Why chemically defended amphibians are more likely to face extinction: a phylogenetic comparative test of hypotheses.

Rachel Haynes
Student number: [redacted]
Swansea University

June 2025

Submitted to Swansea University in fulfilment of the requirements for the Degree of Masters by Research in Biosciences.

Abstract

Chemical defence is an antipredator mechanism employed by many amphibians. Despite this, chemically defended amphibians have been shown to exhibit a 60% higher contemporary extinction risk than non-defended species, with a three-times higher background extinction rate. Using structural equation models (SEMs), this phylogenetic comparative study aimed to disentangle the indirect mechanisms explaining this seemingly counterintuitive link. Three hypotheses were tested: the marginal habitats hypothesis, the slow life history hypothesis and the energetics hypothesis. Using the IUCN Red List, the second Global Amphibian Assessment, AmphiBIO and the Global Amphibian Biodiversity Project, data on chemical defence, threat status and population trajectory were compiled for 855 amphibian species. Data on habitat use, life history and body size were obtained to serve as proxies for each hypothesis. The SEMs show that the benefits of chemical defence enable species to survive in a higher proportion of marginal habitats than non-defended species. However, the intrinsic instability of these habitats will ultimately put these species at higher risk of extinction. Therefore, the marginal habitats hypothesis provides a strong explanation for the indirect effect of chemical defence on extinction risk. There was weaker support for the slow life history hypothesis and no support for the energetics hypothesis. These results underscore the importance of considering an amphibians' full ecological context when assessing extinction risk and allocating conservation resources.

Declarations and Statements

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed: [redacted]
Date: 30/06/2025

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed: [redacted]
Date: 30/06/2025

I hereby give consent for my thesis, if accepted, to be available for electronic sharing.

Signed: [redacted]
Date: 30/06/2025

The university's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

Signed: [redacted]
Date: 30/06/2025

Statement of Expenditure:

N/A

Persons Involved:

Contributor Role	Persons Involved
Conceptualisation	Rachel Haynes, Kevin Arbuckle
Data Curation	Rachel Haynes
Formal Analysis	Rachel Haynes, Kevin Arbuckle
Funding Acquisition	N/A
Investigation	Rachel Haynes
Methodology	Rachel Haynes, Kevin Arbuckle
Project Administration	Rachel Haynes, Kevin Arbuckle
Resources	Rachel Haynes, Kevin Arbuckle
Software	Rachel Haynes, Kevin Arbuckle
Supervision	Kevin Arbuckle, Rory Wilson
Validation	Kevin Arbuckle
Visualisation	Rachel Haynes, Kevin Arbuckle
Writing- original draft preparation	Rachel Haynes
Writing- review and editing	Kevin Arbuckle, Rachel Haynes

Ethical Approval:

Approval Date: 22/07/2024
Research Ethics Approval Number: 2 2024 9093 9826

Action Required on Form	Status	Review Reference	Date Modified
No	Approved	2 2024 9093 9826	22/07/2024 14:55

Health and Safety/Risk Assessments:

N/A- Desk Based Study

Table of Contents

Tables and Figures

Acknowledgements:

I would like to thank Dr Kevin Arbuckle for his unwavering enthusiasm, support and expertise throughout this degree, despite many hurdles along the way. I would also like to thank Professor Rory Wilson for his help in project planning and for being my secondary supervisor. Lastly, I would like to thank everyone in the METALab group for the many coffees and sweet treat runs, Utkarsh for his patience and Isaac and Tiny for keeping me sane.

Introduction

Amphibians are the most widely threatened vertebrate taxonomic group, with 40.7% of species classified as globally threatened by the International Union for Nature Conservation (IUCN) Red List (Luedtke et al 2023). The ongoing, rapid declines of amphibian populations has been described as a 'mass extinction' (Wake and Vredenburg 2008), with global populations continue to deteriorate rapidly (Luedtke et al 2023). Although the leading threat varies across species, populations and regions (Campbell Grant et al 2020), habitat loss and degradation, climate change and disease are responsible for many enigmatic declines (Luedtke et al 2023). Amphibians are particularly vulnerable to environmental changes due to their unique biological traits. As ectotherms, their body temperature directly depends on ambient conditions, making them highly susceptible to shifts in climate. Their biphasic lifecycle, which relies on both suitable aquatic and terrestrial habitat further amplifies this sensitivity; changes in temperature, precipitation or pollution directly impact the suitability of these critical environments (Rohr and Palmer 2013). The impact of environmental changes is further exacerbated by amphibians' often low dispersal capacities, as they are unable to escape to more favourable environments (Winter at al 2016; Deutsch et al 2008).

It is therefore of utmost importance that conservation efforts are effectively targeted towards this taxonomic group, with decisions about resource allocation strategically underpinned by a strong evidence-base (Ausden and Walsh 2020). The IUCN Red List acts as a crucial tool in this effort, by providing rigorous data on population trends and the threat status of species over time (IUCN 2024; Mace et al 2008; Rodrigues et al 2006). Understanding factors correlating with and potentially influencing extinction risk have been investigated in various species, further informing conservation planning and prioritisation (Purvis et al 2000; Bielby et al 2008; Bland 2017; Reed and Shine 2002; Cooper et al 2008; Hutchings et al 2012a; 2012b; Verde et al 2013; Jeppsson and Forslund 2012; Arbuckle 2016).

Amphibians face considerable predation pressure due to their small size, slow movements and thin, permeable skin, which lacks the protective armour found in other species (Stankowich 2011; Peplinski et al 2021). In addition to crypsis (Toledo and Haddad 2009) and behavioural responses such as body inflation and posturing (Toledo et al 2011), many amphibian species have also evolved another deterrent in the form of chemical defence (Brizzi and Corti 2007; Toledo and Jared 1995). Chemical defence can be found across the three amphibian orders (Anura, Caudata and Gymnophiona*)* and is generally passive (Jared et al 2009), as opposed to the active and aggressive envenomation seen in venomous snakes (Barlow et al 2009; Chippaux et al 1991; Hayes et al 2002). However, there are some exceptions to the rule- for example, two species of casque-headed frog inject toxins through dermal glands by headbutting spines into the predator's skin (Jared et al 2015) and three Asian genera of salamanders, *Tylototriton*, *Echinotriton* and *Pleurodeles,* push the sharp tips of their ribs into predators, coated on exit by toxic secretions from epidermal glands (Nowak and Brodie 1978; Heiss et al 2010). Similarly, caecilians possess dental glands that produce enzymes commonly found in snake venoms, creating a viscous secretion around the mouth which helps to break down prey (Jared et al 2018).

A discovery in 2015 found that chemically defended species have a three times higher background extinction rate over evolutionary time than non-defended species (Arbuckle and Speed 2015). Consolidating this result, in 2016, it was found that chemically defended amphibians also have a 60% higher contemporary extinction risk (Arbuckle 2016). This association at first appears counterintuitive as toxins are a highly effective defence mechanism (Heethoff and Rall 2015). However, direct effects are not the only factors influencing extinction risk, with indirect mechanisms also acting as vital components for assessing overall extinction vulnerability (Harnik 2011; Wootton 1994; Shipley 2016; Grace 2006).

Arbuckle and Speed's 2015 paper proposed the following three hypotheses as explanations for why chemical defence may increase extinction risk: the marginal habitats hypothesis, the slow life history hypothesis and the energetics hypothesis (Arbuckle and Speed 2015).

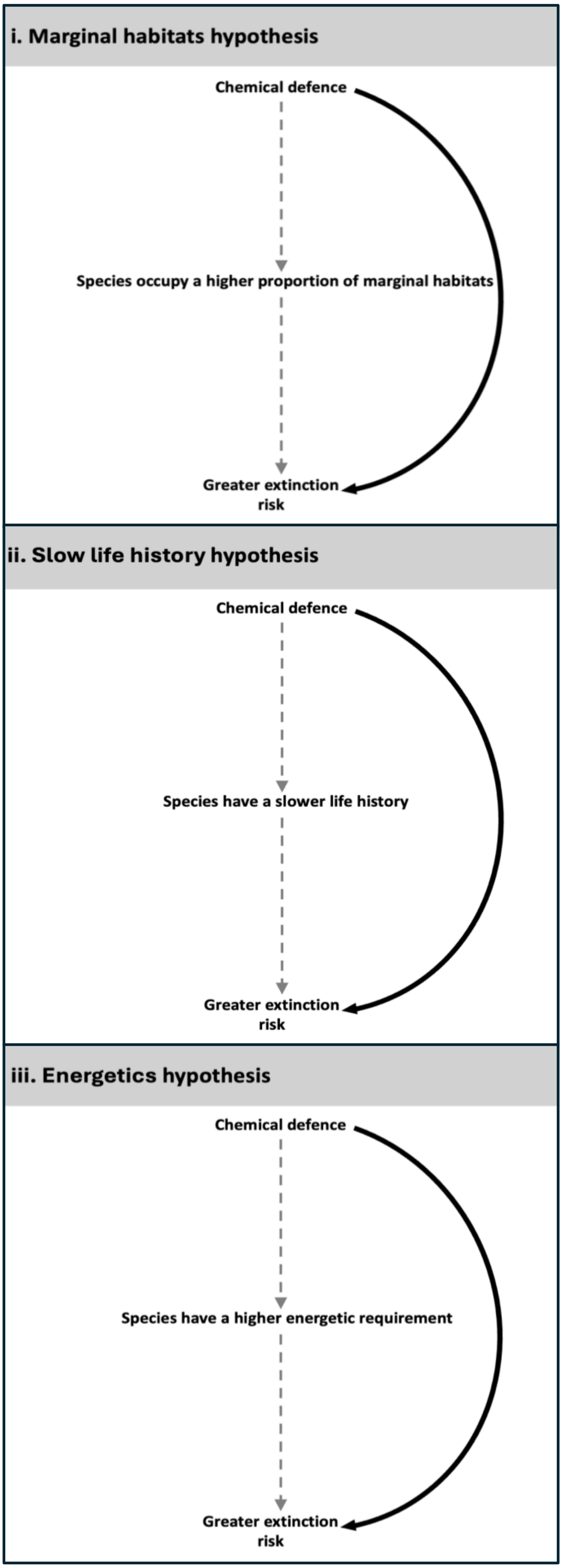

Fig. 1. The simplified versions of each hypothesis, with dashed arrow lines representing the indirect hypothesised relationships between chemical defence and extinction risk, and full black arrows representing direct relationships. (i.) The marginal habitats hypothesis proposes that chemically defended species occupy a higher proportion of marginal habitats, which leads to their higher extinction risk. (ii) The slow life history hypothesis proposes that chemically defended species have a slower life history, which leads to their higher extinction risk and (iii) The energetics hypothesis proposes that chemically defended species have a higher energetic requirement, which leads to their higher extinction risk.

Marginal habitats hypothesis:

The marginal habitats hypothesis (See *Fig. 1.i)* proposes that chemical defence facilitates species' expansion into low carrying capacity ('marginal') habitats (Thompson 1989) as they may be better able to exploit these resource-poor environments (Arbuckle and Speed 2015). Chemical defence provides protection against predation, thus freeing up resources for feeding and reproduction that would otherwise be invested into predator avoidance. As marginal habitats are intrinsically more vulnerable to stochastic events and edge effects (Kawecki 2008), species living in these environments are likely to face a higher extinction risk (Britnell et al 2023). Thus, while species in marginal habitats are likely to face a higher extinction risk due to stochastic events and edge effects, chemical defence may still aid their survival in these environments by buffering against predation, even if the habitats themselves are more vulnerable.

It has been suggested that aposematism, a warning signal that advertises unpalatability or noxiousness (Caro and Ruxton 2019), may enable expansion into new habitats (Speed et al 2010; van den Berg et al 2024), opening further opportunities for foraging, sexual selection and parental care and thereby facilitating diversification (Arbuckle and Speed 2015; Speed et al 2010). This phenomenon is broadly consistent with the 'escape and radiate' hypothesis (Schluter 2000), which suggests that novel traits can open new adaptive zones (Arbuckle and Speed 2015; Speed et al 2010) allowing for range expansion into otherwise adverse (or 'marginal') conditions (Ehrlich and Raven 1964). For example, in poison dart frogs, aposematism facilitates more specialised parental care strategies, such as prolonged tadpole transport to phytotelmata (Carvajal-Castro et al 2021). However, it is crucial to recognise that the macroevolutionary consequences of aposematism can exhibit different evolutionary dynamics compared to the underlying chemical defence alone. Specifically, while aposematism can increase speciation rates without increasing extinction, leading to net diversification, chemical defence itself can be associated with higher extinction rates, resulting in a net reduction in diversification for chemically defended lineages (Arbuckle and Speed 2015).

