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RESEARCH ARTICLE

Behavioural fever reduces ranaviral infection in toads

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Abstract

1. Host behaviour is known to influence disease dynamics. Additionally, hosts often change their behaviours in response to pathogen detection to resist and avoid disease. The capacity of wildlife populations to respond to pathogens using behavioural plasticity is critical for reducing the impacts of disease outbreaks. However, there is limited information regarding the ability of ectothermic vertebrates to resist diseases via behavioural plasticity.
2. Here, we experimentally examine the effect of host behaviour on ranaviral infections, which affect at least 175 species of ectothermic vertebrates. We placed metamorphic (temporal block 1) or adult (block 2) southern toads (*Anaxyrus terrestris*) in thermal gradients, tested their temperature preferences before and after oral inoculation by measuring individual-level body temperature over time, and measured ranaviral loads of viral-exposed individuals.
3. We found significant individual-level variation in temperature preference and evidence for behavioural fever in both metamorphic and adult *A. terrestris* during the first 2 days after exposure. Additionally, we found that individual-level change in temperature preference was negatively correlated with ranaviral load and a better predictor of load than average temperature preference or maximum temperature reached by an individual. In other words, an increase in baseline temperature preference was more important than simply reaching an absolute temperature.
4. These results suggest that behavioural fever is an effective mechanism for resisting ranaviral infections.

KEYWORDS
amphibian declines, behavioural fever, disease ecology, ranavirus, thermal biology, thermoregulation

1 | INTRODUCTION

Pathogens can impose strong selective pressures on their hosts, driving the evolution of host behaviours that reduce disease risk (Han, Bradley, Bradley, & Blaustein, 2008; Moore, 2002). Additionally, many hosts cope with pathogens via phenotypic plasticity, modulation of trait values within the life span of the host (Agrawal, 2001). For example, some ectothermic hosts can respond to pathogen exposure by exhibiting a behavioural fever, which is an acute increase in temperature preference (T_{pref}) (Kluger, Kozak, Kozak, Conn, Leon, & Soszynski, 1998). The slightly warmer environmental temperatures chosen by the host after pathogen exposure are presumably more favourable for the host than the pathogen (Burns, Ramos, Ramos, & Muchlinski, 1996; Ouedraogo, Goettel, Goettel, & Brodeur, 2004; Reynolds, Casterlin, Casterlin, & Covert, 1977). Fever is primarily considered a method for improving host immune function by stimulating immunological defences (Evans, Repasky, Repasky, & Fisher, 2015). However, fever has also been proposed as a mechanism for directly killing or slowing pathogen growth with heat (Richards-Zawacki, 2009). Understanding how phenotypic plasticity, such as

behavioural fever, can limit infection and lower disease risk is crucial for predicting how hosts might be affected by emerging diseases in a changing climate. This is especially true for ectothermic hosts, which cannot regulate body temperature independent of the environment and are especially sensitive to climatic abnormalities.

Studies demonstrating that ectothermic hosts use behavioural fever as an effective method of pathogen resistance, defined as a host strategy that limits or inhibits infection (Roy & Kirchner, 2000), are limited (Adamo & Lovett, 2011; de Roode & Lefèvre, 2012). Here, we test for an effective behavioural fever response to a ranavirus in an amphibian. Ranavirus is an emerging, widespread viral disease caused by viruses in the genus *Ranavirus*. It has caused mass mortality events in amphibian hosts contributing to amphibian population declines in last few decades (Brunner & Yarber, 2018; Chinchar, 2002). Ranaviruses are also known to cause frequent infection in other ectothermic vertebrates, such as in fishes and reptiles (Chinchar, 2002; Duffus et al., 2015). Some of these viruses are even capable of transmission across taxa or interclass transmission (Bandín & Dopazo, 2011; Brunner, Schock, Schock, Davidson, & Collins, 2004). Hosts likely encounter ranaviruses frequently because of their broad distributions and lack of host specificity (Miller, Gray, Gray, & Storfer, 2011), and thus, ranaviruses might impose strong selective pressures on hosts.

