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Putative resistance and tolerance mechanisms have little impact on disease progression for an emerging salamander pathogen

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Abstract

1. Resistance and tolerance are unique host defence strategies that can limit the impacts of a pathogen on a host. However, for most wildlife–pathogen systems, there are still fundamental uncertainties regarding (a) how changes in resistance and tolerance can affect disease outcomes and (b) the mechanisms underlying resistance and tolerance in host populations.
2. Here, we first compared observed patterns of resistance and tolerance and their effects on disease outcomes among salamander species that are susceptible to infection and mortality from the emerging fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*). We then tested whether two putative mechanisms that contribute to host resistance and tolerance, skin sloughing and skin lesion reduction, predicted reduced *Bsal* growth rate or increased host survival during infection, respectively.
3. We performed multi-dose *Bsal* challenge experiments on four species of Salamandridae found throughout North America. We combined the laboratory experiments with dynamic models and sensitivity analysis to examine how changes in load-dependent resistance and tolerance functions affected *Bsal*-induced mortality risk. Finally, we used our disease model to test whether skin sloughing and lesion reduction predicted variability in infection outcomes not described by *Bsal* infection intensity.
4. We found that resistance and tolerance differed significantly among salamander species, with the most susceptible species being both less resistance and less tolerant of *Bsal* infection. Our dynamic model showed that the relative influence of resistance versus tolerance on host survival was species-dependent—increasing resistance was only more influential than increasing tolerance for the least tolerant species where changes in pathogen load had a threshold-like effect on host survival. Testing two candidate mechanisms of resistance and tolerance, skin sloughing and lesion reduction, respectively, we found limited support that either of these processes were strong mechanisms of host defence.
5. Our study contributes to a broader understanding of resistance and tolerance in host–pathogen systems by showing that differences in host tolerance can significantly affect whether changes in resistance or tolerance have larger effects on

disease outcomes, highlighting the need for species and even population-specific management approaches that target host defence strategies.

KEYWORDS

Batrachochytrium salamandrivorans, chytridiomycosis, disease-induced mortality, emerging infectious disease, Integral Projection Model, *Notophthalmus viridescens*, resistance, tolerance

1 | INTRODUCTION

Resistance and tolerance are unique host defence strategies that can limit the impacts of a pathogen on a host (Boots & Bowers, 1999; Råberg et al., 2009; Schneider & Ayres, 2008). Resistance refers to processes that directly affect pathogen load within a host, such as the immune response, and tolerance refers to processes that reduce pathogen-induced damage and immunopathology, such as tissue repair (Medzhitov et al., 2012; Schneider & Ayres, 2008). For emerging pathogens of conservation concern in wildlife hosts, some management strategies have focused on how to augment resistance and tolerance, with the goal of limiting the deleterious effects of an invading pathogen on the host (Langwig et al., 2015; Venesky et al., 2012; Woodhams et al., 2011). For example, the translocation of resistant individuals into declining or extirpated populations can increase average population-level resistance and mitigate disease-induced declines (Joseph & Knapp, 2018; Venesky et al., 2012; Woodhams et al., 2011). Despite this viable management option, there remain fundamental questions on how to best augment resistance and tolerance in wildlife populations. These include, but are not limited to (a) How much do genetics, the environment or their interaction affect resistance and tolerance (Mazé-Guilmo et al., 2014), (b) Are there costs associated with increases in resistance or tolerance (Duffy & Forde, 2009), (c) Which has a larger impact on disease outcomes, proportional changes in resistance or tolerance (Venesky et al., 2012) and (d) What are the mechanisms underlying resistance and tolerance phenotypes and do they have easily observable traits (Medzhitov et al., 2012; Venesky et al., 2012)?

Here, we focus on two of these questions: (a) Which has a larger impact on disease outcomes, proportional changes in resistance or tolerance and (b) What are the mechanisms underlying resistance and tolerance in wildlife hosts? We address these questions in an emerging fungal pathogen of amphibians *Batrachochytrium salamandrivorans* (*Bsal*). *Bsal* infects the skin of amphibian hosts and has been responsible for the declines of salamander species in Europe (Martel et al., 2013, 2014; Stegen et al., 2017; Thomas et al., 2019). *Bsal* is a relative of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), which has caused declines and extinctions in hundreds of amphibian species worldwide (Scheele et al., 2019). While *Bsal* has not successfully invaded North America as of 2020 (Waddle et al., 2020), the high salamander diversity and habitat suitability for *Bsal* in North America has raised substantial concerns about the potential impact of *Bsal* if it does invade (Gray et al., 2015; Yap et al., 2015). Necessary steps for assessing the risk of North American salamander species to *Bsal* include determining how populations and species differ in resistance and tolerance to *Bsal* infection (Carter et al., 2020; Gray et al., 2015), the mechanisms underlying these host defence strategies, and how changes in host defences affect the impacts of *Bsal* on host populations (Canessa et al., 2018).

Previous studies have examined the resistance and tolerance of salamanders in Europe and North America and identified that host defence strategies varied among salamanders, but that lethal impacts of *Bsal* were generally monophyletic within the order Urodela (Martel et al., 2014). Resistance and tolerance of salamander hosts to *Bsal* have been previously considered as binary values—a host is either resistant, tolerant or neither (Martel et al., 2014). In reality, however, a host's response to *Bsal* infection depends on pathogen load and concomitant skin damage (Canessa et al., 2018; Stegen et al., 2017; Van Rooij et al., 2015). Therefore, the underlying host vital rates that determine how *Bsal* infects, grows on and harms a host are functions of pathogen load and chytridiomycosis development, rather than static values (Canessa et al., 2018). A current knowledge gap is how changes in load-dependent resistance or tolerance affect disease outcomes in salamander species. Host–pathogen models are useful tools to address this knowledge gap as parameterized models and concomitant sensitivity analyses can help identify the relative effects of changing host defence strategies on infection outcomes (Restif et al., 2012), with broad implications for understanding the importance of load-dependent resistance and tolerance in wildlife–pathogen systems.

A second knowledge gap in salamander–*Bsal* disease ecology is that we lack a mechanistic understanding of salamander resistance and tolerance. At the coarsest level, differences in resistance between two host populations, for example, may be described by differences in maximum pathogen infection load (Carter et al., 2020; Råberg et al., 2009). More mechanistically, however, these differences in maximum load might be due to differences in the adaptive immune response between these two hosts populations (Medzhitov et al., 2012). While coarser levels of resistance and tolerance are easier to obtain and are thus amenable for comparison across multiple populations or species, a more mechanistic understanding of resistance and tolerance is important to effectively mitigate the negative effects of disease.

