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Impact of heatwave amplitude, duration, and timing on parasite fitness at different baseline temperatures

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Abstract

The frequency and severity of heatwaves are increasing, posing challenges for understanding their effects on host-parasite dynamics. Especially, our understanding of the role of specific heatwave attributes in shaping disease outcomes remains limited. In this study, the *Daphnia magna–Ordospora colligata* host-parasite system, a widely used model for environmentally transmitted diseases, was used to investigate heatwave attributes. The amplitude and duration of heatwaves were manipulated across four baseline temperatures and four distinct time points relative to host exposure to the pathogen. This design resulted in 64 unique heatwave treatments, with *O. colligata* fitness (measured as prevalence and proliferation) recorded at the individual level in temperature-controlled water baths. Results show that heatwaves can alter parasite burden up to 13-fold, whereby amplitude, duration, and timing can interact with baseline temperature. Our results reveal complex interactions between heatwave attributes and baseline temperature, emphasising that heatwaves have context-dependent effects on parasite prevalence and proliferation. Additionally, when compared to other types of temperature variation (for example, cold snaps), heatwaves behave differently. While specific effects may vary across systems, these results demonstrate that interactions between heatwave attributes and baseline temperature can drive substantial variation in infection outcomes. These findings highlight the challenges and complexities involved in understanding and predicting how climate change and extreme weather events may influence disease dynamics in the context of global change. This underscores the need to incorporate thermal fluctuations into disease ecology models, as host-parasite responses to climate extremes are unlikely to be uniform across taxa.

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Introduction

Climate change is increasingly recognised as a pivotal driver of alterations in human health [1], biodiversity [2], and ecosystem changes [3]. The rapid anthropogenic

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change in climate can disrupt host-parasite dynamics [4–6] as it has been well established that the host-parasite relationship can be modified by temperature [7–11]. Indeed, a recent study reported that 58% of human pathogenic diseases have been aggravated by climate change [12]. Temperature can influence host susceptibility by altering host homeostasis [13], immunity [14], and behaviour [15]. For example, in marine ecosystems, elevated sea temperatures can increase the prevalence of coral diseases by impairing coral immune functions and promoting pathogen growth [16]. Moreover, temperature changes can directly affect the parasite by modifying its infectivity [17], proliferation [18], and survival in the environment [19]. For example, the parasitic trematode *Ribeiroia ondatrae* infecting an amphibian host displayed an increase in host penetration but a decrease in infectivity and survival outside of the host depending on temperature [20]. Thus, the outcome of host-parasite relationships in response to temperature can be complex [18, 21, 22]. Yet, climate change is not only a linear increase in mean temperature [23], but it also modifies temperature variability, leading to altered temperature fluctuations and more frequent extreme weather [24].

Climate change is expected to increase the frequency and magnitude of anomalous weather events, such as heatwaves, cold snaps, and droughts [25]. Indeed, the frequency, intensity, and duration of heatwaves have accelerated globally since the 1950s [26,27]. Like rising mean temperatures, these temperature shifts caused by extreme and fluctuating weather can influence the survival of both the host and the parasite [18]. For example, thermal fluctuations at the lower thermal limits of malaria will increase infection rates, while decreasing infection rates at the upper thermal limits [28]. Moreover, climatic extremes are expected to result in biodiversity loss [29], changes in coevolutionary trajectories [21], and shifts in ecosystem structure [30] as the host-parasite relationship is altered. For instance, abnormally cooler temperatures in Oregon led to sea star wasting disease in the sea star *Pisaster ochraceus,* which resulted in the collapse of this keystone predator, leading to a trophic cascade [31]. Regardless of parasite exposure, thermal extremes are expected to influence life history traits [32], including development [33], ageing [34], and reproductive output [35]. Furthermore, temperature variability can disrupt transmission pathways, alter host susceptibility, and shift parasite life cycles in multi-host parasite systems [36,37]. Such effects are particularly relevant for vector-borne and environmentally transmitted pathogens, where hosts and parasites may respond differently to thermal fluctuations. However, not all thermal fluctuations or extreme weather events are the same; different attributes of such temperature events can lead to alterations in physiological responses.

The amplitude and duration of temperature fluctuations and extreme weather events are known to have variable outcomes on host-parasite interactions [38–43]. For example, immune responses in ectothermic hosts can vary depending on the frequency and severity of thermal fluctuations, with some species exhibiting immune suppression under prolonged heat stress while others show resilience through plastic physiological adjustments [44,45]. Extreme temperature events such as heatwaves can also modulate immune function, either by suppressing immune defences or

altering energetic trade-offs [46,47]. Such immune shifts may have cascading effects on disease susceptibility, influencing parasite establishment and proliferation in hosts experiencing thermal stress. Additionally, the temporal scale of multiple stressors (e.g., heatwave attributes) should be considered to add realistic complexity when exploring disease outcomes [48]. Indeed, the timing of heat stress relative to infection can influence which aspects of the host-parasite interaction are most affected as parasites may trigger immune responses or other physiological changes [49]. Moreover, predictive models incorporating thermal performance curves suggest that climate-driven shifts in disease prevalence will depend on complex interactions between temperature, parasite fitness, and host susceptibility [5, 50]. These models emphasise the importance of considering non-linear thermal effects when forecasting future disease risks under climate change. To better understand how these complex interactions will alter the host and parasite, it is important to consider how specific temperature events, like heatwaves, may directly affect physiological responses of the host. Still, while some papers [41, 51, 52] have focused on the effect of heatwaves on the host-parasite relationship, few have comprehensively examined the effect of varying heatwave attributes across multiple temperatures [38, 41].