In fact, mounting evidence suggests that many hosts are adapting immunological and behavioural strategies to combat ranaviral infections (Duffus et al., 2015; Parris, Davis, Davis, & Collins, 2004; Teacher, Garner, Garner, & Nichols, 2009; Whittington, Philbey, Philbey, Reddacliff, & Macgown, 1994). For example, larval amphibians appear to prefer warmer temperatures while infected with ranaviruses (Parris et al., 2004). However, this study did not measure temperature preference before exposure and therefore could not differentiate between pre-existing differences in T_{pref} and differences caused by exposure to ranavirus. Additionally, this study did not measure ranaviral load or prevalence, so there is no evidence that the apparent difference in T_{pref} is effective at resisting infection. Other studies have shown that experimentally warming ranavirus-infected hosts can reduce host mortality and viral loads (Echaubard et al., 2014; Rojas, Richards, Richards, Jancovich, & Davidson, 2005) despite successful ranaviral growth in culture at these higher temperatures (Chinchar, 2002). In these studies, the warming was induced by the experimenters, not the hosts, and exceeded the magnitude and duration of most behavioural fever responses. Additionally, these studies only tested for temperature effects in larval amphibians. Hence, it is unclear whether hosts more generally respond to ranaviral infections with behavioural fever and whether fever is effective at reducing ranaviral loads within hosts.

Here, we test whether metamorphic and adult southern toads, *Anaxyrus terrestris*, adjust their preferred temperature after infection with ranavirus and whether any change in temperature preference reduces ranaviral load within hosts. To accomplish these goals, we exposed *A. terrestris* to a ranaviral isolate in thermal gradients ranging in temperature from 12 to 33°C (Sauer, Sperry, Sperry, & Rohr, 2019) to assess individual T_{pref} before and after exposure to this virus. We also measured viral load within individual hosts to assess whether variation in T_{pref} affected virus replication.

2 | MATERIALS AND METHODS

2.1 | Animal husbandry

Adult and metamorphic *A. terrestris* were collected from Hillsborough County, Florida, from sites where ranavirus has not previously been detected. Individuals were maintained in individual containers (23.5 × 16.8 × 10 cm or 11.7 dia. × 13.5 cm, respectively) on top of folded paper towels soaked with artificial spring water (Cohen, Neimark, Neimark, & Eveland, 1980). These toads were held in a laboratory maintained between 24 and 25°C with a 12-hr photoperiod for at least 2 weeks before the start of the experiment. The toads were fed mineral-dusted crickets ad libitum until the start of the experiment, and their containers and paper towels were changed weekly. Only adult individuals were tested for ranavirus before exposure (see Section 2.3 for details) as metamorphic *A. terrestris* were too small to safely test using the oral swabbing methods described below.

2.2 | Experimental design

Experimental design was based on a prior experimental testing for behavioural fever in amphibians to the chytrid fungus, and thus, we only briefly describe the methods here; see Sauer et al. (2018) for additional details on the design. Experiments were conducted in Tampa, Florida, with two temporal blocks, the first being conducted with metamorphs and the second with adults. In each experiment, we first measured baseline non-infected temperature preference (baseline T_{pref}) in thermal gradient apparatuses (see next paragraph for the frequency of these measurements). These apparatuses were previously shown to provide variation in temperature that is independent of humidity and which does not confound amphibian and prey temperature preferences (see Figure S1 and supplemental methods, and Sauer, Sperry, and Rohr (2016) for thermal gradient construction and validation details). The apparatuses provided an ecologically relevant ambient temperature range of 12–33°C (Fritts et al., 2015; Sauer et al., 2018, 2016). After measuring baseline T_{pref}, individuals were split into two groups of similar mean body masses and baseline T_{pref} (N = 53), (a) a sham-exposed control group (metamorph: n = 12; adult n = 14) and (b) a ranavirus-exposed treatment group (metamorph: n = 13; adult n = 14). While individual-level body mass was consistent across treatments within a block, body mass was much smaller in the metamorphic block (mean mass = 0.48 g ± 0.05 *SE*) than adult block (mean mass = 17.70 g ± 1.42 *SE*).

Throughout the experiment, temperature measurements were taken each day, every four hours, between 10:00 hr and 22:00 hr (two during the lighted part of the photoperiod and two during the dark) at the centre of each animal's dorsum (Rowley & Alford, 2007) (Extech® High Temperature IR Thermometer; accuracy:

±2% < 932°F). The only exception is that these measurements were not taken during feeding periods, during which all individuals were fed 10 live crickets in containment bags to prevent crickets from moving freely within the thermal gradient (see Sauer et al. (2016) and Figure S4 for more details). Temperature measurements were taken for 4 days before ranaviral or sham exposure. The mean T_{pref} of those 4 days for each individual toad is referred to hereafter as the baseline T_{pref} ($T_{pref_{baseline}}$ in equations). Temperature measurements were taken daily for 2 weeks (metamorphic block) or 4 days (adult block) after ranaviral or sham exposure. We shortened our time between exposure and sampling for the adult block after discovering that the metamorphs were uninfected 2 weeks after exposure to ranavirus.

