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Extreme heat alters the performance of hosts and pathogen

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The frequency and intensity of extreme heat in the environment have increased in the last decade. Extreme heating events (EHE) have wide-ranging impacts on biological systems from the molecular to the community level. However, the impacts of EHE have been poorly studied in pathogen–host systems. Here, we explore how EHE affects the interaction among the insect hosts, *Osmia cornifrons* and *Osmia lignaria,* and a protozoan pathogen, *Crithidia mellificae*. We compared changes in the upper limit for locomotion of hosts (Ct_{max}), thermal boldness (voluntary exposure to Extreme Temperature Zones – ETZ) between healthy and infected host exposed to EHE, and the effect of host Ct_{max} on pathogen growth rate. Our results showed that 1-day EHE significantly reduced the upper limit for locomotion of hosts by an average of 4 °C in healthy and 7 °C in infected hosts. Further, EHE significantly reduced the protozoan pathogen growth rate. EHE also reduced the hosts' voluntary exposure to (or transit across) extreme (hot or cold) temperature zones (ETZ). Our results show that EHE reduces both hosts' heat tolerance and pathogen fitness, and shed light on the implications of EHE on host–pathogen dynamics under warmer world.

KEYWORDS

extreme environments, pollinators, stress response, heat tolerance, infection, thermal stress, protozoan infection

Introduction

Anthropogenically-induced global warming is increasing the frequency, magnitude, and duration of different kinds of extreme heating events (EHE) globally, posing high risks to biodiversity (Masson-Delmotte et al., 2021). EHE can have profound effects on organisms' development and behavior as well as species' phenology, community structure and ecosystem function (Buckley and Huey, 2016; Williams et al., 2016; Przybyla et al., 2021; Breedveld et al., 2023). In addition, EHE can potentially modify species' interactions, including how extreme heat alters the interactions between hosts and pathogens (Adamo and Lovett, 2011; Burge and Hershberger, 2020). This is particularly important in ectothermic host-pathogen systems where the thermal performance of microbial parasites or pathogens is entirely dependent on the host body temperature (Thomas and Blanford, 2003). In turn, pathogens have the ability

to alter hosts' thermoregulatory strategies (Elliot et al., 2002). Thus, understanding whether and how EHE may affect physiology, behavior, or fitness of hosts and pathogens is critical to better describe interspecific interactions. For example, there may be mismatches between the thermal tolerance of insect hosts and their pathogens, which may change the relationship, including overall infection and disease dynamics. Understanding how an EHE affects relevant traits of hosts and pathogens is critical to better describe interspecific interactions.

In general, the thermal tolerance of a species results from a complex suite of factors, including cellular, metabolic, hormonal, neuronal, abiotic, and biotic factors (Hutchison, 1976; Nowakowski et al., 2016) and affects both insect hosts and their pathogens. Tolerance to heat can be altered by environmental temperature variation (Brochu et al., 2004), drought (Von Bülow and Beitz, 2015; Beetge and Krüger, 2019), pollution (Cairns Lanza and Parker, 1972; Echavez and Leal, 2021; González-Tokman et al., 2021), diet (Krebs and Loeschcke, 1994; Adamo et al., 2012; Hamad, 2012), as well as symbiotic interactions (Dunbar et al., 2007; Xu et al., 2008; Padfield et al., 2020; Porras et al., 2020), and parasitism (Porras et al., 2021; Sánchez et al., 2021). The degree of heat tolerance can vary among populations of both hosts and pathogens (Sinclair et al., 2012; Paudel et al., 2020). While severe EHE are generally predicted to reduce the performance of any given species, understanding whether pathogens and insect hosts might be differentially affected will help to predict the effects of current and future EHE on host–pathogen interactions (Nowakowski et al., 2016; Sinclair et al., 2016).

Exploring how host–pathogen systems differ in their response to EHE poses an experimental challenge since each organism should be studied separately and while interacting (Elliot et al., 2002; Adamo et al., 2012; Poulin and Randhawa, 2015). For instance, ectothermic hosts presumably have a narrower thermal range and lower thermotolerance than their pathogens (Nowakowski et al., 2016; Rohr et al., 2018; Dudney et al., 2021). Therefore, at least in some systems, hosts are more susceptible to disease at temperatures further away from their optimum temperature. While numerous studies have explored the impacts of stressors such as temperature, drought, and pH, on the species' thermal tolerance, individually characterizing impacts of multiple stressors remains elusive (Hector et al., 2023). Furthermore, the effect of hosts' thermal tolerance limits on pathogens has rarely been studied. A potential approach to studying feedback within the environment–host–pathogen system may come from integrating parameters from physiology and behavior, such as the upper thermal limit for locomotion, voluntary exploration of extreme temperatures, and population growth rate. Measuring key host and pathogen traits while they are alone or within their interaction may provide insights into thermal tolerance changes. The upper thermal limit for locomotion (Ct_{max}) is the temperature at which an organism's neuromuscular junctions break down, resulting in loss of neuromuscular coordination (Hochachka and Somero, 2002). Another parameter of interest is voluntary exposure to zones with extreme temperatures (ETZ) or thermal boldness, which is the tendency to voluntarily enter and cross zones of critical (hot or cold) temperatures. Characterizing thermal boldness can provide insights into the role of behavioral decisions on physiology (Hutchison and Maness, 1979). Both, Ct_{max} and voluntary exposure to ETZ can help better understand the plasticity of thermal biology (Rodrigues and Beldade, 2020) and the adaptive processes of species in variable thermal environments (Mayr, 1983; Huey et al., 2003).

