Acclimation to warmer temperatures can protect host populations from both further heat stress and the potential invasion of pathogens

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Abstract

Thermal acclimation can provide an essential buffer against heat stress for host populations, whilst acting simultaneously on various life-history traits that determine population growth. In turn, the ability of a pathogen to invade a host population is intimately linked to these changes via the supply of new susceptible hosts, as well as the impact of warming on its immediate infection dynamics. Acclimation therefore has consequences for hosts and pathogens that extend beyond simply coping with heat stress – governing both population growth trajectories and, as a result, an inherent propensity for a disease outbreak to occur. The impact of thermal acclimation on heat tolerances, however, is rarely considered simultaneously with metrics of both host and pathogen population growth, and ultimately fitness. Using the host *Daphnia magna* and its bacterial pathogen, we investigated how thermal acclimation impacts host and pathogen performance at both the individual and population scales. We first tested the effect of maternal and direct thermal acclimation on the life-history traits of infected and uninfected individuals, such as heat tolerance, fecundity, and lifespan, as well as pathogen infection success and spore production. We then predicted the effects of each acclimation treatment on rates of host and pathogen population increase by deriving a host's intrinsic growth rate (r_m) and a pathogen's basic reproductive number (R_0). We found that direct acclimation to warming enhanced a host's heat tolerance and rate of population growth, despite a decline in life-history traits such as lifetime fecundity and lifespan. In contrast, pathogen performance was consistently worse under warming, with within-host pathogen success, and ultimately the potential for disease spread, severely hampered at higher temperatures. Our results suggest that hosts could benefit more from warming than their pathogens, but only by linking multiple individual traits to population processes can the full impact of higher temperatures on host and pathogen population dynamics be realised.

Keywords: thermal limits, host-pathogen interactions, heat stress, *Pasteuria ramosa*, aquatic ectotherm, population growth, fitness, virulence, knockdown times

Introduction

Processes at every level of biological organisation are fundamentally shaped by temperature, from the rate at which physiological processes occur within the body, to the growth and persistence of a population or community (Angilletta et al., 2004; Chown et al., 2010; Colinet et al., 2014; Somero, 2010; Vázquez et al., 2015). This is particularly true for host-pathogen interactions (Thomas & Blanford, 2003). For a host, acclimation to rising temperatures can increase heat tolerance, providing individuals with a crucial buffer against future heat stress (Rohr et al., 2018; Sgrò et al., 2016; Sinclair et al., 2016). Warmer temperatures will also typically accelerate the pace of life, leading to earlier reproductive output and shortened lifespans, and changes to population growth rates as a result (Angilletta et al., 2004; Debecker & Stoks, 2019; Hector et al., 2021). In turn, for a pathogen, warming can accelerate the infection process by increasing within-host replication and virulence (Fels & Kaltz, 2006; S. E. Mitchell et al., 2005; Paull et al., 2015; Vale et al., 2008), thereby influencing the rates at which hosts can be encountered and infected (Kirk et al., 2018; Shocket, Strauss, et al., 2018; Shocket, Vergara, et al., 2018), and even reduce the capacity of hosts to respond to both average temperature shifts and extreme heat (Gehman et al., 2018; Greenspan et al., 2017; Hector et al., 2019; Kunze et al., 2022; Porras et al., 2021; Ware-Gilmore et al., 2021).

The response of hosts and pathogens to increasing temperatures will thus depend on the relative effects of thermal acclimation on thermal tolerances, versus on traits that underlie whether a host or pathogen population will increase or decrease under warming (Gehman et al., 2018; Mordecai et al., 2019; Shocket, Ryan, et al., 2018). An understanding of both the individual and population level performance begins by comparing changes in thermal tolerances (e.g., upper thermal limits or immobilisation times, (Hector et al., 2019, 2020)) with host and pathogen vital metrics. For hosts, life tables can be used to estimate the influence of thermal change on the intrinsic rate of population growth, denoted as r_m, and therefore the temperature which is likely to maximise host fitness (Amarasekare & Coutinho, 2013; Amarasekare & Savage, 2012). In contrast, the ability of a pathogen to persist in a host population can be influenced by both host population dynamics and pathogen transmission (Aulsebrook et al., 2023; Civitello et al., 2013; S. R. Hall et al., 2009; Mordecai et al., 2019). Estimates of infection success, proliferation, and the supply of new susceptible hosts can be integrated via an epidemiological model into the basic reproduction number, R_0, which captures the potential of a pathogen to spread through a completely

susceptible host population (Anderson & May, 1986), and its dependence on changing temperatures (Mordecai et al., 2019; Shocket, Ryan, et al., 2018; Shocket, Strauss, et al., 2018).

Despite the importance of thermal tolerances in shaping host and pathogen geographic distributions (e.g., (Blanford et al., 2013; Mordecai et al., 2019; Rohr & Raffel, 2010; Shocket, Ryan, et al., 2018)), the impact of thermal acclimation on heat tolerances is rarely considered in unison with metrics of how well both host and pathogen populations might perform, such as r_m and R_0 (but for a non-disease example, see (Cavieres et al., 2020)). More commonly, changes in thermal tolerances are correlated with the variation induced in an individual's phenotype, such as how temperature impacts host development, fecundity, survival, or immunity, or how temperature alters pathogen proliferation or virulence (e.g., (Fels & Kaltz, 2006; Hector et al., 2019; Laidlaw et al., 2020; S. E. Mitchell et al., 2005; Paull et al., 2015; Raffel et al., 2006, 2013, 2015; Sun et al., 2022; Vale et al., 2008)). Although population-level processes intrinsically depend on these individual responses to temperature, one does not necessarily predict the other (M. D. Hall & Mideo, 2018; Mideo et al., 2008; Penczykowski et al., 2016). Even where one or more measures of host or population growth are available, understanding the potential spread of the pathogen is often the primary goal and more emphasis is thus placed on R_0 or related metrics (Beck-Johnson et al., 2017; Kirk et al., 2018; Mordecai et al., 2019; Paaijmans et al., 2009; Shocket et al., 2019; Shocket, Vergara, et al., 2018); but see (Gehman et al., 2018; Shocket, Ryan, et al., 2018)), rather than an explicit comparison of how warming shapes host and pathogen population growth rates and the temperature most likely to maximise fitness in each (sensu (Amarasekare & Coutinho, 2013; Amarasekare & Savage, 2012)).

