Meta-analysis showing that immunisation is an effective method to reduce amphibian susceptibility to the chytrid fungus

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ABSTRACT

Emerging infectious diseases are increasingly causing mortality in vertebrates, driving widespread population declines in some species. Amphibian chytridiomycosis is a lethal fungal disease that has caused population collapses and extinctions worldwide. Identifying approaches that can effectively enhance host survival is therefore an urgent conservation priority. Here, we investigate whether immunising individuals against chytrid is an effective strategy. We synthesised evidence from 223 effect sizes (53 experiments across 36 studies and 22 species) to evaluate how susceptibility to *Batrachochytrium dendrobatidis* (infection rates, infection intensity, mortality) differed between experimentally immunised and non-immunised individuals that were subsequently exposed to live chytrid. Individuals immunised by exposure to live chytrid, their cell-free compounds, or synthetic antiparasitics consistently had reduced disease susceptibility follow re-exposure, although effectiveness varied across the stages of disease progression, depended on life stage, and varied among taxonomic groups. In contrast, we found no clear benefits of probiotic or dead-pathogen immunisation. These findings highlight three promising immunisation methods that reduce amphibian susceptibility to the chytrid fungus. They also suggest that wild animals exposed to chytrid may benefit from habitat manipulations, such as providing thermal refugia or targeted chemical disinfection, that enable animals to clear infection, thereby inducing live-pathogen immunity that could enhance survival in susceptible species.

KEYWORDS

Batrachochytrium, antifungal, microbe, metabolites, zoospore

INTRODUCTION

The emergence and spread of infectious diseases represent one of the most significant threats to wildlife populations worldwide[1,2]. While many taxa have been impacted by emerging diseases, amphibians have been hardest hit due to the global expansion of two fungal pathogens, *Batrachochytrium dendrobatidis* (Bd)[3] and *B. salamandrivorans* (Bsal)[4]. These pathogens cause chytridiomycosis, a disease that can trigger mass mortality events[5,6]. Since the 1970s, the spread of chytridiomycosis has contributed to population declines in more than 500 amphibian species, with at least 90 species potentially extinct[7]. Consequently, amphibians are now the most imperilled vertebrate class, with more than 40% of species threatened, and disease identified as the major threatening process[7,8].

The challenge for susceptible amphibian species is that, once established in the wild, the disease appears virtually impossible to eradicate. This persistence stems not only from the pathogen's ability to infect non-amphibian hosts[9,10] and remain viable in environments without hosts[11], but also from amphibian species that become infected but rarely develop disease, thereby acting as long-term pathogen reservoirs and continual sources of infection for more vulnerable species[12]. As a result, conventional conservation measures, including translocations and reintroductions, often fail because disease-causing pathogens persist in the environment[13-15]. For Bd and Bsal, the only proven management strategy is to remove susceptible individuals from natural habitats and maintain disease-free captive populations[16], a scenario that risks leaving many amphibian species extinct in the wild. This reality underscores the urgent need to develop approaches that mitigate disease impacts and enable amphibian hosts to coexist with the pathogens.

A promising avenue for reducing disease impacts involves stimulating protective immune responses in susceptible species. In many animals, exposure to live-attenuated pathogens, inactivated pathogens, or their derivatives enables immune cells to recognise pathogens, develop immunological memory, and mount faster and more effective responses upon subsequent exposure[17,18]. For amphibians, successful immunisation strategies that enhance host survival[19-21] could substantially improve conservation outcomes, for example by enabling the release of immunised individuals to bolster threatened populations, or by guiding habitat manipulations that help amphibians clear infection naturally, develop immunity under wild conditions, and thus enable long-term population persistence[22,23].

However, immunisation efforts targeting Bd and Bsal in amphibians have produced mixed outcomes[24,25], with no clear consensus and considerable uncertainty about their overall effectiveness. This inconsistency likely reflects the wide variety of immunisation approaches that have been trialled (Table 1), including exposure to live pathogens[24,26], dead pathogens[21,27], pathogen metabolites[28,29], antifungal microbes[30,31], and synthetic anitfungals[32,33], as well as the broad diversity of host species on which these methods have been tested.

Despite this variability, synthesising these results and identifying the most promising strategies remains a priority, as effective immunisation approaches would represent a major advance in

combatting chytridiomycosis. Here, we conducted a meta-analysis drawing on all available amphibian immunisation studies targeting Bd and Bsal to identify the most effective strategies and to understand the drivers of variation in immunisation success. We focused on how different immunisation approaches influenced subsequent infection rates, infection intensity (when infected), and mortality when immunised individuals were exposed to live pathogens, anticipating that effective strategies would reduce infection rates, lower pathogen loads, and/or decrease mortality. We evaluated how these outcomes varied across immunisation methods (Table 1) and among amphibians differing in life stage, origin (captive or wild bred) and taxonomic family (Table 2).

RESULTS

We screened 1,517 studies and identified 38 containing relevant datasets (Figure 1 and S1). Together, these studies reported 275 effects from 59 experiments, each comparing outcomes following Bd or Bsal exposure between immunised (treated) and control (untreated) animals under common conditions. Most experiments targeted Bd (n = 53) with only six addressing Bsal. All Bsal experiments involved live-pathogen immunisation: exposing animals to either live Bd[24] or live Bsal[34,35] and testing their subsequent susceptibility in a live Bsal challenge. Results from these Bsal experiments were mixed: infection loads and mortality in immunised individuals were reduced relative to controls in some species[24] but not others[34,35], while prevalence was unaffected by immunisation treatment in all cases. Given the limited number of Bsal experiments, we estimated effect sizes only for Bd.

Immunisation treatments reduce susceptibility to Bd across amphibians

We quantified differences in infection risk (prevalence), infection intensity, and mortality between immunised and control groups following a live Bd exposure challenge using multi-level meta-analytic models that included within-study, between-study and phylogenetic random effects (Figure 2). Effect sizes for prevalence (k = 90) and mortality (k = 62) were calculated as the natural logarithm of the risk ratio (logRR), while effect sizes for infection intensity (k = 71) were calculated as the natural logarithm of the response ratio (lnRR).

Mortality is arguably the most consequential outcome because it links directly to population performance and the potential for long-term persistence. Immunising individuals with live pathogens reduced mortality following subsequent live Bd exposure, lowering death rates by an average of 26% (Figure 3). Although the confidence intervals (CIs) slightly overlapped zero (p = 0.054), the magnitude of the effect suggests that live-pathogen immunisation can substantially improve host survival following re-exposure to the Bd pathogen (Table S1).

Live-pathogen immunisation not only reduced mortality but also markedly lowered infection loads (Figure 3b), decreasing infection intensity by an average of 70% (p = 0.04). To test the result robustness, we excluded experiments that included uninfected individuals when calculating Bd loads (k = 15) because these experiments confound effects on infection rates (prevalence) with those on infection intensity. The benefits of live-pathogen immunisation

became stronger when excluding uninfected individuals, reducing pathogen loads by an average of 80% (p = 0.02; Table S2). These findings suggest that live-pathogen immunisation robustly reduces infection loads, irrespective of its effects on prevalence. Align with this, we found no effects of live-pathogen immunisation on prevalence (p = 0.21; Figure 3a).

In contrast, natural-chemical immunisation did not reduce mortality (p = 0.34), but reduced both prevalence and pathogen load by 27% (p = 0.003) and 80% (p = 0.053), respectively (Figure 3; Table S1). However, effects of natural-chemical immunisation on pathogen loads became unclear when we excluded experiments that included uninfected animals (CIs: −96% to +37%; p = 0.11; Table S2). This suggests that the observed effect of natural-chemical immunisation on infection intensity is largely attributable to its effects on prevalence.

Another promising approach is synthetic-antiparasitic immunisation, which reduced mortality risk by an average of 34% (Figure 3c). However, this effect remained uncertain (p = 0.10), likely due to the small sample sizes (k = 5). The CIs were largely skewed towards reduced mortality risk (CIs: −60% to +8%), warranting further investigation of this intervention. The remaining immunisation methods (dead-pathogen, probiotic and other) showed no clear effects on either prevalence, infection intensity, or mortality (Figure 3).

Immunisation effects were correlated among prevalence, infection intensity and mortality

Our results show that different immunisation methods influence infection dynamics at different stages of disease progression, from the probability of becoming infected to the intensity of infection and ultimately morality (Figure 3). To examine whether the effects of immunisation on one disease stage were associated with compensatory or reinforcing effects at other stages, we fitted multivariate models that assessed correlations among effect sizes across the stages of disease progression within studies. The effects of immunisation on prevalence and pathogen load were strongly positively correlated (ρ = 0.95), suggesting a reinforcing effect whereby immunisation that limits initial infection also suppresses within-host pathogen abundance. In contrast, effects on prevalence and pathogen loads were both negatively correlated with effects on mortality (ρ = -0.56 and -0.28, respectively), suggesting a compensatory effect whereby immunisation that enhances resistance (e.g., preventing initial infection and limiting pathogen burden) tends to reduce tolerance (e.g., increasing mortality at a given pathogen burden).

Consistent effects on prevalence but effects were more variable across studies for infection intensity and mortality

There was evidence that some immunisation methods significantly lowered Bd prevalence, infection intensity, and mortality, but the consistency of effects varied across studies. The effects of immunisation on prevalence were highly consistent across studies ($I^2 \approx 0\%$) and showed little variation relative to the overall mean effect (mean-standardised heterogeneity: $CVH^2 \approx 0$), largely because most immunisations did not clearly alter infection rates (Figure S2). In contrast, the effects of immunisation on infection load exhibited extremely high heterogeneity across studies ($I^2 = 99.53\%$), with substantial variation relative to the mean effect

(CVH^2 = 6.37). The effects of immunisation on mortality exhibited small to moderate heterogeneity (I^2 = 30.14%), although effect sizes varied markedly relative to the overall mean effect (CVH^2 = 105.32), reflecting that mortality responses varied considerably across studies despite a small mean effect (Figure S2). Together, these findings indicate substantial variation in how immunisation methods influence infection burden and mortality risk (Table S3).

For infection intensity, most of the heterogeneity was due to between-study differences and within-study variation ($I^2_{between-study}$ = 49%, $I^2_{within-study}$ = 51%, $I^2_{phylogeny} \approx$ 0%). For mortality, heterogeneity primarily arose from between-study differences and phylogeny ($I^2_{between-study}$ = 21%, $I^2_{within-study} \approx$ 0%, $I^2_{phylogeny}$ = 9%), indicating mortality outcomes were linked to evolutionary relatedness. The substantial contribution of between-study differences to overall heterogeneity in both traits suggests that between-study ecological and methodological differences were key drivers of variation in immunisation effects.

Immunisation effectiveness varies by life stage and family, with stronger effects in more susceptible groups

In addition to immunisation methods, we found that host type (life stage and taxonomy) moderated treatment effectiveness. Immunisation was more effective at lowering prevalence in larval amphibians relative to other life-stages (Figure 4a), reducing larval infection risk by 28% (p = 0.01; Table S4). However, immunisation did not reduce larval mortality (p = 0.25) or infection intensity (p = 0.19; Figure 4). In contrast, immunisation reduced infection intensity in juveniles by an average of 58%, although the CIs slightly overlapped zero (p = 0.054; Figure 4b). The effect in juveniles became unclear when we excluded experiments that included uninfected animals to calculate Bd loads (p = 0.15; Table S5). This demonstrates that immunisation reduced infection intensity in juveniles largely because many immunised juveniles cleared their infection. Immunisation had no effect on prevalence or mortality in juveniles, or on any traits in adults (Figure 4; Table S4).