Here, we investigated the impact of varying heatwave attributes (that is timing, amplitude, and duration) across a broad spectrum of baseline temperatures (representing the mean temperature maintained throughout the experimental period). We used the *Daphnia magna – Ordospora colligata* host-pathogen model, a popular model system for environmentally transmitted diseases (characterised by horizontal transmission mechanisms and infection dynamics governed by mass action principles) [53]. The studies by Kunze et al. (2022) and McCartan et al. (2024) were the basis used for the experimental design. Although both studies found alternative patterns in parasite burden in the same *Daphnia-Ordospora* system depending on the heatwave/cold snap treatment (i.e., a pulse of extreme temperature), they did not specifically manipulate heatwave attributes. Building upon this observation, we hypothesised that not all heatwave treatments may result in the same outcome in parasite fitness, and this may depend on the baseline temperature, amplitude and duration of the heatwave, and timing in relation to disease exposure. In fact, parasite performance can be reduced as a result of thermal stress when temperatures become too high (as suggested by the Thermal Stress Hypothesis [54]), while shifts in temperatures may lead to increased parasite performance according to the Thermal Variability Hypothesis [9]. This hypothesis suggests that smaller organisms, such as parasites, have a faster metabolic rate than their larger hosts and consequently will acclimate more quickly to temperature changes compared to their hosts (as outlined by the Metabolic Theory of Ecology [55]). Moreover, as thermal performance curves are inherently non-linear, small changes in temperature can result in disproportionately large changes in host or parasite performance due to Jensen's Inequality [38]. In addition, as shifts in temperature are known to distinctly affect various host and parasite life history traits [11, 20, 41, 54, 56], this may further complicate how parasite life cycles, disease dynamics, and epidemics are impacted by global change. Overall, this means that the effects of temperature fluctuations on the host-parasite relationship may be complicated, as performance can be affected by the variability in temperature, altering expectations regarding disease outcome [57]. As anticipated, our findings revealed that heatwaves can induce unexpected and intricate shifts in parasite infection prevalence and proliferation, which will impede the prediction of host-pathogen relationships under climate change.

Materials and Methods

Two experiments were conducted to explore the effect of heatwaves (i.e., pulse heat treatments) on parasite fitness. Experiment 1 focused on the effect of baseline (constant) temperature, timing, amplitude, and duration on infection prevalence and burden in *Daphnia magna* infected with *Ordospora colligata.* Experiment 2 focused on the repeatability of results using one baseline temperature and one amplitude but altered the timing and duration of heatwave treatments.

Study system

The freshwater crustacean *Daphnia magna* (genotype Fi-OER-3–3) was infected with its microsporidian parasite *Ordospora colligata* (isolate 3) to simulate 64 heatwave treatments (both host and parasite were originally sampled from

Tvärminne, Finland, and maintained in the lab for over 10 years). *Daphnia magna* is a widely studied freshwater crustacean found across the northern hemisphere, which clonally reproduces via parthenogenesis, allowing for genotypic consistency [58]. They are a popular model system for the study of host-pathogen interactions [53] due to their fast generation time, their well-known ecology, and their key role in aquatic ecosystems [59]. *Ordospora colligata* is a microsporidian microparasite (i.e., an infectious disease) which is transmitted horizontally to the host via filter feeding. It invades the upper gut epithelium, where it forms intracellular clusters of 32–64 spores, which burst and are shed into the water via faeces [58]. We may expect to see differences in the performance of the antagonists in this system in response to heatwaves, given that *Daphnia* have a wider thermal range of 6-33.3°C compared to *Ordospora's* narrower range of 11.8-29.7°C [7].

Temperature-controlled water baths

Water baths were used to ensure the water temperature was accurately controlled throughout the experiment. Each bath was fitted with an aquarium chiller (Hailea HC-150A, DC300, or DC750), an aquarium heater (EHEIM JÄGER 300W) and pumps (Micro-Jet Oxy and Oase Optimax 500) to ensure even temperature distribution, and a programmable controller (Inkbird ITC-308 or ITC-310T) for regulation. Temperatures were monitored using a HOBO logger (HOBO UA-001–08) and checked daily. Heatwaves were simulated by moving individual microcosms directly to their assigned temperature bath, while the baths were maintained at a constant baseline temperature for the duration of the experiment. Acclimatisation to the heatwave treatment started immediately upon entering the bath, and microcosms took less than an hour to reach the target temperature. When the heatwave treatments were concluded, the microcosms were returned to their original position.

Experiment 1 setup

The methods used for this experiment were similar to those outlined by McCartan et al. (2024). A fully factorial experimental design was conducted where the timing, amplitude, and duration of heatwaves were altered across four baseline (constant) temperatures (14, 17, 20, and 23°C) using eighteen temperature-controlled water baths ranging from 14-29°C. Heatwave duration was varied by 3 days or 6 days (two durations), and heatwave amplitude was increased by +3°C or +6°C (two amplitudes). These baseline temperatures were chosen to remain within the thermal limits of *Ordospora* (11.8–29.7°C) while ensuring infection success and thermal stress at some baseline temperatures. Heatwave treatments increased temperatures by up to 6°C to reflect realistic warming scenarios [60], representing an extreme but plausible event that pushes conditions toward *Ordospora's* upper thermal threshold. After the heatwave treatment was completed, treatments returned to their baseline temperature. The experiment commenced 10 days prior to exposure to *Ordospora* (day -10). Individual juvenile female *Daphnia* were added to allocated microcosms held in the water baths prior to the start of the first heatwave (day -10), and subsequent heatwave treatments occurred on day 0 (day of exposure to *O. colligata*), day 10, and day 20 (four timings) (Fig 1). In total, there were 64 heatwave treatments (4 baseline temperatures • 4 heatwave timings • 2 amplitudes • 2 durations), each with 15 replicates. A constant temperature treatment (amplitude 0°C) was included for each baseline temperature with additional constant controls at 26°C and 29°C. Likewise, uninfected (placebo) controls were included at each temperature to confirm that no unintended spread of *Ordospora* occurred.

