Torrent frogs have fewer macroparasites but higher rates of chytrid infection in landscapes with smaller forest cover

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Abstract. Deforestation can compromise ecological processes and biotic interactions, including the host–parasite relationship. While some parasites infect only one host, others require multiple hosts to complete their complex life cycles. In this context, different parasites may have different demands and traits and may have specific responses to habitat degradation. Here, we tested whether forest cover has different effects on different frogs’ parasites, as chytrid (Batrachochytrium dendrobatidis; Bd) and helminths (Platyhelminthes and Nematoda). We collected data on two stream frog species (Crossodactylus caramaschii and Crossodactylus schmidti) from nine sites in the Brazilian Atlantic forest, with forest cover ranging from 20% to 99%. Bd presence and load increased with decreasing forest cover, but the opposite was observed for nematodes. Load of monoxenous and heteroxenous helminths increased with forest cover. We suggest that variations in potential host diversity, micrometeorological conditions, and host immune response may be responsible for the contrasting patterns found for micro-(Bd) and macroparasites (helminths, except Platyhelminthes). Our work brings evidence of how habitat reduction can affect host–parasite relationships, including infection with the pathogen responsible for hundreds of global species extinctions.

Key words: Amphibia; Batrachochytrium dendrobatidis; biotic interaction; chytrid; deforestation; helminths; pathogens.

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INTRODUCTION

It has been long recognized that environmental disturbance promoted by human activities drive local climate alterations and biodiversity loss (Wilcox and Murphy 1985, Caughley 1994, Meyer and Sisk 2001, Karlsson and Van Dyck 2005, Hillers et al. 2008, Krauss et al. 2010). Among several anthropogenic changes, deforestation is one of the main concerns in tropical regions (Bowles et al. 1998, Pineda and Halffter 2004). Logging, agricultural clearance, and urban
expansion have fragmented many pristine forests and, generally, bring irreversible damage to biodiversity by compromising ecological processes (Valladares et al. 2006, Wilson et al. 2016). Habitat loss has been shown to jeopardize biotic interactions, affecting ecological functions such as seed dispersal and nutrient transfer (Turner 1989, Metzger 2001, Herrera and García 2010, Bentéz-Maldvado et al. 2016). One important issue in vogue is related to the effects of habitat loss and degradation on host–parasite interactions (Calegaro-Marques and Amato 2014, Bordes et al. 2015).

Current evidences have shown that habitat degradation can affect parasitic diseases in different ways (Chasar et al. 2009). A potential prediction would be greater parasitism in degraded areas, as the host’s immune system may be compromised under stressful conditions (Walsh et al. 1993). However, the opposite effect is also possible (Lafferty and Kuris 2005): Modified environments characterized by reduced species diversity would restrict the availability of alternative hosts, interrupting the life cycle of heteroxenous parasites (King et al. 2008). In this context, with lower diversity and density of potential hosts, parasites should have a lower chance of being successful and surviving. Therefore, a reduced ecological resilience caused by biological diversity simplification in degraded habitats may homogenize possible parasite–host interactions (Laliberté and Tylianakis 2010). Here, we hypothesized that different kinds of parasites may respond differently to varied levels of deforestation, and these responses are involved to particular parasite traits, host diversity and susceptibility, and particular environmental conditions.

Despite the fact that frogs normally carry small parasites species pools, many kinds of parasitic organisms can use them as definitive or intermediate hosts (Nickol 1985, Moravec and Kaiser 1994, Thiemann and Wassersug 2002, Santos and Amato 2010, Imasuen et al. 2012, Pough et al. 2015). One possible explanation for interaction with lots of parasites is that amphibians are a diverse group of vertebrates, especially in the tropics, with complex life cycles, occupying both terrestrial and aquatic environments (Duellman and Trueb 1994, Pyron and Wiens 2013). Many parasites can adversely affect, with different magnitudes, the survival, growth, and reproduction of their hosts (Agnew et al. 2000). In general, macroparasites, such as helminths, are usually long-lived in their hosts and typically cause no apparent (or only minor) symptoms (Goater 1994, MacDonald et al. 2002). In contrast, chytridiomycosis (a fungal disease) is highly virulent in susceptible amphibians, commonly leading to host death (Berger et al. 1998, Voyles 2009, Scheele et al. 2019). As amphibians populations are especially vulnerable to the frog-killing fungus Batrachochytrium dendrobatidis (hereafter Bd), which is one of the most lethal anuran microparasites; this pathogenic fungus causes chytridiomycosis and is one of the major causes of mass mortalities in amphibian populations around the world (Scheele et al. 2019). In adult frogs, the chytrid develops a zoosporangium on the skin, which may potentially damage some physiological processes at the epidermal tissue (Voyles 2009, Fites et al. 2013), may disrupt host’s immune system (Fites et al. 2013, Fernández-Loras et al. 2017), and cause diverse sublethal effects (Salla et al. 2015, 2018, Bovo et al. 2016).

