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LETTER

Experimental warming drives a seasonal shift in the timing of host-parasite dynamics with consequences for disease risk

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Abstract

Multi-species experiments are critical for identifying the mechanisms through which climate change influences population dynamics and community interactions within ecological systems, including infectious diseases. Using a host–parasite system involving freshwater snails, amphibians and trematode parasites, we conducted a year-long, outdoor experiment to evaluate how warming affected net parasite production, the timing of infection and the resultant pathology. Warming of 3 °C caused snail intermediate hosts to release parasites 9 months earlier and increased infected snail mortality by fourfold, leading to decreased overlap between amphibians and parasites. As a result, warming halved amphibian infection loads and reduced pathology by 67%, despite comparable total parasite production across temperature treatments. These results demonstrate that climate–disease theory should be expanded to account for predicted changes in host and parasite phenology, which may often be more important than changes in total parasite output for predicting climate-driven changes in disease risk.

Keywords

Amphibians, climate change, community interactions, disease risk, malformations, mismatch, pathology, phenology, *Ribeiroia ondatrae*, seasonality.

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INTRODUCTION

Climate change is altering the population dynamics and interactions of species in multiple ways, making it challenging to predict the net consequences for complex interaction networks such as infectious disease systems. Recent controversy over the net influence of climate change for disease has highlighted some of the contrasting physiological and community-level mechanisms through which climate and temperature can influence disease risk (Lafferty 2009; Rohr *et al*. 2011; Altizer *et al*. 2013; Paull & Johnson 2013). For instance, temperature increases tend to accelerate not only development and production rates of parasite populations but also their mortality (Lafferty 2009; Paull *et al*. 2012). Similar debate has emerged over the net effect of climate change for community interactions that influence parasite transmission. For instance, warming could elevate disease risk by facilitating novel host–parasite interactions through changes in species ranges or migration patterns (Dobson 2009; Harvell *et al*. 2009; Pascual & Bouma 2009). Alternatively, however, shifts in climate could also dampen infection by disrupting host–parasite interactions if the range shifts of host, parasite or vector do not change concordantly (Lafferty 2009; Randolph 2009). Identifying the population and community-level effects likely to dominate climate-driven changes in disease systems is the next key step in improving climate–disease theory and predictions.

Experiments that explicitly manipulate climate variables can be used to formally test mechanisms suggested by correlational studies, and to develop relevant process-based predictions. For instance, one reason why species differ in their sensitivity to warming is because some rely on climatic cues for the timing of seasonal events such as migration or breeding, whereas others rely on climate insensitive triggers such as photoperiod (Winder & Schindler 2004). While several field studies have suggested this could lead to asynchrony between interacting species (e.g. Winder & Schindler 2004; Both *et al*. 2006), experimental tests of the potential for phenological shifts to drive mismatched interactions remain rare. In an important exception, Berger *et al*. (2010) independently manipulated warming and stratification depth (e.g. light) in aquatic mesocosms, and showed that higher light conditions accelerated both the phytoplankton bloom as well as growth of the primary grazers (*Daphnia hyalina*), such that a mismatch did not occur.

The multiple interacting species and seasonality inherent to disease systems make them one of the most complex and challenging ecological networks in which to conduct semi-natural climate change experiments. Nonetheless, the conservation and public health implications increase the urgency of gaining a mechanistic understanding of climate-driven changes in these systems. Parasites often infect multiple host species, and transmission can be affected by co-occurring members of the community (e.g. Woolhouse *et al*. 2001; Pedersen *et al*. 2005; de Roode *et al*. 2011), suggesting that experiments focused on single-host species may overlook community interactions that are crucial to the transmission process. Because host species vary in temperature sensitivity and competence for infection (Bowden *et al*. 2007; Angilletta 2009), warming could shift the relative importance of hosts to the transmission process. Furthermore, hosts and parasites frequently exhibit seasonal population fluctuations, which can affect the timing, intensity and resultant disease spread associated with infection (Altizer *et al*. 2006). If host phenology advances to a lesser degree than parasite phenology, climate changes could also cause

potential mismatches in the timing of host–parasite interactions (Yang & Rudolf 2010; Paull & Johnson 2013), such that hosts either become infected at more vulnerable stages of development or avoid infection altogether. Collectively, these divergent possible outcomes emphasise the importance of experimental designs that incorporate the community context and seasonality of parasite transmission to understand net effects of climate change on disease risk.

