ISSN 2633-996X

BRITISH HERPETOLOGICAL SOCIETY REPORTS
No. 8, September 2026. Special Report.

From Population Collapse towards Recovery: Protecting Darwin's Frogs in Parque Tantauco

Andrés Valenzuela-Sánchez, Benjamin Tapley, Bastián Santana, Alan Bannister, Pablo Aguilar, Soledad Delgado, Jose Manuel Serrano, David Uribe-Rivera, Claudia Faure, Jaime Beltrand, Diego Peñaloza, Michael Meyerhoff, Claudio Azat, Daniel Kane, Chris Sergeant & Andrew A. Cunningham

Edited by Simon Townson

FRONT COVER: A free-living Darwin's frog *Rhinoderma darwinii* from Parque Tantauco, Chiloé Island, southern Chile (photo: Jaime Beltrand, NGO Ranita de Darwin archive).

Contents

From Population Collapse towards Recovery: Protecting Darwin's Frogs in Parque Tantauco

Andrés Valenzuela-Sánchez[1,2*], Benjamin Tapley[3*], Bastián Santana[2], Alan Bannister[4], Pablo Aguilar[4], Soledad Delgado[2], Jose Manuel Serrano[2], David Uribe-Rivera[2], Claudia Faure[2], Jaime Beltrand[2], Diego Peñaloza[2], Michael Meyerhoff[5], Claudio Azat[6], Daniel Kane[3], Chris Sergeant[1] & Andrew A. Cunningham[1]

[1]Institute of Zoology, Zoological Society of London, UK
[2]ONG Ranita de Darwin, Chile
[3]Zoological Society of London, UK
[4]Fundación Parque Tantauco, Chiloé, Chile
[5]Zoo Leipzig, Germany
[6]One Health Institute, Universidad Andrés Bello, Santiago, Chile

[*]Corresponding authors e-mails: andres.valenzuela-sanchez@ioz.ac.uk; ben.tapley@zsl.org

ABSTRACT – Darwin's frogs *Rhinoderma rufum* and *Rhinoderma darwinii* are Evolutionarily Distinct and Globally Endangered amphibians threatened by habitat loss and chytridiomycosis. Parque Tantauco, located in the Chiloé Island in southern Chile, historically represented a major stronghold for *R. darwinii*, harbouring the largest known populations and remaining free of *Batrachochytrium dendrobatidis* (*Bd*) until 2022. Following *Bd* invasion, we documented rapid and severe population collapse using long-term capture-recapture data and post-invasion occupancy surveys. We estimate that approximately 76% of historically occupied local populations in the Inio sector of the park have been extirpated and that at least 850–1,344 *R. darwinii* individuals were lost within a single year (2023–2024) in this area. In response, the multi-institutional Alianza Tantauco initiated emergency conservation actions, including the establishment of ex-situ survival-assurance colonies, enhanced biosecurity, field monitoring and in-situ disease-mitigation trials. Recognising the potential social and reputational challenges associated with our emergency ex-situ interventions, we implemented strategic communication and stakeholder-engagement activities to build public understanding, transparency and institutional support at local, national and international levels. Together, these efforts seek to reverse recent losses and restore Parque Tantauco as a long-term stronghold for *R. darwinii*, ensuring the persistence of abundant and stable populations across the park.

Figure 1. Unique paternal care in Darwin's frog *Rhinoderma darwinii*. **A.** Eggs on moss at a site in Parque Tantauco, southern Chiloé Island, Chile. **B.** A male has incorporated three of the larvae into the vocal sac and is preparing to incorporate the remaining individual. Hatching appears to occur during this process, as the male ruptures the egg capsule while incorporating the offspring. **C.** Males in some populations can rear more than ten larvae within the vocal sac, resulting in a markedly distended vocal sac.

Introduction

Darwin's frogs *Rhinoderma rufum* and *Rhinoderma darwinii* are unique amphibians due to their remarkable form of parental care, in which males brood and rear tadpoles within their vocal sac (Fig. 1). The southern Darwin's frog *R. darwinii* (Figs. 2–4) occurs in both Chile and Argentina, whereas the northern Darwin's frog, or "sapito vaquero" *R. rufum*, historically had a distribution restricted to Chile's Coastal Range (Soto-Azat et al., 2013a). The latter species, however, has not been observed since 1981 and is currently classified as Critically Endangered (Possibly Extinct), while *R. darwinii* is listed as Endangered (IUCN SSC ASG, 2018). Both species face major threats, including habitat loss and degradation, chytridiomycosis and climate change (IUCN SSC ASG, 2018). Darwin's frogs are considered Evolutionarily Distinct and Globally Endangered (EDGE) species, meaning that they have few close relatives and represent an irreplaceable component of the world's natural heritage. Consequently, they are a global priority for species conservation.

Chytridiomycosis, caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), is an infectious disease implicated in the declines of at least 500 amphibian species worldwide and is thought to have caused the extinction of at least 90 species (Scheele et al., 2019). *Bd* is a significant threat to Darwin's

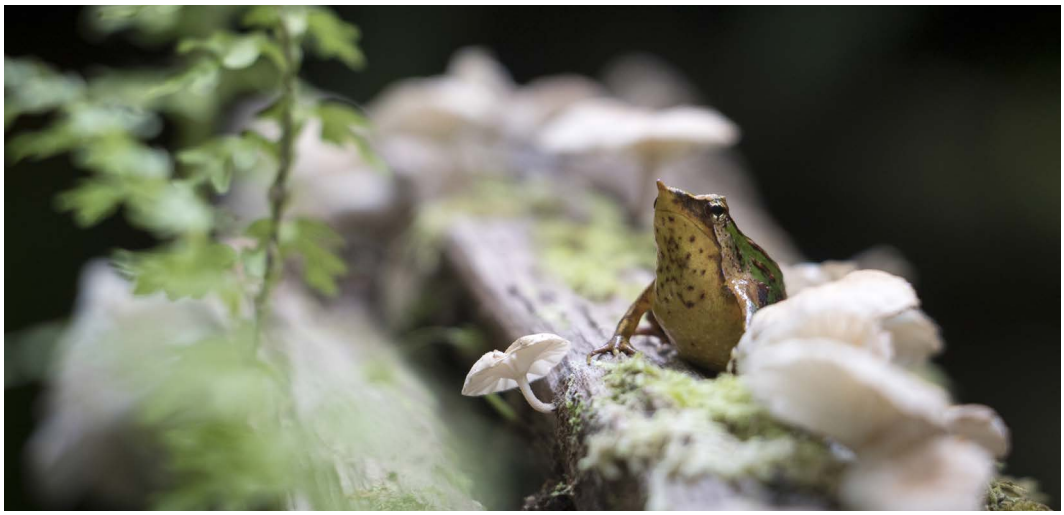

Figure 2. The southern Darwin's frog *Rhinoderma darwinii*. Photograph by Jaime Beltrand, NGO Ranita de Darwin archive.

Figure 3. A Darwin's frog *Rhinoderma darwinii* from Parque Tantauco, Chiloé Island, southern Chile. This individual is held at ZSL's London Zoo as part of a conservation breeding and research programme.

