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Cryptic disease-induced mortality may cause host extinction in an apparently-stable host-parasite system

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Abstract

The decline of wildlife populations due to emerging infectious disease often shows a common pattern: the parasite invades a naïve host population producing epidemic disease and a population decline, sometimes with extirpation. Some susceptible host populations can survive the epidemic phase and persist with endemic parasitic infection. Understanding host-parasite dynamics leading to persistence of the system is imperative to adequately inform conservation practice. Here we combine field data, statistical and mathematical modelling to explore the dynamics of the apparently-stable *Rhinoderma darwinii*-*Batrachochytrium dendrobatidis* (Bd) system. Our results indicate that Bd-induced population extirpation may occur even in the absence of epidemics and where parasite prevalence is relatively low. These empirical findings are consistent with previous theoretical predictions showing that highly pathogenic parasites are able to regulate host populations even at extremely low prevalence, highlighting that disease threats should be investigated as a cause of population declines even in the absence of an overt increase in mortality.

Keywords: chytridiomycosis; Cormack-Jolly-Seber models; Darwin's frogs; epidemic and endemic emerging infectious disease; matrix population models; multi-state capture-recapture models

1. Introduction

In his pioneering work, Anderson [1] used epidemiological models to show that highly pathogenic parasites are likely to induce their own extinction before that of their host. Subsequent theoretical and empirical work showed that, under certain circumstances, parasites can drive host populations to extinction [2–5]. For instance, the chytrid fungus *Batrachochytrium dendrobatidis* (hereafter Bd) [6] has been associated with mass mortality events, the extirpation of local amphibian populations and the extinction of amphibian species on multiple continents [7,8]. As in other host-parasite systems [5], the capability of Bd to have these devastating effects on host populations is largely attributed to the presence of multiple reservoirs, the existence of a free-living infective stage, and the introduction of the parasite into naïve host populations [3,7,9,10].

Diverse emerging host-parasite systems (e.g. morbillivirus disease in mammals, white nose syndrome in bats, Ebola in primates), including the amphibian-Bd system, show a common pattern of disease-induced host population decline: the parasite invades a naïve host population producing a disease outbreak or epidemic, leading to mass mortality, population decline and, eventually, extirpation [4,11–17]. For the amphibian-Bd system, theory predicts and empirical evidence confirms, that populations of highly susceptible hosts (i.e. hosts that develop the fatal, Bd-induced disease chytridiomycosis) can survive the epidemic state and persist in relative stability with endemic Bd infection dynamics [18–23]. The persistence of a population of susceptible hosts with endemic Bd infection could arise as a consequence of several processes that include, but are not restricted to, an increase in recruitment that compensates for the Bd-induced mortality [19,24,25], changes in biotic or abiotic factors that reduce average infection intensity and increase parasite aggregation [21,26], and density-dependent transmission dynamics [24]. As these processes are general, they are

not restricted to the amphibian-Bd interaction but should play a key role in the dynamics of most host-parasite systems [27–29].

As emerging infectious diseases have become a significant threat to biodiversity [3,30], it is urgent to gain a thorough understanding of host-parasite systems that can transit from an epidemic to an endemic state and, therefore, where a population of susceptible hosts is able to persist in the face of recurrent parasite infection and high probability of disease-induced mortality if infection occurs [29]. In this study we combined field data, statistical and mathematical modelling to explore the dynamics of an apparently stable amphibian-Bd system. To this end, we focused on the Southern Darwin's frog (*Rhinoderma darwinii*), an amphibian species that inhabits the austral temperate forest of southern South America [31]. The *R. darwinii*-Bd system is a suitable model for the study of endemic Bd infection dynamics in a susceptible host species because: 1) Bd infection can produce mortality in *R. darwinii* individuals [32]; 2) retrospective and cross-sectional data are consistent with the chytridiomycosis-driven extirpation of local populations of *R. darwinii* and its sister species, the Northern Darwin's frog, *R. rufum* [32]; and 3) prior to the beginning of this study, neither epidemics nor mass die-offs have been observed in Bd-positive *R. darwinii* populations over five years of monitoring [32,33,A. Valenzuela-Sánchez, unpublished data].

Here, we used data from a 24-month capture-recapture (CR) study covering multiple seasons (i.e. spring, early summer and early autumn) in eight wild populations of *R. darwinii* to estimate demographic and epidemiological parameters. We incorporated these parameters into matrix population models in a way that is analogous to classical compartment disease models (e.g. SIR model) [2,34,35], to predict the long-term dynamics of this apparently stable system. We provide evidence suggesting that disease-induced population extinction is possible in the absence of epidemic dynamics and at relatively low infection probabilities in our study system. In fact, our population model predicted that, most

likely, *R. darwinii* populations are in slow decline due to chytridiomycosis and that Bd-infected populations will eventually become extinct. Our findings provide rare empirical support for previous theoretical predictions showing that highly pathogenic parasites can regulate a host population even at extremely low prevalence [2,4].

2. Methods

(a) Model species and study area

Rhinoderma darwinii is a fully terrestrial, forest specialist frog [36]. Its populations are not homogeneously distributed in native forest but clustered in specific sites, with individuals exhibiting high site fidelity and small home ranges [37]. From 2014 to 2016 we surveyed two sites with known presence of *R. darwinii* [31] in each of four geographical areas of Chile (figure 1): 1) Nahuelbuta range (Monumento Natural Contulmo, 'MNC' and Reserva Forestal Contulmo, 'RFC'); 2) the Andes (Reserva Biológica Huilo Huilo, 'HUI1' and 'HUI2'); 3) Chiloé Island (Parque Tantauco, 'TAN1' and 'TAN2'); and 4) Patagonia (Reserva Natural Melimoyu, 'MER1' and 'MER2'). Within an area, the minimum distance between sites was at least 300 m. Considering the short inter-annual movements observed in *R. darwinii* (i.e. mean adult displacement between years = 6.3 m) [38] we considered these sites as independent units and call them 'populations' hereafter. At each site, we defined a rectangular plot of different size (table S1) to demarcate each population and in which to conduct our CR study.

(b) Capture-recapture study

We surveyed northern populations (MNC, RFC, HUI1 and HUI2) on seven occasions. Due to the difficulties reaching southern populations (TAN1, TAN2, MER1 and MER2), which were located in remote areas, we surveyed them only on five occasions. Each captured *R. darwinii* individual was measured (snout-vent-length, SVL), photographed for individual recognition [31] and skin-swabbed for Bd detection following Soto-Azat *et al.* [32]. Details on searching and handling methodology can be found in the electronic supplementary material. Syntopic anurans were captured opportunistically when seen (table S2); these animals were held in individual, disposable plastic bags until the survey was completed, sampled for Bd detection and then released at the site of capture without being marked.

We defined three age classes for *R. darwinii*: recently-metamorphosed frogs (SVL <11 mm), juveniles (SVL ≥11 to 24 mm, but SVL ≥11 to 19.5 for TAN1-TAN2) and adults (SVL > 24 mm, but SVL >19.5 for TAN1-TAN2). The smaller adult size used for frogs on Chiloé Island follows observations that these animals are smaller and reach sexual maturity at ~19.5 mm SVL [36]. Recently-metamorphosed frogs were rarely captured (4.8% of all captures); since their individual ventral markings were not completely developed, we did not include them in our CR analyses.

