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High temperature enhances host pathology in a snail–trematode system: possible consequences of climate change for the emergence of disease

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SUMMARY

1. Disease severity may be altered by the differential responses of hosts and parasites to rising temperatures leading to an increase or reduction in disease. The net effect of climate change on emerging diseases will reflect the effects of temperature on all life history stages of both hosts and parasites.
2. To explore how climate change differentially influences hosts and parasites, we studied the effect of increasing temperatures on different life stages of the multi-host trematode parasite *Ribeiroia ondatrae*, which has been linked to the emerging phenomenon of amphibian limb malformations, and its snail intermediate host *Planorbella trivolvis*. We determined the effects of temperature on the development of *R. ondatrae* eggs and redia larvae and the effects of parasite exposure (exposed and sham-exposed), temperature (13, 20, and 26 °C) and their interaction on snail host vital rates, including growth, mortality and reproduction.
3. *Ribeiroia* eggs developed four times faster at 26 °C than at 17 °C and did not develop at 12 °C. Higher temperatures increased snail growth, egg production and mortality. Infection interacted with temperature to enhance the growth of infected snails while reducing their fecundity at 26 °C. These results suggest that pathology associated with infection is amplified at higher temperatures.
4. The timing of interactions between *R. ondatrae* and *P. trivolvis* may be influenced by their physiological responses to temperature. Temperature-driven increases in the growth of infected snails coupled with the cessation of parasite development at lower temperatures suggest that warming temperatures will change host–parasite dynamics. Taken together, these results indicate that future climate change could alter parasite abundance and pathology by creating a 'phenological mismatch' between snail hosts and parasites, potentially leading to infection of both snail and amphibian hosts in earlier and, in the case of amphibians, more vulnerable stages of development.

Keywords: amphibian deformities, phenological mismatch, *Planorbella trivolvis*, *Ribeiroia ondatrae*, vital rates

Introduction

Anthropogenic environmental changes are frequently cited as primary drivers of disease emergence in human and wildlife populations (Daszak, Cunningham & Hyatt, 2000; Jones *et al.*, 2008). Disturbances of aquatic habitats by eutrophication, urbanisation and invasive species are shifting the dynamics of freshwater diseases (Daszak *et al.*, 2000; Gajadhar & Allen, 2004; Johnson *et al.*, 2007; Okamura *et al.*, 2010;

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Poulin *et al.* 2010). Climate change will likely interact with other forms of global environmental change to further alter pathogen transmission in freshwater habitats (Hakalahti, Karvonen & Valtonen, 2006; Marcogliese, 2008). Evidence already suggests that climate change is influencing the distribution and abundance of freshwater diseases of both medical and conservation significance (Pascual *et al.*, 2006; Atkinson & LaPointe, 2009; Johnson & Paull, 2010). However, the multifaceted interactions between climate change and freshwater environments make predicting the response of freshwater diseases to climate change particularly challenging (Ibelings *et al.* 2011; Paull & Johnson, in press). Climate change will likely lead to both direct (i.e., physiological) and indirect (i.e., interspecific interactions) effects on parasite transmission, some of which may increase disease while others will reduce infection or pathology.

Temperature can act directly on disease by altering the susceptibility of hosts, the virulence of pathogens and the growth rates of both hosts and pathogens, which can in turn influence host pathology and disease emergence (Cairns *et al.*, 2005; Raffel *et al.*, 2006). For instance, Bally & Garrabou (2007) demonstrated that infection with four bacterial pathogens induced complete mortality of corals at temperatures above 24 °C, whereas no disease symptoms were observed at 16 °C. They attributed this result to elevated pathogen virulence and greater host susceptibility at higher temperatures. Increased pathogen growth rates can also lead to population-level changes in host disease incidence. For example, warming temperatures in the cool waters of Finland could cause a crustacean ectoparasite to switch from completing one generation per season to two, potentially leading to higher disease incidence in farmed fish (Hakalahti *et al.*, 2006). These direct effects of temperature on host pathology can be compounded by indirect changes acting on host–parasite interactions.