To add complexity to our understanding of thermal change and host-pathogen interactions, an individual's prior thermal exposure can have a greater influence on fitness than contemporary temperatures. In many species, maternal and developmental acclimation are a vital mechanism for preparing populations to cope with future environmental conditions (Beaman et al., 2016; Hoffmann et al., 2012; Sgrò et al., 2016). In *Drosophila melanogaster*, for example, the temperature experienced during development can have an overriding effect on adult heat tolerance (Kellermann et al., 2017; Slotsbo et al., 2016). Temperature can also impact disease-related traits across generations and infection cycles. Host resistance to infection and the infectivity of pathogen spores have both been shown to increase when past generations experienced warmer conditions (Ferguson & Sinclair, 2019; Garbutt et al., 2014; Paull et al., 2015; Shocket, Vergara, et al., 2018; Sun et al., 2022). It remains unclear, however, how various host and

pathogen fitness components, and therefore population level dynamics, will be shaped by the thermal acclimation of a host before it encounters a pathogen.

In this study, we contrasted how thermal acclimation, before and during infection, shapes both host heat tolerance and the resulting life-history of the host and pathogen. We then expanded our view to consider host population growth and the potential spread of a pathogen. To address these questions, we used the water flea *Daphnia magna* and its bacterial pathogen *Pasteuria ramosa*. *Daphnia* are able to mount strong plastic thermal acclimation responses in their heat tolerance (Burton et al., 2020; Yampolsky et al., 2014), and temperature can mediate their response to infection (Auld & Brand, 2017; Garbutt et al., 2014; Kirk et al., 2018; Kunze et al., 2022; Shocket, Vergara, et al., 2018), and any damage that may follow (Hector et al., 2021). *Pasteuria ramosa* is a natural bacterial pathogen of *Daphnia*, which has distinct genotypes known to vary in various aspects of within-host performance, including infection rates and spore production (Clerc et al., 2015; M. D. Hall & Mideo, 2018), both of which have been shown to be sensitive to temperature stress (Vale et al., 2008; Vale & Little, 2009).

We considered two forms of thermal acclimation (at 25°C) versus a standard temperature (at 20°C): i) a maternal and developmental acclimation treatment that occurred prior to infection, and ii) a direct thermal acclimation treatment that was applied to focal individuals from the infection period onwards. Under combinations of both acclimation treatments (herein maternal and focal), we measured individual-level traits including host heat tolerance (assessed as knockdown time under heat shock, see (Hector et al., 2019), host lifespan, and the fecundity of infected and uninfected *Daphnia*, as well as within-host pathogen spore loads and infection success for each pathogen genotype. From our experiment we then used host life table data (lifespan and fecundity) to evaluate potential host population dynamics, and then parameterised an epidemiological model to estimate a metric for the potential for disease spread through a host population (Aulsebrook et al., 2023; Civitello et al., 2013; Shocket, Vergara, et al., 2018). Together, these measures allow us to contrast how thermal acclimation can impact host and pathogen performance at an individual scale, and, in turn, how they may drive population dynamics.

Methods

Host and pathogen

The cyclically parthenogenic crustacean *Daphnia magna* Straus is commonly found in both fresh and brackish waters, including shallow pools and large lakes, across Eurasia. *Pasteuria ramosa*

Metchnikoff is a Gram-positive bacterial pathogen of *D. magna* that enters the host during filter feeding, before severely reducing host fecundity (via castration) and lifespan (Clerc et al., 2015; Ebert et al., 2016; M. D. Hall et al., 2019). At host death millions of spores are released into the environment where exclusively horizontal transmission takes place, which itself depends on the interplay between the pathogen's ability to produce mature transmission spores and its virulence (M. D. Hall & Mideo, 2018). In this study we used *Daphnia* genotype BE-OMZ-M10 infected with one of three *P. ramosa* genotypes (C1, C14 and C20). These pathogen genotypes were chosen because they display genetic variation in their virulence and transmission potential (Clerc et al., 2015; M. D. Hall & Mideo, 2018), and in the extent to which they reduce host heat tolerances (Hector et al., 2019).

Before the experiments, female *Daphnia* taken from stock culture were placed individually in 70-mL jars filled with 50 mL of Artificial *Daphnia* Medium (ADaM; following (Ebert et al., 1998)) for three generations to minimise trans-generational effects. *Daphnia* were changed into fresh ADaM twice a week and fed with algae (*Scenedesmus sp.*) daily. To meet the growing energy needs of the animals, food levels were increased from one million cells per jar at birth, to eight million by age 14 days. *Daphnia* were maintained under standard conditions (20°C, 16L:8D) and repositioned within the incubator regularly to minimise any positional effects.

Experimental animals, thermal acclimation, and infection

Thermal acclimation began in the maternal generation. On the day of birth, maternal generation (F0) *Daphnia* were taken from clutches 3–5 of the standardised animals and maintained individually at either 20°C or 25°C (maternal/developmental temperature treatment, hereafter maternal acclimation). Experimental (F1) *Daphnia* were then collected from clutches 3–5 of the acclimated mothers on the day of birth and placed at either 20°C or 25°C (focal acclimation) in a fully factorial design, resulting in four thermal acclimation treatments (20-20, 20-25, 25-20 & 25-25°C). The maternal acclimation involved maternal and developmental effects (because *Daphnia* are ovoviviparous), whilst the focal acclimation was experienced directly by experimental animals. Experimental animals were kept at their focal acclimation temperatures from birth until either being used in heat tolerance assays or until death, including over the infection period. The warm acclimation temperature was chosen as it is ecologically realistic for the higher temperatures experienced in summer in *Daphnia* populations (Yampolsky et al., 2014) and below the thermal maxima for both the host and pathogen (Hector et al., 2019; Kirk et al., 2018).

