

## Research



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## Global change biology

# Temperature and pathogen exposure act independently to drive host phenotypic trajectories

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Natural populations are experiencing an increase in the occurrence of both thermal stress and disease outbreaks. How these two common stressors interact to determine host phenotypic shifts will be important for population persistence, yet a myriad of different traits and pathways are a target of both stressors, making generalizable predictions difficult to obtain. Here, using the host *Daphnia magna* and its bacterial pathogen *Pasteuria ramosa*, we tested how temperature and pathogen exposure interact to drive shifts in multivariate host phenotypes. We found that these two stressors acted mostly independently to shape host phenotypic trajectories, with temperature driving a faster pace of life by favouring early development and increased intrinsic population growth rates, while pathogen exposure impacted reproductive potential through reductions in lifetime fecundity. Studies focussed on extreme thermal stress are increasingly showing how pathogen exposure can severely hamper the thermal tolerance of a host. However, our results suggest that under milder thermal stress, and in terms of life-history traits, increases in temperature might not exacerbate the impact of pathogen exposure on host performance, and vice versa.

## 1. Introduction

As a result of global change, populations are experiencing an increase in the occurrence of thermal stress [1,2]. Simultaneously, increasing temperatures are driving a shift in the distribution of pathogens and the severity of infectious disease outbreaks [3–7]. How individual's response to global change will increasingly be determined by the interactions between thermal stress and pathogen exposure [8–10]. For example, infection has been shown to severely hamper a host's thermal tolerance, suggesting individuals exposed to disease will be particularly sensitive to extreme thermal stress such as heat waves [11–13]. Increases in temperature and pathogen exposure, however, will not only shape host thermal tolerance, but will also drive changes in all aspects of a host's phenotype, including development, growth, fecundity and population-level processes (e.g. [9,14–17]). Population persistence will therefore be shaped by how temperature change and pathogen exposure directly interact to shift multiple facets of a host's phenotype, in addition to the complex role that temperature plays in driving pathogen transmission strategies [18–20] and host–pathogen interactions more broadly [21,22].

Several outcomes are possible when an individual experiences both a pathogen and elevated temperatures. These stressors can act additively, where the impact they have on an individual is simply the sum of their independent effects. Alternatively, antagonistic and synergistic interactions can occur, where the cumulative impact of two stressors is less than, or more than, the sum of their independent effects, respectively [23–25]. An antagonistic interaction between thermal stress and pathogen exposure suggests that disease outcomes under

thermal change might be less severe than expected, while any synergistic interaction would instead place a host at greater risk than expected. An understanding of how co-occurring stressors interact to determine shifts in host performance is therefore vital if we are to understand the fitness consequences of thermal change and pathogen exposure acting in concert.

Studies of the impacts of temperature and pathogen exposure often focus on their effects on individual host traits (e.g. [14–17]). However, organisms are comprised of multiple correlated traits that together form the integrated phenotype [26]. While determining how stressors impact individual traits gives insight into how they may affect specific components of individual or population performance, a univariate approach can be misleading if the magnitude or direction of any phenotypic shifts depend on which trait is being assessed or on the correlations between traits [27]. By taking a multivariate view, it is possible to understand phenotypic shifts in response to environmental change in a more holistic manner [27–29]. Indeed a phenotypic shift can manifest via changes in the magnitude and/or the direction of phenotypic change in multivariate space—collectively known as the phenotypic trajectory [30,31]. By assessing the multivariate phenotypic trajectory of a host, we can more fully understand whether the phenotypic impact of a pathogen will be enhanced or lessened under rising temperatures.

In this study, we tested how exposure of *Daphnia magna* to a bacterial pathogen interacts with increased temperatures to drive shifts in a host's phenotype. Across 11 life-history traits, we used phenotypic trajectory analysis to compare the magnitude and direction of temperature-induced phenotypic changes for animals exposed or unexposed to a pathogen. As part of this process, we assessed the independent impacts of elevated temperature during the maternal generation and on the focal generation to dissect trans-generational from direct thermal effects, but found maternal temperature effects to be relatively minor. Traits studied included pace of life traits such as developmental timing and intrinsic population growth rates, in addition to fecundity traits, somatic growth and lifespan. Using this integrated multivariate approach, we assessed whether temperature increases might exacerbate or lessen the impact of pathogens on host performance.