Growing evidence suggests that climate-driven changes in interspecific interactions may lead to important consequences for host–pathogen relationships and disease emergence (Gilman *et al.*, 2010). Because temperature patterns control growth and reproduction in a variety of organisms (Stenseth & Mysterud, 2002), changes in temperature are likely to influence the rate and timing of development of some species more strongly than others. This asymmetrical response to temperature has led to mismatched interactions between plants and pollinators as well as predators and prey (Both & Visser, 2001; Memmott *et al.*, 2007). For example, over the last four decades in Lake Washington, warming temperatures have caused the peak algal bloom to shift 20 days earlier in the season; however, there has been no corresponding shift in peak densities of *Daphnia*, which depend on the algae for food, likely because *Daphnia* use photoperiod more than temperature as a cue for egg hatching (Winder & Schindler, 2004). Such 'phenological mismatch' in a host–parasite system could affect the pathology associated with infection. Small organisms tend to have faster generation times, stronger growth responses to temperature and wider thermal windows (Portner, 2002), suggesting that climate change could cause pathogens to become abundant more quickly than their hosts during warm seasons. This could lead to more severe infections in immature or immunologically naïve hosts, or alternatively to an absence of susceptible hosts during peak parasite abundance, thereby reducing infections.

Parasites with complex life cycles require multiple host species to reach maturity, making them particularly susceptible to the direct and indirect effects of climate change (Harvell *et al.*, 2002; Marcogliese, 2008; Mas-Coma, Valero & Bargues, 2009). The trematode parasite, *Ribeiroia ondatrae* (Price), has recently been linked to widespread observations of limb deformities in amphibians, including missing or extra limbs as well as skin webbings (Johnson *et al.*, 1999). *Ribeiroia*, which is transmitted sequentially from birds to snails to amphibians via free-living infectious stages, causes amphibian mortality and limb deformities in experimental studies (Johnson *et al.*, 1999, 2004). Analysis of historical accounts of amphibian populations suggests that the frequency, severity and distribution of amphibian deformities have likely increased in recent years (Hoppe, 2000; Johnson *et al.*, 2003; Johnson & Chase, 2004). Although not all malformations are because of *Ribeiroia* infection, a diverse suite of anthropogenic influences including eutrophication, pesticide exposure and introduced fish can exacerbate infection and may have contributed to increases in infection (Johnson *et al.*, 2007, 2010; Rohr *et al.*, 2008). As of yet, however, no studies have examined the potential influence of climate change – historical or forecasted – on interactions between *Ribeiroia* and its hosts, despite laboratory findings indicating that the life span of free-living trematode stages and

development within ectothermic hosts is highly temperature dependent (Fried & Ponder, 2003; Poulin, 2006; Yang *et al.*, 2007). The life cycle of *Ribeiroia* is similar to that of other trematodes causing diseases in humans or wildlife, including schistosomiasis, fascioliasis and cercarial dermatitis. In view of this similarity, the effects of temperature on *R. ondatrae* may illustrate the general consequences of climate change for the epidemiology of trematode diseases and the conservation of hosts.

To explore the potential effects of climate change on host pathology and host–parasite interactions, we experimentally evaluated the effects of temperature on the development of *R. ondatrae* eggs and the pathology associated with infection of snail hosts, *Planorbella trivolvis* (Say). In particular, we sought to mechanistically determine (i) the direct effects of temperature on infection pathology of the snail host and (ii) the potential for indirect effects of temperature on host–parasite interactions resulting from differences in the responses of *R. ondatrae* and *P. trivolvis* to changing temperatures. To this end, we assessed the effects of temperature on host growth, fecundity and mortality, as well as parasite development at multiple stages of the life cycle. Extending studies of the direct effects of temperature on disease to explore their ecological implications for host–parasite interactions is important for understanding the complex responses of hosts and parasites to climate change. Our approach allowed us to compare changes in all aspects of snail pathology, including changes in growth, fecundity and mortality between infected and uninfected hosts. Given the role of *R. ondatrae* in causing deformities and mortality in natural amphibian populations (Johnson *et al.*, 1999), understanding the influence of climate change on the physiology of this pathogen and its interactions with intermediate snail hosts is relevant to its overall effects on amphibian populations.