(c) *Batrachochytrium dendrobatidis* detection

Extraction of DNA from skin swabs and subsequent detection of Bd using a specific real-time PCR assay (qPCR) was done following the methods described by Boyle *et al.* [39] as amended by Soto-Azat *et al.* [32]. We assumed that a Bd-positive swab indicated Bd infection of the swabbed animal. By including known concentrations of Bd DNA in serial diluted control wells on each PCR plate, we were able to quantify infection intensity, which we defined as the number of zoospore equivalents (ZE) per swab. For this, infection

intensity was corrected by multiplying the genomic equivalent value obtained from the qPCR assay by 120 (see Hudson *et al.* [40] for further details).

(d) Capture-recapture models

In order to evaluate differences in individual frog survival between Bd-positive and Bd-negative populations, we used Cormack-Jolly-Seber (CJS) models [41]. The Bd-status of each population was defined by the qPCR results: if any animal in a population tested positive for Bd at any time during the course of the study, that population was considered Bd-positive. In the CJS and related non-spatial CR models, mortality cannot be disentangled from emigration, and survival probability is considered to be 'apparent' [41]. Emigration could lead to a sub-estimation of true survival probability [41], however, our estimates of 'apparent' survival probability (ϕ) are likely to be near to the true survival probability because *R. darwinii* individuals move only short distances between seasons and years, and because each study site was centred on a population that was several times larger than the average home range of individuals [37,38]. The step-by-step process used for CJS model construction and the comparison between Bd-positive and Bd-negative populations are described in the electronic supplementary material. In 'Results' we show a CJS model where ϕ was constrained by age (adult and juveniles) and population.

Subsequently, we used multistate CR (MSMR) models to estimate the effect of Bd infection on ϕ [18,34,40,42,43]. In MSMR modelling, ϕ and recapture probability (p) can be separately estimated for different states [42]. We constructed models with two states according to the observed Bd-infection status of individuals (infected or uninfected). Additionally, individuals may change states between survey periods and, therefore, transition probability (ψ) can be estimated. We defined the transition from the uninfected to

the infected state as 'infection probability' (ψ_{UI}) and the transition from the infected to the uninfected state as 'recovery probability' (ψ_{IU}). The return rate of infected frogs (i.e. percentage of infected frogs in Bd-positive populations that were recaptured at least once during the course of the study) was very low (only two frogs), therefore, ψ_{IU} and p of infected frogs (p_I) were unidentifiable parameters with our data [41]. We performed a simulation study to evaluate the likelihood of observing only by chance such a low return rate of infected individuals. To this end, we ran 10,000 simulations where the capture history matrix from Bd-positive populations was held (a total of 388 frogs) but the position of the 31 infections was re-sampled (without replacement) following a flat categorical distribution.

We fitted a first MSMR model to evaluate if the difference in ϕ estimates between Bd-infected (ϕ_I) and uninfected (ϕ_U) frogs was consistent across Bd-positive populations (MSMR model 1). Subsequently, we constructed a model to evaluate any effect of age on ϕ and ψ_{UI} estimates (MSMR model 2; see electronic supplementary material for further details on MSMR model construction). Previous studies on amphibian-Bd systems have found no differences between p of uninfected individuals (p_U) and p_I (*Rana sierra* [21]; *Litoria rheocola* [18]), while for *Leptodactylus fallax* p_U was lower than p_I [40]. Therefore, we constrained the MSMR models in such a way that p_I and p_U were equal. To test the sensitivity of ϕ_I estimates to violations on this assumption, we re-ran the MSMR model 2 several times using a time-constant p_I that ranged from 0.1 to 0.9.

We analysed the CR models in a Bayesian framework, as described by Kéry and Schaub [41], using the package jagsUI in R [44,45], which internally calls and runs the program JAGS [46]. The ϕ and ψ estimates were obtained for the time interval between each survey period for each population, but for simplicity and comparability between populations, all estimates were calculated and are presented as annual probabilities in 'Results' (estimates

194 in the original time intervals are presented in electronic supplementary material, tables S3
195 and S5). All our estimates are presented as the mean of the posterior distribution of the
196 parameter with a 95% credible interval (CRI). We used vague priors for all parameters [41].
197 For most models, we ran three chains of 110,000 Markov Chain Monte Carlo iterations with
198 a burn-in of 10,000 thinning every 10th observation. Otherwise, MCMC were run as long as
199 was necessary to reach convergence in all parameter estimates, which was evaluated
200 using the Gelman-Rubin $\hat{R}$ statistic (i.e. $\hat{R}$ values <1.1) and by a visual inspection of the
201 chains [41].

202

203 **(e) Matrix population models**

204 To estimate and compare the asymptotic population growth rate (λ) and the extinction risk
205 in Bd-positive and Bd-negative populations, we developed deterministic state-structured
206 matrix population models [34,35,47]. In our models, individuals change between states and
207 reproduce in discrete 1-year time steps and the population is sampled just after breeding
208 (i.e. post-breeding census) [47]. Six states were defined based on a combination of Bd-
209 status (uninfected and infected frogs) and age class (1-year old juveniles, 2-year old
210 juveniles and adults). A detailed explanation on the criteria used to classify animals by age,
211 model parameters and full model structure, is shown in the electronic supplementary
212 material.

213 We constructed two matrix population models to represent different epidemiological
214 scenarios. Population model 1 was constructed to represent an average Bd-positive
215 population. Therefore, this model had all above described states and was parameterized
216 with averaged demographic and epidemiological parameters estimated with the MSMR
217 model 2 in the Bd-positive populations. Population model 2 was constructed to represent an

218 average Bd-negative population, thus this model had only uninfected states and was
219 parameterized with averaged demographic parameters estimated with the CJS model in the
220 Bd-negative populations (electronic supplementary material, CJS model 3). Additionally, we
221 made an *ad hoc* split of Bd-negative populations in order to illustrate that the λ and the
222 population size projection produced by population model 2 were largely influenced by ϕ
223 estimates coming from a single Bd-negative population (i.e. TAN1). To this end, we
224 constructed population model 3, which also represents a Bd-negative population and had
225 the same structure as population model 2, but was parameterized with averaged
226 demographic parameters from TAN2-MER1-MER2 only (electronic supplementary material,
227 CJS model 4).

228 Under our study design, ψ_{UI} and ϕ_U could be underestimated because an unknown
229 proportion of Bd infections are not detected if disease-induced mortality takes place in a
230 period of time shorter than 3 months (i.e. both infection and mortality occurred between
231 study visits). Assuming that the differences between mean ϕ_U from individuals at Bd-
232 negative and Bd-positive populations (estimated using CJS models) are only due to
233 unobserved disease-induced deaths, we can use our fully parameterized matrix population
234 model (population model 1) to provide corrected ψ_{UI} estimates. With this single purpose we
235 ran population model 4. This model has the same states and parameter values as
236 population model 1, but two modifications were made. First, we used the highest ϕ_U values
237 (i.e. from TAN2-MER1-MER2). Second, we tested different ψ_{UI} values and checked which
238 of them led to the same mean λ that was observed with the population model 1.

239 To include uncertainty of our parameter estimates in model outputs, we ran 10,000
240 simulations of each matrix population model. In each simulation the parameter values were
241 randomly sampled from different probabilistic functions fitted with parameter values
242 presented in the electronic supplementary material, table S7. We started each simulation

with a Bd-free population and ran the model for a 30-year period. We calculated λ from models 1-4 during each simulation using eigenanalysis, as described by Stevens [48]. Further details on model structure, parameter estimation and simulation setting are provided in the electronic supplementary material.

