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How temperature shifts affect parasite production: testing the roles of thermal stress and acclimation

Sara H. Paull*[,1], Thomas R. Raffel[2], Bryan E. LaFonte[1] and Pieter T. J. Johnson[1]

[1]*Ecology and Evolutionary Biology Department, University of Colorado, Boulder 80309, Colorado, USA; and* [2]*Biology Department, Oakland University, Rochester, Michigan 48309, USA*

Summary

1. Changes in the magnitude and frequency of temperature shifts with climate change will influence species interactions if species have differential acclimation responses. For example, if parasites acclimate to temperature shifts faster than their hosts, as might be expected due to their smaller sizes and faster metabolisms, temperature variability could lead to increased infection. However, this assumption might not hold if benefits of acclimation are counteracted by energetic costs or thermal stress, underscoring the need for empirical efforts to assess how temperature variability will influence host–parasite interactions.

2. We used an array of replicate incubators to test how temperature shifts from five acclimation temperatures (13–25 °C) to five performance temperatures (16–28 °C) influenced release of infective stages by the trematode parasite *Ribeiroia ondatrae* from its snail intermediate host (*Helisoma trivolvis*) at four time-points after a temperature shift.

3. Initially, parasite release was higher at warm temperatures and increased temporarily after infected snails were shifted to higher temperatures, particularly for hosts acclimated to cooler temperatures. However, these effects were transient, such that parasite release at warm temperatures declined steadily over the 7 days following the shift. Warmer temperatures also increased snail mortality.

4. Parasite release was strongly influenced not only by ambient temperature but also by the thermal history of the host. Prior acclimation to warm temperatures reduced parasite release at warm performance temperatures, contrary to the beneficial acclimation hypothesis. Rather, the observed pattern was likely driven by: (i) energetic costs of prolonged exposure to high temperatures ('thermal stress') or (ii) parasites' capacity to 'store' infectious stages at cooler temperatures.

5. The time-dependent nature of thermal effects on parasite performance highlights the importance of considering the amplitude and frequency of temperature variability for understanding future changes to disease dynamics.

Key-words: adaptation, cercariae, climate change, host defence, host–parasite interactions, infection, pseudoreplication, temperature variability, thermal stress, trematode

Introduction

In addition to changing mean temperatures, climate change is predicted to alter temperature variability and the frequency of extreme events, yet only recently have studies begun exploring the physiological and ecological consequences of temperature variability (Easterling 2000; Fischer, Rajczak & Schär 2012; Thompson *et al.* 2013). Because species also differ in their responses to temperature fluctuations, the relative abilities of species to acclimate or adapt to temperature shifts will affect the outcome of interspecific interactions (Stireman *et al.* 2005; Grigaltchik, Ward & Seebacher 2012; Raffel *et al.* 2013). For example, sustained swimming performance of cold-acclimated predators (Australian bass) declines at warm temperatures, whereas this is not the case for their eastern mosquitofish prey, suggesting that the character of this predator–prey relationship could change under differing thermal regimes (Grigaltchik, Ward & Seebacher 2012). Thus, a better understanding of how organisms respond to

*Correspondence author: E-mail: sara.paull@colorado.edu

temperature variability will improve our ability to predict their responses to climate change (Scherm 2004; Dell, Pawar & Savage 2011).

Acclimation theory is a particularly useful framework for considering how temperature shifts will influence organisms and their interactions. Thermal acclimation is the plasticity of thermal performance in response to prolonged exposure to a given temperature (Angilletta *et al.* 2006; Lagerspetz 2006; Angilletta 2009). Organisms often exhibit improved physiological performance at a particular temperature following acclimation to the same temperature, a pattern that has been shown for performance metrics such as swimming speed, mating success and jumping distance (Renaud & Stevens 1983; Glanville & Seebacher 2006; Wilson, Hammill & Johnston 2007). These results support the 'beneficial acclimation hypothesis', which postulates that acclimation should improve physiological performance at the acclimation temperature (Wilson & Franklin 2002). However, acclimation responses are governed by energetically costly biochemical and metabolic adjustments such as enzyme synthesis and cell membrane modification, and thermal stress at high temperatures might impose additional energetic costs, limiting the generality of acclimation as a universally 'beneficial' process for improving physiological performance (Hoffmann 1995; Dewitt, Sih & Wilson 1998; Hoffmann & Hewa-Kapuge 2000; Wilson & Franklin 2002; Angilletta *et al.* 2006; Deere & Chown 2006).

One interaction type for which thermal acclimation might be especially important is that between hosts and parasites. Small organisms tend to have higher mass-specific metabolic rates than large (Gillooly *et al.* 2001), which could allow parasites to acclimate faster than their hosts following a temperature shift. This faster acclimation might give parasites an advantage when temperatures are more variable (Raffel *et al.* 2006, 2013; Rohr & Raffel 2010), assuming that parasite and host acclimation responses both lead to improved performance at the acclimation temperature (i.e. beneficial acclimation). In support of this hypothesis, Raffel *et al.* (2013) found that Cuban treefrogs (*Osteopilus septentrionalis*) subjected to a 10 °C drop in temperature had higher growth rates of the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) than frogs that had been acclimated to the lower temperature prior to exposure.