Methods

Study system

The complex life cycle of *R. ondatrae* involves transmission from bird or mammal definitive hosts to snail intermediate hosts and then to amphibian or fish second intermediate hosts (Johnson *et al.*, 2004). Definitive hosts release parasite eggs into aquatic systems where they hatch into miracidia that live for 12–24 h. Miracidia locate and infect snails in the family Planorbidae, ultimately developing into rediae within the snail over the course of several weeks. At night, mature rediae release mobile cercariae, which have *c.* 1–2 days to locate and encyst within a larval amphibian host, often around the developing limb buds. When larval amphibians are infected early in development, this process can cause developmental abnormalities in amphibians, including extra, missing or deformed limbs (Johnson *et al.*, 1999). *Planorbella trivolvis* (previously *Helisoma trivolvis*) is a pulmonate snail found in fresh waters throughout North America (Russell-Hunter, Browne & Aldridge, 1984). Pulmonate snails are hermaphroditic, although self-fertilisation is rare (Norton & Bronson, 2006). Pulmonate reproductive cycles are typically semelparous; however, there are some cases of iteroparous *P. trivolvis* in mesotrophic habitats (Eversole, 1978; Dillon, 2000). The life span of *P. trivolvis* in wild populations is typically one to two years (Dillon, 2000). Growth in pulmonate snails is indeterminate, with no pre-determined size at maturation, and continues throughout life (Russell-Hunter *et al.*, 1984). These snails serve as one of the main hosts for *R. ondatrae* in the United States (Johnson *et al.*, 2004). Infection of *P. trivolvis* by trematodes leads to reproductive castration and accelerated growth known as gigantism (Lagrue *et al.*, 2007).

Effects of temperature on parasite free-living stages

To obtain eggs of *R. ondatrae*, we exposed five rats to 50 metacercariae removed from laboratory-infected *Lithobates catesbeianus* (Shaw) tadpoles. After two weeks, we collected faeces on wet paper towels below wire rat cages. After filtering the faeces through a series of five sieves (smallest = 47 μm pore size), we placed 12 mL of material (*c.* 11 000 eggs) into 0.5-L glass jars filled with spring water. We covered jars to prevent light exposure, which is a known stimulus for parasite hatching (Johnson *et al.*, 2004). Water baths within temperature-control chambers were used to maintain specific water temperatures. We placed three jars in each temperature bath (12, 17, and 26 °C) and used temperature-control chambers to maintain water baths at the 12 and 17 °C temperature treatments, and 250 Watt heaters (Jager brand) to warm the 26 °C temperature treatment for 30 weeks. Power heads (Rio Mini 50) were placed in each water bath to keep water circulating and at a uniform temperature, and Hobo

underwater dataloggers (Onset Computer Corporation, Bourne, MA, U.S.A.) were placed in one water bath at each temperature to record temperatures every hour during the course of the experiment. We aerated jars continuously to reduce bacterial growth, changed the water weekly and rotated jar locations in the water baths regularly to avoid positional effects.

We sampled 25–30 eggs from each jar twice a week at 200× magnification and characterised the fraction of eggs in each of the following categories: ostensibly viable but without signs of development (stage zero), initial concentration of material towards the centre of the egg (stage one), outline of developing miracidium visible (stage two), developed miracidium with a visible eyespot (stage three), hatched with exit pore visible (stage four) or dead.

Effects of temperature on intra-host development and host pathology

We studied the effects of temperature on *R. ondatrae* intra-snail development and host pathology using a two-by-three factorial experiment with two levels of snail infection (exposed and sham-exposed to *R. ondatrae* eggs) and three temperatures (13, 20 and 26 °C). Snails ranged in shell length from 7.2 to 12.3 mm and came from a laboratory stock of snails originally collected in Minnesota and bred in the laboratory for multiple generations. We randomly assigned 360 snails to each of the six treatments (60 snails per treatment), taking care to divide different size classes (grouped in 1 mm increments) evenly among treatments. To infect the snails, we obtained eggs from the faeces of *R. ondatrae*-infected rats and incubated them at 29 °C. After 17 days of incubation, we quantified the number of *R. ondatrae* mature eggs (with a visible eyespot) per 20 μL subsample. We placed snails from the infected treatment into an 80-L container, exposing them as a group to 100 eggs per snail. Snails from the uninfected treatment were sham-exposed to filtered rat faeces collected from uninfected rats. All snails were held in their exposure tanks for 1 week prior to the start of the experiment.

At the start of the experiment, we marked each snail shell with 'queen bee' tags (Beeworks, Orillia, ON, Canada) for individual identification and placed snails in groups of five within 2.5-L containers (12 containers in each of the six temperature x infection treatments for a total of 72 containers) to ensure reproductive activity since isolated *P. trivolvis* produce few to no eggs (S.H. Paull, pers. observ.). Each 40-L temperature bath held six of these containers (three from each infection treatment), for a total of four replicate temperature baths for each treatment. Temperatures were controlled as described in the egg experiment. We rotated the position of containers and water baths weekly and moved snails among containers to avoid persistent container effects. In addition to the 360 experimental snails described previously, we kept a supplementary 15 'replacement' snails in identical conditions to experimental snails at each temperature and infection treatment to replace any snails that died to maintain equivalent snail densities in each experimental container. Data from replacement snails were only included in the egg analyses because their growth and mortality were not recorded unless they entered the experiment. When all replacement snails had been used, we shifted snails between containers of the same treatment to maintain equal densities. Containers were filled with dechlorinated tap water, which was changed every five days. We fed snails a mixture of lettuce and TetraMin Spirulina flake food ad libitum and changed the water every 5 days.