Here, we present results from a year-long outdoor mesocosm experiment designed to mechanistically test the net effects of forecasted temperature increases on host infection and pathology. By including multiple host species of the virulent parasite *Ribeiroia ondatrae*, and maintaining natural diel and seasonal temperature fluctuations, we evaluated how temperature-driven changes in community dynamics and seasonality influenced both net infection and timing of host–parasite interactions. We tested two competing hypotheses regarding the influence of experimental warming on parasite production. Theoretical and empirical work suggests that warming could either *increase* infection pressure by elevating parasite development rates and output (e.g. Poulin 2006) or *decrease* infection pressure owing to reduced survival of hosts or parasites (e.g. Lafferty 2009). At the community level, we tested the hypothesis that temperature-driven changes in parasite development rates could shift the timing of host–parasite interactions, resulting in either elevated pathology through exposure of hosts at earlier, more vulnerable stages of development, or reduced pathology due to declining temporal overlap between host and parasite (Paull *et al*. 2012). Our primary goal was to extend beyond single-host laboratory studies and develop a more mechanistic understanding of temperature-driven changes in disease dynamics across a larger community, yielding important insights into the potential for changes in the timing of host–parasite interactions.

METHODS

Experimental Set-up

We conducted an outdoor experiment on the east campus of the University of Colorado, Boulder, in 379 L mesocosms with a 2 × 3 factorial design, in which we manipulated water temperature (heated or ambient) and exposure to eggs of the parasite *Ribeiroia ondatrae* (none, low or high doses) to assess how temperature changes within the range predicted by climate models influenced infection dynamics and pathology. This parasite is passed from eggs released in the faeces of definitive hosts (birds or mammals) into snails, which subsequently produce free-swimming cercariae that infect amphibian or fish hosts (Johnson *et al*. 2004). *Ribeiroia* can infect tadpoles after they have hatched from eggs, and the risk of pathology (e.g. malformations or mortality) as a result of infection tends to decline in tadpoles at older developmental stages (Johnson *et al*. 2011). In each mesocosm, we included *Helisoma trivolvis* snails and two amphibian species, *Lithobates catesbeianus* (bullfrogs) and *Pseudacris triserata* (chorus frogs). Treatments were assigned randomly and replicated six times for a total of 36 mesocosms. To preserve natural diel temperature variability and help maintain heating effects through winter, we buried all mesocosms 60 cm into the ground for insulation. We designed and built greenhouse lids that used solar irradiation to warm mesocosms at the surface, maintaining natural thermoclines and temperature variability (Netten *et al*. 2008; Fig. 1a, see Appendix S1). We suspended Hobo data loggers (Onset Computer Corp., Bourne, MA, USA) 10 cm from the bottom of each mesocosm to measure water temperature every 3 h. Greenhouse lids generated a 3 °C water temperature difference relative to unheated treatments when comparing either over the full year ($F_{1,34}$ = 1174, P < 0.01; Fig. 1b) or just spring and fall temperatures (i.e. excluding winter). This selective temperature difference offered a conservative approximation of trends for North American water temperatures in the next 100 years, which could rise by 5 °C if carbon dioxide emissions double (Fang & Stefan 2009). The standard deviation of water temperature was 17% higher in warmed treatments ($F_{1,34}$ = 337.9, P < 0.01), in agreement with predictions for increased climate variability and extreme high-temperature events with climate change (Thompson *et al*. 2013). For the unheated mesocosms, we used lids made of 60% shade cloth, which allowed entry of a similar amount of light relative to the greenhouse lids (as measured in lumens by Hobo data loggers) ($F_{1,34}$ = 0.07,