Slow life history hypothesis:

The slow life history hypothesis (See *Fig. 1. ii*) proposes that chemically defended species face a higher extinction risk as they are more likely to exhibit slow life history strategies (Arbuckle and Speed 2015). Traits associated with a slow life history, such as delayed reproduction and smaller clutch sizes, may not initially contribute to population declines and could even buffer against them by producing higher-quality offspring. However, once a population declines to low levels- where they are more susceptible to high levels of demographic stochasticity- species with slow life histories will remain in a 'high risk' low-abundance state for longer (Cardillo 2005; Davidson et al 2009; Hutchings et al 2012b). This has been suggested to contribute to population decline and extinction of frogs (Cooper at al 2008; Williams and Hero 1998; Lips et al 2003).

Slow life history traits are important correlates of extinction risk (Purvis et al 2000) in mammals (MacArthur and Wilson 1967; Johnson 2002; Brashares 2003; Cardillo 2005), snakes (Webb et al 2002), sharks, skates, rays (Garcia et al 2008) and birds (Bennett and Owens 1997; O'Grady et al 2004; Isaac et al 2009). However, other studies indicate that they do not affect extinction risk in passerines (Pocock 2010) or parrots (Jones et al 2006), and herbaceous perennials with earlier maturation (a fast life history trait) are more vulnerable to extinction (Hernández-Yáñez et al 2022; Jeppsson and Forslund 2012). These different results between taxa might be explained by considering that the impact of slow life histories on extinction risk often becomes most apparent after a population has already declined due to other factors, as their slow recovery rates prolong vulnerability to stochastic events. Therefore, the relevance of these traits as predictors of extinction risk can vary depending on the initial drivers of decline and the specific ecological context of the species.

Slow life history theory predicts that reduced extrinsic mortality (e.g. from predation) can favour the evolution of slower life histories. The rationale for this phenomenon could be that species well-protected by potent chemical defences, like many amphibians, may experience lower predation rates, thereby reducing selection pressure for rapid reproduction and short lifespans. This allows resources to be reallocated towards other traits, potentially leading to increased longevity and delayed maturation (Hossie et al 2013; Blanco and Sherman 2005). Empirical evidence supports this, showing that chemically defended amphibians tend to exhibit slower life histories, such as increased longevity (Hossie et al 2013; Blanco and Sherman 2005). Furthermore, while chemical defences broadly function against predators by reducing extrinsic mortality, production of antimicrobial peptides may also directly influence longevity, and their production can be an area of increased investment for amphibians with slow life histories, particularly against parasites (Woodhams et al 2016). This suggests a potential feedback loop where effective chemical antipredator defence drives the evolution of slow life history traits, which, in turn, can influence further investment in defence. Ultimately, this intrinsic link between potent chemical defences and slow life histories reinforces the slow life history hypothesis, contributing to the elevated extinction risk observed in chemically defended species by hindering their recovery from population declines.

Energetics hypothesis:

The energetics hypothesis (See *Fig. 1. iii*) proposes that the production and maintenance of chemical defence is energetically costly. Therefore, it is likely that a higher quantity and/or quality of nutritional resources will be required to sustain these processes in chemically defended species (Blennerhassett et al 2019). Chemically defended species may therefore have less energy available for other essential functions and may be left vulnerable to attack from threats, such as infectious diseases (Smilanich et al 2009).

Many studies show significant metabolic costs in venom-producing species, including snakes (McCue 2006; Pintor et al 2010; Smith et al 2014), stingrays (Enzor et al 2011) and fish (Harris and Jenner 2019). For this reason, venom optimisation takes place, where species only inject specific quantities into prey, suggesting that it is costly to replenish (Wigger et al 2002; Morgenstern and King 2013; Hayes 2008). In amphibians, toxins are shown to vary between individuals (Bókony et al 2016) and some tentative evidence shows plasticity in toxin production in relation to the presence of predators (Hettyey et al 2019), which could imply resource allocation optimisation. It should be noted that as the vast majority of venoms are proteins produced by the animal itself, they have an energetic cost of protein biosynthesis (Morgenstern and King 2013; McCue 2006). Although many amphibians do synthesise some defensive chemicals (including some proteins), many species also sequester them from the diet (Darst et al 2005). With this method, the transport and alteration of dietary chemicals (Alvarez-Buylla et al 2022), in addition to the toxin resistance required for storage (Coleman and Canatella 2023) may also result in energetic costs. However, as many sequestered toxins come with their own energy from a food item, this may mitigate the cost compared to *de novo* biosynthesis, where the energetic cost must be borne regardless of the energy intake. Studies comparing these strategies in leaf beetles provide evidence that while sequestration involves its own physiological costs, it may alleviate the biosynthetic constraints associated with *de novo* production, allowing for faster replenishment and a more effective defence (Zvereva et al 2017).

Blennerhassett et al reported that the toxins of cane toads (*Rhinella marina*) may be generated at the expense of growth, reproduction and liver stores (Blennerhassett et al 2019). As the liver is where most energy storage and biosynthesis in amphibians takes place (Withers and Hillman 2001), this is strong evidence that toxin production depletes energy. The bufodienolide component of toxin production is synthesised from the same precursor molecules as reproductive hormones (Bókony et al 2019), implying that a trade off with reproduction is likely. Experimental removal of toxins also reduces rates of growth and movement, further suggesting that replenishment of toxins is significantly costly in this species (Blennerhassett et al 2019). Toads in particular display a reluctance to release toxins and will do so only as a last resort, after first attempting deterrence via crypsis or escape (Jared et al 2014; Hayes 1989; Hudson et al 2017; Kowalski et al 2018; Marchisin and Anderson 1978). Furthermore, after expulsion, it takes time for toxins to replenish. One study found that only 68% of toxins had been replenished 76 days after manual expulsion (Chen and Chen 1933).

Structural equation models (SEMs) have been used to tease apart the direct and indirect effects in other evolutionary and ecological contexts and hence shed new light on mechanisms (Thorson and van der Bijl 2023; Lee and Jetz 2010). Addressing the hypotheses proposed by Arbuckle and Speed (2015), this phylogenetic comparative study investigates three indirect mechanisms using SEMs - the marginal habitat hypothesis, the energetics hypothesis and the slow life history hypothesis- to investigate the indirect mechanisms that may explain why chemically defended species exhibit higher extinction risk.

Method

Data collection

Data on the presence or absence of chemical defence in 857 amphibian species were extracted from a previous dataset (Arbuckle and Speed 2015). Within this dataset, data on chemical defence (1) were only recorded for a given species if there was primary literature available confirming the presence of specialised skin glands (e.g. parotoid or granular glands), specific toxins (e.g. alkaloids or bufadienolides) or clear toxic effects on predators. Species were classified as non-defended (0) only if research explicitly found a lack of these defences. Those with ambiguous or missing information were excluded to ensure that the 'non-defended' group was accurate.

The final dataset comprised 855 species, providing broad representation across the three amphibian orders: Anura (620 species across 44 families), Caudata (227 species across 10 families), and Gymnophiona (8 species across 5 families). While sampling density was naturally higher in clades with more extensive primary literature on chemical assays—such as the *Hylidae* (n=102), *Bufonidae* (n=83), and *Plethodontidae* (n=147)—the inclusion of 59 total families ensures that the analysis captures a wide breadth of the amphibian tree of life. A full breakdown of species counts per family involved in analysis is provided in Supplementary Table S1, and the distribution of these taxa is visualized on the phylogenetic tree in *Fig. 2*.

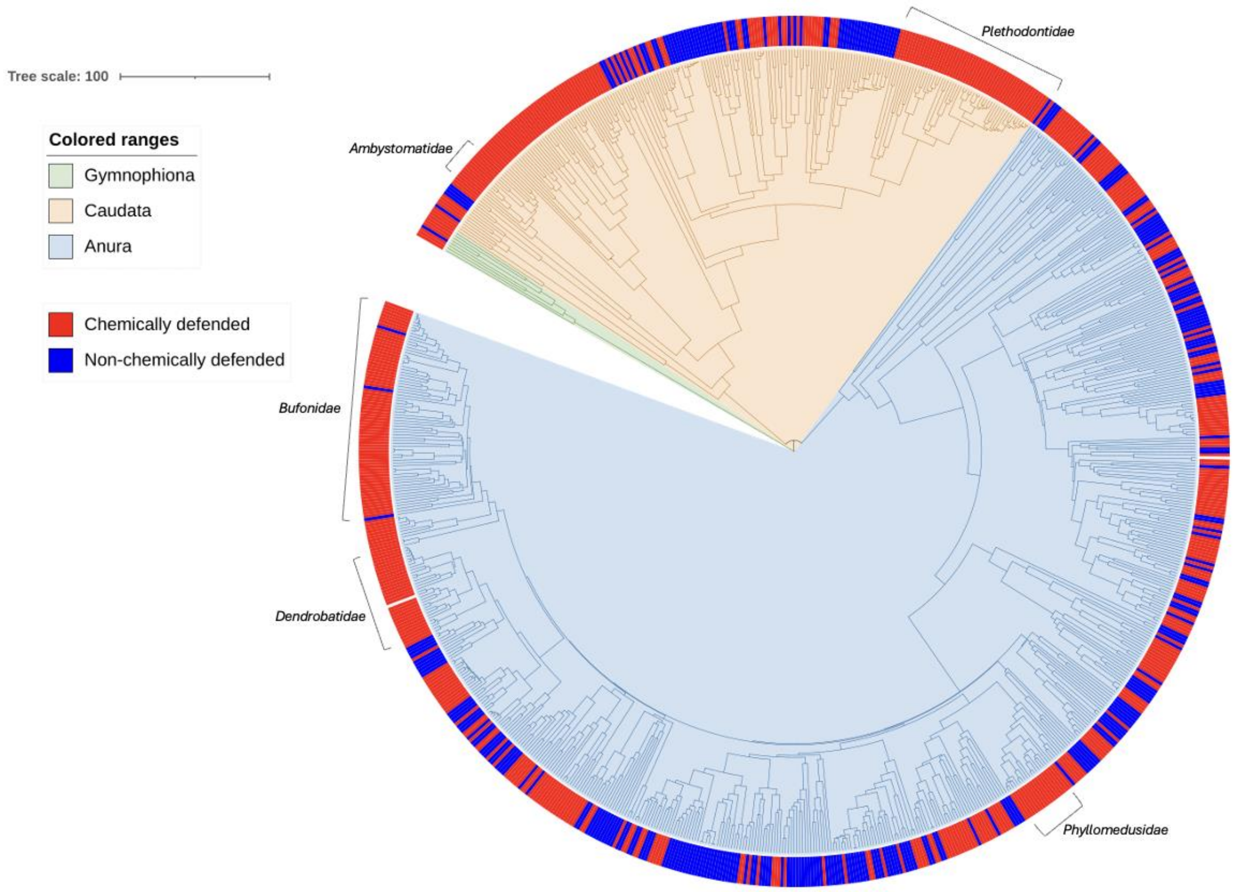

Fig. 2. Circular phylogeny of the 855 amphibian species included in the study. Internal clade colouring represents the three amphibian orders: Anura, Caudata and Gymnophiona. The outer ring maps the presence (red) or absence (dark blue) of chemical defence for each species. Key families with high data availability are labelled to illustrate taxonomic coverage and the distribution of defensive traits.

To avoid pseudoreplication during analysis, two species (*Dendrobates duellmani* and *Rana esculenta*) were removed from the dataset. Reclassification of these species led to their removal from the IUCN database, as they are no longer considered distinct species. Duellman's poison dart frog *(Dendrobates duellmani)* was removed as examination of the holotype of the Amazonian poison frog (*Ranitomeyer ventrimaculatus*) revealed that *D. duellmani* was not a distinct species but a synonym of *R. ventrimaculatus* (Brown et al 2011). Because the species are no longer considered distinct, their entries in the original dataset were identical, and so no decision about which data to retain was necessary. The edible frog *(Rana esculenta)* was excluded, due to its status as a klepton-taxon: a permanent F_1 hybrid between the pool frog (*Pelophylax lessonae)* and the marsh frog (*Pelophylax ridibundus*) (Berger 1971; Spolsky and Uzzell 1986), with reproduction relying entirely on the presence of sympatric *P. lessonae* and *P. ridibundus* populations. With all gametes transmitting the *P.*

ridibundus genome, many taxonomists do not consider *Rana esculenta* to be a distinct species.