We tested two hypotheses regarding the mechanisms of resistance and tolerance in North American salamanders to *Bsal*. First, we tested the hypothesis that skin sloughing is a resistance mechanism. Skin sloughing is a common sign of chytridiomycosis caused by the closely related fungal pathogen *Bd* and has been found to increase with increasing *Bd* load (Ohmer et al., 2015; Voyles et al., 2009). For some amphibian species, skin sloughing has been shown to reduce

the infection load of *Bd*, indicating that variability in skin sloughing could be partially responsible for variability in observed resistance among species (Ohmer et al., 2017). However, we do not know whether skin sloughing is a resistance mechanism for *Bsal* infection. Second, we tested the hypotheses that an increased ability to repair or prevent skin lesions was a mechanism of tolerance. *Bsal* infections are often associated with skin lesions that may disrupt osmoregulatory processes associated with amphibian skin or make hosts more susceptible to infection by bacterial species (Bletz et al., 2018; Stegen et al., 2017; Van Rooij et al., 2015). By combining dynamic disease models with laboratory data collected on four salamander species, we address critical questions regarding the importance of and mechanisms conferring resistance and tolerance to an emerging infectious disease.

2 | MATERIALS AND METHODS

2.1 | Laboratory infection experiments

We performed multi-dose *Bsal* challenge experiments on four Salamandridae species found throughout North America. The species tested included *Notophthalmus viridescens* ($n = 147$ individuals), *N. meridionalis* ($n = 40$), *N. perstriatus* ($n = 50$) and *Taricha granulosa* ($n = 47$). *N. viridescens* were collected from six geographically separated populations: three in Tennessee (subsequently labelled as Middle, Ijams and Roan) and one each in Pennsylvania, Vermont and Michigan. *T. granulosa* were captured by the California Fish and Wildlife Department. *N. meridionalis* and *N. perstriatus* were captive bred at the Fort Worth and Jacksonville Zoos, respectively, and transferred to the University of Tennessee with authorization by U.S. Fish and Wildlife Service. Individuals were acclimated for between 2 and 4 weeks before the experiment began. Animals were not tested for *Bsal* prior to the experiment. They were presumed to be negative as *Bsal* has not been detected in the United States (Waddle et al., 2020).

All animals were housed in environmental chambers set at 15°C and 12-hr light and dark cycles. For each challenge experiment, we randomly assigned animals to one of four exposure doses ranging from 5×10^3 to 5×10^6 *Bsal* zoospores/ml or to a control group where we inoculated animals with autoclaved dechlorinated water ($n = 5$–10 individuals per treatment per species). We exposed an animal to *Bsal* by placing it in a 237-ml container with 9 ml of autoclaved dechlorinated water and 1 ml of the desired exposure dose. We mock inoculated control animals by placing them in the same size container with 10-ml dechlorinated water. All animals were exposed for 24 hr. Following exposure, each animal was housed individually during the experiment inside 710 cm^3 enclosures, containing a moist paper towel and a 7.6 cm PVC cover object. We replaced each animal's container and water every 3 days. We swabbed each animal every 6 days beginning 4 days post-exposure until the end of each experiment (40–70 days, depending on the species). We chose 6 days as this time step allowed us to capture *Bsal* growth dynamics on individual hosts while minimizing the amount of time we handled the animals (Robinson et al., 2020). During each swabbing period, we examined the animals for signs of skin lesions and skin sloughing. Skin lesions were counted grossly along the body during each swabbing period. We counted lesions as visible skin erosions and ulcers, occasionally with the aid of a magnifying lamp. We also recorded presence or absence of skin sloughing during each check by visually examining the animal and the animal's container for signs of skin sloughing. All experimental procedures followed approved University of Tennessee Institutional Animal Care and Use Committee protocol #2395.

We extracted genomic DNA from skin swabs using a Qiagen DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany). We then performed *Bsal* quantitative polymerase chain reaction (qPCR) using methods similar to Blooi et al. (2013). All qPCRs were performed on an Applied Biosystems Quantstudio 6 Flex qPCR instrument (Thermo Fisher Scientific, USA). Using qPCR, we determined the infection status of each animal and estimated the *Bsal* zoospore copies/μl recovered from each swab. Each qPCR was run in duplicate and samples were considered positive if both reactions amplified prior to 50 cycles.

2.2 | Estimating resistance and tolerance functions using Integral Projection Models

We quantified the differences in load-dependent resistance and tolerance within and among salamander species using Integral Projection Models for host–parasite systems (IPM; Figure 1; Easterling et al., 2000; Metcalf et al., 2015). The IPM is composed of five resistance, tolerance and transmission functions that depend on pathogen load and initial pathogen exposure (Figure 1; Metcalf et al., 2015; Wilber et al., 2016). We used the IPM in Figure 1 to model the observed *Bsal* infection trajectories from our laboratory experiments (Appendix S1, Figure S1). In the IPM, x refers to $\log_{10}$ *Bsal* load and a time step is 6 days (to match the frequency of sampling in the laboratory infection experiments). The vital rate functions associated with the host–parasite IPM are defined below. The statistical models are described in Appendix S1.

The transmission function, ϕ: The transmission function describes the probability that an uninfected host at time t becomes infected by time $t + 1$ (Figure 1A). Our experimental design put three constraints on ϕ. First, because we only exposed animals to *Bsal* at the start of the experiment and changed the tank water every 3 days, true transmission could only occur during the first time step. Second, because contact with *Bsal* zoospores at exposure was guaranteed, observed transmission was a resistance function that captured the probability of infection given contact. Third, because we housed all animals separately we could not estimate an effect of host density on contact.

The initial infection function, $G_0(x')$: The initial infection function is a resistance function that describes the probability density that an uninfected host at time t that has encountered the pathogen