The experimental generation included a total of 1008 female *Daphnia* with 63 individuals per treatment, in a fully factorial design (2 maternal temperatures x 2 focal temperatures x [3 pathogens + uninfected controls]). For infection, individual *Daphnia* were exposed to 40,000 *P. ramosa* spores starting three days after birth. Pathogen exposure took place in 70-mL jars filled with 20 mL of artificial media for three days, after which all animals were transferred to 50 mL fresh media and maintained as described above.

Heat tolerance assays

Static heat shock assays were used to measure the heat tolerance, quantified via knockdown times, of *Daphnia* from all treatments described above. Knockdown times measure the capacity of an animal to avoid physical incapacitation during thermal extremes (Hector et al., 2019; K. A. Mitchell & Hoffmann, 2010). Individual *Daphnia* were placed in 5-mL glass fly vials covered in mesh and immersed in a constantly agitated water bath filled with media and set to 37°C, which is an acute heat stress that is lethal to animals after several hours or less (Hector et al., 2019; Yampolsky et al., 2014). Starting from when they were first placed in the water bath, time until knockdown was recorded for each *Daphnia* when there was no visible movement (Hector et al., 2019; Yampolsky et al., 2014). A total of 36 *Daphnia* per treatment were chosen at random to measure heat tolerance. Three individuals per treatment could be measured per assay run, so 12 runs were conducted over three consecutive days. All animals were between 19- and 21-days post-infection at the time of the assays.

Measuring the characteristics of individual hosts and pathogens

Daphnia that were not used in the heat tolerance assays were kept at their respective focal acclimation temperatures until death. From birth, these animals were checked daily for deaths, and any dead animals were frozen in 500 µL of RO water for later bacterial spore counting (see below). Offspring were counted and removed twice weekly for all experimental individuals. This gave us four important metrics of individual host and pathogen performance for each temperature by pathogen treatment combination: host lifespan, host age-specific fecundity, pathogen spore loads at host death, and infection rates.

Bacterial spore counts were quantified using an Accuri C6 Flow Cytometer (BD Biosciences, San Jose, California). Infected animals were thawed and homogenised in 500 µL of RO water. Then, 10 µL of this sample was pipetted into 190 µL of 5mM EDTA in a 96-well plate. A combination of gates based on fluorescence (via the 670 LP filter) and side scatter (cell granularity) were used to identify mature spores based on their distinct size, morphology, and fluorescence, compared to immature

spores, algae, or animal debris. Each sample was counted twice, and the average used to calculate total spore load per infected individual.

Analysis of individual-based metrics

All analyses were conducted in R (v. 3.6.2; R Development Core Team, www.R-project.com). For all traits, maternal acclimation temperature (2 levels: 20°C or 25°C), focal acclimation temperature (2 levels: 20°C or 25°C), pathogen treatment (4 levels: pathogen genotype C1, C14 and C20, or uninfected controls) and their interactions were fitted as fixed effects and analysed via an analysis of variance (ANOVA Type III; *car* package: (Fox & Weisberg, 2011)). For this analysis, age-specific fecundity data were summed to generate a metric of lifetime fecundity for each individual host. Due to differences in early survival, handling errors, and male individuals set up unintentionally, sample sizes for the different treatment combinations and disease traits varied between 17 and 26. All exposed individuals were included in the analysis of host traits in order to capture the changes in a host's phenotype that can manifest even when infection isn't ultimately "successful" for the pathogen in producing mature transmission spores (Butterworth et al., 2024; M. D. Hall et al., 2024).

For heat knockdown times used a linear mixed effect model (*nlme* package; (Pinheiro & Bates, 2000) with assay run treated as a random effect. We allowed residual variance to vary independently at the level of the focal acclimation temperature to account for heteroscedasticity using the '*VarIdent*' function. Least-squared linear models were then used to analyse host lifespan, host lifetime fecundity, and pathogen spore loads, with host lifespan and fecundity both log-transformed before analysis. For spore loads, we additionally used a white-corrected analysis of variance to account for residual heteroscedasticity. Finally, for infection rates (i.e., the probability that each pathogen genotype would infect and go on to produce mature transmission spores), we used a binomial generalized linear model, with mean infection rates and standard errors extracted using *emmeans* package (see github.com/rvlenth/emmeans).

Modelling host population growth in the absence of the pathogen

To quantify the impact of thermal acclimation on the population growth potential of the host, we calculated the intrinsic rate of increase (r_m) using lifespan and age-specific fecundity data from the unexposed (i.e., control) animals in each treatment (Fig. S1). Following the methodology of Shocket *et al.* (2018) we used a simplified version of the Euler-Lotka equation to calculate the intrinsic rate of increase for each individual (rather than for each whole population),

$$1 = \sum_t e^{-r_m t} l_t F_t \quad (1)$$

where for each single individual, l_t (i.e., the proportion of individuals in a cohort surviving to day *t)* always equals 1, while the animals remain alive, and F_t is the fecundity of each individual at day *t*.

From these data, we also estimated additional demographic metrics that play an important role in understanding the likely spread of the pathogen, which assume the density-dependence of host birth rates (see below). We first calculated the instantaneous death rate (*d*) for our control hosts in each temperature treatment assuming time until death followed an exponential distribution, where the likelihood of a constant death rate (*d*) is calculated from our time until death (lifespan) data under each temperature treatment (Civitello et al., 2013; Shocket, Strauss, et al., 2018) as per

$$\ell(d|t_d) = de^{-dt_d}. \quad (2)$$

We then estimated the birth rate (*b*) of hosts as the sum of their intrinsic rate of increase (r_m) and death rate (*d*) for the control animals (where $b = r_m + d$, (Civitello et al., 2013; Shocket, Strauss, et al., 2018)).