Species in different families may vary in responses to disease and immunisation due to taxa-specific physiology[36] and ecological niches[37], which are not necessarily captured by phylogenetic relatedness. Indeed, we found that immunisation effects varied by amphibian family, reducing infection risk in Hylidae by an average of 9% (p = 0.03), but not in other families (Figure 4; Tables S6-7). Hylids are known to be particularly susceptible to Bd and often experience high Bd-induced mortality[38]. To test whether immunisation provides greater benefits in more susceptible groups, we fitted a meta-regression examining the moderating effect of inherent susceptibility (i.e., the mortality rate of the control group) on immunisation effects. Inherent susceptibility did not predict immunisation effects on prevalence (t_{82} = 1.24, p = 0.22) or infection intensity (t_{60} = -0.71, p = 0.48). However, immunisation reduced mortality more strongly in groups with higher inherent susceptibility (t_{60} = -2.04, p = 0.046), suggesting that more susceptible species or populations gained greater survival benefits from immunisation (Table S8).

Immunisation effectiveness did not vary by host origin (i.e., wild or captive bred) (Figure 4; Tables S9-10) or by methodological procedure (i.e., the time intervals between immunisation, Bd challenge, and trait measurement, as well as measurement effort) (Tables 1 and S11). Therefore, immunisation was similarly effective in wild-bred and captive-bred amphibians across studies with different experimental durations and measurement frequencies.

Publication bias

We assessed two forms of publication bias: (1) small-study effect, whereby studies with small sample sizes and significant results are more likely to be published[39] and (2) time-lag bias, whereby significant findings are published earlier than non-significant ones[40]. Neither effective sample size nor publication year (from 2009 to 2024) predicted immunisation effectiveness (all slopes overlapping zero; all $p > 0.20$; Table S12), indicating no publication bias. Publication bias was not evident from visual inspection of funnel plot asymmetry (Figure S3).

DISCUSSION

Identifying approaches that reduce chytrid-associated mortality is essential for safeguarding threatened amphibians and strengthening global conservation efforts. Given the ongoing persistence of Bd in the wild, efforts to enhance individual resistance are widely regarded as a critical first step in preventing extinctions and enabling successful reintroductions[22]. Our meta-analysis shows that prior exposure to live Bd, its natural chemical products, or synthetic antiparasitics consistently reduced host susceptibility to Bd. These methods therefore appear especially promising for immunising animals prior to translocation or population supplementation.

Exposure to live Bd or its natural chemical reduced host susceptibility to Bd, while exposure to dead Bd had no effect

Live-pathogen immunisation effectively reduced infection intensity and mortality upon re-exposure. This result aligns well with the concept of vaccination, which stimulates adaptive immunity and lessens the severity of later infections[17,18]. In addition to live-pathogen immunisation, we found clear evidence of natural-chemical immunisation in reducing infection risk and pathogen load. Most experiments of natural-chemical immunisation applied Bd metabolites (cell-free by-products) to induce resistance[28,29,41]. We therefore highlight that prior exposure to live Bd or its metabolites can reduce disease susceptibility. Interestingly, we did not detect any effects of dead-pathogen immunisation, consistent with epidemiological evidence suggesting that non-infectious vaccines elicit weaker protection than live vaccines[18]. What remains unclear, however, is why Bd metabolites – another form of non-infectious vaccines – were effective. One possible explanation is that inhibitory compounds produced by Bd stimulate host immune responses (e.g., methylthioadenosine[42], spermidine[43]). These immunomodulatory factors are produced by Bd zoosporangial walls rather than zoospore cells[44], yet dead-Bd immunisation typically exposed individuals to purified dead zoospores without zoosponrangia[21,27,45,46]. In contrast, Bd metabolites were usually prepared by filtering

culture media containing both zoosporangia and zoospores[29]. The inclusion of zoosporangia during preparation might explain the strong effects of Bd metabolites. Consistent with this explanation, antibody production was detected when frogs were treated with a mixture of dead zoospores and zoosporangia[47], but not when treated with purified dead zoospores[46,48].

Is pathogen-related immunisation practical to establish herd immunity in nature?

The goal of developing an immunisation strategy is to administer it broadly to large numbers of individuals, increase the number of resistant individuals, and reduce pathogen transmission. However, not all effective approaches can achieve this goal. For example, immunisation using Bd metabolites are labour-intensive, with studies dosing animals daily or every other day for periods ranging from weeks to months[19,21,29], making them impractical for real-world conservation. Recent studies have begun testing the minimal doses and duration needed to produce a protective effect of Bd metabolites[28], but these trials are still in their infancy and largely limited to laboratory conditions. Alternatively, resistance may be enhanced through habitat manipulation that produces effects similar to effective immunisation methods. For example, the establishment of hotspot shelters[20] or saline satellite ponds[20,49] allows wild frogs to clear infection, thereby creating natural live infection-clearance processes analogous to live-pathogen immunisation. In our meta-analysis, a few live-pathogen immunisation (7 of 24 experiments) did not involve clearance because the live Bd strains did not cause sickness. These low-virulence strains are assumed to colonise available cutaneous niches, preventing invasion by more virulent strains encountered later[24]. Despite their low virulence, these live Bd strains remain transmissible and can be spread naturally in untreated wild individuals, potentially accelerating the establishment of herd immunity[50]. Notably, however, low-virulence strains can be still lethal to some susceptible species[24] and may increase the risk of co-infection with other pathogens[51]. The use of live strains without clearance hence requires careful assessment of population-level disease dynamics, given their transmissibility.

Exposure to synthetic antiparasitics: a convenient and promising method to reduce susceptibility to Bd

Contrary to the aim of vaccination to stimulate adaptive immunity, exposure to synthetic antiparasitics were often used as sham procedures to control for clearance effects following live-pathogen immunisation[25,52], or arose from interest in the antifungal effects of common pesticides[33,53]. Studies have reported mixed effects on host susceptibility (decreased[53,54] or increased[19,33]), but our meta-analysis revealed a strong tendency in reduce host mortality. While the effect remained uncertain due to a small sample size, it underscores the potential practical value of synthetic antiparasitics. First, synthetic drugs are cheaper and more transferrable than live Bd and its natural chemical (which require zoospores quantification and immediate use), making synthetic-antiparasitic treatments easier to prepare in remote areas[28]. Second, synthetic antiparasitics pose no risk of introducing the pathogen into the wild. Because synthetic antiparasitics do not contain Bd, the induced defence is likely mediated by innate immunity (e.g., epidermal trauma due to drug exposure that mitigate

susceptibility[55]). One key unknown is how long protection persists after exposure to synthetic antiparasitics. For example, the beneficial effects of antifungal treatment were lost after one year[56]. In contrast, combining individual-level antifungal treatment with habitat manipulation (chemical disinfection) successfully mitigated infection outcomes for up to two years[57]. This success might reflect the combined effect of synthetic-antiparasitic immunisation and reduced Bd abundance in the environment. Currently, drug administration (especially itraconazole) is the most widely applied therapeutic intervention to mitigate chytrid[58]. However, its use remains highly controversial due to unintended impacts on animal performance (e.g., reduced growth[53]) and ecosystems, warranting further evaluation before broader application.

Effects on mortality were negatively correlated with effects on prevalence and infection load, suggesting function redundancy between tolerance and resistance

We revealed a negative correlation between effects on mortality and those on prevalence or infection intensity, but a positive correlation between prevalence and infection intensity. Positive effect correlations are intuitive, as immunisation that inhibit initial infection (prevalence) should also limit pathogen growth within the host (infection intensity) and prevent it from reaching lethal thresholds (mortality). On the other hand, it is well established that some amphibians can carry high pathogen loads without sickness or mortality[59]. Hosts can combat pathogens through two mechanisms[60,61]: (1) limiting pathogen invasion and growth (resistance) or (2) minimising pathology without reducing pathogen burden (tolerance). Amphibians may therefore coexist with chytrid by being either highly resistant (e.g., not infected)[32,62] or highly tolerant (e.g., not sick when infected)[59], implying functional redundancy between these two defences[60]. Trade-offs between resistance and tolerance are expected if both are costly and provide additive benefits[63]. In our meta-analysis, prevalence and infection intensity reflect resistance, whereas mortality reflects tolerance. This framework may explain the positive correlation within resistance outcomes and the negative correlation between resistance and tolerance outcomes. Among approach types (Figure 3), natural-chemical immunisation reduced prevalence and infection intensity, but not mortality; likewise, synthetic-antiparasitic immunisation reduced mortality, but not prevalence or infection intensity. However, an exception was live-pathogen immunisation, which reduced infection intensity and mortality but not prevalence, indicating some uncertainty in which outcomes are affect by different methods.

To explain where immunisation methods exert their effects, we hypothesised different cases based on the concepts of resistance and tolerance (Figure 5). In case [1], untreated animals carry high pathogen loads, with some high enough to cause death. Immunisation reduces pathogen loads, and thus mortalities, but not to the point of infection clearance, so infection prevalence remains unchanged (Figure 5a). Case [1] aligns with the effects of live-pathogen immunisation and the high prevalence observed in their datasets (Figure S4). In case [2], untreated animals carry low pathogen loads below disease-causing lethal thresholds, so few individuals are at risk of dying. Immunisation further reduce pathogen loads leading to more animals clearing infection and hence reduced prevalence, but the mortality rate remains unchanged (Figure 5a). Case [2] aligns with the effects of natural-chemical immunisation and

the low mortality observed in their datasets (Figure S5). Case [3] illustrates increased tolerance after immunisation, where immunised individuals become more tolerant of higher infection loads, and thus mortality is reduced without changes in pathogen load or prevalence (Figure 5b). Case [3] is consistent with the effects of synthetic-antiparasitic immunisation. Case [4] indicates increased tolerance after immunisation, which is not detectable in experiments.

Future conservation efforts need to consider the timing and life-stage of immunisation

Immunisation were particularly effective at reducing prevalence in larvae and infection intensity in juveniles (Figure 4). In tadpoles (larvae), keratinised tissues are restricted to the mouthparts[64], where infection rarely cause mortality[38,61]. This aligns with our hypothesis that when pathogen loads are far below lethal thresholds, immunisation is more likely to reduce prevalence (Case [2] in Figure 5). In contrast, post-metamorphic juveniles have fully keratinised skin[64] but incomplete adult immune systems[65], and often experience high mortality following infection[38,61,65]. Indeed, the Bd-caused lethal threshold is typically lower for juveniles than for larvae or adults (55% and 87% lower, respectively)[38]. This finding is important because pathogen growth was slower in immunised juveniles, plausibly reducing the risk of reaching lethal thresholds at this most vulnerable life stage. While infection-caused mortality is uncommon in tadpoles[61], infected tadpoles often die during or shortly after metamorphosis[66] because Bd spreads as keratin appears across the skin covering the entire body[64]. Tadpoles also act as reservoirs that carry Bd and increase disease transmission[67]. Evidence that immunisation reduced infection rates in tadpoles is likewise encouraging because if fewer tadpoles are infected, the risk of them dying after metamorphosis or transmitting disease to other individuals is likely lower. Field investigations further showed that low Bd prevalence in tadpoles was associated with rapid recruitment and population recovery despite high mortality in adults[68,69].

Most studies investigated immunisation success within a single life stage, so it remains unclear whether effects in the larval stage can persist through metamorphosis. This is a critical question that remains to be addressed, especially given our findings show significant effectiveness in reducing infection prevalence within the larval phase. Larval immunisation offers several advantages: (1) a single adult breeding pair often produce hundreds to thousands of embryos, allowing easy collection of large cohorts, and (2) tadpoles can be reared at higher densities on inexpensive plant-based diets rather than the carnivorous diets post-metamorphic frog require. Importantly, (3) tadpoles are far more tolerant of Bd infection than post-metamorphic frogs[61,70]. This heightened tolerance makes tadpoles particularly well suited for live-pathogen immunisation. If increased resistance in immunised larvae transfers across metamorphosis into more disease-resistant adults, larval manipulation would offer a powerful tool to immunise larger numbers of individuals at lower costs and enable significant immunised-head starting for reintroduction or supplementation programs.