Figure 4. Diversity of dorsal colouration and patterning in Darwin's frogs *Rhinoderma darwinii*. These individuals were photographed as part of monitoring of a captive-assurance population from Parque Tantauco held at ZSL's London Zoo.

frogs. This pathogen is suspected to be linked to the disappearance of *R. rufum* and has also led to the local extinction of several *R. darwinii* populations (Soto-Azat et al., 2013b; Valenzuela-Sánchez et al., 2017; 2026). *Rhinoderma darwinii* individuals are highly susceptible to developing fatal chytridiomycosis, with disease-induced mortality rates estimated to exceed 85% (Valenzuela-Sánchez et al., 2026).

Natural history of the southern Darwin's frog

Rhinoderma darwinii is a small amphibian, with adults averaging approximately 24 mm snout-vent length and females generally being larger than males (Valenzuela-Sánchez et al., 2015). Dorsal colouration is highly variable, ranging from green to brown, and individuals can change colour over periods of weeks to months, helping them to camouflage themselves against forest substrates (Bourke et al., 2011).

The species is best known for its remarkable reproductive biology. During the breeding season, which generally extends from September to April depending on geographical location, males call actively during the day, producing the soft whistling advertisement calls characteristic of the species (Serrano et al., 2020). Amplexus generally appears to occur at the site from which the male had been calling, where the female deposits relatively large eggs, approximately 5 mm in size, in small refuges on the damp forest floor among leaf litter, mosses and ferns (Busse, 2004).

Embryonic development initially proceeds outside the male for approximately two weeks. Once the embryos begin muscular movement, the male takes them into his vocal sac and may actively assist hatching by breaking the egg membranes with his mouth (Cabeza-Alfaro et al., 2021). The larvae usually complete development within the vocal sac over six to eight weeks, although development can occasionally extend over several months. During this period, the male supplies nutrients and oxygen through the vocal-sac epithelium, and as many as 22 larvae may be brooded simultaneously (Wilhelm, 1927; Jorquera et al., 1972). Fully metamorphosed juveniles are ultimately released directly into the terrestrial environment, so *R. darwinii* does not require a water body to complete its life cycle.

Rhinoderma darwinii is a native-forest specialist, with a preference for old-growth and structurally complex native temperate rainforests (Valenzuela-Sánchez et al., 2019a). Within forests, individuals are not distributed uniformly but occur in spatially clustered local populations that generally occupy areas smaller than 0.5 ha (Crump, 2002; Soto-Azat et al., 2013a; Valenzuela-Sánchez et al., 2014;

2017; 2026). Contemporary local populations are generally small, with published abundance estimates ranging from 10 to 145 individuals (Soto-Azat et al., 2013a; Valenzuela-Sánchez et al., 2017; 2026).

Adults are highly sedentary and show strong site fidelity, but there is no evidence of territorial behaviour (Valenzuela-Sánchez et al., 2014). In southern Chiloé, the mean home range estimated over three months during the breeding season was only 1.82 m² (Valenzuela-Sánchez et al., 2014). In Andean populations, adults moved a mean of 3.64 m annually, whereas juveniles moved over longer distances and are likely to represent the life stage during which dispersal occurs most frequently (Valenzuela-Sánchez et al., 2019b). Model-based estimates suggested that 1% of juveniles might travel 146 m over one year (Valenzuela-Sánchez, 2017). Together, these characteristics result in strongly spatially structured populations with limited exchange among neighbouring local populations (Valenzuela-Sánchez et al., 2026).

The mean lifespan in the wild is around seven to eight years, although populations differ substantially in their life-history strategies: some have lower longevity but higher annual fecundity, whereas others contain longer-lived individuals with lower annual fecundity (Valenzuela-Sánchez et al., 2022; 2023). Long-term capture-recapture observations also suggest that some individuals may reach at least 15 years of age in the wild (A. Valenzuela-Sánchez, personal observation). In studied populations, population growth rate is particularly sensitive to adult survival (Valenzuela-Sánchez et al., 2017).

Individuals of the species are sit-and-wait predators, capturing prey that move within reach rather than actively searching for food (Crump, 2002). The diet is relatively generalist. Stable-isotope and stomach-content analyses indicate

Figure 5. Habitat of Darwin's frog *Rhinoderma darwinii* in Parque Tantauco, Chiloé Island, southern Chile. This fully terrestrial amphibian primarily occupies old-growth forests that maintain high humidity levels throughout the year.

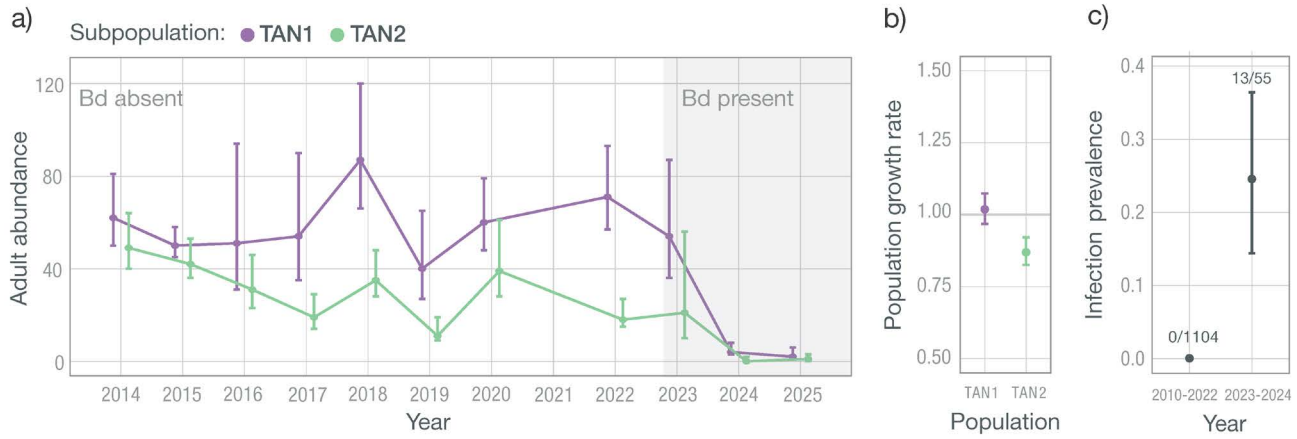

Figure 6. *Batrachochytrium dendrobatidis* (*Bd*) invasion and collapse of two *Rhinoderma darwinii* local populations in Parque Tantauco. **a)** Adult abundance estimated using a closed capture-recapture model and **b)** geometric mean population growth from 2014 to 2022 showing both populations were close to stability (i.e. growth rate close to 1) prior the first detection of *Bd*. **c)** Prevalence of *Bd* infection in *R. darwinii* grouped by the period before the first detection of *Bd* (i.e. 2010 to 2022) and when *Bd* was present (i.e. 2023 and 2024). In 2025 only one *R. darwinii* individual was found, captured at TAN1, suggesting TAN2 is extinct. Parameters in **a)** and **b)** were estimated using a Bayesian closed capture–recapture model fitted to data from 657 *R. darwinii* individuals (TAN1: 418; TAN2: 239). Parameters in **c)** were estimated using a Bayesian binomial model. The numbers in **c)** represent the number of *Bd*-positive individuals over the total number of individuals sampled. Points represent posterior means, and error bars indicate 95% Bayesian credible intervals. Adapted from Valenzuela-Sánchez et al. (2026).

that herbivorous invertebrates account for 68.1% of assimilated food, with mosquitoes, flies, grasshoppers, crickets and ants among the most commonly consumed prey; detritivorous and carnivorous invertebrates occur at lower proportions (Molina-Burgos et al., 2018).