In addition to the chemical defence data, data was collected for all available species from the IUCN Red List for several traits, including threat, decline and the number and proportion of marginal habitats. The IUCN Red List categories are a widely used and standardised proxy of extinction risk (Sutherland et al 2015). Threat status was recorded as a binary (threatened vs non-threatened) trait, based on the IUCN Red List categories, where least concern (LC) and Near Threatened (NT) were considered 'non-threatened' (0) and all other categories (VU, vulnerable; EN, endangered; CR, critically endangered; EW, extinct in the wild; and EX, extinct) were considered 'threatened' (1), as used by Arbuckle (2016), in line with recommendations made by the IUCN 2024-2 (IUCN 2024). It is therefore the internationally recognised standard for what constitutes a threatened species. While the Red List itself is ordinal, the binary classification used in this study (threatened or non-threatened) reflects the functional threshold used in global conservation policy. Additionally, from a modelling perspective, this approach minimises the risk of over-parameterization in the SEM while focusing on the ecological shift toward extinction vulnerability.

The second Global Amphibian Assessment (Luedtke et al 2023) provided an opportunity to independently test the association between chemical defence and extinction risk in amphibians, based on Red List status changes since the original dataset was compiled (Arbuckle and Speed 2015). Therefore, a preliminary analysis was undertaken to assess the number of species changing Red List status between the 2015 study and the time of my data collection (2024). I hypothesised that for species that have had changes to their Red List status, chemically defended species should be disproportionately common amongst those with increased threat status. Two binomial tests were used to investigate whether an increase or a decrease in status (by one or more categories) was more frequent for chemically defended species than non-chemically defended species. Of the 855 species in my dataset, 65% (n= 552) were chemically defended and 35% (n= 303) were not chemically defended. Therefore, the proportion of changes to threat status for chemically defended species since the 2015 study should be 0.646, if there is no difference between the two defence categories. The same total proportions were also found if calculated just from the dataset of species which have had changes to threat status. Species with insufficient data or that had not changed category since the 2015 study were classed as 'NA' and excluded from the counts.

As predicted, chemically defended species were disproportionately more likely to increase in threat status than non-chemically defended species. 83% of the species with newly increased threat status were chemically defended (binomial test, number of successes= 25, number of trials= 30, p= 0.035). Decreases in threat status between chemically defended and non-defended species were proportional to their relative abundance in the dataset (binomial test, number of successes= 21, number of trials= 32, $p \approx$ 1).

Values for 'decline' were obtained from the 'trend' section of each IUCN Red List species profile, stating whether a species' population was currently 'increasing', 'decreasing' or 'stable'. This was recorded as a binary variable, with 1 for decreasing, 0 for increasing or stable and 'NA' for those with insufficient data.

The total number of different habitat types occupied was obtained from the finer-scale 'Habitat' codes of each Red List profile. The number of marginal habitats was also recorded from this section, by only counting habitats whose suitability was classed as 'marginal' rather than 'suitable'. This allowed for calculation of the proportion of marginal habitats each species occupied. In the case of species with habitats of 'unknown' suitability, the proportion was calculated classing 'unknown' habitats as 'marginal'.

A sensitivity analysis was performed where habitats of 'unknown' suitability were instead re-coded as 'suitable', to ensure the results were not biased by the method of classification. These alternative coding methods produced very similar results (see Table S2 for a comparison of path coefficients), confirming that the choice of coding did not influence the outcome.

For the energetics hypothesis, body size was used as a proxy for energy requirements, as data on diets of most species is lacking. Data on body size (mm) (n= 563) was obtained from a recent and comprehensive publication (Cejp and Griebeler 2024) that used data from AmphiBIO (Oliveira et al 2017), Stark and Meiri (2018) and the datasets available from the Global Amphibian Biodiversity Project Website (Pincheira-Donoso 2017). These body size measurements consisted of mean adult snout to vent length (SVL) for Anura and Caudata species and mean total length (TL) of Gymnophiona species, which included SVL plus tail length, as recommended by Oliveira et al (2017). Clutch size (n= 438) and egg size (mm) (n=

387) were acquired from Allen et al (2017) to provide data for the slow life history hypothesis.

While geographic range size is a known significant component of extinction risk, it was not included in the models due to the substantial time requirements for global data compilation across 855 species. Furthermore, excluding range size prevented over-parameterization of the SEMs, ensuring that the models remained focused on the primary biological and ecological predictors (chemical defence, life history, and habitat type) without compromising statistical parsimony.

Data analysis

Analyses were performed in R Studio v. 4.4.2 (R Studio 2024) for basic manipulation and other packages were used as stated for particular methods.

Structural equation models

An extensive phylogeny of amphibians was obtained from Pyron and Wiens (2013; Arbuckle and Speed 2015). After pruning the phylogenetic tree to exclude the two species no longer included in the dataset using the R package ‘ape’ (version 5.8) (Paradis and Schliep 2018), the package ‘phylolm’ (version 2.6.5) (Tung Ho and Ané 2014) was used to fit structural equation models (SEMs) according to the structure shown in *Fig. 1*, with both indirect and direct pathways for each hypothesis. Missing data were handled using complete case analysis, where species lacking information for a particular variable were excluded only from the models requiring that variable. Consequently, final sample sizes varied across the three analyses as follows: Marginal habitats hypothesis (threat model: n= 851, decline model: n=760), slow life history hypothesis (threat model: n=438, decline model: n=395), and the energetics hypothesis (threat model: n=562, decline model: n=510).

The decision to create separate SEMs rather than combining them all into one global model was a strategic choice to provide higher statistical power and to avoid overparametising the model. As data availability was varied across traits (e.g. n = 851 for the marginal habitats threat model compared to n = 395 for the slow life history decline model), a global model would have excluded over 450 species. By using separate models, we ensured that each model included the most taxonomically diverse sample possible, thereby avoiding the biases and loss of power that would have arisen from a single combined model. While a global approach could potentially identify indirect inter-relationships between ecological and life history variables, our prioritised focus was on maintaining robust sample sizes. For this reason, as global databases expand, future research should aim to re-test these relationships within an integrated framework.

Phylogenetic path analysis and structural equation models (SEMs) are well-suited to teasing apart the causes of extinction risk, as they consider both direct and indirect causal relationships, whilst accounting for phylogeny (Reed and Shine 2002; Pugesak et al 2003), which assists in finding a parsimonious and statistically supported model (Texeira et al 2012; Reed and Shine 2002). Many traits influencing extinction risk exhibit phylogenetic signal (Fisher and Owens 2004; McKinney 1997), and amphibian declines often show phylogenetic dependence, where related species frequently share similar vulnerabilities to extinction. Although these approaches have been used in various extinction risk studies (Yang et al 2022; Lee and Jetz 2011; Iriondo et al 2003; Malaeb et al 2000; Allen et al 2017), none to date have attempted to understand the indirect effect of chemical defence on amphibian extinction risk.

While regression models such as GLMMs can account for multiple interacting variables, they are limited to evaluating their impact on a single response variable. SEMs however, allow variables to be arranged in causal chains. This allows us to determine if chemical defence leads to extinction risk indirectly, as a consequence of facilitating expansion into marginal habitats. In a standard regression, these cascading effects are often masked. Furthermore, the outputs of SEMs provide path coefficients which indicate the strength and direction of each link, which can show how well the proposed causal structure fits the data.

Phylogenetic path analysis (PPA) was used to analyse the relationships between chemical defence and potential explanatory variables, allowing for the phylogenetic relatedness between species to be considered (Bergman et al 2024). Two phylosems (Thorson and van der Bijl 2023; Hardenberg and Gonzalez-Voyer 2013) were created for each of the three hypotheses (See *Fig. 3),* one using ‘decline’ and a second using ‘threat’ as the variable used to proxy extinction risk.

Results

Marginal habitats hypothesis

Chemical defence positively and significantly predicted the proportion of marginal habitats in both models (threat model: n = 851, t = 1.979, p = 0.047; decline model: n = 760, t = 1.991, p = 0.047), however it did not significantly predict either threat (t = 0.926, p = 0.355) or decline directly (t = 0.225, p = 0.822). The proportion of marginal habitats significantly positively predicted decline (t = 2.183, p = 0.029) but did not predict threat (t = 1.931, p = 0.053) (See *Fig.3. i.)*. These relationships remained significant regardless of mode of habitat coding (see Table S2 for sensitivity analysis).

Slow life history hypothesis

Chemical defence did not directly predict threat (threat model: n = 438, t = 1.858, p = 0.063) or decline (decline model: n = 395, t = 1.133, p = 0.257), nor did it influence egg size in either model (threat model: t = 0.329, p = 0.063; decline model: t = 1.130, p = 0.258). Chemical defence positively predicted clutch size in both models (threat model: t = 2.383, p = 0.017; decline model: t = 2.461, p = 0.012).

Egg size and clutch size significantly and negatively predicted each other in both models (threat model: t = 4.382, p = 0.000; decline model: t = 4.391, p = 0.000). Clutch size did not significantly predict decline (t = 1.361, p = 0.174), but it did negatively predict threat (t = 4.382, p = 0.000). Egg size positively predicted both threat (t = 2.794, p = 0.005) and decline (t = 2.337, p = 0.02) (See *Fig. 3. ii.)*. The support for this hypothesis remains weaker than for the marginal habitats hypothesis, as, despite linking some traits to extinction risk, chemical defence failed to predict egg size and also showed an unexpected positive relationship with clutch size, which contradicts the concept of a consistently slow strategy.

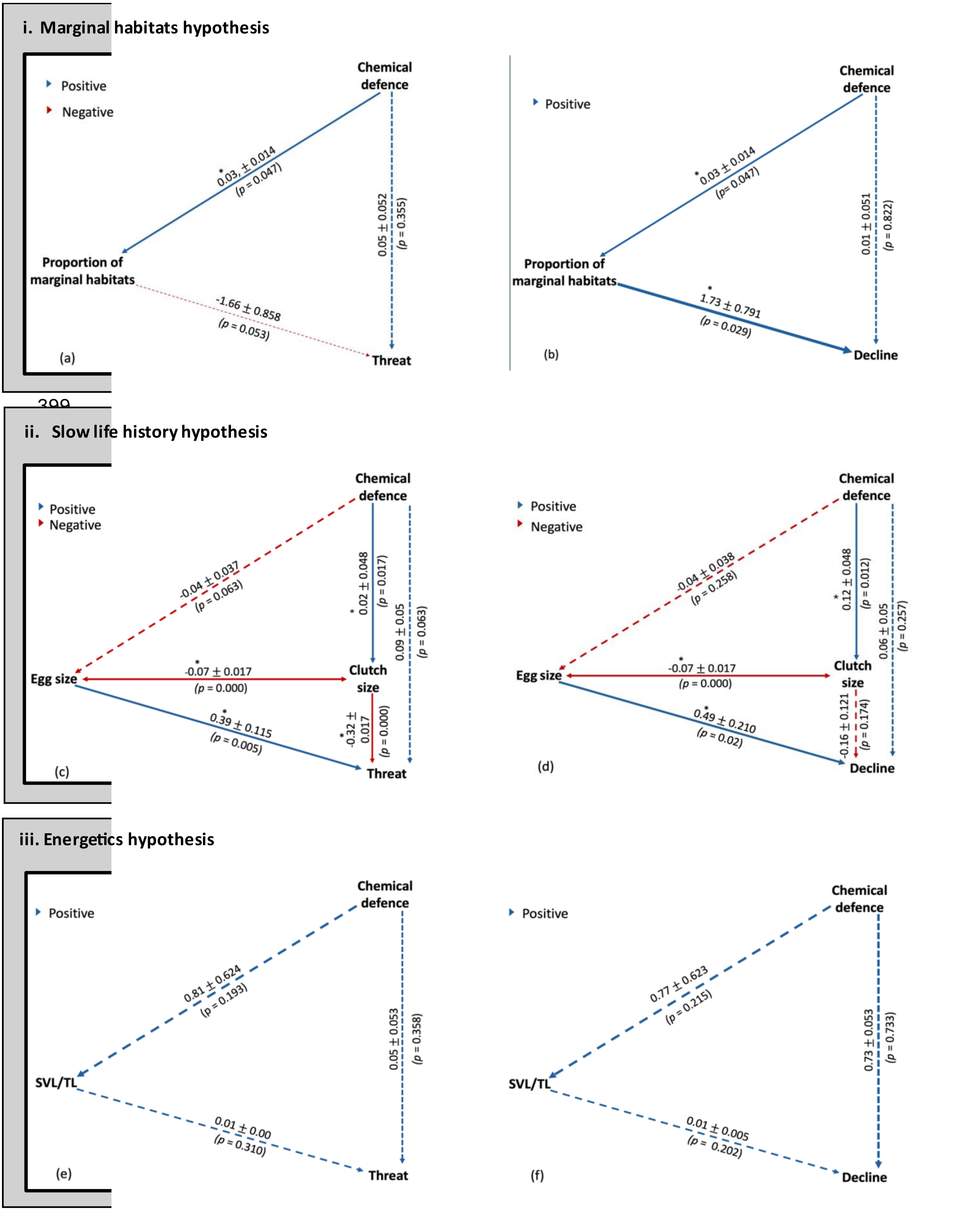

Fig. 3. Path diagrams including path coefficients (generated using package 'phylosem' in R Studio v. 4. 4. 2) for structural equation models (SEMs) demonstrating the direct and indirect influence of chemical defence on extinction risk (threat and decline) for i. Marginal habitats hypothesis: (a) threat and (b) decline; ii. Slow life history hypothesis: (c) threat and (d) decline; and iii. Energetics hypothesis: (e) threat and (f) decline. Positive relationships are indicated by blue arrows. Negative relationships are indicated by red arrows. Statistically significant (p<0.05) paths are indicated by solid arrow lines and an asterisk (*) above the coefficient. Statistically insignificant paths are indicated by dashed arrow lines. Arrow line thickness is proportional to the standardised path coefficient.