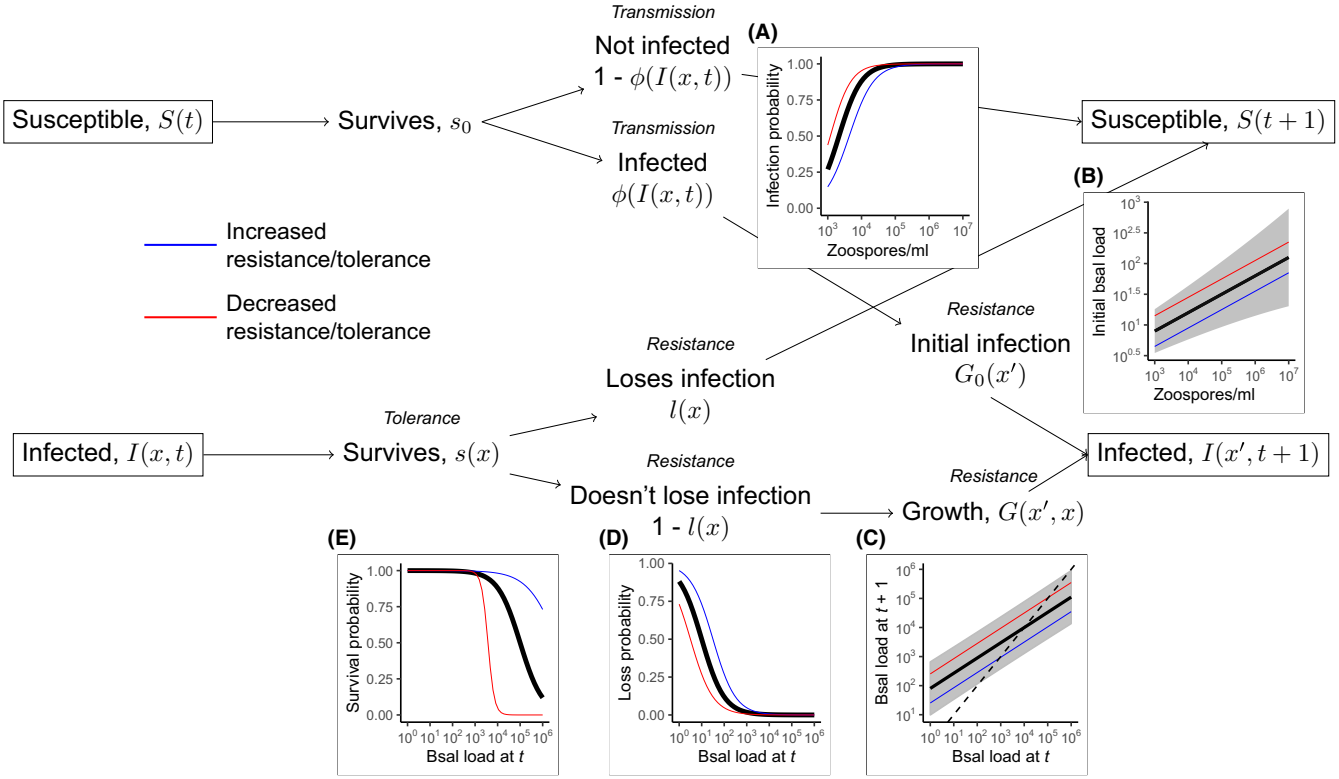

FIGURE 1 An integral projection model (IPM) for salamander–*Bsal* interactions. The IPM tracts how the number of susceptible (*S*) hosts and infected hosts with a $\log_{10}$ *Bsal* load of *x* ($I(x, t)dx$) change over a time step *t* to *t* + 1 (Appendix S1). The IPM is composed of underlying vital rate functions that relate to transmission (A), resistance (B–D) and tolerance (E). Inset plots display how these vital rate functions might look. Shaded regions on the growth function $G(x', x)$ and initial infection function $G_0(x')$ are 95% prediction intervals. The dashed line on the growth function is the one-to-one line. Red and blue lines indicate how changes in the vital rate functions affect resistance or tolerance. The axis 'Zoospores per ml' refers to the exposure dose. These vital rate functions can be parameterized from individual-level *Bsal* load trajectories observed in the laboratory infection experiments (Figure S1)

acquires an infection of x' by time $t + 1$ (Figure 1B). A more resistant host would acquire a lower *Bsal* load upon initial infection (Figure 1B). As with transmission, true initial infection could only occur during the first time step.

***Bsal* growth function, $G(x', x)$:** The *Bsal* growth function is a resistance function that describes the probability density that a host transitions from a pathogen load of *x* at time *t* to a pathogen load of x' at time $t + 1$, given that a host is infected (Figure 1C). A more resistant host would have a lower equilibrium *Bsal* load predicted by the growth function. This could be due to a reduced intercept or slope of $G(x', x)$ (Figure 1C). Reducing the intercept of $G(x', x)$ is similar to reducing *Bsal* growth rate.

Initial data analysis showed that the *Bsal* growth function varied with time since initial infection (see Appendix S1). We found that allowing the *Bsal* growth function to vary as a piecewise function before and after day 11 of the experiment (two time steps) adequately captured the time-varying effects of dose on *Bsal* growth (Appendix S1).

Loss of infection function, $l(x)$: The loss of infection function is a resistance function that describes the probability that a host loses a *Bsal* infection by time $t + 1$, given a pathogen load of *x* at time *t* (Figure 1D). A more resistant host would have an increased ability to lose infection as *Bsal* load increases.

The host survival function, $s(x)$: The host survival function is a tolerance function and describes the probability of a host surviving from time *t* to $t + 1$, given an infection load of *x* at time *t* (Figure 1E). We modeled host survival as a tolerance function with two parameters, one describing host tolerance and one describing host vigor (Appendix S1). Decreasing tolerance means that increasing pathogen load leads to a larger reduction in host survival (Figure 1E).

2.2.1 | Model fitting

We modelled between species differences in resistance and tolerance functions using random effects (Appendix S1). We also included an additional set of random effects that accounted for population-level differences in resistance and tolerance in the six different populations of *N. viridescens* (Appendix S1). When fitting all vital rate functions, we accounted for detection error—that is, the failure to detect *Bsal* infection when it was truly present (Appendix S2; DiRenzo et al., 2018). All of the vital rate functions were fit using a Bayesian framework in the probabilistic programming language Stan (Appendix S1; Carpenter et al., 2017) and we standardized dose and *Bsal* load covariates to a mean of zero and

standard deviation of one before fitting. We compared the fitted vital rate parameters across all species using pairwise comparisons and testing whether the 95% credible of the posterior distribution between the parameters of two different species contained zero. As all of the species-specific parameters were shrunk towards the mean using random effects, we did not correct for multiple comparisons (Gelman et al., 2012).

2.3 | The effects of resistance and tolerance on host survival

We used the parameterized IPM to understand how perturbations in resistance and tolerance functions affected the mean survival time of an initially uninfected individual for different host species (Appendix S3). Because we did not have estimates for the relative contributions of direct and indirect transmission or the influence of host density on transmission (but see Malagon et al., 2020), we assumed that individuals persisted in an environment with a constant source of *Bsal*. This is not an unreasonable null assumption as the presence of a resistant *Bsal* spore stage that can transmit through the environment and the potential presence of other amphibian species that can maintain and shed infectious spores without succumbing to chytridiomycosis could largely decouple the force of infection from focal host density (Stegen et al., 2017). We used the model to explore scenarios in which the focal host species were in four constant *Bsal* environments and the exposure levels in these environments corresponded with the four dose treatments. Unlike our experiment where individuals were only exposed at the first time step, our simulations allowed individuals to be repeatedly exposed each time step, more closely mimicking presumptive natural conditions if *Bsal* were present in an amphibian community. We then performed a local elasticity analysis to examine how sensitive the predicted mean survival time of an initially uninfected salamander host was to proportional changes in the lower-level parameters of the resistance and tolerance functions (Appendix S3).