Our work represents an important advance for understanding the effect of habitat reduction on host–parasite interactions, considering the perspective of different kinds of amphibian parasites (Belasen et al. 2019). Given the particular traits of micro- and macroparasites, we expected to find different responses of these parasites to hosts occurring in areas with different levels of forest cover in the surrounding landscape. We expected that macroparasites, specially with complex life cycle (Heteroxenous), would be negatively affected by forest loss, due to the reduction of host diversity (resource) and potential interactions. Conversely, we expected that B. dendrobatidis, as an opportunistic and epizootic organism, would have higher transmission performance in deforested landscapes, where hosts are potentially more stressed and immunologically susceptible.

**Materials and Methods**

**Study species and sites**

We collected 78 frogs of two species: Crosso
dactylus caramaschii (51) and Crosso
dactylus schmidtii (27) from nine localities (in six small to large forest blocks; ranging from 6,680 to 694,000 ha) in the Atlantic forest of South and Southeastern Brazil (Table 1). These localities cover 30% of all municipalities where these
species have been recorded in Brazil (based in the speciesLink data). Both species are active and reproduce in small streams, with males exhibiting calls on rocks during the day (Haddad and Prado 2005, Haddad et al. 2013). The two species are ecologically equivalent and allopatric; *C. caramaschii* has a distribution associated with dense ombrophilous forest, occurring between southern São Paulo state and localities in the states of Paraná and Santa Catarina, Brazil (Frost 2019); *C. schmidti* occurs in semideciduous forest, ranging from Misiones, Argentina, and adjacent southeastern Paraguay; western Paraná, and Santa Catarina states and northern and western Rio Grande do Sul state, Brazil (Frost 2019). *Crossodactylus schmidti* is currently listed as Near Threatened by IUCN (2018), and its population trend follows a pattern of decline. Whereas *C. caramaschii* populations are distributed in a well-preserved area (a single large continuous extent of forest), *C. schmidti* in some regions, such as Paraná and western Santa Catarina, occupies severely fragmented environments. Such condition allowed we sampled different sites in order to capture a deforestation gradient, with forest cover ranging from 20% to 99% (Fig. 1). Our samples from *C. schmidti* populations were more restricted because of their threat status and limited and patched distribution.

**Parasite sampling**

Frog specimens were collected individually and placed in plastic bags. After capture, we swabbed individuals for detection of the fungus *B. dendrobatidis* (Hyatt et al. 2017). We extracted DNA from each swab using Prep-Man UltraR (Life Technologies, Carlsbad, California, USA). We used TaqMan qPCR (Boyle et al. 2004) with standards of 0.1, 1, 10, 100, and 1000 zoospore genomic equivalents (g.e.) to measure the Bd loads in each frog. As a quantitative standard for all qPCRs, we prepared standards using the Bd isolate CLFT 159. We considered samples positive for Bd when zoospore g.e. was \( \geq 1 \) (Kriger et al. 2007).

We euthanized each specimen soon after swabbing procedures and necropsies were carried out to sample internal macroparasites. We searched for the presence of parasites in all organs and body cavities. After this procedure, individuals were fixed and deposited at Museu de Zoologia “prof. Adão José Cardoso,” Universidade Estadual de Campinas (Unicamp), Campinas, São Paulo, Brazil (ZUEC 24007-38; 24043-60; 24284-8; 24291-5). Sample collections had been approved by the Chico Mendes Institute for Biodiversity Conservation (ICMBio permit #57611-2).