Modelling the spread and population growth of the pathogen

For a pathogen, an analogous measure to the hosts intrinsic rate of increase (r_m) is the basic reproduction number, R_0, which informs us about a pathogens potential to spread through an entirely susceptible population (Anderson & May, 1986). Larger values of R_0 suggest the potential for larger epidemics. For the *Daphnia-Pasteuria* disease system, R_0 can be derived from a compartmental model that tracks changes in the density of susceptible hosts, infected hosts, and environmental pathogen spores ((S. R. Hall et al., 2009) see also (Aulsebrook et al., 2023; Civitello et al., 2013). From this model, R_0 is calculated as

$$R_0 = \left(\frac{b-d}{bc}\right)\left(\frac{\sigma\beta}{m}\right) \quad (3)$$

which is conditional on the density-dependent dynamics of the host population in the absence of disease, $(b-d)/(bc)$, and three epidemiological traits, $(\sigma\beta/m)$. R_0 will increase if there are increases in host birth rate, *b*, environmental transmission rate, β, or pathogen spore loads, σ. R_0 decreases if there are increases in host death rate, *d*, the rate of pathogen loss from the environment, *m*, or the strength of density-dependence on population growth, *c* (Civitello et al., 2013; S. R. Hall et al., 2009).

Parameterization of host and pathogen rates of increase

For all measures of host and pathogen population growth (r_m and R_0), we used JAGS as implemented in R (*R2jags* package: (Su & Yajima, 2009) to calculate Bayesian posterior distribution estimates for each underlying trait, following equations 1 and 3 above. Our standard JAGS settings included 75000 iterations, 30000 burn-in, thinning of 16, and 3 individual chains. We used semi-informative priors and set the Bayesian posteriors to follow the appropriate distributions for each trait following Shocket et al (2018). To calculate R_0, we first estimated the instantaneous death rate for each treatment (d), following equation 2, and used this to estimated instantaneous birth rates (b) for each acclimation treatment under the assumption that r_m equals the difference between instantaneous birth and death rates (($r_m = b - d$, (McCallum, 1999)). We then estimated environmental transmission rates (β) using the numbers of infected and uninfected individuals from each temperature and pathogen treatment using a binomial distribution in a likelihood function to model the number of uninfected hosts in each jar, where the probability of remaining uninfected (P) is

$$P = e^{-\beta Z t} \quad (2)$$

where Z is the density of pathogen spores and t is the length of the infection period (see (Shocket, Strauss, et al., 2018) and the supplementary material therein for details of how this likelihood function is derived). In our estimates of environmental transmission rate (β), individuals were only scored as being infected if they became infected and went on to produce mature transmission spores. For the Bayesian estimate of environmental transmission rate (β) and the GLM for infection probability, one treatment achieved a 100% infection rate in our experiment (pathogen C20, temperature treatment 20°C and 20°C), so to allow more reasonable point estimates and error to be calculated we adjusted this treatment to include one uninfected individual.

Finally, to calculate R_0 for each pathogen and temperature treatment, we incorporated the Bayesian posterior estimates of each of the estimated parameters into our derived equation for R_0 (eqn. 1). By incorporating the posterior estimates for each calculated trait in turn, we allowed the propagation of error in our estimates of each trait into our final estimates of the potential for disease spread, R_0. Two parameters that contribute to our indicator of the potential for disease spread (R_0), the strength of density-dependence on birth rates, c, and spore degradation rate, m, were set as constants for all treatments (c = 0.01 and m = 0.9, see (Civitello et al., 2013; Shocket, Vergara, et al., 2018). Whilst it is conceivable that temperature will alter the strength of density-dependence and spore degradation, and the population dynamic of the host and pathogen in nature, neither was possible to quantify in these experiments (but see (Shocket et al., 2019)).

Results

Acclimation improves thermal tolerance for both uninfected and infected hosts

Individuals directly exposed to 25°C showed a clear improvement in knockdown times, regardless of whether they were infected by a pathogen or not (Fig. 1 and Table 1). For example, both control and infected individuals exposed to a focal temperature of 25°C saw a two-fold increase in knockdown times compared to hosts acclimated to 20°C. However, infection by a pathogen significantly reduced the thermal tolerance of a host compared to controls, but only in the focal 25°C treatments (Fig. 1, and a significant focal by pathogen treatment interaction in Table 1). Here the influence of maternal acclimation also become most apparent, with the relative difference between the control and pathogen treatment groups depending on the maternal thermal history (20°C or 25°C), leading to a three-way interaction between maternal temperature, focal temperature, and pathogen treatment in shaping overall knockdown times (Table 1).

Warmer temperatures decrease host and pathogen individual performance

Host lifespan and lifetime fecundity were considerably lower at warm focal temperatures (Fig. 2A, B). Uninfected hosts, for example, saw almost a fifty percent reduction in both traits at 25°C compared to their counterparts at 20°C. Pathogen exposure severely reduced both traits at each acclimatisation temperature, however, the greatest difference between healthy and infected hosts occurred at the standard focal temperatures (20°C), accounting for the significant interaction between pathogen exposure and focal temperature treatments (Table 1). Maternal acclimation prior to infection also subtly modified this interaction, contributing to the three-way interactions for both traits (Table 1), as the difference between healthy and infected hosts was smallest when both maternal and focal acclimation occurred at 25°C (Figure 2A, B).

For the pathogen, the probability of successfully infecting a host, and the resulting production of mature transmission spores, was also reduced when directly acclimated to warmer temperatures (Fig. 2C, D). However, variation in the probability of infection depended on a significant three-way interaction between both acclimation treatments and pathogen genotype (Table 1). Infection rates were significantly lower for individuals reared directly at a 25°C and it is at this temperature that differences in infection rates emerged between the pathogen genotypes. Yet the pathogen that performed best or worse depended on the maternal acclimation temperature. At a focal temperature of 25°C, for example, pathogen C20 had the lowest infection success when maternal acclimation occurred at 20°C but outperformed all other pathogen genotypes when mothers were

311 acclimated at the warmer temperature (Fig. 2C). In contrast, mature spore loads were determined
312 by the independent effects of focal temperature and pathogen genotype (Table 1). Overall, we
313 saw a reduction in mature transmission spores under 25°C focal acclimation, with pathogen
314 genotype C1 generally producing more spores than C14 and then C20 (Fig. 2D).

