Temperature alters pathogen dynamics of a salamander pathogen on eastern newts

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1 The biological problem

Chytridiomycosis, a disease induced by a fungal pathogen, is considered a global panzoonotic and is the main driver of amphibian extinctions globally [1]. The pathogenic chytrid fungi, Batrachochytrium dendrobatidis (Bd) and Batrachochytrium salamandrivorans (Bsal), are responsible for causing the disease chytridiomycosis in frogs and salamanders, respectively, on six continents and 54 countries [1]. Both Bd and Bsal originated in Asia but have spread with amphibian populations around the world as a part of the pet trade [2]. Despite widespread invasion of chytrid, there are many populations of susceptible amphibians still at risk of infection. Unlike Bd, Bsal has yet to reach North America, making salamanders in the US at risk for a potential invasion event. It was previously unknown whether North American salamanders had resistance to Bsal, but recent Bsal inoculation experiments on several North American salamanders have shown that all species tested are highly susceptibility to the pathogen [3, 4]. Considering the vast range and diversity of salamander species in the US that are probable to have susceptibility to Bsal, an introduction is predicted to be devastating to North American salamander populations [4]. Researcher have already conducted intense surveys of salamander species across the US and have not found any evidence of pathogen presence [5]. Even though there has been no detection of Bsal in the US, preparation for a potential invasion is essential to protecting salamander populations in North America.

The first step in preparation for a potential invasion is determining individual susceptibility of newts in regions where the habitat is suitable for Bsal growth. There are many abiotic and biotic factors that can determine disease outcomes [6]. An abiotic factor that affects both host susceptibility and chytrid fungi virulence is temperature [6–8]. Here, we use results from a Bsal inoculation experiments to model infection dynamics among the eastern newt, Notophthalmus viridescens at different temperatures.

Tracking the dynamics of Chytridiomycosis (Chytrid) induced by Bsal in newts poses a challenge to matrix modelling. Chytrid cannot be characterized as either a micro or macro parasite because it is considered a slow growing microparasite, where the burden of infection is directly linked to the health

and mortality of the host [9]. Slow growing fungal microparasites like Chytrid fall somewhere in the middle of traditional disease modelling that characterize pathogens as either micro or macro [10]. In order to properly track infection dynamics of a slow-growing microparasites, its imperative to 1) incorporate pathogen behavior at the individual level and 2) model the pathogen growth on individuals as a continuous variable. Integral projection models provide an excellent tool to paramaterize host traits that are influenced by a very important continuous variable, in this case pathogen growth, to predict population-level outcomes [11]. The aim of the model presented in this paper is to utilize empirical data to paramaterize *Bsal* growth on individual hosts, using basic linear models, to predict population level infection and population dynamics of a North American newt, *Notophthalmus viridescens* at different temperatures.

2 The Model

The Bsal IPM model here is an extension of the IPM described in Wilber et al. 2016 [9], which is an adaptation of a basic S-I-S model (Figure 1). There are four main assumptions of the model which include: all individuals recover from infection and return to the susceptible class, population dynamics are tracked for one season (no breeding), and that transmission is density independent.

With all the assumptions considered our equations are as follows:

Susceptible equation

$$S\left(t+1\right)=S\left(t\right)s_{0}\left(1-\phi\left(T\right)\right)+\int_{L_{load}}^{U_{load}}I\left(x,t\right)s\left(x\right)l(x)dx,\left(1\right)$$

Infected equation

$$I\left(x',\ t+1\right)=S\left(t\right)s_{0}\phi(T)G_{0}(x',\ T)+\int_{L_{load}}^{U_{load}}I\left(x,t\right)s\left(x\right)\left(1-l\left(x\right)\right)G\left(x',x,T\right)dx,\left(2\right)$$

Equation one represents newt hosts in the susceptible class, the first term represents hosts who remain uninfected until t + 1, the second step represents hosts that were previously infected, lost their infection and returned to the susceptible class at t+1. For both equations, U is the upper limit and L is the lower limit, where x' (pathogen load) predicts the outcomes at t +1. Equation two represents newt hosts in the infected class, the first term represents hosts that were in the susceptible class and move to the infected class with load size being x'. The second term represents represents hosts that were previously infected with load x and transition to load x' in the next time step.