3. Results

(a) Captures

We made a total of 1,182 captures of 723 different frogs (figure 1b). Of these, 284 (39.3%) were recaptured at least once across survey periods. Adults presented a slightly, but consistently, higher number of recaptures than juveniles (electronic supplementary material, figures S1 and S2). Despite the large number of individuals captured, we did not observe dead frogs or individuals with abnormal behaviour or other possible signs of chytridiomycosis during the course of this study.

(b) Bd-infected frogs and infection intensity

Only the four northern populations were found to be Bd-positive (i.e. at least one positive sample during the study; figure 1b). Of the 338 individual frogs captured in these populations, only 8.9% were positive for Bd at least once (30 individuals and one re-infection). The return rate of infected frogs was 6.6%; lower than would be expected by chance (95% CI= 29.0% – 64.5%; figure 2b). Only two frogs, both having low infection intensities (10 and 13 ZE per swab), were recaptured as uninfected. One of these frogs subsequently gained a heavy infection (27,649 ZE) and was never captured again.

The infection intensity ranged from 3 to 326,786 ZE per swab (mean =15,498, bootstrapped 95% CI = 2,810 – 38,719; median = 365, bootstrapped 95% CI = 71 – 1,380). Of infected

frogs, 36.7% had >1,000 ZE per swab at least once. The distribution of log-transformed ZE per swab was bell-shaped (figure 2a), slightly right skewed (bootstrapped skewness = 0.26) with a variance to mean ratio of 1.45.

Eighty-six individuals of six other amphibian species were captured from seven sites; no syntopic amphibians were found at site HUI1 (table S2). One syntopic anuran species (*Eupsophus contulmoensis*) was positive for Bd. This species was found only in the two most-northern sites of MNC and RFC, where 6 of 59 (10.2%) animals tested positive for Bd, but with a low infection intensity (mean = 287 ZE, bootstrapped 95% CI = 43 – 624; median = 71 ZE, bootstrapped 95% CI = 21 – 769; table S2).

(c) Capture-recapture models

The CJS models showed that adult mean annual ϕ was, on average, lower in Bd-positive than in Bd-negative populations (mean difference = -0.354, 95% CRI = -0.506 – -0.173), although it was similar between the Bd-positive populations and TAN1 (figure 1a; electronic supplementary material, table S4). The same pattern was observed for juveniles (mean difference = -0.397, 95% CRI = -0.560 – -0.196), but with considerably larger credible intervals for ϕ estimates from Bd-negative populations (figure 1a).

The MSMR models showed that ϕ was considerably lower in infected frogs in comparison to uninfected frogs (figure 2a). This difference was consistent across all study populations (electronic supplementary material, figure S3a) and between age classes (figure 2a). Infected adults and juveniles were 55.0% and 43.5% less likely to survive one-year than uninfected adults and juveniles, respectively (figure 2a). The difference between ϕ_U and ϕ_I was consistent across all the tested p_I values (figure 2b).

Annual ψ_{UI} was higher in juveniles than in adults (26.8% [95% CRI = 9.1 – 52.5%] vs 14.7% [95% CRI = 7.2 – 25.2%]), and was similar across all Bd-positive populations (electronic supplementary material, figure S3b).

(d) Matrix population models

Matrix population models suggest that Bd infection has a profound impact at the population level (figure 3): mean λ in the Bd-positive population model (model 1) was 0.77 (95% CI = 0.51 – 1.13), while in the Bd-negative population model (models 2) it was 0.99 (95% CI = 0.648 – 1.473). The percentage of simulations with population decline (i.e. $\lambda < 1$) dropped from 90.1 % to 54.7% between the Bd-positive and the Bd-negative population models (figure 3). The mean λ in the Bd-negative population model 2 was close to stability, but it was largely influenced by survival rates in the Bd-negative population TAN1. Excluding this population from the analysis (i.e. population model 3) results in a λ of 1.18 (95% CI = 0.78 – 1.66) and a percentage of simulations with population decline of 24.1%. Median extinction time (i.e. fewer than two frogs) was predicted at year 17 in model 1, while the other populations (model 2 and 3) did not go extinct during the 30-year period (figure 4).

Model 4 indicated that a one-fold increase in the estimated annual ψ_{UI} led to an equal mean λ as observed in model 1, therefore, the corrected annual ψ_{UI} was 53.6% for juveniles and 29.4% for adults.

4. Discussion

As in other wildlife populations threatened by infectious diseases [e.g. 11–13,43], well-documented amphibian population declines due to chytridiomycosis have been characterized by the occurrence of disease outbreaks and mass mortalities following pathogen introduction into naïve populations [14–17]. With such epidemics, the pathogen

obviously threatens population survival. A less evident pattern of disease-induced population decline was predicted by Anderson [2]: if the probability of disease-induced mortality is sufficiently high when infection occurs, a parasite can regulate a host population even at extremely low prevalence. Empirical evidence supporting this important theoretical prediction, however, has remained largely elusive [4]. Our current study provides empirical support to this prediction. Our results suggest that *R. darwinii* populations are unlikely to persist where Bd infection is endemic, even in the absence of mass mortalities and where infection prevalence is low. At current fecundity, infection and survival probabilities, our matrix population models predicted slow population decrease and eventual extirpation of Bd-positive populations in most of the simulations (figures 3 and 4).

Less obvious than epidemics and rapid local population extirpations, a slow population decline might go unnoticed in short-term studies or might be attributed to other causes (such as a change in climatic conditions) [49]. Low infection probability (i.e. parasite transmission) could be a characteristic of some terrestrial amphibian-Bd systems, preventing the occurrence of epidemic spread and mass mortalities, even if Bd-induced population declines are occurring. For instance, in this study we found a lower Bd infection probability in *R. darwinii* compared with that observed in other amphibian species [e.g. 18,21,40,50], even when corrected for an assumed underestimation due to our sampling design. This might have implications for current understanding of the biological and environmental factors that predict host susceptibility to Bd, such as the findings that amphibian species with an aquatic life-stage are more likely to suffer Bd-induced population declines than terrestrial species [7,10].

The low mean survival probability observed at Bd-positive populations suggests that Bd-induced mortality is not compensatory to other natural causes of mortality in our model species. Being aware that many extrinsic and intrinsic factors can drive amphibian

337 population dynamics, and that we have not quantified such effects, we propose lines of
338 evidence that support our conclusion that Bd infection is the main reason for the observed
339 difference in mean survival probability between Bd-positive and most Bd-negative
340 populations. First, we detected a strong negative effect of Bd infection on individual survival
341 (figure 2a); this effect being consistent across age classes, Bd-positive populations, and at
342 a wide range of p_I values. Second, even though climatic conditions in TAN2 and MER1-
343 MER2 populations differ considerably [36], mean survival estimates for frogs in each of
344 these Bd-negative populations were similar. Third, population size in fully terrestrial
345 amphibians shows a relatively low temporal variance, likely due to a relatively low
346 environmental stochasticity [51], adding support to our suggestion that it is unlikely that the
347 observed differences in survival probabilities were associated with environmental
348 differences across sites. Finally, our results are consistent with retrospective and cross-
349 sectional evidence suggesting chytridiomycosis as an explanation for the documented
350 recent extirpation of several northern populations of *R. darwinii*, particularly those within
351 protected areas where other recognized threats (habitat loss/degradation, pollution, over-
352 extraction) are unlikely to operate [31,32]. The lower mean survival probability estimated for
353 frogs in TAN1 compared to that observed in the other Bd-negative populations (figure 1a)
354 could be due to density-dependent mortality and/or dispersal. At the beginning of this study,
355 this population had the largest known population density of *R. darwinii*, which was around
356 10 times higher than the population density observed in most of the study populations
357 (electronic supplementary material, table S1). Our ongoing spatial capture-recapture work
358 suggests the presence of density-dependent mortality in this species [A. Valenzuela-
359 Sánchez, unpublished data]; long-term time-series data might be necessary to confirm this
360 hypothesis.