2.4 | Testing the mechanisms of resistance and tolerance

We tested two hypotheses regarding the mechanisms underlying resistance and tolerance. Our first hypothesis was that skin sloughing was a resistance mechanism that could reduce *Bsal* infection. However, as skin sloughing can also be a sign of chytridiomycosis (Ohmer et al., 2015; Voyles et al., 2009), we first analysed the relationship between skin sloughing and *Bsal* infection load, with the prediction that increased *Bsal* infection load would correlate with increased skin shedding. We then tested skin sloughing as a resistance mechanism by including our observed variable of sloughed skin presence (1) or absence (0) in a tank/on a host as a covariate in the *Bsal* growth function (Appendix S1), with species and population-level random effects of sloughing. Our indicator variable of the presence of sloughed skin is an imperfect measure of sloughing. For uninfected hosts, sloughing events cannot be detected using presence of shed skin as amphibians tend to slough skin in one piece and then consume it (Weldon et al., 1993). However, chytridiomycosis can disrupt the skin sloughing process such that skin is shed in pieces (Ohmer et al., 2015; Voyles et al., 2009). In this situation, the presence or absence of skin in the environment can correlate with true sloughing events under a broad range of conditions (Appendix S4). Thus, we use the presence/absence of skin in the environment as a proxy for skin sloughing in infected hosts.

We predicted that the presence of sloughed skin in the current or previous time step would predict a reduction in *Bsal* load at the current time step, after accounting for *Bsal* load in the previous time step. We also performed a similar analysis on loss of infection in a time step, where we predicted that presence of sloughed skin in the current or previous time step would increase the probability that a host would lose an infection in the next time step.

The second hypothesis we tested was that the repair or prevention of skin lesions was a tolerance strategy. Before testing this hypothesis, we examined whether *Bsal* exposure and load were predictive of observing lesions. We compared the number of lesions on control and *Bsal*-exposed animals as well as the relationship between *Bsal* load and lesion number on exposed animals. We then tested our hypothesis that lesion repair and prevention were tolerance strategies by examining the relationship between *Bsal* load, lesion quantity and host survival. We predicted that reducing lesion quantity would increase host survival probability for a fixed *Bsal* load. We tested this prediction by including $\log_{10}$ lesion quantity + 1 at time t as an additional covariate in the host survival function (Appendix S1). We included the lesion coefficients as species and population-level random effects.

3 | RESULTS

3.1 | Load-dependent resistance and tolerance

Across different dimensions of host defence, resistance to *Bsal* infection varied among salamander species. Regarding *Bsal* growth on a host $G(x', x)$ after day 11, *N. meridionalis* showed the lowest level of resistance—when *Bsal* infection load was low, it increased most rapidly on *N. meridionalis*, relative to *N. perstriatus*, *N. viridescens* and *T. granulosa* (Figure 2A). In addition, increasing initial *Bsal* dose significantly increased *Bsal* growth on *N. meridionalis* and *N. perstriatus*, but had no effect on *Bsal* growth on *N. viridescens* and a small effect on *T. granulosa* (Figure 2B). In contrast, before day 11, *Bsal* showed statistically similar growth rates and increased in a similar magnitude with increasing dose for all four species (Figure 2C,D).

Overall, the differences in the *Bsal* growth function meant that at high doses, *N. meridionalis* and *N. perstriatus* had significantly higher

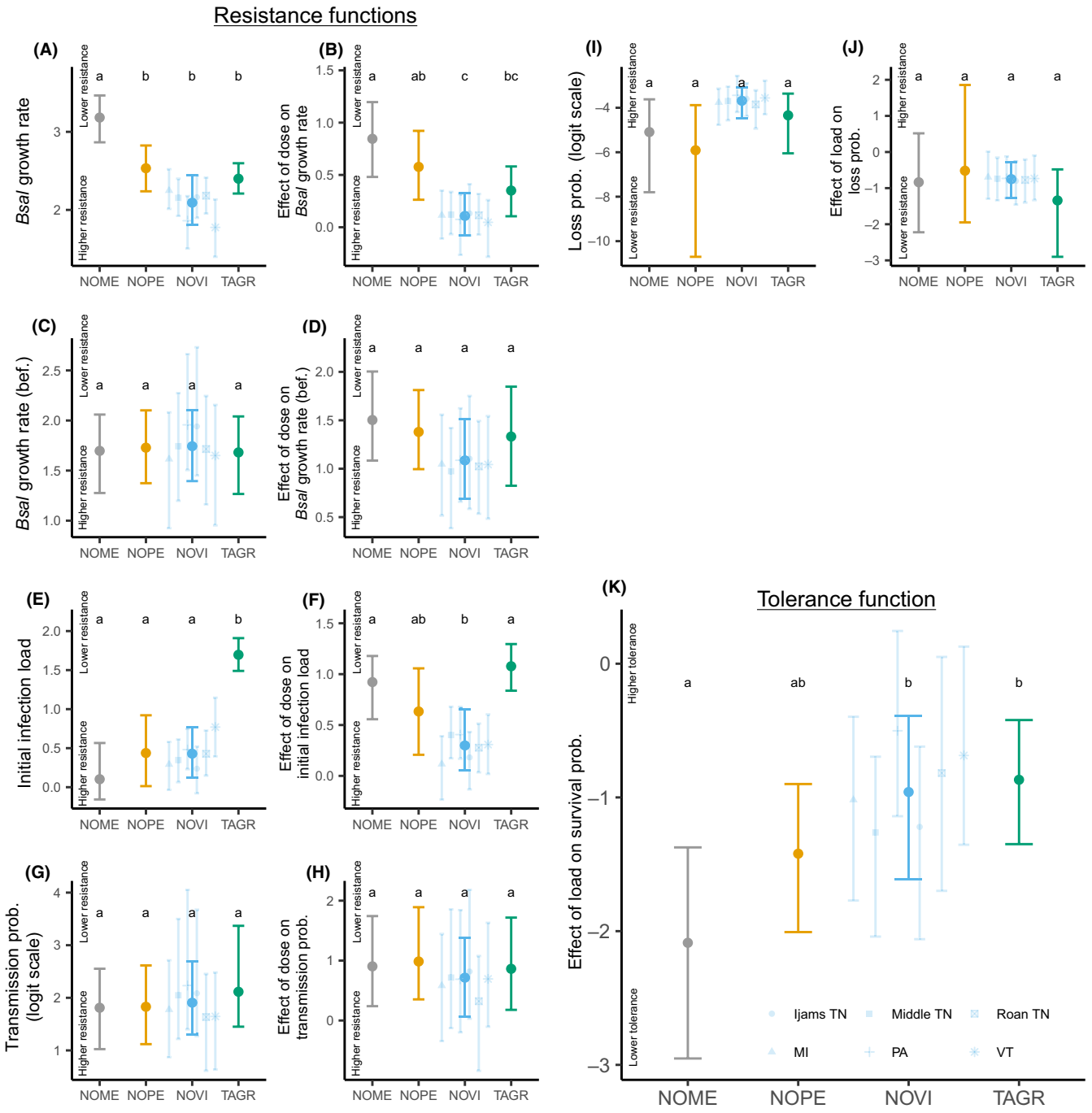

FIGURE 2 Parameter estimates for resistance and tolerance vital rate functions. (A–J) Parameter estimates for five different resistance functions, where each row is a resistance function. The resistance functions are *Bsal* growth function $G(x', x)$ after day 11 (A, B), *Bsal* growth function $G(x', x)$ before day 11 (C, D), initial infection function, $G_0(x')$ (E, F), transmission function, ϕ (G, H), and loss of infection function $l(x)$ (I, J). For a given resistance function, each column is a different estimated parameter (Appendix S1). (K) The tolerance estimates for four salamander species. The given estimates are effects of *Bsal* load on host survival probability in a time step ($s(x)$) as described in Appendix S1. Coloured circles given the median parameter estimates for a species and blue shapes give the median parameter estimates for different *Notophthalmus viridescens* populations. Error bars are 95% credible intervals around the median estimates. If parameter estimates share a letter, then they are not significantly different based on a 95% credible interval. NOME: *N. meridionalis*; NOPE: *N. perstriatus*; NOVI: *N. viridescens*; TAGR: *T. granulosa*

