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Original Contribution

Introduction of Ranavirus to Isolated Wood Frog Populations Could Cause Local Extinction

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Abstract: Amphibian declines and extinction have been attributed to many causes, including disease such as chytridiomycosis. Other pathogens may also contribute to declines, with ranavirus as the most likely candidate given reoccurring die-offs observed in the wild. We were interested in whether it is possible for ranavirus to cause extinction of a local, closed population of amphibians. We used susceptibility data from experimental challenges on different life stages combined with estimates of demographic parameters from a natural population to predict the likelihood of extinction using a stage-structured population model for wood frogs (*Lithobates sylvaticus*). Extinction was most likely when the larval or metamorph stage was exposed under frequent intervals in smaller populations. Extinction never occurred when only the egg stage was exposed to ranavirus. Under the worst-case scenario, extinction could occur in as quickly as 5 years with exposure every year and 25–44 years with exposure every 2 years. In natural wood frog populations, die-offs typically occur in the larval stage and can reoccur in subsequent years, indicating that our simulations represent possible scenarios. Additionally, wood frog populations are particularly sensitive to changes in survival during the pre-metamorphic stages when ranavirus tends to be most pathogenic. Our results suggest that ranavirus could contribute to amphibian species declines, especially for species that are very susceptible to ranavirus with closed populations. We recommend that ranavirus be considered in risk analyses for amphibian species.

Keywords: amphibian declines, carrying capacity, closed populations, iridoviridae, *Lithobates sylvaticus*, matrix model

Introduction

Disease has been implicated as a factor potentially affecting the sustainability of many species (IUCN 2013). However, there has been a lot of debate about how frequently disease actually contributes to local and global extinction. Smith et al. (2006) noted that infectious disease not only contributed to less than 4% of extinctions and 8% of critical endangerment but also indicated that there is a great deal of uncertainty in identifying causal agents and their contribution to species declines. According to theoretical models, disease is most likely to cause extinction when populations are small, transmission is not density dependent, or reservoir hosts are present (de Castro and Bolker 2005). Most empirical cases of disease-induced extinction have been related to small population size (de Castro and Bolker 2005), and seem to be taxonomically biased toward birds

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and amphibians (Smith et al. 2006). Amphibians are considered the most imperiled taxonomic class of vertebrates (Wake and Vredenburg 2008), and disease may be playing a role in their declines (Vredenburg et al. 2010).

Amphibian declines and extinctions have alarmed conservation biologists for more than two decades (Stuart et al. 2004). According to the IUCN (2013), 30% of amphibian species are threatened or endangered with another 25% having too little data for an accurate assessment. Many potential threats to amphibians and causes of amphibian declines have been identified, ranging from climate change to habitat destruction to pesticides (Kiesecker et al. 2001; Collins and Storfer 2003). Disease is one threat that has been shown to definitively cause amphibian extinction (Collins and Crump 2009). The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*; etiologic agent of chytriodiomycosis) has moved through Central America causing massive frog die-offs and species extinctions (Lips et al. 2006; Skerratt et al. 2007). *Bd* has also been implicated in declines of amphibians in western North America and Australia (Daszak et al. 2003; Skerratt et al. 2007; Vredenburg et al. 2010).

Other amphibian pathogens have been proposed as potential contributors to amphibian declines. The water mold *Saprolegnia* (Blaustein et al. 1994) and the parasite *Ribeiroia* (Johnson et al. 1999) can cause stress and death in amphibians, but it is unclear whether they can cause major population-level effects (Daszak et al. 2003). Ranaviruses are a pathogen that may be emerging (Gray and Miller 2013), and are known to cause infection and disease in >70 amphibian species across 14 taxonomic families (Miller et al. 2011). Ranaviruses are double-stranded DNA viruses that cause systemic hemorrhagic disease in amphibians (Gray et al. 2009). Species that have fast developing larvae seem to be most susceptible (Hoverman et al. 2011; Brenes 2013). In very susceptible species, mortality can be as quick as three days following exposure to ranavirus in water (Hoverman et al. 2011). Miller et al. (2011) suggested that ranaviruses could contribute to species declines, because the pathogen infects multiple hosts with different susceptibilities, environmental persistence of virions can be long, and clustering behavior by amphibians occurs—all which can facilitate density-independent transmission (Anderson and May 1979). However, others have suggested that ranaviruses play a minor role in the persistence of amphibian species (Collins and Crump 2009).

There is some evidence that ranavirus could affect amphibian population dynamics. In the United States, disease-associated amphibian die-offs are most frequently caused by ranaviruses (43–58%; Green et al. 2002; Muths et al. 2006). Teacher et al. (2010) reported that adult populations of the common frog (*Rana temporaria*) in England had median declines of 81% over 10 years after experiencing reoccurring ranavirus-associated die-offs. Petranka et al. (2003) reported no recruitment of wood frogs (*Lithobates sylvaticus*) in several consecutive years due to die-offs from ranavirus. In this study, wood frog populations only persisted due to immigration from adjacent source populations without ranavirus (Petranka et al. 2007). Thus, it appears that ranavirus could contribute to amphibian declines, but it is still largely unclear whether ranavirus could cause extinction in isolated, local populations.

