

How robust are signals of local adaptation? Exposure to a common fungal pathogen does not disrupt clinal trends in host thermal tolerance

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ABSTRACT

The adaptation of populations to their local thermal environment often gives rise to latitudinal clines in innate thermal tolerance, where low-latitude populations are more tolerant of the heat. These clinal signals can be used to identify at-risk populations under scenarios of warming, but only if coinciding challenges, such as food stress or infection, do not modify or erode the rate at which thermal tolerances have been shown to change across geographic space. Here we explored how a fungal pathogen might affect the thermal tolerance of a freshwater organism, *Daphnia carinata*, sampled from populations along a latitudinal gradient, and in the presence and absence of warming. We predicted that infection would reduce a host's thermal tolerance in a population-specific manner, altering or eroding clinal signals, and that infection would also reduce any capacity to acclimate to warmer conditions. However, while infection reduced host knockdown times by approximately 2 min on average, this effect was consistent across hosts, and the latitudinal cline in thermal tolerance remained robust to infection. Acclimation to warming also increased thermal tolerances, particularly for low-latitude clones, and thus enhanced the strength of the clinal signal, but again this plastic response was also not limited by infection. Our results suggest that for this host and pathogen system, the relatively small and consistent impact of infection does not disrupt strong clinal signals or plastic responses underlying acclimation. However, pathogens with longer infection durations, or more population-specific effects, may still threaten the reliability of clinal signals.

1. Introduction

With global change leading to an increase in the frequency of extreme heat events, the persistence of populations likely hinges on their capacity to endure warmer conditions or adapt their phenotypes plastically (Deutsch et al., 2008; Angilletta Jr, 2009; Hoffmann and Sgrò, 2011). Yet not all populations will be equally at risk (Huey and Kingsolver, 1989; Deutsch et al., 2008; Somero, 2010). Variation in the selective pressure of temperature commonly results in populations becoming locally adapted to their thermal environment (Hoffmann et al., 2002; Sgrò et al., 2010). Across large geographic regions, this process gives rise to a clinal relationship for thermal tolerances, where populations closer to the equator are better able to withstand high temperatures (Hoffmann et al., 2002; Sgrò et al., 2010; Yampolsky et al., 2013; Pereira et al., 2017; Lush et al., 2024), via an improved capacity to remain physiologically active at higher temperatures (i.e., higher critical thermal maxima) or persist for longer when faced with extreme heat stress (i.e., longer knockdown times (Sgrò et al., 2010; Terblanche et al., 2011)). These latitudinal clines help identify which populations may be

most at risk to rising temperatures (Hoffmann et al., 2003; Deutsch et al., 2008; Somero, 2010; De Frenne et al., 2013; Moran et al., 2016; Kingsolver and Buckley, 2017). However, any predictions regarding at risk populations are only as sound as the signals are reliable (Lush et al., 2024). Signals of local adaptation could be modified (Hector et al., 2020), or even eroded (Thawley et al., 2019), when populations are faced with additional challenges that coincide with warmer conditions.

For many populations, rising temperatures will coincide with an additional threat of infection (Gehman et al., 2018; Tesla et al., 2018; Mordecai et al., 2019; Price et al., 2019). Given the ubiquity of parasites and pathogens in every ecosystem and trophic level (Chrétien et al., 2023; Dobson et al., 2008; Lafferty et al., 2006), many individuals will be exposed to the threat of infection at some stage of their life, and in extreme cases, infected individuals can be widespread within a population (e.g. up to 78 % in safari parks; Hossain et al., 2021; Ferdous et al., 2023). Importantly, both exposure to a pathogen and subsequent infection can alter a host's innate thermal tolerance (Greenspan et al., 2017; Hector et al., 2019; Laidlaw et al., 2020; Siddons and Searle, 2021). These effects, however, are highly variable. In some systems

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infection can lead to increases thermal tolerance via improved knock-down times (Bates et al., 2015) or higher critical thermal maxima (Sasaki et al., 2023), while systems that do feature reductions in either metric can observe substantial intra-specific variation between populations (Greenspan et al., 2017; Hector et al., 2019, 2020). For example, infection of both aphids (*Acyrtociphom pisum*) and ladybugs (*Hippodamia convergens*) by a fungal pathogen reduces their upper critical thermal limits by 10–20 % (Porrás et al., 2021). By contrast, the copepod *Acartia tonsa*, infected with the larvae of a bopyrid isopod, experiences a small but significant increase in the same metric (Sasaki et al., 2023).