Prior exposure to high or low temperatures might also influence the future physiological performance of parasites and hosts if these temperatures are energetically stressful and lead to depleted host energy stores, thereby influencing the resource availability and hostility (e.g. host physiological defences) of the parasite's environment. This type of effect, which we refer to as the 'thermal stress hypothesis', could lead to reduced performance of parasite and/or host following exposure to an extreme temperature. For example, if acclimation to a high temperature causes energetic stress, sustained exposure to warmer temperatures would lead to a progressive reduction in performance, opposite the prediction of beneficial acclimation. By extension, acclimation to non-stressful temperatures at which energy for host maintenance is not limiting could lead to increased parasite production over time. Such energetic costs of thermal stress might be especially important for parasites that consume a high percentage of the host's biomass and energy budget, such as trematode parasites in snails (Gerard & Theron 1997).

Here, we systematically evaluated predictions of the beneficial acclimation and thermal stress hypotheses in assessing how temperature shifts influence the interaction between a larval trematode (*Ribeiroia ondatrae*) and its snail intermediate host (*Helisoma trivolvis*), using the release of parasite infective stages (cercariae) from snail hosts as an index of parasite productivity. The beneficial acclimation hypothesis predicts that both parasite and host should have reduced performance immediately following a temperature shift in either direction, relative to those already acclimated to the performance temperature. It therefore predicts that cold-acclimated snails would release more cercariae at cold performance temperatures than would warm-acclimated snails shifted to a cooler temperature, and conversely that warm-acclimated snails would release more cercariae at warm performance temperatures (Fig. 1a). With progressive time following the temperature shift, however, unacclimated parasites and hosts should gradually obtain the same performance levels as acclimated parasites and hosts. Importantly, the beneficial acclimation hypothesis predicts that parasite and host performance should be optimal for acclimated parasites and hosts, such that acclimated infected snails should maintain similar parasite release levels through time if kept at the same temperature (Fig. 1a). The beneficial acclimation hypothesis has mixed support in the literature and may be most applicable at intermediate temperatures (Angilletta 2009).

The thermal stress hypothesis, in contrast, predicts that an energetically stressful thermal environment reduces both parasite and host performance over time, with performance continuing to decline the longer they are exposed to this unfavourable environment. Thus, at temperatures that are warm enough to be energetically stressful, this hypothesis predicts a progressive and sustained decline in parasite production, possibly due to a reduction in resource availability from stressed hosts (Fig. 1b). It also predicts that energy accumulation in snails acclimated to cooler, but non-stressful temperatures will lead to (i) increased parasite production over time, and (ii) greater parasite release when shifted to a stressful temperature than snails already acclimated to the stressful temperature (Fig. 1b). Because snails at cooler temperatures require less energy for maintenance, energy accumulation is expected to be greater at cooler temperatures, provided these are not cold enough to be stressful.

We tested these predictions using a crossed experimental design, in which parasite release from infected snails was measured before and after a shift from one of five

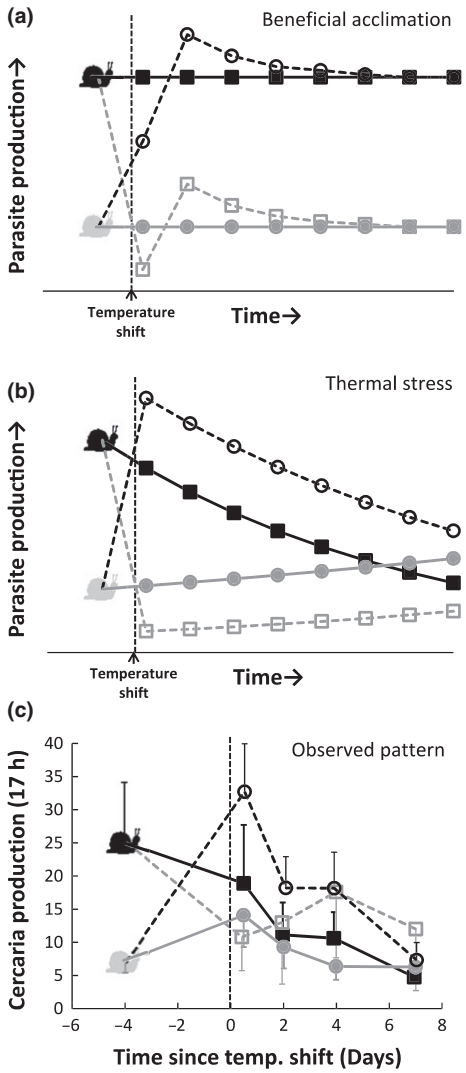

Fig. 1. Predicted patterns and corresponding results for parasite release from warm-acclimated (squares) and cold-acclimated (circles) snails following a temperature shift (vertical dotted line). (a) The beneficial acclimation hypothesis predicts that parasite release will be initially lower for unacclimated (open symbols and dotted lines) relative to acclimated (closed symbols and solid lines) snails. Because metabolic theory predicts that parasites should acclimate faster than their hosts, parasite release should increase temporarily as parasites acclimate to the new temperature, followed by an asymptotic decrease as acclimation of the host immune response brings parasite production back down to an equilibrium level. (b) The energetic stress hypothesis predicts that snails acclimated to an energetically stressful (i.e. the warmer temperatures in this experiment) environment will exhibit a steady decrease in parasite release and that cold-acclimated snails moved to a warm environment will initially exceed the release rates of warm-acclimated snails. (c) Cercaria release by cold-acclimated (13 and 16 °C; circles) and warm-acclimated (25 °C; squares) infected snails in the experiment, both before and after being shifted to either a cold (16 °C; gray) or warm (25 and 28 °C; black) temperature.