## 2. Methods

*Daphnia magna* Straus is a freshwater cyclically parthenogenic crustacean from the northern hemisphere [32]. *Pasteuria ramosa* is a natural Gram-positive bacterial pathogen of *Daphnia* that infects during filter feeding before entering the body and reproducing, causing reduced fecundity and lifespan of its host [33–35]. Here, we used *Daphnia* genotype BE-OMZ-M10 infected with one of three *P. ramosa* genotypes (C1, C14, C20), which vary in their rates of within-host proliferation as well the virulence they cause to their host [34,36]. Prior to the experiment female *Daphnia* were raised individually for three generations in 70 ml jars filled with 50 ml of artificial *Daphnia* Medium (ADaM; [37]) and maintained under standard conditions (see electronic supplementary material).

### (a) Temperature treatments and infection

On their day of birth, maternal generation females were taken from clutches 3–5 of the standardized animals and placed at either 20°C or 25°C (maternal temperature treatment). Experimental individuals were then collected from clutches 3–5 of the acclimated

mothers on their day of birth and placed at either 20°C or 25°C (focal temperature treatment) in a fully factorial design. The first temperature represents maternal and developmental acclimation (*Daphnia* experience early development within the brood pouch of their mothers) and the second temperature represents direct thermal acclimation on the focal individuals from birth, which lasted over the rest of their lives, including during growth, maturity and the infection period.

A total of 496 females were set up individually in the experimental generation in a fully factorial design, with 31 individuals per treatment (2 maternal temperatures  $\times$  2 focal temperatures  $\times$  [3 pathogens + uninfected controls]). For the infection treatments, individual *Daphnia* was exposed to 40 000 *P. ramosa* spores from one of the three pathogen genotypes over 2 days (20 000 per day) starting 3 days after birth. The infection took place over 3 days in 70 ml jars filled with 20 ml ADaM, after which all animals were transferred to 50 ml of fresh ADaM and maintained at their respective temperatures.

### (b) Life-history traits

All individuals were monitored daily from birth until death and various life-history traits were recorded: age and body size at maturity, body size at day 20, age at and size of the first clutch, lifetime fecundity, total number of clutches, mean clutch size and lifespan. Body size measures the length of *Daphnia* from the top of the head above the eye to the base of the tail spine. Two additional traits were also calculated, including, somatic growth rate ( $G$ ) and individual intrinsic rate of increase ( $r$ ), calculated by solving the Euler–Lotka equation (see electronic supplementary material).

### (c) Statistical analysis

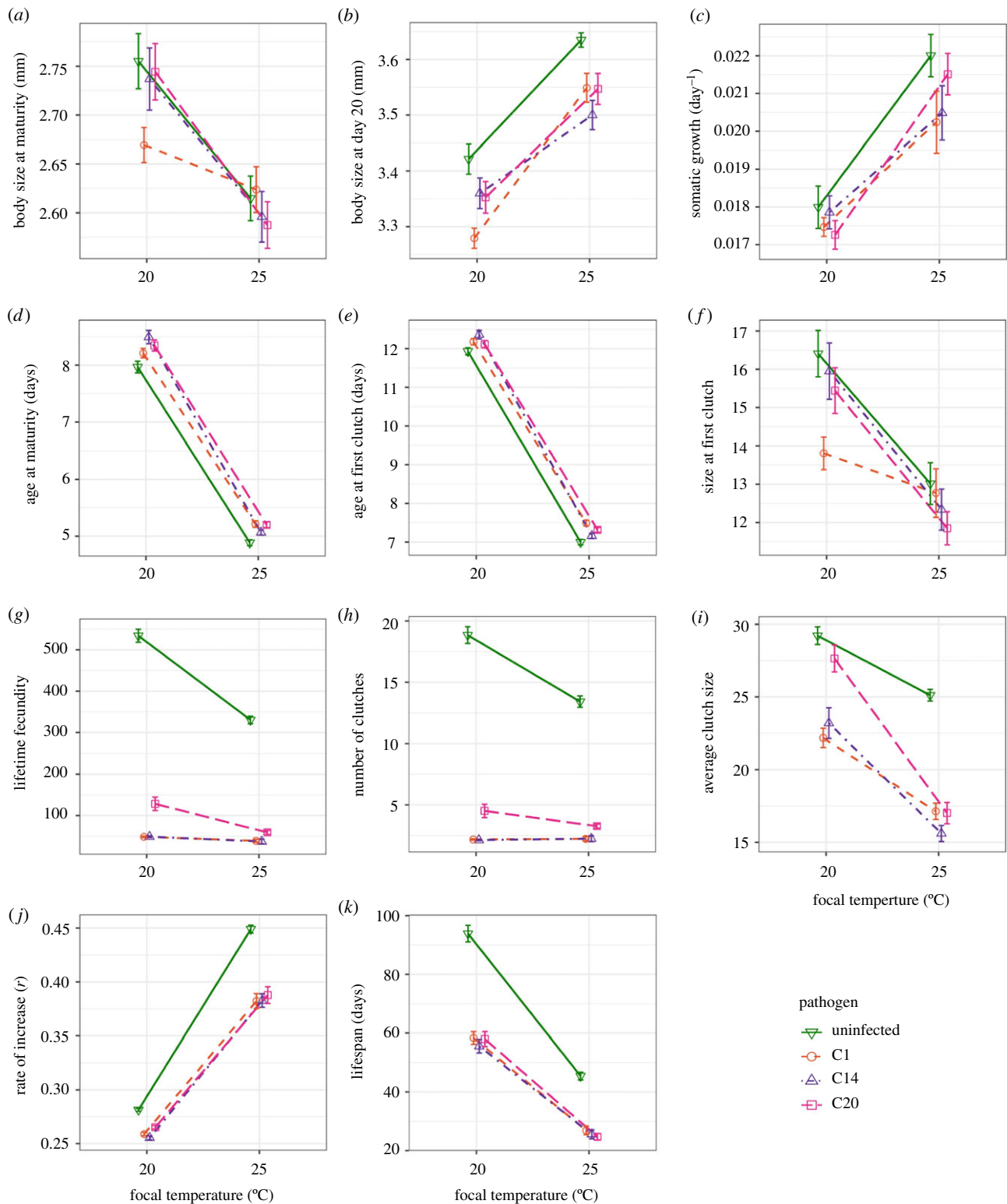
All analyses were conducted in R (v. 3.6.2; R Development Core Team). Figures were produced using *ggplot2* [38] and *cowplot* [39].