To examine this possibility, we investigated whether the introduction of ranavirus to closed populations of wood frogs could cause population declines or extinctions using stage-structured population matrix models (Caswell 2000). We chose wood frogs, because they are very susceptible to ranavirus (Hoverman et al. 2011), the mostly widely distributed amphibian species in North America (Lanoo 2005), and commonly associated with ranavirus die-offs in the wild (e.g., Greer et al. 2005; Harp and Petranka 2006; Gahl and Calhoun 2010; Brunner et al. 2011). Further, wood frogs have been intensively studied (Berven 1990, 1995, 2009), and robust estimates of vital rates and fecundity are available and have been used previously in population models (Harper et al. 2008; Al-Asuoad et al. 2012). In our model, we used experimental challenge data for wood frogs during different pre-metamorphic life stages (Haislip et al. 2011), and examined the effects of pathogen exposure interval and adult carrying capacity. For this initial modeling effort, we chose to use closed populations, because we were interested in whether ranavirus-induced extinction was possible. Further, amphibians tend to have low vagility, and their movement is negatively affected by anthropogenic land use (Guerry and Hunter 2002), often creating isolated populations (Marsh and Trenham 2001). Although wood frog populations often have a meta-population structure (Peterman et al. 2013), closed populations likely occur as evidenced by high genetic differentiation in geographically isolated breeding ponds (Newman and Squire 2001; Julian and King 2003). Isolated populations are especially likely in areas of habitat fragmentation and along the periphery of the wood frog range.

Materials and Methods

Wood frogs are pond-breeding amphibians with a large geographic range, including large portions of Canada and the United States. They are explosive breeders that typically mate and lay eggs (often as many as 1,000 in one clutch) over a period of a few nights in early spring (Redmer and Trauth 2005). Larvae develop over the next few months and metamorphose in summer. Females mature in 1–4 years depending on the population (Bellis 1961; Berven 1990, 1995). Adults generally breed only one or two times in their lifetime (Berven 1990).

We created a four-stage female only, discrete time (1-year increments) population matrix model (parameter values in Table 1; Caswell 2000) to examine the effects of ranavirus on closed populations of wood frogs. In this model, the population size for stage i (where i = pm, 1, 2, and 3+ for pre-metamorphosis and years 1, 2 and $\geq$3) at time t [$N_i(t)$] is calculated by multiplying the population matrix at time $t-1$ by the transition matrix containing parameter estimates for survival (p) and fecundity (F; Eq. 1, Table 1).

$$\begin{pmatrix} N_{\mathrm{pm}}(t) \\ N_1(t) \\ N_2(t) \\ N_{3+}(t) \end{pmatrix} = \begin{pmatrix} 0 & F_1 & F_2 & F_3 \\ p_1 & 0 & 0 & 0 \\ 0 & p_2 & 0 & 0 \\ 0 & 0 & p_3 & p_4 \end{pmatrix} \times \begin{pmatrix} N_{\mathrm{pm}}(t-1) \\ N_1(t-1) \\ N_2(t-1) \\ N_{3+}(t-1) \end{pmatrix} \quad (1)$$

Table 1. Parameter estimates used in wood frog population model derived from Harper et al. (2008) and Haislip et al (2011).

Parameter[a]	Parameter estimate	Standard deviation
p_1 (Pre-metamorphic to year 1)	0.0350	0.0245
p_2 (Year 1 to 2)	0.3775	0.1020
p_3 (Year 2 to 3+)	0.2490	0.1575
p_4 (Remain year 3+)	0.1200	0.0500
F_1 (Year 1)	0.215	0.0548
F_2 (Year 2)	334.5	31.944
F_3 (Year 3)	338.3	31.6228
Ranavirus survival: egg	0.570	0
Ranavirus survival: hatchling	0.170	0
Ranavirus survival: larvae	0	0
Ranavirus survival: metamorph	0	0

[a]p survival probabilities and F fecundity (the average number of female eggs produced per female modified by probability of breeding) that were used in the transition matrix.

We parameterized the model using the same survival and fecundity estimates in Harper et al. (2008), which were from a robust population of wood frogs in Beltsville, Maryland (USA) that was monitored for over 7 years (Berven 1990). We used parameter estimates for female wood frogs only, because they generally produce one clutch per year, whereas males can fertilize multiple clutches and are thus not considered to limit population growth. The model is a post-breeding model, where 1 year in the model spanned from the end of one mating season to the next. Following Harper et al. (2008), estimates were drawn at a specified frequency to simulate stochasticity. We took weighted averages of these estimates to create a normal distribution for each parameter in order to simulate stochasticity in a continuous manner, which is more realistic (Table 1). In each year of the model simulations, a random value was drawn from the distribution for each parameter.