'acclimation temperatures' to one of five 'performance temperatures' (25 treatments) using an array of experimental incubators to ensure independent replication. This design allowed us to assess the full range of potential responses of parasite release to temperature changes of varying directions and magnitudes as well as the persistence of these effects over time.

Materials amd methods

STUDY SYSTEM

Ribeiroia ondatrae (hereafter *Ribeiroia*) is a trematode that infects planorbid snails after being released into the water from a definitive bird or mammal host. Parasitic rediae develop inside the snail, eventually castrating this host before producing cercariae, which emerge from the snail to infect larval amphibians, which subsequently pass the infection back to a definitive host when consumed (Johnson *et al.* 2004). Larval trematodes, which have important effects for both human health and wildlife conservation, offer a useful model for understanding thermal acclimation effects on parasite release because output of these parasites from their snail intermediate hosts has been shown to be strongly temperature-dependent and is easily quantified (Poulin 2006; Thieltges & Rick 2006; Studer, Thieltges & Poulin 2010). Additionally, the life cycle of *Ribeiroia* resembles that of other trematodes that cause a variety of diseases of humans (e.g. schistosomiasis, cercarial dermatitis) and domestic animals (fascioliasis), emphasizing the importance of understanding trematode acclimation responses.

EXPERIMENTAL DESIGN

We measured release of parasite infective stages from snail hosts and host mortality twice at five 'acclimation temperatures' (13, 16, 19, 22 and 25 °C) and at four time-points after hosts were switched to five new 'performance temperatures' (16, 19, 22, 25 and 28 °C) in a fully crossed design (25 treatments; Fig. 2). We chose to measure the response to directional temperature shifts while controlling any smaller scale diel temperature variability, because the focus of our study was to gain insight into the relative acclimation rates of hosts and parasites independent of smaller scale fluctuations. We chose the temperature range based on water temperatures measured every 2 h at 21 California field sites using Hobo dataloggers (Onset Computer Corp, Bourne, MA, USA), placed 50 cm below the water surface from June through early August. Daily average temperatures recorded at these sites ranged from 15·5 to 29·3 °C. Temperatures also showed large changes between weeks, with the maximum observed fluctuation between 1 week and the next being 5·9 °C. Given that our data set excludes the more climatically variable spring-time temperatures, we anticipate that temperature shifts between weeks likely exceed 6 °C in natural ponds.

Throughout the manuscript, the 'acclimation temperature' refers to the temperature to which organisms were exposed prior to a temperature shift, whereas 'performance temperature' refers to the time period after the temperature shift. This design allowed us to assess the full range of potential responses of parasite release to temperature changes of varying directions and magnitudes as well as the persistence of these effects over time. Parasite release is a particularly informative response variable for determining the net effects of acclimation on parasite exposure rates, because it is jointly regulated by host condition and parasite production capacity. We assessed parasite output before, immediately after, and up to 7 days post-shift. To generate infected hosts, we exposed 500 laboratory-raised snails to *c.* 30 *Ribeiroia* eggs per snail (mean ± SE shell size = 8·55 ± 0·06 mm) 3 months prior to the start of the experiment. Parasite eggs were obtained from the faeces of surrogate rat hosts that were passed through a 45 μm sieve and embryonated at 30 °C for 2 weeks (Johnson *et al.* 2007). By constructing an array of 50 individual incubators using Styrofoam coolers, heat tape and an adjustable thermostat (see Raffel *et al.*

