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Authors for correspondence:
Andrés Valenzuela-Sánchez
e-mail: andresvalenzuela.zoo@gmail.com
Claudio Soto-Azat
e-mail: csoto@unab.cl

†These authors contributed equally to this study.

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

Andrés Valenzuela-Sánchez[1,2,3], Benedikt R. Schmidt[4,5], David E. Uribe-Rivera[2], Francisco Costas[2], Andrew A. Cunningham[3,†] and Claudio Soto-Azat[1,†]

[1]Centro de Investigación para la Sustentabilidad, Facultad de Ecología y Recursos Naturales, Universidad Andres Bello, República 440, Santiago, Chile
[2]ONG Ranita de Darwin, Nataniel Cox 152, Santiago, Chile
[3]Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK
[4]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[5]Info Fauna KARCH, Passage Maximilien-de-Meuron 6, 2000 Neuchâtel, Switzerland

AV-S, 0000-0002-0445-9156; BRS, 0000-0002-4023-1001; AAC, 0000-0002-3543-6504

The decline of wildlife populations due to emerging infectious disease often shows a common pattern: the parasite invades a naive 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.

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 naive 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 naive 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

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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: (i) Bd infection can produce mortality in *R. darwinii* individuals [32]; (ii) 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 (iii) prior to the beginning of this study, neither epidemics nor mass die-offs have been observed in Bd-positive *R. darwinii* populations over 5 years of monitoring [32,33] (A. Valenzuela-Sánchez 2016, 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 *R. darwinii* populations are probably 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): (i) Nahuelbuta range (Monumento Natural Contulmo, 'MNC' and Reserva Forestal Contulmo, 'RFC'); (ii) the Andes (Reserva Biológica Huilo Huilo, 'HUI1' and 'HUI2'); (iii) Chiloé Island (Parque Tantauco, 'TAN1' and 'TAN2'); and (iv) 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* (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 (electronic supplementary material, 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 (electronic supplementary material, 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 and TAN2) and adults (SVL >24 mm, but SVL >19.5 for TAN1 and TAN2). The smaller adult size used for frogs on Chiloé Island follows observations that these animals are smaller and reach sexual maturity at approximately 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*

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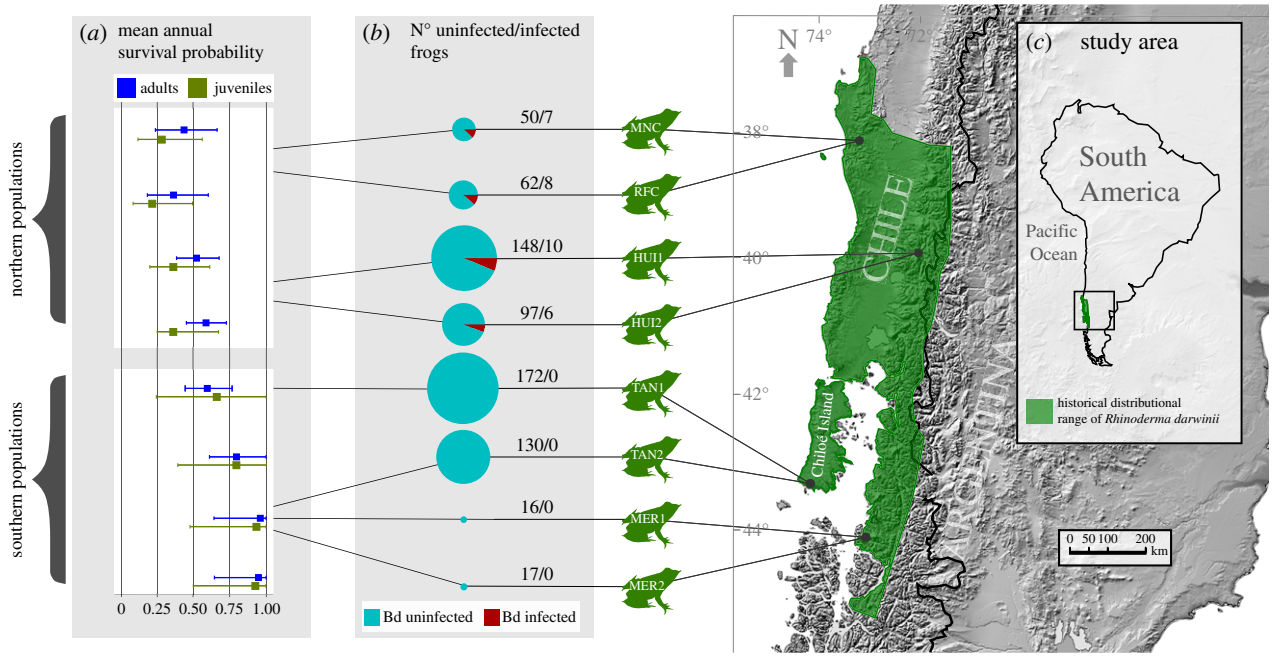

