and have low intensity. Further studies could explore what drives this unusual incidence of midges on females.

Despite their small area, the nostrils were particularly targeted by midges, along with the gular region, corroborating our fifth hypothesis. Given that corethrellids do not detect frog hosts by potential CO_2 emission, which is mainly done via the whole body rather than the nostrils (Amaral & Pinho, 2015), this is likely due to the thinner skin in these regions, which allows for easier access to blood (Silva et al., 2014). This has been demonstrated for other species, with nostrils and other thinner, vascularized regions being preferred by midges (Silva et al., 2014). The strong incidence on the nostrils has also been linked to them being especially exposed to species calling from the water (Virgo & Eltz, 2022). However, this is not the case with males of *H. asper,* as they call from rocks and logs (Haddad & Giaretta, 1999). Furthermore, even though it has been hypothesized that males are targeted mainly in the gular region due to the presence of a vocal sac, which is a potential visual cue, we found the gular region was equally targeted for both sexes. This suggests that for *H. asper* this region may be particularly bitten by midges due to its vascularization and not because of male calling activity. Additionally, variation in the attractiveness to frog-biting midges has been attributed to species-specific differences in the vascular characteristics of the skin (Silva et al., 2014). Further research may explore if sympatric *H. phyllodes* have a thicker skin, especially around the nostrils and gular region, thus being less prone to midge biting.

We found that the abundance of frog-biting midges increased with precipitation, particularly under higher temperatures, consistent with their breeding requirements (Legett et al., 2018). Precipitation has previously been shown to directly influence midge abundance, as standing water is required for egg laying and larval development (Borkent, 2008). For example, Legett et al. (2018) reported that Corethrellidae abundance peaked during rainy seasons in a Neotropical rainforest, correlating with increased water availability for larval development. Similar patterns have been observed in other blood-feeding midges, such as *Culicoides* spp., which exhibit higher abundance in warmer months and at sites with persistent water sources (Neupane et al., 2025). Furthermore, distance from the main stream flow strongly influenced the presence of midges, supporting our third hypothesis. The higher concentration of calling and foot-flagging males near the main stream flow, compared to more marginal areas, may explain this pattern.

In addition to climatic and spatial factors, we found that midge activity was strictly diurnal, with no frog-biting midges recorded at night, despite the continuous presence of inactive *H. asper*. This temporal restriction may be associated with intrinsic limitations of the midges, as well as with host behavior. For example, at night, many stream-dwelling frogs reduce surface activity or remain sheltered and less exposed (e.g., Narins et al., 2005; Grafe et al., 2018), which could lower the chances of host encounter. Moreover, micropredation activity often shows temporal overlap with peak host signaling behavior (Bernal et al., 2006; Bernal & de Silva, 2015), and in our study the alignment of diurnal midge activity with the calling period of males suggests a co-occurrence that may increase micropredation success. At present, the mechanisms driving this strict diurnality remain unclear, highlighting an important gap for future investigation.

Our findings likely reinforce the under-explored link between midge presence and infectious diseases such as chytridiomycosis, as previously suggested by Toledo et al. (2021a). For instance, the potential relationship between midge abundance and infection rates may be modulated by environmental factors. We showed that midge abundance increased with precipitation and maximum temperature, which is not coincident with higher infection rates observed during winter in the same *H. asper* population (Ruggeri et al., 2015). Notably, higher temperatures combined with lower precipitation have been linked with declines in abundances of *H. asper* (Ruggeri et al., 2015).

Even though *H. asper* is an abundant stream-dwelling frog in the study region, its populations exhibit near-complete annual turnover (Ruggeri et al., 2015), making them particularly susceptible to even minor demographic shifts. This vulnerability is exemplified by historical local declines of *Hylodes* spp. in the 1980s, including a population of *H. asper* (Heyer et al., 1988; Toledo et al. 2023). In addition to direct mortality caused by chytridiomycosis in the region, infection could have sublethal impacts on individual fitness. Infected frogs may invest more in sperm or egg production (perhaps as a terminal investment; see Chatfield et al. 2013), usually tend to be in poorer body condition (Brannelly et al., 2021), and are less likely to call than uninfected individuals, although this effect can be absent when body condition is high (Brannelly et al., 2021). Additionally, infected frogs have been reported to spend more time in the water (Roznik et al., 2013) and to exhibit reduced locomotor performance (Chatfield et al. 2013). Considering that *H. asper* strongly relies on foot-flagging displays for courtship, Bd infection could indirectly compromise reproductive success, ultimately affecting population-level fitness.

There are several instances of syntopy of two species of *Hylodes* in streams throughout the large distribution of the genus (at least 11 degrees of latitude along the Atlantic Forest; Pereira, 2017), offering valuable opportunities for further exploring the host-micropredation dynamics identified here. While our study was limited to a single population and lacked direct experimental tests of pathogen transmission, it nevertheless provides novel evidence that midge-frog interactions are structured by host traits, environmental factors, and temporal patterns. Considering that precipitation and temperature strongly influenced midge abundance, ongoing climate change is likely to alter the frequency and intensity of these interactions. From a conservation perspective and given the potential role of midges in Bd transmission, further studies on the behavior of midges and syntopic *Hylodes* species, coupled with local Bd dynamics, will be critical to anticipate disease impacts. More broadly, our findings highlight frog-biting midges as overlooked components of Neotropical stream ecosystems, small but persistent antagonists that may shape amphibian behavior, reproduction, and ultimately, vulnerability to disease.

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Author contributions

FCS, JCDR, LFT and MM planned the manuscript. FS, JCDR and MM led the study. LRVA, MPG, IM, LFT and MM collected the data. FCS analyzed the data. FCS, JCDR and MM wrote the original draft; FCS, JCDR, LFT and MM reviewed and edited the manuscript. All authors approved the final version for publication.

Conflict of Interest

The authors declare that there is no conflict of interest related to the publication of this manuscript.

Data Availability Statement

The data and code that support the findings of this study are available on request from the corresponding author.

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