All treatments were organised into trays where a tray contained twenty-seven 100 mL microcosms (filled with 80 mL of Artificial *Daphnia* Medium (ADaM [61]). A single tray contained two constant temperature treatments, one uninfected control and five replicates of a single heatwave treatment. Each temperature had three baths, and each bath held four trays. One tray per bath corresponded to a single heatwave timing (i.e., all heatwave treatments in the same tray began a simulated heatwave treatment at the same time) (Fig 1). Trays were rotated every day in a clockwise direction within each bath. In total, there were 1176 animals, each placed in a separate microcosm (i.e., 960 heatwave treatments, 144 constant treatments and 72 uninfected controls).

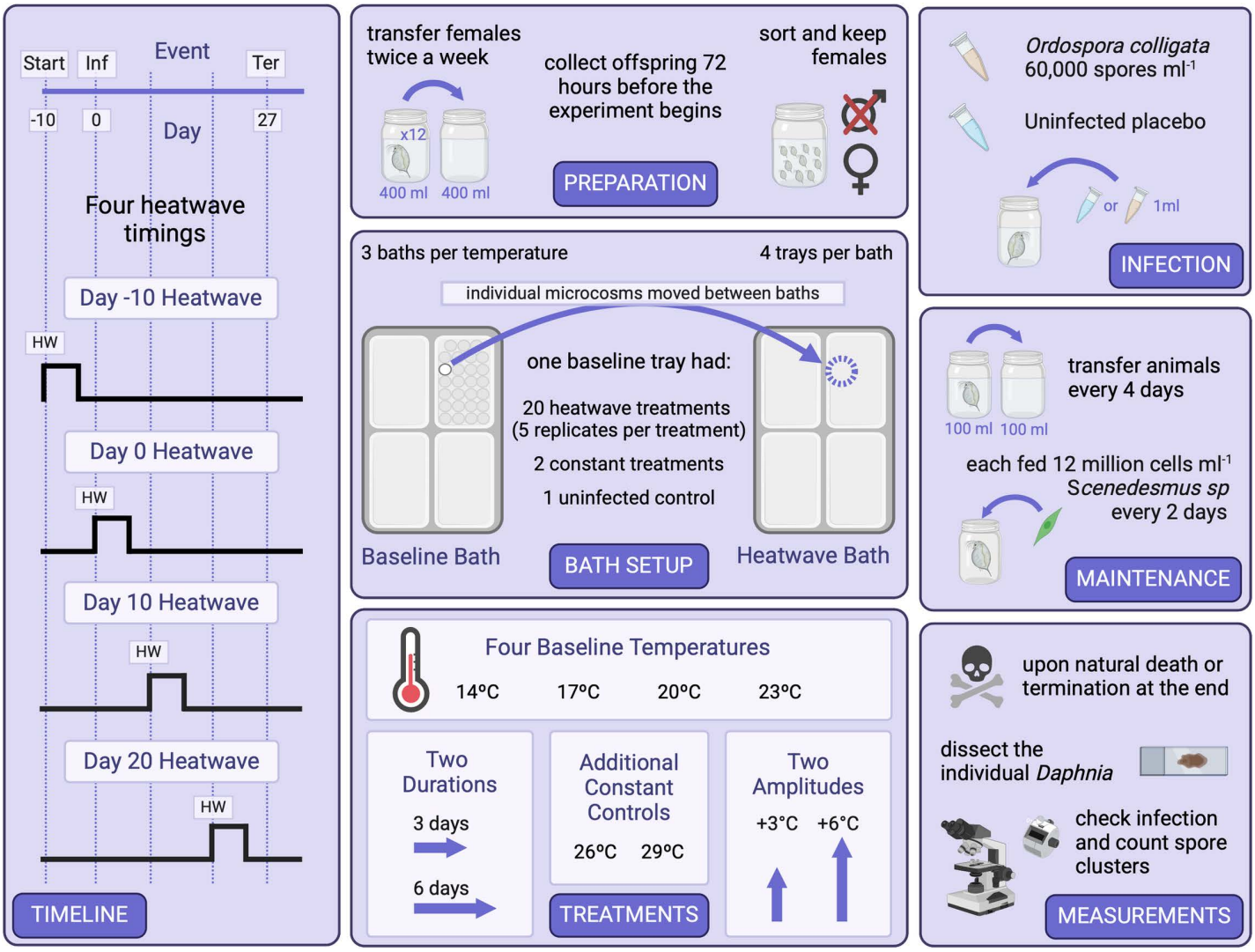

Fig 1. ***Experimental Setup.*** *TIMELINE; illustrates the timeline for the experiment starting at day -10 and terminating at day 27, exposure occurred on day 0, and heatwaves began on day -10, 0, 10, and 20. PREPARATION; preparation started three weeks prior to exposure when 10-12 mothers were added to 400 mL microcosms, 72 hours before the experiment began, offspring were collected and females were kept. INFECTION; a dose of ~60,000 Ordospora colligata spores was given to each exposed individual, while unexposed controls were given a placebo dose made of crushed-up uninfected individuals. BATH SETUP; each temperature had three baths, and each bath held four trays with 27 microcosms. Each bath contained 80 heatwave-treated individuals (five replicates per heatwave treatment), eight constant temperature treatments, and four uninfected controls. To simulate a heatwave, microcosms were moved between baths and then returned to the baseline bath when the heatwave finished. TREATMENTS; 64 treatments were included in the experiment (four baseline temperatures • four heatwave timings • two amplitudes • two durations). Additionally, at the heatwave-specific temperatures (26°C and 29°C), a tray held 27 microcosms but only two constant treatments and one uninfected control, the remaining microcosms contained no Daphnia, so these could be moved when the heatwave occurred. MAINTENANCE; maintenance and measurements were carried out between days -8 and day 27. Figure created on Biorender.com.*