If exposure to a pathogen changes a host's thermal tolerances, then our understanding of how populations respond to global change will be disrupted when pathogens are inevitably encountered (see also Hector et al., 2019), regardless of whether the net change is positive or negative. This effect could manifest in several ways. First, each population could be affected equally such that the average thermal tolerance changes, but not the predictable change in thermal tolerance that is expected across latitudes (i.e., the elevation not the slope). Second, the extremes of the latitudinal cline may respond most strongly, leading to increases or decreases in strength of the clinal trends. In both cases, the best performing population in the heat will remain the same whether infected or not. Finally, if the effect of infection is population-specific and random across space, clinal signals may be disrupted or completely eroded (Thawley et al., 2019; Hector et al., 2020), such that any population now has the potential to be the best or worst performer in the heat, changing our understanding of thermal tolerance across geographic space. One of the few studies to explore the impact of infection across a latitudinal cline found support for this last scenario, but focused on immune activation and wounding, drawing into question what might happen when natural infections arise (Hector et al., 2020).

In addition to potentially reducing the innate thermal tolerance of an organism, pathogens may be capable of disrupting the ability of their hosts to immediately respond to rising temperatures. Phenotypic plasticity describes the capacity of genotypes to create multiple phenotypes in response to environmental variation (Scheiner, 1993; Whitman and Agrawal, 2009; Sgrò et al., 2016). For thermal tolerance, exposure to even brief periods of elevated temperatures can lead to a rapid increase in upper thermal limits (Rohr et al., 2018; Oliveira et al., 2021). In many cases, a population's ability to plastically respond to thermal stress will be key to persistence through a warming climate in the short term (Sgrò et al., 2016). Just like its ability to reduce innate thermal tolerance, however, infection by a pathogen can limit a host's plastic response to the environment (Vale et al., 2008; Hector et al., 2024). Hector et al. (2024), for example, showed that uninfected hosts achieved a far greater increase in thermal tolerances than infected hosts when exposed to warmer temperatures. Despite this, studies investigating the role of infection in limiting a thermal plastic response remain limited, particularly with hosts sampled from multiple populations.

In this study, we sought to explore if the costs of infection on innate thermal tolerance and phenotypic plasticity were consistent amongst hosts sampled from different locations along a cline, and consequently, if clinal signals would remain robust to the threat of infection. To address these questions, we used the Australian water flea, *Daphnia carinata*, and a local fungal pathogen, *Metschnikowia* spp. The thermal tolerance of *Daphnia* is strongly linked to their local thermal environment (Williams et al., 2012; Yampolsky et al., 2013; Seefeldt and Ebert, 2019; Lush et al., 2024), and exhibits clinal trends similar in nature to those observed across species (Sunday et al., 2011). Additionally, *Daphnia* are capable of plastically improving their thermal tolerance in response to warming (Yampolsky et al., 2013; Burton et al., 2020; Hector et al., 2024), even given relatively short acclimation periods (Williams et al., 2012). *Metschnikowia* provides an ideal test case of the robustness of thermal tolerances to infection. *Metschnikowia* is a generalist pathogen that indiscriminately infects hosts after ingestion during filter feeding (Hall et al., 2007). Needle-like spores pierce the gut wall of

the host, before proliferating in the haemolymph, and ultimately increasing the rate of mortality (Hall et al., 2007; Stewart Merrill and Cáceres, 2018).