In this study, we asked about the effect of extreme heat on the performance of insect hosts, a pathogen, and their interaction. Our question arises from the lack of knowledge about the links between the effects of extreme heat and infection in host–pathogen system and the feedback mechanisms within their interactions. To address this question, we conducted laboratory experiments with two solitary mason bee species, *Osmia cornifrons* and *Osmia lignaria* (Hymenoptera: Megachilidae), hosts of a protozoan pathogen, *Crithidia mellificae* (Trypanosomatida). Both solitary bees are cavity-nesting and univoltine emerging in early spring. However, *O. cornifrons* is an introduced Asian species, while *O. lignaria* is native to North America. Both species are susceptible to *C. mellificae* (Strobl et al., 2019; Ngor et al., 2020), a unicellular, eukaryotic flagellate that attaches to the host's hindgut cells using its flagellum (Langridge and McGhee, 1967; Schwarz et al., 2015). The pathogen forms hemidesmosomes causing lesions in the intestinal cells (Hubert et al., 2017), which reduce both survival and fecundity (Strobl et al., 2019; Gómez-Moracho et al., 2020). We hypothesize that EHE reduces heat tolerance and inhibits thermal boldness in the hosts, as well as reduces pathogen population growth rate. We predict that EHE will reduce host and pathogen performance in ways that could induce a mismatch in the host-pathogen interaction. To answer this question and test these hypotheses, we assessed the effects of EHE, pathogen infection, and the combination of both factors on the hosts' heat tolerance and voluntary exploration of cold and hot ETZ. Next, we explored how the changes induced by extreme heat on host thermal tolerance altered the pathogen growth rate.

Materials and methods

EHE

Overwintering adults of *O. cornifrons* and *O. lignaria* in cocoons were obtained from Watts Bees (Bothell, WA) and stored at 5°C before the experiment (1,200 cocoons total). In March 2022, groups of 100 cocoons of each species were transferred daily to plastic cups (50 cocoons/cup, 5 cm DI × 15 cm length) with a napkin introduced inside the cup to aid eclosion and provide a substrate for chewing, which reduced mason bee stress. Two plastic cups with cocoons were placed inside an insect cage (BugDorm MegaView Science Co., Ltd., Taichung, Taiwan) with a 10 mL feeder containing 50% sucrose solution, and held for 4 days to ensure mating at 23°C with 70% RH and photoperiod 10 L (low intensity): 14 D. Groups of 12 females were transferred from the mating cages to plastic cups with a 4 mL feeder containing 50% sucrose solution and napkin (3 cm × 10 cm) simulating the setting of the insect cage. These groups of females were exposed to increasing temperatures at a rate 0.5°C/ 3 min from 23°C to 30 ± 1°C, and held at this constant temperature for 25 h in a climate-controlled chamber. We chose this temperature based on heat wave temperature records for spring in Washington and California in 2021 recorded by NOAA and AccuWeather platforms (Glahn and Ruth, 2003), and by comparison with the expected average temperature during spring (McEvoy and Hatchett, 2023) given that our experiments were

conducted using specimens from the west coast of USA. The main methods are described in **Supplementary Figure 1**.

Pathogen inoculations (*Crithidia mellificae*)

Females of *O. cornifrons* and *O. lignaria* were fed with 50% (w/v) sucrose solution prepared with a final concentration of approximately 100,000 living *C. mellificae* cells/bee following a protocol modified from Schwarz and Evans (2013) and Williams et al. (2019). Each group of females (12 individuals) was caged with 1 mL of the *C. mellificae* sucrose solution or only with sucrose (controls) and allowed to feed for 6 h. Food consumption was checked every 2 h, and when the bees consumed the entire volume, an uncontaminated 50% (w/v) sucrose solution was provided *ad libitum* until the end of the experiment.

Host upper temperature limit for locomotion (Ct_{max}) of healthy and infected solitary bees

To determine Ct_{max} of healthy and infected individuals of each species, we employed a protocol modified from Ribeiro et al. (2012), using a hotplate with a programmable heating rate controlled by a computer interface (Sable Systems, North Las Vegas, NV, USA). The temperature was monitored by independent type K thermocouples connected to a HOBO® Onset® UX 120-014 M 4-channel data logger (Bourne, MA, USA). One thermocouple was attached to the surface of the hotplate, another sensor was attached inside a glass tube plugged by a cotton ball in which we placed an individual insect; this tube was placed in a water bath on the hot plate. This equipment was located inside an automated thermal chamber kept at 23°C (interior dimensions: width 40.5 cm × 35 cm length × 40 cm height). Each bee was transferred into the glass tube and exposed to gradual heating at a rate of 0.3°C min^{-1} until its locomotion stopped. Ct_{max} was recorded when the insect turned upside down. Bee behavior was constantly monitored, and when the insect turned upside down and could no longer return to the upright position within 5 s we removed it from the experiment and recorded the temperature at which righting response was lost as its Ct_{max} (Ribeiro et al., 2012). The insect was returned to a 15 mL Eppendorf with sucrose solution in cotton for recovery (n = 20 individuals per treatment). Data were only considered valid if the insect displayed normal activity 2 h after a Ct_{max} test.

Voluntary exposure by solitary bees to extreme thermal zones

To examine how voluntary exposure to ETZ was affected by EHE, pathogen infection, and the combination of both factors, we transferred 10 females from the mating cages to a black plastic