We recorded snail mortality daily, and snail growth weekly, using digital callipers to measure shell length. At five-day intervals, we counted the number of eggs in each container. Beginning three weeks post-infection, dead snails were dissected to look for evidence of developing infections (i.e. rediae). One month post-exposure, we checked for mature infections in all surviving snails weekly by placing snails individually into 50-mL centrifuge tubes overnight at their respective temperature treatments. The following morning, we removed the snails from their vials and quantified released cercariae.

Temperature range

We used a total temperature range spanning 12–26 °C to reflect both within-season variation in pond temperatures as well as projected changes to lake water temperatures over the next century. The mean spring–summer temperature measured every four hours by a datalogger in 2006 in the Minnesota pond from which these snails were collected was 19 ± 7 °C (SD). Over the course of a full year, the temperature in the pond ranged from 0 to 30 °C. Most projections for changes

in the temperature of small lakes across the United States and Canada (the known range of this parasite) predict a temperature change of between 5 and 10 °C by the year 2100 with lakes at higher latitudes expected to exhibit the greatest degree of change (Sharma *et al.*, 2007; Fang & Stefan, 2009). Thus, we chose temperature intervals that would encompass expected changes owing to climate change between each temperature category while still representing the wide range of seasonal temperatures in ponds where the parasite occurs.

Analyses

We used a repeated-measures ANOVA to test the effect of temperature on *R. ondatrae* egg development over time (mean developmental stage per sampling date). Because we rotated snails randomly among containers and water baths during the course of the experiment, we used fixed effects models for the analyses of snail growth, mortality, fecundity and parasite development. In addition, we ran the analyses with models that included each snail's initial water bath and container identity as random nested factors and the findings were the same (results not shown). The growth (mm day^{-1} per snail) and fecundity (eggs day^{-1} per container) data were also square-root transformed to reduce positive skew and improve homogeneity of variance. The effects of temperature and infection on snail growth were analysed using a factorial two-way ANOVA with infection status and temperature treatment as fixed factors. Because we expected infection to reduce fecundity over time, we used a two-way repeated-measures ANOVA to analyse the influence of infection and temperature on snail egg production. The first four intervals (20 days) of egg data were used in this analysis since mortality in the warmest treatment precluded the use of longer-term data. To analyse the effects of treatment on snail mortality, we used a proportional hazards test with infection status and temperature as fixed factors, and snails that were sacrificed or that did not die during the course of the experiment labelled as 'censored'. We tested the effect of temperature on the time required for infected snails to begin releasing cercariae (patency) using a proportional hazards test with individuals that never shed labelled as 'censored'. We used JMP 8.0 (SAS Institute, Cary, NC, U.S.A.) for all statistical analyses.

To compare the relative influence of temperature on infected and uninfected snail growth rates, we calculated physiological Q_{10} rates using the formula: $\log Q_{10} = \frac{10\log(R_2 - R_1)}{T_2 - T_1}$ (Poulin, 2006). In this formula, R_1 and R_2 are growth rates (mm day^{-1}), at the 20 °C (T_1) and 26 °C (T_2) temperature treatments, respectively. Conceptually, a Q_{10} rate is the relative change in a physiological rate over a ten degree change in temperature and describes the temperature sensitivity of physiological rates. The 13 °C treatment was excluded from the Q_{10} analyses because growth was negligible.

Results

Parasite egg development

Temperature significantly enhanced the rate of *R. ondatrae* egg development (RM-ANOVA, $F_{2,6} = 300.66$, $P < 0.001$). Eggs in the 26 °C treatment began to hatch within 15 days, while eggs in the 17 °C treatment did not begin hatching until 58 days after the experiment began. Eggs incubated at 12 °C never developed beyond stage 0 after 52 days of observation, even after being warmed to the 26 °C treatment for the final 7 days of observation.