To introduce ranavirus into the population, we used data from Haislip et al. (2011) who performed experimental challenges for four pre-metamorphic life stages (egg, hatchling, larva, metamorph) with a *Frog Virus 3* (FV3)-like isolate (Miller et al. 2007); FV3 is the type species of *Ranavirus* (King et al. 2012). FV3-like ranaviruses are responsible for most of the ranavirus die-offs in North America, and FV3 is the species of *Ranavirus* most often found in the wood frog range (Miller et al. 2011). Haislip et al. (2011) exposed individuals to 10^3 plaque-forming units (PFU)/mL of FV3 in a 0.5-L water bath for three days, and monitored survival for 14 days. This concentration is considered to be an environmentally relevant approximation of virus in water during a die-off (Gray et al. 2009). The survival rates from Haislip et al. (2011) ranged from 0–57% depending on life stage (Table 1). This is consistent with wood frog die-offs in natural populations where researchers often observe greater than 90% mortality (Green et al. 2002) or fail to detect any live individuals despite intensive sampling (Petranka et al. 2003; Todd-Thompson 2010).

Simulations were run in a factorial design varying three factors: life stage of ranavirus exposure (egg, hatchling, larvae, or metamorph), exposure interval (none and every 50, 25, 10, 5, 2, and 1 years), and adult female carrying capacity (50, 100, 500, 1,000 and 1,500). Life stages were delineated as in Haislip et al. (2011), where egg, hatchling, larvae and metamorph corresponded to pathogen exposure starting at Gosner stage 11, 21, 30, and 41, respectively (Gosner 1960). To simulate mortality in years with ranavirus exposure, we multiplied the pre-metamorph to year

one transition probability (p_1) times the ranavirus survival probability for the appropriate stage. For these runs, we assumed that individuals that were exposed and survived were not exposed again during a subsequent life stage. For the pathogen interval exposure, we treated intervals probabilistically such that years with pathogen exposure were randomly determined using a binomial distribution appropriate for the exposure interval. Thus, ranavirus was not introduced exactly every 10 years but on average every 10 years for the 10-year exposure interval. The exposure intervals that were included likely represented realistic intervals in wood frog populations inasmuch as die-offs at the same site have been recorded in multiple consecutive years (e.g., Green et al. 2002; Petranka et al. 2007; Todd-Thompson 2010). Adult female carrying capacity was set as an upper population threshold, where the combined abundance of 2 and 3+ year olds could not exceed the carrying capacity. Carrying capacities were based on the number of egg masses detected at ponds, which is thought to represent the number of females visiting the pond. The adult female carrying capacities used in our study were from Raithel et al. (2011), where the average number of clutches per pond over 16 years ranged from 30 to 1,264. Because more clutches have been occasionally observed in other areas (Berven 2009), we used 1,500 as our largest carrying capacity.

For each scenario, we performed 1,000 model runs of 1,000 years each. We chose 1,000 years as the end-point for simulations, because we were most interested in whether it was possible for ranavirus to cause extinction and how long it would take for extinction to occur. We also defined extinction in a very conservative manner, where there had to be zero individuals remaining for extinction to occur. For each model run, we determined whether the population went extinct, the extinction time, and the total number of adult females (i.e., 2 and 3+ year classes) at 1,000 years. Because we could generate a large amount of data, we chose to present the data graphically and not perform statistical analyses. All models were run in Matlab release 2010a (Mathworks, Natick, Massachusetts, USA).

Sensitivity analyses were conducted to determine which model parameters influenced the model the most and, thus, how important they were for population dynamics. We conducted sensitivity analyses by running 1,000 simulations of 1,000-year runs, where parameter values were randomly generated as in previous simulations but then fixed for all years of the model run. The total population size and whether the population went extinct in each 1,000-year period were used as the response variables. We assessed the influence of parameters on response variables by running simple and logistic regressions for population size and extinction, respectively, using R version 2.7.2 to produce R^2 values (R Development Core Team 2008).

Results

Simulated exposure to ranavirus was able to cause extinction within 1,000 years in local populations of wood frogs in some scenarios, but it never occurred without ranavirus exposure (Fig. 1). Extinction also never occurred if individuals were exposed to ranavirus in the egg stage and occurred rarely if the exposure interval was on average every 50 years. Extinction probability was greater when ranavirus exposure occurred during metamorph and larval stages (ca. 36% across all simulations) when compared to the hatchling stage (ca. 17% across all simulations). Extinction probability also increased with more frequent pathogen exposure intervals (e.g., 0.01% extinction for every 50 years and 67% extinction for every year across all simulations), and in populations with lower carrying capacities (e.g., ca. 20% extinction for $K = 50$ and ca. 16% for $K = 1500$ across all simulations). All populations went extinct if exposure to ranavirus was every 1 or 2 years during the larval or metamorph stage regardless of the carrying capacity, and also if hatchlings were exposed every year (Fig. 1).