Figure 1. (*a*) Annual apparent survival probability from a Cormack–Jolly–Seber model applied to capture–recapture data from eight wild populations (*c*) of *Rhinoderma darwinii* located in Chile. In (*b*) the proportion of frogs uninfected and infected with the fungus *Batrachochytrium dendrobatidis* is shown. The size of the chart is proportional to the number of frogs captured in each population. Error bars in (*a*) represent the 95% credible interval. (Online version in colour.)

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

(e) Matrix population models

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

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

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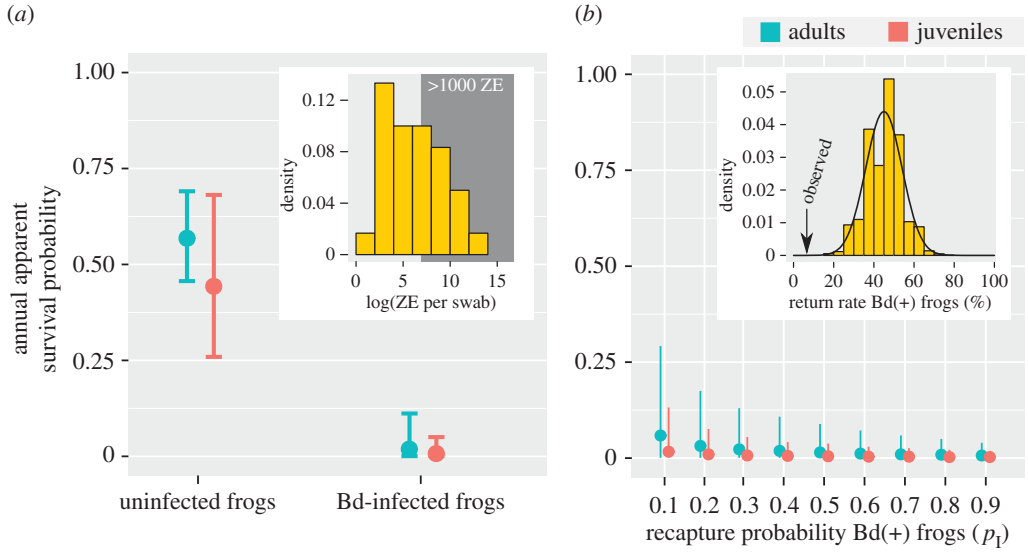

Figure 2. (*a*) Annual apparent survival probability of uninfected and Bd-infected frogs from four wild populations of *Rhinoderma darwinii* and infection intensity (zoospores equivalents per swab) of infected frogs (inset). In (*b*) we show the survival probability of Bd-infected frogs at different recapture probabilities and the distribution of the observed and simulated return rate of the 30 Bd-infected frogs (inset). Error bars represent the 95% credible interval of the posterior distribution of the parameter estimated using Markov chain Monte Carlo in a multi-state capture–recapture model. (Online version in colour.)