315 **Thermal acclimation has opposing effects on host and pathogen population growth**

316 To predict the likely rate of population growth for the host under the different acclimation
317 treatments, we incorporated the age-specific fecundity and lifespan of each control individual into
318 an estimate of the intrinsic rate of increase (r_m). In stark contrast to the negative influence of
319 warmer temperatures on the fecundity and lifespan on individuals (Fig. 2A, B), we found that
320 direct acclimation to temperatures promoted a statistically clear increase in r_m values (Fig. 3A,
321 non-overlapping 95% CIs). Maternal acclimation led to a slight decrease in r_m for individuals
322 experiencing 25°C for both the maternal and focal acclimation treatments. Overall, the potential
323 for population growth appears to be maximised by the acceleration of early reproduction when
324 the host directly experienced warmer temperatures (see also Fig. S1).

325 In contrast, when we estimated the pathogen's potential to spread in a susceptible population
326 (R_0), and thus increase in population size, we saw a considerable decrease in R_0 when infecting
327 hosts directly acclimated to 25°C (Fig. 3B, focal 25°C). Indeed, for most pathogen genotypes, the
328 potential for disease spread was around an order of magnitude lower (with separation of 95% CIs),
329 when the focal temperature experienced was 25°C compared to 20°C. As above, the maternal
330 acclimation temperature experienced by hosts – before they encountered any pathogens – most
331 notably influenced the rank order of R_0 across pathogen genotypes when infections subsequently
332 took place at 25°C (Fig. 3B).

333 This severe reduction in R_0 at warmer focal temperatures appears to be driven entirely by the
334 effects of temperature on pathogen environmental transmission rates and spore production.
335 Environmental transmission rates (which capture the rate at which host become infected via the
336 ingestion of free-living spores from the environment) and spore loads (which fuel the free-living
337 spores pool) were generally higher when infections took place at 20°C, compared with 25°C (Fig.
338 3D, F). The contributions of an increased supply of susceptible hosts, on which R_0 also depends
339 (see Eqn. 3), did not offset these within-host disadvantages. Despite the sensitivity of the host's
340 intrinsic rate of increase to temperature (r_m, Fig. 3A), the slight increase in death rates associated
341 with warmer focal temperatures (Fig. 3C) was not sufficiently strong enough to alter the rate at
342 which carrying capacity might be reached (i.e., (b – d)/b, Fig. 3E). Thus, the density-dependent

dynamics of the susceptible host population and the relative contribution of births and deaths in our model of disease spread, (b – d)/b, remain unaffected by thermal acclimation and unable to compensate for the poorer within-host performance of the pathogen at 25°C.

Tables and figures

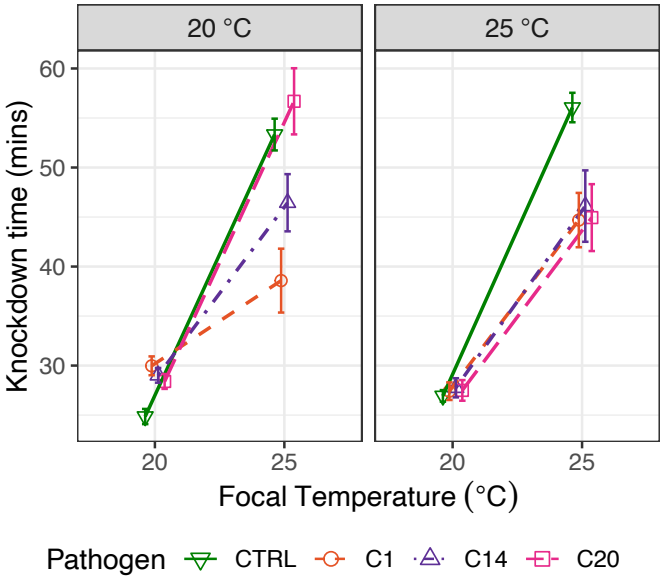

Figure 1: The effect of thermal acclimation on heat knockdown times. Knockdown time was measured for *Daphnia* infected with one of three pathogen genotypes (C1, C14 or C20) or uninfected (CTRL). Each facet represents the maternal acclimation temperature treatment pre-infection, while the focal temperature was experienced by experimental animals from birth, including over the duration of the infection. Points represent treatment means (± SE).