Immunisation effects differ among taxonomic families and depend on inherent susceptibility

While phylogenetic relatedness did not explain much of the observed heterogeneity, immunisation effects differed among taxonomic families. Immunisation was particularly effective in reducing prevalence in Hylidae. In fact, high Bd-induced mortality in Hylidae has been reported in a previous meta-analysis[38], suggesting that immunisation may be more effective in more susceptible species. Consistent with this assumption, we found that baseline mortality rates significantly predicted immunisation effectiveness: greater reductions in mortality occurred in groups with higher baseline mortality. Conversely, Bufonidae are also recognised as highly susceptible to Bd[7,38,71], yet immunisation was not particularly effective in this family. We noted that these estimates were based on only three Bufonidae species (Figure 2), mostly involving probiotic treatments with less precise effects (Figure 3). We therefore encourage more studies testing effective approaches in Bufonidae, such as exposure to live-pathogen, natural chemical or synthetic antiparasitics.

CONCLUSION

This meta-analysis reveals that prior exposure to live Bd, natural chemical (with datasets mostly comprising Bd metabolites) or synthetic antiparasitics shows the strongest tendency to reduce susceptibility to Bd. These approaches act at different stages of infection progression, with effects varying across life stages and taxonomic groups. This underscores the importance of application life stage and species-specific targeting for successful chytrid mitigation. In addition, we detected a significant effect in reducing larval prevalence, highlighting the need for cross-stage research to test whether the benefits of immunisation in larvae persist into adulthood. Our results further suggest that habitat manipulation providing benefits similar to effective approaches – such as establishing suitable microhabitats that enable wild animals to clear infection (vaccination through live infection-clearance) – is more likely to succeed than those involving ineffective approaches (e.g., environmental augmentation of microbiomes). Importantly, any unintended effects of immunisation treatments (e.g., toxicity of synthetic antiparasitics) must be carefully evaluated before real-world implantation.

METHODS

We conducted a systematic review and meta-analysis following, as closely as possible, the PRISMA-EcoEvo guidelines[72]. A checklist is provided as a supplementary file. For our full PRISMA diagram detailing our search and selection criteria see Figure S1.

Literature searches

We aimed to retrieve a large representative sample of studies measuring changes in amphibian susceptibility to chytridiomycosis after applying different interventions. We searched publications from ISI Web of Science (Core Collection), Scopus, ProQuest and ScienceDirect in April 2025. The search string developed included “chytridiomycosis” (OR synonyms) AND “amphibian” (OR synonyms) AND terms for individual-based interventions

(e.g. immunisation, vaccine) (see *Supplementary Material* for full details). To validate our search string, we generated a list of ten gold-standard papers[19-21,25,26,28,29,41,46,52] that contained suitable datasets and ensured that our search strings retrieved all ten. To capture recent research, we included grey literature (dissertations, theses, preprints and non-English publications). We focused on empirical data and did not retrieve reviews, book chapters, meeting abstracts, editorials, and corrections. Furthermore, we found all papers citing or cited in four reviews[73-76] using Web of Science and Scopus in May 2025. All of the above details were provided in the supplementary material. We identified 3,538 records and removed 2,053 duplicates using the R package *litserchr*[77], the website *Rayyan*[78] as well as manual checks (Figure S1). The remaining 1,485 records included 45 PhD and 26 MSc theses, from which we identified an additional 31 papers and 1 PhD thesis (total 1,517 records).

Inclusion criteria

For the 1,517 records, we first screened the title and abstract and then their full text. To be included, the study needed to meet all the following inclusion criteria:

1. ***Be an in vivo experiment on amphibians****:* We excluded non-experimental studies, experimental studies on non-amphibians, and *in vitro* studies on amphibians (e.g. isolated cells, skin secretions).
2. ***Conduct a treatment aimed at reducing individual-level susceptibility to chytridiomycosis, with a comparable control***: *A comparable control* comprises individuals with the same initial infection states, from the same population and subjected to identical procedures (except for the active treatment components). This ensures that observed differences are attributable to the focal treatment rather than variation in initial infection states, populations or handling procedures. Accordingly, we excluded (a) studies aiming to enhance host susceptibility[47] and (b) studies aiming to mitigating host susceptibility by creating chytrid-unfavourable environments. We excluded studies identified as (b) because treatment differences can result from reduced chytrid performance rather than increased host resistance.
3. ***Have comparable treatment conditions***: We included studies where untreated and treated individuals were (a) uninfected at the onset of experimental infection to avoid confounding by prior infection, (b) exposed to the same chytrid strain and (c) kept in a common-garden setting during the period of experimental infection.
4. ***Present the correct infection outcomes***: We only included studies that reported (a) infection prevalence, (b) infection intensity and/or (c) mortality.
5. ***Complete an infection assessment***: We included studies in which infection outcomes were measured in all individuals, or for a randomly selected subset. We excluded mark-recapture studies that failed to assess released individuals (e.g., those with <27% recapture rate[79]), because treatment effects cannot be reliably assessed without knowing the infected states of non-recaptured animals.

Data collection and classification

We identified 38 eligible studies (Figure S1; supplementary files–study summary) and classified the number of experiments within each study. An experiment was defined as a comparable pair of animal groups whose resistance was experimentally enhanced (treatment) or left unchanged (control), with all other conditions held constant. For instance, a study testing the effect of probiotics independently at two temperatures[80], using four different samples of animals, yielded two experiments.

Within each experiment, we recorded the research location (Figure 1), methodological and biological variables (Tables 1 & 2). For taxonomy, species were consolidated into family-level groups to increase sample sizes (Figure 2). For life stage, we categorised them into (a) larva (no forelimbs), (b) metamorph (forelimb emergence to tail resorption), (c) juvenile (tail resorption to sexual maturation) and (d) adult (after sexual maturation)[81]. However, there were no eligible studies on metamorphs. We extracted the life stage both at immunisation and at live pathogen challenge to clarify cross-stage effects. However, cross-stage experiments were rare (6 of 53), so these two life stages were largely overlapping. We therefore considered only the life stage at challenge for analyses. We also recorded the time elapsed between immunisation and live pathogen challenge (Table 1) to clarify effect persistence.

We focused on treatment differences in prevalence, infection intensity and mortality. Mortality rate was defined as the cumulative proportion of deaths following live pathogen challenge, for which we extracted the final measure closest to the end of the challenge period. In contrast, prevalence and infection intensity could either increase or decrease over time as individuals transition between infection states. For studies that tested prevalence and/or infection intensity multiple times throughout the experiment, we extracted the peak value (indicating the most severe outcome caused by the chytrid). Peak prevalence was defined as the greatest proportion infected at a given time point. Peak infection intensity was calculated by identifying the maximum pathogen loads for each individual across all measures and then averaging individual peak values. A single value was extracted if prevalence and/or intensity was only measured once. To quantify variation in measurement effort, we recorded the total number of trait measurements (Table 1) for the datasets of prevalence and infection intensity.

We extracted the mean, measures of error (SD, standard error (SE), 95% CI), sample size, and the time elapsed between live pathogen challenge and outcome measurement (Table 1). Data were extracted from text, tables, figures or supplementary materials. If raw data were available we extracted the necessary data using the R package *tidyverse*[82] for calculation of summary statistics. If figures were used, we used the R package *ShinyDigitise*[83] to extract values. The data source is listed in the supplementary files–metadata.

SEs and 95% CIs were first converted to SDs before calculating effect statistics. For studies reporting $\log_{10}$-scale summaries, we converted the data to natural-log mean (Z) and SD (SD_Z) and then back-transformed these to the raw-scale mean (m) and SD (SD_m) following Higgins et al[84]:

$$m = \exp\left(Z + \frac{{SD_Z}^2}{2}\right)$$

$$SD_m = \sqrt{\left(\exp\left({SD_Z}^2\right) - 1\right)\exp\left(2Z + {SD_Z}^2\right)}$$

Back-transformation can be unreliable when SD_Z is missing or large. For such cases, we approximated $m \approx \exp(Z)$ and considered SD_m as unavailable. We ran a sensitivity analysis excluding these transformed data (k = 18). The moderating effects of immunisation method and host life stage became unclear in this sensitivity analysis (Table S13), suggesting that the observed immunisation effects on infection intensity were largely driven by these transformed data. Studies reporting transformed data typically involved highly variable or extreme values, which in our dataset tended to show larger treatment contrasts. Notably, live-pathogen immunisation still tended to reduce infection intensity when transformed data were excluded (CIs: –93% to +6%; p = 0.06), which align with the main conclusion (CIs: –90% to –6%; p = 0.04).

Effect size calculation

Effect sizes were calculated only for *Bd* datasets. For both prevalence and mortality, we converted percentages into counts of events ('infection' for prevalence; 'death' for mortality) and non-events. We then calculated the log risk ratio (logRR), which compares the risk (probability) of an event in the treatment compared to the control group[85]:

$$\log \mathrm{RR} = \ln\left(\frac{T_{event} \,/\, (T_{event} + T_{non-event})}{C_{event} \,/\, (C_{event} + C_{non-event})}\right)$$

We calculated sampling variance, v(log RR), for prevalence and mortality using the following formulas:

$$v(\log RR) = \frac{1}{T_{event}} - \frac{1}{T_{event} + T_{non-event}} + \frac{1}{C_{event}} - \frac{1}{C_{event} + C_{non-event}}$$

Here, T_{event} and C_{event} are the count of events for treatment (immunised) and control groups, respectively, whereas $T_{non\text{-}event}$ and $C_{non\text{-}event}$ are the count of non-events. Cells with a zero value generate undefined estimates. In the cases with at least one zero-count, we added 0.5 to all cells[86]. We excluded experiments with non-events: that is, no infections for prevalence (k = 2) and no deaths for mortality (k = 27). These experiments were excluded because there were no infection outcomes to be compared.

For infection intensity, we calculated the log response ratio (lnRR), which represents the log of proportional difference in the means between groups[87]:

$$\mathrm{lnRR}_1 = \ln\left(\frac{m_T}{m_C}\right)$$

$$v(\mathrm{lnRR})_1 = \frac{{SD_T}^2}{n_T {m_T}^2} + \frac{{SD_C}^2}{n_C {m_C}^2} = \frac{{CV_T}^2}{n_T} + \frac{{CV_C}^2}{n_C}$$

where m is the mean, n is the sample size, SD is the standard deviation, and CV (SD/m) is the coefficient of variation for treatment (T) and control (C) groups, respectively. However, lnRR_1 and $v(\mathrm{lnRR})_1$ can be biased when sample size are small to moderate[88]. We further corrected for such bias[89]:

$$\mathrm{lnRR}_2 = \ln\left(\frac{m_T}{m_C}\right) + \frac{1}{2}\left(\frac{{\mathrm{CV}_T}^2}{n_C} - \frac{{\mathrm{CV}_C}^2}{n_T}\right)$$

$$v(\mathrm{lnRR})_2 = \frac{{CV_T}^2}{n_T} + \frac{{CV_C}^2}{n_C} + \frac{{CV_T}^4}{2{n_T}^2} + \frac{{CV_C}^4}{2{n_C}^2}$$

lnRR_2 and $v(\mathrm{lnRR})_2$ cannot be estimated when CVs are unavailable. Only few (6 of 71) observations lacked CVs, so we applied the 'missing-cases' method[89] – using the weighted average CVs from datasets that report CVs to estimate effect sizes and sampling variances for missing cases (i = 1, 2, ..., K):

$$\mathrm{lnRR}_3 = \ln\left(\frac{m_T}{m_C}\right) + \frac{1}{2}\left(\frac{\left[\frac{\sum_{i=1}^{K}(n_{Ti}\,CV_{Ti})}{\sum_{i=1}^{K} n_{Ti}}\right]^2}{n_T} - \frac{\left[\frac{\sum_{i=1}^{K}(n_{Ci}\,CV_{Ci})}{\sum_{i=1}^{K} n_{Ci}}\right]^2}{n_C}\right)$$

$$v(\mathrm{lnRR})_3 = \frac{\left[\frac{\sum_{i=1}^{K}(n_{Ti}\,CV_{Ti})}{\sum_{i=1}^{K} n_{Ti}}\right]^2}{n_T} + \frac{\left[\frac{\sum_{i=1}^{K}(n_{Ti}\,CV_{Ci})}{\sum_{i=1}^{K} n_{Ci}}\right]^2}{n_C} + \frac{\left[\frac{\sum_{i=1}^{K}(n_{Ti}\,CV_{Ti})}{\sum_{i=1}^{K} n_{Ti}}\right]^4}{2{n_T}^2} + \frac{\left[\frac{\sum_{i=1}^{K}(n_{Ci}\,CV_{Ci})}{\sum_{i=1}^{K} n_{Ci}}\right]^4}{2{n_C}^2}$$

All types of lnRR and v(lnRR) were calculated following the *func.R* script provided by Nakagawa et al[89]. To test the result robustness, we ran a sensitivity analysis using the 'all-cases' method[89] to estimate sampling variance. Under the 'all-cases' estimates, the effect of live-pathogen immunisation became non-significant, with p-values changing from 0.04 to 0.10 (Figure 3b; Table S14). In contrast, immunisation effects became significant in Hylidae (p = 0.02) and in captive individuals (p = 0.046), reducing infection loads by 71% and 73%,

respectively (Table S14). These findings under the 'all-cases' estimates partly align with the main conclusions, which showed strong benefits of immunisation in Hylidae.