2.3 | Ranaviral exposures and quantification

We used a ranavirus strain isolated from infected wood frogs in Michigan with 99% similarity to *Frog Virus 3* (GenBank accession number: PRJNA504607). We cultured the virus on fathead minnow cells and Eagle's minimum essential media containing 5% foetal bovine serum (MEM) to a titre of 3.6×10^5 plaque-forming units (PFU) ml^{-1}. The virus was stored at −80°C until used in the experiments. Before exposure, virus stock was thawed and homogenized, and then, each individual was dosed orally with 77 µl of the virus (2.8×10^4 PFU) or the same volume of a sham inoculum of MEM. The individuals from the metamorphic block were euthanized 2 weeks after ranavirus exposure and dissected to remove spleen, kidney and liver for quantification of ranaviral loads. The individuals in the adult block were sampled prior to exposure, to ensure they were not already infected, then sampled again 4 days after exposure by inserting and twirling a sterile swab in their mouth for thirty seconds and then freezing these swabs at −80°C (Allender, Mitchell, Mitchell, McRuer, Christian, & Byrd, 2013). Non-destructive sampling via oral swab was preferred because it allowed us the option to sample individuals multiple times. However, we had to destructively sample metamorphic individuals because they were too small to swab orally.

Ranaviral DNA was extracted from each metamorphic and adult sample using the Qiagen DNeasy Blood and Tissue protocol (Qiagen, Inc.). To determine viral load, we used qPCR methods based on Forson and Storfer (2006), with a 250-bp fragment of the major capsid protein (MCP) gene used as a standard (gBlocks® plasmid-based standards; Integrated DNA Technologies). The qPCR mixture contained 10 µl TaqMan® Universal Master Mix (Applied Biosystems), 0.6 µl of primer (Thermo Fisher Scientific), 0.625 µl of TaqMan® TAMRA fluorescent probe (Applied Biosystems) and 6.275 µl of nuclease-free water per well with 2.5 µl of either DNA sample, standard or nuclease-free water added to each well. All samples and standards were run in duplicate, and thus, an individual-level load represents the mean DNA copies of these two subsamples. The duplicates agreed in all but one instance where one of the two did not amplify. For that individual, we ran a third subsample, which did amplify and used the mean of the two positive subsamples.

2.4 | Data analysis

All statistics were conducted with R 3.4.0 (Team, 2017). To test for repeatability in baseline T_{pref} within individuals and variation in baseline T_{pref} among individuals, we conducted a one-way repeated-measures ANOVA (*stats* package, *aov* function). This analysis tested whether baseline T_{prefs} of individuals varied significantly across days (main effect of day) and whether they varied among individuals (among-individual variance). Using the ANOVA table from this analysis, we calculated repeatability or the variance explained by individual-level behaviour: $r = \frac{MS_W}{MS_W + \left(\frac{MS_W - MS_A}{n}\right)}$, where MS_W is the within-group variance component, and MS_A is the among-groups variance component (Lessells & Boag, 1987).

To test for behavioural fever, we conducted multiple two-factor (treatment and time) repeated-measures linear mixed-effects models with individual treated as a random effect (*lme4* package, *lmer* function) followed by log-likelihood ratio tests to determine significance (*car* package, ANOVA function). For each model, we paired baseline T_{pref} with each post-exposure day T_{pref} (time; one model for each post-exposure day) and looked for an interaction between treatment (ranavirus-exposed or sham-exposed) and time on the z-score of ΔT_{pref}. Change in T_{pref} (ΔT_{pref}) is calculated as:

$$\Delta T_{pref} = T_{pref_{i,j}} - \overline{T_{pref_{baseline,i,j}}}$$

where $T_{pref_{i,j}}$ is the temperature preference for individual *i* at time point *j*, and $\overline{T_{pref_{baseline,i,j}}}$ is the mean temperature preference of all baseline time points for individual *i*. Before statistical analysis, we transformed our data to standardized deviations away from the mean of the ΔT_{pref} of all individuals in a day. This ensured that temperature preferences were independent of any change in room temperature over time and that we were comparing T_{prefs} between treatments within rather than across days. A significant interaction between treatment and time would mean that the two treatments behaved differently after ranaviral or sham exposure.