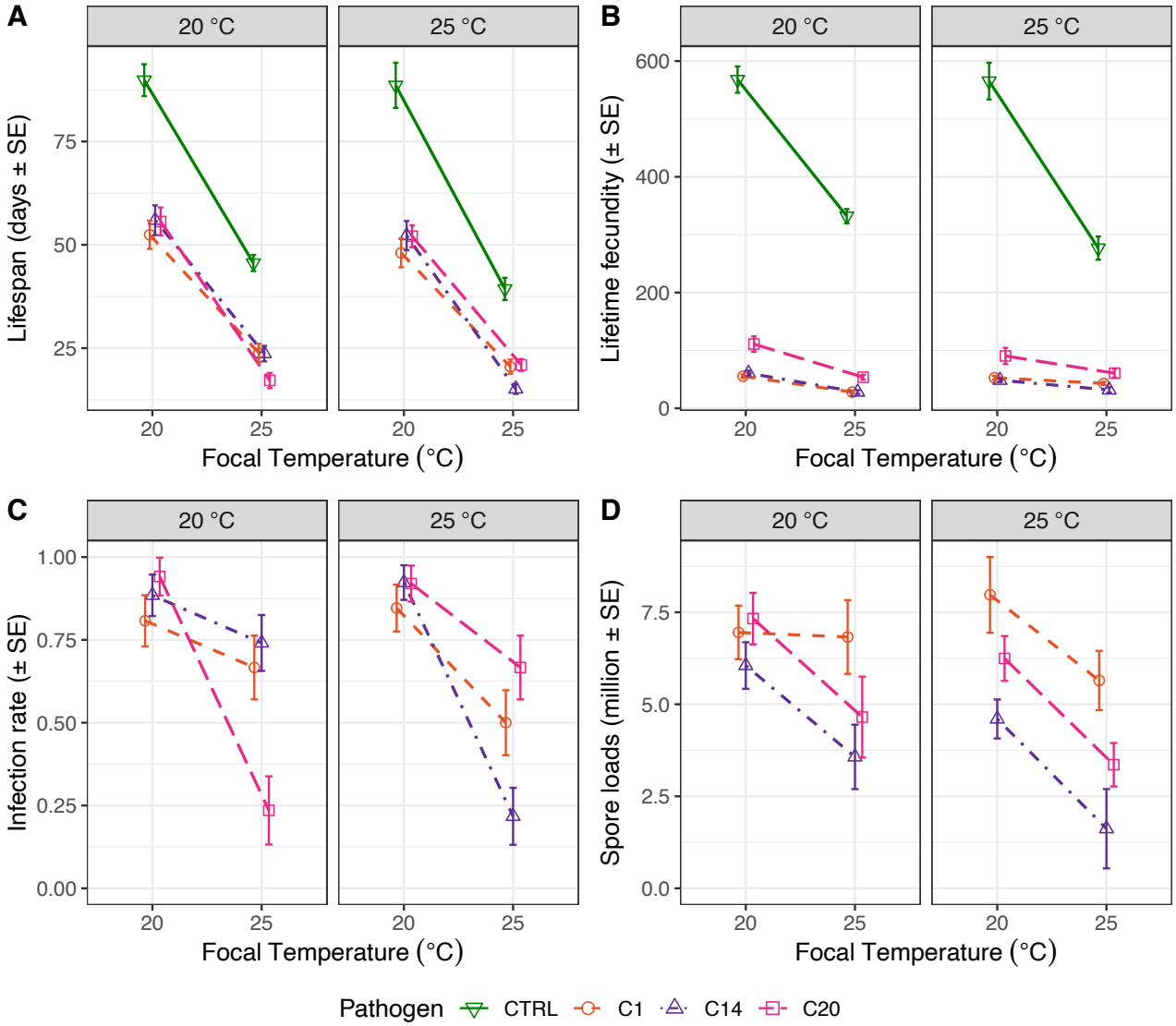

Figure 2: The effect of thermal acclimation and infection on host and pathogen life-history traits as measured for *Daphnia* exposed to one of three pathogen genotypes (C1, C14 or C20) or maintained as unexposed control animals (CTRL). Shown are the treatment means (± SE) for A) host lifespan, B) host fecundity over their lifetime, C) the proportion of hosts infected by the pathogen, and D) the subsequent production of mature spores at host death. Each facet represents the maternal thermal acclimation temperature. The focal temperature was experienced by experimental animals from birth, including over the duration of the infection.