In all calculations, a negative effect size indicates a reduced infection outcome in immunised individuals compared with control individuals. All analyses were conducted in R[90] (version 4.5.1) using the *escalc* function in the *metafor* package (version 4.8-0)[91]. Literature searches, data collection and effect size calculation were conducted by a researcher (MHJC).

Data analysis

For each outcome, we ran a multi-level meta-analysis (MLMA) using residual maximum likelihood (REML) and *t*-based interference for parameters. We reported both the confident intervals and prediction intervals of effect size estimates[92-94]. To assess effect heterogeneity, we calculated I^2, defined as the proportion of total variance among effect sizes unexplained by the known sampling variance, as well as mean-standardised heterogeneity (CVH^2) and variance-mean-standardised heterogeneity (M^2)[95]. We used the *orchaRd* package (version 2.1.3)[96] to calculate heterogeneity and visualise results.

We included three random factors in each MLMA: (1) 'observation ID' to capture within-study variance beyond the expected from sampling error; (2) 'study ID' to account for similar experimental setups within studies; and (3) 'phylogenetic relatedness' to account for phylogenetic distances among species. We pruned a recently published amphibian phylogeny[97] to species in our datasets (Figure 2) and generated a corresponding phylogenetic correlation matrix using the *vcv* function in the *ape* package (version 5.8-1)[98].

Multiple effect sizes within an experiment were often computed using a shared treatment group (such as 'high-dose Bd metabolites' vs a sham control, and 'low-dose' vs the same control[28]). To account for such non-independence[99], we adjusted the sampling variance *v*(lnRR) by creating a sampling (co)variance matrix using the *vcalc* function in the *metafor* package[91], and we included this matrix in our models to account for shared-control effects.

Meta-regression

We tested whether *immunisation method* (6 levels; Table 1), *host life stage* (3 levels: larva, juvenile, adult), *host origin* (2 levels: captive, wild) and *taxonomic family* (9 levels; Figure 2) moderated immunisation effectiveness. Each moderator was analysed separately along with the random factors and correlation matrix described above. There were no sufficient data on host sex (48 of 53 experiments = 91% missing) and pathogen characteristics [lineage (77% missing), virulence (79% missing) and passage history (85% missing)], so we did not test their moderating effects.

For *host origin*, we used juvenile and adult datasets because only these datasets included both captive-bred and wild-bred individuals; in contrast, larvae were all wild-bred. To test whether *origin* moderated effects on infection intensity, we further restricted the analysis to juvenile

datasets only because intensity data in adults were mostly derived from wild-bred individuals (k = 15 of 16). For *taxonomic family*, we only interpreted results for Hylidae, Ranidae and Bufonidae because the other families had very small datasets (k < 5; Figure 2). For transparency, results for all families were reported in the supplementary material.

We mean-centred three methodological variables (Table 1): (1) immunisation-to-challenge interval (0–896 days; mean = 38 days), (2) challenge-to-measurement interval (5–241 days; mean = 43 days) and (3) measurement effort (1–33; mean = 6) and included them as separate moderators in the meta-regressions estimating overall effects. None of these variables predicted immunisation effects (Table S11), so they were not further considered when testing the focal moderators given the relatively small datasets in our meta-analysis.

To test whether more susceptible populations or species benefit more strongly from immunisation, we included the mortality rates of the untreated group (i.e., inherent susceptibility) as a moderator in the meta-regressions estimating overall effects.

Infection progression

Studies often reported multiple outcome measures. To quantify how effects on different outcomes covary within studies, we ran a multivariate meta-analytic model using *metafor* package[91]. We considered infection outcome as a moderator with a study-level random effect (outcome -1 | study ID) to estimate within-study effect correlation. Effect size and sampling variance were calculated as in the univariate models. We allowed heterogeneity to differ among the three outcomes by using an unstructured (co)variance matrix for the true effects. We tested overall effect correlations rather than correlations within specific immunisation methods or host types because of limited sample sizes (n = 23 studies reporting both prevalence and infection intensity, n = 21 reporting both infection intensity and mortality, n = 26 reporting both prevalence and mortality).

Publication bias

We ran additional meta-regressions that included the square root of the inverse of effective sample size and the mean-centred publication year as separate moderators to test for the small-study effect[39] and the time-lag bias[40], respectively. We also visually evaluated funnel asymmetry using funnel plots, which showed the residuals of a meta-regression against effect size precision (1/SE).

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DATA AVAIABILITY: Metadata and supplementary files can be downloaded from https://doi.org/10.5281/zenodo.18844488

Table 1. Methodological sources of variation that may affect estimates of immunisation effectiveness in experiments assessing amphibian susceptibility to chytridiomycosis. Factors likely to alter observed outcomes include the immunisation method with the expected immune pathways involved, and key experimental design features (immunisation-to-challenge interval, challenge-to-measurement interval, and measurement effort).

Factor	Details
Immunisation method	1. **Live pathogen**: Exposure to live fungus, either followed by a clearance procedure[19,20,25,26,62,100] or not (in cases without clinical signs[24,34,52], or when individuals are given time to self-cure[35]). This approach aims to stimulate adaptive immunity. 2. **Dead pathogen**: Exposure to killed fungus[21,27,45,46], commonly administered through repeated doses[21,27,45]. This approach aims to stimulate adaptive immunity. 3. **Natural chemical**: Exposure to chemicals produced by live organisms, including skin peptides from resistant frog species[101] or cell-free pathogen compound (Bd metabolite)[28,29,41]. Exposure to Bd metabolites often involves repeated dosing[28,29,41]. The immunity stimulated by this approach is unclear. 4. **Synthetic antiparasitic**: Exposure to synthetic antifungals (e.g., itraconazole[54] or terbinafine[32]) or pesticides (e.g., lvermectin[53]). The immunity stimulated by this approach is unclear. 5. **Probiotic:** Exposure to antifungal microbes[30,31,80,102-104]. Before adding microbes, amphibians are often washed in antimicrobial agents[30,80,102,103] or natural media[31,104] This step aims to remove existing bacteria to increase successful colonisation of the introduced microbes and also minimise unintended bacterial interactions. Probiotics are expected to enhance innate immunity[105,106], whereas recent studies suggest their defences to specific pathogens[107], aligning with adaptive immunity. 6. **Other:** Other approaches, such as enriching skin mast cells (immune cells that mediate neutrophil recruitment and mucin production)[108] or exposing amphibians to metyrapone (an inhibitor of corticosterone synthesis that may reduce stress-related immunosuppression and thus stabilise microbiota)[109]. These two examples are expected to enhance innate immunity.
Immunisation-to-challenge interval	Time elapsed between immunisation and live pathogen challenge. If immunisation-induced protection declines with time, any benefits should be reduced when challenge occurs long after immunisation. In contrast, if protection is long-lasting, immunised individuals should remain less susceptible over longer periods.
Challenge-to-measurement interval	Time elapsed between live pathogen challenge and outcome measurement. Because infection unfolds over time, the stage of disease reached at the point of measurement can strongly influence observed outcomes. Longer intervals allow greater divergence in infection trajectories, potentially magnifying differences among treatment groups depending on how rapidly disease progresses.
Measurement effort	Disease progression involves moving through different infection states and severities[20,24,26,32], generating temporal variation in observed outcomes. Studies with regular measurements (e.g., regular skin swabs) may therefore be more likely to detect treatment differences than studies with low measurement effort (e.g., skin swabbing only at the end of the experiment).

Table 2. Biological factors that can influence the effectiveness of immunisation treatments in reducing host susceptibility to chytridiomycosis. Factors marked with an asterisk (*) had sufficient data for quantitative synthesis and were included in the meta-analysis.

Factor	Details
Host life stage*	The chytrid fungus infects keratinized tissues, which are restricted to the mouthparts of tadpoles and then extend to the entire skin after metamorphosis[61,64]. Therefore, tadpoles are typically more disease-resistant than post-metamorphic stages, with metamorphs or newly metamorphosed juveniles the most susceptible[38]. The increased susceptibility during metamorphosis is further linked to transient immune downregulation associated with organ reorganisation and the incomplete transition from larval to adult immune systems[65,110].
Host origin*	Captive-bred amphibians have reduced contact with other species and natural substrates, and their microbial communities often differ from those of wild-bred individuals[111]. Whether these differences translate into different responses to Bd infection or to immunisation is unknown.
Host Taxonomy*	Taxa-specific biology (e.g., physiology[36], behaviour[112], habitat[37]) could influence susceptibility to Bd and may drive species-specific responses to immunisation[24].
Host sex	Males and females use different strategies to reproduce. Males often increase fertilisation success by investing more in sexual traits (sperm, courtship) that are immunosuppressive[113]. In contrast, females usually increase breeding success by investing in soma that promote fecundity[114]. Amphibians have sex-specific reproductive strategies[115], and males are often be more susceptible to Bd than females[116,117]. It is currently unclear, however, whether immunisation benefits males more strongly than females.
Pathogen characteristics	Chytrid virulence varies with genotype, phenotype and passage history[29,71]. For example, the global pandemic Bd lineage is highly virulent and linked with widespread amphibian declines, whereas the impacts of other chytrid lineages remain unclear[5]. Virulence also tends to diminish in strains subject to extensive *in vitro* passage[118].