2.1 Vital rate functions and their parameterization

There are six major host vital rate functions that built the IPM and track individual hosts in a time step (t to t+1) that either remain uninfected, become

infected, lose infection, experience pathogen growth, or die. Each vital rate function was estimated using linear regression models and parameterized using data from inoculation experiments on *Notophthalmus viridescens* at University of Tennessee described in Carter et al. 2021. Uninfected individual newts were either kept at 6, 14 or 22°C and initially inoculated with a *Bsal* zoospore dose of either $5x10^3, 5x10^4, 5x10^5, 5x10^6$ Bsal zoospores per 10 mL. *Bsal* zoospore growth was tracked via swabs tested by qPCR every six days. Parameterization techniques from Wilber et al. 2016 were utilized in this analysis. Below are descriptions of each vital rate function coupled with the method/results of parameterization from the laboratory experiments.

2.1.1 The survival functions

The survival of individuals from t to t+1 was modeled for uninfected and infected newts separately. Survival of uninfected individuals s_o, was modeled as a constant 1. Uninfected individuals did not experience mortality throughout the span of the experiment and thus did not have an effect on the survival probability. The survival function of infected individuals, $s(x)$, gives the probability of a host with *Bsal* zoospore load x surviving from t to t +1. The survival probability of infected individuals was estimated using logistic regression with the link function, where $b_{0,0}$ is the intercept, and $b_{1,0}$ is the coefficient estimating the effect of a unit change in log zoospore load on the logit-transformed probability of survival from t to t + 1.

Temperature was not included as a covarite in the survival function because temperature was not a significant predictor of survival. This is surprising given that when plotting the predicted survival probabilities over time at each temperature separately, the projected survival probability at 22°C was much higher than the the probabilities at 6 and 14°C (Fig. 1). Regardless, increased infection load was a significantly affected individual survival (Fig. 4; P = 2.63e-09; Fig. 1). Whereby increasing the zoospore load significantly reduced the survival of infected individuals (Fig. 1&4). Initial dose inoculate was a significant predictor of survival, but was not incorporated into the model because ultimately initial dose and log zoospore size both incorporate the zoospore load into the model.

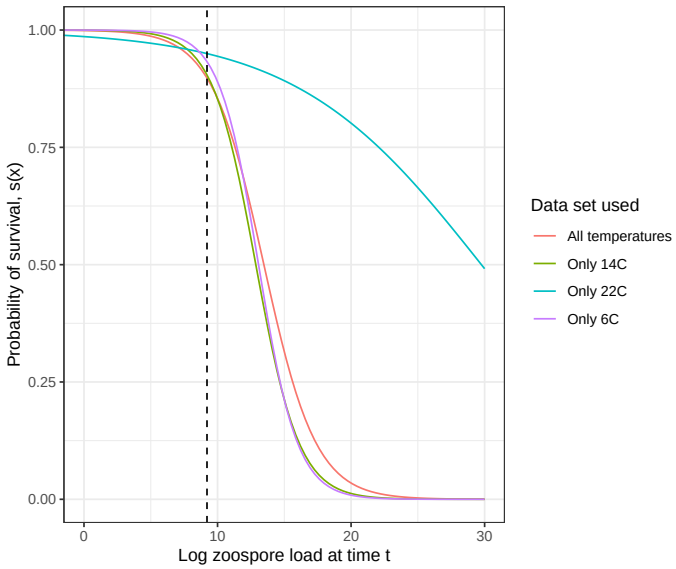

Figure 1: Survival probabilities as a function of log zoospore load at 6, 14, and 22°C

2.1.2 The transmission function

The transmission function ϕ is defined as the probability of an individual being uninfected at time t and then becoming infected at time t + 1. In this model, infection transmission is density-independent because newts were stored in individual containers. ϕ was modeled as a logistic regression with the link function (Fig. 4), where b_{04} is the slope of the link function on the logit scale and b_{14} is the effect of a unit change of temperature on the logit-transformed probability of becoming infected in a time step(Fig. 4).