Host growth

We found a significant, positive main effect for temperature on host growth (2-way ANOVA, $F_{2,303} = 115.13$, $P < 0.001$). Snails grew over five times faster at 26 °C than at 13 °C (Fig. 1). While there was no main effect for infection ($F_{1,303} = 0.72$, $P = 0.397$), we observed a significant infection-by-temperature interaction, such that warmer temperatures enhanced the growth of infected snails more than uninfected snails ($F_{2,303} = 9.27$, $P < 0.001$). The Q_{10} value for infected snail growth was 4.21 relative to 1.77 for uninfected snails. Among snails maintained at 26 °C, infected snails grew nearly twice as much per day relative to uninfected snails.

Host fecundity

Host egg production within each container increased with time and was significantly enhanced by temperature (RM-ANOVA, time: $F_{3,64} = 9.30$, $P < 0.001$; temperature: $F_{2,66} = 50.113$, $P < 0.001$). Snails in the 26 °C treatment produced, on average, 7.5 times more eggs

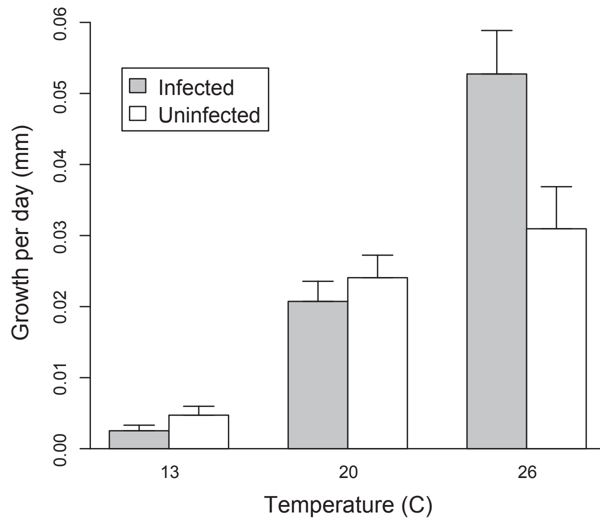

Fig. 1 Influence of temperature and infection by *Ribeiroia ondatrae* on mean growth per day (±1 SE) of snail host (*Planorbella trivolvis*). Snail growth was calculated as the total shell growth for each snail divided by the number of days the snail survived.

per day than snails in the 13 °C treatment. This is reflected in the greater total numbers of eggs per snail per day at the different temperatures (Fig. 2). We found a significant temperature-by-infection interaction ($F_{2,66}$ = 10.40, P < 0.001), such that the fecundity of infected snails was significantly reduced only in the 26 °C treatment ($F_{1,22}$ = 76.72, P < 0.001), but not at 20 °C ($F_{1,22}$ = 1.24, P = 0.277) or 13 °C ($F_{1,22}$ = 1.69, P = 0.207). On average, snails in the uninfected 26 °C treatment produced nearly 3 times more eggs than snails in the infected 26 °C treatment.

Host mortality

Higher temperatures significantly reduced snail survival, whereas infection had no effect on time to death (Proportional Hazards, temperature: χ^2 = 272.56, P < 0.001; infection: χ^2 < 0.01, P = 0.986). The median survival time of snails at 13 °C was 130 days, which was five times longer than for snails at 26 °C (Fig. 3). There was no significant temperature-by-infection interaction on snail survival (temperature-by-infection: χ^2 = 5.41, P = 0.067).

Host and parasite developmental rates

We estimate that at least 95% of exposed snails and 0% of sham-exposed snails became infected. Among snails that died during the experiment, rediae were visible upon dissection between 1 and 3 months after exposure, depending on the temperature treatment. Of the exposed snails that survived to the point that rediae could reliably be detected in each temperature, 95% had visible rediae upon dissection. Exposed snails maintained at 26 °C released cercariae significantly earlier than those at 20 °C (Proportional Hazards: χ^2 = 81.76, P < 0.001). Snails at 26 °C first began releasing cercariae 28 days after the experiment began, compared with 50 days in the 20 °C treatment (Fig. 4). Snails maintained at the 13 °C treatment did not produce cercariae during the entire duration of the 7-month experiment, despite the fact that dissection of these snails revealed pre-patent infections after 3 months (Fig. 4). When snails in the 13 °C treatment failed to produce cercariae after 4 months, we randomly selected 15 infected snails from this treatment and warmed them to 20 °C. Two of these snails survived long enough at this temperature to produce cercariae 22 and 29 days after warming, suggesting that, while *R. ondatrae* infection can develop below 13 °C, snails do not release cercariae until temperatures are warmed past this threshold.