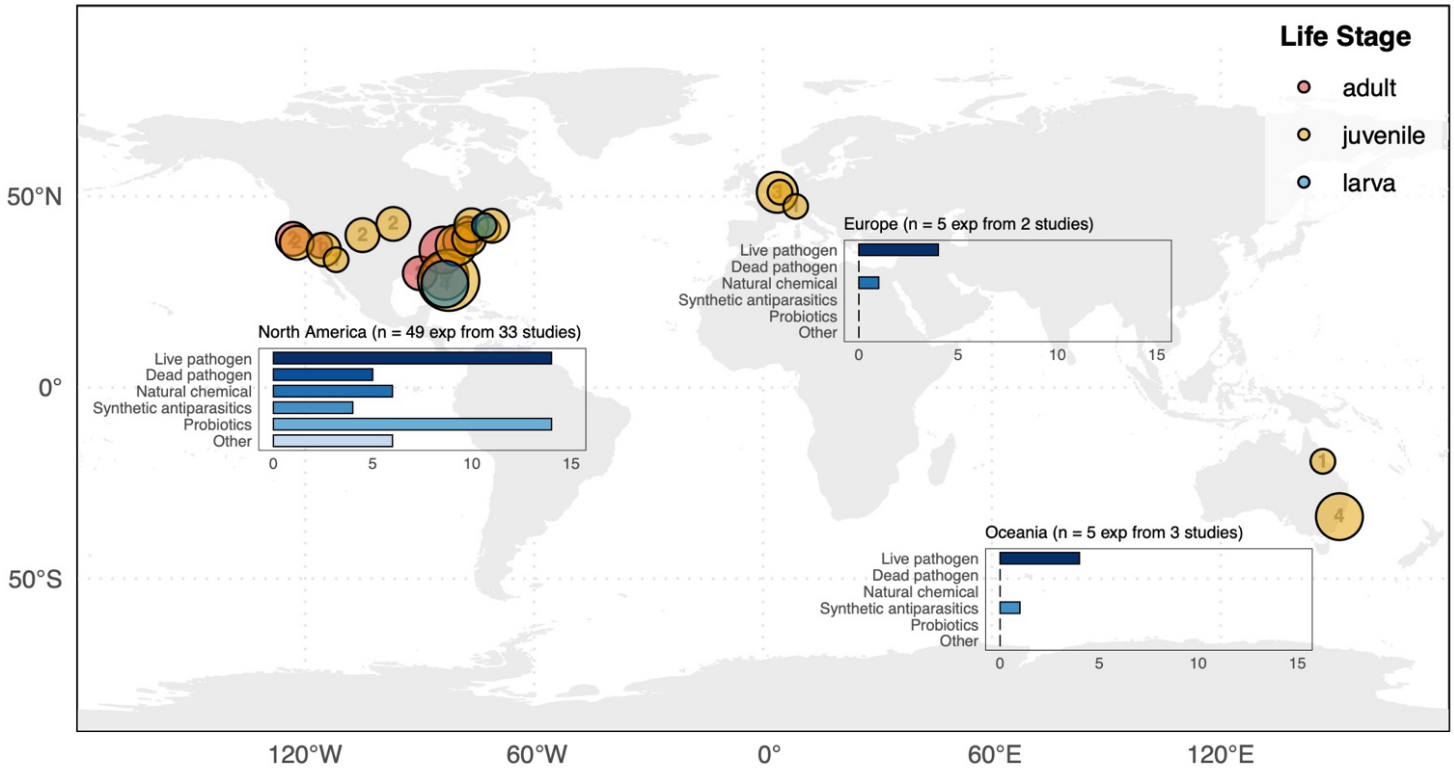


Figure 1. Geographic locations of eligible studies. There are usually multiple independent experiments in a study, so counts represent the number of experiments. Bubble size indicates the number of experiments per location, and colour represents the life stage at chytrid (Bd/Bsal) infection challenge. Histograms show the number of experiments for each immunisation approach on each continent. Experiments that tested multiple approaches were counted separately for each approach.

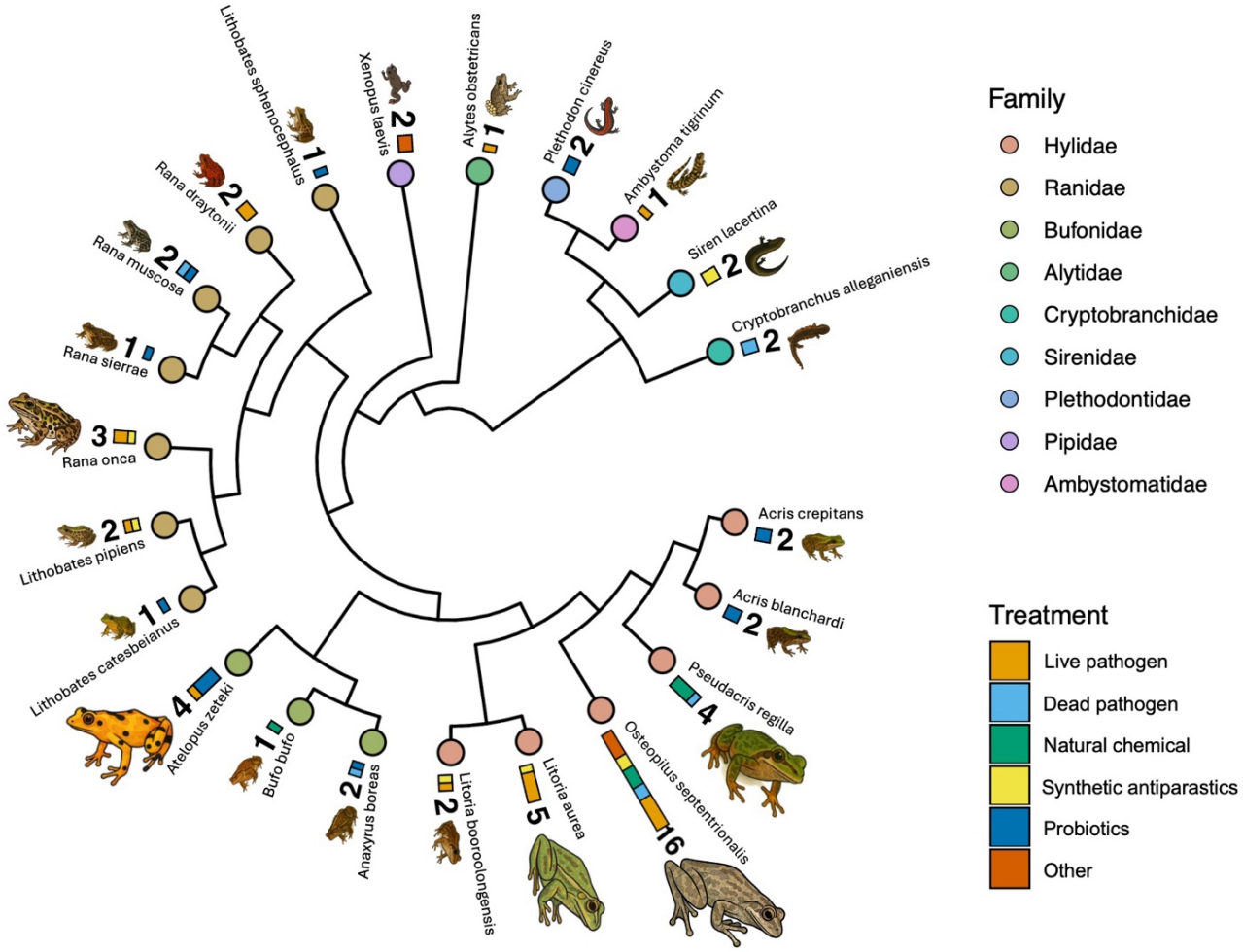


Figure 2. Phylogenetic relatedness of species used in Bd experiments. This tree was pruned according to the recently published phylogeny[97]. Node colours indicate family, and rectangle colours indicate approach types. The sizes of each rectangle and animal silhouette reflect the number of experiments per immunisation approach, with counts indicating the total number of experiments per species. Experiments that tested multiple approaches were counted separately for each approach.

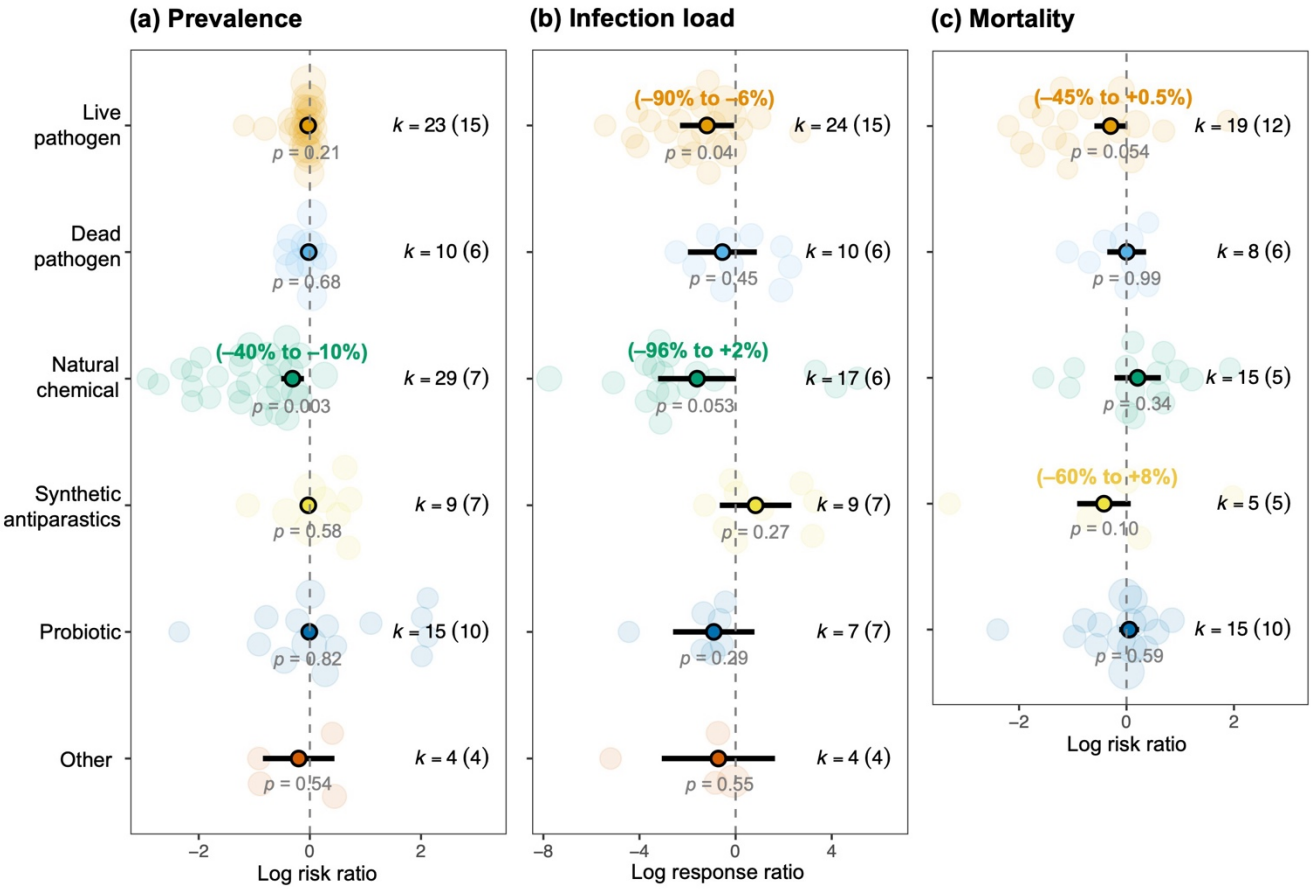


Figure 3. Moderating effects of immunisation method on (a) prevalence, (b) infection intensity and (c) mortality. Darker dots and lines indicate means and 95% confidence intervals, along with *p*-values. Confidence intervals are further presented as percentage changes in outcome measures for specific methods showing a clear effect. Lighter dots indicate individual effect sizes, and dot sizes reflect the relative precision (1/SE) of each effect size. Counts represent the number of effect sizes (*k*), with the number of experiments in parentheses. Full model outputs (including *t*-values and prediction intervals) are reported in Table S1.