Parque Tantauco: A stronghold for Darwin's frog conservation

Parque Tantauco, a 250,000-acre privately protected area in southern Chile's Chiloé Island, has been an important refuge for *R. darwinii* (Fig. 5); until 2022, *Bd* was inferred to be absent based on intensive monitoring of wild amphibians (>1,000 samples) (Fig. 6; Valenzuela-Sánchez et al., 2026), and species distribution models predicted low environmental suitability for the pathogen (Bacigalupe et al., 2019). In addition, this protected area – particularly its southernmost sector at the locality of Inio – harboured the largest known contemporary populations of *R. darwinii*, with densities approaching an order of magnitude higher than those observed in other well-monitored populations (Soto-Azat et al., 2013a; Valenzuela-Sánchez et al., 2022; 2026). This quantitative evidence from field monitoring is further supported by consistent anecdotal observations from tourists, scientists and park rangers, who frequently reported sightings of the species or hearing its characteristic whistling call in the Inio sector.

In 2023, we detected *Bd* infection in *R. darwinii* and syntopic amphibians for the first time in Parque Tantauco (Valenzuela-Sánchez et al., 2026). Between 2023 and 2024, the abundance of *R. darwinii* declined by 91.3% (Bayesian 95% Credible Interval [CRI]: 82.0–96.1%) and 98.0% (CRI: 84.6–100.0%), in two previously stable local populations that had been monitored using capture-recapture methods since 2014 (Fig. 6; Valenzuela-Sánchez et al., 2026). This abrupt decline was also independently perceived by park rangers in the Inio sector, who reported that they suddenly stopped hearing the frogs, coincident with the arrival of *Bd* in the area.

Extent and severity of population loss in *R. darwinii* at Parque Tantauco

Our team began monitoring *R. darwinii* in Parque Tantauco in 2010 (Soto-Azat et al., 2013a; Valenzuela-Sánchez et al., 2014). In 2014, we decided to focus our efforts exclusively on the Inio sector (Fig. 7), where the largest populations in the park had been detected and where capture-recapture surveys for long-term population monitoring were logistically feasible. These surveys required repeated visits to the same sites over multiple consecutive days (typically four days per site) to collect data for abundance estimation using closed-population models, which assume demographic closure (i.e. no births, deaths or dispersal) during the sampling

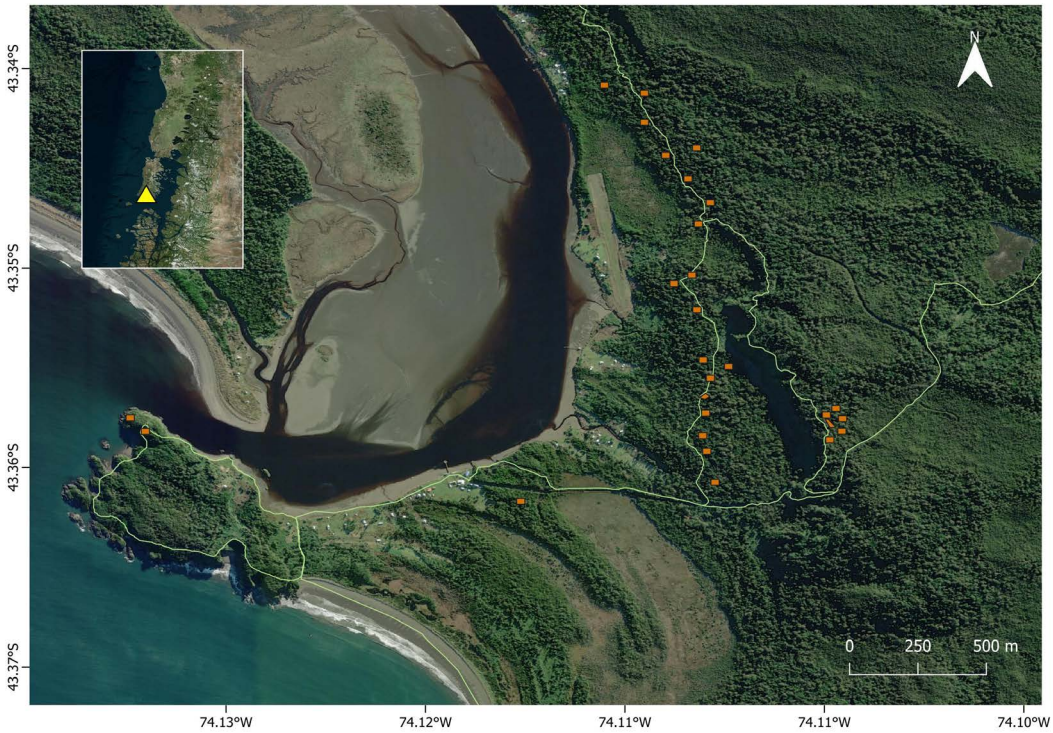

Figure 7. Study area in the Inio sector of Parque Tantauco, located in the Chiloé Island, southern Chile. The orange squares represent the 28 plots used in the occupancy survey, which began in 2024. Eight of these plots contained local *Rhinoderma darwinii* populations prior to the *Batrachochytrium dendrobatidis* invasion in 2023, including populations TAN1 and TAN2 shown in Figure 6. The green lines represent foot trails. The inset shows the location of the study area (yellow triangle) within the Chiloé Island.

period (Kéry & Schaub, 2012). This approach was necessary given the absence of roads in the park and the complex topography along the foot trails. Specifically, we focused our efforts on a study area of approximately 202 ha (500 acres) near to Caleta Inio (Fig. 7). Caleta Inio is a small artisanal harbour located at the mouth of the Inío River, with a permanent population of fewer than 100 human inhabitants.

In this study area, we have evidence for the presence of at least 14 local populations of *R. darwinii* since 2011. Here, we define a local population (or subpopulation) as a demographic unit connected to other local populations by annual dispersal rates of less than 10%. Given the extremely limited vagility of *R. darwinii* – for example, a median annual displacement of 3.64 m in adults in a studied population in the Andean region of Chile (Valenzuela-Sánchez et al., 2019) – such population units can occur at very small spatial scales. Indeed, similarly low dispersal rates have been observed between local populations of the species separated on average by only 60–80 m (Valenzuela-Sánchez et al., 2026).

To operationalise population monitoring, we arbitrarily delineated the area occupied by each population by establishing an approximately 30 × 30 m plot centred on the area of highest observed individual density, reflecting field observations that these animals typically occur in spatially clustered populations (Soto-Azat et al., 2013a; Valenzuela-Sánchez et al., 2014). We estimated the abundance of *R. darwinii* in five local populations (TD1 to TD5) using data collected in 2014 (Fig. 8). We fitted a Bayesian closed capture-recapture model with constant detectability to the data, implemented in JAGS via the R package jagsUI, following Kéry & Schaub (2012). All Bayesian models described in this study were fitted using vague or uninformative priors. The estimated total population size across the five populations was 421 frogs (CRI: 371–482), with a mean of 84 frogs per local population (CRI: 74–96; Fig. 8).