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Daphnia preparation and exposure to *Ordospora*

A group of 10–12 females were added to 400 mL microcosms with ~300 mL of ADaM, transferred twice a week, and fed *ad libitum* with batch culture algae (*Scenedesmus* sp.) to stimulate the production of offspring [62]. The juvenile offspring used to initiate the experiment were collected in a 72-hour period and sexed (using a dissecting microscope at 8x to 12x

magnification). Only females were kept to remove variation in sex [58], which corresponds to the dominant state of the natural populations, which are female-biased. The experiment began on day -10 when *Daphnia* juveniles were individually added to a 100 mL microcosm filled with 50 mL of ADaM and a pinch of Cetyl alcohol to break the surface tension. The individuals remained unexposed until day 0 when *Daphnia* in the exposed treatments received a 1 mL dose of ADaM containing ~60,000 *Ordospora* spores. The spore dose was created by crushing ~3580 individuals with a known average spore burden, and the slurry was diluted to 1300 mL using ADaM. Uninfected control treatment *Daphnia* received a placebo dose made up by crushing uninfected *Daphnia.* Individuals were transferred to fresh ADaM every four days (to avoid the accumulation of offspring produced and waste products) and fed every two days (from 5 million algae/mL on day -10–12 million algae/mL by day 5, which remained constant until the end of the experiment). Day 0 also coincided with the start of a heatwave, which commenced after all *Daphnia* received either an infective or placebo dose.

Experiment 2 setup

A second experiment was carried out to test the reproducibility of the results. Here, timing and duration were altered at the same amplitude (+6°C) and a single baseline temperature (17°C). Heatwaves occurred 10 days prior to exposure, the day of exposure (day 0) and 10 days post-exposure. A heatwave occurring 20 days post-exposure was excluded to focus on timings before, during, and after exposure at equal intervals. Duration was also manipulated, and individuals were subjected to either a 3-day or a 6-day heatwave at 23°C (two durations). Constant temperature controls and uninfected individuals to control for accidental infections were also present in this experiment. In total, there were six treatments (3 heatwave timings • 2 durations • 1 amplitude). There were two baths at the baseline temperature (17°C) and two heatwave baths (23°C). Each baseline bath held four trays (with 27 microcosms in each tray), and each bath held 90 heatwave-treated individuals (thirty replicates per heatwave treatment), fourteen constant treatments, and three uninfected controls. In total, experiment 2 contained 248 individual *Daphnia,* each in an individual microcosm. The bath and microcosm setup was the same as in experiment 1. Likewise, infection preparation was similar, but ~1148 *Daphnia* were used to make an infective dose of ~60,000 spores in a 400 mL dilution, while uninfected individuals received a placebo dose. The feeding regime was also identical to experiment 1.

Measurement of parasite fitness for both experiments

To measure parasite fitness, the infection prevalence (i.e., presence or absence of spores) and burden (i.e., the number of spore clusters in the host) were examined in each individual. *Daphnia* were checked for infection upon natural death or upon termination at the end of the experiment (within five days of day 27). If infected, spore clusters (each cluster holding up to 64 individual spores) were counted using bright field or phase-contrast microscopy (with 400x magnification). For experiment 1, deaths occurring before day 9 (n = 98) were omitted due to the lack of accuracy in the diagnosis of infection status at this early stage. Any inconclusive infections (n = 35) and misidentified males (n = 2) were also omitted. For experiment 2, deaths occurring before day 7 (n = 25) were omitted due to the lack of accuracy in diagnosis, and inconclusive infections (n = 7) and males (n = 1) were also omitted.

Data analysis for both experiments

Analysis was performed using R version 4.0.3 [63]. Infection prevalence (i.e., presence or absence of infection), exposure (i.e., number of *Ordospora* spore clusters, including zeros for uninfected exposed individuals), and burden (i.e., the number of *Ordospora* spore clusters excluding zeros) were the response variables. Experiment 1 used baseline temperature, timing, amplitude, and duration as the explanatory variables, while Experiment 2 used experiment, timing, and duration as the explanatory variables. Infection prevalence and exposed data included all exposed individuals, while burden data included confirmed infections only. Exposure data can be found in the supplement (Tables D and E in S1 Text) and corresponded largely to the output of the model for 'burden'. Furthermore, 'bath' was excluded as a variable as 'temperature'

was treated as a continuous centred variable to reduce collinearity issues in experiment 1, and was not significant in experiment 2 (GLM, analysis of deviance $\chi^2_{(2,275)}$ $p = 0.8618$).

For experiment 1, baseline temperature was treated as a continuous centred variable to address collinearity concerns, with a cubic polynomial added to the burden model to accommodate the non-linear temperature response, while infection prevalence was modelled with a linear relationship. For variable selection and regression modelling, elastic net regression was applied using the "glmnet" package [64] as a Generalised Linear Model (GLM) with a Poisson distribution, a GLM with a negative binomial distribution (glm.nb), and a Generalised Linear Mixed Model (GLMM), all had problems with multicollinearity. Elastic net regression combines L1 (lasso) and L2 (ridge) regularisation by shrinking coefficients and preventing overfitting, thus mitigating the impact of collinearity. While elastic net regression is effective for handling multicollinearity and high-dimensional data, some limitations should be noted. It does not support random effects, so batch-specific variability (e.g., 'bath') could not be accounted for. Additionally, while it helps manage overfitting, elastic net may still have limitations in fully capturing complex random effects that might be present in the data. Therefore, while mixed-effects models, like GLMMs, were considered, 'glmnet' was chosen for its effectiveness in managing multicollinearity and high-dimensional data.