Here, we explored the ability of a generalist pathogen to both disrupt clinal signals of thermal tolerance and limit the ability of hosts to form a plastic response to warming. We selected six host clones of *Daphnia carinata* from latitudinally distinct populations and raised them both in the absence (20 °C) and presence of warming (25 °C; similar to that of the high-risk climate scenario, Calvin et al., 2023). Hosts were then exposed to the generalist pathogen *Metschnikowia* spp. and their upper thermal limits were determined using static heat shock assays (Hoffmann et al., 2002; Yampolsky et al., 2013; Hector et al., 2019; Lush et al., 2024). Based on our knowledge of how infection interacts with host innate thermal tolerance (Hector et al., 2019, 2020), we predicted that any reduction in host thermal fitness would be population-specific. As a consequence, we anticipated that the clinal relationship would be altered either in its strength, signified by a change in the slope, or be eroded if the response of populations was independent of latitude. Additionally, we predicted that infection would reduce the plastic capacity of hosts.

2. Methods

Daphnia carinata is a freshwater cladoceran found throughout Australia, India, and New Zealand. Clones of *D. carinata* were sampled from populations along a latitudinal cline in eastern Australia during 2017 ((Drapes et al., 2021; Lever et al., 2021); see Fig. S1 and Table S1 for sampling locations). Each clonal line was established from a single female. This approach assumes that each clonal line captures the broader patterns of local adaptation to local temperatures and can be used as a proxy for the study of latitudinal clines. Supporting this assumption, *Daphnia*, as a clonal organism, typically exhibit populations dominated by a limited number of founder clones that possess numerical superiority within both the active population and the diapausing egg bank (Hebert and Crease, 1983). Moreover, studies using these approaches in *Daphnia* spp. (Yampolsky et al., 2013; Lush et al., 2024) have repeatedly found variation in thermal tolerances across large geographic areas to be of similar magnitude to those found in other model organisms, including *Drosophila melanogaster* (Hoffmann et al., 2002; Sgrò et al., 2010). We selected six populations based on their position along the cline (Lush et al., 2024), and their ability to be infected by our fungal pathogen at a sufficient rate.

2.1. Experimental maintenance of study animals

Experimental lines were maintained at 20 °C, a 16:8 L:D light regime, and at low densities, to ensure *Daphnia* undergo asexual reproduction and maintain the clonal lines. From stock populations, single females presenting eggs were sampled and maintained in individual 70 mL jars containing 40 mL of Artificial *Daphnia* Medium (ADaM, Klüttgen et al., 1994; modified in Ebert et al., 1998). *Daphnia* were maintained individually for three generations to minimise any maternal effects, before experimental animals were taken from clutches three to seven, correlating with animals allocated to experimental block numbers one to four. Parental clutches were sexed at birth, with only females selected. We are aware that *Daphnia* sexes differ in their thermal tolerance and interactions with disease (Laidlaw et al., 2020), however inclusion of males was outside the scope of this study.

Once sexed, parental clutches were evenly split among all treatment groups, with each animal maintained individually throughout the experiment. Animals were kept in 70 mL glass jars with 40 mL of ADaM and fed green algal cells daily (*Scenedesmus* sp.). Food quantity was ramped up from birth, increasing from one million cells at 1 day old to five million cells by 7 days old to meet the growing needs of animals. *Daphnia* medium was changed twice weekly, ensuring that any excess food or clutches were removed. The position of animals in controlled

climate chambers was shuffled daily, to prevent any positional effects.

2.2. Basic experimental design

Experimental animals were randomly allocated at birth to one of two thermal environments for the duration of the experiment (20 °C or 25 °C). Individuals were maintained under standard laboratory conditions until 10 ± 1 days old, where individuals were denoted as either unexposed controls or exposed to the fungal pathogen. This resulted in the four treatments groups per *Daphnia* clone. From pilot data, we ascertained that successful infection of the pathogen was difficult to determine visually prior to any heat shock assay. Consequently, we exposed (and assayed) 100 individuals from each treatment with the expectation that 25 % of exposed individuals would become infected (based on unpublished, pilot data). All animals were assayed for thermal tolerance through a heat shock assay at 20 ± 1 days old. Animals were then frozen, and their infection status was later determined via haemocytometer. A total of 1536 experimental animals were set up over four temporal blocks, with a total of 28 controls and 100 exposed individuals per treatment group (6 populations $\times$ 2 thermal acclimations $\times$ 4 blocks [7 unexposed controls + 25 individuals exposed to the pathogen]).