Energetics hypothesis

Chemical defence did not significantly predict threat (threat model: $n = 562$, $t = 0.919$, $p = 0.358$), decline (decline model: $n = 510$, $t = 0.342$, $p = 0.733$) or SVL/TL (threat model: $t = 1.301$, $p = 0.193$; decline model: $t = 1.241$, $p = 0.215$). SVL/TL also did not predict threat ($t = 1.015$, $p = 0.310$) or decline ($t = 1.275$, $p = 0.202$) (See *Fig. 3. iii.).*

Data availability: The full dataset, phylogeny and R-script are hosted on Figshare and can be accessed at https://figshare.com/s/fce9c3b4a80c166030e2.

Discussion

My results corroborated Arbuckle's (2016) finding of a link between chemical defence and contemporary extinction risk and subsequently found support for one of the proposed mechanisms for this relationship. The structural equation models suggests that the marginal habitats hypothesis is the most plausible, as chemical defence results in occupation of a higher proportion of marginal habitats, ultimately leading to a higher extinction risk. I found weaker support for the slow life history hypothesis and no significant support for the energetics hypothesis.

Marginal habitats hypothesis:

My findings support the hypothesis that chemically defended species are more likely to occupy marginal (low carrying capacity) habitats which in turn leads to their greater extinction risk (Arbuckle 2016). In both the threat and decline models, chemically defended species were consistently associated with a higher proportion of marginal habitats. Notably, chemical defence itself did not directly predict extinction risk in either model. This suggests that the relationship is indirect, with chemical defence facilitating the occupancy of marginal habitats, which then increases vulnerability. The specific mechanisms by which chemical defence facilitates survival in marginal habitats are explored below.

Habitats can be considered marginal (or low carrying capacity) for a variety of reasons, often relating to lower food availability, increased predation or disease (Kawecki 2008). Firstly, low or unstable food availability can be a prominent feature of marginal habitats but species with chemical defence may be better equipped to withstand these conditions. Some species have been shown to upregulate toxins in response to low food availability (Üveges et al 2017). This upregulation may allow amphibians to remain defended during periods of resource scarcity, thus minimising the energy required for predator avoidance when the amphibian is already physiologically stressed due to malnutrition. This strategy, while advantageous in the short term, could lead to long term physiological stress and lowered reproductive output, further contributing to their extinction risk. These findings suggest that chemical defence may play a multifaceted role in adapting animals to marginal habitats, but that these adaptations may come at a cost, potentially contributing to their elevated extinction risk.

As mentioned, high predation can also be a feature of marginal habitats. For instance, low carrying capacity environments may have lower predator diversity, but potentially more intense predation as predators are resource-limited and therefore have to be more thorough in their use of prey resources that exist in the habitat. Chemical defence acts as a highly effective method of predator deterrence (Heethoff and Rall 2015; Toledo and Jared 1995), freeing up energy for vital functions like feeding and reproduction. This provides a clear advantage over non-defended species that are restricted from moving into predator-rich marginal habitats, due to predation pressure. Some toxic amphibian species have the ability to flexibly upregulate toxin production (Bucciarelli et al 2017), in some instances in response to the presence or density of predators (Hettyey et al 2019; Benard and Fordyce 2003; Hagman et al 2009), However, other studies find no influence (Üveges et al 2017; Üveges et al 2019; Bókony et al 2016). It is suggested that a lack of inducible defences in some tadpole studies could be explained by the high level of unpredictability of predator presence (as predators can move between ponds whereas larvae are restricted to their pond) combined with the high cost of incorrectly downregulating toxin production (i.e. death of tadpoles). Therefore, despite the immediate benefits of chemical defence in predator-rich marginal habitats, the observed inconsistencies in toxin plasticity and the high costs of mismanaging this defence highlight a potential vulnerability that could exacerbate extinction risk for these species in unpredictable environments.

Marginal habitats can also be defined by suboptimal environments, such as those with high pathogen loads. Several studies have revealed the diverse arsenal of amphibians' chemical defence mechanisms to be highly resistant against a variety of pathogens and parasites (Calhoun et al 2016; Calhoun et al 2017; Barnhart et al 2017). For example, some species secrete antimicrobial peptides (AMPs) onto their skin, inhibiting bacterial, viral and fungal growth (Rollins-Smith et al 2023; Chinchar et al 2004). While these defences significantly enhance survival in pathogen-rich environments, producing and maintaining these compounds can impose physiological costs that ultimately increase extinction risk, outweighing the initial benefits of pathogen protection.

Marginal habitats predicted decline, but they did not predict threat**.** One explanation for this could be that chemical defence may enable defended species to occupy a wider range of habitats overall, leading to initially larger populations or a broader geographic spread compared to non-defended species (Arbuckle et al 2013). Consequently, even if these more robust species experience declines, they may not yet meet the criteria for a change in IUCN threat status (IUCN Standards and Petitions Committee 2024). This situation is exacerbated if a higher proportion of habitats occupied by defended species are marginal, making them inherently more susceptible to declines (Kawecki 2008). This dynamic can be explained by source-sink dynamics, whereby a species exists in a network of high-quality ‘source’ habitats and lower-quality ‘sink’ habitats, where the sink population would decline without immigration from source populations (Amarasekare and Nisbet 2001). Marginal habitats might function as ‘sink’ habitats due to their various increased stressors, with declining populations sustained by immigration from ‘source’ or ‘suitable’ habitats. This is a precarious situation, because if environmental changes cause the connection between the source and the sink to be disrupted, the sink populations will be vulnerable to rapid decline and eventual extinction (Kawecki 2008). The constant influx from suitable or ‘source’ habitats could potentially mask the true extent of the decline in the marginal habitats, maintaining populations at a level that doesn't yet trigger a change in threat status, thus leading to underestimation of a species’ vulnerability.

Furthermore, while geographic distribution and range size are a critical component of these assessments, they were not included as a variable in the SEMs. This was so the focus remained on intrinsic biological traits and so the models avoided over-parameterization. Future research incorporating geographic range as a mediating variable would clarify whether chemical defence and marginal habitat use influence risk independently or if they are primarily correlates of a species’ total spatial footprint.

In addition to influencing population dynamics, marginal habitats can also have significant evolutionary consequences, as they can lead to species becoming isolated from the source population, preventing gene flow, which allows for independent evolution (Kawecki et al 2008). Organisms in marginal habitats face different environmental pressures to those in source populations, forcing them to adapt to new niches, which can lead to significant divergence from the original population (Kawecki et al 2008). For this reason, the costs of producing chemical defences could be countered by facilitating increased speciation opportunities.

Future studies should investigate potential nuances in the hypothesis by considering which specific habitat types of marginal and suitable habitats are associated with extinction risk of defended and non-defended amphibians, moving beyond solely focusing on the proportion of marginal to suitable. It could be that this hypothesis is contingent on ecological conditions that vary across habitat types. An alternative approach could be to investigate if there are habitat types which are inherently challenging for amphibians to survive in, and if so, if chemically defended species are more likely to exist and persist in those habitats. Elucidating high-risk habitat types in this way could provide valuable insight that could refine the application of this hypothesis, leading to more impactful conservation strategies.

Slow life history hypothesis:

My results found partial support for the slow life history hypothesis, which proposes that chemically defended species are more likely to exhibit a slow life history, leading to a higher extinction risk due to a weaker ability to recover after declines. The life histories of declining species are usually characterised by low fecundity and long periods before maturity (Purvis et al 2000; Williams and Hero 1998; Hero et al 2005), whilst non-declining species tend to demonstrate a fast life history (Williams and Hero 1998; Pianka 1970).

I found both the expected negative inverse relationship between clutch size and egg size and that egg size positively predicted extinction risk in both models. In line with r-K selection theory (Pianka 1970), a trade-off must be made between clutch size and egg size, due to females' finite egg-carrying capacity and reproductive reserves (Gould et al 2022; Smith and Fretwell 1974). This generally results in an inverse relationship between egg size and clutch size, with selection favouring either the production of a few large eggs or many small eggs (Liedtke et al 2014; Gould et al 2022; Gould et al 2020). Species with larger eggs (and therefore smaller clutch sizes) may have lower reproductive rates, potentially hindering recovery from population declines (Pincheiro-Donoso et al 2021; Cardillo 2005; Isaac et al 2009; Purvis et al 2000; Bennett and Owens 1997; Pimm et al 1988). This likely explains why my results showed large eggs to result in a higher extinction risk.

An unexpected finding in my results was the positive relationship between chemical defence and clutch size in both models, which contradicts the egg size result when considering the egg size and clutch size trade-off. It also contrasts previous findings in mustelids and invertebrates (Arbuckle et al 2013; Ivanisevic et al 2011; Nazareth and Machado 2015) and goes against the slow life history hypothesis. It could be that chemically defended amphibians are able to invest in larger clutches, as the smaller amount of energy invested in predator avoidance frees up resources for reproduction. In addition to adults, the eggs (Hayes et al 2009; Gall et al 2014) and larvae (Bucciarelli et al 2017; Üveges et al 2019; Bókony et al 2016; Gall et al 2011) of many chemically defended amphibian species also contain toxins. Research shows that strawberry poison dart frog (*Oophaga pumilio*) mothers provision tadpoles with alkaloids for many weeks after hatching and that the quantity of alkaloids increases with development (Stynoski et al 2014b). In this species, maternally-provisioned alkaloids reduce predation against some, however not all species of predator (Stynoski et al 2014b; 2014a), so they may still rely on having large clutch sizes to counteract the reduced, albeit still present effect of predation.

Alternatively, or in conjunction with this, it is possible that species capable of investing in costly chemical defences could also be those with higher overall resource acquisition abilities, effectively masking a potential trade-off with reproduction. This explanation is supported by literature demonstrating that total resource availability is a key confounding factor in correlative studies of life-history trade-offs (van Noordwijk and de Jong 1986; Congdon et al 2001). These findings underscore the complex interplay of factors influencing clutch size and chemically defended species, highlighting the need for further research to fully understand the ecological and evolutionary implications.

Clutch size partially predicted extinction risk, as it negatively predicted threat but did not predict decline. Similarly, Cooper et al (2008) found no direct link between clutch size and extinction risk in frogs, once range size was controlled for. It was suggested that a direct link between clutch size and extinction risk is often obscured by other factors, particularly geographical range size. Cooper et al also emphasised the importance of including phylogeny as a factor when considering extinction risk, as done in my study, as only some of the existing studies do so (Hero et al 2005; Murray and Hose 2005). Additionally, previous research may only reflect regional not global patterns, as studies focused exclusively on Australia (Hero et al 2005; Murray and Hose 2005; Williams and Hero 1998) or Central America (Lips et al 2003). It could therefore be the case that on a global scale and when considering phylogeny, clutch size is not as strongly linked to extinction risk as previous studies have implied. This could explain the differing results between my threat and decline models. The lack of a predictive relationship with decline could suggest that long term declines are driven by factors operating on different timescales than those influenced by clutch size. It is crucial to acknowledge that factors such as habitat loss or disease may still drive species declines, even in species with large clutch sizes, as these threats may operate on longer timescales and could override the effects of reproductive output. As discussed, species with smaller clutch sizes are likely to exhibit slower recovery periods in response to adverse conditions and so may experience declines as they are unable to bounce back after stochastic events. Alternatively, there might be other complex interactions between chemical defence and other life history traits that are not yet fully understood. Further research is needed to explore these potential explanations, to determine the precise role of chemical defence in shaping life history strategies.