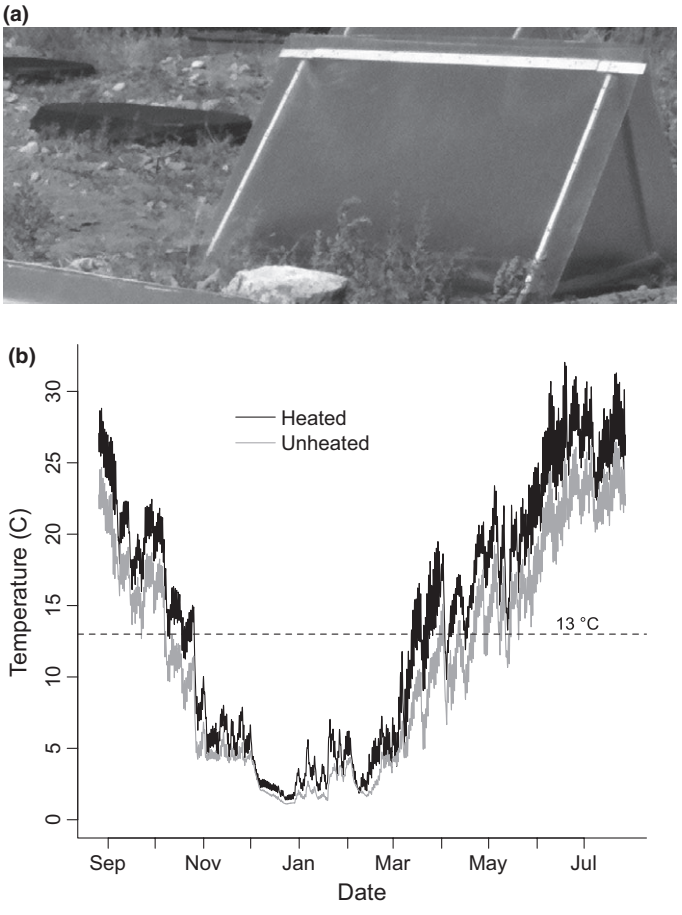

Figure 1 Photo of (a) and seasonal water temperature profile in (b) outdoor mesocosms (379 L) either unmanipulated or heated using greenhouse lids. The dashed line at 13 °C indicates the threshold temperature below which infected snails stop releasing parasites (Paull & Johnson 2011).

$P = 0.79$). Results of a short-term pilot experiment comparing 'sham' heating lids with shade cloth lids also showed no differences between measured abiotic and biotic variables as a function of the lid type, with the exception of some members of the aquatic non-host community (see Appendix S1).

Outdoor mesocosms are particularly good representations of small pond ecosystems because they allow for experimental manipulations of ecological communities with natural changes in season, photoperiod and temperature (Semlitsch & Boone 2010). We sought to maximise the realism of our aquatic communities by adding a sand substrate, nutrients, microbiota and zooplankton from local pond sources (see Appendix S1 for details). Two weeks later, on 6 August 2011, we added 50 field-collected *H. trivolvis* snails to each mesocosm, followed 1 and 2 weeks later by additions of embryonated *R. ondatrae* eggs (~ 14 000 and 28 000 total in the low- and high-exposure dose treatments, respectively) or sham material (unexposed treatment; see Appendix S1). We chose a density of snails within the natural range encountered at California field sites and used parasite egg additions that yielded a moderate-to-high snail infection prevalence based on field data (Johnson *et al.* 2013). We added parasite eggs in August because many of the more susceptible amphibian host species emerge naturally in mid-summer, after which they are consumed by definitive bird hosts, which, following a brief incubation period, transmit the infection back into pond ecosystems. This timing is supported by the observation that among *Ribeiroia*-infected snails observed at over 70 sites in both 2009 and 2010, the percentage of infections that were new (as determined through dissection) rose substantially in July and August relative to earlier in the summer (unpublished data). *Ribeiroia* infection dynamics also tend to be cyclical across years at sites with moderate-to-heavy infection prevalence (unpublished data).

Beginning the following April, we added larvae of two amphibian species to each mesocosm. Because one goal of the study was to determine how seasonal changes in host–parasite dynamics could influence infection risk, we added one 'sentinel' amphibian to each mesocosm (all treatments) every month for a 72 h period as a standardised method to track seasonal changes in amphibian parasite exposure. We used *L. catesbeianus* (Gosner stage 25) as the sentinel species because they suffer little infection-induced pathology (Johnson *et al.* 2012), yet overwinter as larvae. This makes them representative of anurans with long larval periods, in contrast to more seasonal species such as chorus frogs. To determine how seasonal changes in infection risk could influence amphibian pathology, we also added 20 larval *P. triseriata* to each mesocosm (infected and uninfected) in late May. Egg masses of *P. triseriata* were collected from two wetlands in Boulder, Colorado, hatched in the laboratory, and tadpoles were randomly assigned to mesocosms once they were large enough to transport (mean ± SE stage: 27.7 ± 0.21). We used this species because they are moderately susceptible to mortality and malformations following *Ribeiroia* infection (Johnson *et al.* 2012) and are broadly representative of amphibian species with an intermediate larval period.

We elected to add *P. triseriata* to mesocosms in accordance with their local phenology and at the same time to all treatments, rather than artificially accelerating their input within heated mesocosms. This was based on empirical observations that, over the last four decades in Ontario, Canada, the *P. triseriata*/*maniculatus* complex has not begun calling or emerging earlier, despite significant warming (Klaus & Lougheed 2013). This is likely because breeding in this species – and in many amphibians – is co-regulated by precipitation and temperature. Nonetheless, by including larvae of both *P. triseriata* (a seasonal species) and *L. catesbeianus* (the sentinel species), we aimed to assess how patterns of parasite exposure risk would vary over a range of development times. All surviving tadpoles with front limbs were removed daily from mesocosms, measured (snout-vent length and mass) and examined for malformations. The experiment was terminated after all metamorphosing frogs had been removed from the mesocosms because no new snail infections would have been possible without additional input of parasite eggs.