For populations that went extinct, time to extinction decreased with more frequent ranavirus exposure intervals and tended to occur more quickly when exposure occurred during the larval or metamorph stages than the hatchling stage (Fig. 2). However, there was a great deal of variability, particularly with less frequent ranavirus exposure, due to the inherent stochasticity in the model and the lower sample size of extinct populations. Extinction also occurred more quickly in populations with lower carrying capacity (Fig. 2). Extinction occurred most quickly when exposure happened during the larval or metamorph stage every 1 or 2 years, with extinction occurring on average between 25 and 44 years for the two-year exposure interval and in 5 years for exposure every year (Fig. 3).

For populations that did not go extinct within the 1,000-year period, many experienced population declines. Those populations without ranavirus exposure or with infrequent exposure (i.e., every 50 or 25 years) maintained

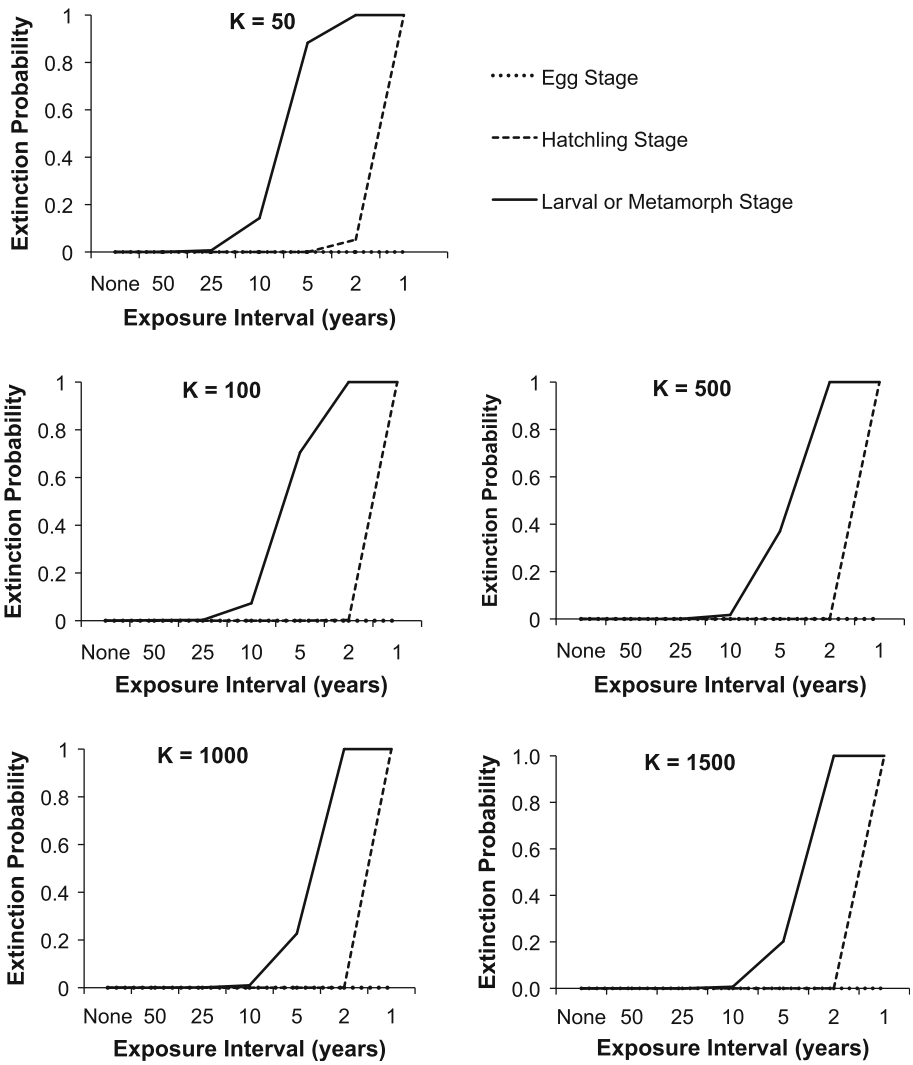

Fig. 1. Extinction probability over 1,000 years of wood frogs exposed to ranavirus at different intervals in different life stages for populations with different adult female carrying capacities (K). The larval and metamorph stages are the same, because they experienced the same mortality rate when exposed to ranavirus in controlled experiments. Note that extinction never occurred with ranavirus exposure in the egg stage.

populations at or near their carrying capacity (Fig. 4). Exposure in the egg stage also resulted in population levels near carrying capacity with minor declines of 3–4% when exposure occurred every one to 2 years. As with the extinction probability and time to extinction, population declines were greater with exposure in the larval or metamorph stages than the hatchling stage and with more frequent ranavirus exposure intervals. When the larval or metamorph stages were exposed to ranavirus every 5 years or when hatchlings were exposed every 2 years, population declines were around 20% (Fig. 4).