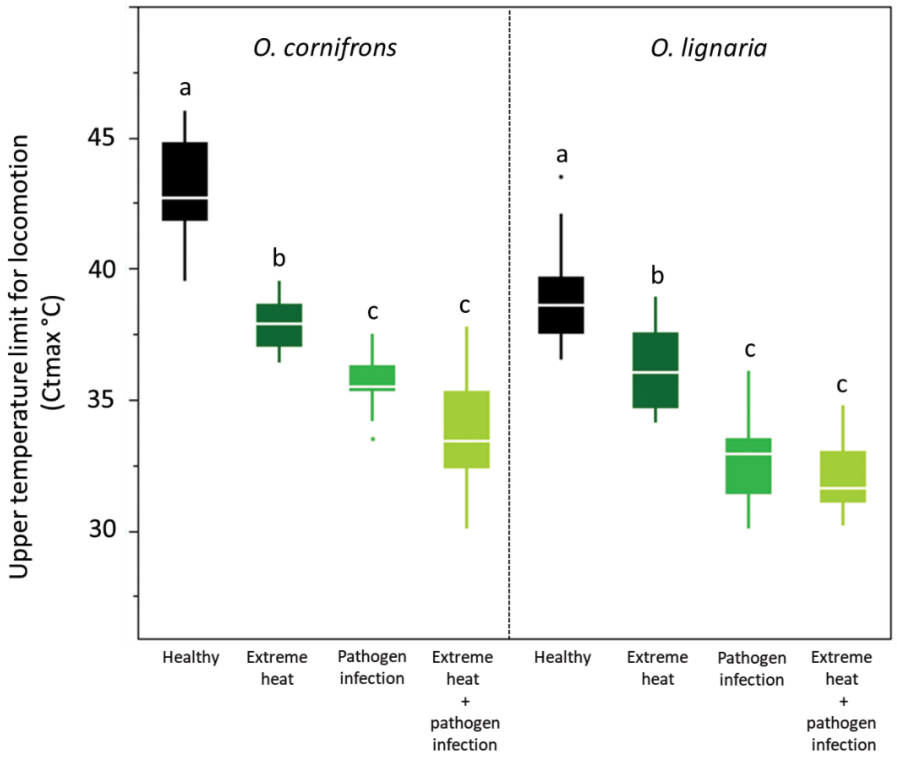

FIGURE 1
Upper temperature limit for locomotion [critical thermal maximum (Ct_{max})] for *Osmia cornifrons and O. lignaria* exposed to extreme heat, pathogen infection, and the combination of extreme heat and pathogen infection. ANOVA [boxplots display median line, interquartile range (IQR) boxes, 1.5 × IQR whiskers; n = 20 females per treatment]. Lowercase letters represent pairwise comparisons among treatment.

bottle, then attached the bottle to a choice test arena following a modified protocol from Navas et al. (2022). This experimental arena allowed insects to freely move across zones with extreme temperatures (hot and cold) after which they have access to sterilized pollen moistened with sugar water (50%) (Navas et al., 2022). To reach the pollen, individuals had to cross either a hot or cold ETZ. The hot and cold extreme temperatures for each species were determined during preliminary Ct_{max} and lower limit of locomotion experiments for each species. The location of each insect was observed after 60 min and was classified as: "exploration" for individuals that left the initial black bottle but did not cross ETZs, and hot or cold ETZ "crossings." The experiment was replicated ten times for each species and treatment condition (*O. cornifrons*: healthy, infected; *O. lignaria*: healthy, infected).

Pathogen growth and sub-culturing

Crithidia mellificae RS 3212 was cultured from frozen stock provided by Dr. Ryan Schwarz, and was maintained in Brain Heart Infusion (BHI) medium (37 g/L), supplemented with 0.3 g/L KCl, 3.5 μg/mL hemin and 2% (v/v) anti-contamination cocktail (Maser et al., 2002; Ravoet et al., 2015) pH 6.5 (Runckel et al., 2011) at 27°C (Engel and Parodi, 1985; Runckel et al., 2011). Parasites were maintained by subculture passage every 4 days (Runckel et al., 2011). The contents of the tubes were thawed and then centrifuged at 1,000 rpm for 10 min. After discarding the supernatant, the pellet was washed with 500 mL of fresh medium, centrifuged at 1,000 rpm for 10 min, and the supernatant was discarded. Each pellet was then re-suspended in a small amount of fresh medium and eventually transferred into a culture flask containing 5 mL of medium (Runckel et al., 2011).

Growth parameter determination after heat stress

To determine the growth rates of *C. mellificae*, pathogen inoculums were cultured at an initial concentration of 0.8×10^6 parasites/mL and incubated at 27°C for 96 h, this temperature was identified as optimal based on preliminary results. Next, to assess pathogen ability to recover after thermal treatments, we exposed