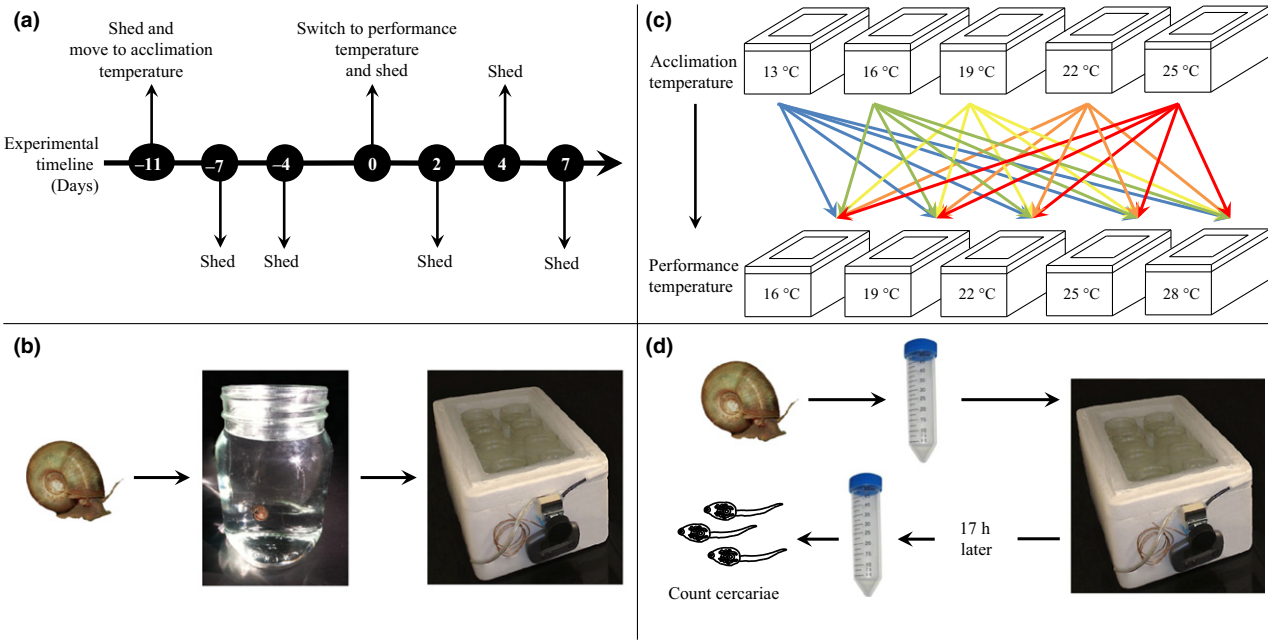

Fig. 2. The timeline of experimental events (a), the experimental housing for the snails (b), the temperature shift process (c) and the shedding process used to quantify parasitic cercariae (d).

2013), we were able to avoid the common pitfall of pseudoreplication that occurs when replicates within a single incubator are treated as independent. The incubators maintained the target temperature with a standard deviation of 1·2 °C averaged across all treatments (range: 0·7–1·6). We placed each snail individually into 800 mL mason jars with eight jars in each incubator (two uninfected, six infected); ten incubators were maintained at each of the five acclimation temperatures (for a total of 300 infected and 100 uninfected snails). Snails were fed *ad libitum* with a gel consisting of a mixture of Tetramin fish food (5 g), agar (1 g), calcium (0·25 g) and water (40 mL). We used filtered tap water and changed the water every 3 days, always using new water of the appropriate temperature for that treatment to avoid temperature shocks.

SAMPLING METHODS

Prior to the experiment, snails were maintained at room temperature (*c.* 21 °C). We obtained a baseline value for the numbers of parasites released by placing each snail into a 50 mL vial of water overnight (4 PM–9 AM) and preserving any cercariae in 70% ethanol for later counting using a gridded Petri dish and stereomicroscope. Next, we randomly assigned snails to each incubator and used the same shedding technique to quantify parasite release four and 7 days after being held at these 'acclimation temperatures' (Fig. 2). After 11 days at the acclimation temperatures, we assigned snails to new temperatures in a randomized block design such that the original group of six infected snails within each incubator divided and moved to one of the five new temperatures (with one extra replicate at one temperature determined at random). We quantified cercariae release 12 h later and then again at two, four and 7 days after the temperature shift. Snails were recorded as dead when they had fully retracted into their shells and failed to respond to mechanical stimuli, and mortality was recorded daily.

ANALYSES

To evaluate the relationship between temperature and parasite release prior to the temperature shift, we tested for linear and quadratic effects of temperature on parasite output after snails had been at their acclimation temperatures for four and 7 days as well as for changes in parasite release from 4 to 7 days. To do this, we used generalized linear mixed effects models (GLMM) with negative binomial distributed responses and log link functions, including incubator as a random effect. Next, to determine how temperature shifts affected parasite release and over what time duration, we ran linear regressions (after determining that the data met the appropriate assumptions) testing for effects of acclimation temperature, performance temperature and their interaction on the difference in parasite release from individual snails across different time-points. We used this approach rather than analysing the effect of acclimation temperature and magnitude of the shift directly because these two variables were strongly collinear. Treating the day of the temperature shift as Day 0, we did this first for a comparison of parasite release 4 days before (Day −4) and 12 h after the temperature shift (Day 0 minus Day −4). We ran a similar analysis using as the response the difference in parasite output from Day 7 minus Day 0. We chose the first interval to represent the change in output from just prior to immediately after the temperature shift, and the second interval because it is the longest interval possible following the shift.

To test our hypotheses about the influence of acclimation status on parasite release, we analysed whether acclimated hosts (snails that did not undergo a temperature shift) released more or fewer parasites than unacclimated hosts (snails that switched temperatures), and whether the effect was dependent upon performance temperature or time since the temperature shift (i.e. 0·5, 2, 4 or 7 days). To do this, we used a generalized linear model with a negative binomially distributed response to look for effects of performance temperature, acclimation status, time since the shift and their interactions on the number of cercariae released. Some snails were chronically poor shedders and were excluded from the analyses if there was only one nonzero count for parasite output (n = 127 snails removed). To determine which factors influenced snail mortality, we ran two binomial glms on mortality of snails before and after the temperature shift. We tested for effects of acclimation temperature, performance temperature and infection status and all two-way interactions (excluding performance temperature from the pre-shift mortality analysis).

Results

Prior to the temperature shift, temperature had a strong, positive effect on cercariae release from snails (4 days: $\chi^2_1 = 76{\cdot}19$, $P < 0{\cdot}01$; 7 days: $\chi^2_1 = 29{\cdot}73$, $P < 0{\cdot}01$, Fig. 3). Snails acclimated for 7 days to 25 °C released a mean ± SE of 21·4 ± 6·5 cercariae over the 17-h shedding period, compared to only 6·7 ± 1·2 released from snails at 13 °C. Additionally, snails released more cercariae the longer they were maintained at 13 °C (13 °C: $\chi^2_1 = 44{\cdot}10$, $P < 0{\cdot}01$), while there was no difference in cercaria release after four and 7 days at the other acclimation temperatures. Snails released a mean ± SE of 0·6 ± 0·2 cercariae after 4 days of being maintained at 13 °C, which grew to 6·7 ± 1·2 after 7 days at this temperature.