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

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

To include uncertainty of our parameter estimates in model outputs, we ran 10 000 simulations of each matrix population model. In each simulation the parameter values were randomly sampled from different probabilistic functions fitted with parameter values 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 1182 captures of 723 different frogs (figure 1*b*). 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, nor 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 1*b*). 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 2*b*). 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 = 2810–38 719; median = 365, bootstrapped 95% CI = 71–1380). Of infected frogs, 36.7% had more than 1000 ZE per swab at least once. The distribution of log-transformed ZE per swab was bell-shaped (figure 2*a*), 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 (electronic supplementary material, 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%

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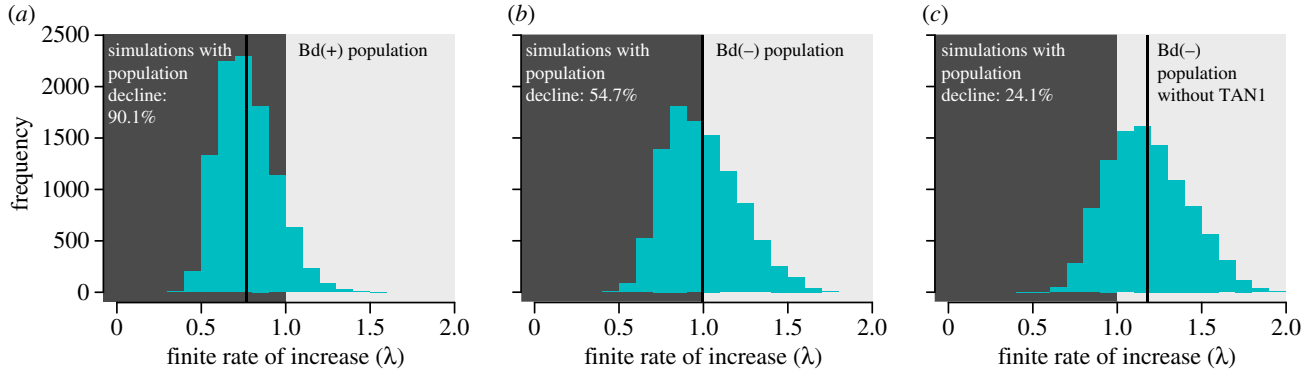

Figure 3. Distribution of the finite rate of increase (λ) for 10 000 simulations of three matrix population models of *Rhinoderma darwinii*. The Bd-positive population model (*a*) uses parameters estimates obtained from a multi-state capture–recapture model of four wild populations, while the Bd-negative models use parameters estimates obtained from Cormack–Jolly–Seber models of another four (*b*) or three (*c*) Bd-negative wild populations. The black lines represent the mean. The darker grey area represents simulation with population decline (i.e. $\lambda < 1$). (Online version in colour.)

CI = 43–624; median = 71 ZE, bootstrapped 95% CI = 21–769; electronic supplementary material, 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 to −0.173), although it was similar between the Bd-positive populations and TAN1 (figure 1*a*; electronic supplementary material, table S4). The same pattern was observed for juveniles (mean difference = −0.397, 95% CRI = −0.560 to −0.196), but with considerably larger credible intervals for ϕ estimates from Bd-negative populations (figure 1*a*).

The MSMR models showed that ϕ was considerably lower in infected frogs in comparison with uninfected frogs (figure 2*a*). This difference was consistent across all study populations (electronic supplementary material, figure S3*a*) and between age classes (figure 2*a*). Infected adults and juveniles were 55.0% and 43.5% less likely to survive 1 year than uninfected adults and juveniles, respectively (figure 2*a*). The difference between ϕ_U and ϕ_I was consistent across all the tested p_I values (figure 2*b*).