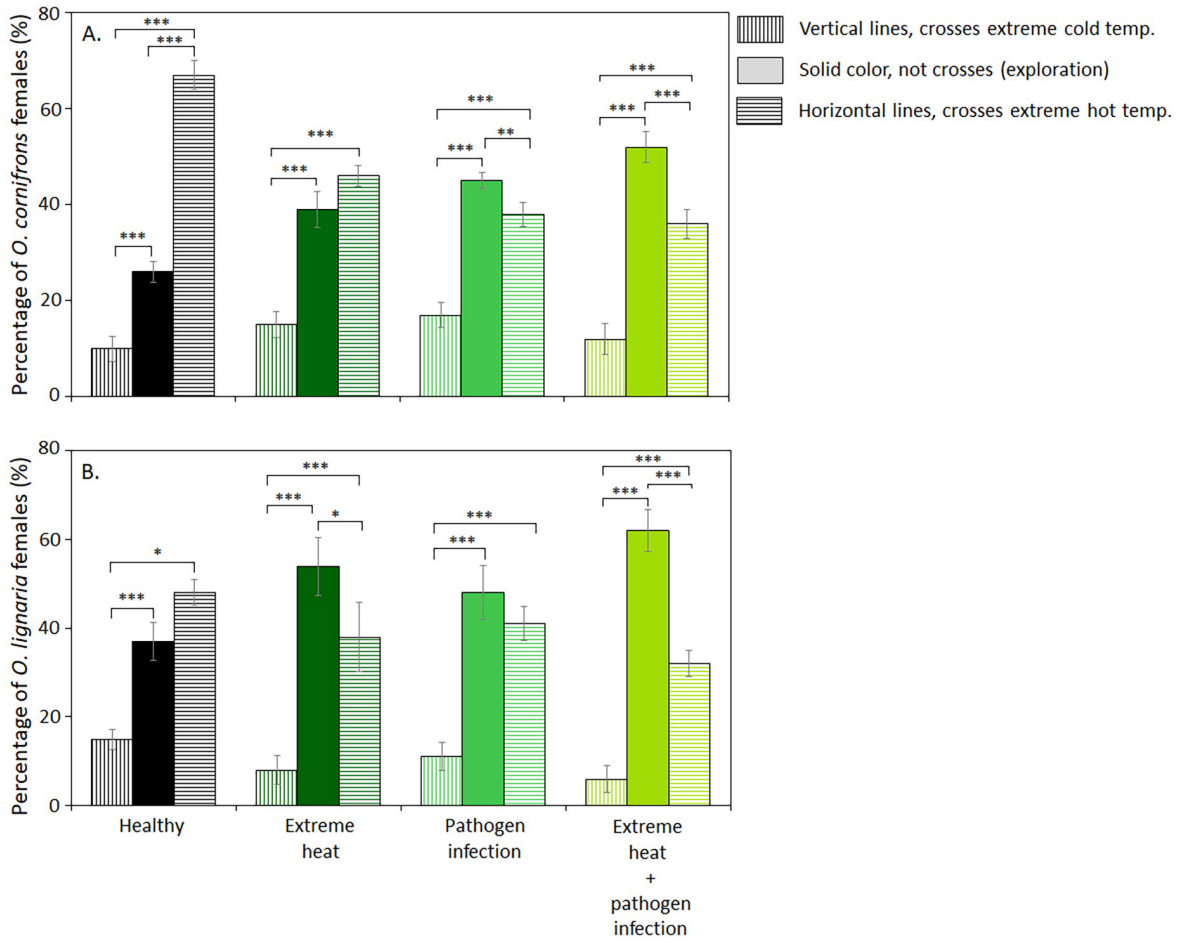

FIGURE 2
Thermal boldness of *Osmia* species exposed to extreme heat, pathogen infection, and the combination of both factors. The behavior was measured as the percentage of individuals in a sample that voluntarily crossed extreme thermal zones (ETZs, cold, or hot). **(A)** *O. cornifrons*. **(B)** *O. lignaria*. Warm and cold ETZs were set at 54°C and 13°C. Bars represent mean ± SE, $^*P \leq 0.05$, $^{**}P \leq 0.001$, $^{***}P < 0.0001$ differences within treatments (n = 10, 10 individuals per replicate, 100 individuals in total per treatment).

a subculture to heat stress by giving them three shocks with the Ct_{max} (heat shocks Ct_{max}/min^{-1} three times) of healthy solitary bees [*O. cornifrons* – *O.c.* = 43°C (n = 4 replicates), *O. lignaria* – *O.l.* = 39°C (n = 8 replicates)], Ct_{max} of solitary bees exposed to EHE [*O.c.* = 37°C (n = 4), *O.l* = 36°C (n = 8)], and Ct_{max} of infected solitary bees [*O.c.* = 35°C (n = 4), *O.l* = 32.5°C (n = 8)]. We did not assess the pathogen growth rate at the solitary bees' Ct_{max} that corresponds to the combination of infection + EHE because Ct_{max} of both species was nearly the same (*O.c.* = 33.5°C and *O.l* = 32.03°C). To measure the effects of EHE we exposed the pathogen inoculums to the same thermal regime to which bees were exposed, increasing temperature at a rate 0.15°C min^{-1} until reaching 30 ± 1°C, constant temperature for 25 h (n = 8). As a control we measured the pathogen growth at the optimum temperature of 27°C (n = 4). Each treatment was replicated four times (n = 4) or eight times (n = 8). We then recorded the parasite density immediately after the heat shock (time zero) and every 24 h, parasite density was determined by absorbance at 600 nm. The growth rates (r) were calculated using the 24 h measurements and the equation $r = \frac{\ln(OD_{t1}/OD_{t0})}{\triangle t}$, where r represents relative growth rate (h^{-1}), OD_{t0} represents initial net OC of pathogen, OD_{t1} represents OD at the time of measurement (24 h), and $\triangle t$ is the time between the t_0 and the measurement (Palmer-Young et al., 2018).

Data analysis

All data were tested for statistical test assumptions using a qqplot, Levene's homogeneity test and the Shapiro–Wilk normality test at alpha = 0.05 significance level. For critical thermal limits (Ct_{max}) experiments, we conducted ANOVAs followed by *post-hoc* parametric multiple comparisons. For voluntary exposure to ETZs, we used a generalized linear model with treatment (healthy, infected, infected + EHE, and EHE) with Poisson distribution, followed by comparisons within each treatment group. For healthy insects, we used a *t*-test to compare crosses between hot or cold ETZs; for infected insects, we conducted ANOVAs for comparisons among 23°C, hot or cold ETZs. For the effects of temperature on *C. mellificae* growth rates, we conducted a general linear mixed model using package lme4 (Bates et al., 2009, 2014) with experiment round as a random effect. F-tests were used to evaluate significance of model terms (Bates et al., 2014), followed by pairwise comparisons "lsmeans" (Lenth and Lenth, 2018). Analyses were performed in the R programming environment (v. 3.4.3., CRAN project) (R Core Team, 2013).

Results

Upper temperature limit for locomotion (Ct_{max})

Prior exposure to 1-day EHE reduced tolerance of *O. cornifrons* and *O. lignaria* by an average 5 and 3°C, respectively. The post-treatment average Ct_{max} of *O. cornifrons* was 42.5°C and *O. lignaria* was 38°C. Pathogen infection reduced heat tolerance of both solitary bee species by 8 and 6.5°C, and the combination of both stressors reduced heat tolerance by an average of 10 and 7°C, respectively (*O. cornifrons*: F = 113.95, DF: 3.74, $P < 0.001$; *O. lignaria*: F = 84.54, DF: 3.74, $P \leq 0.001$: **Figure 1**). The Ct_{max} were similar in females exposed to infection or the combination of EHE + infection in both species.

Impacts of infection on voluntary transit across extreme thermal zones

Voluntary exposure to zones with extreme temperatures (ETZ), or thermal boldness, in both species was reduced by EHE, pathogen infection and the combination of both factors (**Figures 2A, B**). In healthy *O. cornifrons,* 67% of females crossed the hot ETZ. After 1-day EHE, the percentage of *O. cornifrons* females that crossed hot ETZ was reduced by 24 points, while the percentage of females that did not cross ETZ increased by 13 percentage points (from 26 to 39%). Pathogen infection reduced the percentage of crosses to hot ETZ by 28 percentage points (from 67 to 39%), and the crosses through cold ETZ increased by 7%. The combination of both factors reduced the percentage of individuals that crossed hot ETZ by 31 percentage points (from 67 to 36%); under this condition, we recorded the highest percentage of individuals that did not cross ETZs, which was equivalent to 52% (χ^2 = 35.29, DF = 11.108; $P \leq 0.0001$; **Figure 2A**; **Table 1**).