In other amphibian-Bd systems, the survival of Bd-infected individuals is also lower than the survival of uninfected frogs [19,40], including in a population of *Litoria pearsoniana* that had co-existed with the parasite for approximately 30 years [50]. In some cases, however, individuals from species that have been documented as being highly susceptible to Bd can develop a decreased susceptibility when their populations have been infected with the parasite for a few decades [18,52]. Although Bd was most likely introduced into Chile in the 1970s [32,38], our results suggest that *R. darwinii* individuals from all the studied Bd-positive populations are highly susceptibility to chytridiomycosis. The time of Bd introduction into these populations, however, is unknown and we cannot discard the possibility of a gradual, long-term decrease in host susceptibility in our study species.

When parasite-induced mortality occurs in a parasite-load-dependent fashion, parasite aggregation (i.e. a small proportion of hosts holding high parasite burdens while most hosts have low burdens) may allow populations of susceptible hosts to persist in the face of recurrent infection [2,21,26]. It is worth noting, however, that even under the presence of strong parasite aggregation, theory predicts that a parasite can regulate a host population if the turnover of heavily infected hosts occurs at high rates [2]. Our field data do not allow us to discern between individuals that are in an early infective stage from those holding long-lasting, low parasite burdens. This situation limits our capability to draw strong conclusions from the distribution of parasite burdens in the host population. Even though we were not able to incorporate parasite load as a covariate in our modelling (3-month infection probability and survival probability of infected frogs were very close to zero, therefore, the effect of any covariate cannot be modelled reasonably), our results suggest that, regardless of parasite burden, case mortality is extremely high in our model host-parasite system.

An additional mechanism that may allow host populations to persist with endemic parasitic infection, even though the parasite has detrimental effects on host survival, is

compensatory recruitment. This population response to disease has been observed in the badger-*Mycobacterium bovis* system [29] as well as in the amphibian-Bd system [24,25]. In *R. darwinii*, paternal care may inhibit the occurrence of compensatory recruitment, as offspring are carried internally by adult males until metamorphosis and, therefore, Bd-induced death in brooding males also leads to the death of developing offspring. The small egg clutch size in *R. darwinii* [37], along with the limited number of larvae that each male is able to brood, likely decrease further the species' ability to offset chytridiomycosis-induced mortality through compensatory recruitment. Additionally, our results indicate that over a half of *R. darwinii* juveniles in Bd-positive populations become infected and die from chytridiomycosis before reaching adulthood.

Parasite transmission is a central component of the host-parasite interaction [28]. The mode of Bd transmission in *R. darwinii* is unknown and should be studied in more detail, but, as this species lives in moist vegetation and substrate, indirect transmission is possible as well as transmission via direct contact with conspecifics and syntopic amphibian species [53,54]. We used a constant infection probability in our modelling and, therefore, other models of parasite transmission such as density-dependent or frequency-dependent were not considered here. This choice is supported by three lines of evidence. First, parasite transmission is low and *R. darwinii* individuals are highly sedentary and commonly live at low densities [37,38,A. Valenzuela-Sánchez, unpublished data], suggesting that intra-specific transmission is unlikely to be important for Bd transmission and persistence within local populations. Additionally, the low survival rate of infected *R. darwinii* individuals further decreases the chances of intra-specific transmission [1]. Second, we found a similar infection probability across the four Bd-positive populations, which have different population sizes and densities. Third, reservoir hosts may lead to constant infection probability. In our system, syntopic species are likely important for the introduction and maintenance of Bd

411 within *R. darwinii* populations. As with previous reports [32,33], we detected Bd only in
412 northern populations of *R. darwinii*. This is coincident with a spatial pattern of a markedly
413 higher Bd prevalence in sympatric anurans towards the northern distribution of the range of
414 *R. darwinii* [32]. For instance, *E. contulmoensis* could be acting as a Bd reservoir in our
415 study system. As for most anurans syntopic to *R. darwinii*, *E. contulmoensis* individuals
416 have a higher vagility than our model species and use both aquatic (where individuals can
417 easily come into contact with the infective stage of Bd) and terrestrial (where individuals
418 overlap spatially and temporally with *R. darwinii* individuals) environments. The role of
419 syntopic species in the transmission of Bd to *R. darwinii* requires further investigation, as
420 management interventions, such as exclosures, to limit inter-specific contact might be a
421 feasible (short-term) mitigation measure for the conservation of *R. darwinii*, especially as
422 discrete local populations of this species exist within small, manageable areas.

423

424

425 **5. Conclusion**

426 Epidemics and mass die-offs are tacitly or explicitly assumed as a pre-requisite for the
427 occurrence of disease-induced extirpation, even though theory predicts that a parasite with
428 extremely low prevalence can regulate host populations if case mortality is sufficiently high
429 [2]. We showed, with empirical evidence, that a cryptic pattern of disease-induced host
430 population decline is an alternative route to population extirpation. Our findings challenge
431 the way we conceive pathogen threats to host populations and show that disease should be
432 investigated as a cause of population regulation even in the absence of an overt increase in
433 mortality.

434

435 **Ethics.** This research project was approved by the Animal Welfare Committee at the
436 Universidad Andrés Bello, Chile (N°13/2015) and by the Zoological Society of London's
437 Ethics Committee (WLE709), and was conducted in accordance with Chilean law under
438 permits N°5666/2013, N°230/2015, and N°212/2016 of the Servicio Agrícola y Ganadero de
439 Chile, and N°026/2013 and N°11/2015 IX of the Corporación Nacional Forestal de Chile.

440 **Data accessibility.** Complete dataset supporting our results are available at:
441 https://doi.org/10.5281/zenodo.583629.

442 **Authors' contributions.** AV-S, AAC and CS-A conceived the study. AV-S, BRS, AAC and
443 CS-A formulated the ideas. AV-S, DU-R and FC performed fieldwork. AV-S and BRS
444 analysed samples and data. AV-S wrote the first draft of the manuscript, and all authors
445 contributed to revisions.

446 **Competing interests.** We declare we have no competing interests.

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References

1. Anderson RM. 1979 Parasite pathogenicity and the depression of host population equilibria. *Nature* **279**, 150–152. (doi:10.1038/279150a0)
2. Anderson RM. 1995 Evolutionary pressures in the spread and persistence of infectious agents in vertebrate populations. *Parasitology* **111**, S15–31. (doi:10.1017/S003118200007579X)
3. Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, Speare R. 1999 Emerging infectious diseases and amphibian population declines. *Emerg. Infect. Dis.* **5**, 735–748. (doi:10.3201/eid0506.990601)
4. Tompkins DM, Dobson AP, Arneberg P, Begon ME, Cattadori IM, Greenman JV, Heesterbeek JAP, Hudson PJ, Newborn D, Pugliese A et al. 2002 Parasites and host population dynamics. In *The Ecology of Wildlife Diseases* (eds. Hudson PJ, Rizzoli A, Grenfell BT, Heesterbeek H, Dobson AP), pp. 45–62. Oxford, UK: Oxford University Press.
5. De Castro F, Bolker B. 2005 Mechanisms of disease-induced extinction. *Ecol. Lett.* **8**, 117–126. (doi:10.1111/j.1461-0248.2004.00693.x)
6. Longcore JE, Pessier AP, Nichols DK. 1999 *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* **91**, 219–227. (doi:10.2307/3761366)
7. Berger L, Roberts AA, Voyles J, Longcore JE, Murray KA, Skerratt LF. 2016 History and recent progress on chytridiomycosis in amphibians. *Fungal Ecol.* **19**, 89–99. (doi: 10.1016/j.funeco.2015.09.007)
8. Gascon C, Collins JP, Moore RD, Church DR, McKay JE, and Mendelson III JR. 2007 *Amphibian conservation action plan*. Gland, Switzerland and Cambridge, UK: IUCN/SSC Amphibian Specialist Group.