Sampling

We recorded the number of snail egg masses, hatchlings and dead snails (adults only) every 14–21 days throughout the ice-free period of the experiment (no measurements from 18 October 2011 to 30 March 2012). We subsampled counts of egg masses and hatchlings from a plexiglass sheet (20 × 25 cm) on the bottom west side of each mesocosm, and recorded and removed any dead adult snail shells. When night-time temperatures were consistently above the 13 °C threshold for parasite release (Paull & Johnson 2011; Fig. 1b), we checked for mature infections every 3 weeks by placing up to 25 snails individually into 50 mL vials in their mesocosms overnight and recording the proportion of snails that released or 'shed' parasites the following morning (*Ribeiroia* is released from snails only at night). To calculate the number of adult snails with mature infections at each time point, we multiplied the number alive in each mesocosm (determined from the most recent mortality survey) by the percentage of adult snails that shed. At the culmination of the experiment on 14 August 2012, we shed all snails > 5 mm. We also estimated the total number of cercariae produced in each mesocosm over the duration of the experiment using the formula: $\sum_{i=1}^{i} P_i * C_i * A_i$, where P_i is the proportion of adults shedding on day i, C_i is the number of cercariae shed per snail, A_i is the number of live adults and j is the number of days in the experiment. P_i was set to 0 on nights when that mesocosm's temperature went below the 13 °C threshold for shedding, and the daily values for each variable were estimated using values that were collected on the nearest time point to that day (either in the past or future). We also measured phytoplankton fluorescence, periphyton biomass and zooplankton abundance to assess other temperature-driven changes in the aquatic community (see Appendix S1).

Analyses

Our primary analytical approach involved mixed-effects models with mesocosm as a random effect to test for interactive effects of water temperature, exposure dose and, when applicable, time for our response variables. For all analyses in

which the response was measured at more than two time points (number of snail egg masses, hatchling snails, infected adults and bullfrog infections), we plotted the autocorrelation function to test for temporal autocorrelation in the fitted model and determine the appropriate process and lag. When present, we incorporated an autoregressive term of the order indicated by the plots. Quadratic terms for time were also added to the models for adult snail and bullfrog infections because we anticipated unimodal effects of time on these variables. Depending on the distribution of the response variable, we used either linear mixed-effects models (Gaussian distribution using the nlme package, Pinheiro *et al*. 2013) or generalised linear mixed-effects models (binomial distribution glmms using the lme4 package, Bates *et al*. 2013, or negative binomial distribution glmms using the MASS package, Venables & Ripley 2002). We also used a parametric survival analysis with a Weibull distribution (survival package, Therneau 2013, and survreg function) to test for interactive effects of water temperature and exposure dose on snail mortality, again including mesocosm as a random effect. To test for an overall effect of temperature on net bullfrog infection intensities and parasite production, we used an ANOVA. We used a *t*-test to test for differences in mean and standard deviation of water temperature and light levels between temperature treatments. For these tests, variables were transformed when needed to meet assumptions. The mean light measurement was calculated for only the first week of data because surface algal growth blocked light from the suspended data loggers as the experiment progressed. We excluded one mesocosm in the high-exposure unheated treatment from all analyses because all the live organisms in it died over the winter for unknown reasons. For all analyses, we removed non-significant interactions from models before reporting results, and we report only variables with significant effects (Zuur *et al*. 2009). We performed all analyses in R 2.14.1 (R Development Core Team 2012).

RESULTS

Snail population dynamics

Snail mortality increased more quickly within parasite-exposed mesocosms that were also heated compared with either the unheated or unexposed mesocosms (temperature-by-exposure interaction: $X^2 = 751.35$, d.f. = 2, $P < 0.01$). By the end of winter, four times as many snails had died in the exposed + heated mesocosms relative to other treatments, ultimately leading to strong differences in the seasonality of infection dynamics between temperature treatments (Fig. 2a, Fig. S1). Temperature, exposure dose and time all interacted to influence snail reproduction, such that total snail eggs increased through time in unexposed treatments, but the increase was sharper in unheated relative to heated mesocosms (temperature-by-exposure-by-time interaction: $t = -2.60$, d.f. = 449, $P = 0.01$; Appendix Fig. S2). Unexposed snails produced ~ 2.5 times more eggs than snails from parasite-exposed mesocosms (high and low treatments combined). Temperature also negatively influenced the number of hatchlings found in mesocosms ($t = -2.97$, d.f. = 32, $P = 0.01$). Experimental warming decreased both snail egg production and snail hatchlings by 62% and 61%, respectively, relative to unheated mesocosms.