With increasing temperature, individuals were significantly more likely to move from uninfected to infected(Fig. 4; P = 017698). This result does not may not mimic infection dynamics in the wild. Most individuals that gained an infection during the course of the experiment were at 22°C, which could be due to the lower undetectable growth rate at this temperature. Essentially, the growth rate could have been so slow that detection error in the qPCR detection may not have picked up the initial infection, delaying detectable growth at this temperature. Overall, the parameters of this function are only useful for this specific lab experiment and does not reflect pathogen dynamics in a natural disease transmission scenario.

2.1.3 The initial infection function

The initial infection function, $G_0(x)$, estimates the initial zoospore load at t + 1 when an individual was uninfected at t. Initial infection burden was estimated

on a log scale, which allowed for negative values, so $G_0(x)$, as a normal distribution $X \sim N(\mu(T)), \sigma^2(T)$. This type of estimation calculated the mean initial pathogen load at different temperatures (Table 1). In the estimate, $b_{0,2}$ is the intercept and $b_{1,2}$ calculates the effect of a unit change in temperature on the initial log zoospore load at time t + 1. This type of parametrization also allows for a calculation of variance or σ^2, where v_1 is a constant and c_{01} determines the effect of the initial log zoospore infection load on the variance.

Initial zoospore load was not significantly different among temperatures, but there was a decreasing trend of zoospore load from 14°C to 22°C. For this reason, $G_0(x)$ was still paramaterized with temperature as a predictor variable, indicating that the initial zoospore load tends to decrease with increasing temperature(Fig. 4).

2.1.4 The pathogen growth function

The continuous host trait within the IPM is the measure of zoospore growth on individual newts. The growth function, $G(x', x)$, estimates the growth of zoospores from t to t+1. In a similar fashion to initial infection function, zoospore growth was estimated on a log scale as a normal distribution $X \sim N(\mu(T)), \sigma^2(T)$. The mean log zoospore load was calculated at temps 6, 14 and 22°C, with $b_{0,1}$ as the intercept while $b_{1,1}$ and $b_{1,2}$ calculate the effect of a unit change in log zoospore load (at t) and temperature on the log zoospore load at time t + 1.

In the growth estimation, increasing zoospore size at time t was a significant predictor of zoospore growth at time t + 1 (Fig.4; $P < .001$; Fig. 2&3), while increasing temperature significantly limited zoospore growth at time t + 1(Fig. 4; $P < .001$; Fig. 2&3). It should be noted that there were no discernible differences in pathogen growth rates between 6 and 14°C, indicating that there may be a potential temperature threshold on growth somewhere between 14 and 22°C. Since temperature was only a significant predictor of growth at 22°C, model projection can only estimate future model projections at this temperature.

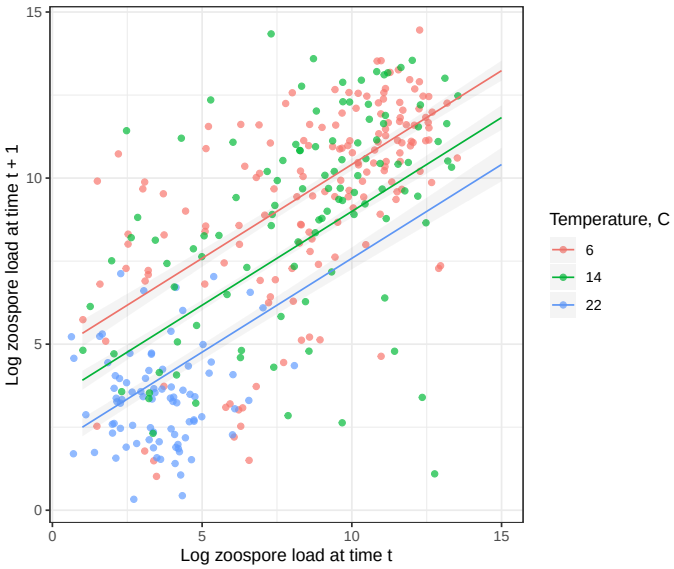

Figure 2: The growth kernel - zoospore growth from t to t+1 at temperatures 6, 14, and 22°C