Owing to the relatively minor influence of maternal temperature across most traits, we controlled for any effect of maternal temperature (two levels: 20°C or 25°C) by including it as an additive term and focussed instead on the interaction between focal temperature (two levels: 20°C or 25°C) and infection treatment (four levels: control, C1, C14 or C20). Lifetime fecundity and total number of clutches were both log-transformed and intrinsic rate of increase ( $r$ ) was squared before all analyses. For the univariate analyses, age at maturity and age at first clutch were analysed using a generalized linear model with Poisson distributed errors. All other traits were analysed using linear models. The significance of fixed effects was then tested using analysis of variance (Type III, white corrected to account for residual heterogeneity; *car* package [40]).

Prior to multivariate analysis, all traits were standardized to have a mean of zero and a standard deviation of one. A permutation-based MANOVA was used to analyse how temperature and pathogen exposure influence multivariate host phenotypes using the fixed effect structure described above [41,42]. We then used phenotypic trajectory analysis to calculate the relative magnitude ( $D$ ) and angle ( $\theta$ ) of any shift in host phenotype (phenotypic trajectory) across temperature for each infection treatment [30,31,41] and visualized these patterns using principal component analysis (PCA) on all 11 traits across the two treatments. All multivariate analyses were conducted using the *RRPP* package [42]. See the electronic supplementary material for details on the sample sizes for each treatment combination.

## 3. Results

We observed three distinct outcomes for the effects of temperature and pathogen exposure on individual traits (figure 1; electronic supplementary material, table S1 for ANOVA results). First, for traits like body size and age at maturity, and size and