Snail infection dynamics

Higher temperatures dramatically accelerated parasite development within snails; snails in heated mesocosms began releasing parasites just 43 days after exposure, which was 9 months earlier than snails in unheated mesocosms began releasing parasites (Fig. 2b, time-by-temperature interaction: $t = -6.57$, d.f. = 89, $P < 0.01$). The number of adult snails with mature infections was highest in late fall and early spring in the heated mesocosms, whereas it was highest later in the spring and summer in the unheated mesocosms (time^2: $t = -3.63$, d.f. = 89, $P < 0.01$, time: $t = 4.93$, d.f. = 89, $P < 0.01$). Among snails that hatched within mesocosms (i.e. second-generation individuals), temperature positively influenced infection prevalence (Fig. 3, $Z = -3.01$, $P < 0.01$). While a mean of only 4% of hatchlings shed cercariae in unheated mesocosms, 36% shed in the heated mesocosms by the end of the experiment. Despite this pattern, there were more total snails shedding in unheated relative to heated mesocosms due to the greater number of adult snails remaining alive in that treatment at the end of the experiment, and the low number of infected hatchlings (Appendix Fig. S3). Neither the temperature nor exposure dose treatments influenced the number of cercariae that were shed per infected snail (temperature: $t = 0.88$, $P = 0.38$; exposure: $t = 0.19$, $P = 0.85$; Appendix Fig. S4). There was a trend for the number of cercariae released by each snail to increase over the season ($t = 1.86$, d.f. = 364, $P = 0.06$).

The combined effects of an earlier peak in snail infections, yet higher snail mortality within heated mesocosms yielded no net difference in the estimated total numbers of cercariae produced in heated compared with unheated mesocosms over the year-long experiment ($t = -0.957$, d.f. = 21, $P = 0.35$). Simply stated, the increase in mortality approximately offset the increase in parasite development rate. Based on examination of parasite release patterns from adult snails, an estimated mean ± SE of 122 711 ± 22 267 cercariae was produced per mesocosm over the duration of the experiment in unheated mesocosms, compared to 102 296 ± 26 896 in heated mesocosms. Changes in the mean monthly sum of parasites released by adult snails broadly mirrored the seasonal infection patterns observed for sentinel amphibians (Fig. S5, Fig. 2c).

Amphibian infection and pathology

Following the trends in snail infection dynamics, *Ribeiroia* infection within sentinel bullfrogs was also non-linear and peaked 2 months earlier in the heated mesocosms relative to unheated mesocosms (Fig. 2c, time-by-temperature: $t = -8.51$, d.f. = 137, $P < 0.01$, time^2: $t = -9.58$, d.f. = 137, $P < 0.01$, time: $t = 12.58$, d.f. = 137, $P < 0.01$). When bullfrog infection loads were summed across time points to compare total amphibian infection (as distinct from net parasite production by snails described above), bullfrogs in the unheated mesocosms had over twice as many total parasites as those in heated mesocosms ($F_{1,20} = 8.85$, $P = 0.01$). Corre-