We also had capture-recapture data available from an additional population monitored within an area of approximately 250 m² from October 2014 to February 2015, as part of a study of acoustic and reproductive behaviour

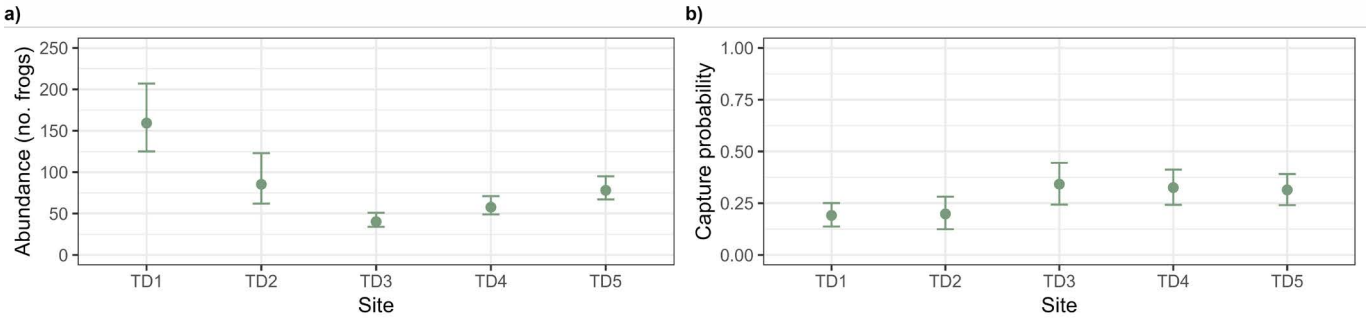

Figure 8. Abundance and capture probability of *Rhinoderma darwinii* in five local populations from Parque Tantauco prior to *Batrachochytrium dendrobatidis* invasion. Estimates are derived from a Bayesian closed model fitted to capture-recapture data collected in 2014 in the Inio sector of Parque Tantauco. **a)** Posterior mean abundance of all post-metamorphic life stages. **b)** Posterior estimates of capture (detection) probability, assumed constant across survey occasions and individuals within each population (i.e. model M0). Points indicate posterior means and vertical bars show 95% Bayesian credible intervals.

in *R. darwinii* (Serrano et al., 2020). For this population, capture histories were available at monthly intervals over a five-month period; therefore, we fitted a Bayesian Jolly-Seber open-population capture-recapture model to estimate abundance (Kéry & Schaub, 2012). The estimated total abundance (i.e. superpopulation size; Kéry & Schaub, 2012) of this local population was 173 frogs (CRI: 153–199). These estimates are not directly comparable to those obtained from the closed-population model above, as the superpopulation size of the Jolly-Seber model includes individuals that immigrated into the population as well as those born during the study period. Nevertheless, the results suggest that abundance in this local population was at least as high as that estimated for TD1–TD5.

Following the invasion of *Bd* in the area, in 2024 we initiated an occupancy study across 28 plots (30 × 30 m) within the study area (Fig. 7). Plots were pseudo-randomly selected using QGIS according to three criteria: (i) proximity to foot trails (maximum distance of 150 m from the plot centre); (ii) presence of suitable habitat (i.e. native forest); and (iii) inclusion of known local *R. darwinii* populations in at least one third of the plots. Consequently, the estimated occupancy should not be interpreted as occupancy across the entire study area, but rather as being biased towards locations with a high probability of species occurrence. This design was chosen to provide robust occupancy estimates and to enable detection of population extirpations and potential recovery (i.e. recolonisation) over the long term.

Eight of the 28 plots had confirmed presence of *R. darwinii* at least until 2014. Although these local populations were not monitored regularly thereafter, independent anecdotal evidence suggests that they persisted until at least 2023, with two corresponding to populations TAN1 and TAN2 shown in Figure 6. In March 2024, all 28 plots were surveyed daily for three consecutive days. In March 2025, 11 plots were surveyed on three days, eight plots on two days, and nine plots on a single day. In 2024, *R. darwinii* was detected in 4 of the 28 plots, whereas in 2025 it was detected in 5 of the 28 plots. During each survey occasion, each plot was searched for 30 mins, typically by two researchers.

We used a Bayesian single-season occupancy model (Kéry & Schaub, 2012) to estimate detection probability and occupancy as constant parameters within each year (Fig. 9), explicitly accounting for imperfect detection. Local population extirpation was quantified as a derived quantity within the same Bayesian model. Specifically, extirpation probability was defined as the posterior probability that a historically occupied site was unoccupied in both post-invasion survey years (i.e. 2024 and 2025). For each posterior realisation, a site was classified as extirpated if its latent occupancy state was estimated by the model as absent in both years. Extirpation probability was then calculated as the proportion of historically occupied sites inferred to be extirpated, averaged across posterior samples. This approach provides a conservative estimate of extirpation risk, as it requires consistent absence across multiple survey years rather than a single

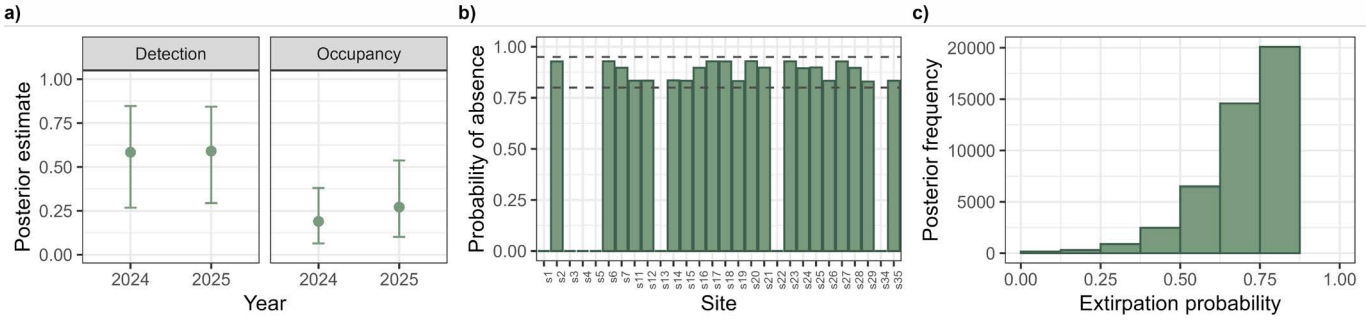

Figure 9. Post-invasion occupancy, detection and extirpation probability of *Rhinoderma darwinii* local populations in the Inio sector of Parque Tantauco. Estimates are derived from a Bayesian occupancy model fitted to surveys conducted in 2024 and 2025 across 28 plots. **a)** Posterior mean estimates of species detection probability and occupancy probability for each survey year; points indicate posterior means and vertical bars show 95% Bayesian credible intervals. **b)** Posterior probability of two-year absence for each plot (i.e. unoccupied in both 2024 and 2025). Bars represent the proportion of posterior draws from the MCMC chains in which the latent occupancy state was estimated as absent in both years. Higher values therefore indicate greater posterior support that a plot was truly unoccupied following invasion, rather than the species being present but undetected; dashed horizontal lines indicate thresholds of support for true absence (0.80 and 0.95, which we interpret as strong and very strong support for absence, respectively). **c)** Posterior distribution of the proportion of eight historically occupied local populations inferred to have been extirpated following *Batrachochytrium dendrobatidis* invasion. For each posterior draw, this proportion was calculated as the number of these eight plots estimated to be unoccupied in both 2024 and 2025, divided by eight.

non-detection event, while explicitly accounting for uncertainty in detectability. This approach assumes persistence of the 14 known historical populations until *Bd* invasion, an assumption that cannot be directly verified but is supported by the absence of indications that the species was detected less frequently prior to invasion.