To test whether individual-level T_{pref} affects ranaviral load (log-transformed ranaviral DNA copies divided by mass of the individual), we conducted multiple linear regressions (*stats* package, *glm* function; normal error distribution). Each analysis tested for an effect of one of three metrics for body temperature on ranaviral load during a specified time interval. Our three metrics for body temperature were as follows: (a) ΔT_{pref} ($\Delta T_{pref} = T_{pref_{i,j}} - \overline{T_{pref_{baseline,i,j}}}$), (b) mean T_{pref} (mean of the four body temperature measurements per day) and (c) maximum T_{pref} (maximum of the four body temperature measurements per day). We conducted an analysis for each of the first 4 days after exposure (one model per independent variable, per day; 12 total models) and pooled across all 4 days (one model per independent variable; three total models). Additionally, we tested for an effect of baseline T_{pref} on ranaviral load to check for underlying differences in susceptibility that happened to be correlated with baseline T_{pref}.

3 | RESULTS

There was no mortality during either block of this experiment. Before ranaviral exposure, we were able to detect consistency in the baseline T_{pref} of individuals (repeatability: $r > .98$; Figure S2) and variation in baseline temperature preferences among individuals (all blocks and treatments combined; main effect of individual on baseline T_{pref}: $F_{52,1537} = 7.60$, $p < 2.0 \times 10^{-16}$). *Anaxyrus terrestris* metamorphs and adults did not have significantly different mean T_{prefs} ($F_{1,51} = 3.90$, $p = .54$), and together, their mean T_{pref} was 23.07°C ± 0.66 *SE*. For both temporal blocks, we found evidence of behavioural fever after ranaviral exposure (effect of the interaction between treatment and time; metamorphs: day 1: $\chi^2 = 22.0$, $p < .001$ and day 3: $\chi^2 = 5.70$, $p = .017$; adults: day 2: $\chi^2 = 5.51$, $p = .019$; Figure 1 and Table S1). One day after exposure, metamorphs exposed to ranavirus increased their preferred temperature by 3.52°C ± 0.78 *SE* relative to controls (Figure 1a). Two days after exposure, adults exposed to ranavirus significantly increased their preferred temperature by 1.43°C ± 0.59 *SE* relative to controls (Figure 1b). We did not test whether the magnitude of behavioural fever between metamorphs and adults was different, because the two life stages were not tested simultaneously.

For metamorphs, we were unable to detect ranavirus in sampled toads 2 weeks after exposure. For adults, we intentionally sampled after 4 days rather than 14 days in the hopes that we would detect ranavirus before it was cleared. We detected ranavirus in 92.9% of the adult toads. We found that ΔT_{pref} ($t_{11} = -4.89$, $p < .001$; Figure 2b), mean T_{pref} ($t_{11} = -3.11$, $p = .01$; Figure S3) and max T_{pref} ($t_{11} = -3.1$, $p = .004$; Figure S4) 2 days after exposure were all associated negatively with ranaviral load 4 days after exposure. Additionally, ΔT_{prefs} on day 1, 3 and 4 post-exposure were also significant negative predictors of ranaviral load on day 4 ($t_{11} = -3.19$, $p = .009$; $t_{11} = -2.42$, $p = .03$; and $t_{11} = -3.78$, $p = .003$, respectively; Figure 2). Finally, we found a positive effect of mean ΔT_{pref} ($t_{11} = -4.77$, $p < .001$) and mean T_{pref} ($t_{11} = -2.42$, $p = .03$) on ranaviral load but no effect of overall max T_{pref} ($t_{11} = -1.23$, $p = .25$), and baseline T_{pref} ($t_{11} = 1.04$, $p = .32$) on ranaviral load.