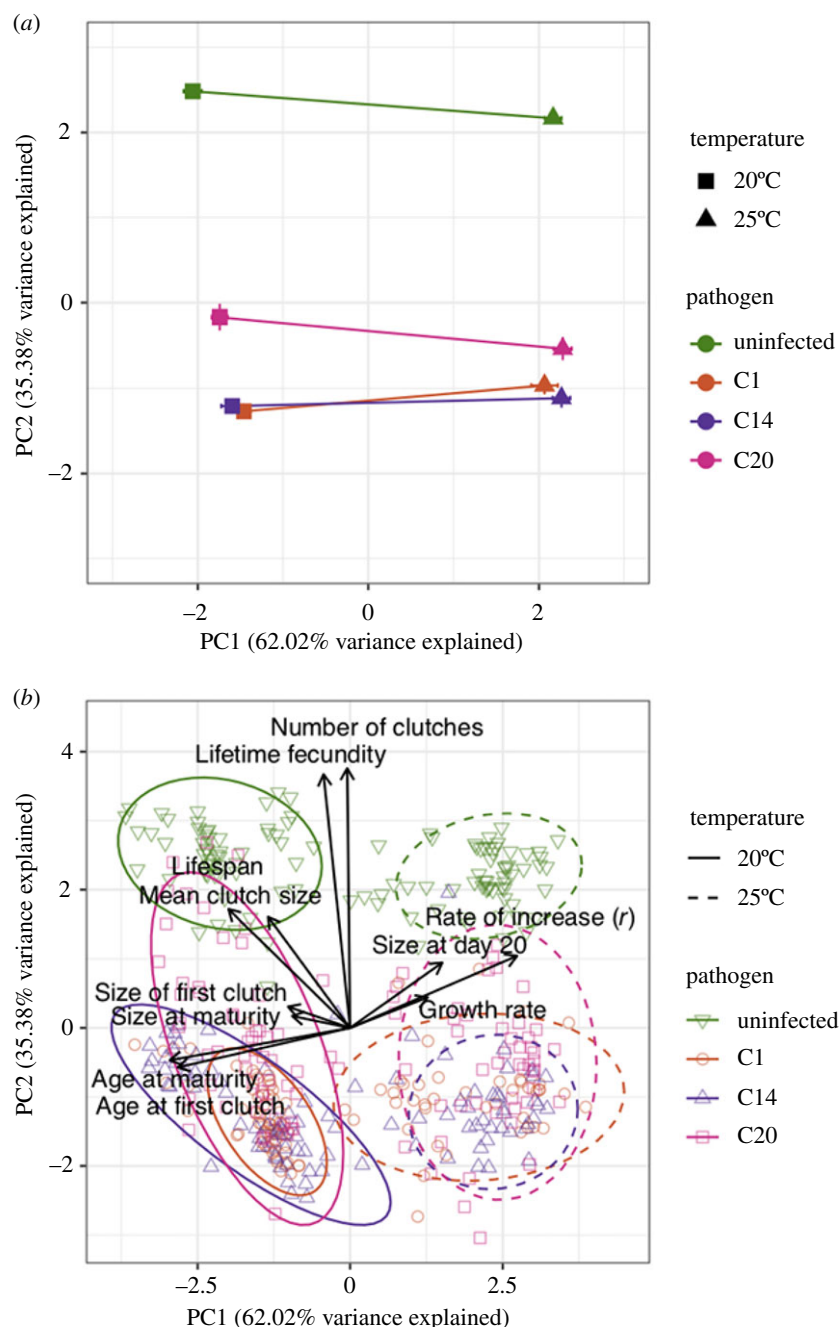


**Figure 1.** Univariate responses to infection treatment and focal temperature, showing the mean ( $\pm$ s.e.) for each treatment group. Due to generally small effects of maternal temperature treatment on most variables, for visual clarity data were pooled across maternal temperature. See electronic supplementary material, figure S1 for data split across maternal temperature.

age of the first clutch, trait values decreased as temperature increased, with patterns dominated by the effect of temperature alone. Second, for somatic growth rate and body size at day 20, trait values increased with temperature, with temperature again being the dominant driver of variation, but with pathogen exposure also leading to a reduction in trait values. For all other traits, any response depended on an interaction between temperature and pathogen exposure. With lifetime fecundity, the total number of clutches and lifespan, trait values decreased at warmer temperatures, but much more so for uninfected

animals. By contrast, the intrinsic rate of increase increased at higher temperatures, where the detrimental impact of pathogen exposure was also greatest. Average clutch size was lowest at warmer temperatures, with the greatest reduction seen in infected individuals.

The MANOVA and phenotypic trajectory analysis results reveal that, after accounting for correlations among trait responses, temperature and pathogen exposure act largely independently to drive host phenotypic trajectories (electronic supplementary material, table S2; table 1 and figure 2a).



**Figure 2.** Principal component plots showing: (a) the phenotypic trajectories in response to temperature and pathogen exposure across 11 life-history traits ( $\pm$ s.e.) and (b) PCA plots showing all observations grouped by temperature and infection treatment. Length of arrows represent the relative magnitude of the contribution each trait made towards PC1 and PC2, while their orientation represents the direction of any shift, and relative direction shows trait covariation. Ellipses represent 95% confidence bands.