Energetics hypothesis:

My results indicate that the energetics hypothesis, is not a strong explanation. Neither model found any significant relationships between chemical defence, body size or extinction risk. This was unexpected, as there is strong prevailing evidence that chemical defence is costly (Bucciarelli et al 2017), as toxin replenishment requires synthesis of complex compounds, involving mobilisation and breaking down of precursors and secretion of final products (Hayes 2008; Nisani et al 2012). Additional energy costs arise as resources are allocated away from other functions (Bucciarelli et al 2017; Blennerhasset et al 2019; Fordyce et al 2006; Smith et al 2014) and more oxygen is consumed after depletion of venom stores (Enzor et al 2011; Harris and Jenner 2019; Morgenstern and King 2013; Hayes 2008, McCue 2006, Smith et al 2014; Nisani 2012), further emphasising the metabolic strain chemical defence puts on an organism. Due to the wealth of existing knowledge that biosynthesis and maintenance of chemical defences are indeed energetically costly, it is possible that the metric employed to assess these energetic requirements was not sufficiently representative.

The use of an imperfect proxy of energetic requirement (body size: SVL for Anura and TL for Gymnophiona and Caudata) could have impacted on why this hypothesis did not show significance. This proxy was used due to the established allometric relationship between body size and metabolic rate (as exemplified by the ¾ power law) (Brown et al 2004; Brown et al 2005) which suggests that SVL and TL could serve as reasonable, if imperfect proxies in this context, where there is a paucity of nutritional data for the vast majority of species. Nutritional research is biased towards species held in captivity, especially anurans (Ferrie et al 2014; Livingston et al 2014). Data on wild diets, which would likely be more useful, is even more scarce (Ferrie et al 2014). However, this data is unlikely to be made available on the scale needed for a study of this size.

Another explanation for the lack of significance in the energetics hypothesis is that geographic range size mediates the effect of body size (Cooper et al 2008), thus making body size alone a less accurate predictor of extinction risk. There are strong positive associations between body size and range size, where smaller species are more likely to be constrained to narrow distributions, making them more vulnerable to stochastic events (Cooper at al 2008). Additionally, species with wider ranges may be less vulnerable to localised threats, as population declines in some areas may not affect their overall threat status if populations persist elsewhere (Cooper at al 2008). A negative size-threat association is seen in amphibians even after controlling for the effects of range size (Cardillo et al 2021). This suggests that other intrinsic features or ecological interactions are also involved in increasing extinction risk, beyond mere geographic distribution.

Furthermore, a critical distinction should be made- many studies documenting high costs focus on venoms, which are typically *de novo* biosynthesised proteins. In contrast, a significant proportion of amphibian toxins are sequestered from dietary sources or their metabolites. This dietary acquisition strategy provides an inherent ‘energy bonus’, as the energy contained within these ingested compounds contributes to the overall energy budget (Pough and Taigen 1990; Savitzky et al 2012). This could, to some extent, ease the apparent trade-off between defence and other life history strategies and potentially mask the underlying energetic costs that would be more apparent if the toxins were solely synthesized internally.

Conclusions

This phylogenetic comparative study aimed to disentangle the indirect mechanisms that could explain the seemingly counterintuitive link between chemical defence and elevated extinction risk in amphibians. Structural equation models (SEMs) tested three key hypotheses: the marginal habitats hypothesis, the slow life history hypothesis and the energetics hypothesis.

My findings provide strong support for the marginal habitats hypothesis. The SEMs consistently showed that chemical defence positively predicts the proportion of marginal habitats occupied by amphibian species and, ultimately, that presence in these inherently unstable habitats significantly predicts population declines. This suggests that chemical defence, despite offering the benefit of reduced predation, indirectly increases extinction risk by enabling species to exist in environments that are more prone to stochastic events. The indirect nature of this relationship highlights that the benefits of defence in marginal habitats may ultimately be outweighed by the intrinsic instability of these environments, potentially masked in the short-term by source-sink dynamics.

Support for the slow life history hypothesis was weaker and more nuanced. Whilst egg size positively predicted both threat and decline, there was an unexpected positive relationship between chemical defence and clutch size in both models, which contradicted the theory that defended species would exhibit slower life histories. This suggests a more complex interplay, where chemical defence may be beneficial by freeing up resources for reproduction or simply that chemically defended species may possess a larger overall energy budget. The lack of a direct link between clutch size and decline, particularly when accounting for phylogeny and global scale, also indicates that other factors may obscure or override this relationship.

The energetics hypothesis received no significant support in this study. Despite previous studies providing evidence for metabolic costs of toxin production, this outcome suggests that the proxy used (body size) could potentially have been insufficient, however the ideal data is currently not available. Alternatively, the energy dynamics from sequestered toxins may differ from *de novo* biosynthesis, potentially mitigating perceived costs.

In conclusion, these results confirm the link between chemical defence and increased amphibian extinction risk, with the marginal habitats hypothesis emerging as a compelling indirect explanation. This underscores the importance of considering a species' full ecological context, particularly habitat dynamics when assessing their vulnerability and allocating conservation resources.

Future research could explore the extinction risk of chemically defended species in the context of specific marginal habitat characteristics, in order to elucidate particularly high-risk habitat types. Additionally, incorporating geographic range size as a mediating variable could clarify whether these traits influence risk directly or via the facilitation of broader spatial distributions. This is especially warranted due to its central role within IUCN Red List assessments. Further investigation is also warranted into chemical defence and life history traits, particularly the unexpected relationship with clutch size. Finally, future energetics studies should differentiate between *de novo* and sequestered toxins, to understand the nuances between species' extinction risks more clearly.

References:

Allen, W. L., Street, S. E., & Capellini, I. (2017). Fast life history traits promote invasion success in amphibians and reptiles. *Ecology Letters*, *20*(2), 222–230. https://doi.org/10.1111/ele.12728

Alvarez-Buylla, A., Payne, C. Y., Vidoudez, C., Trauger, S. A., & O'Connell, L. A. (2022). Molecular physiology of pumiliotoxin sequestration in a poison frog. *PLOS ONE*, *17*(3), e0264540. https://doi.org/10.1371/journal.pone.0264540

Amarasekare, P., & Nisbet, Roger M. (2001). Spatial Heterogeneity, Source-Sink Dynamics, and the Local Coexistence of Competing Species. *The American Naturalist*, *158*(6), 572–584. https://doi.org/10.1086/323586

Arbuckle, K. (2016). Chemical antipredator defence is linked to higher extinction risk. *Royal Society Open Science*, *3*(11), 160681. https://doi.org/10.1098/rsos.160681

Arbuckle, K., Brockhurst, M., & Speed, M. P. (2013). Does chemical defence increase niche space? A phylogenetic comparative analysis of the Musteloidea. *Evolutionary Ecology*, *27*(5), 863–881. https://doi.org/10.1007/s10682-013-9629-z

Arbuckle, K., & Speed, M. P. (2015). Antipredator defenses predict diversification rates. *Proceedings of the National Academy of Sciences*, *112*(44), 13597–13602. https://doi.org/10.1073/pnas.1509811112

Ausden, M., & Walsh, J. C. (2020). *The use of evidence in decision-making by practitioners*. Research.monash.edu; Cambridge University Press. https://research.monash.edu/en/publications/the-use-of-evidence-in-decision-making-by-practitioners

Barlow, A., Pook, C. E., Harrison, R. A., & Wüster, W. (2009). Coevolution of diet and prey-specific venom activity supports the role of selection in snake venom evolution. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1666), 2443–2449. https://doi.org/10.1098/rspb.2009.0048

Barnhart, K. L., Forman, M. E., Umile, T. P., Kueneman, J. G., McKenzie, V. J., Salinas, I., Kevin, & Woodhams, D. C. (2017). Identification of Bufadienolides from the Boreal Toad, Anaxyrus boreas, Active Against a Fungal Pathogen. *Microbial Ecology*, *74*(4), 990–1000. https://doi.org/10.1007/s00248-017-0997-8

Benard, M. F., & Fordyce, J. A. (2003). Are Induced Defenses Costly? Consequences of Predator Induced Defenses in Western Toads, Bufo boreas. *Ecology*, *84*(1), 68–78. JSTOR. https://doi.org/10.2307/3107998

Bennett, P. M., & Owens, I. P. F. (1997). Variation in extinction risk among birds: chance or evolutionary predisposition? *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *264*(1380), 401–408. https://doi.org/10.1098/rspb.1997.0057

Berger, L. (1971). Sex ratio in the F1 progeny within forms of Rana esculenta complex. *Genetica Polonica*, *12*(1-2). https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-5067700b-5ace-41b0-a0a2-cbdad2cab089

Bergman, J., Pedersen, R. Ø., Lundgren, E. J., Trepel, J., Pearce, E. A., Szymon Czyżewski, Tietje, M., Lemoine, R. T., Moisès Coll Macià, Schierup, M. H., & Jens-Christian Svenning. (2024). Trade-offs beget trade-offs: Causal analysis of mammalian population dynamics. *BioRxiv (Cold Spring Harbor Laboratory)*, *2024-08. Biological Conservation*(113), 367–377. https://doi.org/10.1101/2024.08.16.608243

Bielby, J., Cooper, N., Cunningham, A. A., Garner, T. W. J., & Purvis, A. (2008). Predicting susceptibility to future declines in the world's frogs. *Conservation Letters*, *1*(2), 82–90. https://doi.org/10.1111/j.1755-263x.2008.00015.x

Blanco, M. A., & Sherman, P. W. (2005). Maximum longevities of chemically protected and non-protected fishes, reptiles, and amphibians support evolutionary hypotheses of aging. *Mechanisms of Ageing and Development*, *126*(6-7), 794–803. https://doi.org/10.1016/j.mad.2005.02.006

Bland, L. M. (2017). Global correlates of extinction risk in freshwater crayfish. *Animal Conservation*, *20*(6), 532–542. https://doi.org/10.1111/acv.12350

Blennerhassett, R. A., Bell-Anderson, K., Shine, R., & Brown, G. P. (2019). The cost of chemical defence: the impact of toxin depletion on growth and behaviour of cane toads (Rhinella marina). *Proceedings of the Royal Society B: Biological Sciences*, *286*(1902). https://doi.org/10.1098/rspb.2019.0867

Bókony, V., Móricz, Á. M., Zsófia Tóth, Zoltán Gál, Anikó Kurali, Zsanett Mikó, Katalin Pásztor, Márk Szederkényi, Zoltán Tóth, János Ujszegi, Bálint Üveges, Dániel Krüzselyi, Capon, R. J., Hoi, H., & Attila Hettyey. (2016). Variation in Chemical Defense Among Natural Populations of Common Toad, Bufo bufo, Tadpoles: the Role of Environmental Factors. *Journal of Chemical Ecology*, *42*(4), 329–338. https://doi.org/10.1007/s10886-016-0690-2

Bókony, V., Üveges, B., Verebélyi, V., Ujhegyi, N., & Móricz, Á. M. (2019). Toads phenotypically adjust their chemical defences to anthropogenic habitat change. *Scientific Reports*, *9*(1), 3163. https://doi.org/10.1038/s41598-019-39587-3

Brashares, J. S. (2003). Ecological, Behavioral, and Life-History Correlates of Mammal Extinctions in West Africa. *Conservation Biology*, *17*(3), 733–743. https://doi.org/10.1046/j.1523-1739.2003.01592.x

Britnell, J. A., Zhu, Y., Kerley, G. I. H., & Shultz, S. (2023). Ecological marginalization is widespread and increases extinction risk in mammals. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(3), e2205315120. https://doi.org/10.1073/pnas.2205315120

Brizzi, R., & Corti, C. (2007). Cutaneous antipredatory secretions and pheromones in anurans and urodeles. *Marine and Freshwater Behaviour and Physiology*, *40*(3), 225–231. https://doi.org/10.1080/10236240701557016

Brown , J. H., West, G. B., & Enquist , B. J. (2005). Yes, West, Brown and Enquist's Model of Allometric Scaling Is Both Mathematically Correct and Biologically Relevant. *Functional Ecology*, *19*(4), 735–738. JSTOR. https://doi.org/10.2307/3599236

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*(7), 1771–1789. https://doi.org/10.1890/03-9000

Brown, J. L., Twomey, E., A. Amézquita, Souza, M. B., Caldwell, J. P., S. Lötters, May, R., P.R. Melo-Sampaio, D. Mejía-Vargas, P. Perez-Peña, Pepper, M., Poelman, E. H., M. Sanchez-Rodriguez, & Summers, K. (2011). A taxonomic revision of the Neotropical poison frog genus Ranitomeya (Amphibia: Dendrobatidae). *Zootaxa*, *3083*, 1–120. https://research.wur.nl/en/publications/a-taxonomic-revision-of-the-neotropical-poison-frog-genus-ranitom