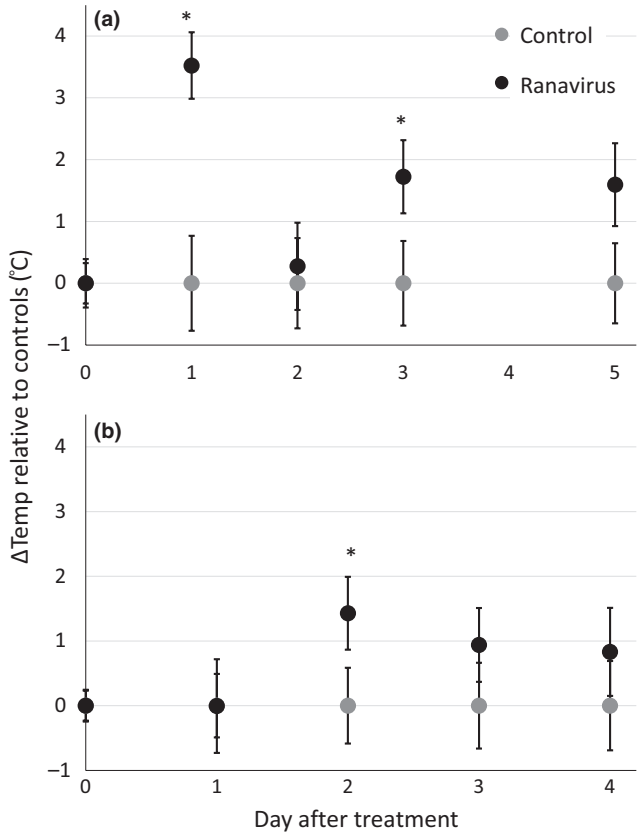

FIGURE 1 Difference between the daily mean for each treatment group and sham-exposed (control) treatments ($\Delta T_{pref,i,j} - \overline{\Delta T_{pref,control,j}}$) in change in individual-level temperature preference ($\Delta T_{pref} = T_{pref_{i,j}} - \overline{T_{pref_{baseline,i,j}}}$) through time for (a) metamorphic and (b) adult *Anaxyrus terrestris* toads, where $T_{pref_{i,j}}$ is the temperature preference for individual *i* at time point *j*, and $\overline{\Delta T_{pref,control,j}}$ is the mean temperature preference of all control animals from time point *j*. Asterisks denote time points where the exposed and sham-exposed groups differ significantly ($p < .05$); error bars represent ± 1 *SE*

4 | DISCUSSION

We set out to determine whether *A. terrestris* responded to ranaviral exposure with behavioural fever and whether fever facilitated ranaviral resistance by limiting pathogen loads. By measuring the thermal preference of individuals in thermal gradients both before and after exposure, we found that *A. terrestris* individuals responded to ranaviral exposure with behavioural fever. We also demonstrated that individual-level change in T_{pref} during the first 2 days after exposure was the greatest predictor of ranaviral load in adult toads. Individuals that increased their T_{pref} the most had the lowest ranaviral loads. These results suggest that behavioural fever appears to be effective at resisting ranaviral infections.

We demonstrated that variation in baseline T_{pref} among individuals before ranaviral exposure was greater than the variation in baseline T_{pref} within individuals (Figure S2). In other words, individuals showed consistency in their preferred temperature through time and individual toads exhibited different preferred temperatures. However, once exposed to ranavirus, we found that individuals moved from their baseline T_{pref} to warmer locations. For *A. terrestris* metamorphs, this behavioural fever response peaked 1 day after exposure, while for adults, it peaked 2 days after exposure (Figure 1). These differences might be partly because of large differences in body size. As both blocks were exposed to the same dose, the dose per g was higher in the metamorphic than adult block. Additionally, given that there is a negative relationship between body size and metabolic rates and processes, immune and fever responses to ranavirus might have been triggered more quickly in the smaller-bodied metamorphs than adults (Garner, Rowcliffe, Rowcliffe, & Fisher, 2011; Rohr et al., 2018).

We did not sample metamorphic *A. terrestris* for ranavirus until 14 days after exposure, and by that time, no individuals were infected. Thus, we do not discuss the effects of thermoregulatory