The 'cv.glmnet' function was used with a binomial distribution for infection prevalence. The regression fit (alpha = 0.5) was selected based on the lowest cross-validation error, while the regularisation strength (lambda = 0.0467) was chosen via cross-validation with one standard error 'lambda.1se'. For exposed data, the 'glmnet' function was chosen with a regression fit (alpha = 0.85) based on the lowest AIC score. The regularisation strength of the model was also chosen by the lowest AIC score (lambda = 0.0050). The negative binomial family (theta = 0.7260) was selected to account for overdispersion. Burden also used the same 'glmnet' function and has a regression fit (alpha = 0.7) and regularisation strength (lambda = 0.0248) based on the lowest AIC score. A negative binomial family (theta = 1.3112) was also used in this model. Bootstrapping (50,000 iterations) was used for infection prevalence, exposure, and burden to assess the model stability and significance, resulting in the estimated coefficients (effect) and their 95% confidence intervals. Finally, feature importance was assessed for each factor by calculating the absolute value of the effect coefficients from the elastic net regression model. This approach allowed us to identify the most influential features in predicting the outcome (response variable). The process was performed separately for each polynomial degree, and the results were then aggregated to determine the overall importance of each variable. Custom contrasts were created with the "emmeans" package [65] to compare the average infection prevalence and burden of the heatwave treatments. For this, timing, amplitude, and duration were combined into a new variable 'treatment', and modelled against temperature for clarity of interpretation. The contrast p-values were then adjusted for multiple comparisons using the 'Benjamini-Hochberg' method [66].

Data analysis to compare experiments 1 and 2 focused on burden using a GLM with a negative binomial distribution ('glm.nb') to observe the individual effects of timing, duration, and experiment (the difference between experiments 1 and 2), with no interaction between the variables as these were not significant. An analysis of deviance (χ^2) was then carried out to assess the overall significance of the predictor variables. Finally, to observe the effect of specific treatments in experiments 1 and 2, the "emmeans" package was used to compare burden against the predictor variable 'treatment' (combined variables: experiment, timing, and duration). In other words, experiment 1 treatments were compared to one another, and experiment 2 treatments were compared, but all in the same model (Table K in S1 Text). Like with experiment 1, contrast p-values were adjusted for multiple comparisons using the 'Benjamini-Hochberg' method.

Results

Infection prevalence

All factors (i.e., the timing, amplitude, and duration of the heatwave, and baseline temperature) influenced infection prevalence. Timing of the heatwave (before, during, or after infection) led to variable outcomes (Fig 2). Infection prevalence was reduced in heatwaves beginning on the day of exposure (day 0) that had a strong amplitude of +6°C above the baseline