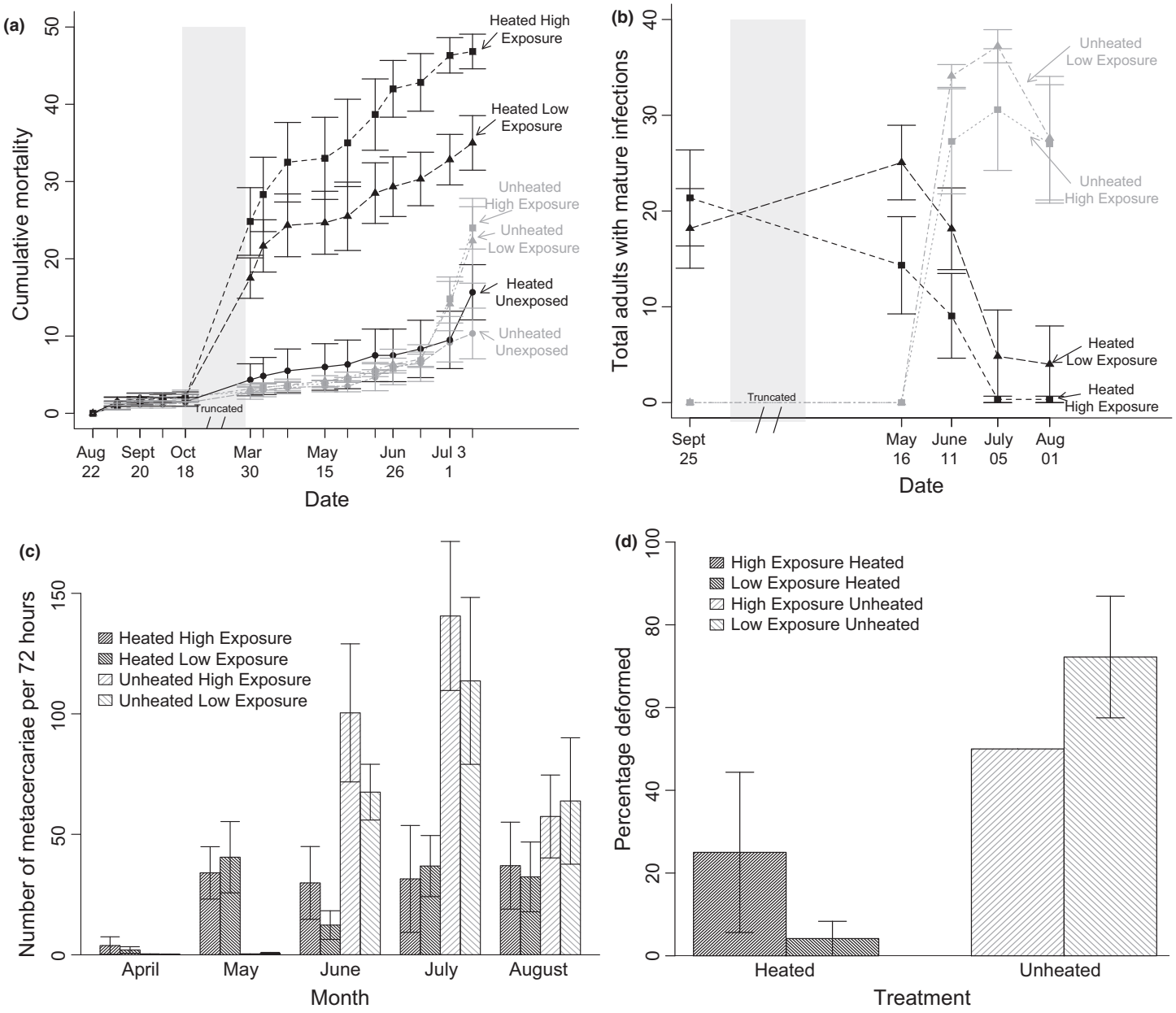

Figure 2 Seasonal changes in mortality and *R. ondatrae* infection dynamics within first intermediate adult snail hosts help to explain patterns of infection and pathology in second intermediate amphibian hosts. Mean ± SE (a) mortality of adult snail (*H. trivolvis*) hosts and (b) total number of adult snails actively releasing parasites over time. These influence (c) infection dynamics in sentinel amphibian hosts (*L. catesbeianus*) and (d) pathology in the amphibian *P. triseriata*. The grey area in panels a and b represents the winter time during which mesocosms were not sampled. There is no error bar for the high-exposure heated treatment in panel d because all values were the same in this treatment. Note that *L. catesbeianus* were also added to the control (unexposed) mesocosms, and confirmed to be free of *Ribeiroia* infections.

spondingly, temperature, but not exposure dose, had a strongly negative effect on the percentage of *P. triseriata* tadpoles that emerged deformed (Fig. 2d, $Z = -2.99$, $P < 0.01$), with an overall mean of 16% of emerging frogs deformed in the heated treatment compared to 63% in the unheated. However, there was no effect of either temperature or exposure dose on *P. triseriata* survival (exposure: $Z = 0.76$, $P = 0.44$, temperature: $Z = 1.2$, $P < 0.23$), length (exposure: $t = -0.12$, d.f. = 19, $P = 0.90$, temperature: $t = 1.20$, d.f. = 19, $P = 0.25$) or mass (exposure: $t = -0.11$, d.f. = 19, $P = 0.91$, temperature: $t = 0.57$, d.f. = 19, $P = 0.58$) at metamorphosis.

DISCUSSION

Experiments that manipulate climate variables in complex communities can provide key insights into the net effects of climate-driven changes and push forward our capacity for prediction and theory development (e.g. Hansson *et al.* 2013). Despite growing interest in climate–disease linkages, semi-nat-

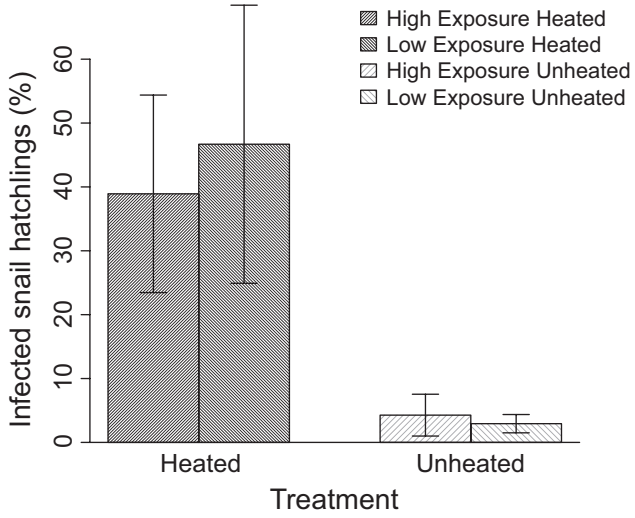

Figure 3 Differences in the percentage of hatchling snails (those that were > 5 mm and not marked as being from the original cohort of snail additions) that were infected with *Ribeiroia* at the end of the experiment as a function of whether mesocosms were heated or unheated and exposed to low or high dosages of parasite eggs. Error bars are ± SE.