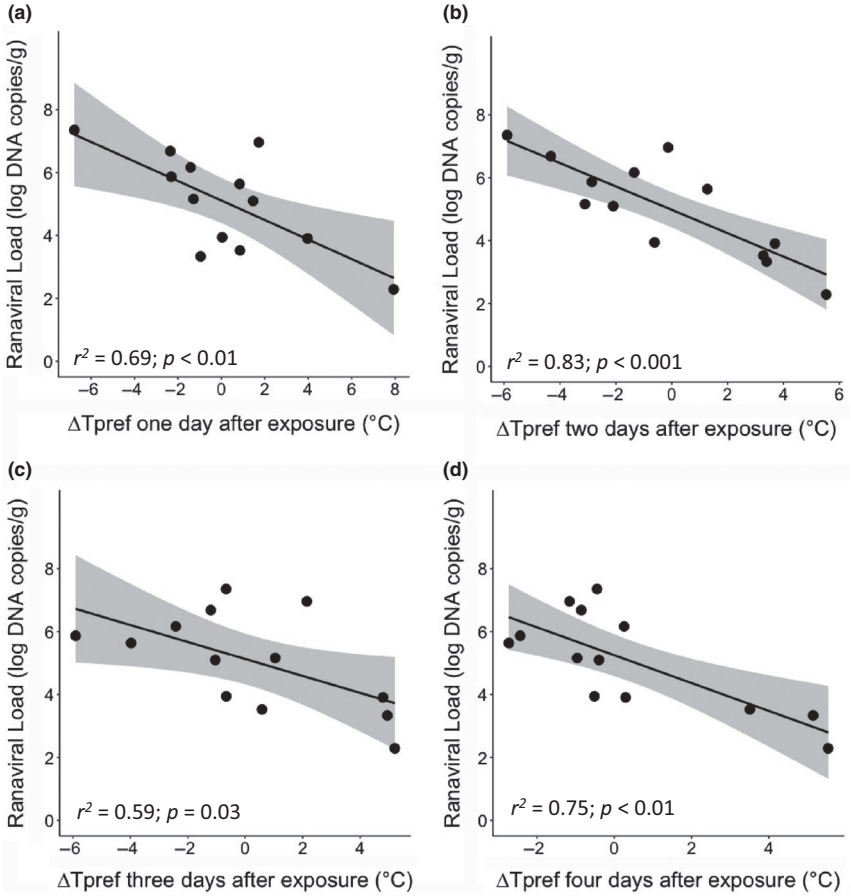

FIGURE 2 Relationship between change in individual-level temperature preference ($\Delta T_{pref} = T_{pref_{i,j}} - \overline{T_{pref_{baseline,i}}}$) (a) one, (b) two, (c) three and (d) four days after ranaviral exposure and ranaviral loads on adult *Anaxyrus terrestris*, where $T_{pref_{i,j}}$ is the temperature preference for individual i at time point j, and $\overline{\Delta T_{pref,baseline,i}}$ is the mean temperature preference of individual i prior to ranaviral exposure. Frogs that exhibited the greatest increase in T_{pref} had the lowest ranaviral abundance ($t_{11} = -4.89$, $p < .001$). The shaded grey area represents the 95% confidence band

behaviour on ranaviral loads in the metamorphic block. For adult *A. terrestris*, which were sampled for ranavirus 4 days after exposure, there was a significant negative correlation between ranaviral load 4 days post-exposure and ΔT_{pref}, mean T_{pref} and maximum T_{pref} during the second day post-exposure (Figure 2). We also found that ΔT_{pref} was negatively correlated with ranaviral loads across all 4 days post-exposure. Thus, an increase in temperature, regardless of an individual's baseline T_{pref}, helped to reduce ranaviral infection. We did not find the same overall effect of mean T_{pref} or maximum T_{pref} on ranaviral load; in fact, maximum T_{pref} was the worst predictor of ranaviral load of the three measurements (ΔT_{pref}, mean T_{pref} and maximum T_{pref}). This result supports the hypothesis that the main purpose of fever is to increase the immune system's efficiency by raising body temperature to promote both innate immunity and adaptive immunity (Boltana et al., 2018; Evans et al., 2015; Rakus, Ronsmans, Ronsmans, & Vanderplasschen, 2017), not to maximize absolute preferred temperature within the range that a host can tolerate. Though fever or increased temperature can slow or stop pathogen growth directly for some host–pathogen systems (Anderson, Blanford, Blanford, Jenkins, & Thomas, 2013; Sauer et al., 2018), any increase in body temperature from baseline T_{pref} should benefit the host. Behavioural fever should be beneficial to the host even if the pathogen tends to grow better in warmer temperatures, as is the case for ranaviruses and many other viral and bacterial pathogens, to which fever is a common method of resistance (Evans et al., 2015), assuming the temperature increase falls within the bounds of the host's thermal performance breadth (Cohen et al., 2017; Evans et al., 2015; Sauer et al., 2018). For example, some ectothermic host species that are adapted to cooler climates cannot tolerate temperature increases when infected with ranavirus (Bayley, Hill, Hill, & Feist, 2013; Brand et al., 2016) and other pathogens (Cohen, Civitello, Venesky, McMahon, & Rohr, 2018; Thomas & Blanford, 2003).