Using a stereomicroscope, we identified macroparasites belonging to the phyla:

| Site | Elevation (m) | Min temp (°C) | PREA       | FSS     | Coordinates (lat; long) |
|------|--------------|---------------|------------|---------|-------------------------|
| *Crossodactylus schmidti* | | | | | |
| Parque Estadual do Rio Guarani | 456 | 16.4 | Livestock | Secondary | −25.445242°; −53.160522° |
| Parque Nacional do Iguacu | 669 | 16.7 | Grains | Primary | −25.151121°; −53.841327° |
| Parque Estadual do Turvo | 237 | 18.7 | Livestock | Primary | −27.142135°; −53.877599° |
| Frederico Westphalen | 563 | 18.6 | Urban | Secondary | −27.362047°; −53.410647° |
| São Miguel do Oeste | 650 | 17.2 | Urban | Secondary | −26.760030°; −53.525131° |
| *Crossodactylus caramaschii* | | | | | |
| Parque Estadual Carlos Botelho | 780 | 16.6 | Mining, fruits | Primary | −24.062317°; −47.992336° |
| Parque Estadual Intervales | 808 | 16.5 | Mining, fruits | Primary | −24.274903°; −48.417346° |
| Parque Estadual da Caverna do Diabo | 483 | 20.4 | Mining, fruits | Primary | −24.636329°; −48.403442° |
| Parque Estadual do Turístico do Alto Ribeira | 270 | 18.7 | Mining, fruits | Primary | −24.533561°; −48.699261° |

**Notes:** Min temp, minimum temperature; PREA, principal regional economic activity; FSS, forest succession stage.
Nematoda, Acanthocephala, and Platyhelminthes. In addition, we classified these macroparasites according to their life cycle as monoxenous (one host necessary) and heteroxenous (more than one obligatory host).

Calculation of forest cover, temperature, and elevation

For each landscape, we obtained data of forest cover for a circular buffer with a radius of 5 km centered on the location of the sampled populations. We calculated forest cover based on color intensity, by considering that darker pixels correspond to forest areas, and whiter pixels to open areas (including large rivers). We exported images from Google Earth to the Gap Light Analyzer 2.0 (GLA) software (Forest Renewal BC., Simon Fraser University (SFU), Burnaby, British Columbia, Canada & Institute of Ecosystem Studies (IES), Millbrook, New York, USA), where we transformed our color images into black-and-white images by manually selecting the threshold that best represented the difference between forest and non-forest for each landscape. We then exported these images to the Paint.net v4.1.6 software (dotPDN LLC, Rick Brewster, and contributors), where we measured the number of pixels corresponding to black (forest) and white (non-

Fig. 1. Sampled landscapes for each locality. Each circle has 5-km radius from centered populations, representing a total area about 78 km². Abbreviations are TH, threshold adjust; FW, Frederico Westphalen; SMO, São Miguel do Oeste; PERG, Parque Estadual do Rio Guarani; PNI, Parque Nacional de Iguacu; PECB, Parque Estadual Carlos Botelho; PET, Parque Estadual do Turvo; PECV, Parque Estadual da Caverna do Diabo; PETAR, Parque Estadual Turistico do Alto Ribeira; PEI, Parque Estadual Intervales.
forest) areas (Fig. 1). Forest cover was calculated by dividing the number of black pixels by the total number of pixels and multiplying by 100.

We measured elevation by direct visualization of our geographic points on Google Earth. Based on a grid format of 30 seconds of spatial resolution (~1 km), we extracted the average minimum annual air temperature for each locality using the bioclimatic database from WorldClim (http://www.worldclim.org/) visualized in the software R.

Data analysis
We analyzed how the presence/absence and load of macroparasites (classified by different subgroups: heteroxenous and monoxenous macroparasites; platyhelminthes and nematodes; as well as all helminths; we excluded Acanthocephala from these analyses due to the small number of individuals found) and microparasites (Bd infection) are related to forest cover. We used each sampled individual as the sampling unit in all analyses and included the sampling site as a random factor. For the presence/absence data, we adjusted generalized linear mixed models (GLMM) with a binomial distribution and logit link function. For macroparasite data, we adjusted GLMMs with a negative binomial distribution and a log link function (Zuur et al. 2009). For Bd load, we first attributed a value of zero to loads between 0 and 1 GE. We then used a log-10 transformation on the non-zero values to reduce the effect of extreme values. Finally, we adjusted GLMMs with a gamma distribution and an inverse link function; as the gamma distribution does not allow for values of zero, we replaced the zeroes by a small value (0.00001); biologically, this small value would mean that it is possible that the frog would have traced of Bd which were too small to be detected or considered an infection.

We then used bootstrap (Manly 2007) to calculate 95% confidence intervals for the slope coefficients of each GLMM; positive slopes indicate a positive effect of forest cover on the response variable, except for the effects of forest cover on Bd load: As these effects were modeled with a Gamma distribution and an inverse link function, negative slopes indicate positive effects and vice versa. We thus repeated the analysis on each of 4999 bootstrapped samples and kept only the first 4999 models that converged for each response variable. For each bootstrap iteration, we randomly resampled, with replacement, our sampling units, with the restriction that each bootstrapped sample had to contain at least one individual per site to ensure that the bootstrapped samples were ecologically meaningful. We calculated 95% confidence intervals as the 2.5 and 97.5 percentiles of the 5000 datasets (original data plus the 4999 bootstrapped datasets) and considered that there was a significant effect of forest cover on the response variable when the confidence interval did not include zero. We visually assessed the effects by plotting all the bootstrapped curves in a single figure (Figs. 2, 3).