The sensitivity analysis showed that wood frog population dynamics were most sensitive to the pre-metamorph to 1-year old transition probability (p_1). This was true for both the total population size ($R^2 = 0.19$, $P < 0.001$) and the probability of extinction ($R^2 = 0.35$, $P < 0.001$). The transition probability from year 1–2 (p_2) and the fecundity of 3+ year olds (F_{3+}) showed significant sensitivity, but correlations were extremely low (total N: $R^2 < 0.002$; extinction probability: $R^2 < 0.04$). All other parameters appeared to have very little influence on total population size and extinction probability (all $R^2 < 0.002$, all $p > 0.18$).

Discussion

Pathogens can cause extinction in a limited number of scenarios (de Castro and Bolker 2005), and very few cases of disease-induced extinction have been reported (Smith et al. 2006). It has been hypothesized that ranaviruses might be able to cause extinctions, because several characteristics of this host-pathogen system may lead to density-independent transmission (Miller et al. 2011). For highly susceptible hosts, such as the wood frog, our results demonstrate that extinction is possible by ranavirus in

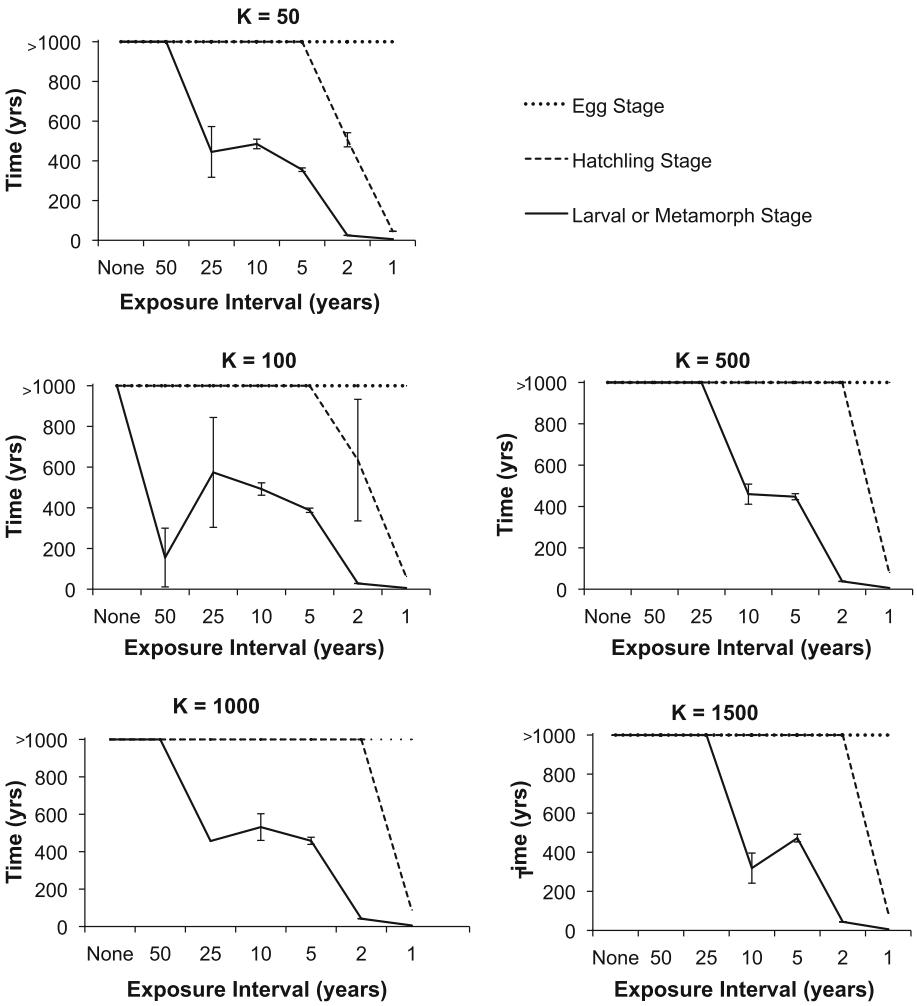

Fig. 2. Time to extinction of wood frogs exposed to ranavirus at different intervals in different life stages for populations with different adult female carrying capacities (*K*). The larval and metamorph stages are the same, because they experienced the same mortality rate when exposed to ranavirus in controlled experiments. Note that extinction never occurred within 1,000 years with ranavirus exposure in the egg stage. *Error bars* represent standard error.