Bsal loads compared to *N. viridescens* and *T. granulosa* (Figure S2). At the highest dose treatment of 5×10^6 zoospores/ml, the predicted equilibrium $\log_{10}$ *Bsal* loads were as follows: 5.65 for *N. meridionalis* (95% credible interval: [4.45, 7.94]), 3.71 for *N. perstriatus* (95% CI: [2.71, 4.95]), 2.29 for *N. viridescens* (95% credible interval: [1.75, 2.96]) and 3.29 for *T. granulosa* (95% CI: [2.77, 3.84]).

T. granulosa was the least resistant species in terms of preventing high initial *Bsal* infections when dose treatment was high (Figure 2E,F).

At the highest dose treatments, *T. granulosa* acquired an average initial infection $\log_{10}$ *Bsal* load of 3.17, compared to 1.38, 1.32 and 0.85 for *N. meridionalis*, *N. perstriatus* and *N. viridescens*, respectively (Figure S3). The ability to clear infection also, on average, decreased most rapidly with increasing *Bsal* load for *T. granulosa*, but this was not significantly different than other species (Figure 2I,J; Figure S4). Finally, all species experienced statistically similar probabilities of infection during the experiment and this infection probability increased with dose in a similar way for all species (Figure 2G,H; Figure S5).

Notophthalmus meridionalis was the least tolerant species, with the probability of survival in a time step decreasing the most quickly with increasing *Bsal* infection load (Figure 2K; Figure S6). *N. perstriatus* tolerance was on average lower than *N. viridescens* and *T. granulosa*, but was not statistically different (Figure 2K).

3.2 | Sensitivity of host survival time to resistance and tolerance

Starting as uninfected, our model predicted that *N. viridescens* and *T. granulosa* survived longer than *N. perstriatus* and *N. meridionalis* across all simulated exposure environments (Figure 3A). From the highest exposure environment to the lowest exposure environment, mean predicted survival time for an uninfected host ranged from 23

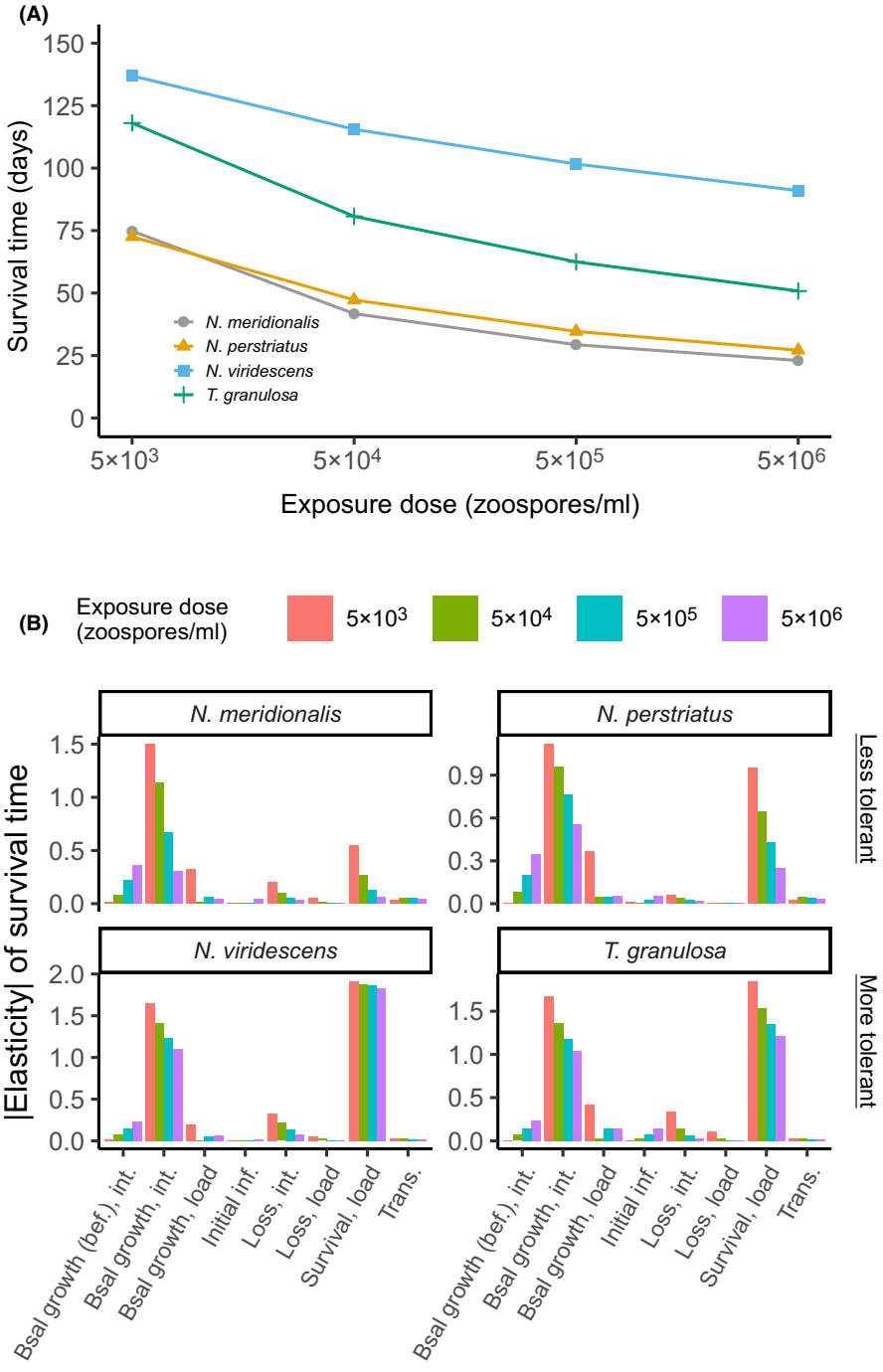

FIGURE 3 (A) The predicted mean survival time of an initially uninfected host continually exposed to *Bsal* in four different dose environments. The mean survival times are computed from the parameterized IPM model (Appendix S3). (B) The absolute value of the change in mean host survival time (i.e. elasticity) given proportional changes in lower-level resistance and tolerance parameters. A larger bar means that a proportional change in the given parameter has a larger effect on mean survival time. Colours indicate the elasticity given different doses of *Bsal*. For the elasticity analysis, parameters that described the effect of dose on the vital rate functions were included in either the intercept or the *Bsal* load parameter (Appendix S3). The resistance parameters are as follows: *Bsal* growth ($G(x', x)$) intercept (int.) and load, initial infection ($G_0(x')$), loss of infection ($l(x)$) intercept and load effect, and transmission (ϕ). 'Survival, load' is the tolerance parameter