In healthy *O. lignaria*, only 45% of females crossed hot ETZ. Extreme heat reduced crosses through hot ETZ by 10 percentage points (from 48 to 38%), and the individuals that did not cross either ETZ increased by 17%. Pathogen infection slightly reduced the crosses through hot and cold ETZ by 7 and 4%, respectively. The combination of extreme heat and pathogen infection increased the number of individuals that remain in the home bottle by 25 percentage points, and the crosses to hot and cold ETZ were reduced 9 percentage points (from 15 to 6%) and 16 percentage points (from 48 to 32 %), respectively (χ^2 = 80.45, DF = 3.2; $P \leq 0.0001$; **Figure 2B**; **Table 2**).

Pathogen growth rates

To examine whether thermal shocks altered pathogen growth rate, we conducted an unbalanced experiment with *C. mellificae* cells maintained inside temperature-controlled incubators. EHE induced the lowest growth rate for *C. mellificae*, followed by exposures at 43°C (Ct_{max} healthy *O. cornifrons*) and 39°C (Ct_{max} healthy *O. lignaria*). Pathogens exposed to the constant regime at 27°C had the highest growth rate as expected (χ^2 = 44.245, DF = 55; $P \leq 0.0001$; **Table 3**; **Figure 3**). Exposure to heat shock increased the lag phase and slowed the growth rate of stressed pathogens.

Discussion

Exposure to a EHE reduces the heat tolerance of two solitary bee host species, *Osmia cornifrons* and *O. lignaria*, with analogous effects on its protozoan pathogen, *C. mellificae*. We also documented changes in the voluntary exposure to ET of

both solitary bee species by EHE and infection, prior exposure to extreme heat and infection, as well as the combination. Moreover, *C. mellificae* mortality was also affected by the extreme heat at temperatures corresponding to the upper thermal limits for locomotion (Ct_{max}) of healthy bee hosts. Overall, these results suggest that EHE alters heat tolerance of both hosts and pathogen, resulting in shifts in physiological and behavioral responses.

Here we consider whether EHE and pathogen infection may drive changes in thermal tolerance and behavior of two pollinator species. We estimated that EHE could reduce tolerance (as measured by Ct_{max}) to heat by an average of 5°C in females of *O. cornifrons* and 3°C in *O. lignaria*. Pathogen infection further reduced the heat tolerance in both *Osmia* species. For instance, temperatures between 37 and 45°C are detrimental to *O. cornifrons* (White et al., 2009; McKinney et al., 2017) and *O. lignaria* development (Kemp and Bosch, 2005; Sgolastra et al., 2011; Kral, 2019). In *Osmia* species, warming has been shown to reduce fat content, body mass, and survival, while also altering phenology (CaraDonna et al., 2018). Complex physiological mechanisms underlie heat tolerance in both insects and protozoan pathogens, including

TABLE 1 Parameter estimates of analysis of deviance (type II) of an unbalanced model from experiments testing the effect EHE and infections on *Osmia cornifrons* voluntary exposure to hot or cold ETZ (Acc: 409.91).

Term	Estimate	Std error	L-R Chi square	Prob > Chi sq.	Lower CL	Upper CL
Intercept	3.3583333	0.1672905	403	<0.0001	3.0410327	3.6969731
Condition [Cold]	−2.008333	0.1980811	84.172382	<0.0001	−2.396325	−1.617065
Condition [Home bottle]	0.6916667	0.2484675	8.2950293	0.0040	0.2162874	1.1922947
Treatment [*O. cornifrons*]	0.0749999	0.2919047	0.0666693	0.7962	−0.477134	0.6701594
Treatment [*O. cornifrons* + *C. mellificae*]	−0.025	0.2890358	0.0074565	0.9312	−0.571861	0.5642577
Treatment [*O. cornifrons* + *C. mellificae* + extreme heat]	−0.025	0.2890358	0.0074565	0.9312	−0.571861	0.5642577
Treatment [*O. cornifrons*]*Condition [Cold]	−0.425	0.3363406	1.5947301	0.2067	−1.091649	0.2370466
Treatment [*O. cornifrons*]*Condition [Home bottle]	−1.525	0.4028544	12.932109	0.0003	−2.307827	−0.717738
Treatment [*O. cornifrons* + *C. mellificae*]*Condition [Cold]	0.375	0.3508917	1.1517768	0.2832	−0.309872	1.0775472
Treatment [*O. cornifrons* + *C. mellificae*]*Condition [Home bottle]	0.475	0.4385107	1.2142906	0.2705	−0.360696	1.3682354
Treatment [*O. cornifrons* + *C. mellificae* + extreme heat]*Condition [Cold]	−0.125	0.3388092	0.1359645	0.7123	−0.793085	0.5460607
Treatment [*O. cornifrons* + *C. mellificae* + extreme heat]*Condition [Home bottle]	1.175	0.4516175	7.3787142	0.0066	0.3186715	2.0982508

TABLE 2 Parameter estimates of analysis of deviance (type II) of an unbalanced model from experiments testing the effect EHE and infections on *Osmia lignaria* voluntary exposure to hot or cold ETZ (Acc: 436.66).

Term	Estimate	Std error	L-R Chi square	Prob > Chi sq.	Lower CL	Upper CL
Intercept	3.3333333	0.1666667	400	<0.0001	3.0172551	3.6707507
Treatment [*O. lignaria* + *C. mellificae*]	2.2157e-8	0.2886751	5.684e-14	1.0000	−0.546071	0.588627
Treatment [*O. liganria* + *C. mellificae* + extreme heat]	−6.3e-8	0.2886751	3.979e-13	1.0000	−0.546071	0.588627
Treatment [*O. lignaria*]	2.0283e-8	0.2886751	5.684e-14	1.0000	−0.546071	0.588627
Condition [Cold]	−2.333333	0.1900292	119.75748	<0.0001	−2.707824	−1.960285
Condition [Home bottle]	1.6916666	0.2639181	48.509509	<0.0001	1.1885287	2.2247049
Condition [Cold]-treatment [*O. lignaria* + *C. mellificae*]	0.1	0.3316625	0.0908767	0.7631	−0.554905	0.7562698
Condition [Cold]-treatment [*O.liganria* + *C. mellificae* + extreme heat]	−0.4	0.3188521	1.5935875	0.2068	−1.037747	0.2208555
Condition [Cold]-treatment [*O. lignaria*]	0.5	0.341565	2.1536522	0.1422	−0.168508	1.18239
Condition [Home bottle]-treatment [*O. lignaria* + *C. mellificae*]	−0.225	0.4529993	0.2426031	0.6223	−1.089311	0.6961553
Condition [Home bottle]-treatment [*O. lignaria* + *C. mellificae* + extreme heat]	1.1749999	0.4780603	6.6066504	0.0102	0.2706846	2.1530191
Condition [Home bottle]-treatment [*O. lignaria*]	−1.325	0.4322904	8.5273128	0.0035	−2.157143	−0.452098