Immediately following the temperature shift (12 h post-shift), acclimation temperature (i.e. the temperature at which hosts had been maintained for the previous 7 days) had a negative effect on cercariae release, whereas performance temperature had a positive effect (acclimation temperature: $t = -4{\cdot}44$, $P < 0{\cdot}01$; performance temperature: $t = 4{\cdot}53$, $P < 0{\cdot}01$; Fig 4a,b). For example, snails moved to the hottest temperature of 28 °C released nearly 3·5 times more parasites 12 h after the temperature shift than just before; in contrast, snails moved to 16 °C released fewer than half as many parasites as they did prior to the shift. Importantly, however, the positive effect of performance temperature on change in parasite release was transient; 1 week after the temperature shift, parasite release declined with higher performance temperatures relative to just after the shift (e.g. Day 7–Day 0) ($t = -5{\cdot}13$, $P < 0{\cdot}01$, Fig. 4c,d). Thus, despite a strong initial increase in parasite release, snails maintained at 28 °C for a week released only one-fifth as many parasites as immediately following the temperature shift.

In comparing snails that did not undergo a temperature change (acclimated) to those that did (unacclimated), we found that the effects of performance temperature varied both by time and acclimation status (Fig. 5; See also Fig. S1, Supporting Information). Time and performance temperature interacted ($\chi^2_1 = 17{\cdot}88$, $P < 0{\cdot}01$, Table S1), such that performance temperature positively affected parasite release only at the first time-point (12 h post-shift, $\chi^2_1 = 22{\cdot}18$, $P < 0{\cdot}01$). Acclimation status also interacted with performance temperature to influence parasite release ($\chi^2_1 = 8{\cdot}15$, $P < 0{\cdot}01$, Table S1). When analysed separately, acclimated snails had lower parasite release at higher performance temperatures ($\chi^2_1 = 5{\cdot}33$, $P = 0{\cdot}02$, Table S2), driven largely by low release rates at 25 °C (Fig. 5). Unacclimated snails, in contrast, had overall higher parasite release at higher performance temperatures (Table S3), but also exhibited a significant interaction between time and performance temperature such that this positive effect of performance temperature declined through time ($\chi^2_1 = 15{\cdot}58$, $P < 0{\cdot}01$, Table S3; Fig. S1). Immediately following the temperature shift, unacclimated snails shifted to 25 °C released nearly eight times more parasites than snails already acclimated to 25 °C (Fig. 5). When results are analysed as cumulative parasite release, the results are similar, with performance temperature positively influencing cumulative parasite output in unacclimated but not acclimated snails, and the effect declining over time since the shift (Fig. S2, Tables S4–S7).

Host mortality was also affected by both infection status and temperature. Infected snails had higher mortality both before and after the temperature shift compared to uninfected snails (before: $\chi^2_1 = 42{\cdot}49$, $P < 0{\cdot}01$; after: $\chi^2_1 = 10{\cdot}17$, $P < 0{\cdot}01$). During the course of the experiment, nearly 40% of infected snails died ($N = 113$), whereas only 8% of uninfected snails died ($N = 8$), and all uninfected mortality occurred after the temperature shift. Snail mortality also increased at higher performance temperatures after the temperature shift, with a similar, but non-significant trend for warm-acclimated snails to have elevated mortality prior to the temperature shift (before: $\chi^2_1 = 3{\cdot}02$, $P = 0{\cdot}08$; after: $\chi^2_1 = 7{\cdot}76$, $P = 0{\cdot}01$; Fig. S3). There were no significant interactive effects of temperature and infection status on mortality for any of the time periods analysed.

Discussion

Our results revealed strong effects of both temperature and temperature shifts on the release of *Ribeiroia* parasites by

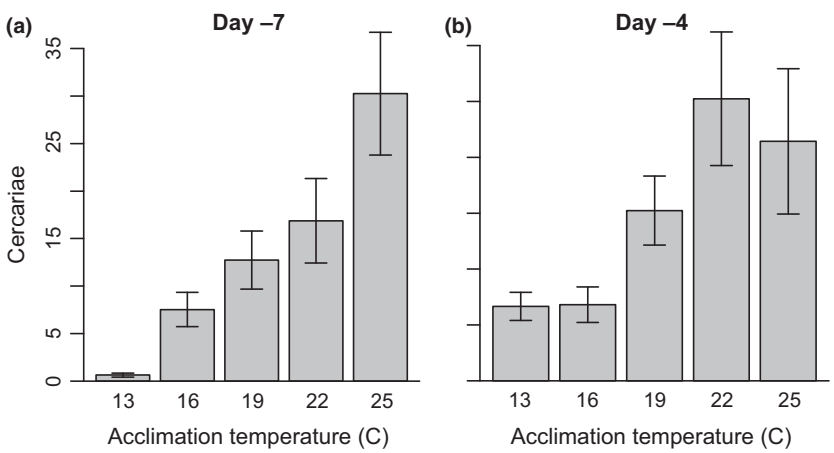

Fig. 3. Number of *Ribeiroia ondatrae* cercariae released from snails overnight (4PM–9AM) after (a) four and (b) 7 days of being at acclimation temperatures.