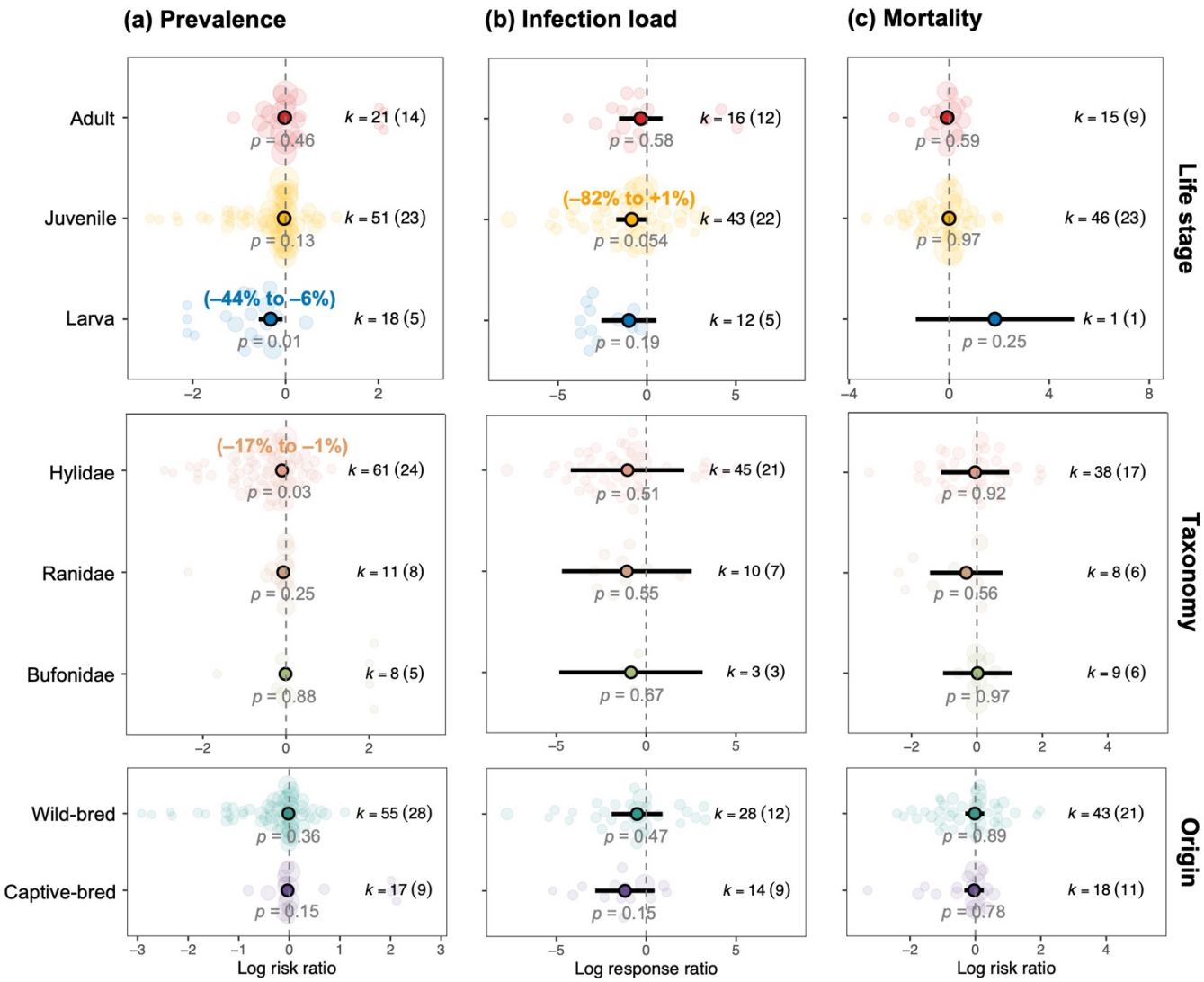
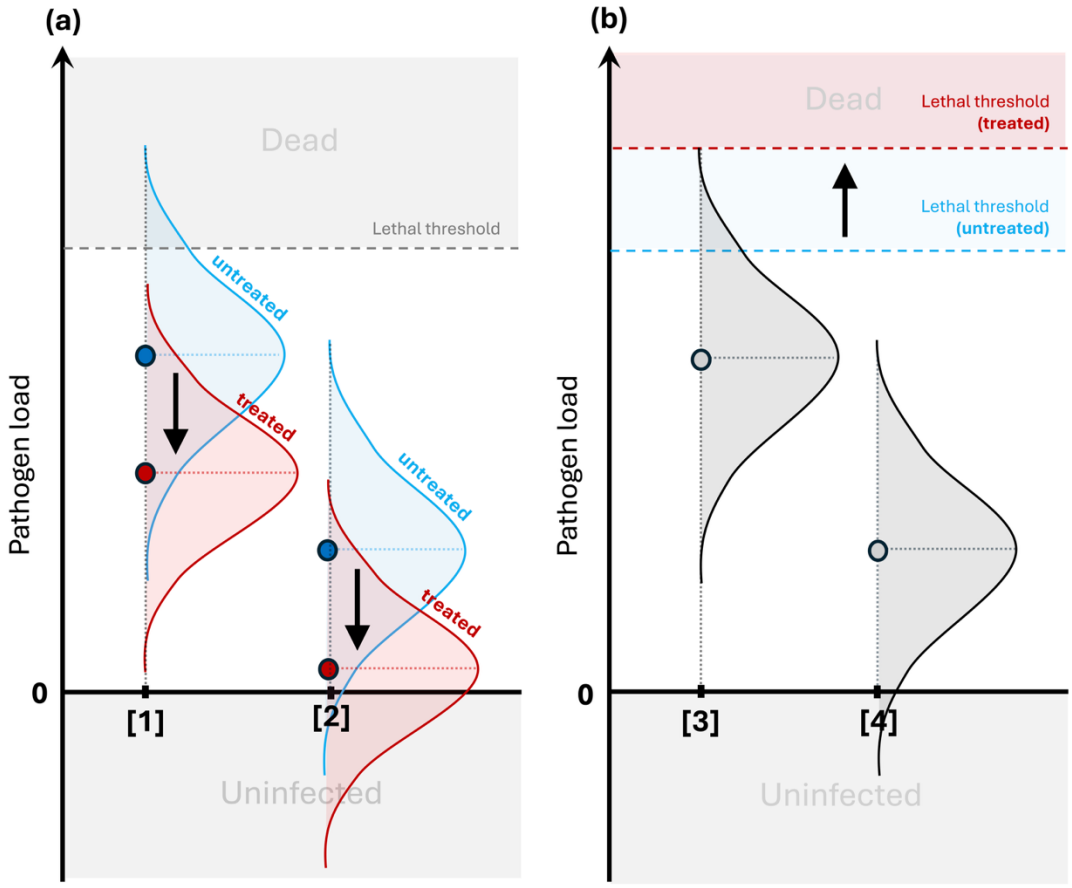


Figure 4. Moderating effects of host type (life stage, taxonomy, origin) on (a) prevalence, (b) infection intensity and (c) mortality. Darker dots and lines indicate means and 95% confidence intervals, along with *p*-values. Confidence intervals are further presented as percentage changes in outcome measures for categories showing clear patterns. Lighter dots indicate individual effect sizes, and dot sizes reflect the relative precision (1/SE) of each effect size. Counts represent the number of effect sizes (*k*), with the number of experiments in parentheses. Full model outputs (including *t*-values and prediction intervals) are reported in Tables S4, S6, and S9.



Outcome \ Case	[1]	[2]	[3]	[4]
Prevalence	×	↓	×	×
Infection load	↓	↓	×	×
Mortality	↓	×	↓	×

Figure 5. Hypothetical illustration of immunisation-induced (a) enhanced resistance (reduced pathogen load) or (b) enhanced tolerance (reduced mortality without reducing pathogen load). The y-axis represents pathogen load from zero (uninfected) to lethal levels. The x-axis represents studies with different infection outcomes (e.g. [1] vs [2]), potentially resulting from differences in focal species (e.g. high vs low inherent susceptibility), pathogen characteristics (e.g., high vs low virulence) or testing environment (e.g., winter vs summer).

Reference

(*) indicates eligible *Bd* studies, and (‡) indicates eligible *Bsal* studies used in this meta-analysis

1 Baker, R. E. *et al.* Infectious disease in an era of global change. *Nat Rev Micriobiol* **20**, 193–205 (2022).

2 Tompkins, D. M., Carver, S., Jones, M. E., Krkošek, M. & Skerratt, L. F. Emerging infectious diseases of wildlife: a critical perspective. *Trends Parasitol* **31**, 149–159 (2015).

3 Berger, L. *et al.* Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc Natl Acad Sci USA* **95**, 9031–9036 (1998). https://doi.org/10.1073/pnas.95.15.9031

4 Martel, A. *et al. Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proc Natl Acad Sci USA* **110**, 15325–15329 (2013). https://doi.org/10.1073/pnas.1307356110

5 Lips, K. R. Overview of chytrid emergence and impacts on amphibians. *Philos Trans R Soc B* **371**, 20150465 (2016). https://doi.org/10.1098/rstb.2015.0465

6 Yap, T. A., Nguyen, N. T., Serr, M., Shepack, A. & Vredenburg, V. T. *Batrachochytrium salamandrivorans* and the risk of a second amphibian pandemic. *Ecohealth* **14**, 851–864 (2017). https://doi.org/10.1007/s10393-017-1278-1

7 Scheele, B. *et al.* Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* **363**, 1459–1463 (2019). https://doi.org/10.1126/science.aav0379

8 Luedtke, J. A. *et al.* Ongoing declines for the world's amphibians in the face of emerging threats. *Nature* **622**, 308-314 (2023). https://doi.org/10.1038/s41586-023-06578-4

9 Oficialdegui, F. J., Sanchez, M. I., Monsalve-Carcaño, C., Boyero, L. & Bosch, J. The invasive red swamp crayfish (*Procambarus clarkii*) increases infection of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). *Biol Invasions* **21**, 3221–3231 (2019). https://doi.org/10.1007/s10530-019-02041-6

10 Liew, N. *et al.* Chytrid fungus infection in zebrafish demonstrates that the pathogen can parasitize non-amphibian vertebrate hosts. *Nat Commun* **8**, 15048 (2017). https://doi.org/10.1038/ncomms15048

11 Johnson, M. L. & Speare, R. Survival of *Batrachochytrium dendrobatidis* in water: quarantine and disease control implications. *Emerg Infect Dis* **9**, 922–925 (2003).

12 Burns, T. J. *et al.* Indirect terrestrial transmission of amphibian chytrid fungus from reservoir to susceptible host species leads to fatal chytridiomycosis. *Anim Conserv* **24**, 602–612 (2021). https://doi.org/10.1111/acv.12665

13 Stockwell, M., Clulow, S., Clulow, J. & Mahony, M. The impact of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* on a green and golden bell frog *Litoria aurea* reintroduction program at the Hunter Wetlands Centre Australia in the Hunter Region of NSW. *Aust Zool* **34**, 379–386 (2008).