Bucciarelli, G. M., Shaffer, H. B., Green, D. B., & Kats, L. B. (2017). An amphibian chemical defense phenotype is inducible across life history stages. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-08154-z

Calhoun, D. M., Bucciarelli, G. M., Kats, L. B., Zimmer, R. K., & Johnson, P. T. J. (2017). Noxious newts and their natural enemies: Experimental effects of tetrodotoxin exposure on trematode parasites and aquatic macroinvertebrates. *Toxicon*, *137*, 120–127. https://doi.org/10.1016/j.toxicon.2017.07.021

Calhoun, D. M., Woodhams, D., Howard, C., LaFonte, B. E., Gregory, J. R., & Johnson, P. T. J. (2016). Role of Antimicrobial Peptides in Amphibian Defense Against Trematode Infection. *EcoHealth*, *13*(2), 383–391. https://doi.org/10.1007/s10393-016-1102-3

Campbell Grant, E. H., Miller, D. A. W., & Muths, E. (2020). A Synthesis of Evidence of Drivers of Amphibian Declines. *Herpetologica*, *76*(2), 101. https://doi.org/10.1655/0018-0831-76.2.101

Cardillo, M. (2005). Multiple Causes of High Extinction Risk in Large Mammal Species. *Science*, *309*(5738), 1239–1241. https://doi.org/10.1126/science.1116030

Cardillo, M. (2021). Clarifying the relationship between body size and extinction risk in amphibians by complete mapping of model space. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1944), 20203011. https://doi.org/10.1098/rspb.2020.3011

Caro, T., & Ruxton, G. (2019). Aposematism: Unpacking the Defences. *Trends in Ecology & Evolution*, *34*(7), 595–604. https://doi.org/10.1016/j.tree.2019.02.015

Carvajal-Castro, J. D., Vargas-Salinas, F., Casas-Cardona, S., Rojas, B., & Santos, J. C. (2021). Aposematism facilitates the diversification of parental care strategies in poison frogs. *Scientific Reports*, *11*(1), 19047. https://doi.org/10.1038/s41598-021-97206-6

Cejp, B., & Griebeler, E. (2024). Body Mass Shapes Most Life History Traits and a Fast-Slow Continuum in Amphibians. *Ecology and Evolution*, *14*(10). https://doi.org/10.1002/ece3.70377

Chen, K. K., & Chen, A. (1933). Notes on the poisonous secretions of twelve species of toads. *The Journal of Pharmacology and Experimental Therapeutics*, *47.3*, 281–293.

Chinchar, V. G., Bryan, L., Silphadaung, U., Noga, E., Wade, D., & Rollins-Smith, L. (2004). Inactivation of viruses infecting ectothermic animals by amphibian and piscine antimicrobial peptides. *Virology*, *323*(2), 268–275. https://doi.org/10.1016/j.virol.2004.02.029

Chippaux, J.-P. ., Williams, V., & White, J. (1991). Snake venom variability: methods of study, results and interpretation. *Toxicon*, *29*(11), 1279–1303. https://doi.org/10.1016/0041-0101(91)90116-9

Coleman, J. L., & Cannatella, D. C. (2023). The molecular basis and evolution of toxin resistance in poison frogs. *Evolutionary Ecology*, *38*(5), 747–780. https://doi.org/10.1007/s10682-023-10258-0

Congdon, J. D., Dunham, A. E., Hopkins, W. A., Rowe, C. L., & Hinton, T. G. (2001). Resource allocation-based life histories: A conceptual basis for studies of ecological toxicology. *Environmental Toxicology and Chemistry*, *20*(8), 1698–1703. https://doi.org/10.1002/etc.5620200811

Cooper, N., Bielby, J., Thomas, G. H., & Purvis, A. (2008). Macroecology and extinction risk correlates of frogs. *Global Ecology and Biogeography*, *17*(2), 211–221. https://doi.org/10.1111/j.1466-8238.2007.00355.x

Darst, Catherine R., Menéndez-Guerrero, Pablo A., Coloma, Luis A., & Cannatella, David C. (2005). Evolution of Dietary Specialization and Chemical Defense in Poison Frogs (Dendrobatidae): A Comparative Analysis. *The American Naturalist*, *165*(1), 56–69. https://doi.org/10.1086/426599

Davidson, A. D., Hamilton, M. J., Boyer, A. G., Brown, J. H., & Ceballos, G. (2009). Multiple ecological pathways to extinction in mammals. *Proceedings of the National Academy of Sciences*, *106*(26), 10702–10705. https://doi.org/10.1073/pnas.0901956106

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, *105*(18), 6668–6672. https://doi.org/10.1073/pnas.0709472105

Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and Plants: A Study in Coevolution. *Evolution*, *18*(4), 586. https://doi.org/10.2307/2406212

Enzor, L. A., Wilborn, R. E., & Bennett, W. A. (2011). Toxicity and metabolic costs of the Atlantic stingray (Dasyatis sabina) venom delivery system in relation to its role in life history. *Journal of Experimental Marine Biology and Ecology*, *409*(1), 235–239. https://doi.org/10.1016/j.jembe.2011.08.026

Ferrie, G. M., Alford, V. C., Atkinson, J., Baitchman, E., Barber, D., Blaner, W. S., Crawshaw, G., Daneault, A., Dierenfeld, E., Finke, M., Fleming, G., Gagliardo, R., Hoffman, E. A., Karasov, W., Klasing, K., Koutsos, E., Lankton, J., Lavin, S. R., Lentini, A., & Livingston, S. (2014). Nutrition and health in amphibian husbandry. *Zoo Biology*, *33*(6), 485–501. https://doi.org/10.1002/zoo.21180

Fisher, D. O., & Owens, I. P. F. (2004). The comparative method in conservation biology. *Trends in Ecology & Evolution*, *19*(7), 391–398. https://doi.org/10.1016/j.tree.2004.05.004

Fordyce, J. A., Nice, C. C., & Shapiro, A. M. (2006). A Novel Trade-off of Insect Diapause Affecting a Sequestered Chemical Defense. *Oecologia*, *149*(1), 101–106. https://doi.org/10.1007/s00442-006-0428-x

Gall, B. G., Stokes, A. N., French, S. S., Schlepphorst, E. A., Brodie, E. D., & Brodie, E. D. (2011). Tetrodotoxin levels in larval and metamorphosed newts (Taricha granulosa) and palatability to predatory dragonflies. *Toxicon*, *57*(7-8), 978–983. https://doi.org/10.1016/j.toxicon.2011.03.020

Gall, B. G., Stokes, A. N., Pett, J. J., Spivey, K. L., French, S. S., Brodie, E. D., & Brodie, E. D. (2014). Tetrodotoxin concentrations within a clutch and across embryonic development in eggs of the rough-skinned newts (Taricha granulosa). *Toxicon*, *90*, 249–254. https://doi.org/10.1016/j.toxicon.2014.08.060

García, V. B., Lucifora, L. O., & Myers, R. A. (2008). The importance of habitat and life history to extinction risk in sharks, skates, rays and chimaeras. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1630), 83–89. https://doi.org/10.1098/rspb.2007.1295

Gould, J., Beranek, C., Valdez, J., & Mahony, M. (2020). Quality versus quantity: The balance between egg and clutch size among Australian amphibians is related to life history and environmental conditions. *BioRxiv (Cold Spring Harbor Laboratory)*, *03*. https://doi.org/10.1101/2020.03.15.992495

Gould, J., Beranek, C., Valdez, J., & Mahony, M. (2022). Quantity *versus* quality: A balance between egg and clutch size among Australian amphibians in relation to other life-history variables. *Austral Ecology*, *47*(3), 685–697. https://doi.org/10.1111/aec.13154

Grace, J. B. (2006). *Structural Equation Modeling and Natural Systems*. https://doi.org/10.1017/cbo9780511617799

Hagman, M., A, H. R., J, C. R., & Shine, R. (2009). Alarm Cues Experienced by Cane Toad Tadpoles Affect PostMetamorphic Morphology and Chemical Defences. *Functional Ecology*, *23*(1), 126–132. JSTOR. https://doi.org/10.2307/40205509

Hardenberg, A. von, & Gonzalez-Voyer, A. (2013). Disentangling evolutionary cause-effect relationships with phylogenetic confirmatory path analysis. *Evolution*, *67*(2), 378–387. https://doi.org/10.1111/j.1558-5646.2012.01790.x

Harnik, P. G. (2011). Direct and indirect effects of biological factors on extinction risk in fossil bivalves. *Proceedings of the National Academy of Sciences*, *108*(33), 13594–13599. https://doi.org/10.1073/pnas.1100572108

Harris, R. J., & Jenner, R. A. (2019). Evolutionary Ecology of Fish Venom: Adaptations and Consequences of Evolving a Venom System. *Toxins*, *11*(2), 60. https://doi.org/10.3390/toxins11020060

Hayes, F. E. (1989). Antipredator Behavior of Recently Metamorphosed Toads (Bufo a. americanus) during Encounters with Garter Snakes (Thamnophis s. sirtalis). *Copeia*, *1989*(4), 1011. https://doi.org/10.2307/1445987

Hayes, R. A., Crossland, M. R., Hagman, M., Capon, R. J., & Shine, R. (2009). Ontogenetic Variation in the Chemical Defenses of Cane Toads (Bufo marinus): Toxin Profiles and Effects on Predators. *Journal of Chemical Ecology*, *35*(4), 391–399. https://doi.org/10.1007/s10886-009-9608-6

Hayes, W. (2008, January 1). *The Snake Venom-Metering Controversy: Levels of Analysis, Assumptions, and Evidence*. https://www.researchgate.net/publication/259337962_The_Snake_Venom-Metering_Controversy_Levels_of_Analysis_Assumptions_and_Evidence

Hayes, W. K., Herbert, S. S., Rehling, G. C., & Gennaro, J. F. (2002, January 1). *Factors that influence venom expenditure in viperids and other snake species during predatory and defensive contexts*. Biology of the Vipers (pp.207-233). https://www.researchgate.net/publication/259338085_Factors_that_influence_venom_expenditure_in_viperids_and_other_snake_species_during_predatory_and_defensive_contexts

Heethoff, M., & Rall, B. C. (2015). Reducible defence: chemical protection alters the dynamics of predator–prey interactions. *Chemoecology*, *25*(2), 53–61. https://doi.org/10.1007/s00049-014-0184-z

Heiss, E., Natchev, N., Salaberger, D., Gumpenberger, M., Rabanser, A., & Weisgram, J. (2010). Hurt yourself to hurt your enemy: new insights on the function of the bizarre antipredator mechanism in the salamandrid Pleurodeles waltl. *Journal of Zoology*, *280*(2), 156–162. https://doi.org/10.1111/j.1469-7998.2009.00631.x

Hernández-Yáñez, H., Kim, S. Y., & Che-Castaldo, J. P. (2022). Demographic and life history traits explain patterns in species vulnerability to extinction. *PLOS ONE*, *17*(2), e0263504. https://doi.org/10.1371/journal.pone.0263504

Hero, J.-M., Williams, S. B., & Magnusson, W. E. (2005). Ecological traits of declining amphibians in upland areas of eastern Australia. *Journal of Zoology*, *267*(3), 221–232. https://doi.org/10.1017/s0952836905007296

Hettyey, A., Üveges, B., Móricz, Á. M., Drahos, L., Capon, R. J., Van Buskirk, J., Tóth, Z., & Bókony, V. (2019). Predator-induced changes in the chemical defence of a vertebrate. *Journal of Animal Ecology*, *88*(12), 1925–1935. https://doi.org/10.1111/1365-2656.13083

Hossie, T. J., Hassall, C., Knee, W., & Sherratt, T. N. (2013). Species with a chemical defence, but not chemical offence, live longer. *Journal of Evolutionary Biology*, *26*(7), 1598–1602. https://doi.org/10.1111/jeb.12143

Hudson, C. M., Brown, G. P., & Shine, R. (2017). Evolutionary shifts in anti-predator responses of invasive cane toads (Rhinella marina). *Behavioral Ecology and Sociobiology*, *71*(9). https://doi.org/10.1007/s00265-017-2367-4

Hutchings, J. A., Butchart, S. H. M., Collen, B., Schwartz, M. K., & Waples, R. S. (2012a). Red flags: correlates of impaired species recovery. *Trends in Ecology & Evolution*, *27*(10), 542–546. https://doi.org/10.1016/j.tree.2012.06.005

Hutchings, J. A., Myers, R. A., García, V. B., Lucifora, L. O., & Kuparinen, A. (2012b). Life-history correlates of extinction risk and recovery potential. *Ecological Applications*, *22*(4), 1061–1067. https://doi.org/10.1890/11-1313.1

Iriondo, J. M., AlbertM. J., & Escudero, A. (2003). Structural equation modelling: an alternative for assessing causal relationships in threatened plant populations. *Biological Conservation*, *113*(3), 367–377. https://doi.org/10.1016/s0006-3207(03)00129-0

Isaac, J. L., Vanderwal, J., Johnson, C. N., & Williams, S. E. (2009). Resistance and resilience: quantifying relative extinction risk in a diverse assemblage of Australian tropical rainforest vertebrates. *Diversity and Distributions*, *15*(2), 280–288. https://doi.org/10.1111/j.1472-4642.2008.00531.x

IUCN Standards and Petitions Committee. (2024). *Guidelines for Using the IUCN Red List Categories and Criteria Version 16*. Prepared by the Standards and Petitions Committee. https://www.iucnredlist.org/documents/RedListGuidelines.pdf

IUCN Version 2024-2. (2024). *The IUCN Red List of Threatened Species*. IUCN Red List of Threatened Species. https://www.iucnredlist.org.