TABLE 3 Parameter estimates of analysis of deviance (type II) tables of unbalanced model from experiments testing the effect EHE and infections on *Crithidia mellificae* growth rate (Acc: 1844.17).

Term	Estimate	Std error	L-R Chi square	Prob > Chi sq.	Lower CL	Upper CL
Intercept	0.0076086	0.0007333	93.387809	<0.0001	0.0061673	0.00905
Treatment [Ct_{max} extreme heat *O. lignaria* 36°C]	0.0026307	0.0021999	1.4269211	0.2323	−0.001693	0.0069548
Treatment [Ct_{max} extreme heat *O. cornifrons* 37°C]	0.0002744	0.0021999	0.0155546	0.9007	−0.00405	0.0045985
Treatment [Ct_{max} healthy *O. lignaria* 39°C]	−0.00574	0.0016397	12.037498	0.0005	−0.008963	−0.002517
Treatment [Ct_{max} healthy *O. cornifrons* 43°C]	−0.008055	0.0021999	13.146687	0.0003	−0.012379	−0.003731
Treatment [Ct_{max} infected *O. cornifrons* 35°C]	0.0053712	0.0021999	5.90881	0.0151	0.0010471	0.0096953
Treatment [Ct_{max} infected *O. lignaria* 32.5°C]	0.0042817	0.0016397	6.750175	0.0094	0.0010587	0.0075047
Treatment [extreme heat]	−0.016028	0.0016397	84.087437	<0.0001	−0.019251	−0.012805
Hours [0 h]	−0.02504	0.0017962	153.3521	<0.0001	−0.028571	−0.02151
Hours [24 h]	4.4989e-5	0.0017962	0.0006273	0.9800	−0.003486	0.0035756
Hours [48 h]	−0.001334	0.0017962	0.5510219	0.4579	−0.004865	0.0021967
Hours [72 h]	0.010547	0.0017962	32.820651	<0.0001	0.0070163	0.0140776
Hours [120 h]	0.0085767	0.0017962	22.058804	<0.0001	0.0050461	0.0121073
Hours [192 h]	0.0045058	0.0017962	6.2342747	0.0125	0.0009752	0.0080364
Hours [0 h]-treatment [Ct_{max} *EHEO. lignaria* 36°C]	0.0158522	0.0053887	8.5443961	0.0035	0.0052603	0.0264441
Hours [0 h]-treatment [Ct_{max} extreme heat *O. cornifrons* 37°C]	0.0108139	0.0053887	4.003235	0.0454	0.000222	0.0214058
Hours [0 h]-treatment [Ct_{max} healthy *O. lignaria* 39°C]	−0.02154	0.0040165	27.596037	<0.0001	−0.029435	−0.013645
Hours [0 h]-treatment [Ct_{max} healthy *O. cornifrons* 43°C]	−0.030203	0.0053887	30.031393	<0.0001	−0.040795	−0.019611
Hours [0 h]-treatment [Ct_{max} infected *O. cornifrons* 35°C]	0.0265078	0.0053887	23.36646	<0.0001	0.0159159	0.0370997
Hours [0 h]-treatment [Ct_{max} infected *O. lignaria* 32.5°C]	0.029268	0.0040165	49.299465	<0.0001	0.0213733	0.0371627
Hours [0 h]-treatment [extreme heat]	−0.056122	0.0040165	153.92508	<0.000	−0.064017	−0.048228
Hours [24 h]-treatment [Ct_{max} *EHEO. lignaria* 36°C]	−0.002681	0.0053887	0.2474573	0.6189	−0.013273	0.0079108
Hours [24 h]-treatment [Ct_{max} extreme heat *O. cornifrons* 37°C]	−0.004127	0.0053887	0.5860738	0.4439	−0.014719	0.0064648
Hours [24 h]-treatment [Ct_{max} healthy *O. lignaria* 39°C]	−0.000879	0.0040165	0.0478696	0.8268	−0.008774	0.0070159
Hours [24 h]-treatment [Ct_{max} healthy *O. cornifrons* 43°C]	0.0014436	0.0053887	0.0717591	0.7888	−0.009148	0.0120355
Hours [24 h]-treatment [Ct_{max} infected *O. cornifrons* 35°C]	−0.002761	0.0053887	0.2623735	0.6085	−0.013353	0.0078311
Hours [24 h]-treatment [Ct_{max} infected *O. lignaria* 32.5°C]	−0.006743	0.0040165	2.8067302	0.0939	−0.014638	0.0011517
Hours [24 h]-treatment [extreme heat]	0.0037601	0.0040165	0.8752664	0.3495	−0.004135	0.0116548
Hours [48 h]-treatment [Ct_{max} *EHEO. lignaria* 36°C]	−0.003645	0.0053887	0.4571785	0.4989	−0.014237	0.0069471
Hours [48 h]-treatment [Ct_{max} extreme heat *O. cornifrons* 37°C]	−0.002307	0.0053887	0.1832036	0.6686	−0.012899	0.0082851
Hours [48 h]-treatment [Ct_{max} healthy *O. lignaria* 39°C]	−0.000529	0.0040165	0.017369	0.8951	−0.008424	0.0073654
Hours [Plus 48 h]-treatment [Ct_{max} healthy *O. cornifrons* 43°C]	0.0024452	0.0053887	0.2058371	0.6500	−0.008147	0.0130371
Hours [Plus 48 h]-treatment [Ct_{max} infected *O. cornifrons* 35°C]	−0.004137	0.0053887	0.5888695	0.4429	−0.014729	0.0064549