Discussion

The net effect of climate change on infectious disease dynamics depends on the full spectrum of direct and indirect effects of climate on host and pathogen life histories. Importantly, these effects will extend beyond simple changes in host or parasite geographical distributions to include significant shifts in the physiology and temporal interactions between hosts and parasites which could alter disease dynamics in natural populations. By experimentally manipulating temperature and multiple stages in the life cycle of the pathogenic trematode, *R. ondatrae*, we demonstrate that increases in temperature enhance the growth, mortality and development of both hosts and parasites in different ways, leading to elevated pathology in snail hosts. Given the role of *R. ondatrae* in causing amphibian limb deformities, our results have additional implications for amphibian conservation and disease emergence.

Climate change, host pathology and disease emergence

Parasite-induced pathology in snail (*P. trivolvis*) hosts, including castration and accelerated growth, known

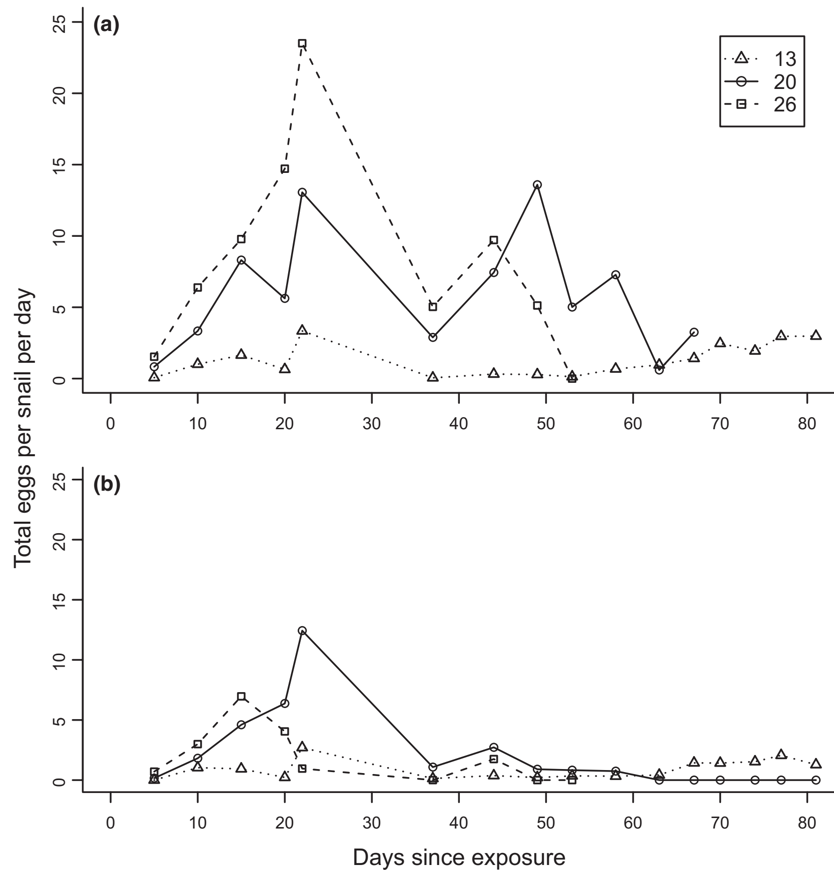

Fig. 2 Total number of eggs produced per snail (*Planorbella trivolvis*) per day in different temperature treatments over time in the (a) uninfected and (b) infected treatments. Eggs are shown for the first 80 days of recording.

as 'gigantism' (Sorensen & Minchella, 2001; Johnson *et al*., 2004), was exacerbated at warmer temperatures. Infected snails in the 26 °C treatment ceased reproduction within 1.5 months, whereas those in the 13 °C treatment continued to reproduce for the duration of the 7-month experiment, regardless of whether they were exposed to parasites. Such temperature-mediated pathology likely occurs through one of two main mechanisms: higher temperatures facilitate parasite production and virulence (Mouritsen & Jensen, 1997; Kocan *et al*., 2009), or temperature suppresses host immune responses (Cairns *et al*., 2005). Our results are consistent with faster development of trematode rediae within snails at higher temperatures, which lead to castration of infected snails before they reached peak egg output (Fig. 2). The energy saved by this reduction in host reproduction can be allocated to snail growth and parasite biomass (Mouritsen & Jensen, 1994), which likely contributed to the larger influence of temperature on the growth rates of infected snails. While the role of snail immunity in these patterns cannot be discounted, immune defenses such as phagocytosis in other mollusks occur efficiently at temperatures ranging from 5 to 37 °C (Prieur *et al*., 1990).