**Table 1.** Phenotypic trajectory analysis comparing the difference in magnitude ( $D$ ) and angle ( $\theta$ ) of phenotypic shifts caused by temperature across infection treatments based on multivariate changes in 11 life-history traits. Significant  $p$ -values are in *italics*.

| treatment comparisons | magnitude difference ( $D$ ) | $Z$    | $p$ -value       | angle difference ( $\theta$ ) | $z$   | $p$ -value   |
|-----------------------|------------------------------|--------|------------------|-------------------------------|-------|--------------|
| uninfected–C1         | 0.703                        | 4.206  | <i>&lt;0.001</i> | 17.201                        | 3.217 | <i>0.006</i> |
| uninfected–C14        | 0.386                        | 1.621  | 0.077            | 18.784                        | 3.519 | <i>0.004</i> |
| uninfected–C20        | 0.211                        | 0.285  | 0.349            | 18.185                        | 3.548 | <i>0.006</i> |
| C1–C14                | 0.318                        | 1.055  | 0.163            | 17.997                        | 3.089 | <i>0.008</i> |
| C1–C20                | 0.492                        | 2.434  | <i>0.027</i>     | 19.266                        | 3.64  | <i>0.002</i> |
| C14–C20               | 0.174                        | −0.039 | 0.437            | 11.702                        | 1.127 | 0.134        |

The magnitude of the multivariate response to temperature ( $D$  in table 1) was consistent across most exposure treatments indicating that the strength of temperature-driven phenotypic change is not altered by infection (figure 2a). The direction of each trajectory in multi-trait space was also relatively aligned ( $\theta$  in table 1 and figure 2a), with any difference in angles between two trajectories all less than  $20^\circ$ , indicative of a very mild convergence of the phenotypic trajectories for each exposure treatment at warmer temperatures (figure 2a; electronic supplementary material, table S2 for the MANOVA results).

Visualization of the traits contributing to the phenotypic trajectories revealed that temperature and infection shape phenotypic trajectories in very different ways (figure 2b). Temperature primarily shifted phenotypes across 'pace of life' traits, including developmental timing traits and population growth rates. By contrast, pathogen exposure impacted reproductive potential, causing large reductions in both lifetime fecundity traits and lifespan.

## 4. Discussion

Rising temperatures have the potential to either reduce or exacerbate the impact of infection on a host's phenotype, depending on whether the interaction between these two stressors is antagonistic or synergistic [23,24]. Our results, however, suggest that changes in host life-history traits, at least, may not be subject to such complex interactions. A phenotypic trajectory analysis of 11 different life-history traits in *Daphnia* showed that trait changes driven by temperature were not substantially modified by infection (table 1 and figure 2). Temperature generally influenced 'pace-of-life' traits, with warmer temperatures accelerating rate-based traits, such as age at maturity and population growth rate ( $r$ ), potentially reflecting the intrinsic link between temperature and metabolic rate (see also [28,43]). Pathogen exposure, by contrast, shaped traits related to the reproductive potential and longevity of its hosts. This may be expected in this system, as the pathogen *P. ramosa* severely reduces the fecundity and lifespan of its host [15,16,34].

The lack of a synergistic interaction, whereby phenotypic differences between infected and uninfected individuals are increased at warmer temperatures, suggests that the fitness costs of infection for a host might not be universally worse under warmer temperatures [4,5,8]. Instead, mild thermal stress and pathogen exposure appear to have largely independent and predictable effects on the phenotypic trajectory of a host. Thus, while pathogen exposure is known to make individuals far more sensitive to extreme thermal stress [11,12], we show the reverse is not necessarily true—elevated temperature may not always exacerbate the damage pathogens cause. We stress that this does not mean that the severity of an infectious disease outbreak for a host population will remain unchanged under warmer temperatures (e.g. [44]). Rising temperatures will also alter the likelihood

a host becomes infected and the transmission dynamics for a pathogen [7,16,18,19,45], and changes in the reproductive capacity of a host, as documented here, will inevitably feed back into the supply of susceptible hosts on which the spread of infectious diseases depend [46].

But what about other host–pathogen systems? Here comparisons remain difficult as rarely are a full suite of host life-history traits, or host population growth rates, measured simultaneously under infection and thermal stress. Instead the focus is often on pathogen transmission and rates of increase [9]. In a similar *Daphnia*–fungus system, for example, infection reduced lifespan, but the relative reduction was reasonably consistent across temperatures [19], consistent with our broad results. But equally, studies in this same system have found that both shifts to cooler temperatures and warmer temperatures can lead to a smaller relative reduction in fecundity or lifespan of infected individuals [17,47]. These results reinforce how only a simultaneous assessment of multiple life-history traits offers a complete picture of the way environmental change will shift host phenotypes [27–29].