2.3. Infection of experimental animals

The isolate of *Metschnikowia* spp was initially sampled from Jock Marshall reserve, Clayton, Australia in 2022 (see Table S1 for location details) and originated from a single infected female and has since been propagated using a single host genotype of *Daphnia carinata*. The infectivity of mature *Metschnikowia* spores has been found to deteriorate with age as well as due to freezing (Duffy and Hunsberger, 2019). Consequently, pathogen stocks were continuously maintained in live hosts, and spores were collected one day prior to the inoculation of experimental animals. At 10 ± 1 days old, all animals were transferred into 20 mL of fresh ADaM. Each jar containing animals allocated to the exposed treatments were then exposed to 20,000 *Metschnikowia* spores (1000 spores/mL). Animals were left within this medium for a period of four days, after which they were again placed into 40 mL of fresh ADaM.

2.4. Heat shock assays

We used static heat shock assays to determine the time to immobilisation (T_{imm}), herein referred to as knockdown times, which have been commonly used as a measure of the ability of individuals to persist through extreme thermal events. We used a temperature of 37 °C in our assays, based on previous studies for *Daphnia* spp (Yampolsky et al., 2013; Hector et al., 2019; Lush et al., 2024). This temperature is similar to the 39 °C used in studies of *D. melanogaster* (Hoffmann et al., 2002; Sgrò et al., 2010) and allows us to detect measurable differences in thermal tolerance between treatment groups within a few hours.

At 20 ± 1 days old, all experimental animals were assayed for thermal tolerance. Even amounts of each treatment group were randomly allocated to one of eight assays that would be performed for each block, before being randomly allocated to one of 48 positions within the assay (i.e., approximately 1 control and 3 exposed animals from each treatment group per assay). Animals were then placed in 5 mL glass vials enclosed with a mesh lid to ensure sufficient transfer of oxygen, before assays commenced within a 70-litre glass aquarium. Assays commenced as soon as vials were submerged and were monitored visually by one observer throughout the assay. Each animal was scored as immobile as soon as all visible movement ceased, including filtration (as per Hector et al., 2019). All assays were constantly agitated with oxygen bubblers to ensure oxygen saturation throughout the duration of the assay. Immediately following heat shock assays, animals were measured for body size using a stereo microscope. Measurements were from the individual's eyespot to the base of the tail (as per Laidlaw et al.,

2020).

2.5. Determination of infection status

All animals were assessed for infection using a haemocytometer. Infection by the fungal pathogen results in the infecting bodies developing through several non-infective developmental stages, before eventually producing the infective ascospores (Metschnikoff 1884). For this study, we were primarily interested in the costs of infection on host performance, and as such animals were considered infected when spores of any developmental stage were present. The median infection rate observed across all *Daphnia* clones was 0.23 (with a range from 0.06 to 0.58), allowing for the inclusion of knockdown times from 20 to 28 unexposed control animals (median 26) and from 5 to 37 exposed and infected animals (median 19) in subsequent analyses for each clone and temperature combination. Further details are provided in Table S2.

2.6. Statistical analyses

All analyses were performed using R (v.4.4.1, 2024; www.R-project.com). Using approaches that have previously been used for latitudinal clines of this system (Lush et al., 2024), as well as in *Drosophila* (Sgrò et al., 2010; Cockerell et al., 2014), we first analysed the clone-specific effects of infection on host innate thermal tolerance (model 1: clone identity as a fixed effect), before determining how infection altered the observed latitudinal cline (model 2: now modelling the latitude of a clone's sampling location instead of clone identity). The relative costs of infection on each clone's innate thermal tolerance were first assessed using a linear mixed-effects model via the *lme4* package (Bates et al., 2015), evaluated via an analysis of variance (Type III Wald F tests with Kenward-Roger degrees of freedom adjustments). We modelled host knockdown times as a response to host clone genotype (six levels), thermal environment (20 °C or 25 °C), infection status (uninfected controls or infected individuals), and all two and three-way interactions as fixed effects. Body size at the time of assay was included as a covariate, while experimental block was also included as a random effect. This analysis (model 1) assesses the absolute ability of infection to reduce a clone's innate thermal tolerance without considering the location of each host.