While we do not have any load data for metamorphic *A. terrestris*, the ranaviral load results from the adults suggests that behavioural fever increased *A. terrestris* resistance to ranavirus. Interestingly, previous studies have shown that ranavirus grows in vitro and in vivo up to temperatures even higher than those reached here by feverish *A. terrestris* (Ariel et al., 2009; Chinchar, 2002). Thus, the decrease in ranaviral load associated with behavioural fever might be the result of a host-mediated mechanism and not a direct effect of increased temperature. Behavioural fever might be one way that ectothermic hosts resist ranaviral infections, but it is unclear how widespread that response is given the paucity of studies examining behavioural fever as a mechanism for resistance to ranaviruses (Parris et al., 2004), especially if the fairly heat-tolerant *A. terrestris* exhibits thermal regulatory behaviour that is not representative of more cold-adapted species (Sauer et al., 2018). Nevertheless, assuming that behavioural fever is, in part, used as a method of improving immune efficiency and not simply as a method of heat-killing the pathogen, this strategy

could be employed more broadly by ectothermic hosts than a strategy of simply reaching an absolute temperature to heat-kill ranavirus. That is, ectothermic hosts with critical thermal maxima lower than that of the pathogen should still benefit from behavioural fever as method to improve immunological resistance, assuming the appropriate temperatures are available and the host does not have a very small thermal safety margin. However, there are likely costs of behavioural fever that might be balanced against its benefits (Lochmiller & Deerenberg, 2000; Sheldon & Verhulst, 1996). For example, behavioural fever can increase predation risk in ectothermic hosts if it requires hosts to increase activity or leave refugia (Bundey et al., 2003; Han et al., 2008; Otti, Gantenbein-Ritter, Jacot, & Brinkhof, 2012; Parris et al., 2004; Todd, Jodrey, Jodrey, & Stahlschmidt, 2016).

In summary, both metamorphic and adult *A. terrestris* responded to ranaviral exposure with behavioural fever. Additionally, we found that adult *A. terrestris* were successful at reducing their viral loads by increasing their body temperature after exposure. Hence, for *A. terrestris*, behavioural fever is a successful method of ranaviral resistance, a host strategy for limiting or inhibiting infection (Roy & Kirchner, 2000). Our results support the idea that behavioural fever is primarily used as a method of improving immunological resistance rather than simply damaging pathogens with heat (Evans et al., 2015). Thus, behavioural fever could be an effective mechanism of resistance used by ectothermic hosts that are less heat-tolerant than the infecting pathogen. More experimental work is needed to determine how widespread the use of behavioural fever as a method of pathogen resistance is in ectothermic vertebrates. Understanding how ectothermic hosts rapidly respond to ranaviruses and other emerging pathogens and how changes to environmental temperature affect these host–parasite interactions is crucial given that ectotherms are increasingly experiencing population declines and die-offs due, in part, to increases in emerging diseases that often appear to be exasperated by abnormal temperatures (Grayfer, Edholm, Edholm, Andino, Chinchar, & Robert, 2015; Harvell, Altizer, Altizer, Cattadori, Harrington, & Weil, 2009; Raffel et al., 2013).

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AUTHORS' CONTRIBUTIONS

E.L.S., J.T.H. and J.R.R. conceived ideas and designed experiments; J.T.H. provided the ranavirus isolate; E.L.S. and N.T. oversaw experiments; E.L.S. and J.R.R. conducted statistical analyses; and E.L.S. and J.R.R. wrote the paper with comments and edits from N.T. and J.T.H.

ETHICAL APPROVAL

Anaxyrus terrestris were collected under permit with the Florida Fish and Wildlife Conservation Commission. Experimental methods were approved by the University of South Florida International Animal Care and Use Committees (W IS00000548).

DATA AVAILABILITY STATEMENT

The data supporting the results are archived in the Dryad Digital Repository: https://doi.org/10.5061/dryad.gc6p546 (Sauer, Trejo, Hoverman, & Rohr, 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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