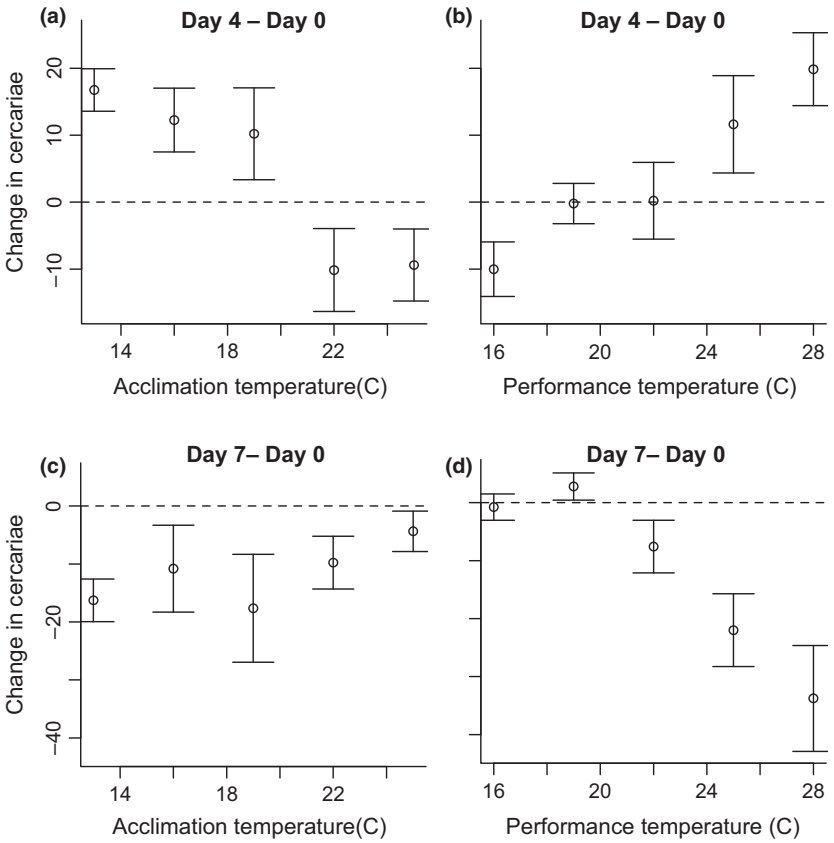

Fig. 4. Acclimation and performance temperatures influenced the difference in the number of *Ribeiroia ondatrae* cercariae released from *Helisoma trivolvis* snails at different time intervals after a temperature shift. Effect of (a) acclimation and (b) performance temperatures on the difference in the number of parasites released before the temperature shift (Day −4) subtracted from the number released immediately following the temperature shift (Day 0), and effect of acclimation (c) and performance (d) temperature on the difference in parasites just after the temperature shift (Day 0) subtracted from the number released a week after the shift (Day 7).

their snail hosts. At constant mean temperatures, release of *Ribeiroia* cercariae increased with temperature. This effect is broadly consistent with previous work demonstrating that as temperatures warm towards a thermal optimum, the development rates, growth and release of parasites are enhanced in a variety of taxa (Lv *et al.* 2006; Poulin 2006; Macnab & Barber 2012). Comparatively little is known, however, about how parasites and their hosts respond to thermal shifts. Our results indicated that temperature not only had a transiently positive influence on trematode release, as reported for a range of other parasites with infectious free-living stages (Lyholt & Buchmann 1996; Mouritsen & Jensen 1997; Studer, Thieltges & Poulin 2010), but also demonstrated that the effect of a temperature change depended on both the performance temperature and the time since acclimation occurred.

Collectively, these findings support predictions of the thermal stress hypothesis as to how temperature shifts should influence parasite production and release, rather than the beneficial acclimation hypothesis (Fig. 1). Soon after the temperature shift (12 h), snails moved from colder to warmer temperatures released more parasites than those already acclimated to warm temperatures. This is consistent with previous studies showing increases in trematode parasite release following shifts to warmer temperatures that exceed the typical thermal-dependent rate of increase for physiological processes (Poulin 2006; Morley, Adam & Lewis 2010). They also corroborate a recent study's finding that trematode-infected snails shifted temporarily from 25 to 30 °C released considerably more parasites than those maintained consistently at 30 °C (Studer, Thieltges & Poulin 2010). However, our study also shows that this increase was transient, with cercariae release declining steadily the longer snails were held at warmer temperatures (Figs 1c, 4 and 5). In contrast, snails moved from warmer to colder temperatures experienced an initial decline in parasite release, but subsequently maintained parasite release levels through time. Consistent with the thermal stress hypothesis, parasite release declined steadily in warm-acclimated snails the longer they were held at higher temperatures (Fig. 1c), whereas release increased among snails over the 7 days they were held at 13 °C (Fig. 1c). Finally, we observed elevated mortality in snails held at warmer performance temperatures (Fig. S3), providing further support for the thermal stress hypothesis.