9. Catenazzi A. 2015 State of the World's Amphibians. *Annu. Rev. Env. Resour.* **40**, 3.1–3.29. (10.1146/annurev-environ-102014-021358)
10. Bielby J, Cooper N, Cunningham AA, Garner TWJ, Purvis A. 2008 Predicting susceptibility to future declines in the world's frogs. *Conserv. Lett.* **1**, 82–90. (doi:10.1111/j.1755-263X.2008.00015.x)
11. Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GG, Butchkoski CM, Kunz TH. 2010 An emerging disease causes regional population collapse of a common North American bat species. *Science* **329**, 679–682. (doi:10.1126/science.1188594)
12. Di Guardo G, Marruchella G, Agrimi U, Kennedy S. 2005 Morbillivirus infections in aquatic mammals: a brief overview. *J. Vet. Med. A* **52**, 88–93. (doi: 10.1111/j.1439-0442.2005.00693.x)
13. Walsh PD, Abernethy KA, Bermejo M, Beyersk R, De Wachter P, Akou ME, Huijbregts B, Mambounga DI, Toham AK, Kilbourn AM, *et al*. 2003 Catastrophic ape decline in western equatorial Africa. *Nature* **422**, 611–614. (doi:10.1038/nature01566)
14. Hudson MA, Young RP, D'Urban Jackson J, Orozco-terWengel P, Martin L, James A, Sulton M, Garcia G, Griffiths RA, Thomas R, Magin C, Bruford MW, Cunningham AA. 2016 Dynamics and genetics of a disease-driven species decline to near extinction: lessons for conservation. *Sci. Rep.* **6**, 30772. (doi:10.1038/srep30772)
15. Gillespie GL, Hunter D, Berger L, Marantelli G. 2014 Rapid decline and extinction of a montane frog population in southern Australia follows detection of the amphibian pathogen Batrachochytrium dendrobatidis. *Anim. Conserv.* **18**, 295–302. (doi:10.1111/acv.12174)
16. Cheng TL, Rovito SM, Wake DB, Vredenburg VT. 2011 Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen

Batrachochytrium dendrobatidis. *Proc. Natl. Acad. Sci. USA* **108**, 9502–9507. (doi:10.1073/pnas.1105538108)

17. Vredenburg VT, Knapp RA, Tunstall TS, Briggs CJ. 2010 Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proc. Natl. Acad. Sci. USA* **107**, 9689–9694. (doi:10.1073/pnas.0914111107)
18. Sapsford SJ, Voordouw MJ, Alford RA, Schwarzkopf L. 2015 Infection dynamics in frog populations with different histories of decline caused by a deadly disease. *Oecologia* **179**, 1099–1110. (doi:10.1007/s00442-015-3422-3)
19. Phillott AD, Grogan LF, Cashins SD, McDonald KR, Berger L, Skerratt LF. 2013 Chytridiomycosis and seasonal mortality of tropical stream-associated frogs 15 years after introduction of *Batrachochytrium dendrobatidis*. *Conserv. Biol. 27*, 1058–1068. (doi: 10.1111/cobi.12073)
20. Tobler U, Borgula A, Schmidt BR. 2012 Populations of a susceptible amphibian species can grow despite the presence of a pathogenic chytrid fungus. *PLoS ONE* **7**, e34667. (doi:10.1371/journal.pone.0034667)
21. Briggs CJ, Knapp RA, Vredenburg VT. 2010 Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proc. Natl. Acad. Sci. USA* **107**, 9695–9700. (doi:10.1073/pnas.0912886107)
22. Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ. 2005 investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. *Ecology* **86**, 3149–3159. (doi:10.1890/04-1428)
23. Retallick RWR, McCallum H, Speare R. 2004 Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *PLoS Biol.* **2**, e351. (doi: 10.1371/journal.pbio.0020351)

534 24. Scheele BC, Hunter D, Skerratt LF, Brannelly L, Driscoll DA. 2015 Low impact of
535 chytridiomycosis on frog recruitment enables persistence in refuges despite high
536 adult mortality. *Biol. Conserv.* **182**, 36–43. (doi:10.1016/j.biocon.2014.11.032)
537 25. Muths E, Scherer RD, Pilliod DS. 2011 Compensatory effects of recruitment and
538 survival when amphibian populations are perturbed by disease*. J. Appl. Ecol.* **48**,
539 873–879. (doi:10.1111/j.1365-2664.2011.02005.x)
540 26. Grogan LF, Phillott AD, Scheele BC, Berger L, Cashins SD, Bell SC, Puschendorf
541 R6, Skerratt LF. 2016. Endemicity of chytridiomycosis features pathogen
542 overdispersion. *J. Anim. Ecol.* **85**, 806–816. (doi: 10.1111/1365-2656.12500)
543 27. Anderson RM, May RM. 1978. Regulation and stability of host-parasite population
544 interactions: I. Regulatory processes. *J. Anim. Ecol.* **47**, 219–247.
545 28. Lloyd-Smith JO, Cross PC, Briggs CJ, Daugherty M, Getz WM, Latto J, Sanchez
546 MS, Smith AB, Swei A. 2005. Should we expect population thresholds for wildlife
547 disease? *Trends Ecol. Evol.* **20**, 511–519. (doi:10.1016/j.tree.2005.07.004)
548 29. McDonald JL, Bailey T, Delahay RJ, McDonald RA, Smith GC, Hodgson DJ. 2016.
549 Demographic buffering and compensatory recruitment promotes the persistence of
550 disease in a wildlife population. *Ecol. Lett.* **19**, 443–449. (doi:10.1111/ele.12578)
551 30. Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging Infectious Diseases of
552 Wildlife—Threats to Biodiversity and Human Health. *Science* **287**, 443–449. (doi:
553 10.1126/science.287.5452.443)
554 31. Soto-Azat C, Valenzuela-Sánchez A, Collen B, Rowcliffe MC Veloso A,
555 Cunningham AA. 2013 The population decline and extinction of Darwin's frogs.
556 *PLoS ONE* **8**, e66957. (doi:10.1371/journal.pone.0066957)
557 32. Soto-Azat C, Valenzuela-Sánchez A, Clarke BT, Busse K, Ortiz JC, Barrientos C,
558 Cunningham AA. 2013 Is chytridiomycosis driving Darwin's Frogs to extinction?
559 *PloS ONE* **8**, e79862. (doi:10.1371/journal.pone.0079862)