ural experiments involving multi-host communities and multiple seasons remain rare. Here, we used an outdoor experiment to test (1) whether warming would lead to a net increase or decrease in parasite abundance, and (2) whether temperature-driven changes in the timing of amphibian infection would elevate or reduce pathology. Warming involving ~ 3 °C temperature increase led to a dramatic acceleration in parasite development: Infected snails in heated mesocosms released parasites 9 months earlier than in unheated mesocosms. However, warming also intensified pathology within snail hosts, reducing snail reproduction through parasitic castration (Paull & Johnson 2011) and increasing overwinter mortality in infected snails. As a result of these conflicting effects, the primary influence of warming was not on total parasite production but on snail hosts and their infection dynamics, which resulted in changes in the timing of amphibian infections. Parasites developed faster and snail hosts died so much more quickly in heated mesocosms that the peak in parasite output occurred before many amphibian species deposit eggs. As such, despite a lack of overall difference in the total number of parasites released in heated and unheated mesocosms, amphibian infection and pathology were greater in unheated mesocosms. This suggests that temperature-driven changes in the seasonality of host infection dynamics, rather than changes in the total number of parasites alone, will be crucial for determining how temperature will influence host infection and pathology.

Thus far, most experiments have focused on the short-term effects of warming on aspects of parasite transmission; however, manipulations that show changing dynamics over multiple seasons are crucial to expand our understanding of climate-driven changes in disease dynamics. Many disease systems are characterised by seasonality of transmission that can be driven by host population dynamics or climate (Altizer *et al.* 2006; Grassly & Fraser 2006). Because of this, climate change experiments performed over very short timescales are likely to miss critical seasonal changes in host infection prevalence and parasite abundance. For instance, after the first 2 months of the current experiment, 41% of adult snails in heated mesocosms were releasing parasites, compared to none in unheated treatments; after a year, however, 92% *fewer* adult snails were releasing parasites in heated mesocosms relative to unheated. We would therefore have drawn opposing conclusions about the influence of temperature on *R. ondatrae* and its hosts had we based our assessment on a single time point from early in the study. Comparisons of the influence of temperature on net total parasite production by snails would have yielded similarly misleading conclusions regarding changes in disease risk for the focal amphibian hosts. Thus, predictions of climate-driven changes in disease risk could be inaccurate when based on measurements from a single time point or even estimates of aggregated totals for parasite output over time without considering host dynamics. Rather, the timing of parasite exposure should be considered a fundamental component of disease risk, necessitating the tracking of climate-driven changes in *both* host and parasite dynamics over time.

Climate-driven mismatches in the timing of interspecific interactions have been explored for a variety of predator–prey and plant–pollinator relationships (e.g. Stenseth & Mysterud 2002; Memmott *et al.* 2007), but it has only recently been discussed in the context of host–parasite interactions and disease (Brown & Rohani 2012; Paull *et al.* 2012). For instance, Brown & Rohani (2012) used a theoretical model to show that if migratory ruddy turnstones arrive earlier or later to Delaware Bay as climate change progresses, the percentage infected with Avian Influenza viruses could increase because of greater overlap with peak viral prevalence in other species of resident birds. The timing of host–parasite dynamics can be crucial not only for host infection but also for pathology, given that hosts infected at earlier, more vulnerable stages of development frequently experience greater pathology (Schotthoefer *et al.* 2003; Kelly *et al.* 2010; Patankar *et al.* 2011). Because disease dynamics are inherently seasonal in a large number of systems (Altizer *et al.* 2006; Grassly & Fraser 2006), future climate–disease work needs to include greater recognition of the fundamental importance of climate-driven changes in host and parasite dynamics, phenologies and the timing of host–parasite interactions.

Our results suggest that changes in the timing of host–parasite interactions are likely to be critical drivers of climate-driven changes in host infection and pathology in natural systems. Warmer temperatures altered the timing of infections of both hatchling snails and larval amphibians. Heating accelerated snail reproduction thereby leading to increased infection among newly hatched snails relative to unheated mesocosms, in which snail reproduction was slower. Ultimately, the resulting difference in hatchling infection prevalence did not translate into higher amphibian infections or pathology, likely because the total number of hatchlings was low, and the accelerated mortality of snails in the heated infection treatments shortened the time horizon over which they released parasites infectious to amphibians. Despite the absence of any difference in the estimates of net parasite output between treatments, *P. triseriata* in heated mesocosms experienced a reduced prevalence of deformities owing to

changes in the timing of exposure. These results therefore highlight the critical importance of phenological concordance in determining the outcome of host–parasite interactions.