We then assessed the ability of infection to alter known clinal trends for this system, by modelling the observed knockdown times across latitude (model 2), using a two-factor analysis of covariance via the *lme4* package, again evaluated via an analysis of variance (Type III Wald F tests with Kenward-Roger degrees of freedom adjustments). The model included thermal environment and infection status as fixed effects, latitude (°S) as an interacting covariate, and experimental block as a random effect (as per Lush et al., 2024). Analyses of this model would provide evidence for how infection interacted with signals of local adaptation for innate thermal tolerance. A significant effect of infection alone would signify that any costs of infection were ubiquitous across space, while a significant interaction term would indicate that infection was either altering the clinal slope or eroding the relationship. Alternatively, interactions between infection status and thermal environment would demonstrate that changes to the clinal trend were due to interactions between infection and the plastic capabilities of hosts. We also extended this approach to model a clinal change in thermal plasticity (model 3). Each individual's absolute plastic capacity was measured by a difference in the knockdown times under warming minus the observed knockdown times at standard laboratory conditions.

3. Results

We first analysed the role infection and thermal environment play in altering each clone's ability to tolerate heat stress. We found that both treatment groups altered knockdown times, albeit in different direction. On average, exposure to the fungal pathogen decreased host thermal

performance, with infection leading to a 2 min reduction in knockdown times (Table 1; Fig. 1). Although it appears there is some variation between clones in their infection responses, particularly in the most northerly clone, we found no statistically clear evidence that the costs of infection uniquely altered their hosts thermal tolerance in any combination of host clone or acclimation temperature (Table 1; $p > 0.10$ for all interactions involving infection). In contrast, acclimation to the warmer environment led to a strong and positive increase in thermal tolerance, but with the relative effect of warming entirely population specific (i.e., two-way interaction term between host genotype and acclimation, Table 1; Fig. 1). For example, acclimation to the 25 °C treatment increased the knockdown time of low latitude population PLD by 3.5 times more than high latitude population YYD (Fig. 1).

Next, we aimed to determine how interactions between infection and temperature might alter the clinal relationship for thermal resistance in *D. carinata*. Similar to previous studies within this system, knockdown times had a significant negative relationship with latitude (Fig. 2), with lower latitude clones having greater thermal resistance than those from higher latitudes (Lush et al., 2024). This broad relationship was robust to any effects of infection within both thermal environments, as shown by neither a decrease in the elevation of the clinal trend nor a change in the slope (Table 2; Fig. 2). We also found little evidence that infection was uniquely limiting the capacity of populations to plastically alter their thermal tolerance (Table 3; $p > 0.10$ for both the interaction between infection and latitude, and its polynomial). Instead, all clones benefitted from the acclimation to warmer conditions, as captured by a vertical shift in the clinal relationship (Fig. 2). This effect, however, was greatest for low latitude clones (i.e., an acclimation by latitude interaction term; Table 2; Fig. 2). This resulted in nearly a two-fold change in the knockdown times of populations as latitudes tended towards the equator (slope change of $3.23 \pm \text{SE}$ min per degree south to $5.59 \pm \text{SE}$ min per degree south).

4. Discussion

Latitudinal clines in thermal tolerance provide an invaluable tool for identifying at risk populations under future warming conditions (Deutsch et al., 2008; Moran et al., 2016; Kingsolver and Buckley, 2017). However, the clinal signals upon which our understanding of the differences in each population's thermal biology depends on, could be modified, or even eroded, if external factors have strong population-specific effects (e.g. Thawley et al., 2019; Hector et al., 2020; but also see Lush et al., 2024). This is especially true for infection, where the costs imposed by a pathogen commonly depend on the genetic background of the host (Lambrechts et al., 2006; Grech et al., 2006; Vale and Little, 2009; Bryner and Rigling, 2011). Variation in the resistance of host genotypes and the infectivity of pathogen genotypes, for example, can alter both the infection duration and the costs imposed on the host (Schmid-Hempel and Ebert, 2003; Grech et al., 2006; Hall et al., 2017), which have shown to be linked to the resulting capacity of hosts to cope with warming (Hector et al., 2019). Here, we explored the

consistency with which pathogens might disrupt the innate thermal tolerances of their hosts, as well as their plastic responses to temperature, using clones of *D. carinata* sampled from populations along a latitudinal cline.