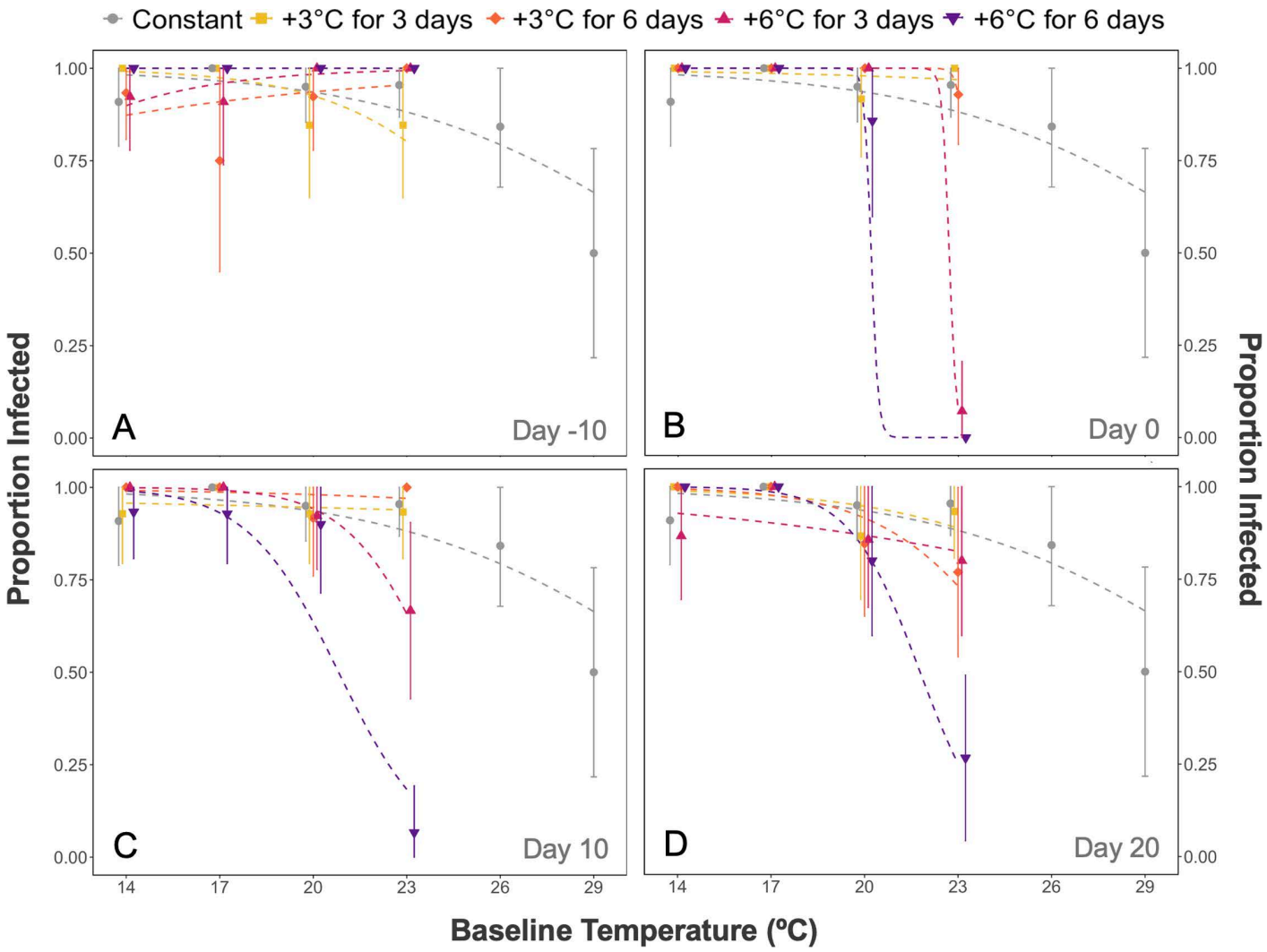

Fig 2. ***The effect of heatwave treatments on infection prevalence in Daphnia magna infected with Ordospora colligata.*** *Each spore cluster contains 32-62 individual Ordospora. Error bars represent the standard error, and each dashed line is the linear fit of the elastic net regression (see Table A in S1 Text for statistical results). Grey; constant treatment, yellow; heatwave lasting 3 days where the temperature was increased by 3°C, orange; heatwave lasting 6 days where the temperature was increased by 3°C, pink; heatwave lasting 3 days where the temperature was increased by 6°C, purple; heatwave lasting 6 days where the temperature was increased by 6°C. A; heatwave treatments which began on day -10, B; heatwave treatments which began on day 0, C; heatwave treatments which began on day 10, and D; heatwave treatments which began on day 20. All factors (heatwave timing, amplitude, duration and baseline temperature) were important for predicting infection prevalence. A strong amplitude treatment (which increased temperature by 6°C) occurring on the day of exposure influenced infection prevalence depending on baseline temperature. Furthermore, the heatwave treatment, which increased temperature by 6°C for 6 days specifically, had distinct effects on infection prevalence depending on baseline temperature. Finally, baseline temperature, regardless of any heatwave attributes, was also important for predicting infection prevalence.*

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temperature (*most important predicting infection prevalence, linear effect, glmnet, effect = -0.44, 95% CI: -0.49 to -0.31 and feature importance = 0.44*, Table 1). These heatwaves only had a single infection at 23°C (Fig 2B) when the heatwave co-occurred during parasite exposure. Indeed, here, the strong and short heatwave treatment (a heatwave of +6°C for 3 days) was different from the constant treatment (*emmean, z(inf) = -3.848, p < 0.008, see Table C in S1 Text for p-values of all custom contrasts*). Yet, when the same heatwave occurred 10 days prior to exposure, no reduction in infection prevalence was observed (Fig 2A). Furthermore, only a small reduction (not significant) was observed when a heatwave

Table 1. Coefficient estimates and feature importance of infection prevalence. A) Coefficient estimates and B) feature importance of infection prevalence in Daphnia magna infected with Ordospora colligata. Coefficient Effect (Effect) represents the estimated coefficient of the 'glmnet' elastic net regression model. Positive values indicate a positive relationship between the variable and infection prevalence, while negative values indicate a negative relationship (i.e., a positive value means the explanatory variables increased prevalence). Effect sizes represent the strength of these relationships, with larger absolute values indicating stronger effects. Lower (Lower CI) and Upper (Upper CI) Confidence Intervals indicate the range (95th percentile) from bootstrapping. Non-significant values have CIs encompassing 0, suggesting no important relationship to the response variable. Temperature was modelled as a linear relationship. Feature importance (Importance) is calculated by reporting the absolute values of coefficient effects for each variable. For clarity, only the most impactful variables are shown here. Refer to Table A in S1 Text for full coefficient estimate output and feature importance variables.

A	Explanatory Variable	Effect	Lower CI	Upper CI
	Temperature: Timing 0: Amplitude +6	-0.44	-0.49	-0.31
	Temperature: Amplitude +6: Duration 6	-0.17	-0.25	-0.06
	Temperature	-0.08	-0.14	-0.02
B	**Explanatory Variable**	**Importance**		
	Temperature: Timing 0: Amplitude +6	0.44		
	Temperature: Amplitude +6: Duration 6	0.17		
	Temperature	0.08		

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occurred 10- and 20-days post-exposure for the short 3-day heatwave with a strong amplitude of +6°C (Fig 2C and 2D). However, regardless of the timing of the heatwave, amplitude and duration also influenced the host-parasite interaction. The strong amplitude and long duration heatwave (heatwave of +6°C for 6 days) differentially influenced prevalence compared to other treatments depending on the baseline temperature and was important in predicting prevalence whereby prevalence decreased with increasing temperature (*linear effect, glmnet, effect = -0.17, 95% CI: -0.25 to -0.06,* Table 1). For example, at 23°C, a strong and long heatwave (heatwave of +6°C for 6 days) occurring 10 days post-exposure was different from the constant temperature treatment (*emmean, z(inf) = -3.904, p < 0.008, see Table C in S1 Text*, Fig 2C). Finally, the baseline temperature was also important for predicting infection prevalence (*linear effect, glmnet, effect = -0.08, 95% CI: -0.14 to -0.02 and feature importance = 0.08,* Table 1*).* Infection prevalence declined as baseline temperatures increased across all heatwave timings (Fig 2). The high feature importance of two complex 3-way interactions present in the infection prevalence model (i.e., indicating how much these variables contribute to the model's predictions) indicates that baseline temperature, timing of the heatwave, amplitude, and duration all influence infection prevalence in the *Daphnia-Ordospora* system.