(Continued)

TABLE 3 (Continued)

Term	Estimate	Std error	L-R Chi square	Prob > Chisq.	Lower CL	Upper CL
Hours [Plus 48 h]-treatment [Ct_{max} infected *O. lignaria* 32.5°C]	−0.010067	0.0040165	6.2244276	0.0126	−0.017962	−0.002173
Hours [Plus 48 h]-treatment [extreme heat]	0.0071459	0.0040165	3.1505	0.0759	−0.000749	0.0150406
Hours [Plus 72 h]-treatment [Ct_{max} *EHEO. lignaria* 36°C]	−0.002556	0.0053887	0.2248528	0.6354	−0.013148	0.0080362
Hours [Plus 72 h]-treatment [Ct_{max} extreme heat *O. cornifrons* 37°C]	−0.003345	0.0053887	0.3850298	0.5349	−0.013937	0.0072472
Hours [Plus 72 h]-treatment [Ct_{max} healthy *O. lignaria* 39°C]	0.0050098	0.0040165	1.5521969	0.2128	−0.002885	0.0129045
Hours [Plus 72 h]-treatment [Ct_{max} healthy *O. cornifrons* 43°C]	0.0058221	0.0053887	1.1653042	0.2804	−0.00477	0.016414
Hours [Plus 72 h]-treatment [Ct_{max} infected *O. cornifrons* 35°C]	−0.005033	0.0053887	0.8713211	0.3506	−0.015625	0.0055586
Hours [Plus 72 h]-treatment [Ct_{max} infected *O. lignaria* 32.5°C]	−0.001842	0.0040165	0.2102084	0.6466	−0.009737	0.0060529
Hours [Plus 72 h]-treatment [extreme heat]	0.0076933	0.0040165	3.6490189	0.0561	−0.000201	0.0155881
Hours [Plus 120 h]-treatment [Ct_{max} *EHEO. lignaria* 36°C]	−0.002395	0.0053887	0.1974593	0.6568	−0.012987	0.008197
Hours [Plus 120 h]-treatment [Ct_{max} extreme heat *O. cornifrons* 37°C]	−0.000301	0.0053887	0.0031134	0.9555	−0.010893	0.0102912
Hours [Plus 120 h]-treatment [Ct_{max} healthy *O. lignaria* 39°C]	0.006106	0.0040165	2.3031868	0.1291	−0.001789	0.0140007
Hours [Plus 120 h]-treatment [Ct_{max} healthy *O. cornifrons* 43°C]	0.0053171	0.0053887	0.9722151	0.3241	−0.005275	0.015909
Hours [Plus 120 h]-treatment [Ct_{max} infected *O. cornifrons* 35°C]	−0.004352	0.0053887	0.6515526	0.4196	−0.014944	0.0062401
Hours [Plus 120 h]-treatment [Ct_{max} infected *O. lignaria* 32.5°C]	−0.002243	0.0040165	0.3117297	0.5766	−0.010138	0.0056517
Hours [Plus 120 h]-treatment [extreme heat]	0.0098603	0.0040165	5.9733315	0.0145	0.0019655	0.017755
Hours [Plus 192 h]-treatment [Ct_{max} *EHEO. lignaria* 36°C]	−0.002054	0.0053887	0.1453196	0.7030	−0.012646	0.0085375
Hours [Plus 192 h]-treatment [Ct_{max} extreme heat *O. cornifrons* 37°C]	−0.000143	0.0053887	0.0007031	0.9788	−0.010735	0.010449
Hours [Plus 192 h]-treatment [Ct_{max} healthy *O. lignaria* 39°C]	0.00608	0.0040165	2.283655	0.1307	−0.001815	0.0139747
Hours [Plus 192 h]-treatment [Ct_{max} healthy *O. cornifrons* 43°C]	0.0073983	0.0053887	1.8796583	0.1704	−0.003194	0.0179902
Hours [Plus 192 h]-treatment [Ct_{max} infected *O. cornifrons* 35°C]	−0.004799	0.0053887	0.7920979	0.3735	−0.015391	0.0057931
Hours [Plus 192 h]-treatment [Ct_{max} infected *O. lignaria* 32.5°C]	−0.004019	0.0040165	0.9998827	0.3173	−0.011914	0.0038755
Hours [Plus 192 h]-treatment [extreme heat]	0.0127198	0.0040165	9.8824594	0.0017	0.0048251	0.0206145

*Represents statistical significance.

heat shock protein synthesis and metabolic compensation. Across other host–pathogen systems, similar responses might be observed in different traits than heat tolerance (McClure et al., 2014; Porras et al., 2021; Breedveld et al., 2023), but such changes may be promoted by ecological rather than evolutionary processes. Exposure to stress can increase tolerance to a second stressor. For example, fungal infection increased fruit fly (*Drosophila melanogaster*) longevity (McClure et al., 2014). Heat tolerance can also be context dependent and vary locally among solitary bee populations, possibly affecting host–pathogen interactions.

