UNIVERSIDADE ESTADUAL DE CAMPINAS

INSTITUTO DE BIOLOGIA

JOELMA SANTOS DO PRADO

TRANSPORT OF A LETHAL AMPHIBIAN PATHOGEN (*Batrachochytrium dendrobatidis*) THROUGH FOG

TRANSPORTE PASSIVO DE UM PATÓGENO LETAL PARA ANFÍBIOS (*Batrachochytrium dendrobatidis*) POR MEIO DE NEBLINA

CAMPINAS

2022

JOELMA SANTOS DO PRADO

TRANSPORT OF A LETHAL AMPHIBIAN PATHOGEN (*Batrachochytrium dendrobatidis*) THROUGH FOG

TRANSPORTE PASSIVO DE UM PATÓGENO LETAL PARA ANFÍBIOS (*Batrachochytrium dendrobatidis*) POR MEIO DE NEBLINA

Dissertation presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements for the degree of Masters, in the area of Ecology

Dissertação apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do Título de Mestra, na área de Ecologia

Orientador: PROF. DR. LUIS FELIPE DE TOLEDO RAMOS PEREIRA

ESTE ARQUIVO DIGITAL CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO DEFENDIDA PELA ALUNA JOELMA SANTOS DO PRADO E ORIENTADA PELO DR. LUIS FELIPE DE TOLEDO RAMOS PEREIRA.

CAMPINAS

2022

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca do Instituto de Biologia
Mara Janaina de Oliveira - CRB 8/6972

P882t

Prado, Joelma Santos do, 1997-
Passive transport of a lethal amphibian pathogen (*Batrachochytrium dendrobatidis*) through fog / Joelma Santos do Prado. – Campinas, SP : [s.n.], 2022.

Orientador: Luis Felipe de Toledo Ramos Pereira.
Dissertação (mestrado) – Universidade Estadual de Campinas, Instituto de Biologia.

1. Neblina. 2. Anfibio - Doenças. 3. DNA ambiental. 4. Aerobiologia. 5. *Batrachochytrium dendrobatidis*. I. Toledo, Luís Felipe, 1979-. II. Universidade Estadual de Campinas. Instituto de Biologia. III. Título.

Informações Complementares

Título em outro idioma: Transporte passivo de um patógeno letal para anfíbios (*Batrachochytrium dendrobatidis*) por meio de neblina
Palavras-chave em inglês:
Fog
Amphibians - Diseases
Environmental DNA
Aerobiology
Batrachochytrium dendrobatidis
Área de concentração: Ecologia
Titulação: Mestra em Ecologia
Banca examinadora:
Luis Felipe de Toledo Ramos Pereira [Orientador]
Carolina Lambertini
Marcelo José Sturaro
Data de defesa: 15-07-2022
Programa de Pós-Graduação: Ecologia

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0003-4929-9253
- Currículo Lattes do autor: http://lattes.cnpq.br/6210146301405018

Campinas, 15 de julho de 2022.

COMISSÃO EXAMINADORA

Prof. Dr. Luis Felipe de Toledo Ramos Pereira

Dra. Carolina Lambertini

Prof. Dr. Marcelo José Sturaro

Os membros da Comissão Examinadora acima assinaram a Ata de defesa, que se encontra no processo de vida acadêmica do aluno.

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa de Pós-Graduação em Ecologia do Instituto de Biologia.

A todos os educadores que

pavimentaram meu caminho até aqui.

AGRADECIMENTOS

Ao meu orientador Felipe Toledo pela instrução, confiança, oportunidades e conselhos de vida.

Aos professores que compuseram o comitê de acompanhamento, bancas de qualificação, exame prévio e de defesa, pela disponibilidade e atenção em contribuir, enriquecendo este trabalho.

Aos colegas de laboratório e pesquisadores colaboradores por todo o apoio profissional e emocional ao longo dos últimos dois anos.

Às agências de fomento pelo financiamento à pesquisa: CNPq e processo nº2020/ 02991-8 da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Ao meu companheiro de vida, Filippe Eduardo, por sempre fazer parte de todas as minhas conquistas. À minha família e amigos, meus alicerces.

Agradeço a todos os educadores que se esforçam diariamente para garantir a educação pública de qualidade e que justamente me permitiram alçar vôo. Se hoje tenho a oportunidade de retornar conhecimento ao mundo é graças a todos os professores que me guiaram até aqui.

RESUMO

A biodiversidade global está em crise. Os anfíbios são o grupo animal vertebrado mais ameaçado da atualidade. Dentre as principais causas de declínio e extinção de espécies, estão as epidemias de doenças infecciosas, como é o caso da quitridiomicose, uma doença causada pelo fungo *Batrachochytrium dendrobatidis* (Bd). O fungo quitrídeo está associado a corpos hídricos, que representam sua principal via de dispersão. Contudo, são reportados casos de anfíbios de desenvolvimento estritamente terrestre com altas cargas do Bd. Permanece não elucidado o processo pelo qual essas espécies, que possuem pouco ou nulo contato com a água, são expostas ao Bd. A neblina é um importante componente hídrico e carreador de microrganismos, inclusive de patógenos, entre ecossistemas. Hipotetizamos que a neblina carregue o Bd em suspensão e que zoósporos ativos em suspensão infectem anfíbios hospedeiros. Realizamos a coleta de neblina e de água de chuva, nove locais na Mata Atlântica, no Brasil e investigamos a presença de Bd através da técnica de qPCR. Também conduzimos experimentos de infecção da espécie de desenvolvimento direto *Eleutherodactylus johnstonei*, através da exposição de indivíduos à neblina artificial e natural. Nós reportamos a primeira evidência de DNA de Bd na neblina e sugerimos que hospedeiros suscetíveis podem ser infectados e desenvolver quitridiomicose letal por meio do transporte passivo de zoósporos de Bd pela neblina. Nossos resultados ampliam a rede de dispersão do Bd entre reservatórios ambientais, adicionando a neblina como um novo mecanismo de transporte passivo de alcance regional. Além disso, abrem novas vias de insvestigação para elucidar mecanismos de exposição das espécies de anfíbios ao patógeno. Assim, sugerimos que futuros estudos epidemiológicos de Bd incluam as vias aéreas de transporte passivo.

Palavras-chave: neblina; doença em anfíbios; eDNA; aerobiologia; *Eleutherodactylus johnstonei*; *Batrachochytrium dendrobatidis*

ABSTRACT

The global biodiversity is in crisis. Amphibians are currently the most threatened vertebrate group. Epidemics of infectious diseases are among the main causes of population declines and species extinction. Which is the case of chytridiomycosis, a disease caused by the fungus *Batrachochytrium dendrobatidis* (Bd). The chytrid fungus is associated with water bodies, its main means of dispersion. However, cases of strictly terrestrial amphibians with high Bd loads are reported. The mechanisms by which these species (which have little or no contact with water) are exposed to Bd remains unclear. Fog is an important water component and carrier of microorganisms, including pathogens, between ecosystems. We hypothesized that fog carries Bd in suspension and that active zoospores in suspension infect host amphibians. We collected fog and rainwater from nine locations along the Atlantic Forest in Brazil and investigated for the presence of Bd in natural fog by qPCR assay. We also conducted infection experiments with the direct developing species *Eleutherodactylus johnstonei*, through individual exposure to artificial and natural fog. We report the first evidence of Bd DNA in the fog. We found that susceptible hosts can become infected and develop lethal chytridiomycosis through the passive transport of Bd zoospores by the fog. Our results extend the network of Bd transport pathways between environmental reservoirs by adding fog as a new regional-range dispersion pathway. In addition, the results open new avenues of investigation to elucidate exposure mechanisms of amphibian species to the pathogen. Thus, we suggest that future epidemiological studies of Bd include passive transport airways.

Keywords: fog; amphibian disease; eDNA; aerial biology; *Eleutherodactylus johnstonei*; *Batrachochytrium dendrobatidis*

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

SUMÁRIO

INTRODUÇÃO GERAL

Anfíbios são o grupo de vertebrados mais ameçado da atualidade e vem sofrendo declínios massivos de populações e extinção de espécies desde 1989 (Wake & Vredenburg, 2008). Doenças infecciosas compreendem uma das principais ameaças à biodiversidade. Dentre elas, a quitridiomicose causada pelo fungo *Batrachochytrium dendrobatidis* (Bd) (filo Chytridiomycota, classe Chytridiomycetes, ordem Rizophydiales) (Longcore et al., 1999) é atualmente uma das doenças de vida silvestre mais severas (Scheele et al., 2019).

O quitrídeo causa a infecção da pele dos anfíbios por todo o corpo do indivíduo, podendo estar mais concentrado na região inguinal e nas membranas interdigitais dos membros posteriores em adultos e na região bucal em girinos. A infecção prejudica os processos fisiológicos essenciais realizados através da pele, como troca de gases e osmorregulação, o que nos anfíbios torna-se letal (Longcore et al., 1999; Voyles et al., 2009).

A quitridiomicose já levou ao declínio de populações e à extinção de espécies de anfíbios em várias regiões do planeta, sendo registrada em todos os continentes (Kriger et al. 2007; Lips et al., 2006; Cheng et al., 2011; Carvalho et al., 2017; Scheele et al., 2019). O Bd é distribuído ubiquamente nos mais variados ecossistemas (Bower et al., 2017; Ruggeri et al., 2018), inclusive em várias regiões da Mata Atlântica (Toledo et al., 2006a; Vieira et al., 2012), bioma que possui relevante riqueza e endemismo de anfíbios (Haddad et al., 2013) e no qual o declínio de populações de anfíbios foi pela primeira vez associado à quitridiomicose (Carvalho et al., 2017).

A dinâmica de dispersão do patógeno Bd é complexa. Os principais mecanismos de transmissão são representados pelo contato direto entre hospedeiros, e através do contato com corpos hídricos, devido ao ciclo de vida composto por uma fase de

zoosporângio séssil e outra fase infectante de zoósporo aquático livre-natante (Kilpatrick et al., 2010). Além disso, a dispersão pode se dar entre reservatórios ambientais (i.e. um reservatório abiótico que abriga o patógeno e permite seu transporte (Hoyt et al., 2020)), envolvendo o movimento da água em corpos hídricos permanentes, atráves do carregamento por sedimento, por outros animais vetores vertebrados (peixes, aves aquáticas, e lagartos) (Liew et al., 2017; Johnson & Speare 2003; Pontes et al., 2018), invertebrados como insetos hematófagos e crustáceos (Toledo et al., 2020; Prahl et al., 2020) e também através da chuva (Garmyn et al. 2012; Kolby et al., 2015). A transmissão terrestre tanto direta quanto indireta, de um reservatório para uma espécie hospedeira sucetível, pode resultar em quitridiomicose letal (Burns et al., 2020). De modo que a transmissão pode ocorrer mesmo que os dois hospedeiros ocorram em determinado reservatório ambiental em momentos diferentes. Ademais, é difícil identificar rotas de dispersão do Bd que ocorrem independentemente de anfíbios hospedeiros e é provável que existam mecanismos ainda não caracterizados (Kolby et al., 2015).

Um importante reservatório ambiental para microrganismos é a neblina (Joung et al., 2017). A neblina é composta além de outras coisas, por aerossóis em suspensão, estes que são um conjunto de partículas atmosféricas de materia orgânica, inorgânica e de gotículas de água. Muitas vezes no processo de aerossolização, microrganismos são arremessados além da camada límitrofe de ar (i.e. a camada de ar de 0.1 a 9 mm imediatos à uma dada superfície), formando os chamados bioaerossóis (Gollakota et al., 2021; Luisetto et al., 2021). Comunidades microbianas estão presentes na neblina de áreas costeiras e esta pode configurar-se como potencial mecanismo de dispersão microbiana de longa distância entre ecossistemas terrestres, marinhos e de água doce adjacentes (Evans et al., 2019). A neblina se configura um importante componente de

input hídrico em diferentes ecossistemas (Torregrosa et al., 2014), principalmente em matas nebulares e em elevadas altitudes, como por exemplo na Mata Atlântica (Bittencourt et al., 2019). Além disso, a neblina tem potencial de manter a viabilidade de microorganismos presentes em aerossóis, tanto por processo físicos quanto biológicos, como por exemplo pela diminuição da dessecação (Duecker et al., 2012b), podendo ser uma fonte contínua de carreamento de patógenos em locais com alta frequência desse evento (Evans et al., 2019). Portanto, caso a neblina possa carregar o Bd entre ambientes, esta seria uma rota indireta alternativa, ainda não considerada em estudos epidemiológicos recentes.

Atualmente, pouco se sabe sobre a viabilidade de zoósporos de Bd em sistemas não controlados em laboratório ou em possíveis reservatórios ambientais de transmissão (Burns et al., 2020). Mas entender como esses mecanismos de transmissão funcionam é fundamental para compreender a doença e mitigar seus impactos. Entender como funcionam as complexas rotas de transporte do patógeno é importante para estudos epidemiológicos e para compreender os mecanismos que levam a declínios atuais causados por Bd (Burns et al., 2020). O transporte passivo do Bd embora ainda seja pouco caracterizado quanto a seus mecanismos, é uma via de investigação cada vez mais relevante (Kolby et al., 2015). Principalmente com relação à vias de transmissão para espécies com baixa associação aquática. Este é o caso de mais de mil espécies do clado Brachycephaloidea (Frost, 2022), que possuem desenvolvimento direto (i.e. sem fase larval), e tem pouco ou nulo contato com corpos hídricos durante o seu desenvolvimento. Espécies de desenvolvimento direto tem se mostrado vulneráveis à quitridiomicose. No entento, permanecem ainda não elucidadas as diferentes rotas pelas quais essas espécies são expostas ao Bd (Mesquita et al., 2017).

Desse modo, frente ao cenário preocupante de perda de diversidade de anfíbios pelo Bd (Hoffmann et al., 2010), os esforços para a conservação devem incluir as espécies sensíveis a esse patógeno (Mesquita et al., 2017). Ademais, detectar e caracterizar a ocorrência de um patógeno é relevante para identificar as condições ambientais que levam a surtos, para desenvolver medidas de controle e evitar epidemias (Hall et al., 2015). Neste estudo, hipotetizamos que i) a água na forma de neblina transporta zoósporos de Bd e ii) zoósporos de Bd na neblina podem infectar novos hospedeiros. Nesse contexto, o presente estudo irá fornecer importante subsídio para uma compreensão de como ocorre a contaminação pelo Bd das espécies terrestres e de como se dá a transmissão do quitrídeo em escala local. Este estudo contribui para maior compreensão das vias de transporte do fungo Bd e levanta questões para próximas perguntas.

ARTIGO

Chytrid in the clouds: an alternative passive transport of a lethal pathogen for amphibians

Joelma S. Prado[1,2,3]; Julia R. Ernetti[1,2]; Mariana R. Pontes[1,2]; L. Felipe Toledo[1]

[1]Laboratório de História Natural de Anfíbios Brasileiros (LaHNAB), Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil, 13083-862.

[2]Programa de Pós-Graduação em Ecologia, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil, 13083-862.

[3]Corresponding author: s.joelmaprado@gmail.com

Abstract

Fog is an important water input in ecosystems and a carrier of microorganisms, including unicellular pathogens. The aquatic amphibian-killing fungus, *Batrachochytrium dendrobatidis* (Bd), has a complex transport dynamic. Understanding how the exposure of amphibians to Bd can occur is important for the development of control measurements and for preventing die-offs. Therefore, we tested if the fog water may transport Bd. We collected fog and rainwater from nine –sites in Brazil's Atlantic Forest and diagnosed (with qPCR assays) the presence of Bd in such water sources. We also experimentally tested if Bd from artificial and natural fog exposures would infect amphibians. As main results we report the first evidence of Bd DNA in fog and corroborate previous data documenting Bd DNA in rainwater. Furthermore, our results indicate that susceptible hosts can be infected and develop lethal chytridiomycosis through the passive transport of Bd live zoospores by the fog. Our results extend the current knowledge about Bd transport pathways between environmental reservoirs. A new short to medium-range dispersion pathway through fog may explain patterns of pathogen occurrence and opens new avenues of investigation to elucidate exposure mechanisms of direct-development amphibians to aquatic pathogens.