The thermal stress hypothesis suggests two primary mechanisms driving the patterns we observed in parasite release. First, the temporary boost in cercaria release from snails moved to high temperatures may have been caused by an initial increase in parasite replication due to a temperature-dependent metabolic increase, as predicted by metabolic theory (Gillooly *et al.* 2001). Secondly, the subsequent decline in cercaria release over time could have been caused by a decline in host condition (and thus resource quality for the parasite) due to thermal stress, leading in turn to reduced parasite reproduction. Such a thermal stress response, possibly driven by increased host and/or parasite metabolism relative to food intake at

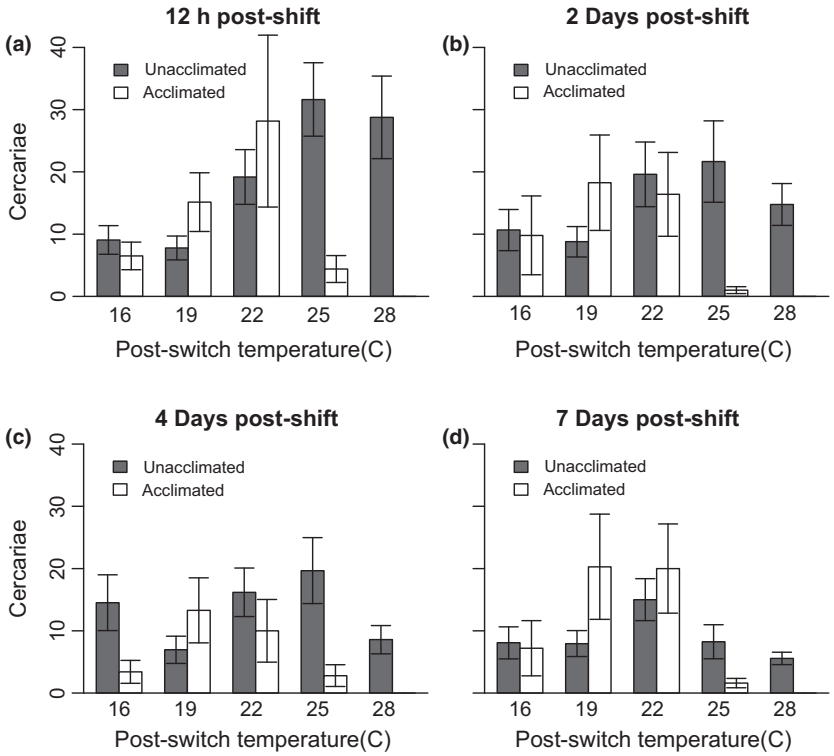

Fig. 5. Parasite release from acclimated (white bars) and unacclimated (grey bars) snails at 12 h (a), 2 days (b), 4 days (c) and 7 days (d) after the temperature shift. Acclimated snails did not experience a temperature shift, while unacclimated snails were shifted to warmer or cooler temperatures. Note that no snails were acclimated to 28 °C, so there are no data for acclimated snails at this temperature.

warmer temperatures, might overwhelm any adaptive acclimation responses occurring at those temperatures (Hoffmann & Hewa-Kapuge 2000; Wilson & Franklin 2002). If snails maintained at cooler temperatures were in better condition and supplied parasites with higher quality resources, this could temporarily boost parasite release when moved to warmer temperatures, until thermal stress reduced snail condition. Such a pattern could be explained relatively simply, if the temperature dependence of parasite metabolism has a slightly greater slope than the temperature dependence of host resource assimilation. This difference in parasite vs. host metabolism would cause the parasite to spend energy at warm temperatures faster than the host can assimilate new energy, whereas the colder temperatures could allow host energy assimilation to equal or exceed parasite energy expenditures. These findings are consistent with the notion of host resource availability functioning as an important determinant of parasite release (Jensen & Munk 1997; Johnson *et al*. 2007; Civitello *et al*. 2013).

An alternative to the thermal stress explanation for the boost in parasite release following a temperature increase is that excess energy accumulated by infected snails at colder temperatures is stored not as host energy reserves but by conversion to parasite infective stages that remain within the snail, as suggested previously (Shim, Koprivnikar & Forbes 2013). This 'storage effect' could occur if temperature dependence of parasite release from the snail has a greater slope than the temperature dependence of parasite reproduction (i.e. cercaria production and development within the snail). In support of this hypothesis, Paull & Johnson (2011) found that production of *Ribeiroia* parasites within snails continued to occur at a temperature of 13 °C, but parasite release did not occur until temperatures were warmed. Similar minimum temperature thresholds for cercarial emergence have been documented in a variety of trematode taxa (Morley & Lewis 2013). The short time-scale of the peak cercarial release following the temperature increase in this study (12 h following the temperature shift) strongly suggests that the excess parasites were already developed and ready for release, lending further support for the storage effect hypothesis. It is possible that the snails held at 13 °C in the present study were able to release parasites (in contrast to the previous study) because they had already been releasing parasites before being moved down to 13 °C, allowing rediae to develop, whereas in the previous study, snails were maintained at 13 °C from the time of infection and the parasite may have been unable to generate mature rediae. If storage of parasites is common across taxonomic systems, it could have strong implications for how temperature changes influence parasite exposure depending on the frequency of the shifts relative to the amount of time it takes for parasite stores to accumulate.