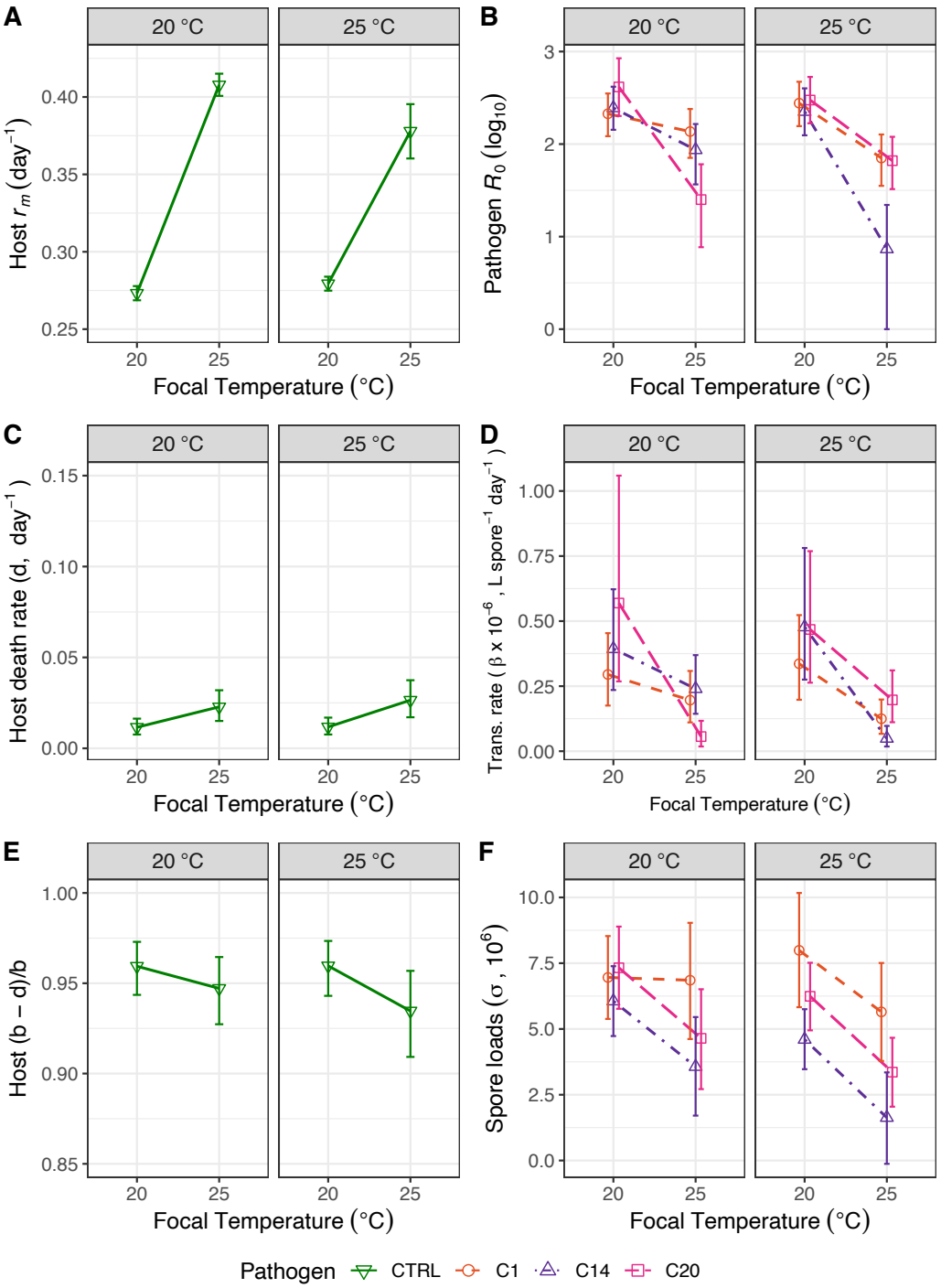

Figure 3: The effect of thermal acclimation on the predicted growth of an unexposed host (r_m) and pathogen (R_0) populations. Shown are the treatment means and 95% credible intervals for A) the host's intrinsic rate of increase (r_m), B) the pathogen's basic reproductive number (R_0), as well as the key parameters required for estimating R_0, including C) death rates of unexposed hosts, D) environmental transmission rates for hosts encountering spores in the environment E) the density-dependent control of population growth for unexposed hosts (i.e., (b-d)/b), , and F) spore loads at host death. For the interrelated metrics of the unexposed host population (A, C, E, in green), we maintained a relatively consistent y-axis scale in each case to facilitate relative effect size comparisons.

Table 1: The effects of maternal thermal acclimation (20°C or 25°C), focal acclimation (20°C or 25°C), pathogen treatment (Controls, C1, C14 or C20) and all interactions on A) host knockdown times under 37°C static heat shock, B) host lifespan (log-transformed), C) host lifetime fecundity (log-transformed), E) pathogen infection success probability, and F) pathogen spore loads.

Term	*F or χ2*	*df*	*p*-value
A) Knockdown times			
Maternal acclimation	0.513	1	0.474
Focal acclimation	399.944	1	**< 0.001**
Pathogen treatment	14.683	3	**0.002**
Maternal x Focal	0.005	1	0.945
Maternal x Pathogen	11.090	3	**0.011**
Focal x Pathogen	32.550	3	**< 0.001**
Maternal x Focal x Pathogen	11.465	3	**0.009**
B) Host lifespan			
Maternal acclimation	7.028	1, 375	**0.008**
Focal acclimation	605.015	1, 375	**< 0.001**
Pathogen treatment	91.240	3, 375	**< 0.001**
Maternal x Focal	0.613	1, 375	0.434
Maternal x Pathogen	2.956	3, 375	**0.032**
Focal x Pathogen	4.837	3, 375	**0.003**
Maternal x Focal x Pathogen	3.160	3, 375	**0.025**
C) Host lifetime fecundity			
Maternal acclimation	0.263	1, 375	0.608
Focal acclimation	156.177	1, 375	**< 0.001**
Pathogen treatment	729.784	3, 375	**< 0.001**
Maternal x Focal	6.324	1, 375	**0.012**
Maternal x Pathogen	3.590	3, 375	**0.014**
Focal x Pathogen	1.241	3, 375	0.295
Maternal x Focal x Pathogen	4.257	3, 375	**0.006**
D) Pathogen infection success			
Maternal acclimation	0.135	1	0.714
Focal acclimation	49.42	1	**< 0.001**
Pathogen treatment	0.292	2	0.864
Maternal x Focal	0.530	1	0.466
Maternal x Pathogen	3.005	2	0.223
Focal x Pathogen	4.573	2	0.102
Maternal x Focal x Pathogen	7.445	2	**0.024**
E) Pathogen spore loads			
Maternal acclimation	3.797	1, 192	*0.053*
Focal acclimation	19.539	1, 192	**< 0.001**
Pathogen treatment	10.445	2, 192	**< 0.001**
Maternal x Focal	0.913	1, 192	0.340
Maternal x Pathogen	0.846	2, 192	0.431
Focal x Pathogen	0.958	2, 192	0.386
Maternal x Focal x Pathogen	0.357	2, 192	0.700

Discussion

Acclimation to warmer temperatures can allow individuals to shift their thermal optima or maxima, potentially acting as a buffer against future heat stress (Rohr et al., 2018; Sgrò et al., 2016; Sinclair et al., 2016), with smaller organisms, such as parasites and pathogens, potentially benefiting more from this process owing to their smaller size (Rohr et al., 2018). We found that warm acclimation improved the heat tolerance of both healthy and pathogen-exposed hosts (Fig. 1). The increase in knockdown times for warm acclimated *Daphnia* was equal, if not greater, than the variation in heat tolerance found across various geographically widespread species (Hector et al., 2020; Hoffmann et al., 2012; Lasne et al., 2018; Lush et al., 2023; Sgrò et al., 2010; Yampolsky et al., 2014). Infected hosts at warmer conditions were also more heat-resistant than both healthy and infected *Daphnia* acclimated to lower temperatures, despite pathogen exposure reducing host heat tolerance at warm temperatures (Fig. 1). Thermal acclimation, therefore, appears to better prepare both infected and uninfected animals for the pressure of extreme heat events through plastic shifts in their thermal performance.

For both the host and pathogen, however, the improvement in heat tolerance came with a significant cost to other measures of individual performance. Exposure to 25°C led to substantial reductions in the lifespan and lifetime fecundity of both healthy and infected individuals (Fig. 1B-C). Simultaneously, at warmer temperatures, within-host pathogen performance was also reduced, as both the probability of infection success and within-host spore loads substantially dropped at 25°C. A decline in infection rates or pathogen load at similar temperatures has been previously observed in studies of different pathogens in *Daphnia* hosts (Kirk et al., 2018; Vale & Little, 2009), as well as in other host-pathogen or vector models (Fels & Kaltz, 2006; Gehman et al., 2018; Mordecai et al., 2019; Raffel et al., 2013, 2015), suggesting that 25°C may lie above the thermal optima for infection success and spore loads in this system (c.f., (Agha et al., 2018; Shocket et al., 2019)). Our results thus highlight how thermal acclimation can have opposing effects on thermal stress resistance versus the other components of host and pathogen life-history (e.g., (Cavieres et al., 2020)).