The influence of warmer temperatures on host–parasite interactions between *Planorbella* and *Ribeiroia* populations in natural systems will also depend on the net difference in reproduction and mortality of both the host and the parasite (Harvell *et al*., 2002; Lafferty, 2009). We found that despite temperature-driven increases in snail mortality, total egg production by uninfected snails over the duration of the experiment was still highest in the 26 °C treatment, suggesting that, over the temperature range measured in this study, warmer temperatures could lead to a net increase in uninfected snail populations. Trematode infection could, however, reduce this positive influence of temperature on snail fecundity by castrating snail hosts, consistent with observations that trematode infections frequently have negative effects on snail population densities (Lafferty, 1993; Fredensborg, Mouritsen & Poulin, 2005).

Whether climate change is contributing to the current emergence of amphibian limb deformities remains an open question. Environmental stressors

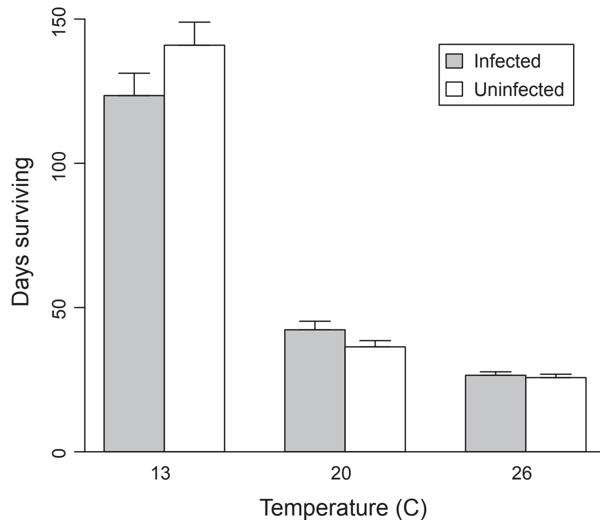

Fig. 3 Influence of temperature and infection by *Ribeiroia ondatrae* on snail host survival. Depicted is the mean number of days (±1 SE) that snails survived as a function of experimental treatment.

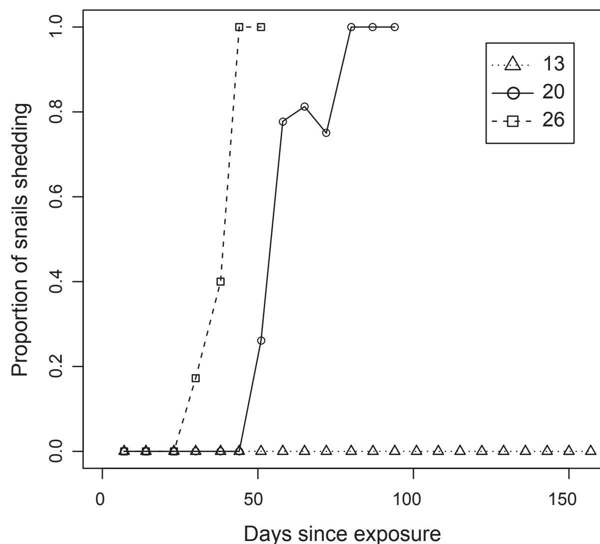

Fig. 4 Proportion of snails releasing cercariae in different temperature treatments. All infected snails died by day 50 in the 26 °C treatment and by day 70 in the 20 °C treatment.

such as pesticides, elevated UV-B radiation and eutrophication occurring alone or in combination with *R. ondatrae* have all been proposed as possible explanations for increased observations of malformed amphibians (Blaustein & Johnson, 2003; Johnson *et al*., 2007; Rohr *et al*., 2008). No studies, however, have yet comprehensively examined the role of climate change in the emergence of amphibian limb deformities. Our experiment demonstrates that warmer temperatures drive faster parasite development, increased growth of infected snails and greater net production of susceptible snail hosts. Further mesocosm and field studies that include the full host community for this complex life cycle parasite will help to determine whether these effects in snail hosts translate into greater pathological effects on amphibians.