14 Hammond, T. T. *et al.* Overwinter behavior, movement, and survival in a recently reintroduced, endangered amphibian, *Rana muscosa*. *J Nat Conserv* **64**, 126086 (2021). https://doi.org/10.1016/j.jnc.2021.126086

15 Joseph, M. B. & Knapp, R. A. Disease and climate effects on individuals drive post-reintroduction population dynamics of an endangered amphibian. *Ecosphere* **9**, e02499 (2018). https://doi.org/10.1002/ecs2.2499

16 Harding, G., Griffiths, R. A. & Pavajeau, L. Developments in amphibian captive breeding and reintroduction programs. *Conserv Biol* **30**, 340–349 (2016). https://doi.org/10.1111/cobi.12612

17 Excler, J. L., Saville, M., Berkley, S. & Kim, J. H. Vaccine development for emerging infectious diseases. *Nat Med* **27**, 519–600 (2021).
18 Andey, T., Soni, S. & Modi, S. *Conventional vaccination methods: inactivated and live attenuated vaccines* in *Advanced Vaccination Technologies for Infectious and Chronic Diseases* pp. 37-50 (Academic Press, 2024).
19 *McMahon, T. A. *et al.* Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. *Nature* **511**, 224–227 (2014). https://doi.org/10.1038/nature13491
20 *Waddle, A. W. *et al.* Hotspot shelters stimulate frog resistance to chytridiomycosis. *Nature* **631**, 344–349 (2024). https://doi.org/10.1038/s41586-024-07582-y
21 *Nordheim, C. L. *et al.* Metabolites from the fungal pathogen *Batrachochytrium dendrobatidis* (bd) reduce Bd load in Cuban treefrog tadpoles. *J Appl Ecol* **59**, 2398–2403 (2022). https://doi.org/10.1111/1365-2664.14242
22 Scheele, B. C. *et al.* Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. *Conserv Biol* **28**, 1195–1205 (2014). https://doi.org/10.1111/cobi.12322
23 Bletz, M. C., Grant, E. H. C. & Direnzo, G. Quantitative support for the benefits of proactive management for wildlife disease control. *Conserv Biol* **39**, e14363 (2025). https://doi.org/10.1111/cobi.14363
24 *‡Greener, M. S. *et al.* Presence of low virulence chytrid fungi could protect European amphibians from more deadly strains. *Nat Commun* **11**, 5393 (2020). https://doi.org/10.1038/s41467-020-19241-7
25 *Cashins, S. D. *et al.* Prior infection does not improve survival against the amphibian disease chytridiomycosis. *PLoS One* **8**, e56747 (2013).
26 *Adams, A. J., Bushell, J. & Grasso, R. L. To treat or not to treat? Experimental pathogen exposure, treatment, and release of a threatened amphibian. *Ecosphere* **13**, e4294 (2022). https://doi.org/10.1002/ecs2.4294
27 *Kaganer, A. W. *et al.* Immune priming prior to pathogen exposure sheds light on the relationship between host, microbiome and pathogen in disease. *R Soc Open Sci* **10**, 220810 (2023). https://doi.org/10.1098/rsos.220810
28 *McMahon, T. A. *et al.* Efficacy of Bd metabolite prophylaxis dose and duration on host defence against the deadly chytrid fungus *Batrachochytrium dendrobatidis*. *J Appl Ecol* **61**, 3139–3147 (2024). https://doi.org/10.1111/1365-2664.14818
29 *Barnett, K. M., Detmering, S. E., McMahon, T. A. & Civitello, D. J. Asymmetric cross-strain protection for amphibians exposed to a fungal-metabolite prophylactic treatment. *Biol Lett* **17**, 20210207 (2021). https://doi.org/10.1098/rsbl.2021.0207
30 *Becker, M. H. *et al.* Composition of symbiotic bacteria predicts survival in Panamanian golden frogs infected with a lethal fungus. *Proc R Soc B* **282**, 20142881 (2015). https://doi.org/10.1098/rspb.2014.2881
31 *Weeks, D. M., Parris, M. J. & Brown, S. P. Recovery and resiliency of skin microbial communities on the southern leopard frog (*Lithobates sphenocephalus*) following two biotic disturbances. *Anim Microb* **2**, 35 (2020). https://doi.org/10.1186/s42523-020-00053-5
32 *Towe, A. E. *et al.* Pilot study of intracoelomic terbinafine implants in greater sirens (*Siren lacertina*). *J Zoo Wildl Med* **55**, 453-461 (2024). https://doi.org/10.1638/2023-0010
33 *Rohr, J. R., Brown, J., Battaglin, W. A., McMahon, T. A. & Relyea, R. A. A pesticide paradox: fungicides indirectly increase fungal infections. *Ecol Appl* **27**, 2290–2302 (2017). https://doi.org/10.1002/eap.1607

34 ‡Barnhart, K. *et al. Batrachochytrium salamandrivorans* elicits acute stress response in spotted salamanders but not infection or mortality. *Anim Conserv* **23**, 533–546 (2020). https://doi.org/10.1111/acv.12565

35 ‡McCartney, J. A. *Fool me once: characterizing the response of Notophthalmus viridescens to multiple exposures of Batrachochytrium salamandrivorans*. (MSc thesis) University of Massachusetts Boston (2022).

36 Woodhams, D. C. *et al.* Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. *Anim Conserv* **10**, 409–417 (2007).

37 Jervis, P. *et al.* Post-epizootic microbiome associations across communities of neotropical amphibians. *Mol Ecol* **30**, 1322-1335 (2021).

38 Sauer, E. L. *et al.* A meta-analysis reveals temperature, dose, life stage, and taxonomy influence host susceptibility to a fungal parasite. *Ecology* **101**, e02979 (2020). https://doi.org/10.1002/ecy.2979

39 Nakagawa, S. *et al.* Methods for testing publication bias in ecological and evolutionary meta-analyses. *Methods Ecol Evol* **13**, 4–21 (2022).

40 Koricheva, J. & Kulinskaya, E. Temporal instability of evidence base: a threat to policy making? *Trends Ecol Evol* **34**, 895–902 (2019).

41 *Barnett, K. M., Hilgendorff, B. A., Civitello, D. J. & McMahon, T. A. Fungal metabolites provide pre-exposure protection but no postexposure benefit or harm against *Batrachochytrium dendrobatidis*. *J Wildl Dis* **59**, 217–223 (2023). https://doi.org/10.7589/Jwd-D-22-00073

42 Rollins-Smith, L. A. *et al.* Immunomodulatory metabolites released by the frog-killing fungus. *Infect Immun* **83**, 4565–4570 (2015). https://doi.org/10.1128/Iai.00877-15

43 Rollins-Smith, L. A. *et al.* Metabolites involved in immune evasion by *Batrachochytrium dendrobatidis* include the polyamine spermidine. *Infect Immun* **87**, e00035-19 (2019). https://doi.org/10.1128/IAI.00035-19

44 Fites, J. S. *et al.* The invasive chytrid fungus of amphibians paralyzes lymphocyte responses. *Science* **342**, 366–369 (2013). https://doi.org/10.1126/science.1243316

45 *McMahon, T. A. *et al. Pseudacris regilla* metamorphs acquire resistance to *Batrachochytrium dendrobatidis* after exposure to the killed fungus. *Dis Aquat Org* **155**, 193–198 (2023). https://doi.org/10.3354/dao03753

46 *Stice, M. J. & Briggs, C. J. Immunization is ineffective at preventing infection and mortality due to the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. *J Wildl Dis* **46**, 70–77 (2010). https://doi.org/10.7589/0090-3558-46.1.70

47 Ramsey, J. P., Reinert, L. K., Harper, L. K., Woodhams, D. C. & Rollins-Smith, L. A. Immune defenses against *Batrachochytrium dendrobatidis*, a fungus linked to global amphibian declines, in the South African clawed frog, *Xenopus laevis*. *Infect Immun* **78**, 3981–3992 (2010). https://doi.org/10.1128/Iai.00402-10

48 Poorten, T. J., Stice-Kishiyama, M. J., Briggs, C. J. & Rosenblum, E. B. Mountain yellow-legged frogs (*Rana muscosa*) did not produce detectable antibodies in immunization experiments with *Batrachochytrium dendrobatidis*. *J Wildl Dis* **52**, 154–158 (2016).

49 Stockwell, M. P., Clulow, J. & Mahony, M. J. Evidence of a salt refuge: chytrid infection loads are suppressed in hosts exposed to salt. *Oecologia* **177**, 901–910 (2015).

50 Bull, J. J., Smithson, M. W. & Nuismer, S. L. Transmissible viral vaccines. *Trends Microbiol* **26**, 6–15 (2018).

51 Longo, A. V., Fleischer, R. C. & Lips, K. R. Double trouble: co-infections of chytrid fungi will severely impact widely distributed newts. *Biol Invasions* **21**, 2233–2245 (2019). https://doi.org/10.1007/s10530-019-01973-3

52 *Waddle, A. W. *et al.* Amphibian resistance to chytridiomycosis increases following low-virulence chytrid fungal infection or drug-mediated clearance. *J Appl Ecol* **58**, 2053–2064 (2021). https://doi.org/10.1111/1365-2664.13974
53 *McMahon, T. A., Fernandez-Denmark, S. & Grim, J. M. Early-life exposure to Ivermectin alters long-term growth and disease susceptibility. *PLoS One* **16**, e0258185 (2021). https://doi.org/10.1371/journal.pone.0258185
54 *Waddle, A. W. *Boosting amphibian resilience to the pandemic fungal disease chytridiomycosis using vaccines and artificial environmental refugia* (PhD thesis) University of Melbourne (2022).
55 Brem, F. & Parris, M. Epidermal trauma reduces the impact of *Batrachochytrium dendrobatidis* in Fowler's toads (*Anaxyrus fowleri*). *The Open Zoology Journal* **6**, 1–7 (2013).
56 Knapp, R. A. *et al.* Effectiveness of antifungal treatments during chytridiomycosis epizootics in populations of an endangered frog. *PeerJ* **10**, e12712 (2022).
57 Bosch, J. *et al.* Successful elimination of a lethal wildlife infectious disease in nature. *Biol Lett* **11**, 20150874. (2015).
58 Goodyear, L. E., Concha-Toro, V. & Pincheira-Donoso, D. Global assessment of interventions for mitigation of amphibian fungal disease is dominated by geoeconomic trends and antifungal success. *bioRxiv* **2025-07** (2025).
59 Brannelly, L. A. *et al.* Non-declining amphibians can be important reservoir hosts for amphibian chytrid fungus. *Anim Conserv* **21**, 91–101 (2018). https://doi.org/10.1111/acv.12380
60 Råberg, L., Graham, A. L. & Read, A. F. Decomposing health: tolerance and resistance to parasites in animals. *Philos Trans R Soc B* **364**, 37–49 (2009).
61 Grogan, L. F., Mangan, M. J. & McCallum, H. I. Amphibian infection tolerance to chytridiomycosis. *Philos Trans R Soc B* **378**, 20220133 (2023). https://doi.org/10.1098/rstb.2022.0133
62 *Sauer, E. L. *et al.* Variation in individual temperature preferences, not behavioural fever, affects susceptibility to chytridiomycosis in amphibians. *Proc R Soc B* **285**, 20181111 (2018).
63 Fornoni, J., Núñez-Farfán, J., Valverde, P. L. & Rausher, M. D. Evolution of mixed strategies of plant defense allocation against natural enemies. *Evolution* **58**, 1685–1695 (2004).
64 McMahon, T. A. & Rohr, J. R. Transition of chytrid fungus infection from mouthparts to hind limbs during amphibian metamorphosis. *Ecohealth* **12**, 188–193 (2015). https://doi.org/10.1007/s10393-014-0989-9
65 Rollins-Smith, L. A. Metamorphosis and the amphibian immune system. *Immunol Rev* **166**, 221–230 (1998). https://doi.org/10.1111/j.1600-065X.1998.tb01265.x
66 Humphries, J. E., Lanctôt, C. M., McCallum, H. I., Newell, D. A. & Grogan, L. F. Chytridiomycosis causes high amphibian mortality prior to the completion of metamorphosis. *Environ Res* **247**, 118249 (2024). https://doi.org/https://doi.org/10.1016/j.envres.2024.118249
67 Narayan, E. J., Graham, C., McCallum, H. & Hero, J. M. Over-wintering tadpoles of *Mixophyes fasciolatus* act as reservoir host for *Batrachochytrium dendrobatidis*. *PLoS One* **9**, e92499 (2014).
68 Crawford-Ash, J. *et al.* Defying decline: very low chytrid prevalence in tadpoles, yet high infection in adults in a naturally recovering frog species. *Anim Conserv* **28**, 567–581 (2025). https://doi.org/10.1111/acv.13006
69 Scheele, B. C., Hunter, D. A., Skerratt, L. F., Brannelly, L. A. & Driscoll, D. A. Low impact of chytridiomycosis on frog recruitment enables persistence in refuges despite

high adult mortality. *Biol Conserv* **182**, 36–43 (2015). https://doi.org/https://doi.org/10.1016/j.biocon.2014.11.032

70 Waddle, A. W. *et al.* Population-level resistance to chytridiomycosis is life-stage dependent in an imperiled anuran. *Ecohealth* **16**, 701–711 (2019). https://doi.org/10.1007/s10393-019-01446-y

71 Sauer, E. L. *et al.* Are novel or locally adapted pathogens more devastating and why? *Ecol Lett* **27**, e14431 (2024).