This contrast between the response of heat tolerance versus other traits indicates that the damage a pathogen causes is context-dependent, and not purely predicted by temperature. The decline in host fitness due to infection, known as virulence, is normally assessed in terms of reductions in lifespan or reproduction (Cressler et al., 2016; Day, 2002). In this context, we found the greatest reductions in lifespan and fecundity relative to uninfected hosts, and thus virulence,

occurred at lower temperatures. Individuals exposed to a pathogen at 20°C, for example, experienced a reduction in lifespan of approximately 15 days greater than that experienced at the higher temperature (Fig 2B). In contrast, individuals acclimated at 25°C experienced up to a 15-minute reduction in their knockdown times due to infection (Fig 1). These results highlight how warming can increase one aspect of pathogen virulence via a loss of heat tolerance, but negate others related to a host's life-history. In the context of escalating heat events the most crucial component of a pathogen's virulence may well be these change made to a host's heat tolerance (Hector et al., 2023).

Our results so far suggest that improved heat tolerance under warming temperatures comes with costs to the individual performance of both hosts and pathogens. Yet, as we show, individual performance metrics can be misleading when population persistence instead depends on vital rates such as growth rates for a host population or the between-host spread of a pathogen (i.e., (Mideo et al., 2008). For the host, the reduction in lifetime fecundity and lifespan (with an associated increase in intrinsic death rate, Fig. 3C) was typical of warmer temperatures increasing "pace of life" traits (see also (Adamo & Lovett, 2011; Aulsebrook et al., 2022; Debecker & Stoks, 2019; Hector et al., 2021)). As a result, warming favoured earlier reproduction and led to an increase in the intrinsic rate of increase (r_m) of the host population, rather than coming with a cost to population growth. The temperature at which r_m is highest is considered to be the optimal temperature for fitness (Amarasekare & Coutinho, 2013; Amarasekare & Savage, 2012). Acclimation to warmer temperatures thus improves host fitness both in terms of thermal tolerance and population growth, despite negatively affecting the expression of key life-history traits at the level of the individual.

In contrast to the host, warmer temperatures reduced the capacity of a pathogen population to expand, as the basic reproduction number, R_0, for each pathogen was around an order of magnitude lower at 25°C compared to 20°C (Fig. 3B). The decline in the capacity of the pathogen to spread between hosts was driven entirely by the reductions in environmental transmission rates (Fig. 3D) and the production of spores at host death (Fig. 3F). Lower spore loads were expected as an increase in pace-of-life cuts short the duration that a pathogen can proliferate within a host (Clerc et al., 2015; Gipson et al., 2019; M. D. Hall & Mideo, 2018). However, feeding rates were expected to increase with temperature, driving higher contact rates between hosts and pathogens, and thus higher infection rates (Shocket et al., 2019; Shocket, Strauss, et al., 2018; Shocket, Vergara, et al., 2018). Warmer temperatures may instead have afforded a host an

improved immune response ((Adamo & Lovett, 2011; Ferguson et al., 2016); but see (Raffel et al., 2006), or otherwise reduced per spore infectivity via an unknown mechanism. Changes in the supply of susceptible hosts did not offset these disadvantages. The relative combination of birth and death rate in our model meant that the control of density-dependent population growth for susceptible hosts, (b-d/b) was equivalent across all temperatures (as indicated by overlapping credible intervals in Fig. 3C).

Finally, our results suggest that when a host is faced with a pathogen, contemporary temperatures experienced during infection may swamp any carryover effects of maternal or developmental acclimation (e.g., (Sun et al., 2022)). This contrasts with terrestrial insects such as *Drosophila* where developmental temperatures drive heat tolerance and fitness (Kellermann et al., 2017; Slotsbo et al., 2016). Here, the maternal thermal environment appears to play a more subtle role in shaping which pathogen genotype performs best at any given temperature (Table 1). The largest effects of maternal acclimation in this context were revealed when the offspring of 20°C acclimated mothers subsequently experienced warm focal conditions (i.e., the 20°C maternal and 25°C focal combination). For example, the relative impact of pathogen exposure on host thermal tolerance varied across maternal acclimation treatments (Fig. 1), as did the rank order of pathogen genotypes in their infection success (Fig. 3F) and, as a result, R_0 (Fig. 3B). Prior thermal environments, via maternal or developmental host effects, may thus have the potential to maintain genetic variation in pathogen populations via changes to both within- and between-host infection dynamics (Fels & Kaltz, 2006; Garbutt et al., 2014; Vale et al., 2008; Vale & Little, 2009).

In conclusion, we have shown that acclimation at warmer temperatures can buffer both hosts and pathogens alike against further heat stress, by improving the thermal tolerance of both uninfected and infected hosts – but this comes with a cost to individual trait performance. For hosts, warming caused severe reductions to overall lifespan and fecundity, but by accelerating the pace-of-life of the host, facilitated an overall increase in predicted rates of population growth and thus ultimately fitness (Amarasekare & Coutinho, 2013; Amarasekare & Savage, 2012). The outlook for a pathogen under warmer temperatures may be bleaker. Within-host pathogen success, and ultimately the potential for disease spread, was severely hampered at warmer temperatures. If true for other species, hosts may hold an advantage over pathogens in warmer and more variable environments, both in terms of their heat tolerance and the capacity to maintain stable populations (but see Shocket et al. 2019). Projections for the eco-evolutionary dynamics of host-pathogen systems could benefit from considering the joint impacts of warming and infection on multiple host and

pathogen traits, and how individual-level traits link to population processes (Kirk et al., 2018; Mordecai et al., 2019; Shocket et al., 2019)

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