Keywords: fog; amphibian disease; eDNA; aerial biology; *Eleutherodactylus johnstonei*; *Batrachochytrium dendrobatidis*.

Introduction

Fog is defined as liquid water suspended in the air at ground level (National Oceanic & Atmospheric Administration, 1995). It is a frequent event in tropical mountain forests at high altitudes, the cloud forests (Bruijnzeel, 2001; Bittencourt et al., 2019). Furthermore, in coastal areas, deserts, mountainous areas and cloud forests, fog is a frequent phenomenon during which the amount of water delivered to an ecosystem can exceed that of annual rainfall (Bruijnzeel, 2001; Torregrosa et al., 2014; Wang et al., 2017; Carmichael et al., 2020). In addition to the water itself, fog transports microorganisms and aggregates of organic particles (Gultepe et al., 2007; Wang et al., 2017; Evans et al., 2019) over long distances between adjacent terrestrial, marine, and freshwater ecosystems (Evans et al., 2019). Fog carrying has the potential to perpetuate the viability of microorganisms present in aerosols, as it has a higher water content, reducing desiccation and has also nutrients in suspension that support microbial ecology (Fuzzi et al., 1997; Dueker et al., 2012; Evans et al., 2019). Thus, fog can frequently introduce pathogens into terrestrial ecosystems (Douwes et al., 2003; Wang et al., 2017; Evans et al., 2019), which is the case of the plant pathogens Tobamovirus (Castello et al., 1995) and the fungus *Gibberella zeae* (Maldonado-Ramirez et al., 2005). Other pathogenic microorganisms from the genera Acinetobacter, Stenotrophomonas, Pseudomonas, and Empedobacter were found in fog water (Wei et al., 2016). Likewise, one of the pathogens that fog could carry is the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), which causes chytridiomycosis, a major disease of amphibians that has caused catastrophic declines across the globe (Scheele et al., 2019).

The chytrid fungus is transported by several routes in the environment, which can be direct (between two amphibian hosts) or indirect, involving environmental reservoirs, such as water from ponds and streams, rainwater, or plant surfaces (Rachowicz & Vredenburg, 2004; Kolby et al., 2015; Burns et al., 2021), or other carriers and vectors, such as midges, lizards, fish and birds (Johnson & Speare, 2005; Liew et al., 2017; Pontes et al., 2018; Toledo et al., 2021). Thus, if fog could carry Bd across environments, it would be an additional indirect route, not yet considered in recent Bd epidemiological studies (see Toledo et al., 2021).

Different mechanisms may be related to the aerosolization process that could generate Bd bioaerosols. Small-scale propulsion mechanisms are splash movements, bubble bursting, and water aspersion by waterfalls (Butterworth & McCartney, 1991; Money, 2016). Rainfall events can promote intense emission of bioparticles into the atmosphere (Huffman et al., 2013). The impact of raindrops can

cause the transfer of microorganisms from soil and leaf surfaces to the air (Butterworth & McCartney, 1991; Joung et al., 2017). When biological material is dispersed by water sources, it is usually surrounded by a thin layer of water that helps the microorganism survive while airborne (Stetzenbach, 2009; Magyar et al., 2016). After bioaerosols are generated, they can be transported over long distances through air currents (Joung & Buie, 2015; Money, 2016), through rain and fog (Evans et al., 2019), or remain suspended in the air for a few days before deposition. Fog and rain events also carry out the wet deposition of microorganisms that are already suspended in the air. Rain can also act on the deposition of microorganisms present on the surface of plants, directly into the soil (Aylor, 1999; Joung et al., 2017). Thus, rain is not a mechanism that can only forms Bd bioaerosols, but it can also contain Bd DNA that was previously suspended in the air before undergoing wet deposition. Thus, rain and fog could also deposit Bd on the surface of plants, in which amphibian hosts could be in contact with the fungus.

If fog transports Bd, this would be relevant to explain the chytrid infection in species with a fully terrestrial life cycle that rarely come into contact with water bodies (Kolby et al., 2015; Mesquita et al., 2017). This is the case of more than a thousand species of the clade Brachycephaloidea, including *Eleutherodactylus johnstonei* (Eleutherodactylidae) (Barbour, 1914; Frost, 2022). Direct developers carry low Bd prevalence in the wild and often show low resistance to chytridiomycosis (Mesquita et al., 2017) maybe due the infrequent contact with water bodies during their life. Consequently, when get infected, they are prone to occasional and quick die-offs (e.g., Longo & Burrowes, 2010; Moura-Campos et al., 2021).

In this context, we tested the hypotheses that i) water in the form of fog and rainwater transport Bd; ii) Bd zoospores in fog infect amphibians; and iii) amphibians infected by Bd transported by the fog water can develop the chytridiomycosis. Our prediction is that water in the form of fog and rainwater can transport Bd, and in the case of live zoospores, they can infect amphibians and lead to chytridiomycosis. Investigating these hypotheses expands the knowledge about the dynamics of Bd in natural and fragile environments, such as the Brasil's Atlantic Forest, which harbor an enormous diversity of amphibians, including threatened species.

Methods

Field sampling

We collected fog and rainwater samples in 9 sites in Brazil's Atlantic Forest (Table 1; Fig.1a; Fig.S1), during expeditions between March 2020 and December 2021.

We collected fog using a fog catcher (passive method), and with an aspirator (active method). The fog catcher was constructed using a fabric net, which is a plastic shading screen with 70% retention of solar luminosity (Fig.1b), 2.0 x 1.5 m size. The net was placed vertically and tensioned between two metal rods, 1 m from the floor. We placed a plastic groove in the bottom of the net, slightly angled where a sterile water collection bottle was attached (Fig.1c-d). We installed the nets in high elevations, preferably close to water bodies (ponds and streams), in flat areas with high probability of winds, so that in the event of a fog, the wind would run through the nets. We installed the fog catchers at dusk and removed them before dawn in each sampling night, in open areas without the presence of canopy above the nets to avoid cross-contamination. We washed the nets and the groove with soap and abundant water between samples. We registered the time extent the catchers were sampling, the temperature and air relative humidity. The mean temperature and mean relative humidity (RH) of the collected events were 21.2 ºC (14.5–29.3 ºC) and 81.1 % (61.2–96.2 %), respectively.

For the active fog sampling, we used a portable aspirator (vacuum cleaner TEDGE 7.4 V), with an attached sterile permeable membrane (47 mm diameter, 0.45 µm pore) (Sartorius Stedim Biotech) (Fig.1e-h). We recorded relative humidity, temperature, and the time extent the aspirator was on. Between samples, the aspirator was cleaned with distilled water and 70 % alcohol. Since this cleaning method does not destroy DNA, we included random control samples of nebulized distilled water during the aspirator experiment. All the blank samples were negative for Bd, showing that the cleaning method with 70% ethanol was sufficient to remove the DNA from the equipment between samples. However, for future replications of this experiment, the cleaning method must be with a 10% bleach solution which is more effective to avoid DNA contamination (Nilsson et al., 2022).

We collected rainwater using a rain trap composed of two 8 l buckets, suspended 1 m above the ground, attached to a tripod (Fig.1i). Four rain traps were installed at dusk, on flat ground, in open areas without canopy, and were removed before dawn. We filtered at least 81 ml of the rainwater samples with a membrane (47 mm diameter and 0.45 µm pore size).

DNA analyses

We extracted all Bd DNA samples with PrepMan ULTRA (Life Technologies®), using 50 µl for swabs and 100 µl for membranes. For each membrane, we used 15% of the total area, due to the necessary amount of reagent for the extraction.

We performed qPCR analyses following Boyle et al. (2004) with the adaptations by Lambertini et al. (2013). To the qPCR reaction we used for each well 20 µl of master mix and 5 µl of the extracted DNA diluted in DNA free water (1:10). The master mix contained 1250 µl of Taqman Master Mix (Applied Biosystems®), 375 µl of distilled water, 125 µl of the probe ChytrMGB2 (5'-6FAM CGAGTCGAACAAAAT MGBNFQ-3') (5 µM), 125 µl of the primer ITS1-3 Chytr (5'-CCTTGATATAATACAGTGTGCCATATGTC-3') (18 µM) and 125 µl of the primer 5.8S Chytr (5'-AGCCAAGAGATCCGTTGTCAAA-3') (18 µM).

To make the standards we used the Bd isolated CLFT 159 of the lineage GPL, following the protocol of Lambertini et al. (2013). In each reaction plate there was DNA free water as the negative control, and the genomic standards of 10^3, 10^2, 10, 1 and 10^{-1} zoospores. We ran the negative control, the standards of 10^3, 10^2 and 10 in duplicate, and the standards of 1 and 10^{-1} in quadruplicates.

Membranes and swab samples were run in singlicates (Kriger et al., 2006a). We rounded zoospores genomic equivalents (g.e.) values to integers, and we considered as a positive sample when loads were ≥ 0.1 zoospore g.e., since environmental DNA samples can have low DNA concentrations.

Aspirator experiment

We grew pure cultures of Bd in the laboratory for a couple weeks. This strain was isolated from *Aquarana catesbeiana* (Ranidae), collected in the municipality of São Paulo, state of São Paulo, Brazil (CLFT 280) and then kept in stock. We then harvested Bd zoospores by flooding Petri dishes with 3 ml of autoclaved distilled water and waiting for 40 minutes for zoospore release from zoosporangia (Greenspan et al., 2018, Ribeiro et al., 2019). We then quantified zoospores in a Neubauer hemocytometer and standardized the inoculum concentrations of 10, 10^2 and 10^3 zoospores/ml.

To test the detectability of Bd DNA in the fog collected with the aspirator, we nebulized 8 ml of the prepared solutions with known concentrations of Bd zoospores (10^1, 10^2 and 10^3 zoospores / ml), which were immediately sampled with the aspirator with the microbiological membrane. The experiment was conducted in laboratory, with temperature control of 22ºC, inside a laminar flow cabinet. We nebulized four replicates for each concentration with two different durations: five and ten minutes of nebulization.

We cleaned the aspirator between samples with ethanol 70%. We also included 3 random control membranes using nebulized distilled water to be sure that the cleaning method using ethanol 70% was

sufficient to remove the DNA from the aspirator. We measured the solution volume before and after the nebulization, to estimate the number of zoospores nebulized (zoospores/ml), which were compared with the zoospore g.e (as indicated by subsequent qPCR analysis) captured by the membranes. We ran samples in singlicate.

For data analysis, we removed zeros and zoospores g.e. values were log transformed. We tested the normality of the data and used the Spearman correlation test to analyze the correlation between the number of nebulized zoospores and the detected zoospores. We verified the assumptions for applying the linear regression model using graphical analysis. Then, we tested the normality of the residuals using the Shapiro-Wilk test to validate the model. In this study, all statistical analyses were conducted with RStudio 1.3.1 software.

Frog infection experiment

We collected 19 frogs of the direct-developing exotic species *Eleutherodactylus johnstonei* in Brooklin neighborhood, state of São Paulo, Brazil (23°36'56" S, 46°40'50" W), in February 2020. Due to the direct development this species has low resistance to chytridiomycosis and is an ideal model organism for infection trials. The collected frogs were transported to Universidade Estadual de Campinas (Unicamp), where they were individually housed in plastic terraria (22 x 15 x 8 cm) with an autoclaved moist *Sphagnum* substrate and were fed with calcium-fortified pinhead crickets. We swabbed all collected frogs and to confirm they were free of Bd (Bd^-), we tested them with the previously described TaqMan® qPCR analysis.

We grew pure cultures of Bd using the same strain and protocol described above. We then quantified zoospores in a Neubauer hemocytometer and standardized the inoculum concentration (2.44 x 10^6 zoospores/ml). Nineteen Bd^- frogs were individually exposed to a nebulized solution using an air compressor nebulizer NB090 (Incoterm, Porto Alegre, RS, Brazil), for 10 min a day, for five consecutive days. The frogs were divided into following treatments: i) a Bd exposed group, that was subjected to a nebulized solution with Bd (average concentration of 2.44 x 10^6 zoospores / ml (n = 9 frogs), and ii) a control group, exposed to a solution of autoclaved distilled water, poured into a Petri dish containing 1 % tryptone-agar (n = 10 frogs). For each exposure, 8 ml of solution were used, the maximum volume of the nebulizer container.

We carried out the experiment in a temperature-controlled room (25º C) and inside the laminar flow cabinet. The experimental design (Fig.S2) consisted of a plastic bottle, where one frog was placed, attached to the nebulizer output. There were holes at the bottom of the bottle, so that the fog could escape and not cause excessive condensation in the bottle. Bottles were individually used for each frog, and for each exposure, the entire nebulizer system was washed with distilled water, cleaned with alcohol 70 %, and refilled with new solution to be nebulized. We swabbed each frog 10 and 20 days post Bd exposure, which is sufficient time for multiple Bd generations (Longcore et al., 1999). We monitored each frog daily and swabbed dead or dying individuals. Afterwards they were submitted to qPCR diagnosis. We log-transformed the genomic equivalents data and performed paired *t-test* to assess the difference between mean infection burdens between 10 and 20 days post-fog exposure. We euthanized surviving individuals with Lidocaine 5% and deposited them at the Museu de Diversidade Biológica (MDBio), at Unicamp, Campinas, SP, Brazil.

Nebulization experiment of water bodies from field

We conducted an experiment in laboratory of nebulization of water samples from field water bodies collected in September 2020, in São Francisco Xavier, where we know there are infected frogs (unpublished data). Six Bd^- frogs of *E. johnstonei* species were individually exposed 6 times for 10 min each, to 8 ml of a nebulized water from different water bodies (2 streams and 1 pond). We swabbed all frogs at the end of the last day of exposition, and we used the same DNA extraction and qPCR protocols described above to detect and quantify Bd load. After the experiment, the animals were euthanized. We also collected 250 ml water samples from the water bodies. The water samples were filtered by permeable membrane and submitted to qPCR analysis. We ran all samples in singlicate.

Cage experiment

In September 2020, in São Francisco Xavier district (where there is a high occurrence of fog), we exposed 30 frogs of *E. johnstonei* to natural fog. The healthy frogs were kept, 10 hours per night, in individual cages (with 5 cm diameter, made of a steel mesh) spaced apart by 50 cm, and suspended 1.7 m above the ground, under a tent. Fog could pass through the mesh (Fig.S3). The tent was installed next to a pond, where there are frogs infected with Bd (unpublished data). We monitored daily, removing the cages during the day. Out of the 30 frogs, 13 were exposed to fog for 2 consecutive nights and died at the 3rd

day due to unknown causes. Immediately after the death, we collected skin swabs, which were submitted to qPCR analysis. The other 17 frogs were exposed to fog for 4 consecutive nights. Soon after that, we collected skin swab samples, and subjected to qPCR Bd diagnosis. We ran samples in singlicate. After the experiment, the animals were euthanized and deposited at the MDBio museum.

Ethics and permits

Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio #73857-3), Instituto Florestal (IF #004408/2020-66), and Sistema Nacional de Gestão do Patrimônio Genético (SisGen #AC5E659) provided the sampling and access permits. The study was approved by Unicamp animal care and ethics committee (CEUA #5535-1/2020, #5866-1/2021).