The estimated probability of local population extirpation was 0.761 (CRI: 0.375–0.875; Fig. 9c). For plots in which the model inferred absence of *R. darwinii* as the most likely state, the unoccupied latent state was supported in more than 80% of posterior draws for each plot (Fig. 9b), indicating a relatively high posterior certainty of true absence rather than non-detection. Based on this estimate, we infer that approximately 10 (CRI: 5–12) of the 14 known local populations historically present in the Inio sector were extirpated following *Bd* invasion in 2023.

Population counts in surviving populations after *Bd* invasion were also markedly reduced compared with historical data from the area, indicating widespread and severe population declines. In 2024, across the four plots where the species was detected, we recorded only 11 *R. darwinii* individuals. In 2025, we counted 21 individuals across the five plots with species detections. These numbers are more than one order of magnitude lower than counts from the five populations monitored in 2014, when a total of 274 *R. darwinii* individuals were found.

Using the lower and upper limits of the credible interval (CRI) for population decline (i.e. 82–100%) reported by Valenzuela-Sánchez et al. (2026), together with the CRI of average population abundance estimated from TD1–TD5 (i.e. 74–96 individuals), and assuming similar population sizes across the 14 known historical populations in the area, we estimate that approximately 850–1,344 *R. darwinii* individuals were lost in the study area in the Inio sector of Parque Tantauco due to the *Bd* outbreak only between 2023 and 2024. This estimate reflects population loss attributable to the outbreak rather than directly observed carcasses, which are unlikely to be detected given the structural complexity of the habitat (Fig. 5) and the small body size of the species (individuals in this area generally measure < 24 mm and weigh < 1.5 g; Valenzuela-Sánchez et al., 2015). The reported range incorporates uncertainty in both pre-outbreak abundance and decline severity. Moreover, this estimate is likely conservative, as additional local populations may have existed in the area but were undocumented prior to the outbreak.

Figure 10. Alianza Tantauco in the field during a rescue operation to bring Darwin's frogs *Rhinoderma darwinii* to ZSL London Zoo. From left to right: Alison Guerrero, Bastián Poblete, Pablo Aguilar, Paul Glynn, Andrés Valenzuela-Sánchez, Chris Sergeant and Alan Bannister.

Alianza Tantauco: A collaborative project to rescue and recover a Darwin's frog stronghold

In response to the emergency caused by the invasion of *Bd* into Parque Tantauco, the "Alianza Tantauco" was formed, comprising the NGO Ranita de Darwin, the Zoological Society of London (ZSL), Fundación Parque Tantauco, Zoo Leipzig, Universidad Andrés Bello, Universidad de Concepción, Zoológico Nacional del Parquemet and the IUCN SSC Amphibian Specialist Group (Fig. 10). All of these organisations are members of the Binational (Chile-Argentina) Conservation Strategy for Darwin's Frogs (CSDF). The CSDF brings together 24 governmental, non-profit and private organisations from Chile, Argentina, Germany and the United Kingdom, and promotes Darwin's frogs as a flagship species for the conservation of Austral temperate forests (Azat et al., 2021). Using an evidence-based approach, this strategy implements priority actions for the conservation of Darwin's frogs and native forests in Chile and Argentina (IUCN SSC ASG, 2018). Alianza Tantauco aims to implement a holistic, long-term project to rescue and recover *R. darwinii* populations in Parque Tantauco, incorporating both in-situ and ex-situ conservation actions. This initiative has also received strong support from the Government of Chile.

Captive survival-assurance colonies are often the only chance to preserve a species or a population at risk of imminent extinction, and if successful, these colonies can play an important component in efforts to recover a species or population to threat-ameliorated habitat (Tapley et al., 2024). Alianza Tantauco initiated its first emergency action with a rescue mission to prevent the imminent extinction of the remaining *R. darwinii* populations in Parque Tantauco by transferring frogs to ZSL London Zoo under loan from the Chilean authorities. Over a five-day period in October 2024, we collected founder individuals for a *R. darwinii* captive survival-assurance colony. At capture, frogs were swabbed twice using MW100 swabs (Medical Wire & Equipment, UK) for *Bd* infection detection, with swabs subsequently analysed using a specific real-time PCR assay (Soto-Azat et al., 2013b). From capture until transport, the animals were housed and closely monitored in a temporary biosecure facility established within Parque Tantauco. Within 48 h of completing frog collection, PCR results were available, and 53 *Bd*-negative frogs – including 12 males carrying developing tadpoles in their vocal sacs – were cleared for transport to Europe. A customised, multi-layer insulated transport crate was designed to maintain stable temperatures and house individuals in separate

Figure 11. Customised, multi-layer insulated transport crate designed to maintain stable temperatures and house individuals in separate containers during the transport of rescued Darwin's frogs *Rhinoderma darwinii* from Parque Tantauco, Chile, to ZSL London Zoo.

containers (Fig. 11). From Inio, the frogs travelled approximately 13,000 km to ZSL London Zoo via combined sea, land and air routes over a 52 h journey. We estimate that at least 51 people from nine institutions participated in this complex operation.

At London Zoo, a dedicated, climate-controlled, biosecure facility was developed for *R. darwinii* (Fig. 12). The facility was designed to replicate environmental conditions in Parque Tantauco. Upon arrival, each frog was examined for signs of disease, swabbed for *Bd* detection and introduced