Voluntary exposure to ETZ, thermal boldness, may result in physiological and behavioral adjustments that confer ecological advantages, such as increasing the opportunity to exploit food resources, escape from a predator, or increase survival (Dillon et al., 2012). The tendency to explore an ET results from

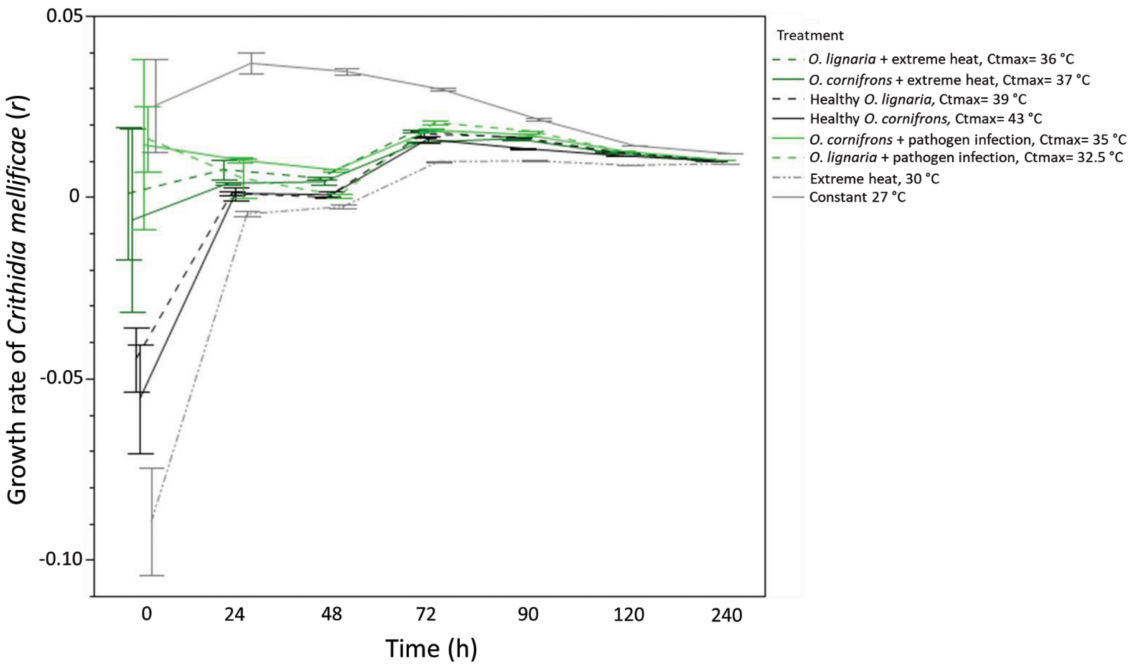

FIGURE 3
Crithidia mellificae growth rates after heat stress by Ct_{max} of *Osmia* species exposed to extreme heat, pathogen infection, the combination of both factors, as well as grown under extreme heat and optimal temperature (27°C). Growth rate was reduced after stress with Ct_{max} of healthy and exposed individual to extreme heat (mean ± SE per treatment per temperature; n = 8 replicates for *O. lignaria* Ct_{max} and n = 3 replicates for *O. cornifrons* per treatment).

thermal sensitivity of performance (via kinetic effects), thermal sensation and integration, and molecular and neural pathways being activated by such stimuli (Johnston and Bennett, 2008). Our results indicate that, despite being closely related, both bee species displayed species-specific thermal boldness behavior, where *O. cornifrons* was in general bolder than *O. lignaria*. These results agree with observations in other ectothermic animals (Myles-Gonzalez et al., 2015; Short and Lucky, 2018; Bensky and Bell, 2022). However, *O. cornifrons* was more sensitive to both EHE and infection than *O. lignaria*, which indicates that new thermal regimes and pathogens may limit its invasive success out of the native range. Thermal boldness of both species was compromised by protozoan infection as observed in another case where insects were infected by a fungal pathogen (Porras et al., 2021), and this effect was exacerbated by EHE. Thus, infectious diseases are predicted to become more severe for insects under climate warming.

Moreover, prior extreme heat exposure reduced the boldness in both solitary bees. Pathogen infection further reduced exploration behavior to cross the ETZ in both hosts species. This suggests that infection amplifies the sensitivity to heat in hosts. Similarly, in other ectotherm host–pathogen systems, infection and environmental temperature modify seasonality and prevalence of hosts (Estrada-Peña et al., 2012). Consequently, interactive stressors might drive ecological processes such a pathogen dispersal. But, the implications of these changes on either hosts or pathogens remain to be explored.

Heat exposures had adverse effects on the stationary and exponential phases of pathogen growth. The increased temperatures we evaluated reduced the *C. mellificae* growth rate for 48 h, which could be a result of energy depletion associated with the high energy demands during and after heat stress (Ketola et al., 2004). We observed an increased population growth rate of about 20% at 72 h after heat exposure (hosts' Ct_{max}), but control cells had a 50% higher growth rate than heat stressed cells. EHE might affect disease severity through different mechanisms, such as altering pathogen physiology, development, and growth rate or some immune response on the part of the host. These changes will also be context dependent, given the new selective pressure that EHE may exert on host's thermal tolerance. Future work could measure the impact of extreme heat on other traits, such as spore induction and survival of pathogens under host thermal constraints.

Overall, we conclude that EHE can reduce heat tolerance, thermal boldness of hosts, as well as pathogen growth rate. Further, EHE exacerbated the adverse effects of infection on hosts' thermal physiology and behavior. At the same time, EHE had harmful effects on the pathogen growth rate, which also proved sensitive to brief exposures to hosts' critical temperatures. Pathogen-induced thermal sensitivity of hosts has been considered a result of several factors, including temperature-dependent susceptibility of individuals, ultimately affecting feedback between host–pathogen interaction (Cohen et al., 2020). Our findings complement prior research showing climate change can alter host–pathogen systems and may influence the evolution and maintenance of species function within the ecosystems like plant pollination. The consequences of EHE may result in phenological asynchrony of host emergence, along with reductions in body mass and fat content

and disruption of pathogen development (Bosch et al., 2008). Finally, we suggest that heat tolerance and thermal boldness of hosts may limit pathogen growth, but the ultimate consequences for disease dynamics shall depend on EHE effects on both hosts and pathogens. Thus, host–pathogen interactions may be very sensitive to climate warming, but more studies are necessary to predict disease outcomes and their effects on ecosystem functioning and services such as pollination.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

MFP and CAN conceived the study. MFP and MGS-M conducted the experiments. MFP, EGR, CAN, and GAA-C wrote the first version of the manuscript. MFP, EGR, CAN, GAA-C, SGC, JGS, VL, and DB discussed the results and commented on the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1186452/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Protozoan pathogens were grown under optimal conditions (27°C), harvested, and used to determine Ct_{max} by exposing them to different temperatures. The same cells were then used to assess recovery after heat stress by following the growth. Healthy and infected hosts were used to evaluate heat stress and behavior.

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