Results

We sampled a total of 433 swabs and 103 membranes samples and deposited in the SLFT collection at Laboratório de História Natural de Anfíbios Brasileiros (LaHNAB), at Unicamp (SLFT 17871–18104; 16105–16579; 18769; 19129–19131), including fog, rainwater, and experimental samples. The sampling effort totaled 19,142 l of aspirated fog and 0.714 l of water collected from the networks. Out of the 50 fog samples (4 from net and 46 from aspirator methods), and only one (collected with the aspirator) was positive for Bd (Bd^+), with 7.26 zoospore g.e. (Table 1; Table S1). This sample was collected on 8 November 2020 (spring), at Parque Estadual da Serra do Mar, núcleo Santa Virginia, municipality of São Luiz do Paraitinga, along the outer edge of a nebular forest fragment. For rainwater, the total collected volume of 36.67 l, ranged from 81 ml to 2.9 l, with an average volume of 817 ml/sample. Out of the 41 rainwater samples, Bd was detected in four (~ 10 %) samples, with loads varying between 4.22 and 44.27 zoospores g.e. (Table S2). Three of the Bd^+ rainwater samples were collected in January 2019 and one in April 2019. All of them were collected at Parque Estadual Campos do Jordão, municipality of Campos do Jordão.

Aspirator experiment

The number of Bd zoospores genomic equivalents detected by the aspirator method was lower than the number of zoospores nebulized in all treatments (Fig. 2; Table S3). Among treatments, with more time of nebulization, the zoospore detection was greater, as showed by a positive correlation between the number of nebulized zoospores and the number of zoospores that were detected (r_s = 0.78, P = 0.01 for 5

min; r_s = 0.88, P = 0.001 for 10 min). The linear regression showed that for each zoospore nebulized, our method was able to detect about 0.525 zoospore g.e.. Out of the 24 replicates, 25 % (n = 6) tested negative for Bd DNA and all of them (Bd^- samples) were from the two lower concentration trials (10^1 and 10^2 zoospores / ml), and from both exposition times.

Infection experiment

All frogs exposed to Bd by fog got infected (since the first swab, 10 days after exposure) and developed chytridiomycosis: we detected a progression in the infection burden as 20 days post-exposure Bd loads were greater than all values detected in the 10th day (t = -14.647; P < 0.001) (Table 2; Fig. 3a). Showing that there is an effect of time of post exposure on infection burden. None of frogs of the control group were Bd^+. All frogs in the control group remained alive until the last day of the experiment (day 31 post-exposure). On the other hand, the infection was lethal for 77 % of the infected frogs, between the 20th to the 31st day after exposure (Fig. 3b), when we interrupted the experiment and euthanized the remaining frogs.

Nebulization experiment of water bodies from field

We detected positive samples for 2 of the 6 exposed frogs (Table S4). One frog was exposed to stream water (zoospore g.e. = 0.14) and another to pond water (zoospore g.e. = 0.1).

Cage Experiment

Out of the 30 *E. johnstonei* individuals, only one was positive for Bd (zoospore g.e. = 0.65). This frog was among the ones exposed to fog for two consecutive nights. All the other individuals were Bd^-.

Discussion

This is the first evidence of an alternative mechanisms of Bd passive transport through fog. Here, we present the first evidence of Bd DNA in the fog. We demonstrate, in a controlled system, that Bd zoospore transmission through air and humid deposition not only can occur, but in high exposure concentrations can also lead to infection of amphibian hosts, which can even develop advanced stages of chytridiomycosis and death. In addition, we found Bd DNA in rainwater, corroborating previous study (Kolby et al., 2015). Although the presence of active Bd zoospores in fog must be yet comproved, our results are suggestive pieces of evidence. While we collected rain and fog events separately, both tend to

occur in association and often in a short time interval (Gultepe, 2007; Bittencourt et al., 2019). It is possible that they act together in a process that causes the pathogen dispersion both on a micro scale (e.g., from a lake to its surroundings) and over greater distances, probably transporting Bd zoospores over several kilometers. Here, we emphasize the need of future studies that detail the mechanisms of Bd aerosolization at the water-air interface.

Bd dispersal by fog could overcome natural barriers. Through the fog, Bd could cross harsh matrices, which both amphibians and the fungus may rarely overcome (Becker et al., 2007; Magyar et al., 2016). For /example, it is common that lakes are disconnected from forest fragments, and this habitat split has been linked to amphibian declines – as the amphibians may die while crossing the inhospitable matrix (Becker et al., 2007). Thus, if Bd could cross such landscapes through air, they could infect new hosts far from the isolated waterbodies. Additionally, such pathway could even explain how Bd-free areas receive the first propagules and infect still naïve frogs (Kolby et al., 2015).

The concentration of microorganisms in bioaerosols varies in time and space (Montero et al., 2016). Although quantitative studies of extensive sampling are necessary to have a more accurate idea of the amount of Bd present in fog or rainwater, they are expected to occur at low concentrations. The concentration of Bd in the fog is expected to be low as it is an aquatic zoospore (Piotrowski et al., 2004), which will depend on mechanisms to be aerosolized from the aquatic source to the air (Magyar et al., 2016). Furthermore, the greater the distance from the origin, the lower the expected concentration of spores due to wind dilution and atmospheric turbulence (Aylor, 2003). Thus, due to the low amounts of the fungus in the fog, a greater sampling effort will be necessary to sample Bd, and we predict that the success of this transport will be directly correlated to the distance of the source.

We were not able to test the viability of the zoospores found in fog or rainwater. However, fog is configured as an ecosystem with high humidity, since most fogs have a liquid water content of 0.01 to 0.4 g/m^3 (Gultepe, 2007), and temperature, which likely coincide with the ideal conditions for the fungus. The average temperature of about 21 ºC of during our sampling is close to the ideal temperatures for growth and reproduction of the fungus (between 17 and 25 ºC), even considering the variation between strains (Piotrowski et al., 2004; Voyles et al., 2017; Muletz-Wolz et al., 2019). Bd zoospores could survive in wet sterile environments for up to seven weeks (Johnson & Speare, 2003), long enough to travel inside the fog or rain. In sterile moist substrate it could persist for up to three months (Johnson &

Speare; 2005). However, information on the viability of Bd zoospores in non-sterile environments are likely reduced (Burns et al., 2021).

Our infection experiment showed that viable Bd zoospores can be transported through humid air and cause infection of the susceptible host, being lethal to *E. johnstonei*. Our data support evidence that indirect transmission from an environmental reservoir to a susceptible host species can result in lethal chytridiomycosis (Burns et al., 2021). Previous exposure or the frequency of exposure to low concentrations of the pathogen may be an important factor in the vulnerability, or tolerance determination of the host species (van Rooij et al., 2015). Considering that, fog may represent an important pathway in this dynamic, as a transport route for the pathogen. Thus, future studies are of interest to investigate the role of frequency of exposure to fog in the tolerance of direct-developing amphibians.

Since viable zoospores can be carried by artificial fog and our infection experiment was carried out with a high concentration of inoculated zoospores, we speculate whether the same occurs with natural fog from water bodies where the amphibians are infected with Bd (Gründler et al., 2012). Although to date there is no assessment of the Bd DNA concentration in water bodies in the Atlantic Forest, loads ranging from 0.5 to 262 zoospores / l have been detected in water bodies in a mountainous region in Spain (Walker et al., 2007) and of 19 g.e. / l in a Colorado mountain park (Kirshtein et al., 2007). The artificial pond and stream water fog experiment revealed Bd DNA in frog's skin. This indicates that, even at low concentrations such as what occurs in natural water from water bodies, Bd DNA can be transported through artificial fog and detected in the skin of frogs.

We detected Bd DNA in amphibians exposed to natural fog. If such diagnostic implies in live forms of the chytrid, even if the animals have not evolved to a condition of chytridiomycosis, they could act in the transmission of Bd to other syntopic hosts (Johnson & Speare, 2005; Sialve et al., 2015). Transport of active zoospores by bioaerosols in fog could occur due to characteristics such as zoospore size and aerosolization of infected cells or aggregated compounds. Since bioaerosol particles range from 0.3 to 100 µm (Luisetto et al., 2021), and the size of chytrid zoospores averaging 2 to 3 µm in diameter (Gleason et al., 2008; Lambertini et al., 2016), it allows them to remain suspended in the air. However, when considering what is being collected in fog samples, it is important to remember that bioaerosols hardly contain individual equivalents of isolated microorganisms, but rather aggregates with particulate matter of organic, inorganic and dust material (Stetzenbach, 2009). Despite the unlikely occurrence of isolated Bd zoospores suspended in the air, fog can carry cell aggregates suspended in humid air, which

could increase the chances of successful dispersal of the still viable pathogen (Luisetto et al., 2021). In addition, ulceration and excessive peeling of the amphibian's skin are clinical signs in advanced stages of chytridiomycosis caused by Bd or Bsal (van Rooij et al., 2015; Thomas et al., 2018). Therefore, the possible aerosolization of cells aggregates infected with the chytrid fungus could cause viable zoospores to be deposited in the environment and even passively transported by the fog.

Bioaerosols associated with larger particles are expected to be locally produced, due to transport range limitations, and they are likely to be viable due to resistance to aerosolization stress (Stetzenbach, 2009; Montero et al., 2016). Thus, the dispersion of bioaerosols containing viable Bd is more likely to happen locally than through long-distances. In turn, aerosols with an aerodynamic size of the order of 1 μm can have a residence time of days to months and can reach a global distribution (Montero et al., 2016). Furthermore, the Bd scattering rate is variable, from 0.7 to 282 km / year and reflects different dispersion routes (Kolby et al., 2015). In this context, the fog could represent a route of local and long-distance dispersion of Bd in regional reach.

The passive collection of fog by the fogcatchers, proved to be an inefficient method, due to the need for large space in an open area without a canopy and with a lot of wind. In addition to the long assembly time, other limitations are the presence of woods or ravines in the site, which are obstacles to the wind preventing the interception of fog by the nets, in addition to sources of contamination by transport and the presence of insects or other animals that could collide with the network. On the other hand, the aspirator method proved to be more efficient than fogcatchers, as it is cheaper, easier to transport, is less susceptible to environmental contaminations, and allows to sample inside the forest, or on ponds margins.

In this study we expanded the knowledge of the Bd fungus epidemiology, adding another environmental route to this system (Fig. 4). As established by Toledo et al. (2021), the route level importance for the transmission of the chytrid fungus is based on the minimum transmission steps necessary to a Bd zoospore shed from one amphibian host to reach another amphibian host through a specific element in the network (such as rain, fog, soil, insect, or other vertebrates). Thus, fog is a pathway of third level of importance, which means it takes at least three steps for the Bd to leave the frog host 1 and reach frog host 2. It is, therefore, less relevant than transmission through water bodies, but may configure a more direct pathway than non-amphibian vectors/carriers (e.g., Johnson & Speare, 2005; Liew et al., 2017; Pontes et al., 2018; Toledo et al., 2021).

Chytrid dispersion through fog depends on aerosolization of the viable pathogen to fog and its deposition in a host or new environmental reservoir. It might be especially important for directly developing species (e.g., Brachycephaloidea), as they have been shown to be susceptible to the pathogen (Mesquita et al., 2017; Moura-Campos et al., 2021). The results of this work reaffirm that the natural dynamics of Bd is complex and dependent on several factors and contexts. Passive transport of Bd in fog becomes even more relevant in the current climate change scenario (IPCC, 2021), as the dynamics of fog formation may be changing (Bittencourt et al., 2019; Ritter et al., 2019; Carmichael et al., 2020), we speculate that the areas with higher fog frequency would be more important for Bd transmission to terrestrial species, for example. Therefore, we recommend the expansion of studies of Bd transmission by the airways also in the epidemiological studies of this pathogen.

Acknowledgments

We thank João P. Bovolon and Joice Ruggeri for support in the field; Ana Clara F. Barbosa for assistance in the laboratory; Domingos S. Leite, Raul C. Pereira, Paulo S. M. C. Oliveira, Rafael S. Oliveira, Carol Lambertini, Marcelo Sturaro, and Martin Pareja for collaboration during the project. Grants and fellowships were provided by São Paulo Research Foundation (FAPESP #2016/25358-3; #2019/18335-5; #2020/02991-8; #2020/00099-0), the National Council for Scientific and Technological Development (CNPq #300896/2016-6; #302834/2020-6), and by the Coordination for the Improvement of Higher Education Personnel (CAPES - Finance Code 001).

References

Aylor, D., 1999. Biophysical scaling and the passive dispersal of fungus spores: relationship to integrated pest management strategies. Agricultural and Forest Meteorology 97: 275–292, https://linkinghub.elsevier.com/retrieve/pii/S0168192399000726.

Barbour, T., 1914. A contribution to the zoogeography of the West Indies, with special reference to amphibians and reptiles. Memoirs of the Museum of Comparative Zoology. Cambridge, Massachusetts 44: 205–359.

Butterworth, J., & H. A. McCartney, 1991. The dispersal of bacteria from leaf surfaces by water splash. Journal of Applied Bacteriology 71: 484–496, https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.1991.tb03822.x.

Aylor, D. E., 2003. Spread of plant disease on a continental scale: role of aerial dispersal of pathogens. Ecology 84: 1989–1997, http://doi.wiley.com/10.1890/01-0619.

Becker, C. G., C. R. Fonseca, C. F. B. Haddad, R. F. Batista, & P. I. Prado, 2007. Habitat Split and the Global Decline of Amphibians. Science 318: 1775–1777, https://www.science.org/doi/10.1126/science.1149374.

Bittencourt, P. R. L., F. de V. Barros, C. B. Eller, C. S. Müller, & R. S. Oliveira, 2019. The fog regime in a tropical montane cloud forest in Brazil and its effects on water, light and microclimate. Agricultural and Forest Meteorology 265: 359–369, https://linkinghub.elsevier.com/retrieve/pii/S016819231830385X.

Bower, D. S., K. R. Lips, L. Schwarzkopf, A. Georges, & S. Clulow, 2017. Amphibians on the brink. Science 357: 454–455, https://www.science.org/doi/10.1126/science.aao0500.

Brannelly, L. A., D. P. Wetzel, M. E. B. Ohmer, L. Zimmerman, V. Saenz, & C. L. Richards-Zawacki, 2020. Evaluating environmental DNA as a tool for detecting an amphibian pathogen using an optimized extraction method. Oecologia 194: 267–281, https://link.springer.com/10.1007/s00442-020-04743-4.

Bruijnzeel L. A. 2001. Hydrology of tropical montane cloud forests: a reassessment. Land use and water resources research 1: 1–1.

Burns, T. J., B. C. Scheele, L. A. Brannelly, N. Clemann, D. Gilbert, & D. A. Driscoll, 2021. Indirect terrestrial transmission of amphibian chytrid fungus from reservoir to susceptible host species leads

to fatal chytridiomycosis. Animal Conservation 24: 602–612, https://onlinelibrary.wiley.com/doi/10.1111/acv.12665.

Burrows, S. M., W. Elbert, M. G. Lawrence, & U. Pöschl, 2009. Bacteria in the global atmosphere – Part 1: Review and synthesis of literature data for different ecosystems. Atmospheric Chemistry and Physics 9: 9263–9280, https://acp.copernicus.org/articles/9/9263/2009/.

Carmichael, M. J., J. C. White, S. T. Cory, Z. C. Berry, & W. K. Smith, 2020. Foliar water uptake of fog confers ecophysiological benefits to four common tree species of southeastern freshwater forested wetlands. Ecohydrology 13, https://onlinelibrary.wiley.com/doi/10.1002/eco.2240.

Carvalho T., C. G. Becker & L. F. Toledo, 2017. Historical amphibian declines and extinctions in Brazil linked to chytridiomycosis. Proceedings of the Royal Society B: Biological Sciences 284: 20162254.

Castello, J.D., D.K. Lakshman, S.M. Tavantzis, S.O. Rogers, G.D. Bachand, R. Jagels, J. Carlisle & Y. Liu. 1995. Detection of infectious tomato mosaic tobamovirus in fog and clouds. Phytopathology, 85: 1409-1412.

Cheng, T. L., S. M. Rovito, D. B. Wake, & V. T. Vredenburg, 2011. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. Proceedings of the National Academy of Sciences 108: 9502–9507, https://pnas.org/doi/full/10.1073/pnas.1105538108.

R Core Team, 2020. R: A language and environment for statis-tical computing. Vienna, Austria: R Foundation for Statistical Computing.