A central question in understanding whether mismatch is likely to occur in nature is whether amphibian hosts will also shift their development in accordance with warming climates. While warming can sharply influence the developmental rate of amphibian larvae (Paull *et al.* 2012), several lines of evidence suggest that temperature increases are less likely to accelerate the timing of amphibian breeding to the same extent as parasites. For many wetland breeding amphibians, the timing of egg deposition is co-regulated by both temperature and precipitation, such that increases in temperature alone may have less pronounced effects if decoupled from precipitation changes. Indeed, Klaus & Lougheed (2013) found that, over a 40-year period in Ontario, Canada, the *P. triseriata*/*P. maniculatus* species complex did not shift the timing of emergence or calling, despite an increase of nearly 3 °C in spring temperatures over the study period. Changes in precipitation in western North America are predicted to be modest, with slightly increased winter wetness and summer dryness (Christensen *et al.* 2007; Cayan *et al.* 2008), suggesting that amphibians with precipitation-dependent breeding are unlikely to shift their breeding phenology substantially. Importantly, we found that the timing of parasite release from snails and amphibian infections was strongly asynchronous between heated and unheated treatments (Fig. 2b,c), indicating that warming could shift infection timing as well as which amphibian species experience pathology. For amphibians with single-season larval periods, warming will likely lead to increased infection pressure among early-breeding or climate-sensitive species, whereas infection loads in climate insensitive species or those with late-season breeding may be reduced. Because deformed frogs are likely more vulnerable to definitive host predators and often support higher infection loads (Goodman & Johnson 2011), net reductions in the abundance of hosts with parasite-induced deformities may reduce regional spread of the parasite as well.

Understanding the net effects of climate change on this and other host–parasite systems can be further refined by addressing several questions of interest. For instance, one remaining question is how plastic host and parasite phenologies are and how quickly selection will operate upon the timing of host–parasite interactions in these systems. Another important consideration for predictions for this system is how parasite egg inputs in natural systems, which may occur more than once in a season, could influence infection of the next generation of snail hosts with consequences for net parasite output. Specific predictions of climate-driven changes in this system should consider (a) how snail population and infection dynamics are likely to change over multiple generations, (b) how the phenologies of a wider variety of amphibian species are likely to respond and (c) how the behaviour and susceptibility of the definitive bird or mammal hosts may be influenced. The degree to which these factors lead to phenological mismatch between amphibians and parasites will play a dominant role in determining the net effect of climate change on this system.

Changes in species interactions are an important component to consider in climate–disease studies. For instance, Hall *et al.* (2006) showed that when predator vital rates scale more strongly with temperature than those of *Daphnia* hosts, parasitism could ultimately decline with warming temperature due to greater selective predation on heavily infected hosts. Here, we saw an 'infection cascade' in which faster parasite development and higher pathology in infected snail hosts at warmer temperatures led to a reduction in pathology among amphibian hosts. Similar patterns may emerge for other disease systems where several host species share a common vector or where parasites share an intermediate host or highly competent host. Even other types of multi-host parasites may show similar community-wide changes in transmission resulting from climate-driven changes in infection dynamics or pathology within a critical host. Recognition of the importance of climate-driven changes in interspecific interactions is growing (e.g. Hall *et al.* 2006; Gilman *et al.* 2010), and future climate–disease work should focus towards developing theory for understanding how climate change will influence transmission in interacting host communities.

Moving beyond the current climate–disease controversy requires realistic experimental manipulations to identify the important mechanisms for climate-driven changes in disease dynamics, which can then be integrated with modelling and field approaches to develop broader predictive theory. Our work highlights the importance of experimental studies focused on identifying how climate change will influence the seasonality and community interactions that are critical aspects of parasite transmission. Furthermore, our results emphasise that predicted changes in the seasonality of parasites and their hosts, rather than net changes in parasite abundance, should be a main focus of understanding climate-driven changes in disease dynamics. In addition, our study demonstrates that disease dynamics can be linked across multiple host species, and more studies are needed that focus on community-level changes in disease risk resulting from climate change. Future efforts in the field should focus on developing more generalisable predictions for phenological changes based on life history or species traits that could then be applied to particular disease systems.

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STATEMENT OF AUTHORSHIP

SHP and PTJJ designed the study. SHP designed the heating structures and performed the research. SHP wrote the first draft and PTJJ contributed substantially to revisions.

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