We initially hypothesised that clinal signals would be vulnerable to being eroded, as interactions between host and pathogen genotypes can often lead to population and genotype specific responses (Vale and Little, 2009; Hector et al., 2019, 2020). In fact, activation of the immune system alone can be enough to alter a host's upper thermal limits in direction and magnitude that is highly population-specific (Hector et al., 2020). Despite this, we found little statistical support for heterogeneity in the response of clones, with infection resulting in an average decrease in knockdown times of 2 min (Fig. 1). Instead for both infected and uninfected animals, we found that low latitude clones had relatively greater knockdown times and thus heat tolerance, decreasing by 3.23 min per degree south at 20 °C and 5.59 min per degree south at 25 °C along the latitudinal cline (Fig. 2). This was comparable to previous work within the same system which observed trends of 3.78 min per degree south at 20 °C (Lush et al., 2024). Variation in thermal limits due to differences between clones and thus sampling locations (Figs. 1 and 2) appears to easily overshadow the relatively small reduction caused by infection, perhaps limiting opportunities for infection to disrupt the observed clinal trend. But in other species, where knockdown times change by a small amount along a cline (e.g., $\Delta 3$ min for *Drosophila* along the same cline, Sgrò et al., 2010), a similar infection effect might be much more impactful.

The ability of clones to plastically alter their thermal tolerances in response to warming was also not influenced by infection. Similar to previous studies, we found that clones sampled from populations closer to the equator had the greatest capacity for plastic change (Sgrò et al., 2010; Williams et al., 2012). While it is often suggested that individuals from high latitude populations should possess a greater ability to thermally acclimate, as these regions typically experience more variable temperatures (climate variability hypothesis, Janzen, 1967; Ghalambor et al., 2006), most supporting evidence is derived from studies comparing different species (see discussion in (Walter et al., 2025)). Our results contribute to a growing recognition that higher latitude populations can sometimes be the most at-risk populations due to both their reduced thermal tolerance and more limited capacity to plastically respond to rising temperatures (Sgrò et al., 2010; Williams et al., 2012). The plastic increases to upper thermal limits when raised in warmer conditions (Fig. 1) were far greater than the impact of infection, in turn limiting any potential for infection-induced changes to the process of acclimation. As a result, for both infection and uninfected animals, acclimation to warmer conditions led to an increase to the signals of local adaptation, with the clinal slope increasing from to 3.23–5.59 min per degree south (Fig. 2). These findings highlight how higher latitude populations can be the most at-risk populations in the heat due to both their reduced thermal tolerance and lower capacity to plastically respond to rising temperatures.

While our results show how exposure to a pathogen like *Metchnikowia* does not necessarily make a population more vulnerable, nor disrupt the use of clines to identify populations at risk, other pathogens, even when faced with the same host, will not behave the same (see also Fig. 1 in Hector et al. 2021). Here, we observed a significantly smaller reduction in host thermal tolerances due to infection in comparison to other pathogens infecting *Daphnia* (e.g., a 2 min reduction was far below the 20 min reduction found in *Pasteuria ramosa* infections; Hector et al., 2019; Laidlaw et al., 2020). The vast difference in effect size may be due to the relative pacing of the infection process, as *Metchnikowia* kills their hosts shortly after infection and before any cost in thermal tolerance may arise. In contrast, infection of hosts in other systems, such as from *Pasteuria* in *Daphnia* and the Chytrid fungus (*Batrachochytrium dendrobatidis*) in frogs, can persist for up to 60 days in a laboratory setting (Berger et al., 1998; Retallick and Miera, 2007; Gipson and Hall, 2018), leaving ample time for any cost to both develop and be detected. This

Table 1

Linear mixed-effect model for the effect of host clone, acclimation (20 °C and 25 °C), infection status (presence or absence of a pathogen) and body size on knockdown times during a 37 °C static heat shock assay. Experimental block was included as a random effect (SD = 0.138).