Douwes J., P. Thorne, N. Pearce & D. Heederik, 2003. Bioaerosol health effects and exposure assessment: progress and prospects. The Annals of occupational hygiene 47: 187-200.

Dueker, M. E., G. D. O'Mullan, K. C. Weathers, A. R. Juhl, & M. Uriarte, 2012. Coupling of fog and marine microbial content in the near-shore coastal environment. Biogeosciences 9: 803–813, https://bg.copernicus.org/articles/9/803/2012/.

Evans, S. E., M. E. Dueker, J. R. Logan, & K. C. Weathers, 2019. The biology of fog: results from coastal Maine and Namib Desert reveal common drivers of fog microbial composition. Science of The Total Environment 647: 1547–1556, https://linkinghub.elsevier.com/retrieve/pii/S0048969718330134.

Fuzzi, S., P. Mandrioli, & A. Perfetto, 1997. Fog droplets—an atmospheric source of secondary biological aerosol particles. Atmospheric Environment 31: 287–290, https://linkinghub.elsevier.com/retrieve/pii/1352231096001604.

Garmyn, A., P. Van Rooij, F. Pasmans, T. Hellebuyck, W. Van Den Broeck, F. Haesebrouck, & A. Martel, 2012. Waterfowl: Potential Environmental Reservoirs of the Chytrid Fungus *Batrachochytrium dendrobatidis*. PLoS ONE 7: e35038, https://dx.plos.org/10.1371/journal.pone.0035038.

Gleason, F. H., M. Kagami, E. Lefevre, & T. Sime-Ngando, 2008. The ecology of chytrids in aquatic ecosystems: roles in food web dynamics. Fungal Biology Reviews 22: 17–25, https://linkinghub.elsevier.com/retrieve/pii/S174946130800002X.

Greenspan, S. E., C. Lambertini, T. Carvalho, T. Y. James, L. F. Toledo, C. F. B. Haddad, & C. G. Becker, 2018. Hybrids of amphibian chytrid show high virulence in native hosts. Scientific Reports 8: 9600, http://www.nature.com/articles/s41598-018-27828-w.

Gründler, M., L. Toledo, G. Parra-Olea, C. Haddad, L. Giasson, R. Sawaya, C. Prado, O. Araujo, F. Zara, F. Centeno, & K. Zamudio, 2012. Interaction between breeding habitat and elevation affects prevalence but not infection intensity of *Batrachochytrium dendrobatidis* in Brazilian anuran assemblages. Diseases of Aquatic Organisms 97: 173–184, http://www.int-res.com/abstracts/dao/v97/n3/p173-184/.

Gultepe, I., R. Tardif, S. C. Michaelides, J. Cermak, A. Bott, J. Bendix, M. D. Müller, M. Pagowski, B. Hansen, G. Ellrod, W. Jacobs, G. Toth, & S. G. Cober, 2007. Fog Research: A Review of Past Achievements and Future Perspectives. Pure and Applied Geophysics 164: 1121–1159, http://link.springer.com/10.1007/s00024-007-0211-x.

Haddad, C. F. B. (ed), 2013. Guia dos anfíbios da Mata Atlântica: diversidade e biologia = Guide to the amphibians of the Atlantic Forest: diversity and biology. Anolis Books, São Paulo.

Hall, E. M., E. J. Crespi, C. S. Goldberg, & J. L. Brunner, 2016. Evaluating environmental DNA -based quantification of ranavirus infection in wood frog populations. Molecular Ecology Resources 16: 423–433, https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12461.

Hoffmann, M., C. Hilton-Taylor, A. Angulo, M. Böhm, T. M. Brooks, S. H. M. Butchart, K. E. Carpenter, J. Chanson, B. Collen, N. A. Cox, W. R. T. Darwall, N. K. Dulvy, L. R. Harrison, V. Katariya, C. M. Pollock, S. Quader, N. I. Richman, A. S. L. Rodrigues, M. F. Tognelli, J.-C. Vié, J. M. Aguiar, D. J. Allen, G. R. Allen, G. Amori, N. B. Ananjeva, F. Andreone, P. Andrew, A. L. A. Ortiz, J. E. M. Baillie, R. Baldi, B. D. Bell, S. D. Biju, J. P. Bird, P. Black-Decima, J. J. Blanc, F. Bolaños, W. Bolivar-G., I. J. Burfield, J. A. Burton, D. R. Capper, F. Castro, G. Catullo, R. D.

Cavanagh, A. Channing, N. L. Chao, A. M. Chenery, F. Chiozza, V. Clausnitzer, N. J. Collar, L. C. Collett, B. B. Collette, C. F. C. Fernandez, M. T. Craig, M. J. Crosby, N. Cumberlidge, A. Cuttelod, A. E. Derocher, A. C. Diesmos, J. S. Donaldson, J. W. Duckworth, G. Dutson, S. K. Dutta, R. H. Emslie, A. Farjon, S. Fowler, J. Freyhof, D. L. Garshelis, J. Gerlach, D. J. Gower, T. D. Grant, G. A. Hammerson, R. B. Harris, L. R. Heaney, S. B. Hedges, J.-M. Hero, B. Hughes, S. A. Hussain, J. Icochea M., R. F. Inger, N. Ishii, D. T. Iskandar, R. K. B. Jenkins, Y. Kaneko, M. Kottelat, K. M. Kovacs, S. L. Kuzmin, E. La Marca, J. F. Lamoreux, M. W. N. Lau, E. O. Lavilla, K. Leus, R. L. Lewison, G. Lichtenstein, S. R. Livingstone, V. Lukoschek, D. P. Mallon, P. J. K. McGowan, A. McIvor, P. D. Moehlman, S. Molur, A. M. Alonso, J. A. Musick, K. Nowell, R. A. Nussbaum, W. Olech, N. L. Orlov, T. J. Papenfuss, G. Parra-Olea, W. F. Perrin, B. A. Polidoro, M. Pourkazemi, P. A. Racey, J. S. Ragle, M. Ram, G. Rathbun, R. P. Reynolds, A. G. J. Rhodin, S. J. Richards, L. O. Rodríguez, S. R. Ron, C. Rondinini, A. B. Rylands, Y. Sadovy de Mitcheson, J. C. Sanciangco, K. L. Sanders, G. Santos-Barrera, J. Schipper, C. Self-Sullivan, Y. Shi, A. Shoemaker, F. T. Short, C. Sillero-Zubiri, D. L. Silvano, K. G. Smith, A. T. Smith, J. Snoeks, A. J. Stattersfield, A. J. Symes, A. B. Taber, B. K. Talukdar, H. J. Temple, R. Timmins, J. A. Tobias, K. Tsytsulina, D. Tweddle, C. Ubeda, S. V. Valenti, P. Paul van Dijk, L. M. Veiga, A. Veloso, D. C. Wege, M. Wilkinson, E. A. Williamson, F. Xie, B. E. Young, H. R. Akçakaya, L. Bennun, T. M. Blackburn, L. Boitani, H. T. Dublin, G. A. B. da Fonseca, C. Gascon, T. E. Lacher, G. M. Mace, S. A. Mainka, J. A. McNeely, R. A. Mittermeier, G. M. Reid, J. P. Rodriguez, A. A. Rosenberg, M. J. Samways, J. Smart, B. A. Stein, & S. N. Stuart, 2010. The Impact of Conservation on the Status of the World's Vertebrates. Science 330: 1503–1509, https://www.science.org/doi/10.1126/science.1194442.

Hyatt, A., D. Boyle, V. Olsen, D. Boyle, L. Berger, D. Obendorf, A. Dalton, K. Kriger, M. Hero, H. Hines, R. Phillott, R. Campbell, G. Marantelli, F. Gleason, & A. Colling, 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 73: 175–192, http://www.int-res.com/abstracts/dao/v73/n3/p175-192/.

Huffman, J. A., A. J. Prenni, P. J. DeMott, C. Pöhlker, R. H. Mason, N. H. Robinson, J. Fröhlich-Nowoisky, Y. Tobo, V. R. Després, E. Garcia, D. J. Gochis, E. Harris, I. Müller-Germann, C. Ruzene, B. Schmer, B. Sinha, D. A. Day, M. O. Andreae, J. L. Jimenez, M. Gallagher, S. M. Kreidenweis, A. K. Bertram, & U. Pöschl, 2013. High concentrations of biological aerosol particles

and ice nuclei during and after rain. Atmospheric Chemistry and Physics 13: 6151–6164, https://acp.copernicus.org/articles/13/6151/2013/.

Johnson, M. L., & R. Speare, 2003. Survival of *Batrachochytrium dendrobatidis* in Water: Quarantine and Disease Control Implications. Emerging Infectious Diseases 9: 915–921, http://wwwnc.cdc.gov/eid/article/9/8/03-0145_article.htm.

Johnson, M., & R. Speare, 2005. Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. Diseases of Aquatic Organisms 65: 181–186, http://www.int-res.com/abstracts/dao/v65/n3/p181-186/.

Joung, Y. S., & C. R. Buie, 2015. Aerosol generation by raindrop impact on soil. Nature Communications 6: 6083, http://www.nature.com/articles/ncomms7083.

Joung, Y. S., Z. Ge, & C. R. Buie, 2017. Bioaerosol generation by raindrops on soil. Nature Communications 8: 14668, http://www.nature.com/articles/ncomms14668.

Kilpatrick, A. M., C. J. Briggs, & P. Daszak, 2010. The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends in Ecology & Evolution 25: 109–118, https://linkinghub.elsevier.com/retrieve/pii/S0169534709002419.

Kirshtein, J., C. Anderson, J. Wood, J. Longcore, & M. Voytek, 2007. Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. Diseases of Aquatic Organisms 77: 11–15, http://www.int-res.com/abstracts/dao/v77/n1/p11-15/.

Kolby, J. E., S. D. Ramirez, L. Berger, K. L. Richards-Hrdlicka, M. Jocque, & L. F. Skerratt, 2015. Terrestrial Dispersal and Potential Environmental Transmission of the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*). PLOS ONE 10: e0125386, https://dx.plos.org/10.1371/journal.pone.0125386.

Kriger, K., J. Hero, & K. Ashton, 2006a. Cost efficiency in the detection of chytridiomycosis using PCR assay. Diseases of Aquatic Organisms 71: 149–154, http://www.int-res.com/abstracts/dao/v71/n2/p149-154/.

Kriger, K., H. Hines, A. Hyatt, D. Boyle, & J. Hero, 2006b. Techniques for detecting chytridiomycosis in wild frogs: comparing histology with real-time Taqman PCR. Diseases of Aquatic Organisms 71: 141–148, http://www.int-res.com/abstracts/dao/v71/n2/p141-148/.

Kriger, K. M., F. Pereoglou, & J.-M. Hero, 2007. Latitudinal Variation in the Prevalence and Intensity of Chytrid (*Batrachochytrium dendrobatidis*) Infection in Eastern Australia. Conservation Biology 21: 1280–1290, https://onlinelibrary.wiley.com/doi/10.1111/j.1523-1739.2007.00777.x.

Lambertini, C., C. G. Becker, T. S. Jenkinson, D. Rodriguez, D. da Silva Leite, T. Y. James, K. R. Zamudio, & L. F. Toledo, 2016. Local phenotypic variation in amphibian-killing fungus predicts infection dynamics. Fungal Ecology 20: 15–21, https://linkinghub.elsevier.com/retrieve/pii/S1754504815001282.

Lambertini, C., C. G. Becker, A. M. Belasen, A. Valencia-Aguilar, C. H. L. Nunes-de-Almeida, C. M. Betancourt-Román, D. Rodriguez, D. da Silva Leite, I. S. Oliveira, J. L. Gasparini, J. Ruggeri, T. Mott, T. S. Jenkinson, T. Y. James, K. R. Zamudio, & L. F. Toledo, 2021. Biotic and abiotic determinants of *Batrachochytrium dendrobatidis* infections in amphibians of the Brazilian Atlantic Forest. Fungal Ecology 49: 100995, https://linkinghub.elsevier.com/retrieve/pii/S1754504820301070.

Lambertini, C., D. Rodriguez, F. B. Brito, S. Leite, & L. F. Toledo, 2013. Diagnóstico do fungo Quitrídio: *Batrachochytrium dendrobatidis*. Herpetologia Brasileira 2: 12–17.

Liew, N., M. J. Mazon Moya, C. J. Wierzbicki, M. Hollinshead, M. J. Dillon, C. R. Thornton, A. Ellison, J. Cable, M. C. Fisher, & S. Mostowy, 2017. Chytrid fungus infection in zebrafish demonstrates that the pathogen can parasitize non-amphibian vertebrate hosts. Nature Communications 8: 15048, http://www.nature.com/articles/ncomms15048.

Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, & J. P. Collins, 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences 103: 3165–3170, https://pnas.org/doi/full/10.1073/pnas.0506889103.

Longcore, J. E., A. P. Pessier, & D. K. Nichols, 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a Chytrid Pathogenic to Amphibians. Mycologia 91: 219, https://www.jstor.org/stable/3761366?origin=crossref.

Longo, A. V., & P. A. Burrowes, 2010. Persistence with Chytridiomycosis Does Not Assure Survival of Direct-developing Frogs. EcoHealth 7: 185–195, http://link.springer.com/10.1007/s10393-010-0327-9.

Longo, A., P. Burrowes, & R. Joglar, 2009. Seasonality of *Batrachochytrium dendrobatidis* infection in direct-developing frogs suggests a mechanism for persistence. Diseases of Aquatic Organisms 92: 253–260, http://www.int-res.com/abstracts/dao/v92/n2-3/p253-260/.

Luisetto, M., B.A. Nili, K. Edbey, G.R. Mashori, A. Y. Rafa, & O. Y. Latishev, 2021. Bioaerosols and Corona Virus Diffusion, Transmission, Carriers, Viral Size, Surfaces Properties and other Factor Involved. International Journal of Medicine and Healthcare Reports 1:1004, https://www.bostonsciencepublishing.us/science-world/articlepdf/ijmhr-1–102.pdf.

Magyar, D., M. Vass, & D.-W. Li, 2016. Dispersal Strategies of Microfungi In Li, D.-W. (ed), Biology of Microfungi. Springer International Publishing, Cham: 315–371, http://link.springer.com/10.1007/978-3-319-29137-6_14.

Maldonado-Ramirez, S. L., D.G.I. Schmale, E.J. Shields, & G.C. Bergstrom. 2005. The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggests the role of long-distance transport in regional epidemics of Fusarium head blight, Agric. For. Meteorol., 132: 20–27.

Mesquita, A. F. C., C. Lambertini, M. Lyra, L. R. Malagoli, T. Y. James, L. F. Toledo, C. F. B. Haddad, & C. G. Becker, 2017. Low resistance to chytridiomycosis in direct-developing amphibians. Scientific Reports 7: 16605, http://www.nature.com/articles/s41598-017-16425-y.

Money, N. P., 2016. Spore production, discharge, and dispersal. In The fungi: Academic Press 67–97.

Montero, A., M. E. Dueker, & G. D. O'Mullan, 2016. Culturable bioaerosols along an urban waterfront are primarily associated with coarse particles. PeerJ 4: e2827, https://peerj.com/articles/2827.

Moura-Campos, D., S. E. Greenspan, G. V. DiRenzo, W. J. Neely, L. F. Toledo, & C. G. Becker, 2021. Fungal disease cluster in tropical terrestrial frogs predicted by low rainfall. Biological Conservation 261: 109246, https://linkinghub.elsevier.com/retrieve/pii/S0006320721002986.

Muletz-Wolz, C. R., S. E. Barnett, G. V. DiRenzo, K. R. Zamudio, L. F. Toledo, T. Y. James, & K. R. Lips, 2019. Diverse genotypes of the amphibian-killing fungus produce distinct phenotypes through plastic responses to temperature. Journal of Evolutionary Biology 32: 287–298, https://onlinelibrary.wiley.com/doi/10.1111/jeb.13413.

National Oceanic & Atmospheric Administration, 1995. Surface weather observations and reports. Federal Meteorological Handbook, vol. 1. pp. 94.