33. Bourke J, Mutschmann F, Ohst T, Ulmer P, Gutsche A, Busse K, Werning H, Boehme W. 2010 *Batrachochytrium dendrobatidis* in Darwin's frog *Rhinoderma* spp. in Chile. *Dis. Aquat. Organ.* **92**, 217–221. (doi:10.3354/dao02239)
34. Cooch EG, Conn PB, Ellner SP, Dobson AP, Pollock KH. 2012 Disease dynamics in wild populations: modelling and estimation: a review. *J. Ornithol.* **152**, S485–S509. (doi:10.1007/s10336-010-0636-3)
35. Oli MK, Venkataraman M, Klein PA, Wendland LD, Brown MB. 2006 Population dynamics of infectious diseases: a discrete time model. *Ecol. Model.* **198**, 183–194. (doi: 10.1016/j.ecolmodel.2006.04.007)
36. Valenzuela-Sánchez A, Cunningham AA, Soto-Azat C. 2015 Geographic body size variation in ectotherms: effects of seasonality on an anuran from the southern temperate forest. *Front. Zool.* **12**, 37. (doi:10.1186/s12983-015-0132-y)
37. Valenzuela-Sánchez A, Harding G, Cunningham AA, Chirgwin C, Soto-Azat C. 2014 Home range and social analyses in a mouth brooding frog: testing the co-existence of paternal care and male territoriality. *J. Zool.* **294**, 215–223. (doi:10.1111/jzo.12165)
38. Valenzuela-Sánchez A. 2017 Is chytridiomycosis a threat to the endangered mouth-brooding Darwin's frog (*Rhinoderma darwinii*)? A multi-approach disease risk assessment. PHD Thesis, Faculty of Ecology and Natural Resources, Universidad Andrés Bello.
39. Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyat AD. 2004 Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Dis. Aquat. Org.* **60**, 141–148. (doi:10.3354/dao060141)
40. Hudson MA, Young RP, Lopez J, Martin L, Fenton C, McCrea R, Griffiths RA, Adams S, Gray G, Garcia G, Cunningham AA. 2016 In-situ itraconazole treatment

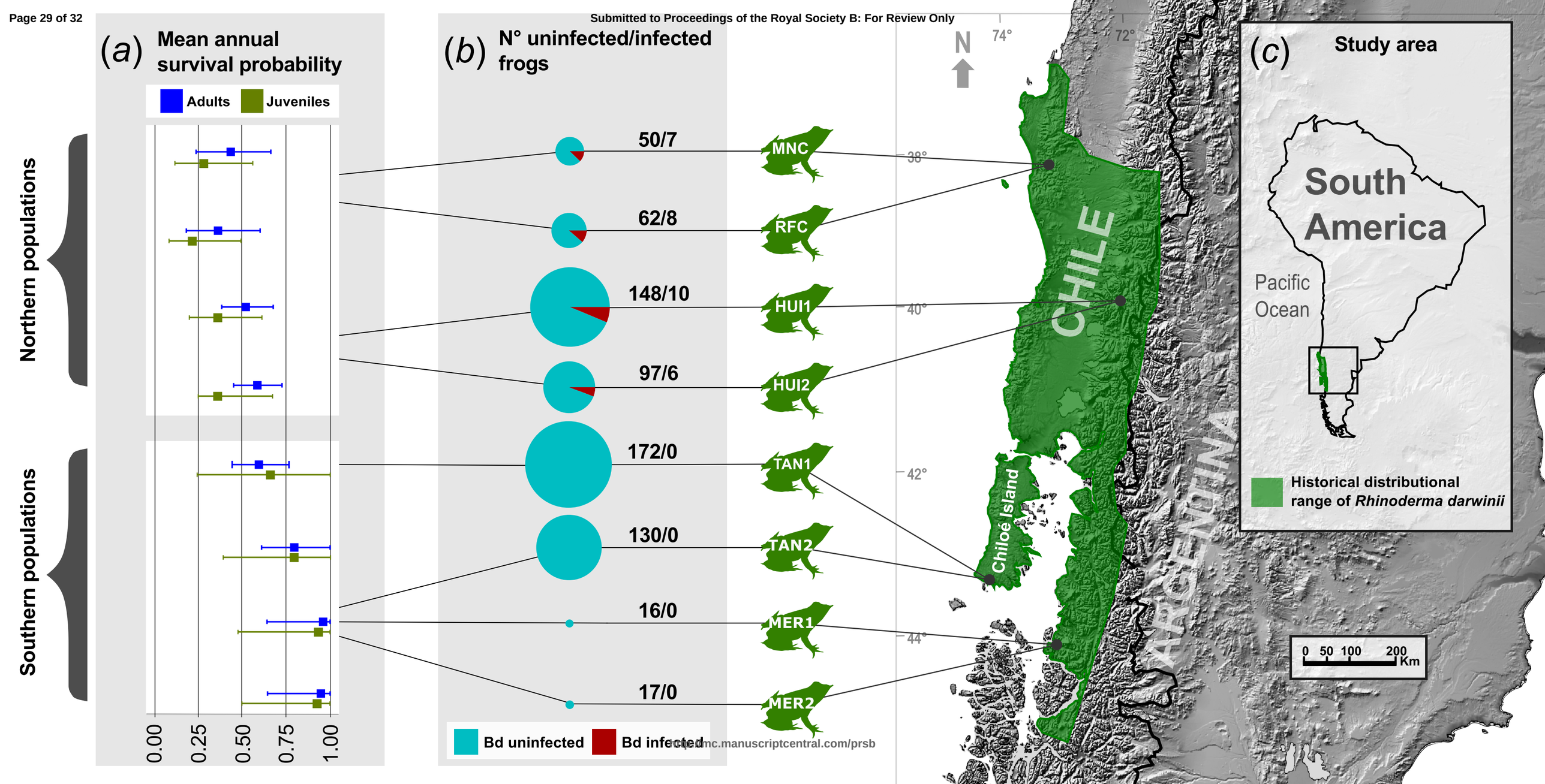
improves survival rate during an amphibian chytridiomycosis epidemic. *Biol. Conserv.* **195**, 37–45. (doi:10.1016/j.biocon.2015.12.041)