To test possible confounding factors in our analysis, we assessed whether elevation and minimum air temperature were correlated with forest cover using Pearson’s correlation coefficient, as these variables are well-established predictors of Bd performance (Bradford 1991, Berger et al. 2004, Kriger and Hero 2008, Raffel et al. 2010, Alemu et al. 2013).

We carried statistical procedures using the packages lme4 (for the generalized linear mixed effects models, Bates et al. 2015) and scales (for making the figure, Wickham 2018) in the software R 3.4.4 (R Core Team 2018; Data S1).

RESULTS
We collected 27 specimens of C. schmidti and 51 of C. caramaschii. Of these specimens, 34 (43.6%) tested positive for Bd. The prevalence of Bd was larger in C. schmidti (48.1%) than in C. caramaschii (41.2%). We found 470 macroparasites in 67 individuals (85.9% of our sampled specimens). The prevalence for macroparasites was larger in C. caramaschii (98.1%) than in C. schmidti (63.0%). Most macroparasites (89.4%) were Nematoda, followed by 9.4% of Platyhelminthes and 1.3% of Acanthocephala, which we found only in specimens of C. caramaschii from primary forest (Table 2).

As forest cover increased, the probability of finding individuals infected with monoxenous macroparasites, nematodes, and helminths in general increased, whereas the probability of Bd infection decreased (Table 3, Fig. 2). Increasing convergence issues, we analyzed more than 4999 bootstrapped samples and kept only the first 4999 models that converged for each response variable. For each bootstrap iteration, we randomly resampled, with replacement, our sampling units, with the restriction that each bootstrapped sample had to contain at least one individual per site to ensure that the bootstrapped samples were ecologically meaningful. We calculated 95% confidence intervals as the 2.5 and 97.5 percentiles of the 5000 datasets (original data plus the 4999 bootstrapped datasets) and considered that there was a significant effect of forest cover on the response variable when the confidence interval did not include zero. We visually assessed the effects by plotting all the bootstrapped curves in a single figure (Figs. 2, 3).

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Forest cover also led to increased loads of both heteroxenous and monoxenous macroparasites, nematodes, and helminths in general, but had no significant effects on Bd load; note, however, that the confidence interval for the effects on Bd load indicates the possibility of a marginally significant decrease with increasing forest cover (Table 3, Fig. 3). Platyhelminthes were not affected by forest cover.

Forest cover was not correlated with either minimum temperature or altitude ($r^2 = 0.17$ and 0.08, respectively), indicating that these variables are not likely to be confounding factors.

**DISCUSSION**

We found evidence that forest cover influences parasite performance in two congeneric amphibian species from streams of the Brazilian Atlantic forest. This find is consistent with the idea that anthropogenic disturbances may affect the relationships between parasites and their (amphibian) hosts (Kiesecker 2002, Johnson et al. 2007, Koprivnikar and Redfern 2012). Our results corroborated our hypothesis, suggesting divergent responses of micro- and macroparasites to habitat loss. It would be expected that anthropogenic
disturbances should increase disease prevalence equally; however, we observed a shift from macroparasites (mostly nematodes) to Bd infection in host–frogs inhabiting deforested landscapes. For many taxonomic groups of hosts, there is no consensus about the relation between habitat amount and infection dynamics (Suzán et al. 2012). Despite that, for many amphibians’ parasites, especially helminths, local environmental conditions are determinant for species occurrences (Aho 1990). In general, this is likely linked to the specific nature of each host–parasite system, as we found in our results. Even among helminths, there was a group (Platyhelminthes) that was not affected by forest cover variation, while for Bd and nematodes, it is possible that changes on biotic complexity, microclimate conditions and host immune response along

Fig. 3. Effect of forest cover on macroparasite (a–e) and Bd (f) load across populations of two species of Crossodactylus from Brazilian Atlantic forest. The two species are represented by different colors: C. caramaschii as red and C. schmidti as purple. The lines represent the effects of forest cover on parasite load, as obtained from generalized linear mixed models, with negative binomial distribution and log link function for macroparasites and gamma distribution and inverse link function for parasite load, adjusted to 4999 bootstrapped samples in addition to the original data.