Such a 'storage' strategy might increase parasite fitness by allowing the parasite to maximize the release of infectious stages at temperatures conducive to infecting the next host, or by ensuring the pulsed release of a large number of cercariae prior to hosts dying from thermal stress. For instance, it would be adaptive for *Ribeiroia* to release cercariae when tadpoles are least able to resist infection. A recent study by Paull, LaFonte & Johnson (2012) found

that, at constant parasite exposure levels, the net burden of *Ribeiroia* infection in amphibians was greatest at an intermediate temperature of 20 °C, suggesting that it might be adaptive for cercariae to be released at this temperature. Interestingly, our results showed that after 7 days of exposure to a constant temperature, parasites released the most cercariae at 22 °C (Fig. 3b), close to the optimal temperature for infecting tadpoles. Such a pattern is consistent with the storage effect hypothesis, suggesting that *Ribeiroia* might retain cercariae until conditions are suitable for infecting the next intermediate host.

The extent to which temperature might influence the snail host's immune regulation of the parasite is also an important factor to consider when interpreting these results. For instance, if warm temperatures enhance the snail immune response, the decline in cercaria output in snails at the warmest temperature could indicate a gradual improvement in the snail host immune response. However, we do not suspect this is the case because (i) freshwater snail immune function may actually decline at extreme warm temperatures (30 °C) (Seppälä & Jokela 2011) and (ii) trematodes are often effective at suppressing snail immune responses once infections are established (e.g. Hanington *et al*. 2010; Kryukova *et al*. 2014). Correspondingly, a recent comparison of the infection of *Lymnaea stagnalis* snails exposed to 15 °C or 25 °C for 7 days demonstrated that infection success with the trematode *Echinoparyphium aconiatum* was higher in snails exposed to the warmer temperature, even when the actual infection was performed at 20 °C (Leicht & Seppälä 2014). Additionally, the observed increase in snail mortality at higher temperatures in the present study is more consistent with host physiological stress than with improved immune function. Together these prior results suggest that temperature-dependent host immune responses are unlikely to have driven the findings of this study.

These results suggest that temperature shifts will have a strong influence on parasite release and thus overall disease risk, but that the intervals between large shifts will play a role in the ultimate outcome. Here, we found that temperature increases caused an immediate large increase in cercaria release, but that this increase was short-lived and followed by a gradual decrease in cercaria release at sustained high temperatures (Fig. 5), such that there is no effect of performance temperature on cumulative parasite release in acclimated snails (Fig. S2). The temperature at which parasites develop does not influence their ability to infect the next host (unpublished data), suggesting that these effects on cumulative parasite release are indicative of the net effects of temperature shifts on parasite fitness and disease risk, although it should be noted that the exposure temperature does have nonlinear effects on tadpole infections (see Paull, LaFonte & Johnson 2012). This could have implications for climate change effects on parasite exposure in this system. For instance, climate models predict future increases in the frequency of extreme high temperatures (Easterling 2000). Given that our results were strongest at the extreme warm temperatures, if parasites require only a short period of time at cooler temperatures to achieve the observed improvement in performance at high temperatures, then increased frequency of temperature extremes could enhance parasite output in the future. However, if increased snail mortality offsets the boost in parasite release, or frequent and sustained exposures to high temperatures reduce cercariae production, then net exposure could decline. The transient nature of the increased parasite release in response to warming temperature emphasizes the importance of considering the amplitude and frequency of temperature shifts predicted to occur with climate change to better understand how parasite exposure rates could change in the future.

Greater emphasis on understanding how temperature change will influence host–parasite interactions is needed, considering that climate change is expected to alter future temperature variability (Easterling 2000). Further work to disentangle parasite acclimation responses from those of their hosts will be important to clarify the mechanisms behind patterns observed in this study, which could have important implications for how other systems respond to climate change. For instance, if the mechanism driving the initial increase and subsequent decline in parasite release is primarily metabolic in nature, it suggests that bouts of extreme high temperature could have adverse consequences for hosts and ultimately parasites as well. On the other hand, if the mechanism is storage of parasites at cooler temperatures, intermittent periods of extreme high temperatures could enhance release of parasites with fewer adverse consequences on hosts. Given the severe pathology that amphibians can experience as a result of *Ribeiroia* infection (Johnson *et al*. 2004), and because the life cycle of *Ribeiroia* is similar to parasitic diseases of significance to the health of domestic animals (fascioliasis) and humans (schistosomiasis), these results are relevant to how temperature variability influences the health of wildlife, humans and livestock. Finally, it will be further useful to complement the research performed here with studies of how diel temperature fluctuations and the frequency of temperature shifts influence acclimation responses and infection in the amphibian hosts. Greater use of thermal biology and metabolic theory to explain patterns in parasite responses to temperature shifts offers a promising foundation for studies of how future temperature changes could influence disease.

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Data accessibility

Data deposited in the Dryad Digital Repository: http://doi.org/10.5061/dryad.h5h3c (Paull *et al*. 2014).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Time and performance temperature effects on parasite release.

Fig. S2. Time and performance temperature effects on cumulative parasite release.
Fig. S3. Effect of temperature and infection on host mortality.
Table S1. Effects of temperature, acclimation and time on parasite release.
Table S2. Effects of temperature and time on parasite release from acclimated snails.
Table S3. Effects of temperature and time on parasite release from unacclimated snails.
Table S4. Effects of temperature, acclimation and time on cumulative parasite release.
Table S5. Effects of temperature and time on cumulative parasite release from unacclimated snails.
Table S6. Effects of temperature and time on cumulative parasite release from acclimated snails.
Table S7. Effects of temperature on cumulative parasite release from unacclimated snails at multiple timepoints.