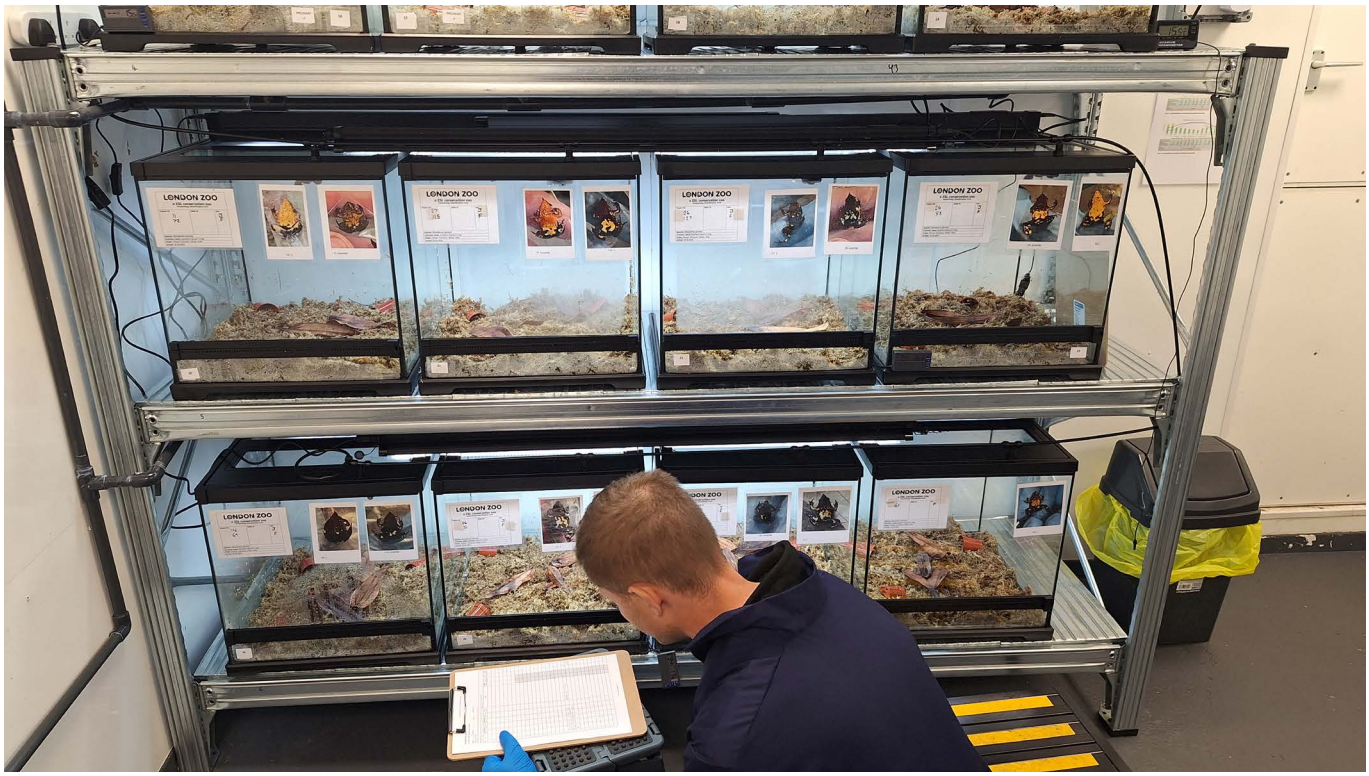

Figure 12. Bio-secure facility for Darwin's frogs *Rhinoderma darwinii* from Parque Tantauco at ZSL London Zoo. Co-author Daniel Kane records monitoring information.

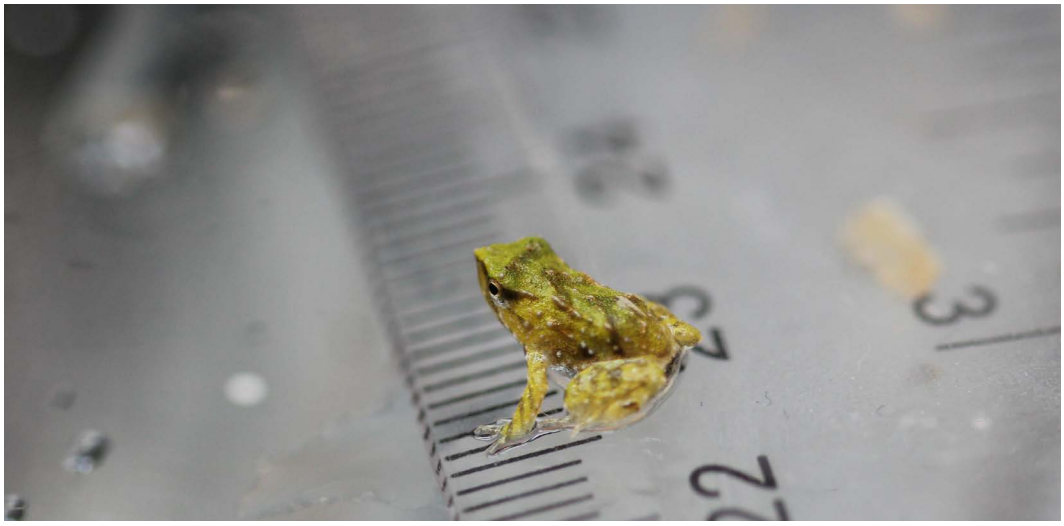

Figure 13. A recently metamorphosed Darwin's frog *Rhinoderma darwinii* at ZSL London Zoo.

to its new enclosure, where vocalisations began shortly thereafter. All frogs were active and clinically healthy on arrival. By January 2025, eleven of the males had successfully brooded 33 metamorphs (Fig. 13). In December 2024 we observed the first egg laying at London Zoo and several males have since succesfully brooded clutches of eggs laid in captivity which is a very encouraging sign for the establishment of a viable captive survival-assurance colony which will, in time, be pivotal to the recovery of populations in Parque Tantauco.

Also in January 2025, Alianza Tantauco carried out a second rescue operation of *R. darwinii* from Parque Tantauco. A second captive survival-assurance colony was established with 32 *Bd* free *R. darwinii* at the Universidad de Concepción's breeding and research station within the native range of the species. This station has focused on the ex-situ conservation of *R. darwinii* for over 15 years in close collaboration with Zoo Leipzig.

Strategic communication and public engagement

The first group of Darwin's frogs, rescued in October 2024, had to be transferred to ZSL London Zoo because no facility in Chile was ready to receive the frogs as part of this emergency conservation action. We recognised early in the project that the international transfer of *R. darwinii* individuals from Chile to Europe could pose a reputational risk, based on anecdotal information suggesting that some people in Chile might oppose the relocation of native species to overseas institutions. To address this, the communications teams at the locally based NGO Ranita de Darwin, in collaboration with ZSL, developed a strategic communications plan aimed at increasing public understanding and support. The plan was designed and led in-country and focused on communicating the project using formats and timelines appropriate for different stakeholders and audiences (Valenzuela-Sánchez et al., 2024).

The social media communications strategy in Chile (implemented through the NGO Ranita de Darwin's platforms) was launched prior to the public announcement of Alianza Tantauco and the project, and focused on explaining the role of ex-situ conservation within a holistic, multisectoral and international framework. It also emphasised ZSL's long-standing involvement (since 2009) in Darwin's frog conservation and the urgent threat posed by chytridiomycosis in Parque Tantauco.
In parallel, we obtained support from key decision-makers in Chile, including a formal letter of endorsement from the Chilean Minister of the Environment. We ensured that all ethical approvals and required permits were in place before proceeding with any animal transfers. Prior to any national or international announcements, we informed and consulted the local community in Inio. Only after this engagement did we release a press statement, in both Spanish and English and under embargo, timed to coincide with a national-level public webinar in Chile launching Alianza Tantauco and the project.