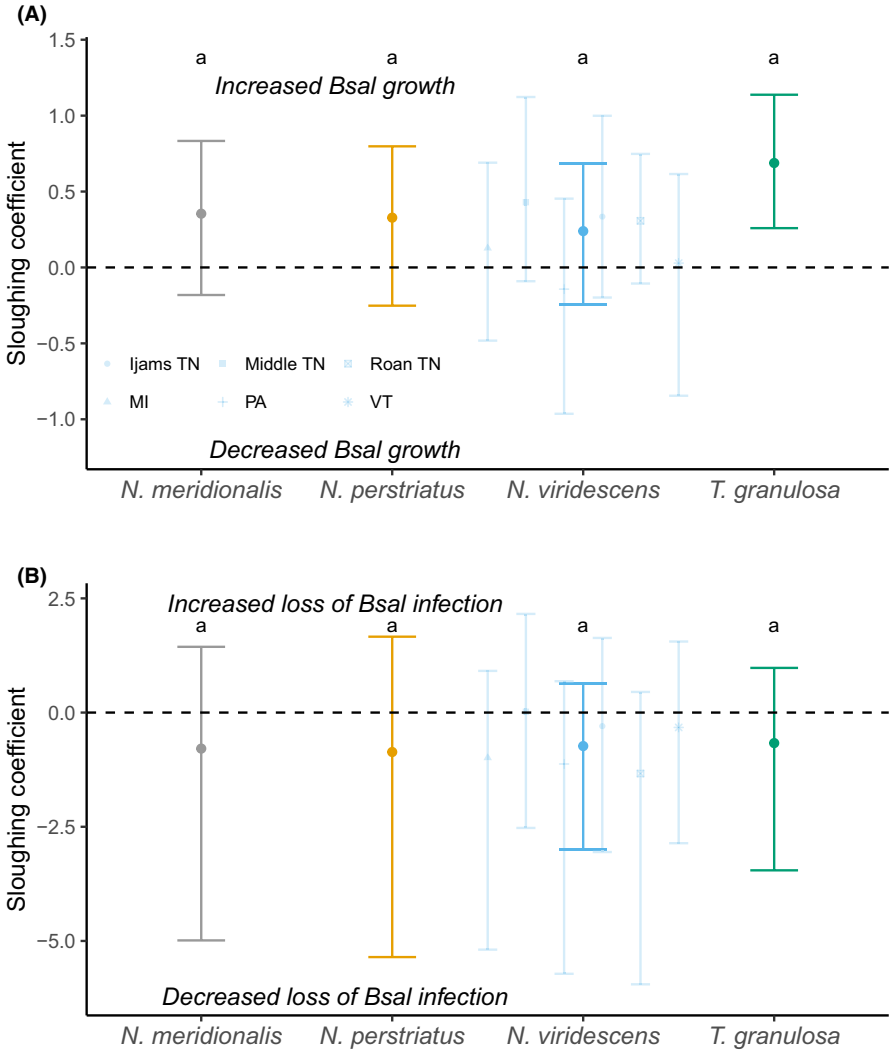

FIGURE 4 (A) The median species-level coefficients indicating whether the presence of sloughed skin at time *t* predicted a change in *Bsal* growth (the intercept of $G(x', x)$) at time $t + 1$. A positive coefficient indicates that the presence of sloughed skin predicted increased *Bsal* growth and a negative coefficient indicates that the presence of sloughed skin predicted decreased *Bsal* growth. (B) The median species-level coefficients indicating whether the presence of sloughed skin at time *t* predicted the probability of losing a *Bsal* infection in time $t + 1$. A positive coefficient indicates that the presence of sloughed skin increased the probability of losing an infection and a negative coefficient indicates that it decreased the probability. For both plots, the error bars are 95% credible intervals about the median. If parameter estimates share a letter, then they are not significantly different based on a 95% credible interval. The different blue shapes give the coefficient estimates for *Notophthalmus viridescens* populations

to 75 days for *N. meridionalis*, 27 to 72 days for *N. perstriatus*, 91 to 136 days for *N. viridescens* and 50 to 118 days for *T. granulosa* (Figure 3A).

Species-level differences in resistance and tolerance led to differences in how perturbations to resistance and tolerance affected disease outcomes (Figure 3B). For the least tolerant species *N. meridionalis* and *N. perstriatus*, proportional changes in resistance, particularly in the *Bsal* growth function, led to larger effects on mean survival time than changes in tolerance (Figure 3B). However, for *N. viridescens* and *T. granulosa*, the more tolerant of the four species on average, proportional changes in resistance and tolerance had similar effects on disease outcomes (Figure 3B). For *N. viridescens* in particular, changes in tolerance had larger effects on mean survival time than changes to resistance in high exposure environments (Figure 3B).

3.3 | Mechanisms of resistance

There was a significant increase in probability that sloughed skin was present in a tank with increasing *Bsal* infection load for all salamander species (Figure S7), suggesting that *Bsal* infection was altering the sloughing process. However, our analysis provided no evidence that skin sloughing was an effective resistance mechanism for the four salamander species we considered (Figure 4A; Figure S8a). The presence of sloughed skin at the previous or current time point did not predict a significant reduction in *Bsal* load in the current time point (95% CIs on sloughing coefficients overlapped 0 or were in the opposite direction of our expectation; Figure 4A; Figure S8a). We also found that the presence of sloughed skin at the previous or current time step had no significant effect on the probability of losing *Bsal* infection in the current time step (Figure 4B; Figure S8b).

3.4 | Mechanisms of tolerance

We did not observe skin lesions on control (i.e. unexposed) animals for any of the four salamander species. We did, however, observe heterogeneous lesion distributions across animals exposed and infected with *Bsal*. The number of observed lesions on exposed hosts ranged from 0 to 700 lesions, depending on the individual and

species, and lesions were only weakly positively correlated with *Bsal* infection load (Figure S9).