Nilsson, M., Maeyer, H.D. and Allen, M., 2022. Evaluation of Different Cleaning Strategies for Removal of Contaminating DNA Molecules. Genes, 13(1): 162.

Olson, D. H., D. M. Aanensen, K. L. Ronnenberg, C. I. Powell, S. F. Walker, J. Bielby, T. W. J. Garner, G. Weaver, The Bd Mapping Group, & M. C. Fisher, 2013. Mapping the Global Emergence of *Batrachochytrium dendrobatidis*, the Amphibian Chytrid Fungus. PLoS ONE 8: e56802, https://dx.plos.org/10.1371/journal.pone.0056802.

Piotrowski, J. S., S. L. Annis, & J. E. Longcore, 2004. Physiology of *Batrachochytrium dendrobatidis*, a Chytrid Pathogen of Amphibians. Mycologia 96: 9, https://www.jstor.org/stable/3761981?origin=crossref.

Pontes, M., G. Augusto-Alves, C. Lambertini & L. F. Toledo, 2018. A lizard acting as carrier of the amphibian-killing chytrid *Batrachochytrium dendrobatidis* in southern Brazil. Acta Herpetologica 13: 201–205.

Rachowicz, L., & V. Vredenburg, 2004. Transmission of *Batrachochytrium dendrobatidis* within and between amphibian life stages. Diseases of Aquatic Organisms 61: 75–83, http://www.int-res.com/abstracts/dao/v61/n1-2/p75-83/.

Ribeiro, L. P., T. Carvalho, C. G. Becker, T. S. Jenkinson, D. da S. Leite, T. Y. James, S. E. Greenspan, & L. F. Toledo, 2019. Bullfrog farms release virulent zoospores of the frog-killing fungus into the natural environment. Scientific Reports 9: 13422, http://www.nature.com/articles/s41598-019-49674-0.

Ritter, A., C. M. Regalado, & J. C. Guerra, 2019. The impact of climate change on water fluxes in a Macaronesian cloud forest. Hydrological Processes 33: 2828–2846, https://onlinelibrary.wiley.com/doi/10.1002/hyp.13523.

Rollins-Smith, L. A., 2017. Amphibian immunity–stress, disease, and climate change. Developmental & Comparative Immunology 66: 111–119, https://linkinghub.elsevier.com/retrieve/pii/S0145305X16302191.

Rollins-Smith, L.A. & D.C. Woodhams, 2012. Amphibian immunity: staying in tune with the environment. In Eco-Immunology (ed. G.E. Demas and R.J Nelson). Oxford, United Kingdom: Oxford University Press 92–143.

Ruggeri, J., J. Ruggeri, T. James, & L. Toledo, 2018. Amphibian chytrid infection is influenced by rainfall seasonality and water availability. Diseases of Aquatic Organisms 127: 107–115, http://www.int-res.com/abstracts/dao/v127/n2/p107-115/.

Scheele, B. C., F. Pasmans, L. F. Skerratt, L. Berger, A. Martel, W. Beukema, A. A. Acevedo, P. A. Burrowes, T. Carvalho, A. Catenazzi, I. De la Riva, M. C. Fisher, S. V. Flechas, C. N. Foster, P. Frías-Álvarez, T. W. J. Garner, B. Gratwicke, J. M. Guayasamin, M. Hirschfeld, J. E. Kolby, T. A. Kosch, E. La Marca, D. B. Lindenmayer, K. R. Lips, A. V. Longo, R. Maneyro, C. A. McDonald, J. Mendelson, P. Palacios-Rodriguez, G. Parra-Olea, C. L. Richards-Zawacki, M.-O. Rödel, S. M. Rovito, C. Soto-Azat, L. F. Toledo, J. Voyles, C. Weldon, S. M. Whitfield, M. Wilkinson, K. R. Zamudio, & S. Canessa, 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363: 1459–1463, https://www.science.org/doi/10.1126/science.aav0379.

Shaw, G., 1802. General Zoology or Systematic Natural History. Volume III, Part 1. Amphibia, Thomas Davison, London.

Sialve, B., A. Gales, J. Hamelin, N. Wery, & J.-P. Steyer, 2015. Bioaerosol emissions from open microalgal processes and their potential environmental impacts: what can be learned from natural and anthropogenic aquatic environments?. Current Opinion in Biotechnology 33: 279–286, https://linkinghub.elsevier.com/retrieve/pii/S0958166915000555.

Skerratt, L. F., L. Berger, R. Speare, S. Cashins, K. R. McDonald, A. D. Phillott, H. B. Hines, & N. Kenyon, 2007. Spread of Chytridiomycosis Has Caused the Rapid Global Decline and Extinction of Frogs. EcoHealth 4: 125, http://link.springer.com/10.1007/s10393-007-0093-5.

Stetzenbach, L. D., 2009. Airborne infectious microorganisms. Encyclopedia of Microbiology, 175.

Thomas, V., M. Blooi, P. Van Rooij, S. Van Praet, E. Verbrugghe, E. Grasselli, M. Lukac, S. Smith, F. Pasmans, & A. Martel, 2018. Recommendations on diagnostic tools for *Batrachochytrium salamandrivorans*. Transboundary and Emerging Diseases 65: e478–e488, https://onlinelibrary.wiley.com/doi/10.1111/tbed.12787.

Toledo, L. F., J. Ruggeri, L. Leite Ferraz de Campos, M. Martins, S. Neckel-Oliveira, & C. P. B. Breviglieri, 2021. Midges not only sucks, but may carry lethal pathogens to wild amphibians. Biotropica 53: 722–725, https://onlinelibrary.wiley.com/doi/10.1111/btp.12928.

Toledo, L.F., C.F.B. Haddad, A.C.O.Q. Carnaval & F.B. Britto, 2006a. A Brazilian anuran (Hylodes magalhaesi: Leptodactylidae) infected by *Batrachochytrium dendrobatidis*: a conservation concern. Amphibian and Reptile Conservation 4:17–21.

Torregrosa, A., T. A. O'Brien, & I. C. Faloona, 2014. Coastal Fog, Climate Change, and the Environment. Eos, Transactions American Geophysical Union 95: 473–474, http://doi.wiley.com/10.1002/2014EO500001.

Van Rooij, P., A. Martel, F. Haesebrouck, & F. Pasmans, 2015. Amphibian chytridiomycosis: a review with focus on fungus-host interactions. Veterinary Research 46: 137, http://www.veterinaryresearch.org/content/46/1/137.

Vieira, C.A. & L.F. Toledo, 2012. Isolamento, cultivo e armazenamento do fungo quitrídio: *Batrachochytrium dendrobatidis*. Herpetologia Brasileira 1: 18–19.

Voyles, J., L. R. Johnson, J. Rohr, R. Kelly, C. Barron, D. Miller, J. Minster, & E. B. Rosenblum, 2017. Diversity in growth patterns among strains of the lethal fungal pathogen *Batrachochytrium dendrobatidis* across extended thermal optima. Oecologia 184: 363–373, http://link.springer.com/10.1007/s00442-017-3866-8.

Walker, S., M. Baldi Salas, D. Jenkins, T. Garner, A. Cunningham, A. Hyatt, J. Bosch, & M. Fisher, 2007. Environmental detection of *Batrachochytrium dendrobatidis* in a temperate climate. Diseases of Aquatic Organisms 77: 105–112, http://www.int-res.com/abstracts/dao/v77/n2/p105-112/.

Wang, L., K. F. Kaseke, & M. K. Seely, 2017. Effects of non-rainfall water inputs on ecosystem functions. WIREs Water 4, https://onlinelibrary.wiley.com/doi/10.1002/wat2.1179.

Wei, M., C. Xu, J. Chen, C. Zhu, J. Li, & G. Lv. 2016. Characteristics of bacterial community in fog water at Mt. Tai: similarity and disparity under polluted and non-polluted fog episodes. Atmospheric Chemistry and Physics Discussions 1-30.

Statements and Declarations

The authors declare that they have no conflict of interest.

Funding

This work was supported by São Paulo Research Foundation (FAPESP #2016/25358-3; #2019/18335-5; #2020/02991-8; #2020/00099-0), the National Council for Scientific and Technological Development

(CNPq #300896/2016-6; #302834/2020-6), and by the Coordination for the Improvement of Higher Education Personnel (CAPES - Finance Code 001).

Data availability

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Table 1. Water sampled sites, years of sampling, sampling method and percentage of *Batrachochytrium dendrobatidis* (Bd) positive samples (number of positive / total samples). Positive samples are in bold.

Sites	Municipality	Elevation range (m)	Sampling period	Sampling method	Bd frequency
National park					
PN Serra da Bocaina	São João do Barreiro	1000–1433	2020	Fog / aspirator	0% (0/2)
State parks					
PECJ: PE Campos do Jordão	**Campos do Jordão**	**1500–1600**	**2018–2020**	**Rain**	**21% (4/19)**
PESM n. Santa Virgínia	São Luiz do Paraitinga	700–1000	2020–2021	Rain	0% (0/15)
PESM n. Santa Virgínia	São Luiz do Paraitinga	700–1000	2020	Fog / fog catcher	0% (0/3)
PESM n. Santa Virgínia	**São Luiz do Paraitinga**	**700–1000**	**2020–2021**	**Fog / aspirator**	**3% (1/30)**
PESM n. Itutinga-Pilões	Bertioga	770	2020	Fog / aspirator	0% (0/2)
PETAR	Iporanga	200–1000	2020	Rain	0% (0/1)
PETAR	Iporanga	200–1000	2020	Fog / aspirator	0% (0/5)
Estação Ecológica Bananal	Bananal	1110–1240	2021	Fog / aspirator	0% (0/3)
Private reserves					
Projeto Dacnis	São Francisco Xavier	720–1000	2020	Fog / fog catcher	0% (0/1)
Reserva Betary	Iporanga	200–1000	2021	Rain	0% (0/6)
Reserva Betary	Iporanga	200–1000	2021	Fog / aspirator	0% (0/2)
Pico do Araçatuba	Tijucas do Sul	1680	2021	Fog / aspirator	0% (0/1)

Table 2. *Batrachochytrium dendrobatidis* (Bd) infection load [mean ± standard deviation (range; number of samples)] from frogs in the treatment group, 10 and 20 days post-exposure, and immediately after death.

Moment	**Infection load (zoospores g.e.)**
10 days after exposure	308 ± 522 (23 – 1764; 9)
20 days after exposure	15,772 ± 11920 (1,595 – 40,539; 9)
Day of death	54,748 ± 56036 (2,195 – 177,445; 9)

Figure legends

Fig. 1 Sampling methods. A) fog over the Atlantic Forest; B) details of the net on which water droplets present in the fog were retained; C and D) installed fog catchers; E) aspirator coupled with a filter membrane; F-H) active fog sampling; I) rainwater collectors

Fig. 2 Relationship between detected and nebulized *Batrachochytrium dendrobatidis* zoospores (log zoospores g.e.) for each exposition time: 5 (r^2 = 0.61; P = 0.007) and 10 min (r^2 = 0.72; P = 0.002). Dashed gray triangles represent 5 min and black circles 10 min

Fig. 3 *Batrachochytrium dendrobatidis* (Bd) zoospores genomic equivalents (g.e.) results detected by qPCR. A) treatment group infection load at 10- and 20-days post-exposure. Boxplots represent the median, upper and lower quartile, and maximum and minimum values. B) survival curve over time after the experiment. Circles indicate Bd load (log zoospores g.e.) collected at death moment

Fig. 4 Transmission pathways of *Batrachochytrium dendrobatidis*, across different elements of the network: hosts, carriers, and environmental reservoirs. Unidirectional routes are represented by dashed lines. Route importance order is the minimum number of steps by which a zoospore from a frog reaches another frog through that element. Novel transmission route through fog is highlighted in red

Figure 1

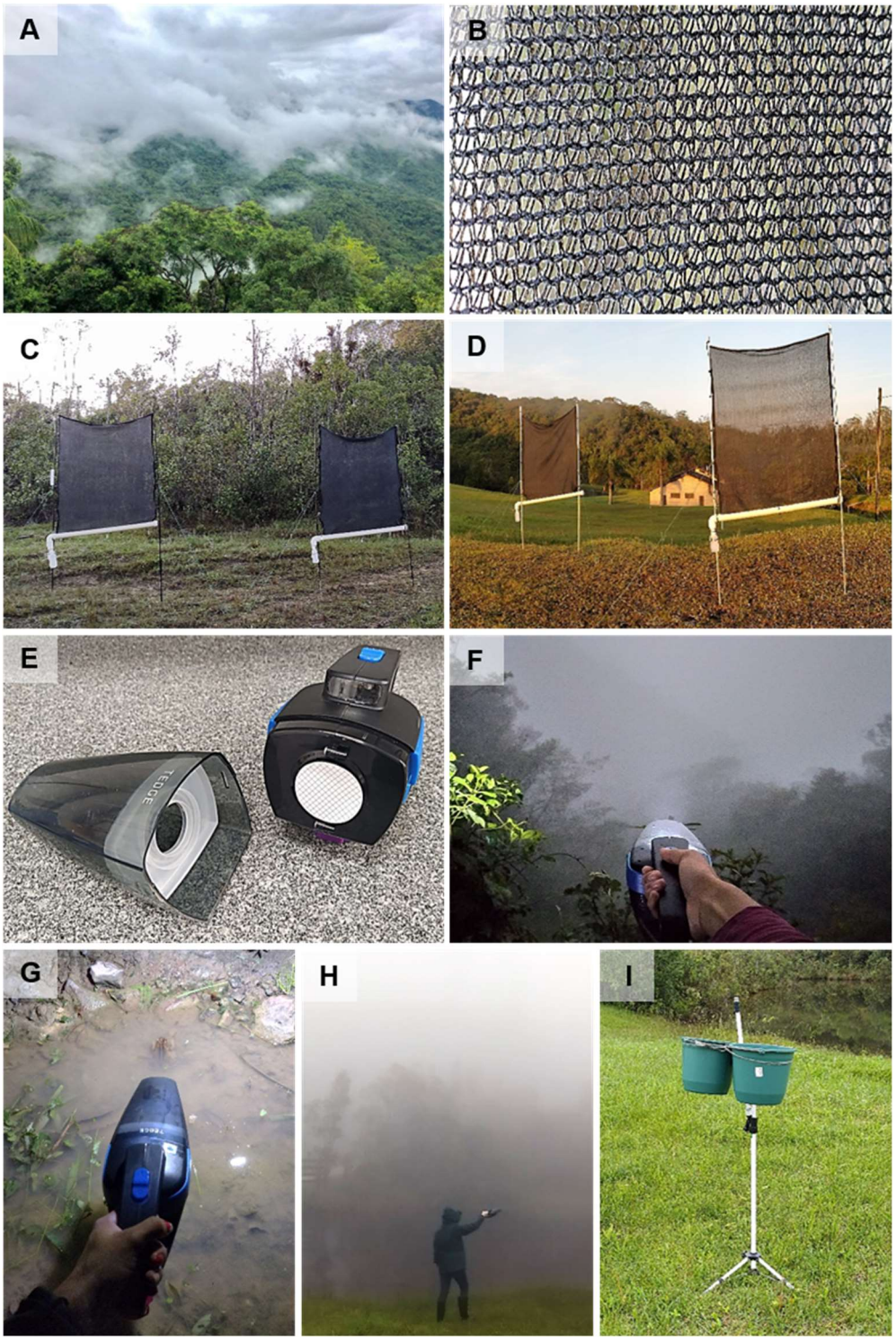


Figure 2

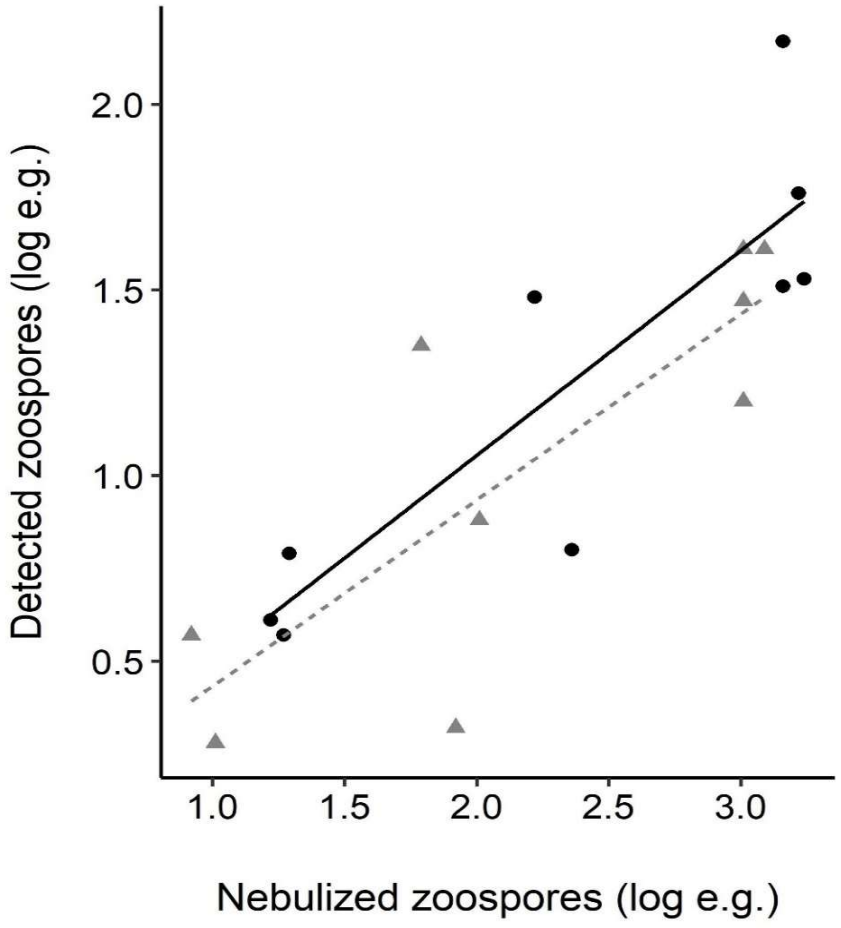

Detected zoospores (log e.g.)
2.0
1.5
1.0
0.5
1.0
1.5
2.0
2.5
3.0
Nebulized zoospores (log e.g.)