Ivanisevic, J., Thomas, O. P., Pedel, L., Pénez, N., Ereskovsky, A. V., Culioli, G., & Perez, T. (2011). Biochemical trade-offs: evidence for ecologically linked secondary metabolism of the sponge Oscarella balibaloi. *PLoS One*, *6*(11), e28059.

Jared, C., Antoniazzi, M. M., Jordão, A. E. C., Silva, J. R. M. C., Greven, H., & Rodrigues, M. T. (2009). Parotoid macroglands in toad (Rhinella jimi): Their structure and functioning in passive defence. *Toxicon*, *54*(3), 197–207. https://doi.org/10.1016/j.toxicon.2009.03.029

Jared, C., Mailho-Fontana, P. L., Marques-Porto, R., Sciani, J. M., Pimenta, D. C., Brodie, E. D., & Antoniazzi, M. M. (2018). Skin gland concentrations adapted to different evolutionary pressures in the head and posterior regions of the caecilian Siphonops annulatus. *Scientific Reports*, *8*(1), 3576. https://doi.org/10.1038/s41598-018-22005-5

Jared, C., Mailho-Fontana, P., Antoniazzi, M., Mendes, V., Barbaro, K., Rodrigues, M., & Brodie, Edmund D. (2015). Venomous Frogs Use Heads as Weapons. *Current Biology*, *25*(16), 2166–2170. https://doi.org/10.1016/j.cub.2015.06.061

Jared, S. G. S., Jared, C., Egami, M. I., Mailho-Fontana, P. L., Rodrigues, M. T., & Antoniazzi, M. M. (2014). Functional assessment of toad parotoid macroglands: a study based on poison replacement after mechanical compression. *Toxicon: Official Journal of the International Society on Toxinology*, *87*, 92–103. https://doi.org/10.1016/j.toxicon.2014.05.020

Jeppsson, T., & Forslund, P. (2012). Can Life History Predict the Effect of Demographic Stochasticity on Extinction Risk? *The American Naturalist*, *179*(6), 706–720. https://doi.org/10.1086/665696

Johnson, C. N. (2002). Determinants of loss of mammal species during the Late Quaternary "megafauna" extinctions: life history and ecology, but not body size. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1506), 2221–2227. https://doi.org/10.1098/rspb.2002.2130

Jones, M. J., Fielding, A., & Sullivan, M. (2006). Analysing Extinction Risk in Parrots using Decision Trees. *Biodiversity and Conservation*, *15*(6), 1993–2007. https://doi.org/10.1007/s10531-005-4316-1

Kawecki, T. J. (2008). Adaptation to Marginal Habitats. *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 321–342. https://doi.org/10.1146/annurev.ecolsys.38.091206.095622

Kowalski, K., Sawościanik, O., & Rychlik, L. (2018). Do Bufonids Employ Different Anti-Predator Behaviors Than Ranids? Comparison among Three European Anurans. *Copeia*, *106*(1), 120–129. https://doi.org/10.1643/ce-16-567

Lee, T. M., & Jetz, W. (2010). Unravelling the structure of species extinction risk for predictive conservation science. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1710), 1329–1338. https://doi.org/10.1098/rspb.2010.1877

Liedtke, H. C., Müller, H., Hafner, J., Nagel, P., & Loader, S. P. (2014). Interspecific patterns for egg and clutch sizes of African Bufonidae (Amphibia: Anura). *Zoologischer Anzeiger - a Journal of Comparative Zoology*, *253*(4), 309–315. https://doi.org/10.1016/j.jcz.2014.02.003

Lips, K. R., Reeve, J. D., & Witters, L. R. (2003). Ecological Traits Predicting Amphibian Population Declines in Central America. *Conservation Biology*, *17*(4), 1078–1088. https://doi.org/10.1046/j.1523-1739.2003.01623.x

Livingston, S., Lavin, S. R., Sullivan, K., Attard, L., & Valdes, E. V. (2014). Challenges with effective nutrient supplementation for amphibians: A review of cricket studies. *Zoo Biology*, *33*(6), 565–576. https://doi.org/10.1002/zoo.21177

Luedtke, J. A., Chanson, J., Neam, K., Hobin, L., Maciel, A. O., Catenazzi, A., Borzée, A., Hamidy, A., Aowphol, A., Jean, A., Sosa-Bartuano, Á., Fong G. , A., de Silva, A., Fouquet, A., Angulo, A., Kidov, A. A., Muñoz Saravia, A., Diesmos, A. C., Tominaga, A., & Shrestha, B. (2023). Ongoing declines for the world's amphibians in the face of emerging threats. *Nature*, *622*(622), 308–314. https://doi.org/10.1038/s41586-023-06578-4

Macarthur, R. H., & Wilson, E. O. (1967). *The Theory of Island Biogeography* (Monographs in Population Biology, Vol. 1, pp. 1–203). Princeton University Press.

Mace, G. M., Collar, N. J., Gaston, K. J., Hilton-Taylor, C., Akcakaya, H. R., Leader-Williams, N., Milner-Gulland, E. J., & Stuart, S. N. (2008). Quantification of extinction risk: IUCN's system for classifying threatened species. *Conservation Biology*, *22*(6), 1424–1442. https://doi.org/10.1111/j.1523-1739.2008.01044.x

Malaeb, Z. A., Summers, J. K., & Pugesek, B. H. (2000). *Environmental and Ecological Statistics*, *7*(1), 93–111. https://doi.org/10.1023/a:1009662930292

Marchisin, A., & Anderson, J. D. (1978). Strategies Employed by Frogs and Toads (Amphibia, Anura) to Avoid Predation by Snakes (Reptilia, Serpentes). *Journal of Herpetology*, *12*(2), 151. https://doi.org/10.2307/1563401

McCue, M. D. (2006). Cost of Producing Venom in Three North American Pitviper Species. *Copeia*, *2006*(4), 818–825. JSTOR. https://doi.org/10.2307/4126548

McKinney, M. L. (1997). Extinction vulnerability and selectivity: Combining Ecological and Paleontological Views. *Annual Review of Ecology and Systematics*, *28*(1), 495–516. https://doi.org/10.1146/annurev.ecolsys.28.1.495

Morgenstern, D., & King, G. F. (2013). The venom optimization hypothesis revisited. *Toxicon*, *63*, 120–128. https://doi.org/10.1016/j.toxicon.2012.11.022

Murray, B. R., & Hose, G. C. (2005). Life-history and ecological correlates of decline and extinction in the endemic Australian frog fauna. *Austral Ecology*, *30*(5), 564–571. https://doi.org/10.1111/j.1442-9993.2005.01471.x

Nazareth, T. M., & Machado, G. (2015). Egg production constrains chemical defenses in a Neotropical arachnid. *PloS one*, *10*(9), e0134908.

Nisani, Z., Boskovic, D. S., Dunbar, S. G., Kelln, W., & Hayes, W. K. (2012). Investigating the chemical profile of regenerated scorpion (Parabuthus transvaalicus) venom in relation to metabolic cost and toxicity. *Toxicon*, *60*(3), 315–323. https://doi.org/10.1016/j.toxicon.2012.04.343

Nowak, R. T., & Brodie, E. D. (1978). Rib Penetration and Associated Antipredator Adaptations in the Salamander Pleurodeles waltl (Salamandridae). *Copeia*, *1978*(3), 424–429. https://doi.org/10.2307/1443606

O'Grady, J. J., Reed, D. H., Brook, B. W., & Frankham, R. (2004). What are the best correlates of predicted extinction risk? *Biological Conservation*, *118*(4), 513–520. https://doi.org/10.1016/j.biocon.2003.10.002

Oliveira, B. F., São-Pedro, V. A., Santos-Barrera, G., Penone, C., & Costa, G. C. (2017). AmphiBIO, a global database for amphibian ecological traits. *Scientific Data*, *4*(1). https://doi.org/10.1038/sdata.2017.123

Paradis, E., & Schliep, K. (2018). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*(3), 526–528. https://doi.org/10.1093/bioinformatics/bty633

Peplinski, J., Malone, M. A., Fowler, K. J., Potratz, E. J., Pergams, A. G., Charmoy, K. L., Rasheed, K., Avdieiev, S. S., Whelan, C. J., & Brown, J. S. (2021). Ecology of Fear: Spines, Armor and Noxious Chemicals Deter Predators in Cancer and in Nature. *Frontiers in Ecology and Evolution*, *9*. https://doi.org/10.3389/fevo.2021.682504

Pianka, E. R. (1970). On r- and K-Selection. *The American Naturalist*, *104*(940), 592–597. https://doi.org/10.1086/282697

Pimm, S. L., Jones, H. L., & Diamond, J. (1988). On the Risk of Extinction. *The American Naturalist*, *132*(6), 757–785. https://doi.org/10.1086/284889

Pincheira-Donoso, D. (2017). *The Global Amphibian Biodiversity Project (GABiP)*. www.amphibianbiodiversity.org.

Pincheira-Donoso, D., Harvey, L. P., Cotter, S. C., Stark, G., Meiri, S., & Hodgson, D. J. (2021). The global macroecology of brood size in amphibians reveals a predisposition of low-fecundity species to extinction. *Global Ecology and Biogeography*, *30*(6), 1299–1310. https://doi.org/10.1111/geb.13287

Pintor, A. F. V., Krockenberger, A. K., & Seymour, J. E. (2010). Costs of venom production in the common death adder (Acanthophis antarcticus). *Toxicon*, *56*(6), 1035–1042. https://doi.org/10.1016/j.toxicon.2010.07.008

Pocock, M. J. O. (2010). Can traits predict species' vulnerability? A test with farmland passerines in two continents. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1711), 1532–1538. https://doi.org/10.1098/rspb.2010.1971

Posit team. (2024). *RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA.* http://www.posit.co/

Pough, F. H., & Taigen, T. L. (1990). Metabolic correlates of the foraging and social behaviour of dart-poison frogs. *Animal Behaviour*, *39*(1), 145–155. https://doi.org/10.1016/s0003-3472(05)80734-1

Pugesek, B. H., Tomer, A., & Alexander von Eye. (2003). *Structural Equation Modeling*. Cambridge University Press.

Purvis, A., Gittleman, J. L., Cowlishaw, G., & Mace, G. M. (2000). Predicting extinction risk in declining species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1456), 1947–1952. https://doi.org/10.1098/rspb.2000.1234

Pyron, R. A., & Wiens, J. J. (2013). Large-scale phylogenetic analyses reveal the causes of high tropical amphibian diversity. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1770), 20131622.

Reed, R. N., & Shine, R. (2002). Lying in Wait for Extinction: Ecological Correlates of Conservation Status among Australian Elapid Snakes. *Conservation Biology*, *16*(2), 451–461. https://doi.org/10.1046/j.1523-1739.2002.02283.x

Rodrigues, A., Pilgrim, J., Lamoreux, J., Hoffman, M., & Brooks, T. (2006). The value of the IUCN Red List for conservation. *Trends in Ecology & Evolution*, *21*(2), 71–76. https://doi.org/10.1016/j.tree.2005.10.010

Rohr, J. R., & Palmer, B. D. (2013). Climate Change, Multiple Stressors, and the Decline of Ectotherms. *Conservation Biology*, *27*(4), 741–751. https://doi.org/10.1111/cobi.12086

Rollins-Smith, L. A. (2023). The importance of antimicrobial peptides (AMPs) in amphibian skin defense. *Developmental & Comparative Immunology*, *142*, 104657. https://doi.org/10.1016/j.dci.2023.104657

Savitzky, A. H., Mori, A., Hutchinson, D. A., Saporito, R. A., Burghardt, G. M., Lillywhite, H. B., & Meinwald, J. (2012). Sequestered defensive toxins in tetrapod vertebrates: principles, patterns, and prospects for future studies. *Chemoecology*, *22*(3), 141–158. https://doi.org/10.1007/s00049-012-0112-z

Schluter, D. (2000). *The Ecology of Adaptive Radiation*. OUP Oxford.