After accounting for *Bsal* load, we found that lesion quantity was either not predictive or only weakly predictive of host survival (Figure 5A,B). Across all species, observed mortality generally began to occur when *Bsal* load was greater than 300 zoospore equivalents and mortality occurred with both high and low lesion quantities (Figure 5A). Despite observing individuals with high lesions and low *Bsal* loads, these individuals did not experience mortality in the following time step (Figure 5A). We confirmed these graphical results with a statistical analysis—for a fixed *Bsal* load, the log10 number of lesions at time *t* did not significantly affect survival probability (all 95% CIs of the lesion parameters overlapped zero, Figure 5B). However, the median coefficient values for *N. meridionalis* and *N. perstriatus* suggested that there was a weak trend that increasing lesion quantity decreased host survival probability for a fixed *Bsal* load.

4 | DISCUSSION

Resistance and tolerance are critical components of host defence, but for many wildlife–pathogen systems, we have a limited understanding of the mechanisms underlying these defence strategies and their relative contributions to disease outcomes. Here, we combined laboratory experiments and dynamic models to examine the relative importance of resistance and tolerance on disease outcomes in four species of salamanders susceptible to the emerging fungal pathogen *Bsal*. We found that, despite all species being susceptible to *Bsal* infection, resistance and tolerance differed significantly among salamander species. These differences in resistance and tolerance translated into significant differences in the proportional effects that host defence strategies had on mean host survival time. In other words, increasing resistance was not always more effective at decreasing disease outcomes than increasing tolerance and vice versa.

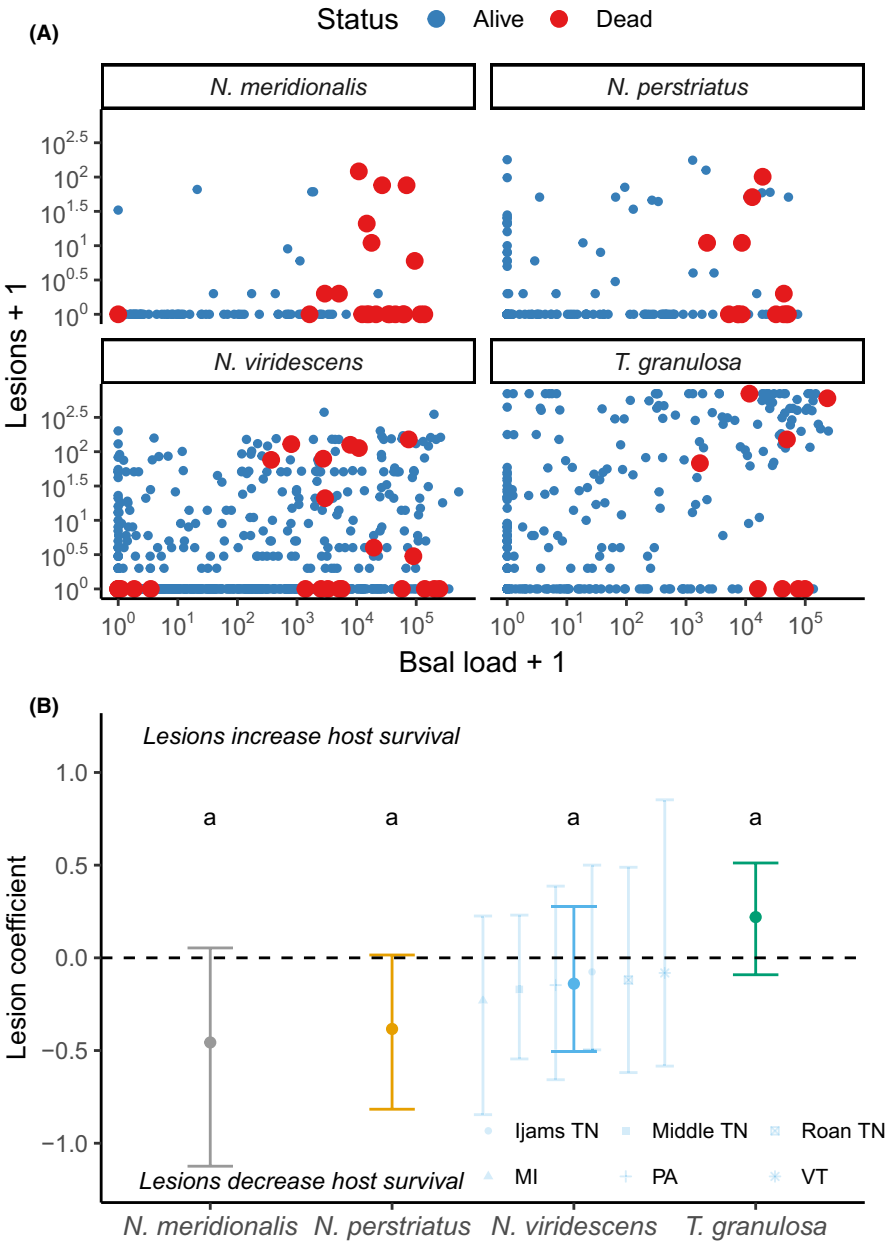

FIGURE 5 (A) The relationship between lesions, load and host survival in a time step for all salamander species. Large, red points indicate when a host died by the following time point, given observed *Bsal* load and lesion abundance. The small, blue points indicate when a host survived in a time step. (B) The predicted effects of lesion abundance + 1 at time *t* on host survival at time *t* + 1, after accounting for *Bsal* load at time *t*. The points are the median coefficient estimates and the error bars are the 95% credible intervals. If parameter estimates share a letter, then they are not significantly different based on a 95% credible interval. The different blue shapes give the coefficient estimates for *Notophthalmus viridescens* populations

The relative importance of resistance versus tolerance depended on the presence of threshold-like effects in a species' tolerance function and the initial exposure to *Bsal*. We also tested two candidate mechanisms of resistance and tolerance, skin sloughing (for which we used presence of sloughed skin as a proxy) and lesion prevention, respectively, and found little empirical support that either of these processes were strong mechanisms of host defence in salamander–*Bsal* interactions. Broadly, our study highlights the importance of considering resistance and tolerance as functions of pathogen load, as this can inform how different host defence mechanisms affect disease outcomes.

In salamander–*Bsal* infection dynamics, resistance and tolerance have thus far been considered as static, one-dimensional traits that describe host susceptibility to *Bsal* infection (Martel et al., 2014). However, to predict the dynamics of *Bsal* upon invasion and how changes in resistance and tolerance might affect the impact of *Bsal* on host populations, we must also account for the load-dependent nature of resistance and tolerance (Canessa et al., 2018). In this study, we found that while all four species suffered *Bsal*-induced mortality, *N. meridionalis* and *N. perstriatus* were generally the least resistant species, with the highest *Bsal* growth rates, particularly when initial infection dose was high. Moreover, *N. meridionalis* and *N. perstriatus* were also, on average, the least tolerant species, with a faster reduction in survival probability with increasing *Bsal* load compared to *N. viridescens* and *T. granulosa*. The lower resistance and tolerance of *N. meridionalis* and *N. perstriatus* to *Bsal* infection compared to *T. granulosa* and *N. viridescens*—two species that have previously been identified as significantly at risk from *Bsal* invasion (Malagon et al., 2020; Martel et al., 2014)—highlights that even among susceptible species there may be hyper-sensitive species to prioritize for conservation. An important caveat, however, is that lower resistance and tolerance in *N. meridionalis* and *N. perstriatus* could be a consequence of these species coming from a captive assurance colony, where it is possible that genetic diversity was lower and microbiome less diverse than the wild populations of *N. viridescens* and *T. granulosa*—both which could affect susceptibility to *Bsal* infection. Regardless, a load-dependent view of resistance and tolerance can help identify the host defence strategies leading to hyper-sensitivity (less tolerant, less resistance or both?).