The story was rapidly covered by major media outlets in both Chile and the United Kingdom, and was featured in the BBC's Weekly Quiz, which highlights prominent news events of the

week. Coverage extended beyond these countries to audiences elsewhere, including France, Italy, Portugal, Argentina, Spain and Australia. We estimate that the project was featured in more than 410 media items, with a potential global audience reach of at least 167 million and an estimated minimum of 2.8 million online reads. The rescue mission to transport southern Darwin's frogs to London was also documented by BAFTA-winning filmmaker Paul Glynn. The documentary, "A Leap of Hope: A Huge Mission to Rescue a Tiny Frog", premiered in February 2025 and is available on ZSL's YouTube channel, with Spanish subtitles hosted on the NGO Ranita de Darwin YouTube channel. As of 19 December 2025, the documentary had accumulated a total of 15,100 views across both platforms.

The project generated substantial public interest and widespread expressions of support. For example, the Chilean Ambassador to the United Kingdom visited ZSL to learn more about the initiative, followed by an outreach event at the Chilean Embassy in London, where "A Leap of Hope" was screened and members of the project team participated in a public talk and round table discussion. Public reaction on social media in Chile was generally positive. However, as anticipated, some negative comments emerged, with a minority of individuals framing the transfer of frogs to London Zoo as a form of neocolonialism. Notably, an unexpected and encouraging outcome followed: rather than requiring direct responses from the project team, members of the public began replying to these comments, explaining the necessity of the intervention, highlighting that the project was led by locally based organisations, and referencing information previously shared through the project's communications. In nearly all negative comments reviewed by our team, multiple individuals provided informed, fact-based responses supporting the project's rationale and conservation value.

Evidence-based conservation actions for the recovery of *R. darwinii*

The ex-situ populations at London Zoo and the Universidad de Concepción will not only provide individuals for conservation reintroductions, but

Figure 14. Fence surrounding a population of Darwin's frogs *Rhinoderma darwinii* in Parque Tantauco, used to exclude syntopic amphibians. This exclusion experiment is being tested as a potential disease-mitigation action against chytridiomycosis in *R. darwinii*. The enclosed area in this replicate of the experiment has a perimeter of 347 m and an approximate surface area of 0.60 ha (1.48 acres).

will also be used to improve our understanding of chytridiomycosis and to develop strategies to mitigate its impacts in Chile and beyond. In-situ, we are implementing biosecurity measures, including footwear disinfection for researchers and visitors at park entry points, as well as continued population and epidemiological monitoring to assess the spread of *Bd* beyond the Inio sector to other areas of Parque Tantauco. In the medium term, we plan to develop a decision-analytical model to guide the selection of optimal strategies for the recovery of *R. darwinii* populations within Parque Tantauco.

Empirical evidence and individual-based modelling indicate that *Bd* transmission in *R. darwinii* is highly localised and strongly mediated by host density and contact with syntopic amphibians (Valenzuela-Sánchez et al., 2026). An individual-based model predicts that reducing contact with *Bd*-tolerant syntopic amphibians can substantially lower *Bd*-driven population depression in *R. darwinii*, particularly when host densities are low (Valenzuela-Sánchez et al., 2026). Together, these findings provide a mechanistic rationale for exclusion experiments and targeted removal of syntopic species as a feasible in-situ disease-mitigation strategy for *R. darwinii*.

Additionally, a key unresolved question is how individuals of a fully terrestrial species such as *R. darwinii* come into contact with a pathogen that has an aquatic infective stage, such as *Bd* (Valenzuela-Sánchez et al., 2017; 2026). Although occasional transfer of *Bd* from aquatic environments into terrestrial habitats via rain (Kolby et al., 2015), fog (Prado et al., 2023) or avian vectors (Hanlon et al., 2017) has been proposed, such events are likely to be infrequent. Instead, syntopic amphibian species that utilise both freshwater and terrestrial habitats throughout their life cycles may play an important role in introducing *Bd* into *R. darwinii* populations and facilitating pathogen spread once established locally (Valenzuela-Sánchez et al., 2017; 2026). To experimentally investigate the role of syntopic species in *Bd* transmission to and within *R. darwinii* populations, we initiated an exclusion experiment in 2025 in the Inio sector of Parque Tantauco using solid fences combined with the removal of syntopic amphibians (Fig. 14).

Together, our ex-situ and in-situ efforts seek to reverse recent losses and restore Parque Tantauco as a long-term stronghold for *R. darwinii*, ensuring the persistence of abundant and stable populations of this unique species across the park.

Acknowledgements
We thank ZSL, Zoo Leipzig, the Rufford Foundation, the Weeden Foundation, the Mohamed bin Zayed Species Conservation Fund, Research England, Wild Lama and the IUCN SSC internal grant scheme for financial support for this project. We also thank Till Ramm, Alison Guerrero, Kevin Tejer, Francoise Cuadra, Diego Peñaloza, Paul Glynn, Claudio Correa, Catalina Martin, Amapola Palacios, Juan Carlos Ortiz, Osvaldo Cabeza, Charif Tala, Marcela Alcaide, Sally Wren, Matthew Perkins, Sam Aberdeen, Unnar Aevarsson, Kim Carter, Jamie Mitchell, Joe Capon, Kanishka Thakur, George Sunter, Jacob Winfield, Jackie Ossowski-Mackie, Stefan Saverimuttu, Amanda Guthrie, Jo Noble, Sheryl Bradley, Robin Fitzgerald, Edward Donovan, Steve Rowland, Perrie Ridsdale, Chloe Walker, Natalie Latham and Rebecca Blanchard for their valuable support of this project.

References

Azat, C., Valenzuela-Sánchez, A., Delgado, S., Cunningham, A.A., Alvarado-Rybak, M., Bourke, J., ... & Angulo, A. (2021). A flagship for Austral temperate forest conservation: an action plan for Darwin's frogs brings key stakeholders together. *Oryx* 55(3), 356–363.

Bacigalupe, L.D., Vásquez, I.A., Estay, S.A., Valenzuela-Sánchez, A., Alvarado-Rybak, M., Peñafiel-Ricaurte, A., ... & Soto-Azat, C. (2019). The amphibian-killing fungus in a biodiversity hotspot: Identifying and validating high-risk areas and refugia. *Ecosphere* 10(5), e02724.

Bourke, J., Barrientos, C., Ortiz, J.C., Busse, K., Böhme, W. & Bakker, T.C. (2011). Colour change in Darwin's frogs (*Rhinoderma darwinii*, Duméril and Bibron, 1841) (Anura: Rhinodermatidae). *Journal of Natural History* 45, 2661–2668. https://doi.org/10.1080/00222933.2011.597885.

Busse, K. (2004). Biología de la reproducción del sapito de Darwin (*Rhinoderma darwinii*) y su cría en cautividad. In: *Cría en cautividad de fauna chilena*. Iriarte, A., Tala, C., González, B., Zapata, B., González, G. & Maino, M. (Eds.). Servicio Agrícola Ganadero. 139–146 pp.

Cabeza-Alfaro, O., Valenzuela-Sánchez, A., Alvarado-Rybak, M., Serrano, J.M. & Azat, C. (2021). First case of male alloparental care in amphibians: Tadpole stealing in Darwin's frogs. *Endangered Species Research* 45, 331–335. https://doi.org/10.3354/esr01138.