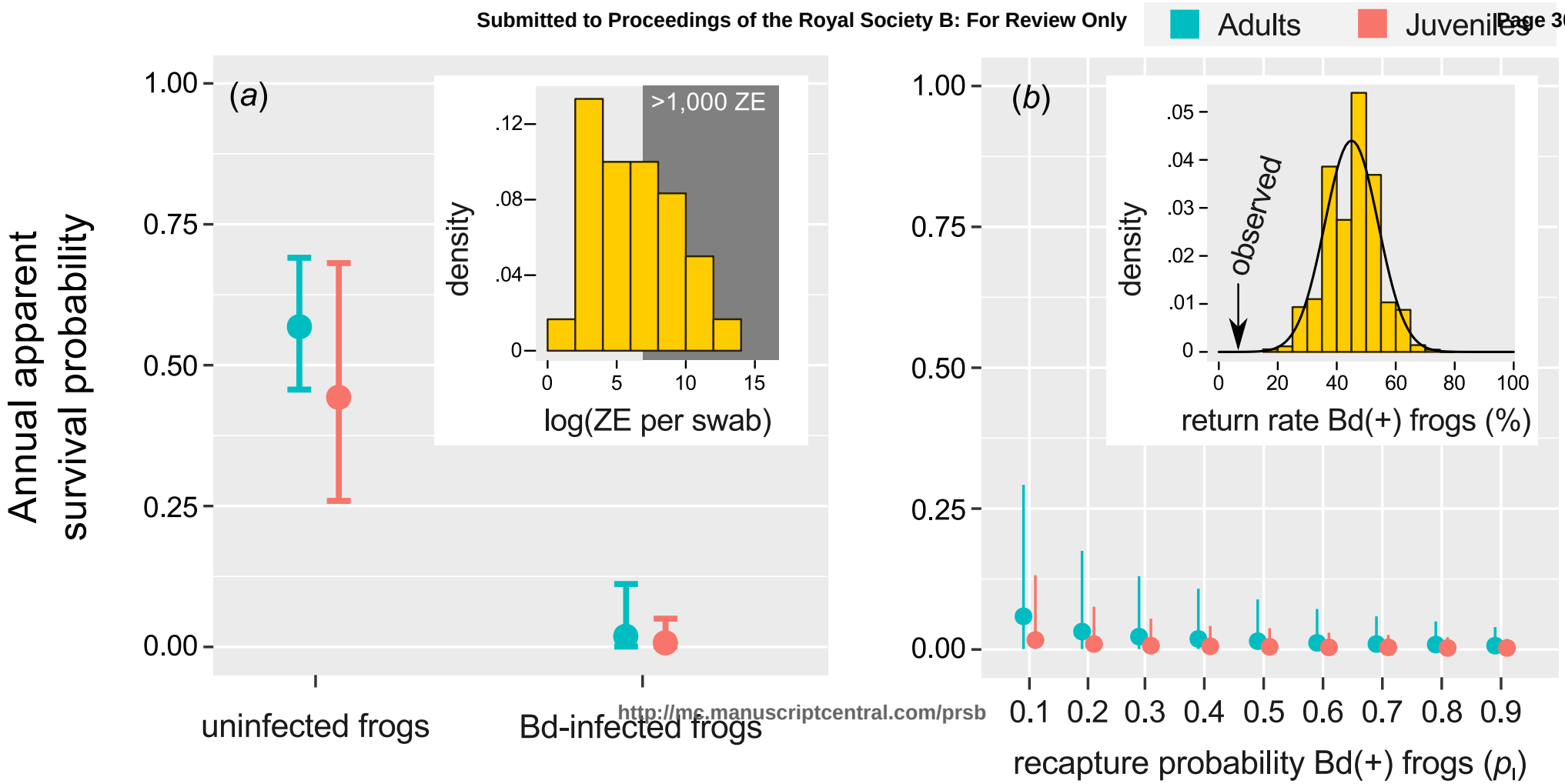
41. Kéry M, Schaub M. 2012 *Bayesian population analysis using WinBUGS. A hierarchical perspective*. Waltham, USA: Academic Press.
42. Lebreton JD, Nichols JD, Barker RJ, Pradel R, Spendelow JA. 2009 Modelling individual animal histories with multistate capture–recapture models. In *Advances in Ecological Research vol. 41* (ed. H Caswell), pp. 87–173. Burlington, USA: Burlington Academic Press.
43. Stegen G, Pasmans F, Schmidt BR, Rouffaer LO, Van Praet S, Schaub M, Canessa S, Laudelout A, Kinet T, Adriaensen C, Haesebrouck F, Bert W, Bossuyt F, Martel A. 2017. Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. *Nature* **544**, 353–356. (doi:10.1038/nature22059)
44. Kellner K. 2015 jagsUI: A Wrapper Around 'rjags' to Streamline 'JAGS' Analyses. R package version 1.3.7. (http://CRAN.R-project.org/package=jagsUI)
45. R Core Team. 2014 *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.
46. Plummer M. 2003 JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling. In *Proceedings of the 3rd International Workshop on Distributed Statistical Computing* (eds. Hornik K, Leisch F and Zeileis A), pp. 1–10. Vienna, Austria.
47. Caswell H. 2001 *Matrix population models: construction, analysis, and interpretation.* Sunderland, USA: Sinauer Associates.
48. Stevens MHH. 2009 *A primer of ecology with R*. New York, USA: Springer.
49. Hefley TJ, Hooten MB, Drake JM, Russell RE, DP Walsh. 2016 When can the cause of a population decline be determined? *Ecol. Lett.* **19**, 1353–1362. (doi: 10.1111/ele.12671)

612 50. Murray KA, Skerratt LF, Speare R, McCallum H. 2009 Impact and dynamics of
613 disease in species threatened by the amphibian chytrid fungus, *Batrachochytrium*
614 *dendrobatidis*. *Conserv. Biol.* **23**, 1242–1252. (doi:10.1111/j.1523-
615 1739.2009.01211.x)
616 51. Green DM .2003 The ecology of extinction: population fluctuation and decline in
617 amphibians. *Biol. Conserv.* **111**, 331–343. (doi:10.1016/S0006-3207(02)00302-6)
618 52. Knapp RL, Fellers GM, Kleeman PM, Miller DAW, Vredenburg VT, Rosenblum EB,
619 Briggs CJ. 2016 Large-scale recovery of an endangered amphibian despite ongoing
620 exposure to multiple stressors. *Proc. Natl. Acad. Sci. USA* Early edition. (doi:
621 10.1073/pnas.1600983113)
622 53. Kolby JE, Ramirez SD, Berger L, Griffin DW, Jocque M, Skerratt LF. 2015 Presence
623 of amphibian chytrid fungus (Batrachochytrium dendrobatidis) in rainwater suggests
624 aerial dispersal is posible. *Aerobiologia* **31**, 411–419. (doi:10.1007/s10453-015-
625 9374-6)
626 54. Kolby JE, Ramirez SD, Berger L, Richards-Hrdlicka KL, Jocque M, Skerratt LF.
627 2015 Terrestrial dispersal and potential environmental transmission of the
628 amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). *PLoS ONE* **10**,
629 e0125386. (doi:10.1371/journal.pone.0125386)
630

631 **Figure 1.** (a) Annual apparent survival probability from a Cormack-Jolly-Seber model
632 applied to capture-recapture data from eight wild population (c) of *Rhinoderma darwinii*
633 located in Chile. In (b) the proportion of frogs uninfected and infected with the fungus
634 *Batrachochytrium dendrobatidis* is shown. The size of the chart is proportional to the
635 number of frogs captured in each population. Error bars in (a) represent the 95% credible
636 interval.
637
638 **Figure 2.** (a) Annual apparent survival probability of uninfected and Bd-infected frogs from
639 four wild populations of *Rhinoderma darwinii* and infection intensity (zoospores equivalents
640 per swab) of infected frogs (inset). In (b) we show the survival probability of Bd-infected
641 frogs at different recapture probabilities and the distribution of the observed and simulated
642 return rate of the 30 Bd-infected frogs (inset). Error bars represent the 95% credible interval
643 of the posterior distribution of the parameter estimated using Markov chain Monte Carlo in a
644 multi-state capture-recapture model.
645
646 **Figure 3.** Distribution of the finite rate of increase (λ) for 10,000 simulations of three matrix
647 population models of *Rhinoderma darwinii*. The Bd-positive population model (a) uses
648 parameters estimates obtained from a multi-state capture-recapture model of four wild
649 populations, while the Bd-negative models use parameters estimates obtained from
650 Cormack-Jolly-Seber models of another four (b) or three (c) Bd-negative wild populations.
651 The black lines represent the mean. The darker grey area represents simulation with
652 population decline (i.e. λ <1).
653

654 **Figure 4.** Predicted variation in the size (median from 10,000 simulations) of a *Rhinoderma*
655 *darwinii* population using matrix population models. The projections are shown for Bd-
656 positive and Bd-negative populations. The dashed black line represents the year of the
657 introduction of Bd (only for the Bd-positive population model). It is worthwhile noting that if
658 Bd is not introduced to the Bd-positive population (i.e. ψ_{UI} = 0; dashed red line), this
659 population is still predicted to decrease, but a much smaller rate (mean λ = 0.93, 95% CI =
660 0.61 – 1.36).