Critically, our model revealed that differences in load-dependent resistance and tolerance functions led to different contributions of resistance and tolerance on disease outcomes. This suggests that, all else being equal, the most effective strategy for enhancing resistance or tolerance will be species-dependent. The key reason for this result relates to the shape of the host tolerance function. All salamander species displayed some level of host vigour in that they could persist with infection loads less than approximately 300 zoospores with little effect on survival probability. However, above this zoospore load, survival probability began to decrease and decreased at the fastest rate in the least tolerant species *N. meridionalis* and *N. perstriatus*. For a nonlinear tolerance function with low levels of tolerance (i.e. a steep negative slope), small increases in host resistance can have large proportional effects on the overall disease outcome because a host can jump from almost certainly dying to almost certainly living with a small change in pathogen load. In contrast, a proportionally similar increase in tolerance will increase host survival, but will not allow for a host to jump across the survival threshold. When tolerance increases, as we saw for *N. viridescens* and *T. granulosa*, the threshold effect induced by a steep tolerance function diminishes such that changes in resistance do not have significantly larger effects on disease outcomes than changes in tolerance. Overall, our results emphasize that it is critical to account for the functional form of resistance and tolerance as their shape can alter the most effective strategy for mitigating the negative impacts of disease invasion on host populations (Gupta & Vale, 2017; Louie et al., 2016).

In addition to differences among species in resistance and tolerance, we also quantified variability among populations in host defence for six populations of *N. viridescens*. While we did observe differences among populations in resistance and tolerance functions, the relative intra-specific variation in resistance and tolerance was generally smaller than inter-specific variation (Table S1). However, variability among populations in the host survival function, relating to both host vigour and host tolerance, was comparable or greater within *N. viridescens* populations compared to among species (Table S1). As our modelling results show that the shape of the tolerance function can dictate the strength of selection for resistance or tolerance, variability among populations in tolerance could lead to *Bsal* invasion selecting for different defence mechanisms among different populations of the same species. In fact, this has been observed in other wildlife–pathogen interactions (e.g. Frick et al., 2017), emphasizing that both species- and population-specific management strategies may be needed following the invasion of novel pathogens (Gray et al., 2015; Langwig et al., 2015). That being said, our results do suggest that resistance-oriented management targeted at reducing *Bsal* infection load, such as the application of fungicide or probiotic treatments at the onset of an epizootic (e.g. Canessa et al., 2018), will typically be more or equally effective for reducing the negative impacts of *Bsal* than strategies focusing on managing tolerance.

While the above results highlight the importance of and variability in particular resistance and tolerance functions for disease outcomes, they tell us little about the processes driving these patterns. In other host–pathogen systems, observable physical processes, such as behavioural fever (Kluger et al., 1975), anorexia (Schneider & Ayres, 2008) and skin sloughing (Ohmer et al., 2017), provide mechanistic explanations as to why a species can reduce pathogen load or reduce the impact of increasing pathogen load on fitness. We examined two physical processes that have been linked to resistance and tolerance in chytridiomycosis: skin sloughing to reduce fungal infection load and lesion prevention or reduction to reduce osmoregulatory effects and opportunities for secondary bacterial infections (Bletz et al., 2018; Martel et al., 2013; Ohmer et al., 2017). We found no evidence that presence of sloughed skin (which we would expect to correlate with sloughing events in infected individuals, Appendix S4) predicted a reduction *Bsal*

infection load, either through direct reductions in load or through the loss of *Bsal* infection. However, we did find strong evidence that the probability of sloughed skin in a tank increased with *Bsal* infection load. Previous studies on the related pathogen *Bd* have shown that skin sloughing can be both a mechanism of resistance and a sign of infection (Ohmer et al., 2015, 2017; Voyles et al., 2009), depending on the species. For four species of salamanders, we found that the presence of sloughed skin was more consistent with a sign of *Bsal* infection, rather than a mechanism of resistance. While we expected the presence of sloughed skin to correlate with sloughing events of infected individuals, quantitatively measuring sloughing events using infrared cameras and skin marks will provide a more direct assessment of the importance of sloughing for *Bsal* resistance (e.g. Ohmer et al., 2015).

Chytridiomycosis induced by *Bsal* is associated with the formation of skin lesions on hosts (Martel et al., 2013), which have been hypothesized to hasten host mortality by promoting secondary infections (Bletz et al., 2018). For the four salamander species we tested, we found only weak evidence that fewer lesions increased host survival for a fixed *Bsal* load and only in two species. In comparison, reducing *Bsal* infection intensity significantly increased host survival probability for all salamander species, even when the observed number of lesions was high. These results suggest that while secondary bacterial infections from *Bsal*-induced skin lesions are a potential threat to salamander survival, the effect on host fitness is smaller than the direct, deleterious effects of *Bsal* infection itself. The necrotizing ulcerations that *Bsal* causes may disrupt skin functions, such as osmoregulation and cutaneous respiration (Van Rooij et al., 2015), and be greater contributors to pathogenesis. However, in a non-laboratory setting, it may be more likely that a salamander could come into contact with a pathogenic bacteria (e.g. *Aeromonas hydrophila*) that could exploit skin lesions. An important next step is to test whether more precise (but harder to obtain) histological measures of skin lesions are predictive of host survival, after accounting for *Bsal* infection intensity.

By considering resistance and tolerance in a load-dependent framework, our study contributes to a broader understanding of resistance and tolerance in host–pathogen systems by identifying that threshold-like effects in host tolerance can make managing for resistance a more effective strategy for reducing disease-induced mortality than managing for tolerance. However, management of resistance and tolerance is aided by a mechanistic understanding of host defence and our results show that putative mechanisms of resistance and tolerance are not predictive of differences in disease outcomes in North American salamanders susceptible to *Bsal*. Identifying alternative mechanisms of resistance and tolerance in salamander hosts is a key next step to prepare for potential *Bsal* invasion of North America.

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AUTHORS' CONTRIBUTIONS

M.J.G. and E.D.C. performed the laboratory experiments; M.Q.W., M.J.G., E.D.C. and C.J.B. developed the conceptual framing of the manuscript; M.Q.W. and C.J.B. built the model and analysed the data; M.Q.W. drafted the manuscript. All authors contributed substantially to revisions.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository at https://doi.org/10.25349/D9JG8C (Wilber et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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