Table 2. Total number of macroparasites found in two species of Crossodactylus† from the Atlantic forest, Brazil.

| Macroparasites    | Crossodactylus caramaschii | Crossodactylus schmidti |
|------------------|---------------------------|------------------------|
| Nematoda         | 355                       | 65                     |
| Platyhelminthes  | 33                        | 11                     |
| Acanthocephala   | 6                         | 0                      |

† C. caramaschii, n = 51 individuals; C. schmidti, n = 27 individuals.
deforestation gradients, have a substantial role in determining their success.

The primer consequence of deforestation is the local biodiversity loss (Burivalova et al. 2014), which may impact important ecological processes, such as parasitism. If we consider that the heteroxenous parasites are dependent on different hosts during their life cycle (Dobson et al. 1992, Cribbet al. 2003, Parker et al. 2003, Koprivnikar et al. 2012), they should be negatively affected by a biotic simplification, which represents a source reduction for such parasites involved in complex interactions. Then, our data corroborated such prediction, once the load of heteroxenous parasites increased in hosts occurring in more forested places. Further, like many organisms, several Atlantic forest amphibian species cannot tolerate deforestation (Becker et al. 2007) and such landscape changes appear to reduce the number of potential host species, affecting most helminths presence and load.

Besides biotic factors, environmental conditions such as wind and local temperature are important to dictate the success in some microparasites. In lowlands, we can say that open habitats have suboptimal temperatures for Bd, which limits their spread and growth (Becker and Zamudio 2011, Stevenson et al. 2013), but small forests, even those which are subjected to external modifications, may maintain favorable climates to the pathogen, due to edge influence on temperature (Arroyo-Rodriguez et al. 2017). It is well acknowledged that edge habitats in Atlantic forest landscapes are more susceptible to stronger winds and higher air temperatures than forest interiors (Magnago et al. 2015); these factors may benefit the pathogen in transitional zones. Humidity transported by strong winds may maximize Bd spread and increase their prevalence, as its environmental transmission is not limited to water (Kolby et al. 2015). Moreover, as for other fundamental condition, larger variation in air temperatures may be stressful for amphibian hosts occupying forest edges, affecting their infection susceptibility (Raffel et al. 2006).

Previous studies found open (and disturbed) habitats acting as shelters from chytrid fungus (Becker and Zamudio 2011, Hossack et al. 2013), which shows that changes on local factors, as canopy cover, may alter microhabitats temperature and rates of evaporative water, acting against Bd performance and survival (Beyer et al. 2015, Roznik et al. 2015). However, our results indicate that, in a landscape perspective, deforestation can increase the exposure to chytrids and that host-populations in smaller forests had higher Bd infection. Our findings have implications for amphibian conservation once their threats are amplified with habitat reduction. Thus, frogs in smaller forests are at lower risk of infection by nematodes; however, they are at greater risk of contracting a potentially lethal pathogen. Our data show that, in addition to other mechanisms, increased risk of infection by a virulent disease can lead to species declines in deforested landscapes. This pattern is opposite to previous finding in amphibians from a European ecosystem, which cooler and wetter conditions in more forested landscapes seem provide more favorable environment for Bd (Scheele et al. 2015).

Nematodes represented the most common anuran macroparasites in our samples, as it has been traditionally found for amphibians (Campiao et al. 2015). In contrast, acanthocephalans were rare. This group of macroparasites has complex life cycles and used C. caramaschii individuals as intermediate hosts, demanding larger terrestrial vertebrates to complete their life cycle (Jones 1986, Amin et al. 1998). In other words,
acanthocephalans success, in this case, demands a predator who can prey on the frog.

Our findings introduce a new phenomenon for disease ecology, in which habitat reduction may culminate in a different relationship of hosts with micro and macro-parasites. Considering the importance of the frog-killing chytrid fungus for amphibian conservation, we highlight an additional concern in which habitat reduction increases the infection by this lethal pathogen in frog species that persists in landscapes with fragmented forests, especially the case of C. schmidtii. Despite we recognize that a more robust sampling including other stream frogs species and locals are needed to give more consistence to our results (and eliminate a possible confusion factor linked to the species), the cautious implication of this finding is that even those species that persist in forest fragments are not out of threat even if deforestation is halted, once changes in landscapes may promote changes in ecological interactions. Based on our findings, we advocate for forest restoration and biodiversity recovery to prevent further disease outbreaks and to curb the risk of ongoing amphibian declines in the Atlantic forest and potentially other forests in the world.

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