Figure 3

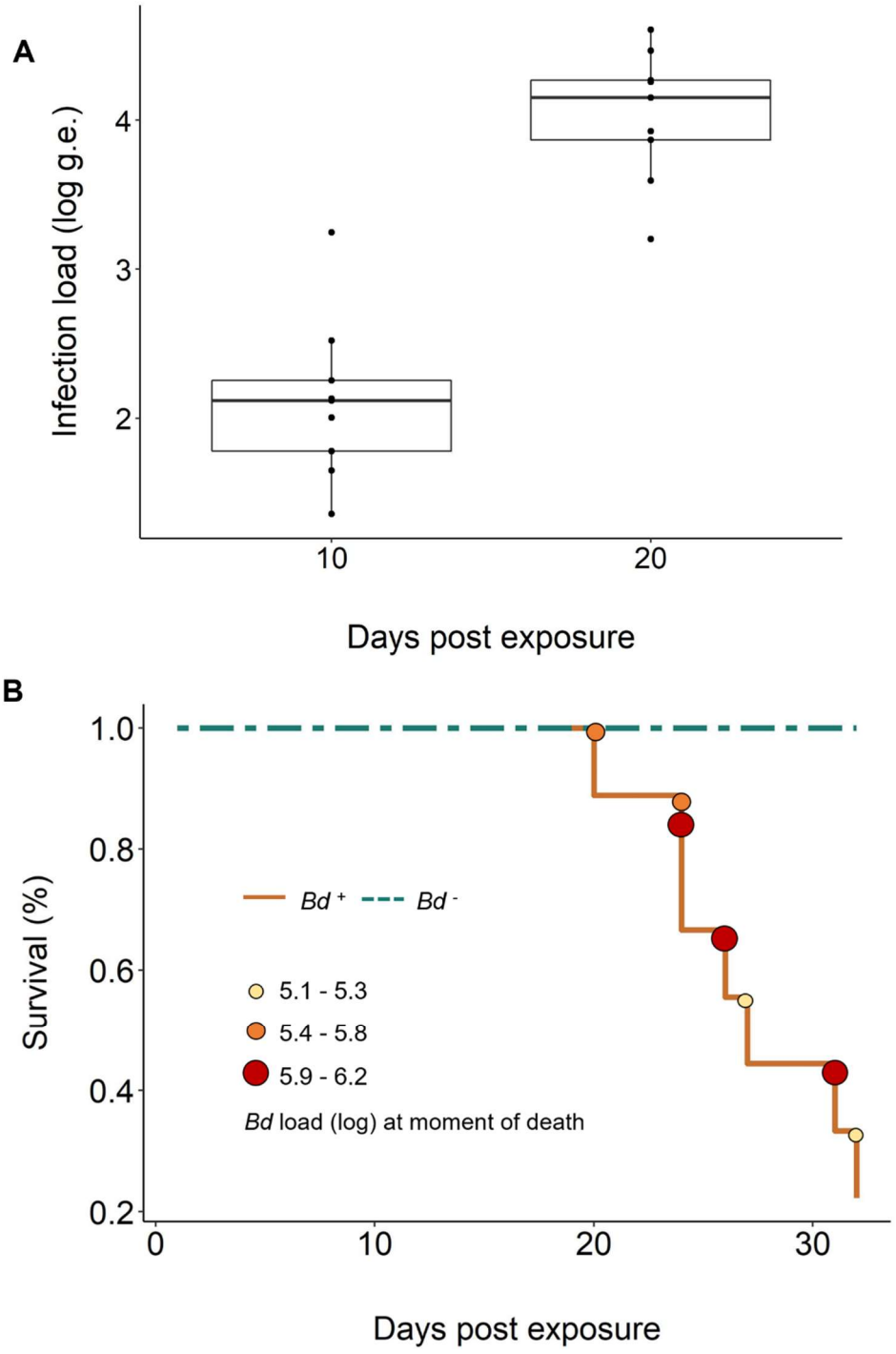
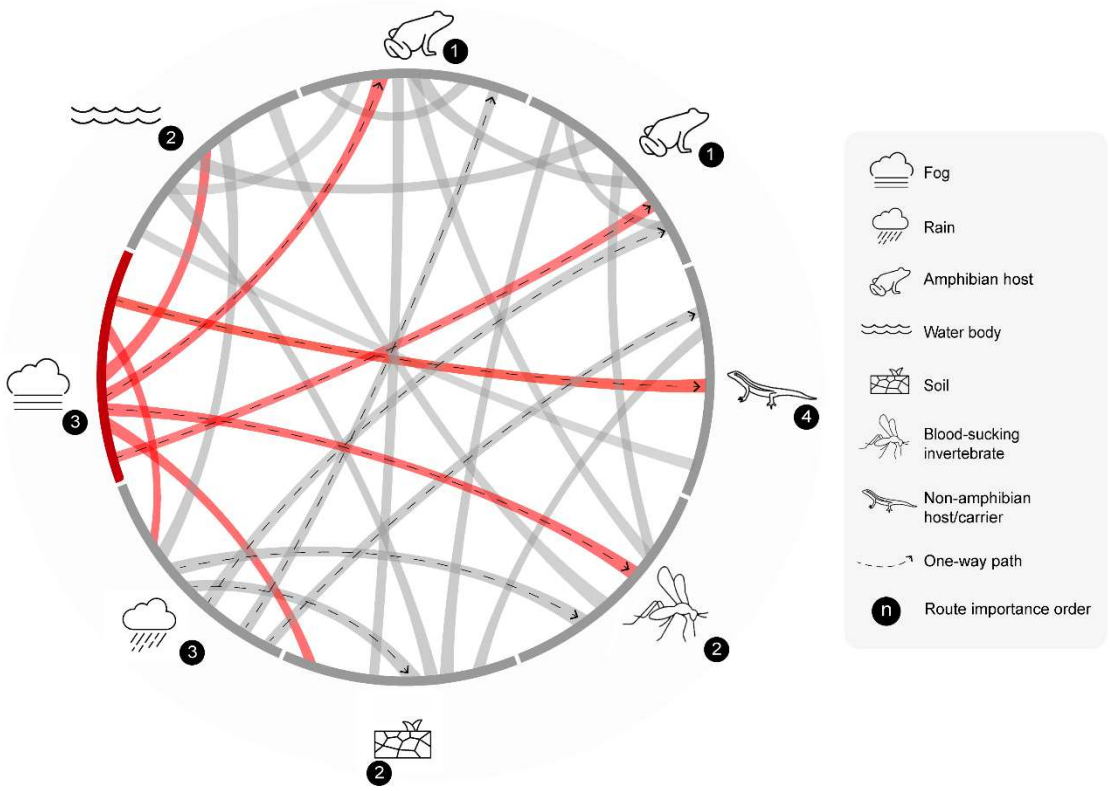

A
Infection load (log g.e.)
4
3
2
10
20
Days post exposure
B
1.0
0.8
0.6
0.4
0.2
Survival (%)
Bd +
Bd -
5.1 - 5.3
5.4 - 5.8
5.9 - 6.2
Bd load (log) at moment of death
0
10
20
30
Days post exposure

Figure 4


1
2
1
3
4
3
2
2
Fog
Rain
Amphibian host
Water body
Soil
Blood-sucking invertebrate
Non-amphibian host/carrier
One-way path
n
Route importance order

SUPPLEMENT

Table S1. Sampled sites and respective elevation range: elevations above sea level.

Site/Protected Area	**Municipality**	**Elevation range (m)**
Projeto Dacnis	São Francisco Xavier, SP	720 –1000
PESM núcleo Itutinga-Pilões	Bertioga, SP	770
PESM núcleo Santa Virginia	São Luiz do Paraitinga, SP	700 – 1000
PNSB: Parque Nacional da Serra da Bocaina	São João do Barreiro, SP	1000 – 1433
PETAR núcleos Caboclos, Santana and Ouro grosso	Iporanga, SP	200 – 1000
Reserva Betary	Iporanga, SP	200 – 700
PECJ: Parque Estadual Campos do Jordão	Campos do Jordão, SP	1500 – 1600
EEB: Estação Ecológica Bananal	Bananal, SP	1110 – 1240
Pico do Araçatuba	Tijucas do Sul, PR	1680

Table S2. Fog samples collected, respective data and *Batrachochytrium dendrobatidis* genomic equivalents (GE) detected by qPCR. SLFT: Swabs collection at Unicamp, SP, Brazil. PNSB: Parque Nacional Serra da Bocaina; PESM: Parque Estadual da Serra do Mar, núcleo Santa Virginia; PETAR: Parque Estadual Turístico do Alto Ribeira; SFX: district of São Francisco Xavier; EEB: Estação Ecológica Bocaina. Duration: the aspiration time extent; Volume: fog volume aspirated, or fog water volume collected; Method: aspirator (A) or net (N); T: temperature; RH: air relative humidity.

Voucher	Method	Site	Latitude (S)	Longitude (W)	Elevation (m)	Sampling date	Duration	Volume (L)	g.e.	T (ºC)	RH (%)
SLFT17895	A	Bertioga	23°42'29.9"	45º7'58.95"	770	10/14/20	20'00"	360	0	21	85
SLFT17896	A	Bertioga	23°42'29.9"	46°02'14.5"	770	10/15/20	20'00"	360	0	NA	NA
SLFT17897	A	PNSB	22º43'43.7"	44º35'48.5"	1433	11/03/20	15'00"	270	0	18	NA
SLFT17898	A	PNSB	22º44'33.8"	44º36'56.6"	1000	11/04/20	15'00"	270	0	NA	NA
SLFT17899	A	PNSB	22º44'33.8"	44º36'56.6"	1000	11/05/20	15'00"	270	0	NA	NA
SLFT17900	A	PESM	23º21'04"	45º08'07"	950	11/06/20	13'00"	234	0	17.07	81.79
SLFT17901	A	PESM	23º21'6.608"	45º8.1'212"	977	11/07/20	32'00"	576	0	23.72	67.42
SLFT17903	A	PESM	23º21'5.58"	45º7'58.95"	963	11/08/20	15'22"	276.6	7.26	17.93	77.6
SLFT17904	A	PESM	23º21'6.608"	45º8.1'212"	977	11/08/20	11'21"	204.6	0	17.93	77.6
SLFT17905	A	PESM	23º20'35"S	45º08'38"	960	11/08/20	15'00"	270	0	18.62	79.1
SLFT17906	A	PESM	23º21'13.17"	45º7'47.42"	974	11/08/20	15'00"	270	0	23.76	67
SLFT17918	A	PESM	23º20'31"	45º8'40"	980	11/11/20	15'00"	270	0	19	86.23
SLFT17919	A	PESM	23º19'11.91"	45º9'48.94"	1034	11/11/20	15'00"	270	0	NA	NA
SLFT17920	A	PESM	23º19'11.91"	45º9'48.94"	1034	11/11/20	30'00"	540	0	NA	NA
SLFT17921	A	PESM	23º21'6.608"	45º8.1'212"	977	11/23/20	16'00"	288	0	16.64	85.33
SLFT17922	A	PESM	23º21'5.58"	45º7'58.95"	963	11/23/20	31'50"	573	0	18.44	82.67
SLFT17923	A	PESM	23º21'5.58"	45º7'58.95"	963	11/24/20	16'20"	294	0	21.36	84.31
SLFT17924	A	PESM	23º21'04"	45º08'07"	950	11/24/20	24'00"	432	0	21.36	84.31
SLFT17925	A	PESM	23º21'5.58"	45º7'58.95"	963	11/24/20	28'20"	510	0	21.36	84.31
SLFT17926	A	PESM	23º21'6.608"	45º8.1'212"	977	11/24/20	30'00"	540	0	21.36	84.31
SLFT17927	A	PESM	23º21'13.17"	45º7'47.42"	974	11/24/20	19'10"	345	0	20.16	89.53
SLFT17933	A	PESM	23º22'3"	45º8'36"	1010	11/25/20	24'09"	434.7	0	29.26	61.19
SLFT17934	A	PESM	23º22'3"	45º8'36"	1010	11/25/20	25'18"	455.4	0	29.26	61.19
SLFT17935	A	PESM	23º21'58"	45º8'27"	980	11/25/20	28'02"	504.6	0	28.14	63.24
SLFT17936	A	PESM	23º21'58" S	45º8'27"	980	11/25/20	26'19"	473.7	0	28.14	63.24
SLFT17937	A	PESM	23º21'6.608"S	45º8.1'212"	977	11/27/20	23'00"	414	0	18.87	86.31
SLFT17938	A	PESM	23º21'6.608"S	45º8.1'212"	977	11/27/20	28'10"	507	0	18.87	86.31
SLFT17939	A	PESM	23º21'6.608"S	45º8.1'212"	977	11/27/20	26'50"	483	0	18.87	86.31
SLFT17940	A	PESM	23º21'6.608"S	45º8.1'212"	977	11/27/20	26'50"	483	0	18.87	86.31
SLFT17941	A	PETAR	24º31'57"	48º42'14"	285	02/03/21	23'38"	425.4	0	23.31	91.65
SLFT17942	A	PETAR	24º31'57"	48º42'14"	285	02/04/21	24'42"	444.6	0	23.31	91.65
SLFT17943	A	Betary	24º35'19"	48º38'15"	283	02/04/21	22'47"	410.1	0	24.71	90.2
SLFT17944	A	Betary	24º35'19"	48º38'15"	283	02/05/21	22'47"	410.1	0	24.24	87.54