Shipley, B. (2016). *Cause and correlation in biology : a user's guide to path analysis, structural equations, and causal inference with R*. Cambridge University Press.

Smilanich, A. M., Dyer, L. A., Chambers, J. Q., & Bowers, M. D. (2009). Immunological cost of chemical defence and the evolution of herbivore diet breadth. *Ecology Letters*, *12*(7), 612–621. https://doi.org/10.1111/j.1461-0248.2009.01309.x

Smith, C. C., & Fretwell, S. D. (1974). The Optimal Balance between Size and Number of Offspring. *The American Naturalist*, *108*(962), 499–506. https://doi.org/10.1086/282929

Smith, M. T., Ortega, J., & Beaupre, S. J. (2014). Metabolic cost of venom replenishment by Prairie Rattlesnakes (Crotalus viridis viridis). *Toxicon*, *86*, 1–7. https://doi.org/10.1016/j.toxicon.2014.04.013

Speed, M. P., Brockhurst, M. A., & Ruxton, G. D. (2010). The dual benefits of aposematism: predator avoidance and enhanced resource collection. *Evolution*, *64*(6), 1622–1633. https://doi.org/10.1111/j.1558-5646.2009.00931.x

Spolsky, C., & Uzzel, T. (1986). Evolutionary history of the hybridogenetic hybrid frog Rana esculenta as deduced from mtDNA analyses. *Molecular Biology and Evolution*. https://doi.org/10.1093/oxfordjournals.molbev.a040376

Stankowich, T. (2011). Armed and dangerous: predicting the presence and function of defensive weaponry in mammals. *Adaptive Behavior*, *20*(1), 32–43. https://doi.org/10.1177/1059712311426798

Stark, G., & Meiri, S. (2018). Cold and dark captivity: Drivers of amphibian longevity. *Global Ecology and Biogeography*, *27*(11), 1384–1397. https://doi.org/10.1111/geb.12804

Stynoski, J. L., Shelton, G., & Stynoski, P. (2014a). Maternally derived chemical defences are an effective deterrent against some predators of poison frog tadpoles (Oophaga pumilio). *Biology Letters*, *10*(5), 20140187. https://doi.org/10.1098/rsbl.2014.0187

Stynoski, J. L., Torres-Mendoza, Y., Sasa-Marin, M., & Saporito, R. A. (2014b). Evidence of maternal provisioning of alkaloid-based chemical defenses in the strawberry poison frogOophaga pumilio. *Ecology*, *95*(3), 587–593. https://doi.org/10.1890/13-0927.1

Sutherland, W. J., Clout, M., Depledge, M., Dicks, L. V., Dinsdale, J., Entwistle, A. C., Fleishman, E., Gibbons, D. W., Keim, B., Lickorish, F. A., Monk, K. A., Ockendon, N., Peck, L. S., Pretty, J., Rockström, J., Spalding, M. D., Tonneijck, F. H., & Wintle, B. C. (2015). A horizon scan of global conservation issues for 2015. *Trends in Ecology & Evolution*, *30*(1), 17–24. https://doi.org/10.1016/j.tree.2014.11.002

Texeira, M., Baldi, G., & Paruelo, J. (2012). An exploration of direct and indirect drivers of herbivore reproductive performance in arid and semi arid rangelands by means of structural equation models. *Journal of Arid Environments*, *81*, 26–34. https://doi.org/10.1016/j.jaridenv.2012.01.017

Thompson, J. N. (1989). Concepts of Coevolution. *Trends in Ecology & Evolution*, *4*(6), 179–183. https://doi.org/10.1016/0169-5347(89)90125-0

Thorson, J. T., & van der Bijl, W. (2023). phylosem: A fast and simple R package for phylogenetic inference and trait imputation using phylogenetic structural equation models. *Journal of Evolutionary Biology*, *36*(10), 1357–1364. https://doi.org/10.1111/jeb.14234

Toledo, L. F., & Haddad, C. F. B. (2009). Colors and Some Morphological Traits as Defensive Mechanisms in Anurans. *International Journal of Zoology*, *2009*, e910892. https://doi.org/10.1155/2009/910892

Toledo, L. F., Sazima, I., & Haddad, C. F. B. (2011). Behavioural defences of anurans: an overview. *Ethology Ecology & Evolution*, *23*(1), 1–25. https://doi.org/10.1080/03949370.2010.534321

Toledo, R. C., & Jared, C. (1995). Cutaneous granular glands and amphibian venoms. *Comparative Biochemistry and Physiology Part A: Physiology*, *111*(1), 1–29. https://doi.org/10.1016/0300-9629(95)98515-I

Tung Ho, L. si, & Ané, C. (2014). A Linear-Time Algorithm for Gaussian and Non-Gaussian Trait Evolution Models. *Systematic Biology*, *63*(3), 397–408. https://doi.org/10.1093/sysbio/syu005

Üveges, B., Fera, G., Móricz, Á. M., Krüzselyi, D., Bókony, V., & Hettyey, A. (2017). Age- and environment-dependent changes in chemical defences of larval and post-metamorphic toads. *BMC Evolutionary Biology*, *17*(1). https://doi.org/10.1186/s12862-017-0956-5

Üveges, B., Szederkényi, M., Mahr, K., Móricz, Á. M., Krüzselyi, D., Bókony, V., Hoi, H., & Hettyey, A. (2019). Chemical defense of toad tadpoles under risk by four predator species. *Ecology and Evolution*, *9*(11), 6287–6299. https://doi.org/10.1002/ece3.5202

van den Berg, C., Santon, M., Endler, J. A., & Cheney, K. L. (2024). Highly defended nudibranchs “escape” to visually distinct background habitats. *Behavioral Ecology*, *35*(5). https://doi.org/10.1093/beheco/arae053 van Noordwijk, A. J., & de Jong, G. (1986). Acquisition and Allocation of Resources: Their Influence on Variation in Life History Tactics. *The American Naturalist*, *128*(1), 137–142. https://doi.org/10.1086/284547

Verde Arregoitia, L. D., Blomberg, S. P., & Fisher, D. O. (2013). Phylogenetic correlates of extinction risk in mammals: species in older lineages are not at greater risk. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1765), 20131092. https://doi.org/10.1098/rspb.2013.1092

Wake, D. B., & Vredenburg, V. T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences*, *105*(Supplement 1), 11466–11473. https://doi.org/10.1073/pnas.0801921105

Webb, J. K., Brook, B. W., & Shine, R. (2002). What makes a species vulnerable to extinction? Comparative life-history traits of two sympatric snakes. *Ecological Research*, *17*(1), 59–67. https://doi.org/10.1046/j.1440-1703.2002.00463.x

Wigger, E., Kuhn-Nentwig, L., & Nentwig, W. (2002). The venom optimisation hypothesis: a spider injects large venom quantities only into difficult prey types. *Toxicon*, *40*(6), 749–752. https://doi.org/10.1016/s0041-0101(01)00277-x

Williams, S. E., & Hero, J.-M. (1998). Rainforest frogs of the Australian Wet Tropics: guild classification and the ecological similarity of declining species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *265*(1396), 597–602. https://doi.org/10.1098/rspb.1998.0336

Winter, M., Fiedler, W., Hochachka, W. M., Koehncke, A., Meiri, S., & De la Riva, I. (2016). Patterns and biases in climate change research on amphibians and reptiles: a systematic review. *Royal Society Open Science*, *3*(9), 160158. https://doi.org/10.1098/rsos.160158

Withers, P. C., & Hillman, S. S. (2001). Allometric and ecological relationships of ventricle and liver mass in anuran amphibians. *Functional Ecology*, *15*(1), 60–69. https://doi.org/10.1046/j.1365-2435.2001.00495.x

Woodhams, D. C., Bell, S. C., Bigler, L., Caprioli, R. M., Chaurand, P., Lam, B. A., Reinert, L. K., Stalder, U., Vazquez, V. M., Schliep, K., Hertz, A., & Rollins-Smith, L. A. (2016). Life history linked to immune investment in developing amphibians. *Conservation Physiology*, *4*(1), cow025–cow025. https://doi.org/10.1093/conphys/cow025

Wootton, J. T. (1994). Predicting Direct and Indirect Effects: An Integrated Approach Using Experiments and Path Analysis. *Ecology*, *75*(1), 151–165. https://doi.org/10.2307/1939391

Yang, L., Zhao, S., & Liu, S. (2022). A global analysis of urbanization effects on amphibian richness: Patterns and drivers. *Global Environmental Change*, *73*(March 2022, 102476), 102476. https://doi.org/10.1016/j.gloenvcha.2022.102476

Zvereva, E. L., Zverev, V., Kruglova, O. Y., & Kozlov, M. V. (2017). Strategies of chemical anti-predator defences in leaf beetles: is sequestration of plant toxins less costly than de novo synthesis? Oecologia, 183(1), 93–106. https://doi.org/10.1007/s00442-016-3743-x

Supplementary material

Table S1. Taxonomic breakdown of amphibian species within the phylogeny (n=855).

Order	Family	Species (*n*)
Anura (620 species, 44 families)	Alsodidae	3
	Alytidae	7
	Aromobatidae	7
	Arthroleptidae	1
	Ascaphidae	2
	Batrachylidae	2
	Bombinatoridae	6
	Brachycephalidae	4
	Brevicipitidae	1
	Bufonidae	83
	Calyptocephalellidae	1
	Centrolenidae	3
	Ceratophryidae	3
	Craugastoridae	5
	Cycloramphidae	2
	Dendrobatidae	70
	Dicroglossidae	8
	Eleutherodactylidae	10
	Heleophrynidae	2
	Hemiphractidae	6
	Hylidae	102
	Hyperoliidae	12
	Leiopelmatidae	4
	Leptodactylidae	27
	Limnodynastidae	15
	Mantellidae	20
	Megophryidae	4
	Microhylidae	16
	Myobatrachidae	12
	Odontophrynidae	9
	Pelobatidae	2
	Pelodryadidae	34
	Phrynobatrachidae	2
	Phyllomedusidae	25
	Pipidae	9

	Ptychadenidae	3
	Pyxicephalidae	10
	Ranidae	66
	Rhacophoridae	10
	Rhinodermatidae	1
	Rhinophrynidae	1
	Scaphiopodidae	7
	Strabomantidae	2
	Telmatobiidae	1
Caudata (227 species, 10 families)	Ambystomatidae	15
	Amphiumidae	3
	Cryptobranchidae	3
	Dicamptodontidae	2
	Hynobiidae	5
	Plethodontidae	147
	Proteidae	4
	Rhyacotritonidae	1
	Salamandridae	43
	Sirenidae	4
Gymnophiona (8 species, 5 families)	Dermophiidae	2
	Ichthyophiidae	1
	Scolecomorphidae	1
	Siphonopidae	2
	Typhlonectidae	2
Total species (*n*)		**855**

Table S2. Sensitivity analysis of Structural Equation Model (SEM) path coefficients under alternative coding of habitat suitability. To test the robustness of the marginal habitats hypothesis, results from the primary analysis (where 'Unknown' habitat suitability was classed as 'Marginal') are compared against a repeat analysis (where 'Unknown' was classed as 'Suitable'). Statistical significance ($p = 0.05$) was maintained for the key relationship between chemical defence and marginal habitat occupancy across both models. This demonstrates that the study's conclusions regarding the mediating role of marginal habitats are not dependent on the mode of coding.

Model	**Path**	**Unknown=marginal (used in analysis)**		**Unknown= suitable (sensitivity test)**	
Threat model	Cd → Propmarg	$t = 1.979$	$p = 0.047$	$t = 2.270$	$p = 0.023$
	Propmarg → threat	$t = 1.931$	$p = 0.053$	$t = 1.653$	$p = 0.098$
	Cd → threat	$t = 0.926$	$p = 0.355$	$t = 0.939$	$p = 0.348$
Decline model	Cd → Propmarg	$t = 1.991$	$p = 0.047$	$t = 2.282$	$p = 0.022$
	Propmarg → decline	$t = 2.183$	$p = 0.029$	$t = 2.110$	$p = 0.035$
	Cd → decline	$t = 0.225$	$p = 0.822$	$t = 0.185$	$p = 0.853$