Burden

For parasite proliferation, the effects of a heatwave ranged from a 13.5-fold decrease in burden to a 2.4-fold increase (Fig 3, Fig 4). Specifically, individuals that experienced a heatwave with a baseline temperature of 23ºC, 10 days post-exposure, with an amplitude of +6ºC for 6 days had a lower burden (mean of 34 spore clusters) than the baseline constant (mean of 458 spore clusters) (Fig 4L), with only one successful infection. Conversely, a heatwave on the day of exposure (day 0) with an amplitude of +6ºC for 6 days and a baseline temperature of 14 ºC resulted in a 2.4-fold increase in burden compared to the baseline constant (mean of 472 vs 197 spore clusters, respectively) (Fig 4E). Thus, similar to infection prevalence, the number of spore clusters within the *Daphnia* depended on the timing of the heatwave, the amplitude and duration, and the baseline temperature (Fig 3). This is supported by the high feature importance of the complex 3-way and 2-way interactions (Table 2), which highlight the intricacy and context-specific nature of the outcomes in burden.

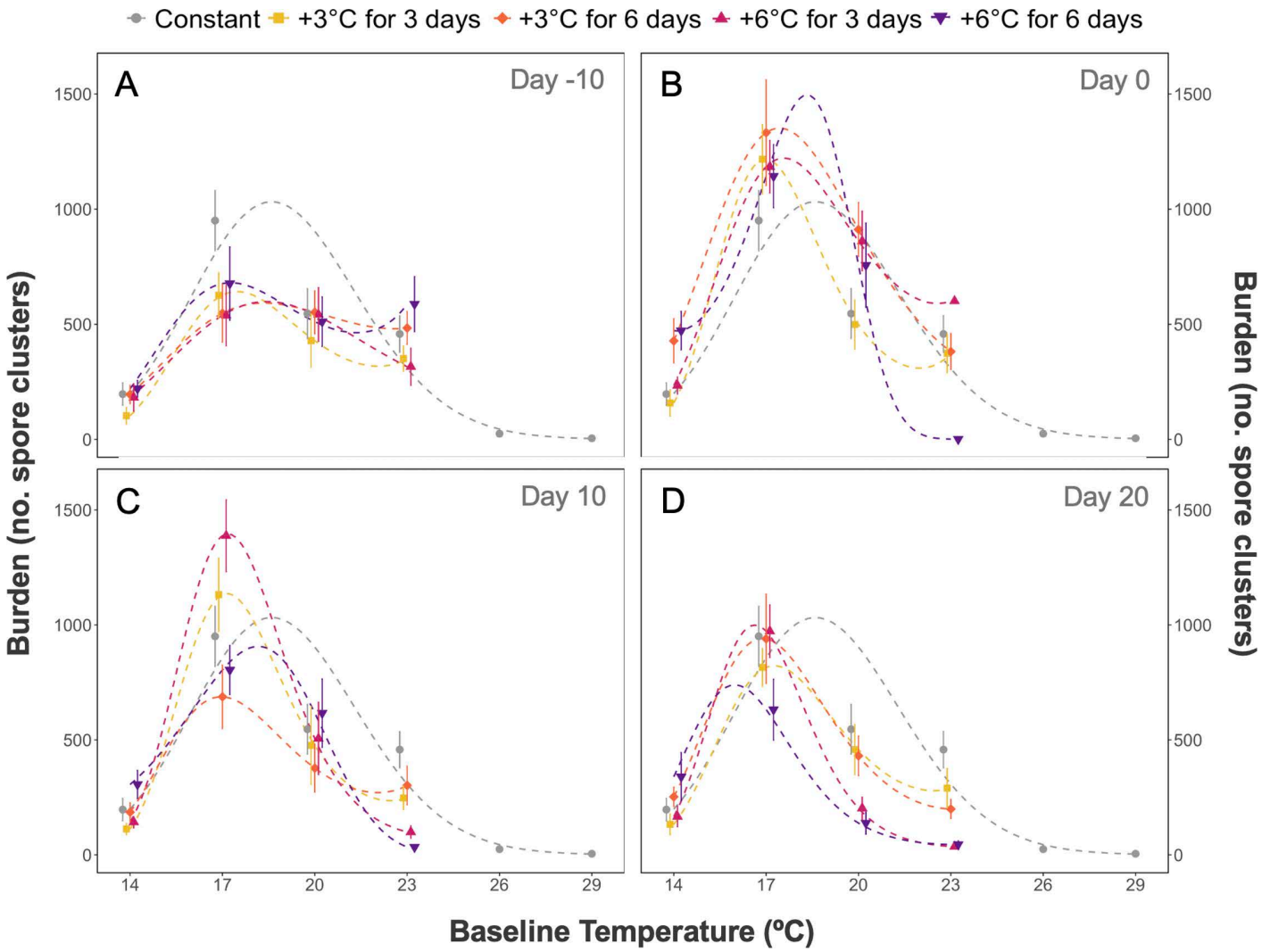

Fig 3. ***The effect of heatwave treatments on burden (average number of spore clusters), in Daphnia magna infected with Ordospora colligata.*** *Each spore cluster contains 32-62 individual Ordospora. Error bars represent the standard error, and each dashed line is the cubic fit of the elastic net regression (see Table F in S1 Text for statistical results). The absence of error bars at any point indicates the presence of a single data point for the treatment. Grey; constant treatment, yellow; heatwave lasting 3 days where the temperature was increased by 3°C, orange; heatwave lasting 6 days where the temperature was increased by 3°C, pink; heatwave lasting 3 days where the temperature was increased by 6°C, purple; heatwave lasting 6 days where the temperature was increased by 6°C. A; heatwave treatments which began on day -10, B; heatwave treatments which began on day 0, C; heatwave treatments which began on day 10, and D; heatwave treatments which began on day 20. No infections occurred in the + 6ºC for 6 days, but the data point (0) was added for clarity in visualisation. Baseline temperature was important for predicting burden. The strong amplitude treatment (which increased temperature by 6°C) occurring 20 days post-exposure influenced burden, but the influence of this effect depended on baseline temperature. Regardless of heatwave timing, a short-duration heatwave (3 days) could also influence burden depending on baseline temperature. Specifically, a heatwave treatment, which increased temperature by 3°C for 3 days, had distinct effects on burden depending on baseline temperature.*