Phenological mismatch in host–parasite interactions

Our results indicate that temperature change will differentially affect the development rates of *P. trivolvis* and *R. ondatrae*. For example, the Q_{10} value for the growth rate of infected snails was more than twice that of uninfected snails. This comparatively high growth rate in infected snails at elevated temperatures is likely driven by the accumulation of both snail and parasitic tissue (Sousa, 1983; Probst & Kube, 1999), which allows for greater parasite production. In a review of the experimental literature, Poulin (2006) noted a dramatic effect of temperature on the Q_{10} rate for the release of several species of trematode cercariae from their snail hosts. Similarly, Yang *et al.* (2007) described an increase in the development rate of schistosome rediae up to the experiment's limit at 30 °C. These studies indicate that trematode parasites are highly sensitive to temperature changes and benefit from increasing temperatures up to relatively high thermal limits. The small size of trematodes may allow them to capitalise on increasing temperatures more efficiently than their hosts, although a thorough comparison of the temperature dependence of host and parasite vital rates requires direct measurement of actual metabolic energy use (i.e. oxygen consumption rates).

Our study also demonstrated differences in threshold temperatures for reproduction and development between *P. trivolvis* and *R. ondatrae* that will likely have important consequences for the timing of host–parasite interactions under scenarios of future climate change. In our study, parasite eggs failed to develop below a threshold temperature of <12 °C. Similarly, cercarial production did not occur in snails raised at 13 °C until the temperature was elevated to 20 °C. By contrast, snails continued to grow and produce eggs at all temperatures experienced within the study. Threshold temperatures for the development of

trematode infections within snails have also been observed at 6 °C for rediae of *Philophthalmus rhionica* (Ataev, 1991) and calculated at 15 °C for schistosome sporocysts (Zhou *et al.*, 2008). These results suggest that localised warming trends could cause trematodes to become active earlier in the season as temperatures rise above the threshold for parasite development. This could lead to interactions with hosts in earlier more vulnerable stages of development, particularly if the hosts experience slower, more consistent (non-threshold driven) increases in developmental rates with temperature.

Mismatched timing of host–parasite interactions could alter pathology or infection prevalence depending on the abundance and developmental stage of hosts during parasite activity. *Planorbella trivolvis* live for between 1 and 2 years and exhibit seasonal egg production in spring (Morris & Boag, 1982), suggesting that developing juvenile snails are the dominant age class when trematode eggs begin to hatch. In our study, snails would have grown more prior to *Ribeiroia* egg hatching in the medium temperature treatment compared to snails at 26 °C because of the increased time for growth before egg hatching at the cooler temperature. These results indicate that earlier spring warming owing to climate change may disproportionately increase the hatching rate of *R. ondatrae* eggs relative to snail development, such that snails may be infected at a smaller size. This could alter the dynamics of *R. ondatrae*, since many aspects of trematode infections in snails are influenced by the size of the snail at infection, including both infection success and snail mortality (Kuris, 1980; Theron, Rognon & Pages, 1998).

Such phenological mismatches could persist or even amplify across different stages in the parasite life cycle. The release of trematode cercariae by infected snails is highly sensitive to temperature in some species (Poulin, 2006; Koprivnikar & Poulin, 2009). For *Ribeiroia*, earlier and more pronounced release of cercariae by infected snails could lead to infection of larval amphibians at earlier stages of development when they are most vulnerable to deformities and mortality (Schotthoefer *et al.*, 2003), thereby enhancing pathology. However, if peak infection precedes amphibian breeding, which is jointly controlled by temperature and rainfall (Duellman & Trueb, 1986), temperature shifts could reduce parasite transmission. Thus, temperature-driven mismatches in the phenology of hosts and parasites in this system may have consequences at multiple stages of the life cycle.

Conclusions

Physiological data can provide a useful framework for understanding not only the effects of climate change on individual species ranges (Jeschke & Strayer, 2008) but also for forecasting likely changes in species interactions, including those of hosts and parasites. While the direct influences of climate change on the geographical range and growth rates of pathogens are the subject of increasing study (Kutz *et al.*, 2005; Pascual *et al.*, 2006), less effort has been focused on identifying the indirect changes in pathogen transmission that are likely to result from temperature-mediated shifts in host–parasite interactions. Threshold temperatures, Q_{10} rates and other measures of temperature sensitivity as well as physiological responses such as thermal limits are a promising way forward in the development of a mechanistic understanding of how climate change could indirectly shift disease dynamics. Climate change will almost certainly cause net increases in some diseases and net decreases in others (Lafferty, 2009). Identifying patterns in the temperature sensitivity of groups of pathogens and hosts may offer insight into both the direct and indirect mechanisms for climate-driven changes in disease. Understanding the potential responses of multiple parasite life stages and host physiological responses to temperature will allow more robust inferences about the influence of temperature on parasite dynamics and may identify which portions of their life cycles will be most susceptible to change.

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