(a) Mean annual survival probability
Adults
Juveniles
Northern populations
Southern populations
0.00
0.25
0.50
0.75
1.00
(b) N° uninfected/infected frogs
50/7
62/8
148/10
97/6
172/0
130/0
16/0
17/0
MNC
RFC
HUI1
HUI2
TAN1
TAN2
MER1
MER2
Bd uninfected
Bd infected
N
74°
72°
38°
40°
42°
44°
CHILE
ARGENTINA
Chiloé Island
(c) Study area
South America
Pacific Ocean
Historical distributional range of Rhinoderma darwinii
0 50 100 200 Km



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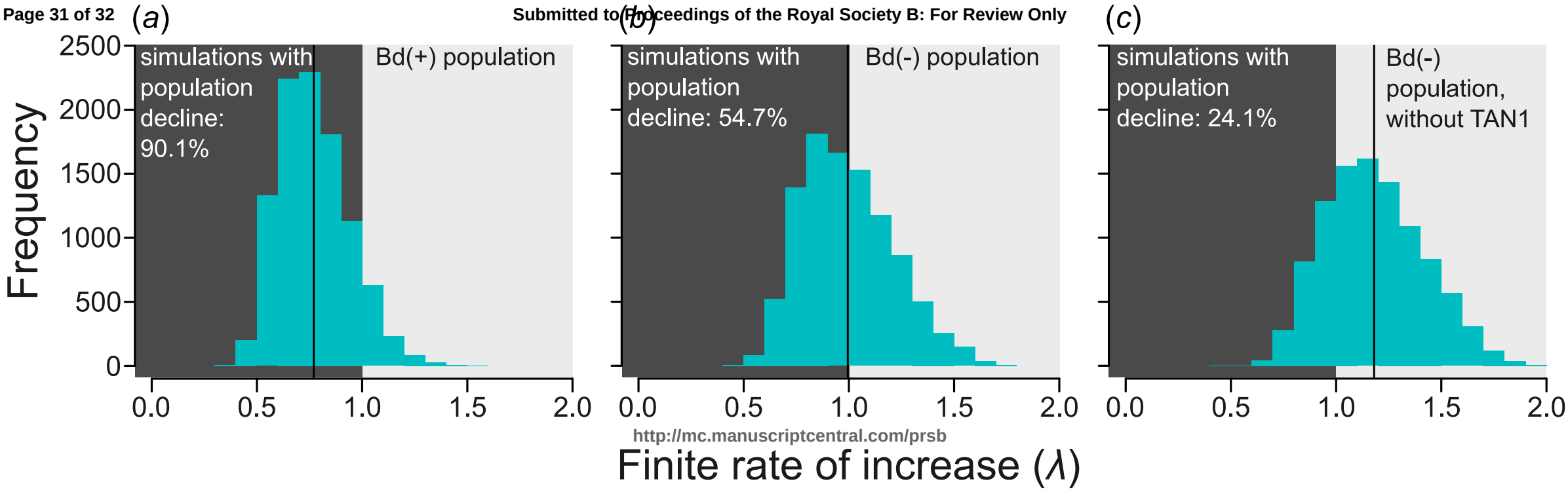

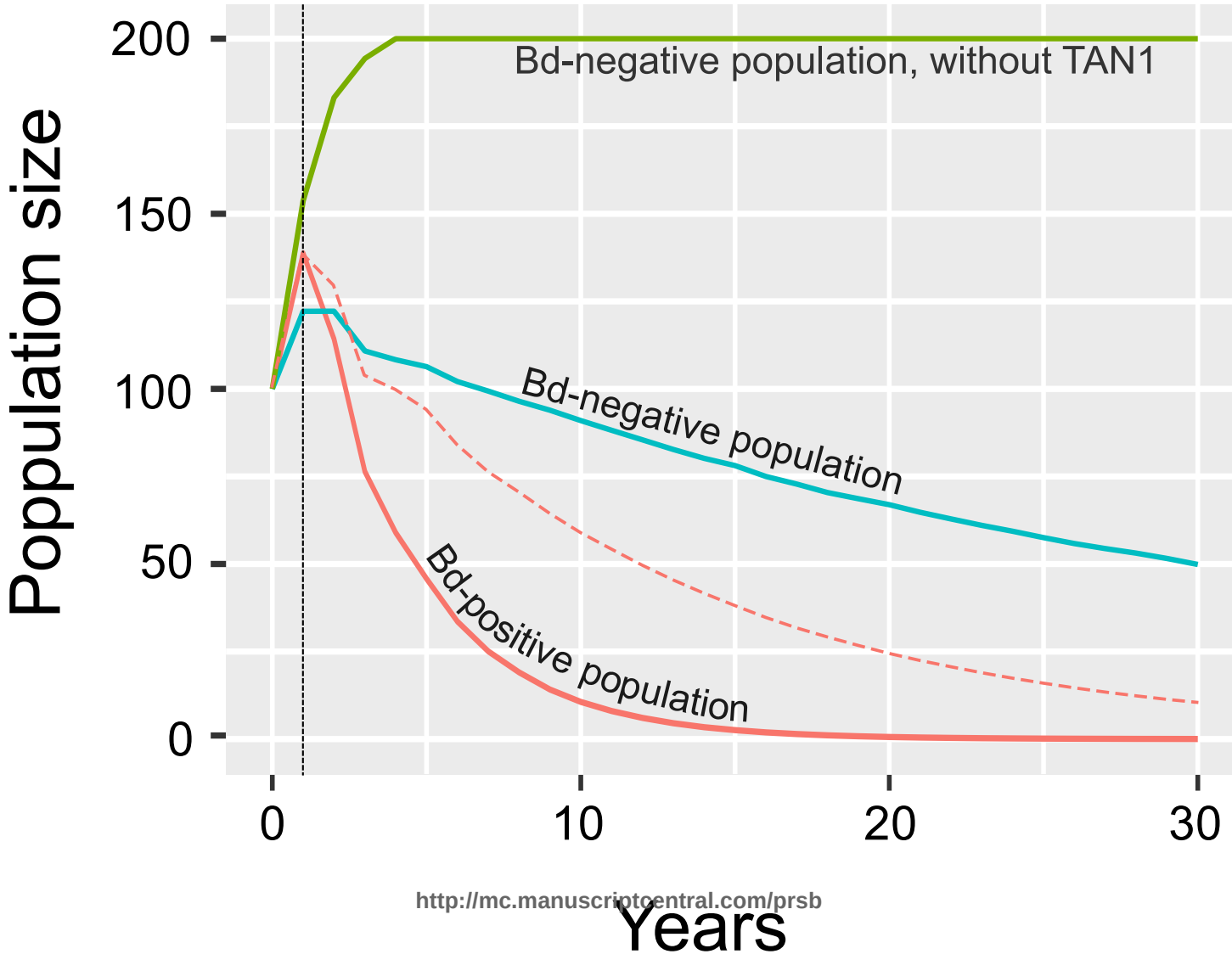
Population size
200
150
100
50
0
0
10
20
30
Years
Bd-negative population, without TAN1
Bd-negative population
Bd-positive population