Term	df	F	p-value
Host clone	5, 500.67	116.02	<0.001*
Acclimation	1, 502.89	541.68	<0.001*
Infection status	1, 366.12	4.88	0.028*
Host clone x Acclimation	5, 500.61	8.97	<0.001*
Host clone x Infection	5, 499.68	1.60	0.157
Acclimation x Infection	1, 499.16	1.54	0.215
Host clone x Acclimation x Infection	5, 499.10	1.68	0.138
Body size	1, 494.25	24.23	<0.001*

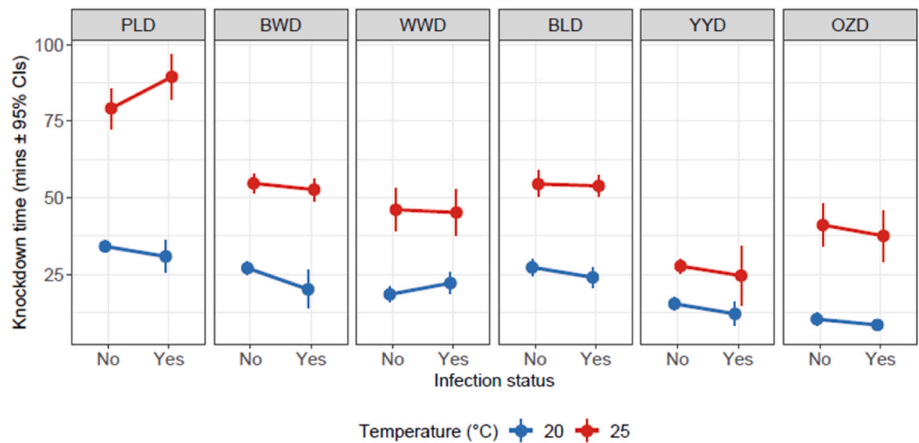


Fig. 1. Observed results for a 37 °C static heat shock assay following infection of host clones to the fungal parasite *Metchnikowia spp.* and then acclimation to one of two thermal environments (20 °C or 25 °C). Each facet represents one the six distinct clones of *Daphnia carinata*, sampled from along a latitudinal cline, and arranged in order from lowest (PLD: 30.92 °S) to highest latitude (OZD: 38.16 °S). Points describe mean values $\pm$ 95 % confidence intervals in all panels. Populations are ordered from most northerly to southern.

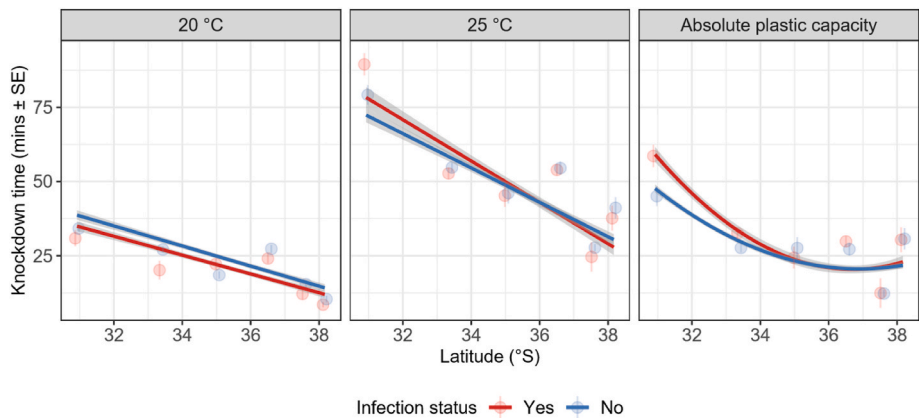


Fig. 2. Predicted clinal trends of thermal tolerance in *Daphnia carinata* following infection by the pathogen *Metchnikowia spp.* and acclimation to one of two thermal environments (20 °C or 25 °C). The first two panels describe the predicted clinal relationships between thermal tolerance and latitude (° S) within each thermal environment. The third panel shows the absolute plastic capacity of clones across space. Each hosts plastic capacity was calculated by subtracting the average thermal tolerance of clones at 20 °C from the observed knockdown times of each individual at 25 °C. Shown are the mean ($\pm$ SE) knockdown times at 37 °C for the latitude at which each *D. carinata* clone was sampled and the predicted relationship thermal tolerance has with latitude ($\pm$ 95 % CIs). Observations in red indicate the means of animals that were confirmed to be infected, while observations in blue indicate unexposed controls.