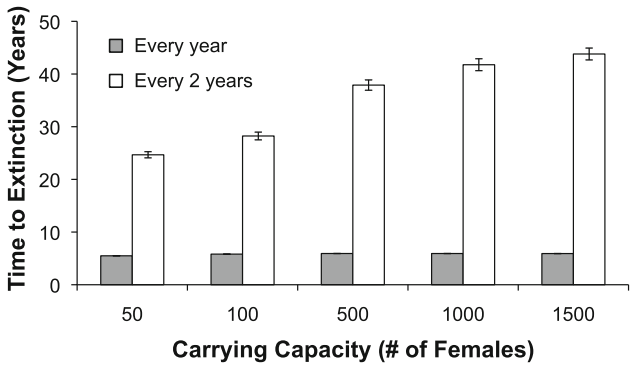

Fig. 3. Time to extinction of wood frogs exposed to ranavirus every year and every 2 years in the larval or metamorph stage at different female carrying capacities. *Error bars* represent standard error.

closed populations. Under the worst-case scenarios, extinction occurred in as little as 25–44 years when exposure was every 2 years, and in 5 years if exposure was every year. These exposure intervals have been reported in some wood frog populations (e.g., Petranka et al. 2007). Thus, it is likely that ranavirus die-offs could be detrimental if a population is demographically isolated (i.e., no immigration). In general, extinction was more likely and occurred sooner if ranavirus exposure occurred in the larval or metamorph stages under frequent exposure intervals in populations with smaller carrying capacities. For populations that did not go extinct, population declines were more likely under these same scenarios.

The life stage when a population was exposed to ranavirus was one of the most important factors determining likelihood of extinction and declines. In experimental trials, the egg stage had a 57% survival rate when exposed to ranavirus (Haislip et al. 2011), which was high enough to prevent extinction under all model scenarios and resulted in almost no change in adult population size even if ranavirus was present every year. Eggs may have a greater survival rate with ranavirus exposure than other stages, because they are protected by a thick gelatinous membrane that may serve as a structural barrier (Berrill et al. 1998; Pauli et al. 1999) or contain anti-viral properties (Han et al. 2008). Wood frog hatchlings, larvae and metamorphs are

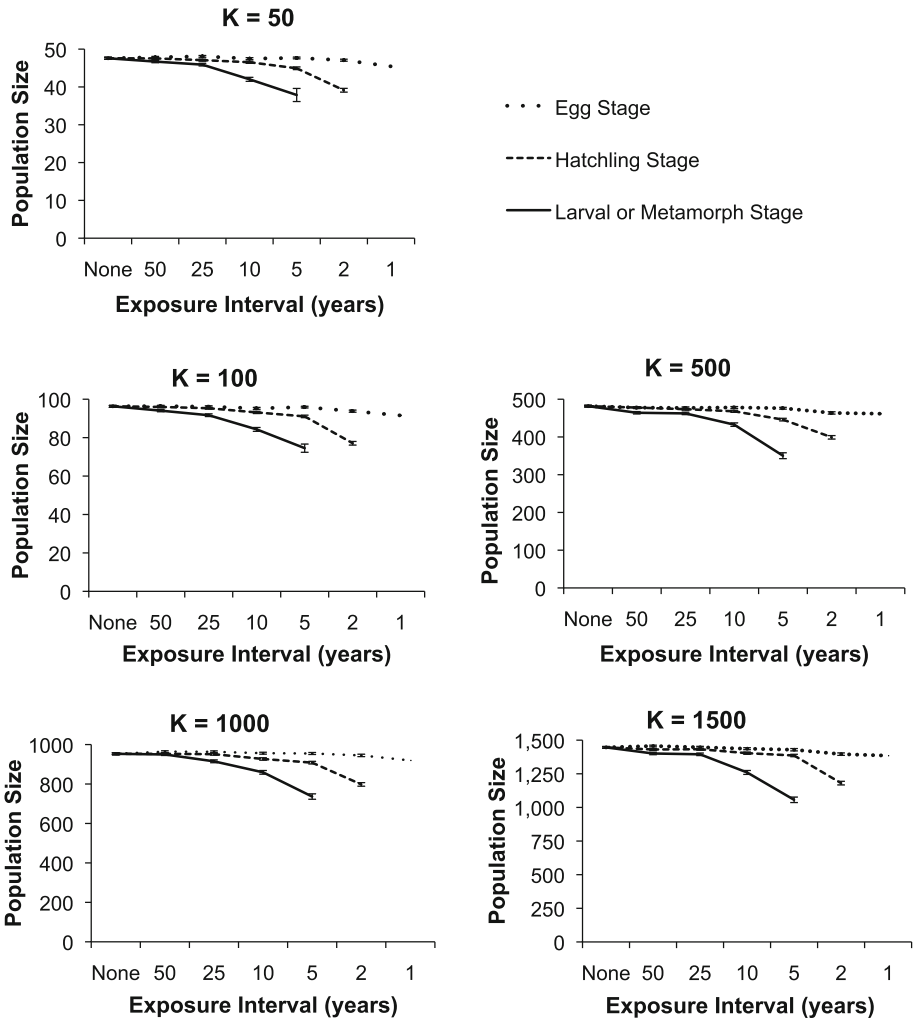

Fig. 4. Female wood frog population size after 1,000 years in populations with no extinction when exposed to ranavirus at different intervals in different life stages for populations with different adult female carrying capacities (*K*). The larval and metamorph stages are the same, because they experienced the same mortality rate when exposed to ranavirus in controlled experiments. Note that in scenarios with no value, all populations went extinct prior to 1,000 years. *Error bars* represent standard error.

much more susceptible, as indicated by their lower survival rates of 0–17% (Haislip et al. 2011), which resulted in extinction in several model scenarios.