In conclusion, the joint effects of thermal stress and pathogen exposure are increasingly being recognized as a key determinant of population persistence for natural populations [8,9,48]. Using a multivariate approach, we have shown that these two stressors may act independently, and that pathogen exposure may not necessarily exacerbate the impacts of global change on host life-history traits. We believe these results, even on a single host genotype, can be generalizable. Our previous work [11,12], and that of others [45], has shown similarities in the way different host genotypes respond to infection and thermal stress (at least in the direction of any response). We also observed that the magnitude and angle of phenotypic shifts due to temperature were remarkably consistent across three pathogen genotypes of varying transmission and virulence [34,36]. Nonetheless, assessing how temperature and pathogens interact to shape host phenotypic trajectories across a broad range of host–pathogen systems, populations and genotypes will help refine the conditions under which an increase in temperature does not exacerbate the impact of pathogen exposure on host performance (see [49]).

**Ethics.** No ethical approval was required for this work.

**Data accessibility.** Data and code from this manuscript can be found via the Figshare repository at <https://dx.doi.org/10.26180/13721266> [50].

**Authors' contributions.** The study was conceived and designed by T.E.H., C.M.S. and M.D.H. Data were collected by T.E.H. Analyses were conducted by T.E.H. and M.D.H. T.E.H. and M.D.H. prepared the first draft of the manuscript. All authors contributed to the final manuscript, approved the final version and agree to be held accountable for the content therein.

**Competing interests.** We declare we have no competing interests.

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## References

1. Buckley LB, Huey RB. 2016 Temperature extremes: geographic patterns, recent changes, and implications for organismal vulnerabilities. *Glob. Change Biol.* **22**, 3829–3842. (doi:10.1111/gcb.13313)
2. Rahmstorf S, Coumou D. 2011 Increase of extreme events in a warming world. *Proc. Natl. Acad. Sci.*