SLFT17951	A	PETAR	24º26'3.9"	48º35'6.9"	637	02/06/21	22'12"	399.6	0	24.28	73.72
SLFT17952	A	PETAR	24º26'3.9"	48º35'6.9"	637	02/06/21	28'29"	512.7	0	24.28	73.72
SLFT17953	A	PETAR	24º23'56"	48º36'12"	831	02/06/21	25'09"	452.7	0	18.87	84.43
SLFT17955	A	PESM	23º21'5.58"	45º7'58.95"	963	02/19/21	27'54"	502.2	0	14.54	96.2
SLFT17956	A	PESM	23º21'5.58"	45º7'58.95"	963	02/19/21	26'54"	484.2	0	14.54	96.2
SLFT17957	A	PESM	23º21'6.3"	45º7'23.27"	963	02/20/21	27'07"	488.1	0	NA	NA
SLFT17958	A	PESM	23º21'6.3"	45º7'23.27"	964	02/20/21	28'34"	514.2	0	NA	NA
SLFT17962	A	PESM	23º22'6"	45º8'38"	968	02/21/21	26'50"	483	0	24.45	77.1
SLFT17963	A	PESM	23º22'6"	45º8'38"	968	02/21/21	28'45"	517.5	0	24.45	77.1
SLFT18769	A	Pico do Araçatuba	25º54'17"	48º59' 52"	1680	23/10/21	24'29"	440.7	0	NA	NA
SLFT19129	A	EEB	22º48'14"	44º22'32"W	1240	10/12/21	27'00"	486	0	NA	NA
SLFT19130	A	EEB	22º48'14"	44º22'32"W	1240	10/12/21	27'55"	502.5	0	NA	NA
SLFT19131	A	EEB	22º48'25"	44º22'4"	1110	10/12/21	27'13"	489.9	0	16	90
SLFT16123	N	SFX	22º54'20.1"	46º00'00.3"	1220	02/18/20		0.069	0	NA	NA
SLFT17907	N	PESM	23º21'5.58"	45º7'58.95"	963	11/08/20		0.194	0	NA	NA
SLFT17908	N	PESM	23º21'5.58"	45º7'58.95"	963	11/08/20		0.085	0	NA	NA
SLFT17909	N	PESM	23º21'5.58"	45º7'58.95"	963	11/09/20		0.366	0	NA	NA

Table S3. *Batrachochytrium dendrobatidis* loads from rainwater samples. SLFT swabs collection at Unicamp, Brazil. PECJ: Parque Estadual Campos do Jordão; PESM: Parque Estadual da Serra do Mar, núcleo Santa Virginia; PETAR: Parque Estadual Turístico do Alto Ribeira, núcleo Ouro Grosso.

Sample	Sampling date	Site	Volume (mL)	Load (zoospore g.e.)
SLFT 10968	13 June 2018	PECJ	131	0.0
SLFT 10969	14 August 2018	PECJ	950	0.0
SLFT 11036	5 September 2018	PECJ	81	0.0
SLFT 11037	9 October 2018	PECJ	650	0.12
SLFT 11038	9 October 2018	PECJ	476	0.0
SLFT 11039	18 October 2018	PECJ	845	0.0
SLFT 13165	8 January 2019	PECJ	1000	11.08
SLFT 13156	8 January 2019	PECJ	908	4.22
SLFT 13157	8 January 2019	PECJ	929	44.47
SLFT 13158	8 January 2019	PECJ	500	0.0
SLFT 13159	8 January 2019	PECJ	450	0.0
SLFT 13160	8 January 2019	PECJ	311	0.0
SLFT 13161	15 January 2019	PECJ	1400	0.0
SLFT 13162	14 February 2019	PECJ	1000	0.0
SLFT 13163	14 February 2019	PECJ	672	0.0
SLFT 13164	18 April 2019	PECJ	575	0.0
SLFT 13166	29 April 2019	PECJ	373	15.10
SLFT 13167	27 May 2019	PECJ	602	0.0
SLFT 13168	12 June 2019	PECJ	290	0.0
SLFT 17910	10 November 2020	PESM	1500	0.0
SLFT 17911	10 November 2020	PESM	1350	0.0
SLFT 17912	10 November 2020	PESM	346	0.0
SLFT 17913	10 November 2020	PESM	1000	0.0
SLFT 17914	10 November 2020	PESM	1480	0.0
SLFT 17915	11 November 2020	PESM	1030	0.0
SLFT 17916	11 November 2020	PESM	745	0.0
SLFT 17917	11 November 2020	PESM	434	0.0
SLFT 17929	24 November 2020	PESM	2907	0.0
SLFT 17930	24 November 2020	PESM	579	0.0
SLFT 17931	25 November 2020	PESM	350	0.0
SLFT 17932	25 November 2020	PESM	384	0.0
SLFT 17945	4 February 2021	Betary Reserve	896	0.0
SLFT 17946	4 February 2021	Betary Reserve	1024	0.0
SLFT 17947	5 February 2021	Betary Reserve	598	0.0
SLFT 17948	5 February 2021	Betary Reserve	960	0.0
SLFT 17949	5 February 2021	Betary Reserve	656	0.0
SLFT 17950	5 February 2021	Betary Reserve	253.8	0.0
SLFT 17954	5 February 2021	PETAR	1150	0.0
SLFT 17959	19 February 2021	PESM	483	0.0
SLFT 17960	20 February 2021	PESM	2070	0.0
SLFT 17961	21 February 2021	PESM	1120	0.0

Table S4. *Batrachochytrium dendrobatidis* zoospore quantity (zoospores/ml) nebulized and detected by qPCR, in different Bd concentrations, and different exposure times using the aspirator. Bd load are the mean ± standard deviation (range).

Exposition time (min)	Bd concentration (z/ml)	Bd load nebulized (z/ml)	Bd load detected (z/ml)
5	$\mathbf{10}^1$	9 ± 1 (8 – 10)	1 ± 2 (0 – 4)
	$\mathbf{10}^2$	82 ± 17 (62 – 103)	8 ± 10 (0 – 22)
	$\mathbf{10}^3$	1082 ± 103 (1030 – 1236)	32 ± 12 (16 – 41)
10	$\mathbf{10}^1$	18 ± 2 (16 – 20)	4 ± 3 (0 – 6)
	$\mathbf{10}^2$	175 ± 36 (144 – 227)	9 ± 14 (0 – 30)
	$\mathbf{10}^3$	1571 ± 155 (1442 – 1751)	68 ± 55 (32 – 149)

Table S5. Zoospore quantity detected by qPCR, for frogs exposed to nebulized water from water bodies.

Voucher	Water body type	Bd load (e.g.)
SLFT 16574	Stream	0.0
SLFT 16575	Stream	0.14
SLFT 16576	Pond	0.1
SLFT 16577	Pond	0.0
SLFT 16578	Pond	0.0
SLFT 16579	Pond	0.0

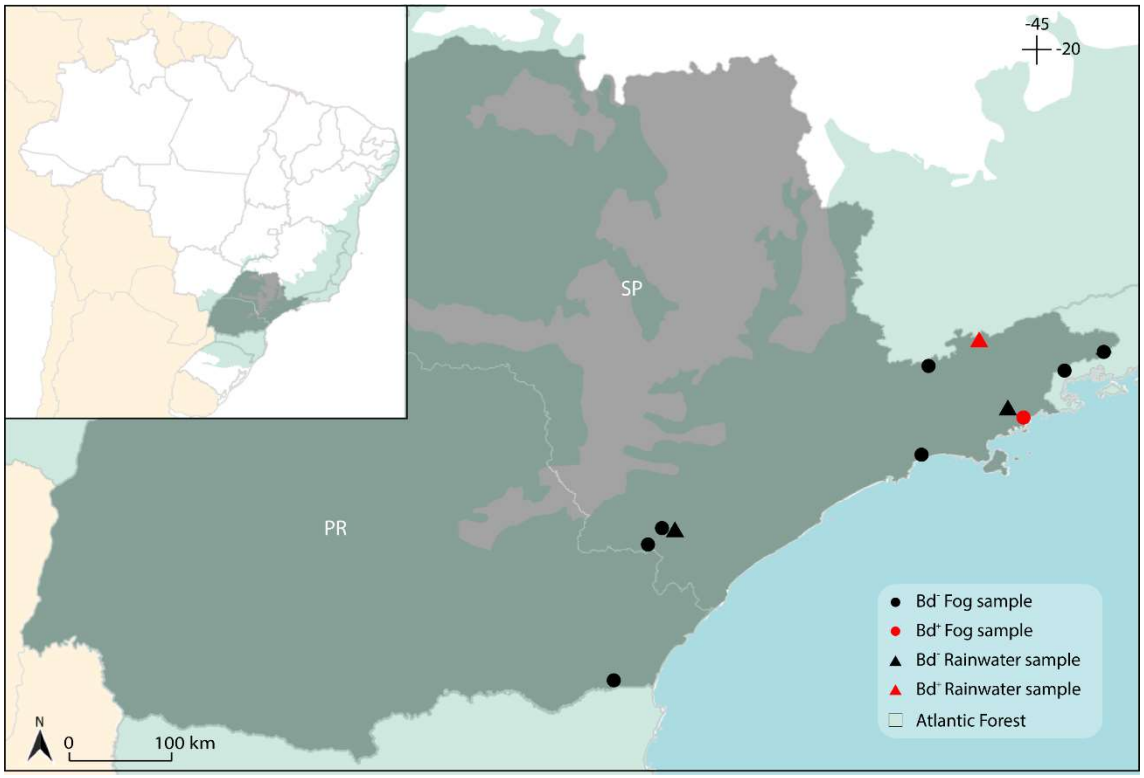


Fig S1 Sampled sites. Black circles and triangles represent sites where we collected fog and rainwater, respectively, but we did not detect Bd DNA (Bd^-). Symbols in red represent Bd positive (Bd^+) samples.

Fig S2 Experimental scheme for the infection assay, including a nebulizer and a bottle where the frog (*Eleutherodactylus johnstonei*) was placed. Schematic figure, not in scale.

Fig S3 Experimental Bd infection through exposure to natural fog. The system scheme contains Bd free *Eleutherodactylus johnstonei* placed inside individual cages, suspended 1.5 m above the ground, and under a tent.

CONCLUSÕES GERAIS

Este estudo ampliou o conhecimento das possíveis rotas de transporte do fungo Bd, acrescentando mais um reservatório ambiental a este sistema. As coletas e experimentos realizados culminaram nas seguintes conclusões:

- DNA de Bd é transportado pela neblina natural e pela água de chuva;

- Bd infecta anfíbios hospedeiros através de neblina artifical, levando à quitridiomicose letal;

- Mesmo em baixas concentrações, como ocorre na água natural de corpos hídricos, o DNA de Bd pode ser transportado através de neblina artificial e detectado na pele de anuros;

- DNA de Bd é transportado pela neblina natural até a pele de anfíbios;

- A neblina configura-se uma possível rota de dispersão de Bd, em escalas local e regional.

CONSIDERAÇÕES FINAIS

Entender que a neblina é uma possível via de transmissão do patógeno quitrídeo suporta novas hipóteses sobre como espécies hospedeiras não aquáticas e vetores podem ter contato com o fungo. Isso é especialmente importante para espécies de anuros de desenvolvimento direto, que são vulneráveis ao patógeno. Os resultados deste trabalho reafirmam que a dinâmica natural do Bd é complexa e dependente de diversos fatores e contextos. O transporte passivo de Bd através da neblina torna-se ainda mais relevante no atual cenário de mudanças climáticas, pois a dinâmica de formação de neblina pode ser alterada e assim, pode-se especular se as áreas de maior concentração desempenhariam um papel na transmissão de Bd para espécies terrestres, por exemplo. Portanto, recomendamos a ampliação dos estudos de transmissão de Bd pelas vias aéreas também nos estudos epidemiológicos desse patógeno. Ademais, foram levantadas as seguintes questões:

O DNA de Bd presente na neblina natural corresponde à zoosporos viáveis?

Qual o tamanho dos bioaerossóis de Bd presentes na neblina natural? E com quais compostos o DNA de Bd tende a estar agregado?

A frequência de exposição ao Bd através da neblina tem efeito sobre a prevalência, carga de infecção ou desenvolvimento de tolerância em populações de hospedeiros anuros de desenvolvimento direto?

REFERÊNCIAS GERAIS

Bittencourt, P. R. L., F. de V. Barros, C. B. Eller, C. S. Müller, & R. S. Oliveira, 2019. The fog regime in a tropical montane cloud forest in Brazil and its effects on water, light and microclimate. Agricultural and Forest Meteorology 265: 359–369, https://linkinghub.elsevier.com/retrieve/pii/S016819231830385X.

Bower, D. S., K. R. Lips, L. Schwarzkopf, A. Georges, & S. Clulow, 2017a. Amphibians on the brink. Science 357: 454–455, https://www.science.org/doi/10.1126/science.aao0500.

Burns, T. J., B. C. Scheele, L. A. Brannelly, N. Clemann, D. Gilbert, & D. A. Driscoll, 2021. Indirect terrestrial transmission of amphibian chytrid fungus from reservoir to susceptible host species leads to fatal chytridiomycosis. Animal Conservation 24: 602–612, https://onlinelibrary.wiley.com/doi/10.1111/acv.12665.

Carvalho, T., C. G. Becker, & L. F. Toledo, 2017. Historical amphibian declines and extinctions in Brazil linked to chytridiomycosis. Proceedings of the Royal Society B: Biological Sciences 284: 20162254.

Cheng, T. L., S. M. Rovito, D. B. Wake, & V. T. Vredenburg. 2011. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen *Batrachochytrium dendrobatidis*. Proceedings of the National Academy of Sciences 108: 9502–9507.

Dueker, M. E., G. D. O'Mullan, K. C. Weathers, A. R. Juhl, & M. Uriarte, 2012. Coupling of fog and marine microbial content in the near-shore coastal environment. Biogeosciences 9: 803–813, https://bg.copernicus.org/articles/9/803/2012/.

Evans, S. E., M. E. Dueker, J. R. Logan, & K. C. Weathers, 2019. The biology of fog: results from coastal Maine and Namib Desert reveal common drivers of fog microbial composition. Science of The Total Environment 647: 1547–1556, https://linkinghub.elsevier.com/retrieve/pii/S0048969718330134.

Frost D.R. (2022) Amphibian Species of the World: An Online Reference. Version 6.1. American Museum of Natural History, New York. Available from: https://amphibiansoftheworld.amnh.org/Amphibia/Anura/Brachycephaloidea (accessed 17 Mar 2022).

Garmyn, A., P. Van Rooij, F. Pasmans, T. Hellebuyck, W. Van Den Broeck, F. Haesebrouck, & A. Martel, 2012. Waterfowl: Potential Environmental Reservoirs of the Chytrid Fungus Batrachochytrium dendrobatidis. PLoS ONE 7: e35038, https://dx.plos.org/10.1371/journal.pone.0035038.

Gollakota, A. R. K., S. Gautam, M. Santosh, H. A. Sudan, R. Gandhi, V. Sam Jebadurai, & C.-M. Shu, 2021. Bioaerosols: Characterization, pathways, sampling strategies, and challenges to geo-environment and health. Gondwana Research 99: 178–203, https://linkinghub.elsevier.com/retrieve/pii/S1342937X21002069.

Haddad, C. F. B. (ed), 2013. Guia dos anfíbios da Mata Atlântica: diversidade e biologia. Anápolis Books, São Paulo.

Hall, E. M., E. J. Crespi, C. S. Goldberg, & J. L. Brunner, 2016. Evaluating environmental DNA -based quantification of ranavirus infection in wood frog populations. Molecular Ecology Resources 16: 423–433, https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12461.

Hoffmann, M., C. Hilton-Taylor, A. Angulo, M. Böhm, T. M. Brooks, S. H. M. Butchart, K. E. Carpenter, J. Chanson, B. Collen, N. A. Cox, W. R. T. Darwall, N. K. Dulvy, L. R. Harrison, V. Katariya, C. M. Pollock, S. Quader, N. I. Richman, A. S. L. Rodrigues, M. F. Tognelli, J.-C. Vié, J. M. Aguiar, D. J. Allen, G. R. Allen, G. Amori, N. B. Ananjeva, F. Andreone, P. Andrew, A. L. A. Ortiz, J. E. M.