Annual ψ_{UI} was higher in juveniles than in adults (26.8% [95% CRI = 9.1–52.5%] versus 14.7% [95% CRI = 7.2–25.2%]), and was similar across all Bd-positive populations (electronic supplementary material, figure S3*b*).

(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).

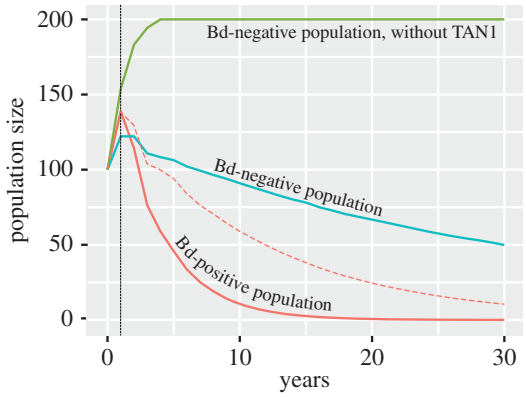

Figure 4. Predicted variation in the size (median from 10 000 simulations) of a *Rhinoderma darwinii* population using matrix population models. The projections are shown for Bd-positive and Bd-negative populations. The dashed black line represents the year of the introduction of Bd (only for the Bd-positive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

Model 4 indicated that a onefold 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 naive 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

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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 correcting 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 population dynamics, and that we have not quantified such effects, we propose lines of evidence that support our conclusion that Bd infection is the main reason for the observed difference in mean survival probability between Bd-positive and most Bd-negative populations. First, we detected a strong negative effect of Bd infection on individual survival (figure 2*a*), this effect being consistent across age classes and Bd-positive populations, and at a wide range of p_I values. Second, even though climatic conditions in TAN2, MER1 and MER2 populations differ considerably [36], mean survival estimates for frogs in each of these Bd-negative populations were similar. Third, population size in fully terrestrial amphibians shows a relatively low temporal variance, probably due to a relatively low environmental stochasticity [51], adding support to our suggestion that it is unlikely that the observed differences in survival probabilities were associated with environmental differences across sites. Finally, our results are consistent with retrospective and cross-sectional evidence suggesting chytridiomycosis as an explanation for the documented recent extirpation of several northern populations of *R. darwinii*, particularly those within protected areas where other recognized threats (habitat loss/degradation, pollution, over-extraction) are unlikely to operate [31,32]. The lower mean survival probability estimated for frogs in TAN1 compared to that observed in the other Bd-negative populations (figure 1*a*) could be due to density-dependent mortality and/or dispersal. At the beginning of this study, this population had the largest known population density of *R. darwinii*, which was around 10 times higher than the population density observed in most of the study populations (electronic supplementary material, table S1). Our ongoing spatial capture–recapture work suggests the presence of density-dependent mortality in this species (A. Valenzuela-Sánchez 2016, unpublished data); long-term time-series data might be necessary to confirm this 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 coexisted 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 probably 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 (three-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, probably decrease further the species's 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

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

5. Conclusion

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

Ethics. This research project was approved by the Animal Welfare Committee at the Universidad Andrés Bello, Chile (no. 13/2015) and by the Zoological Society of London's Ethics Committee (WLE709), and was conducted in accordance with Chilean law under permits no. 5666/2013, no. 230/2015 and no. 212/2016 of the Servicio Agrícola y Ganadero de Chile, and no. 026/2013 and no. 11/2015 IX of the Corporación Nacional Forestal de Chile.

Data accessibility. Complete dataset supporting our results is available at https://doi.org/10.5281/zenodo.583629.

Authors' contributions. A.V.-S., A.A.C. and C.S.-A. conceived the study. A.V.-S., B.R.S., A.A.C. and C.S.-A. formulated the ideas. A.V.-S., D.E.U.-R. and F.C. performed fieldwork. A.V.-S. and B.R.S. analysed samples and data. A.V.-S. wrote the first draft of the manuscript, and all authors contributed to revisions.

Competing interests. We declare we have no competing interests.

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