- USA **108**, 17 905–17 909. (doi:10.1073/pnas.1101766108)
3. Price SJ, Leung WTM, Owen CJ, Puschendorf R, Sergeant C, Cunningham AA, Balloux F, Garner TWJ, Nichols RA. 2019 Effects of historic and projected climate change on the range and impacts of an emerging wildlife disease. *Glob. Change Biol.* **25**, 2648–2660. (doi:10.1111/gcb.14651)
  4. Altizer S, Ostfeld RS, Johnson PTJ, Kutz S, Harvell CD. 2013 Climate change and infectious diseases: from evidence to a predictive framework. *Science* **341**, 514–519. (doi:10.1126/science.1239401)
  5. Rohr JR, Dobson AP, Johnson PTJ, Kilpatrick AM, Paull SH, Raffel TR, Ruiz-Moreno D, Thomas MB. 2011 Frontiers in climate change-disease research. *Trends Ecol. Evol.* **26**, 270–277. (doi:10.1016/j.tree.2011.03.002)
  6. Gehman A-LM, Hall RJ, Byers JE. 2018 Host and parasite thermal ecology jointly determine the effect of climate warming on epidemic dynamics. *Proc. Natl Acad. Sci. USA* **115**, 744–749. (doi:10.1073/pnas.1705067115)
  7. Mordecai EA *et al.* 2019 Thermal biology of mosquito-borne disease. *Ecol. Lett.* **22**, 1690–1708. (doi:10.1111/ele.13335)
  8. Cohen JM, Sauer EL, Santiago O, Spencer S, Rohr JR. 2020 Divergent impacts of warming weather on wildlife disease risk across climates. *Science* **370**, abb1702. (doi:10.1126/science.abb1702)
  9. Claar DC, Wood CL. 2020 Pulse heat stress and parasitism in a warming World. *Trends Ecol. Evol.* **35**, 704–715. (doi:10.1016/j.tree.2020.04.002)
  10. Stoks R, Verheyen J, Van Dievel M, Tuzun N. 2017 Daily temperature variation and extreme high temperatures drive performance and biotic interactions in a warming world. *Curr. Opin. Insect Sci.* **23**, 35–42. (doi:10.1016/j.cois.2017.06.008)
  11. Hector TE, Sgrò CM, Hall MD. 2019 Pathogen exposure disrupts an organism's ability to cope with thermal stress. *Glob. Change Biol.* **25**, 3893–3905. (doi:10.1111/gcb.14713)
  12. Laidlaw T, Hector TE, Sgrò CM, Hall MD. 2020 Pathogen exposure reduces sexual dimorphism in a host's upper thermal limits. *Ecol. Evol.* **10**, 12 851–12 859. (doi:10.1002/ece3.6828)
  13. Greenspan SE, Bower DS, Roznik EA, Pike DA, Marantelli G, Alford RA, Schwarzkopf L, Scheffers BR. 2017 Infection increases vulnerability to climate change via effects on host thermal tolerance. *Sci. Rep.* **7**, 9349. (doi:10.1038/s41598-017-09950-3)
  14. Elliot SL, Blanford S, Thomas MB. 2002 Host–pathogen interactions in a varying environment: temperature, behavioural fever and fitness. *Proc. R. Soc. Lond. B* **269**, 1599–1607. (doi:10.1098/rspb.2002.2067)
  15. Vale PF, Stjernman M, Little TJ. 2008 Temperature-dependent costs of parasitism and maintenance of polymorphism under genotype-by-environment interactions. *J. Evol. Biol.* **21**, 1418–1427. (doi:10.1111/j.1420-9101.2008.01555.x)
  16. Mitchell SE, Rogers ES, Little TJ, Read AF. 2005 Host–parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. *Evolution* **59**, 70–80. (doi:10.1111/j.0014-3820.2005.tb00895.x)
  17. Manzi F, Agha R, Lu Y, Ben-Ami F, Wolinska J. 2019 Temperature and host diet jointly influence the outcome of infection in a *Daphnia*–fungal parasite system. *Freshwater Biol.* **65**, 757–767. (doi:10.1111/fwb.13464)
  18. Fels D, Kaltz O. 2006 Temperature-dependent transmission and latency of *Holospira undulata*, a micronucleus-specific parasite of the ciliate *Paramecium caudatum*. *Proc. R. Soc. B* **273**, 1031–1038. (doi:10.1098/rspb.2005.3404)
  19. Shocket MS, Strauss AT, Hite JL, Slijivar M, Civitello DJ, Duffy MA, Cáceres CE, Hall SR. 2018 Temperature drives epidemics in a zooplankton–fungus disease system: a trait-driven approach points to transmission via host foraging. *Am. Nat.* **191**, 435–451. (doi:10.1086/696096)
  20. Shocket MS, Verwillow AB, Numazu MG, Slamani H, Cohen JM, Moustaid EF, Rohr J, Johnson LR, Mordecai EA. 2020 Transmission of West Nile and five other temperate mosquito-borne viruses peaks at temperatures between 23°C and 26°C. *Elife* **9**, 221–267. (doi:10.7554/eLife.58511)
  21. Thomas MB, Blanford S. 2003 Thermal biology in insect–parasite interactions. *Trends Ecol. Evol.* **18**, 344–350. (doi:10.1016/S0169-5347(03)00069-7)
  22. Rohr JR, Cohen JM. 2020 Understanding how temperature shifts could impact infectious disease. *PLoS Biol.* **18**, e3000938. (doi:10.1371/journal.pbio.3000938)
  23. Marcogliese DJ, Pietrock M. 2011 Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends Parasitol.* **27**, 123–130. (doi:10.1016/j.pt.2010.11.002)
  24. Hall MD, Vettiger A, Ebert D. 2012 Interactions between environmental stressors: the influence of salinity on host–parasite interactions between *Daphnia magna* and *Pasteuria ramosa*. *Oecologia* **171**, 789–796. (doi:10.1007/s00442-012-2452-3)
  25. Hofmann GE, Todgham AE. 2010 Living in the now: physiological mechanisms to tolerate a rapidly changing environment. *Annu. Rev. Physiol.* **72**, 127–145. (doi:10.1146/annurev-physiol-021909-135900)
  26. Steiner UK, van Buskirk J. 2008 Environmental stress and the costs of whole-organism phenotypic plasticity in tadpoles. *J. Evol. Biol.* **21**, 97–103. (doi:10.1111/j.1420-9101.2007.01463.x)
  27. Stoks R, Govaert L, Pauwels K, Jansen B, De Meester L. 2015 Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea *Daphnia magna*. *Ecol. Lett.* **19**, 180–190. (doi:10.1111/ele.12551)
  28. Brans KI, De Meester L. 2018 City life on fast lanes: urbanization induces an evolutionary shift towards a faster lifestyle in the water flea *Daphnia*. *Funct. Ecol.* **32**, 2225–2240. (doi:10.1111/1365-2435.13184)
  29. Plaistow SJ, Collin H. 2014 Phenotypic integration plasticity in *Daphnia magna*: an integral facet of G × E interactions. *J. Evol. Biol.* **27**, 1913–1920. (doi:10.1111/jeb.12443)
  30. Adams DC, Collyer ML. 2009 A general framework for the analysis of phenotypic trajectories in evolutionary studies. *Evolution* **63**, 1143–1154. (doi:10.1111/j.1558-5646.2009.00649.x)
  31. Collyer ML, Sekora DJ, Adams DC. 2015 A method for analysis of phenotypic change for phenotypes described by high-dimensional data. *Heredity* **115**, 357–365. (doi:10.1038/hdy.2014.75)
  32. Ebert D. 2005 *Ecology, epidemiology, and evolution of parasitism in Daphnia*. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information. See <http://ncbi.nlm.nih.gov/entrez/query.fcgi?bd=Books>.
  33. Ebert D, Duneau D, Hall MD, Luijckx P, Andras JP, Pasquier DL, Ben-Ami F. 2016 A population biology perspective on the stepwise infection process of the bacterial pathogen *Pasteuria ramosa* in *Daphnia*. *Adv. Parasitol.* **91**, 265–310. (doi:10.1016/bs.apar.2015.10.001)
  34. Clerc M, Ebert D, Hall MD. 2015 Expression of parasite genetic variation changes over the course of infection: implications of within-host dynamics for the evolution of virulence. *Proc. R. Soc. B* **282**, 20142820. (doi:10.1098/rspb.2014.2820)
  35. Hall MD, Ebert D. 2012 Disentangling the influence of parasite genotype, host genotype and maternal environment on different stages of bacterial infection in *Daphnia magna*. *Proc. R. Soc. B* **279**, 3176–3183. (doi:10.1098/rspb.2012.0509)
  36. Hall MD, Mideo N. 2018 Linking sex differences to the evolution of infectious disease life-histories. *Phil. Trans. R. Soc. B* **373**, 20170431. (doi:10.1098/rstb.2017.0431)
  37. Ebert D, Zschokke-Rohringer CD, Carius HJ. 1998 Within- and between-population variation for resistance of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa*. *Proc. R. Soc. B* **265**, 2127–2134. (doi:10.1098/rspb.1998.0549)
  38. Wickham H. 2016 *Ggplot2*, 2nd edn. New York: Springer.
  39. Wilke CO. 2019 Cowplot: streamlined plot theme and plot annotations for 'ggplot2' [R package cowplot version 1.1.0].
  40. Fox J, Weisberg S. 2018 *An {R} companion to applied regression*, 3rd edn. Thousand Oaks, CA: Sage.
  41. Collyer ML, Adams DC. 2007 Analysis of two-state multivariate phenotypic change in ecological studies. *Ecology* **88**, 683–692. (doi:10.1890/06-0727)
  42. Collyer ML, Adams DC. 2018 RRPP: an rpackage for fitting linear models to high-dimensional data using residual randomization. *Methods Ecol. Evol.* **9**, 1772–1779. (doi:10.1111/2041-210X.13029)
  43. Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. 2004 Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.
  44. Auld SKJR, Brand J. 2017 Simulated climate change, epidemic size, and host evolution across host–parasite populations. *Glob. Change Biol.* **23**, 5045–5053. (doi:10.1111/gcb.13769)