72 O'Dea, R. E. *et al.* Preferred reporting items for systematic reviews and meta-analyses in ecology and evolutionary biology: a PRISMA extension. *Biol Rev* **96**, 1695–1722 (2021). https://doi.org/10.1111/brv.12721

73 Berger, L. *et al.* Advances in managing chytridiomycosis for Australian frogs: *Gradarius Firmus Victoria*. *Annu Rev Anim Biosci* **12**, 113–133 (2024). https://doi.org/10.1146/annurev-animal-021122-100823

74 Grogan, L. F. *et al.* Review of the amphibian immune response to chytridiomycosis, and future directions. *Front Immunol* **9**, 2536 (2018). https://doi.org/10.3389/fimmu.2018.02536

75 Turner, A., Wassens, S., Heard, G. & Peters, A. Temperature as a driver of the pathogenicity and virulence of amphibian chytrid fungus *Batrachochytrium dendrobatidis*: a systematic review. *J Wildl Dis* **57**, 477–494 (2021). https://doi.org/10.7589/Jwd-D-20-00105

76 Woodhams, D. C. *et al.* Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. *Front Zool* **8**, 1–24 (2011).

77 Grames, E. M., Stillman, A. N., Tingley, M. W. & Elphick, C. S. An automated approach to identifying search terms for systematic reviews using keyword co-occurrence networks. *Methods Ecol Evol* **10**, 1645–1654 (2019). https://doi.org/10.1111/2041-210x.13268

78 Ouzzani, M., Hammady, H., Fedorowicz, Z. & Elmagarmid, A. Rayyan—a web and mobile app for systematic reviews. *Syst Rev* **5**, 210 (2016). https://doi.org/10.1186/s13643-016-0384-4

79 Cook, K., Pope, K., Cummings, A. & Piovia-Scott, J. In situ treatment of juvenile frogs for disease can reverse population declines. *Conserv Sci Pract* **4**, e12762 (2022). https://doi.org/10.1111/csp2.12762

80 *Robak, M. J. & Richards-Zawacki, C. L. Temperature-dependent effects of cutaneous bacteria on a frog's tolerance of fungal infection. *Front Microbiol* **9**, 410 (2018). https://doi.org/10.3389/fmicb.2018.00410

81 Gosner, K. L. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190 (1960).

82 Wickham, H. *et al.* Welcome to the Tidyverse. *Journal of Open Source Software* **4**, 1686 (2019).

83 Ivimey-Cook, E. R., Noble, D. W. A., Nakagawa, S., Lajeunesse, M. J. & Pick, J. L. Advice for improving the reproducibility of data extraction in meta-analysis. *Res Synth Methods* **14**, 911–915 (2023). https://doi.org/10.1002/jrsm.1663

84 Higgins, J. P., White, I. R. & Anzures-Cabrera, J. Meta-analysis of skewed data: combining results reported on log-transformed or raw scales. *Statistics in Medicine* **27**, 6072–6092 (2008). https://doi.org/10.1002/sim.3427

85 Borenstein, M., Hedges, L. V., Higgins, J. P. & Rothstein, H. R. *Introduction to meta-analysis*. (John wiley & sons, 2021).

86 Weber, F., Knapp, G., Ickstadt, K., Kundt, G. & Glass, A. Zero-cell corrections in random-effects meta-analyses. *Res Synth Methods* **11**, 913–919 (2020). https://doi.org/10.1002/jrsm.1460

87 Hedges, L. V., Gurevitch, J. & Curtis, P. S. The meta-analysis of response ratios in experimental ecology. *Ecology* **80**, 1150–1156 (1999).
88 Lajeunesse, M. J. Bias and correction for the log response ratio in ecological meta-analysis. *Ecology* **96**, 2056–2063 (2015). https://doi.org/10.1890/14-2402.1
89 Nakagawa, S. *et al.* A robust and readily implementable method for the meta-analysis of response ratios with and without missing standard deviations. *Ecol Lett* **26**, 232–244 (2023). https://doi.org/10.1111/ele.14144
90 R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. (2023).
91 Viechtbauer, W. Conducting meta-analyses in R with the metafor package. *J Stat Softw* **36**, 1–48 (2010).
92 IntHout, J., Ioannidis, J. P., Rovers, M. M. & Goeman, J. J. Plea for routinely presenting prediction intervals in meta-analysis. *BMJ open* **6**, e010247 (2016).
93 Yang, Y., Noble, D. W., Senior, A. M., Lagisz, M. & Nakagawa, S. Interpreting prediction intervals and distributions for decoding biological generality in meta-analyses. *eLife* **14**, RP103339 (2025).
94 Noble, D. W. *et al.* Meta-analytic approaches and effect sizes to account for 'nuisance heterogeneity'in comparative physiology. *J Exp Biol* **225**, jeb243225 (2022).
95 Yang, Y. *et al.* A pluralistic framework for measuring, interpreting and decomposing heterogeneity in meta-- analysis. *Methods Ecol Evol* **16**, 2710–2725 (2025).
96 Nakagawa, S. *et al.* orchaRd 2.0: An R package for visualising meta-analyses with orchard plots. *Methods Ecol Evol* **14**, 2003–2010 (2023). https://doi.org/10.1111/2041-210x.14152
97 Pottier, P. *et al.* Vulnerability of amphibians to global warming. *Nature* **639**, 954–961 (2025). https://doi.org/10.1038/s41586-025-08665-0
98 Paradis, E. & Schliep, K. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**, 526–528 (2019). https://doi.org/10.1093/bioinformatics/bty633
99 Noble, D. W. A., Lagisz, M., O'dea, R. E. & Nakagawa, S. Nonindependence and sensitivity analyses in ecological and evolutionary meta-analyses. *Mol Ecol* **26**, 2410–2425 (2017). https://doi.org/10.1111/mec.14031
100 *Davidson, E. W., Larsen, A. & Palmer, C. M. Potential influence of plant chemicals on infectivity of *Batrachochytrium dendrobatidis*. *Dis Aquat Org* **101**, 87–93 (2012). https://doi.org/10.3354/dao02505
101 *Woodhams, D. C. *et al.* Treatment of amphibians infected with chytrid fungus: learning from failed trials with itraconazole, antimicrobial peptides, bacteria, and heat therapy. *Dis Aquat Org* **98**, 11–25 (2012). https://doi.org/10.3354/dao02429
102 *Harris, R. N. *et al.* Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. *ISME Journal* **3**, 818–824 (2009). https://doi.org/10.1038/ismej.2009.27
103 *Woodhams, D. C. *et al.* Probiotics modulate a novel amphibian skin defense peptide that is antifungal and facilitates growth of antifungal bacteria. *Microb Ecol* **79**, 192–202 (2020). https://doi.org/10.1007/s00248-019-01385-9
104 *Madison, J. D., Ouellette, S. P., Schmidt, E. L. & Kerby, J. L. *Serratia marcescens* shapes cutaneous bacterial communities and influences survival of an amphibian host. *Proc R Soc B* **286**, 20191833 (2019). https://doi.org/10.1098/rspb.2019.1833
105 Bletz, M. C. *et al.* Mitigating amphibian chytridiomycosis with bioaugmentation: characteristics of effective probiotics and strategies for their selection and use. *Ecol Lett* **16**, 807–820 (2013).

106 Woodhams, D. C. *et al.* Symbiotic bacteria contribute to innate immune defenses of the threatened mountain yellow-legged frog, *Rana muscosa*. *Biol Conserv* **138**, 390–398 (2007). https://doi.org/https://doi.org/10.1016/j.biocon.2007.05.004

107 Woodhams, D. C., McCartney, J., Walke, J. B. & Whetstone, R. The adaptive microbiome hypothesis and immune interactions in amphibian mucus. *Dev Comp Immunol* **145**, 104690 (2023). https://doi.org/10.1016/j.dci.2023.104690

108 *Hauser, K. A. *et al.* Amphibian mast cells serve as barriers to chytrid fungus infections. *eLife* **12**, RP92168 (2024). https://doi.org/10.7554/eLife.9216

109 *Knutie, S. A., Gabor, C. R., Kohl, K. D. & Rohr, J. R. Do host-associated gut microbiota mediate the effect of an herbicide on disease risk in frogs? *J Anim Ecol* **87**, 489–499 (2018). https://doi.org/10.1111/1365-2656.12769

110 Humphries, J. E. *et al.* Do immune system changes at metamorphosis predict vulnerability to chytridiomycosis? An update. *Dev Comp Immunol* **136**, 104510 (2022). https://doi.org/10.1016/j.dci.2022.104510

111 Kueneman, J. G. *et al.* Effects of captivity and rewilding on amphibian skin microbiomes. *Biol Conserv* **271**, 109576 (2022).

112 Valencia-Aguilar, A., Toledo, L. F., Vital, M. V. & Mott, T. Seasonality, environmental factors, and host behavior linked to disease risk in stream-dwelling tadpoles. *Herpetologica* **72**, 98–106 (2016).

113 Edler, R., Goymann, W., Schwabl, I. & Friedl, T. W. P. Experimentally elevated testosterone levels enhance courtship behaviour and territoriality but depress acquired immune response in Red Bishops *Euplectes orix*. *Ibis* **153**, 46–58 (2011). https://doi.org/10.1111/j.1474-919X.2010.01075.x

114 Han, X. & Fu, J. Does life history shape sexual size dimorphism in anurans? A comparative analysis. *BMC Evol Biol* **13**, 27 (2013).

115 Janicke, T., Häderer, I. K., Lajeunesse, M. J. & Anthes, N. Darwinian sex roles confirmed across the animal kingdom. *Sci Adv* **2**, e1500983 (2016). https://doi.org/10.1126/sciadv.1500983

116 Adams, A. J. *et al.* Extreme drought, host density, sex, and bullfrogs influence fungal pathogen infection in a declining lotic amphibian. *Ecosphere* **8**, e01740 (2017). https://doi.org/10.1002/ecs2.1740

117 Muths, E., Corn, P. S., Pessier, A. P. & Green, D. E. Evidence for disease-related amphibian decline in Colorado. *Biol Conserv* **110**, 357–365 (2003).

118 Hardy, B. M., Korpita, T., Muths, E., Funk, W. C. & Bailey, L. L. Boreal toad survival varies by degree of attenuation and adaptation of a fungal pathogen. *Ecohealth* (2025). https://doi.org/10.1007/s10393-025-01749-3

*Becker MH, *et al.* The bacterially produced metabolite violacein is associated with survival of amphibians infected with a lethal fungus. *Appl Environ Microbiol*, **75**, 6635–6638 (2009).

Becker MH, et al.* Towards a better understanding of the use of probiotics for preventing chytridiomycosis in Panamanian golden frogs. *Ecohealth* **8**, 501–506 (2011).

Becker MH, et al.* Genetically modifying skin microbe to produce violacein and augmenting microbiome did not defend Panamanian golden frogs from disease. *ISME Commun* **1**, 57 (2021).

*Ellison, AR, *et al.* Fighting a losing battle: vigorous immune response countered by pathogen suppression of host defenses in the chytridiomycosis-susceptible frog *Atelopus zeteki*. *G3* **4**, 1275–1289 (2014)

*Harris RN, *et al.* Addition of antifungal skin bacteria to *salamanders ameliorates* the effects of chytridiomycosis. *Dis Aquat Org*, **83**, 11–16 (2009)

*Korpita T. *Boreal Toad Microbial Communities and Conservation Interventions* (PhD thesis) University of Colorado (2023).
*Rollins-Smith LA, *et al.* Immune defenses of *Xenopus laevis* against *Batrachochytrium dendrobatidis*. *Front Biosci*, **1**, 68–91 (2009)
*Walke JB, *et al.* Community structure and function of amphibian skin microbes: an experiment with bullfrogs exposed to a chytrid fungus. *PLoS One*, **10**, e013984 (2015).