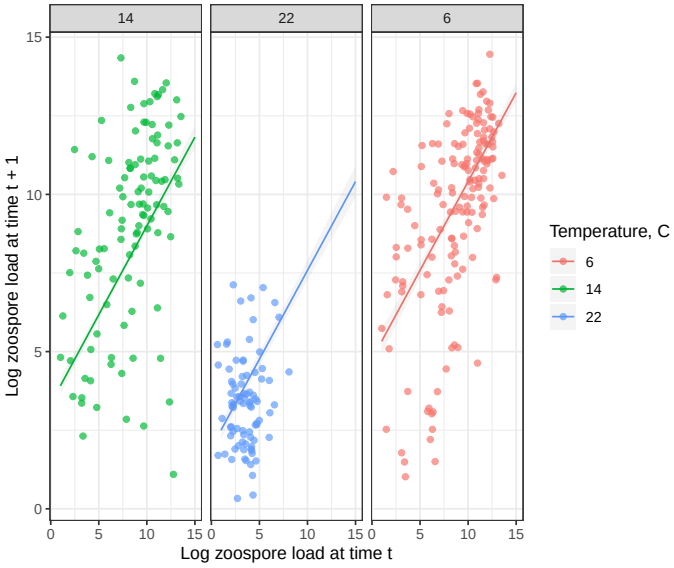

Figure 3: Zoospore growth from t to t+1 at temperatures 6, 14, and 22°C

2.1.5 The loss of infection function

The last vital rate function in the IPM is the loss of infection function, $l(x)$, which estimates the probability of having an infection at time t and losing that infection at time t + 1. Essentially this function tracks the probability of an individual salamander moving from the infected class back to the susceptible class. The loss of infection function was estimated using a logistic regression with the link function, similarly to the infected survival function $s(x)$. Where $b_{0,3}$ is the intercept, and $b_{1,3}x$ is the coefficient estimating the effect of a unit change in log zoospore load on the logit-transformed probability of losing an infection from t to t + 1.

Originally the model including temperature as a predictor variable for $l(x)$, but temperature was not a significant predictor, so we removed it from the model. Otherwise, size at t was a significant predictor of infection loss at t + 1 (Fig. 4; $P = 4.75e - 08$). Overall, individual newts are significantly more likely to lose infection at lower zoospore loads, indicating that there may be a threshold by which individuals may never move back into the susceptible class at high pathogens loads.

Trait	Functional form	Parameters	Details of parameterization
Survival s(x) (infected)	$logit[s(x)] = b_{0,0} + b_{1,0}x$	$b_{0,0} =$ 6.869 $b_{1,0} =$ -0.509	Logistic regression
Survival s_0 (uninfected)	Constant	$s_0 = 1$	Constant
Growth G(x′, x)	$\mu(x,T) = b_{0,1} + b_{1,1}x + b_{2,1}T$ $\sigma^2(x) = v_{0,1}exp(2c_{1,1}x)$	$b_{0,1} =$ 5.821 $b_{1,1} =$ 0.565 $b_{2,1} =$ -0.177 $v_{0,1} =$ 6.021 $c_{0,1} =$ -0.003	Generalized least squares
Initial infection G_0(x′)	$\mu(T) = b_{0,2} + +b_{1,2}T$ $\sigma^2(x) = v_{0,2}exp(2c_{01,2}x)$	$b_{0,2} =$ 3.049 $b_{1,2} =$ -0.011 $v_{0,1} =$ 0.769 $c_{0,1} =$ 0.018	Generalized least squares
Loss of infection l(x)	$logit[l(x)] = b_{0,3} + b_{1,3}x$	$b_{0,3} =$ -0.096 $b_{1,3} =$ -0.662	Logistic regression
Pathogen transmission ϕ	$logit[\phi(T)] = b_{0,4} + b_{1,4}T$	$b_{0,4} =$ -1.792 $b_{1,4} =$ 0.191	Logistic regression

Figure 4: Parameters for the vital rate functions used to simulate the IPM.