Baillie, R. Baldi, B. D. Bell, S. D. Biju, J. P. Bird, P. Black-Decima, J. J. Blanc, F. Bolaños, W. Bolivar-G., I. J. Burfield, J. A. Burton, D. R. Capper, F. Castro, G. Catullo, R. D. Cavanagh, A. Channing, N. L. Chao, A. M. Chenery, F. Chiozza, V. Clausnitzer, N. J. Collar, L. C. Collett, B. B. Collette, C. F. C. Fernandez, M. T. Craig, M. J. Crosby, N. Cumberlidge, A. Cuttelod, A. E. Derocher, A. C. Diesmos, J. S. Donaldson, J. W. Duckworth, G. Dutson, S. K. Dutta, R. H. Emslie, A. Farjon, S. Fowler, J. Freyhof, D. L. Garshelis, J. Gerlach, D. J. Gower, T. D. Grant, G. A. Hammerson, R. B. Harris, L. R. Heaney, S. B. Hedges, J.-M. Hero, B. Hughes, S. A. Hussain, J. Icochea M., R. F. Inger, N. Ishii, D. T. Iskandar, R. K. B. Jenkins, Y. Kaneko, M. Kottelat, K. M. Kovacs, S. L. Kuzmin, E. La Marca, J. F. Lamoreux, M. W. N. Lau, E. O. Lavilla, K. Leus, R. L. Lewison, G. Lichtenstein, S. R. Livingstone, V. Lukoschek, D. P. Mallon, P. J. K. McGowan, A. McIvor, P. D. Moehlman, S. Molur, A. M. Alonso, J. A. Musick, K. Nowell, R. A. Nussbaum, W. Olech, N. L. Orlov, T. J. Papenfuss, G. Parra-Olea, W. F. Perrin, B. A. Polidoro, M. Pourkazemi, P. A. Racey, J. S. Ragle, M. Ram, G. Rathbun, R. P. Reynolds, A. G. J. Rhodin, S. J. Richards, L. O. Rodríguez, S. R. Ron, C. Rondinini, A. B. Rylands, Y. Sadovy de Mitcheson, J. C. Sanciangco, K. L. Sanders, G. Santos-Barrera, J. Schipper, C. Self-Sullivan, Y. Shi, A. Shoemaker, F. T. Short, C. Sillero-Zubiri, D. L. Silvano, K. G. Smith, A. T. Smith, J. Snoeks, A. J. Stattersfield, A. J. Symes, A. B. Taber, B. K. Talukdar, H. J. Temple, R. Timmins, J. A. Tobias, K. Tsytsulina, D. Tweddle, C. Ubeda, S. V. Valenti, P. Paul van Dijk, L. M. Veiga, A. Veloso, D. C. Wege, M. Wilkinson, E. A. Williamson, F. Xie, B. E. Young, H. R. Akçakaya, L. Bennun, T. M. Blackburn, L. Boitani, H. T. Dublin, G. A. B. da Fonseca, C. Gascon, T. E. Lacher, G. M. Mace, S. A. Mainka, J. A. McNeely, R. A. Mittermeier, G. M. Reid, J. P. Rodriguez, A. A. Rosenberg, M. J. Samways, J. Smart, B. A. Stein, & S. N. Stuart, 2010. The Impact of Conservation on the Status of the World's Vertebrates. Science 330: 1503–1509, https://www.science.org/doi/10.1126/science.1194442.

Hoyt, J. R., K. E. Langwig, K. Sun, K. L. Parise, A. Li, Y. Wang, X. Huang, L. Worledge, H. Miller, J. P. White, H. M. Kaarakka, J. A. Redell, T. Görföl, S. A. Boldogh, D. Fukui, M. Sakuyama, S. Yachimori, A. Sato, M. Dalannast, A. Jargalsaikhan, N. Batbayar, Y. Yovel, E. Amichai, I. Natradze, W. F. Frick, J. T. Foster, J. Feng, & A. M. Kilpatrick, 2020. Environmental reservoir dynamics predict global infection patterns and population impacts for the fungal disease white-nose syndrome. Proceedings of the National Academy of Sciences 117: 7255–7262, https://pnas.org/doi/full/10.1073/pnas.1914794117.

Johnson, M. L., & R. Speare, 2003. Survival of Batrachochytrium dendrobatidis in Water: Quarantine and Disease Control Implications. Emerging Infectious Diseases 9: 915–921, http://wwwnc.cdc.gov/eid/article/9/8/03-0145_article.htm.

Joung, Y. S., Z. Ge, & C. R. Buie, 2017. Bioaerosol generation by raindrops on soil. Nature Communications 8: 14668, http://www.nature.com/articles/ncomms14668.

Kilpatrick, A. M., C. J. Briggs, & P. Daszak, 2010. The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends in Ecology & Evolution 25: 109–118, https://linkinghub.elsevier.com/retrieve/pii/S0169534709002419.

Kriger, K. M., F. Pereoglou, & J.-M. Hero, 2007. Latitudinal Variation in the Prevalence and Intensity of Chytrid (*Batrachochytrium dendrobatidis*) Infection in Eastern Australia. Conservation Biology 21: 1280–1290.

Kolby, J. E., S. D. Ramirez, L. Berger, K. L. Richards-Hrdlicka, M. Jocque, & L. F. Skerratt, 2015. Terrestrial Dispersal and Potential Environmental Transmission of

the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*). PLOS ONE 10: e0125386, https://dx.plos.org/10.1371/journal.pone.0125386.

Lambertini, C., J. Ruggeri, R. Rebouças & L. F. Toledo, 2021. Patógenos letais de anfíbios no brasil: Ameaça à Biodiversidade. Herpetologia Brasileira Contemporânea, 85 – 87p.

Liew, N., M. J. Mazon Moya, C. J. Wierzbicki, M. Hollinshead, M. J. Dillon, C. R. Thornton, A. Ellison, J. Cable, M. C. Fisher, & S. Mostowy, 2017. Chytrid fungus infection in zebrafish demonstrates that the pathogen can parasitize non-amphibian vertebrate hosts. Nature Communications 8: 15048, http://www.nature.com/articles/ncomms15048.

Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, & J. P. Collins, 2006. From The Cover: Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences 103: 3165–3170.

Longcore, J. E., A. P. Pessier, & D. K. Nichols, 1999. Batrachochytrium Dendrobatidis gen. et sp. nov., a Chytrid Pathogenic to Amphibians. Mycologia 91: 219, https://www.jstor.org/stable/3761366?origin=crossref.

Luisetto M., B.A. Nili, K. Edbey, G.R. Mashori, A.Y. Rafa, O.Y. Latishev. 2021. Bioaeresols and Coronavirus Diffusion, Transmission, Carriers, Viral Size, Surfaces Properties and Other Factor Involved. Clin Cases Med. 1(1):1004.

Mesquita, A. F. C., C. Lambertini, M. Lyra, L. R. Malagoli, T. Y. James, L. F. Toledo, C. F. B. Haddad, & C. G. Becker, 2017. Low resistance to chytridiomycosis in direct-developing amphibians. Scientific Reports 7: 16605, http://www.nature.com/articles/s41598-017-16425-y.

Piotrowski, J. S., S. L. Annis, & J. E. Longcore, 2004. Physiology of *Batrachochytrium dendrobatidis*, a Chytrid Pathogen of Amphibians. Mycologia 96: 9, https://www.jstor.org/stable/3761981?origin=crossref.Prahl J., Wilson T. P., Giles D., & Craddock J. H. 2020. An overview of research regarding reservoirs, vectors and predators of the chytrid fungus *Batrachochytrium dendrobatidis*. Acta Herpetologica 15(1): 39–45.

Ruggeri, J., Carvalho-e-Silva, S. P., James, T. Y. & Toledo, L. F. 2018. Amphibian chytrid infection is influenced by rainfall seasonality and water availability. Diseases of Aquatic Organisms 127(2): 107−115.

Scheele, B. C., F. Pasmans, L. F. Skerratt, L. Berger, A. Martel, W. Beukema, A. A. Acevedo, P. A. Burrowes, T. Carvalho, A. Catenazzi, I. De la Riva, M. C. Fisher, S. V. Flechas, C. N. Foster, P. Frías-Álvarez, T. W. J. Garner, B. Gratwicke, J. M. Guayasamin, M. Hirschfeld, J. E. Kolby, T. A. Kosch, E. La Marca, D. B. Lindenmayer, K. R. Lips, A. V. Longo, R. Maneyro, C. A. McDonald, J. Mendelson, P. Palacios-Rodriguez, G. Parra-Olea, C. L. Richards-Zawacki, M.-O. Rödel, S. M. Rovito, C. Soto-Azat, L. F. Toledo, J. Voyles, C. Weldon, S. M. Whitfield, M. Wilkinson, K. R. Zamudio, & S. Canessa, 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363: 1459–1463, https://www.science.org/doi/10.1126/science.aav0379.

Toledo, L.F., C.F.B. Haddad. A.C.O.Q. Carnaval & F.B. Britto. 2006a. A Brazilian anuran (Hylodes magalhaesi: Leptodactylidae) infected by *Batrachochytrium dendrobatidis*: a conservation concern. Amphibian and Reptile Conservation, 4:17-21.

Toledo, L. F., J. Ruggeri, L. Leite Ferraz de Campos, M. Martins, S. Neckel-Oliveira, & C. P. B. Breviglieri, 2021. Midges not only sucks, but may carry lethal pathogens to

wild amphibians. Biotropica 53: 722–725, https://onlinelibrary.wiley.com/doi/10.1111/btp.12928.

Torregrosa, A., T. A. O'Brien, & I. C. Faloona, 2014. Coastal Fog, Climate Change, and the Environment. Eos, Transactions American Geophysical Union 95: 473–474, http://doi.wiley.com/10.1002/2014EO500001.

Vieira, C.A., Toledo, L.F., 2012. Isolamento, cultivo e armazenamento do fungo quitrídio: *Batrachochytrium dendrobatidis*. Herpetologia Brasileira 1: 18-19.

Wake, D. B. & V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences 105(Supplement 1): 11466–11473.

ANEXOS

ANEXO I Certificado do comitê de ética 5535-1/2020.

UNICAMP

CEUA/UNICAMP

CERTIFICADO

Certificamos que a proposta intitulada **Transporte passivo de um patógeno letal para anfíbios (Batrachochytrium dendrobatidis) por meio de neblina**, registrada com o nº **5535-1/2020**, sob a responsabilidade de **Prof. Dr. Luís Felipe de Toledo Ramos Pereira e Joelma Santos do Prado**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, do **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**, e com as normas editadas pelo **Conselho Nacional de Controle da Experimentação Animal** (**CONCEA**), **tendo sido** aprovada pela **Comissão de Ética no Uso de Animais** da **Universidade Estadual de Campinas - CEUA/UNICAMP**, em reunião de **21/05/2020**.

Finalidade:	**( ) Ensino (X) Pesquisa Científica**
Vigência do projeto:	01/05/2020 a 01/03/2022
Vigência da autorização para manipulação animal:	21/05/2020 a 01/03/2022
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	15
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	8 Machos 7 Fêmeas
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	15
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	8 Machos 7 Fêmeas
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	15
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	8 Machos 7 Fêmeas
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	15
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	8 Machos 7 Fêmeas
Origem:	Animais invasores que serão coletados nos jardins de residências da cidade de São Paulo
Biotério onde serão mantidos os animais:	LaDiVert, DBA/IB/UNICAMP

A aprovação pela CEUA/UNICAMP não dispensa autorização a junto ao **IBAMA**,**SISBIO** ou **CIBio** e é **restrita** a protocolos desenvolvidos em biotérios e laboratórios da Universidade Estadual de Campinas.

Campinas, **28 de maio de 2020**.

Prof. Dr. Wagner José Fávaro
Presidente

Rosangela dos Santos
Secretária Executiva

IMPORTANTE: Pedimos atenção ao prazo para envio do relatório final de atividades referente a este protocolo: até 30 dias após o encerramento de sua vigência. O formulário encontra-se disponível na página da CEUA/UNICAMP, área do pesquisador responsável. A não apresentação de relatório no prazo estabelecido impedirá que novos protocolos sejam submetidos.

ANEXO II Certificado do comitê de ética 5866-1/2021.

CERTIFICADO CEUA nº 231/2021

UNICAMP

CEUA/UNICAMP

CERTIFICADO

Certificamos que a proposta intitulada **Infecção por um patógeno letal para anfíbios (Batrachochytrium dendrobatidis) por meio de neblina**, registrada com o nº **5866-1/2021**, sob a responsabilidade de **Prof. Dr. Luís Felipe de Toledo Ramos Pereira e Joelma Santos do Prado**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, do **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**, e com as normas editadas pelo **Conselho Nacional de Controle da Experimentação Animal (CONCEA), tendo sido** aprovada pela **Comissão de Ética no Uso de Animais** da **Universidade Estadual de Campinas - CEUA/UNICAMP,** em reunião de **16/09/2021**.

Finalidade:	**( ) Ensino (X) Pesquisa Científica**
Vigência do projeto:	01/08/2021 a 01/07/2022
Vigência da autorização para manipulação animal:	16/09/2021 a 01/07/2022
Espécie / linhagem/ raça:	Espécie silvestre não-brasileira* / Eleutherodactylus johnstonei
No. de animais:	60
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	30 Machos 30 Fêmeas
Origem:	Animais invasores que serão coletados nos jardins de residências da cidade de São Paulo, de acordo com as licenças do SisBio e SISGEN
Biotério onde serão mantidos os animais:	LaDiVert, DBA/IB/UNICAMP

A aprovação pela CEUA/UNICAMP não dispensa autorização a junto ao **IBAMA,SISBIO** ou **CIBio** e é **restrita** a protocolos desenvolvidos em biotérios e laboratórios da Universidade Estadual de Campinas.

Campinas, **20 de outubro de 2021**.

Prof. Dr. Wagner José Fávaro
Presidente

Rosangela dos Santos
Secretária Executiva

IMPORTANTE: Pedimos atenção ao prazo para envio do relatório final de atividades referente a este protocolo: até 30 dias após o encerramento de sua vigência. O formulário encontra-se disponível na página da CEUA/UNICAMP, área do pesquisador responsável. A não apresentação de relatório no prazo estabelecido impedirá que novos protocolos sejam submetidos.

Documento assinado. Verificar autenticidade em sigad.unicamp.br/verifica
Informar código 9717E7B8 EF0E4E20 BEFF5BB2 139264F2

Documento assinado eletronicamente por **WAGNER JOSE FAVARO**, **CORDENADOR CEUA/UNICAMP**, em 21/10/2021, às 13:44 horas, conforme Art. 10 § 2º da MP 2.200/2001 e Art. 1º da Resolução GR 54/2017.

Documento assinado eletronicamente por **ROSANGELA DOS SANTOS**, **SECRETÁRIA EXECUTIVA CEUA/UNICAMP**, em 21/10/2021, às 14:10 horas, conforme Art. 10 § 2º da MP 2.200/2001 e Art. 1º da Resolução GR 54/2017.

A autenticidade do documento pode ser conferida no site: sigad.unicamp.br/verifica, informando o código verificador: **9717E7B8 EF0E4E20 BEFF5BB2 139264F2**

UNICAMP SIGAD

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação de Mestrado, intitulada TRANSPORTE PASSIVO DE UM PATÓGENO LETAL PARA ANFÍBIOS (*Batrachochytrium dendrobatidis*) POR MEIO DE NEBLINA, não infringem os dispositivos da Lei n.º 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 15 de julho de 2022

Assinatura: 

Nome do(a) autor(a): Joelma Santos do Prado

RG n.º 38.036.010-X

Assinatura: 

Nome do(a) orientador(a): Dr. Luis Felipe de Toledo Ramos Pereira

RG n.º 28.465.361-5