Table 2
Two-factor ANCOVA assessing the effect of each clone’s original latitude, acclimation (20 °C and 25 °C), infection status (presence or absence of a pathogen) and body size on knockdown times during a 37 °C static heat shock assay. Experimental block was included as a random effect (SD = 0.213).

Term	df	F	p-value
Latitude	1, 515.91	485.66	<0.001*
Acclimation	1, 513.56	25.98	<0.001*
Infection status	1, 513.64	0.99	0.321
Latitude x Acclimation	1, 513.66	11.22	<0.001*
Latitude x Infection	1, 514.95	1.22	0.270
Acclimation x Infection	1, 513.70	1.61	0.205
Latitude x Acclimation x Infection	1, 513.60	1.29	0.256
Body size	1, 518.81	96.93	<0.001*

coincides with recent studies that show infection does not always lead to substantial reductions in thermal limits (Sasaki et al., 2023), especially considering the bulk of our understanding comes from two disease systems (Greenspan et al., 2017; Hector et al., 2019). Further research into more virulent or faster paced disease systems may allow greater insight into the mechanisms that underlie interactions between host

Table 3
Single factor ANCOVA assessing the effect of infection status (presence or absence of a pathogen), the original latitude of host clones and their body size on their calculated absolute plastic capabilities. Plastic capacity was calculated by the difference in knockdown times under warming (25 °C) versus standard conditions (20 °C), during a 37 °C heat shock assay.

Term	Df	F	p-value
Infection status	1, 258.78	1.57	0.212
Latitude	1, 272.22	26.25	<0.001 *
Latitude ²	1, 272.33	22.9	<0.001 *
Infection status x Latitude	1, 271.38	0.82	0.367
Infection status x Latitude ²	1, 271.46	0.73	0.392
Body size	1, 270.96	53.48	<0.001 *

thermal limits and disease.

In summary, identifying the thermal limits of multiple populations is pivotal not only for the identification of at-risk populations during warming, but also our understanding of thermal biology as a whole (Deutsch et al., 2008; Somero, 2010; Hoffmann and Sgró, 2011). Our results indicated that exposure to a pathogen does not necessarily disrupt our understanding of risk that can be derived from studies of

thermal tolerances along latitudinal clines, nor will all pathogens limit a host's capacity to plastically adjust their thermal tolerance in response to warming (see also Hector et al. 2021). However, a greater understanding of the impact of different types of pathogens, varying in the damage they cause as well as the length of infection, is required to understand when and where infection may play a more detrimental role. Broadening our perspective to encompass the sampling of pathogen themselves from multiple locations, instead of using a single pathogen sampled from the end of the cline as used here, would elevate the study of thermal adaptation to include the pathogens themselves and their potential mismatch with host thermal biology (*sensu* (Cohen et al., 2017)).

On the host side there also remain several critical avenues for future research. Using only a single representative clone per population does not capture the genetic variation within and among populations. Although the biology of the *Daphnia* system makes it possible to represent a location with a limited number of clones (see methods) and observed trends are consistent with findings in other species (Hoffmann et al., 2002; Sgrò et al., 2010), the next step is to formally assess whether variation between populations exceeds that within populations—the primary assumption of this study—and to examine evidence for each population's adaptive potential in response to global change and infectious disease (i.e., (Hoffmann and Sgrò, 2011)). Heat shock assays, upon which our conclusions are derived, are also just one measure of how organisms tolerate temperature (Sgrò et al., 2010; Terblanche et al., 2011). Explorations of different rates of thermal change and formal thermal performance curves along latitudinal clines will help determine how the costs of infection to thermal tolerances may be related to mismatches in host and pathogen thermal optima (Cohen et al., 2017; Mordecai et al., 2019; Cator et al., 2020).

CRedit authorship contribution statement

Jared Lush: Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Connor Bevan:** Project administration, Investigation, Data curation. **Carla M. Sgrò:** Writing – original draft, Supervision, Methodology, Conceptualization. **Matthew D. Hall:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

All Authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2025.104289>.

Data availability

Data are available from the Monash Bridges Digital Repository: <https://doi.org/10.26180/29945108>.

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