3 IPM simulation

The goal of the IPM simulation is to track the infection dynamics and survival of individual newts infected with *Bsal* at different temperatures. Ideally, if temperature acted as a continuous covariate on zoospore growth, the IPM would have the potential to estimate infection dynamics at all temperatures from 6 to 22°C. Unfortunately, the interaction between *Bsal* growth and temperature is non-linear. Zoospore growth at temperatures 6 and 14°C was not significantly different. At 22°C *Bsal* growth was significantly lower than at the lower temperatures. Because of the unusual nature of *Bsal* growth, the IPM simulation can only predict outcomes of the vital rate functions at 22°C, as it was the only temperature that had a significant effect on the response variables in the parameterizations.

In preparation for the simulation, and to implement the IPM numerically, we used the the midpoint rule, a numerical integration method, to discretize the continuous growth function into 100 bins[11]. For the infected class, the upper and lower bounds of the integral of the growth function were set as -5 and 20, which are realistic bounds considering the empirical zoospore load data.

The other simulations executed include calculations of population growth rate, lambda(λ),and mean time to death estimates. These measurements assumed that the paramaterized vital rate functions have a normal multivariate distribution and were calculated by running a simulation that drew random regression paramaters from he IPM using the parameters from the assumed normal distribution of the vital rate functions[9].Because of these assumptions, the population growth estimates and mean time to death were calculated for temperatures 6, 14, and 22°C when those temperatures were fit as a factor.

4 Model simulation results and discussion

The results of the vital rate function parameterizations indicate that increasing temperatures significantly reduce *Bsal* growth on *Notophthalmus viridescens* in a laboratory setting. Although vital rate projections cannot be executed for temperatures 6 and 14°C, the survival plot indicates that newts at 22°C have a higher probability of surviving than newts at lower temperatures(Fig. 1). The enhanced survival in newts at 22°C is most likely outcome of the significantly lower pathogen growth rates on newts at this temperature (Fig. 2&3). This is surprising given that the optimal temperature range for growth of *Bd* on frogs is 17-25°C and can even grow well at 27°C [12,13].

Simulations of various vital rate function generated by the IPM, indicate that there is a growth rate threshold at 22°C (Fig. 56)). The black one to one line on the survival/growth kernel represents equilibrium of the growth function($t = t + 1$), a log zoopore load above the line is growing, whereas below the line, the log zoospore load is shrinking. A zoospore growth threshold exists around 403-1096 individual zoospores ($5 - 7$ log zoospores; Fig. 56)).

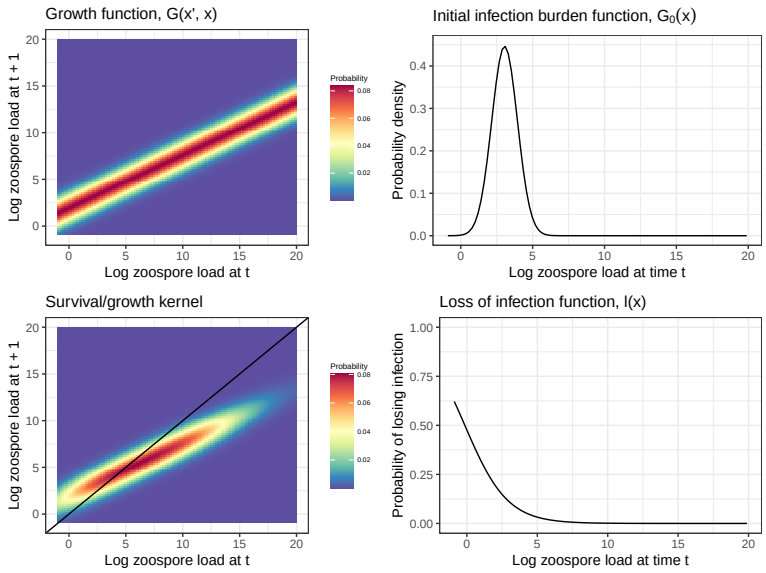

Figure 5: Vital rate function projections for, growth $G(x', x)$, the initial infection function $G_0(x)$, the survival/growth kernel $G(x', x)s(x)$, and the loss of infection function $l(x)$ at 22°C. The black $1:1$ line on $G(x', x)s(x)$ plot represents an equilibrium growth rate for *Bsal*, above this line the *Bsal* zoospore load on a host gets larger in a time step and below this line the *Bsal* zoospore load on a host gets smaller in a time step.