Although our simulations were based on long-term data sets from wild populations in the eastern United States, wood frogs have an extensive range; thus, it is possible that other populations have different survival and fecundity rates. Nonetheless, in all the populations of wood frogs that we have tested, ≥95% mortality of wood frog larvae is common following exposure to ranavirus (Haislip et al. 2011; Hoverman et al. 2011). Moreover, we recently tested wood frog tadpoles from Alaska with the same isolate, and a similar fate occurred (P. Reilly and MJG, University of Tennessee, unpubl. data). Other species or isolates of ranavirus may result in different extinction outcomes. For example, the ranavirus, *Ambystoma tigrinum* virus, causes low mortality in anuran larvae (Schock et al. 2008). For FV3-like isolates, we do not anticipate differences in survival. Hoverman et al. (2011) and Brenes (2013) tested the susceptibility of wood frog tadpoles to other FV3-like isolates, and all isolates resulted in >95% mortality. Of the 35 amphibian species tested by these investigators, tadpoles of 13 species experienced ≥60% mortality when exposed to FV3-like isolates. These results suggest that FV3-like ranaviruses have the capability of causing extinctions in isolated populations, although factors such as population demographics and species sensitivity to ranavirus are important.

Population size is one of the most important factors for the conservation of a species. Smaller populations are known to be more vulnerable to extinction in general (Lande 1993), though even our smallest carrying capacity never went extinct in a 1,000-year period with no exposure to ranavirus. As hypothesized, lower carrying capacities increased probability of extinction and level of population decline with exposure to ranavirus. In most cases where disease is implicated as a cause or contributor to extinction, the population was already small (de Castro and Bolker

2005), as seen in the tree snail (*Partula turgida*) infected by a microsporidian parasite (Cunningham and Daszak 1998) and the Spanish ibex (*Capra pyrenaica hispanica*) with sarcoptic mange (León-Vizcaíno et al. 1999). Species are more likely to be affected by disease if they are in higher conservation risk categories, but it is unclear whether disease is more likely to create greater risk or if species at risk are more susceptible to pathogens (Heard et al. 2013). Pearman and Garner (2005) reported that populations of the Italian agile frog (*Rana latastei*) with lower genetic diversity were more susceptible to ranavirus, suggesting that population bottle necks may cause higher disease vulnerability. Loss of genetic diversity in small, isolated populations is common (e.g., Vrijenhoek 1994); thus, future modeling efforts should incorporate a susceptibility parameter for mixed versus closed populations. Eggs that were collected for Haislip et al. (2011) were from the Royal Blue Unit (Knox County, TN, USA) of the North Cumberland Wildlife Management Area, which is an undisturbed 57,000-ha site with multiple wood frog populations. Thus, our simulations likely represent genetics from a mixed population that recently became isolated.

Our model included a number of assumptions that could be relaxed with further modeling efforts. Many of these assumptions need more empirical data to produce robust predictions. The most important of these assumptions are related to exposure and transmission. For example, we assumed that individuals were exposed to ranavirus during only one life stage as done in Haislip et al. (2011), which might be unrealistic. Given the long persistence of ranavirus outside the host (Nazir et al. 2012) and multiple reservoir species (Brenes et al. 2014b), it is likely that at least some individuals that survive exposure will be re-exposed to virions in subsequent life stages. It is also possible that there are fitness costs associated with harboring subclinical infections, such as lower growth and reproductive rates. Additionally, our model assumed that all individuals of a particular life stage were exposed to die-off concentrations of ranavirus. This assumption needs to be tested. The die-off concentration that we used was from Rojas et al. (2005), who quantified virus in the water being shed by an infected salamander in the laboratory. To date, there are no data on concentrations of ranavirus in the water during wild population die-offs. Nonetheless, ranavirus dose studies (e.g., Brunner et al. 2005; Pearman and Garner 2005) suggest that the concentration we used (10^3 PFU/mL) is a threshold when mortality due to ranavirus increases. Further, we assumed that there were years when individuals were exposed and years when they were not. It is likely that ranavirus exposure in the wild is a continuum, where the concentration of virions and the fraction of the population exposed varies over time and is affected by environmental conditions (e.g., pond size, temperature). Future modeling efforts should attempt to identify and incorporate mechanisms that affect ranavirus transmission dynamics.