Crump, M.L. (2002). Natural history of Darwin's frog

Rhinoderma darwinii. *Herpetological Natural History* 9, 21–31.

Hanlon, S., Henson, J. & Kerby, J. (2017). Detection of amphibian chytrid fungus on waterfowl integument in natural settings. *Diseases of Aquatic Organisms* 126(1), 71–74. https://doi.org/10.3354/dao03160.

IUCN SSC Amphibian Specialist Group (2018). Estrategia Binacional de Conservación de Las Ranitas de Darwin. IUCN SSC Amphibian Specialist Group, Santiago, Chile.

Jorquera, B., Pugín, E. & Goicoechea, O. (1972). Tabla de desarrollo normal de *Rhinoderma darwinii*. *Archivos de Medicina Veterinaria* 4, 1–15.

Kéry, M. & Schaub, M. (2012). *Bayesian population analysis using WinBUGS: A hierarchical perspective*. Academic Press.

Kolby, J.E., Ramirez, S.D., Berger, L., Griffin, D.W., Jocque, M. & Skerratt, L. F. (2015). Presence of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in rainwater suggests aerial dispersal is possible. *Aerobiologia* 31(3), 411–419. https://doi.org/10.1007/s10453-015-9374-6.

Molina-Burgos, B.E., Valenzuela-Sánchez, A., Alvarado-Rybak, M., Klarian, S. & Soto-Azat, C. (2018). Trophic ecology of the Endangered Darwin's frog inferred by stable isotopes. *Endangered Species Research* 36, 269–278. https://doi.org/10.3354/esr00906.

Prado, J.S., Ernetti, J.R., Pontes, M.R. & Toledo, L.F. (2023). Chytrid in the clouds: An alternative passive transport of a lethal pathogen for amphibians. *Hydrobiologia* 850(9), 2061–2073. https://doi.org/10.1007/s10750-023-05218-2.

Scheele, B.C., Pasmans, P., Skerratt, L.F., Berger, L., Martel, A., Beukema, W., Acevedo, A.A., Burrowes, P.A., Carvalho, T., Catenazzi, A. & De la Riva, I. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363, 1459–1463.

Serrano, J.M., Penna, M., Valenzuela-Sánchez, A., Mendez, M.A. & Azat, C. (2020). Monomorphic call structure and dimorphic vocal phenology in a sex-role reversed frog. *Behavioral Ecology and Sociobiology* 74(10), 127.

Soto-Azat, C., Valenzuela-Sánchez, A., Collen, B., Rowcliffe, J.M., Veloso, A. & Cunningham, A.A. (2013a). The population decline and extinction of Darwin's frogs. *PLoS ONE* 8(6), e66957.

Soto-Azat, C., Valenzuela-Sánchez, A., Clarke, B.T., Busse, K., Ortiz, J.C., Barrientos, C. & Cunningham, A.A. (2013b). Is chytridiomycosis driving Darwin's frogs to extinction?. *PLoS ONE*, e79862.

Tapley, B., Johnson, K., Michaels, C.J., Bradfield, K., Barber, D., Randall, L., Vasudevan, K., Paszkowski, C., Griffiths, R.A., Calatayud, N.E. & Griffith, E. (2024). Conservation breeding. In: *Amphibian Conservation Action Plan: A Status Review and Roadmap for Global Amphibian Conservation*. S. Wren, A., Borzée, R., Marcec-Greaves & Angulo, A. (Eds.). IUCN SSC Occasional Paper, No. 57. IUCN. 267–283 pp.

Valenzuela-Sánchez, A., Harding, G., Cunningham, A.A., Chirgwin, C. & Soto-Azat, C. (2014). Home range and social analyses in a mouth brooding frog: testing the coexistence of paternal care and male territoriality. *Journal of Zoology* 294(4), 215–223.

Valenzuela-Sánchez, A., Cunningham, A.A. & Soto-Azat, C. (2015). Geographic body size variation in ectotherms: effects of seasonality on an anuran from the southern temperate forest. *Frontiers in Zoology* 12(1), 37.

Valenzuela-Sánchez, A., Schmidt, B.R., Uribe-Rivera, D.E., Costas, F., Cunningham, A.A. & Soto-Azat, C. (2017). Cryptic disease-induced mortality may cause host extinction in an apparently stable host–parasite system. *Proceedings of the Royal Society B: Biological Sciences* 284, 20171176.

Valenzuela-Sánchez, A. (2017). Is chytridiomycosis a threat to the endangered mouth-brooding frog (*Rhinoderma darwinii*)? A multi-approach disease risk assessment. PhD thesis, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello.

Valenzuela-Sánchez, A., Schmidt, B.R., Pérez, C., Altamirano, T., Toledo, V., Pérez, Í., Teillier, S., Cunningham, A.A. & Soto-Azat, C. (2019a). Assessing habitat quality when forest attributes have opposing effects on abundance and detectability: a case study on Darwin's frogs. *Forest Ecology and Management* 432, 942–948.

Valenzuela-Sánchez, A., Cayuela, H., Schmidt, B.R., Cunningham, A.A. & Soto-Azat, C. (2019b). Slow natal dispersal across a homogeneous landscape suggests the use of mixed movement behaviours during dispersal in the Darwin's frog. *Animal Behaviour* 150, 77–86.

Valenzuela-Sánchez, A., Azat, C., Cunningham, A.A., Delgado, S., Bacigalupe, L.D., Beltrand, J., Serrano, J.M., Sentenac, H., Haddow, N., Toledo, V. & Schmidt, B.R. (2022). Interpopulation differences in male reproductive effort drive the population dynamics of a host exposed to an emerging fungal pathogen. *Journal of Animal Ecology* 91, 308–319.

Valenzuela-Sánchez, A., Schmidt, B.R., Azat, C., Delgado, S., Cunningham, A.A., Lemaître, J.F., Gaillard, J.M. & Cayuela, H. (2023). Variable rate of ageing within species: Insights from Darwin's frogs. *Biological Journal of the Linnean Society* 138, 68–74.

Valenzuela-Sánchez, A., Agostini, G., Maynard, L., Cisternas, J., Hansen-Hendrikx, C.M. & Márquez-García, M. (2024). Communications and education for amphibian conservation. In: *Amphibian Conservation Action Plan: A Status Review and*

Roadmap for Global Amphibian Conservation. Wren, S., Borzée, A., Marcec-Greaves, R. & Angulo, A. (Eds.). IUCN SSC Occasional Paper, No. 57. IUCN. 199–217 pp.

Valenzuela-Sánchez, A., Delgado-Oyarzún, S., Azat, C., Schmidt, B.R., Sentenac, H., Haddow, N., Santana, B., Solano-Iguaran, J.J., Cunningham, A.A. & Bacigalupe, L.D. (2026) Localized transmission of an aquatic pathogen drives hidden epidemics and population collapse in a terrestrial host. *Nature Ecology & Evolution*. https://doi.org/10.1038/s41559-025-02930-1.

Wilhelm, O. (1927). La *Rhinoderma darwinii* D. y B. *Boletín de la Sociedad de Biología de Concepción* 1, 11–39.