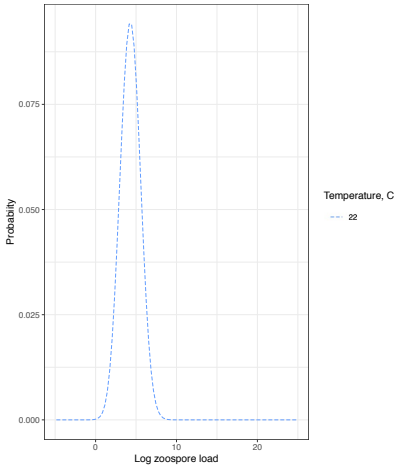

Figure 6: A trajecotry plot predicted by the IPM that estimates the probability of *Bsal* load for 22°C.

The effect of temperature on zoospore growth rate and host survival has implications beyond individual projections. Population growth rate or lambda, λ, was estimated as a function of temperature at 6, 14, and 22°C (Fig. 7). The growth rate calculations show an increasing population growth rate as temperature increasing from 6 to 14°C and 14 to 22°C. Additionally, the IPM projected the mean time to death days as a function log zoospore load at different temperatures, with newt populations 22°C surviving significantly longer than populations at lower temperatures (Fig. 8). These estimates are likely inaccurate because they are not calculated from the empirical data, but it is highly likely that individual infected newts at 22° do survive for longer and experience a higher growth rate.

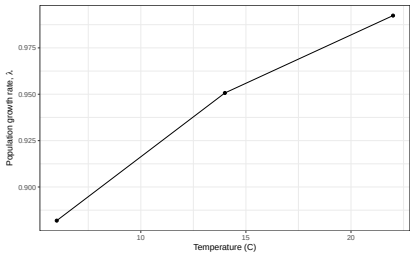

Figure 7: Estimates of population-level growth rate, λ, for *Notophthalmus viridescens* at 6, 14, and 22°C

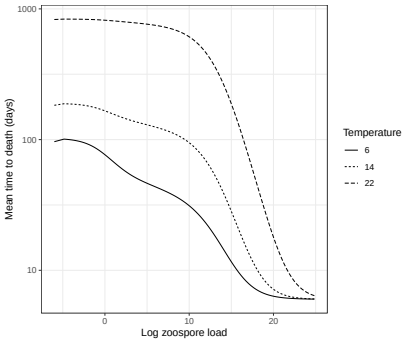

Figure 8: Estimates of population-level mean time to death of infected individuals for *Notophthalmus viridescens* at 6, 14, and 22°C

5 Conclusions and future directions

Overall, the IPM projected that individual *Bsal* infected *Notophthalmus viridescens* experienced slower pathogen growth which in turn enhanced host survival at 22°C. This result would suggest that reducing temperature has different proportional effects on *Bsal* growth depending on the absolute temperature. This result is surprising given that increasing temperature has a linear effect on *Bd* zoospore growth, with an optimal growth temperature range between 17-25°C[12,13]. Potential extensions of this model include adding life stage and dose as additional covariates. Hopefully, these additions would make temperatures at other levels a significant predictor of the vital rate functions. Future laboratory inoculation experiments should continue for multiple North American newt species infected with different strains of *Bsal*, to create an intervention plan if *Bsal* invades North America.

These results inform preparations for a potential *Bsal* invasion in North America for *Notophthalmus viridescens*. The range of *Notophthalmus viridescens*

in North America spans most of the East coast, so monitoring *Bsal* infection on individuals should be prioritized in the colder regions of the Eastern US and during the winter months [7]. This type of preemptive monitoring could prevent *Bsal* entering the US and avoid extinction of North American salamander and newt species in the future.

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