Transmission between hosts is fundamental to the likelihood of a pathogen causing extinction (Anderson and May 1979; McCallum et al. 2001). Transmission of ranavirus can occur through water (Brunner et al. 2005) or direct contact (Brunner et al. 2007), is accelerated when tadpoles scavenge individuals dead from ranavirus infection (Harp and Petranka 2006), and can occur between different amphibian species (Jancovich et al. 2001) and classes of vertebrates (fish and reptiles; Bayley et al. 2013; Brenes et al. 2014a). Epidemiological models suggest that disease can cause extinction if transmission can be density independent (Anderson and May 1979; McCallum et al. 2001) even if most transmission is density dependent (Ryder et al. 2007) or if the system includes a virus reservoir. Harp and Petranka (2006) found that transmission may not be density dependent in wood frogs due to schooling behavior, but density dependence may occur in other species (Greer et al. 2008). Inasmuch as ranaviruses can infect multiple life stages and host species with different susceptibilities (Brunner et al. 2004; Haislip et al. 2011; Hoverman et al. 2011; Brenes 2013) and virions may persist outside of hosts for considerable duration (Nazir et al. 2012), it is possible for density-independent ranavirus transmission to occur (Miller et al. 2011). More research is needed on the mechanisms affecting transmission dynamics of ranavirus. Inclusion of transmission parameters in population models (Briggs et al. 2005) may refine predictions.

Disease can induce rapid evolution of host species as seen in a variety of species, including mollusks, plants and mammals (Altizer et al. 2003). Our model assumed that populations were unable to evolve. Directional selection may occur in populations experiencing die-offs from ranavirus, resulting in subsequent generations with increased immunity (Teacher et al. 2009). Several authors have suggested that tiger salamanders may have coevolved with *A. tigrinum* virus (Huelsenbeck et al. 1997; Storfer et al. 2007; Ridenhour and Storfer 2008). Nonetheless, with the 100% mortality in the larval and metamorph stages that has been observed for wood frogs exposed to ranavirus

(Haislip et al. 2011), there would no individuals remaining to pass on genes that may enhance immunity to a particular type of ranavirus.

Sensitivity analysis is important for determining which vital rates drive population trends. Factors that lower vital rates with high sensitivities are most likely to cause population declines and subsequent extinction (Caswell 2000). Our sensitivity analysis showed that wood frog populations are most sensitive to changes in the transition probability between egg and year 1 (i.e., the pre-metamorphic stages during which ranavirus exposure was possible in our model). Other population models have found that the juvenile stage and highly variable vital rates contribute most to population regulation in pond-breeding amphibians (Biek et al. 2002; Vonesh and De la Cruz 2002). In our model, survival from pre-metamorph to year 1 was the most highly variable parameter, which likely caused the higher sensitivity to this parameter. Our results highlight the importance of the pre-metamorphic stages in predicting disease outcomes for amphibian species with low post-metamorphic survival or that breed infrequently. To date, the susceptibility of post-metamorphic wood frogs to ranavirus has not been tested. Generally, it is assumed that adults have lower susceptibility to ranavirus because of greater immune function, as demonstrated in the African clawed frog (*Xenopus laevis*; Gantress et al. 2003; Robert et al. 2005). However, adults of some amphibian species are known to be very susceptible to ranavirus (Cunningham et al. 1993; Balseiro et al. 2010). Future estimates of adult wood frog susceptibility to ranavirus would improve simulations. Understanding a population's sensitivity to survival probabilities during different life stages will go far in understanding how disease related die-offs affect a population's sustainability (Biek et al. 2002).

Our results show that ranavirus could cause extinction of local, closed populations of amphibians. Many amphibian populations, including wood frogs, occur in a metapopulation structure (Peterman et al. 2013), which may counteract the effects of reoccurring ranavirus die-offs (Petranka et al. 2007). However, we suspect that closed populations of wood frogs (Newman and Squire 2001; Julian and King 2003) and other highly susceptible species (e.g., dusky gopher frog, *L. sevosus*; boreal toad, *Anaxyrus boreas boreas*) occur in highly fragmented landscapes with limited or no immigration. Populations at the edge of species distributions also tend to be more isolated. Future modeling efforts should include a parameter for dispersal, and identify how much immigration is necessary to offset die-offs from ranavirus. Rare species often exist in isolated populations and are more vulnerable to local and global extinction than more widespread species. Thus, ranavirus emergence in populations of rare species should be a concern, especially if they are highly susceptible to ranavirus and if they co-exist with reservoir or amplification species (Gray and Miller 2013). Conservation biologists should consider testing the susceptibility of rare species to ranavirus, and if they are highly susceptible, incorporating ranavirus surveillance into population monitoring. Additionally, given the potential threat of ranaviruses to ectothermic vertebrates, biologists and researchers need to be vigilant about decontaminating footwear and equipment among study sites (Bryan et al. 2009; Green et al. 2009; Gold et al. 2013).

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