45. Allen DE, Little TJ. 2011 Dissecting the effect of a heterogeneous environment on the interaction between host and parasite fitness traits. *Evol. Ecol.* **25**, 499–508. (doi:10.1007/s10682-010-9413-2)
46. Hall SR, Knight CJ, Becker CR, Duffy MA, Tessier AJ, Cáceres CE. 2009 Quality matters: resource quality for hosts and the timing of epidemics. *Ecol. Lett.* **12**, 118–128. (doi:10.1111/j.1461-0248.2008.01264.x)
47. Cuco AP, Castro BB, Gonçalves F, Wolinska J, Abrantes N. 2018 Temperature modulates the interaction between fungicide pollution and disease: evidence from a *Daphnia*–microparasitic yeast model. *Parasitology* **145**, 939–947. (doi:10.1017/S0031182017002062)
48. Cohen JM, Civitello DJ, Venesky MD, McMahon TA, Rohr JR. 2019 An interaction between climate change and infectious disease drove widespread amphibian declines. *Glob. Change Biol.* **25**, 927–937. (doi:10.1111/gcb.14489)
49. Hector TE, Sgrò CM, Hall MD. 2020 The influence of immune activation on thermal tolerance along a latitudinal cline. *J. Evol. Biol.* **99**, e52613. (doi:10.1111/jeb.13663)
50. Hector TE, Sgrò CM, Hall MD. 2021 Data from: Temperature and pathogen exposure act independently to drive host phenotypic trajectories. Figshare Repository. (doi:10.26180/13721266)