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Baseline temperature influenced burden and resulted in the second-highest feature importance (*quadratic effect, glmnet, effect = -19.40, 95% CI: -22.36 to -16.81 and feature importance of the combined polynomial = 19.40,* Table 2). Overall, similar to infection prevalence, burden followed a classic temperature curve with the highest spore counts around the optimal temperature (~17°C) and lower parasite performance at the extremes (Fig 3). However, in heatwave treatments occurring after exposure (day 10 and 20), proliferation generally decreased compared to the constant treatment (Fig 3C

and 3D). Here, with rising baseline temperature, the negative effect on burden accelerated, resulting in a non-linear decelerating relationship. Indeed, alternate baseline temperatures can result in differential outcomes between heatwave treatments. For example, for heatwaves occurring 20 days post-exposure, comparing the strong and long (+6°C for 6 days) and the weak and long (+3°C for 6 days) heatwaves at 14°C and 20°C led to differential outcomes (Fig 3D). At 14°C, the strong and long heatwave had a greater burden (although not significantly greater) than the weak and long heatwave (*emmean, z(inf) = 0.904, p = 0.808, see Table H in S1 Text*, Fig 4M). However, at 20°C, the opposite was true, whereby the weak and long heatwave had the greater burden (*emmean, z(inf) = -3.01, p = 0.048, see Table H in S1 Text*, Fig 4O). Moreover, while baseline temperature can influence the proliferation of *Ordospora*, the timing of the heatwave can lead to alternative outcomes.

Timing of the heatwave relative to parasite exposure influenced burden and affected parasite performance, leading to shifts in thermal performance compared to the constant treatments. For example, there was no difference in burden with any heatwaves occurring 10 days pre-exposure when compared to the baseline constant (Fig 3A, Table F in S1 Text). Yet, heatwaves occurring 20 days post-exposure resulted in a shift in optimal parasite performance towards the colder temperatures (*effect = -0.54, 95% CI: -0.76 to -0.20 and feature importance of the combined polynomial = 0.54,* Table 2, Fig 3D). Timing also interacted with baseline temperature and amplitude to influence proliferation. Specifically, at 20 days post-exposure, a strong amplitude (+6°C) heatwave resulted in a lower burden compared to the constant treatments at and above 20°C (*linear effect, glmnet, effect = -14.65, 95% CI: -21.91 to -0.75 and feature importance of the combined polynomial = 25.52,* Table 2, Fig 3D). For example, at 23°C, both the strong and short (+6°C for 3 days) and the strong and long (+6°C for 6 days) heatwaves had a lower burden than the constant (*emmean, z(inf) = -5.933, p < 0.001 and emmean, z(inf) = -4.099, p = 0.001, respectably, see Table H in S1 Text*, Fig 4P). Thus, the timing of the heatwave can influence parasite burden and result in complex interactions depending on the amplitude and duration of the heatwave and the baseline temperature at which it occurs.

Regardless of timing, amplitude and duration also influenced parasite proliferation. Indeed, burden was influenced by a short-duration (3 days) heatwave mediated by baseline temperature (*cubic effect, glmnet, effect = 9.44, 95% CI: 3.66 to 14.78 and feature importance of the combined polynomial = 9.82,* Table 2). For example, burden increased in a short-duration (3 days) heatwave occurring 10 days post-exposure at 17ºC, while a strong and long heatwave (+6ºC for 6 days) reduced burden (Fig 4J). In other words, a short-duration heatwave mediated by baseline temperature resulted in a non-linear response in burden where an increase in temperature accelerated burden at median temperature ranges and decelerated at others. More specifically, the weak and short heatwave (+3°C for 3 days) can influence burden depending on the baseline temperature (*linear effect, glmnet, effect = 6.77, 95% CI: 0.13 to 12.38 and feature importance of the combined polynomial = 9.82,* Table 2, Fig 3). For example, at 14°C, there was a 1.6-fold decrease in burden in the heatwave treatment (+3°C for 3 days) (mean of 126 spore clusters) compared to the constant treatment (mean of 197 clusters). Furthermore, certain combinations of timing, amplitude, and duration of heatwave treatments also resulted in differential outcomes in burden. A heatwave occurring at 14ºC on the day of exposure (day 0) with a weak amplitude and long duration (+3°C for 6 days) resulted in a 2.7 times higher spore burden than a weak and short heatwave (+3°C for 3 days) (*emmean, z(inf) = 2.970, p = 0.048, see Table H in S1 Text*, Fig 4E). Therefore, heatwave treatments can lead to differing outcomes in parasite fitness, and the impact can depend on the amplitude and duration of the heatwave as well as the timing and baseline temperature at which it occurs.

Experiment comparisons

Analysis of both experiments showed that both the timing and duration of the heatwave altered the *Ordospora* burden compared to constant temperature treatments. However, there was a difference between the burden in experiments 1 and 2 whereby the overall burden of experiment 1 was higher than experiment 2 by a mean of 422 spore clusters (*GLM, analysis of deviance* $X^2_{(1,276)}$ $p < 0.001$) although there were no interactions (Fig 5, Table 3). Duration also influenced burden (*GLM,*

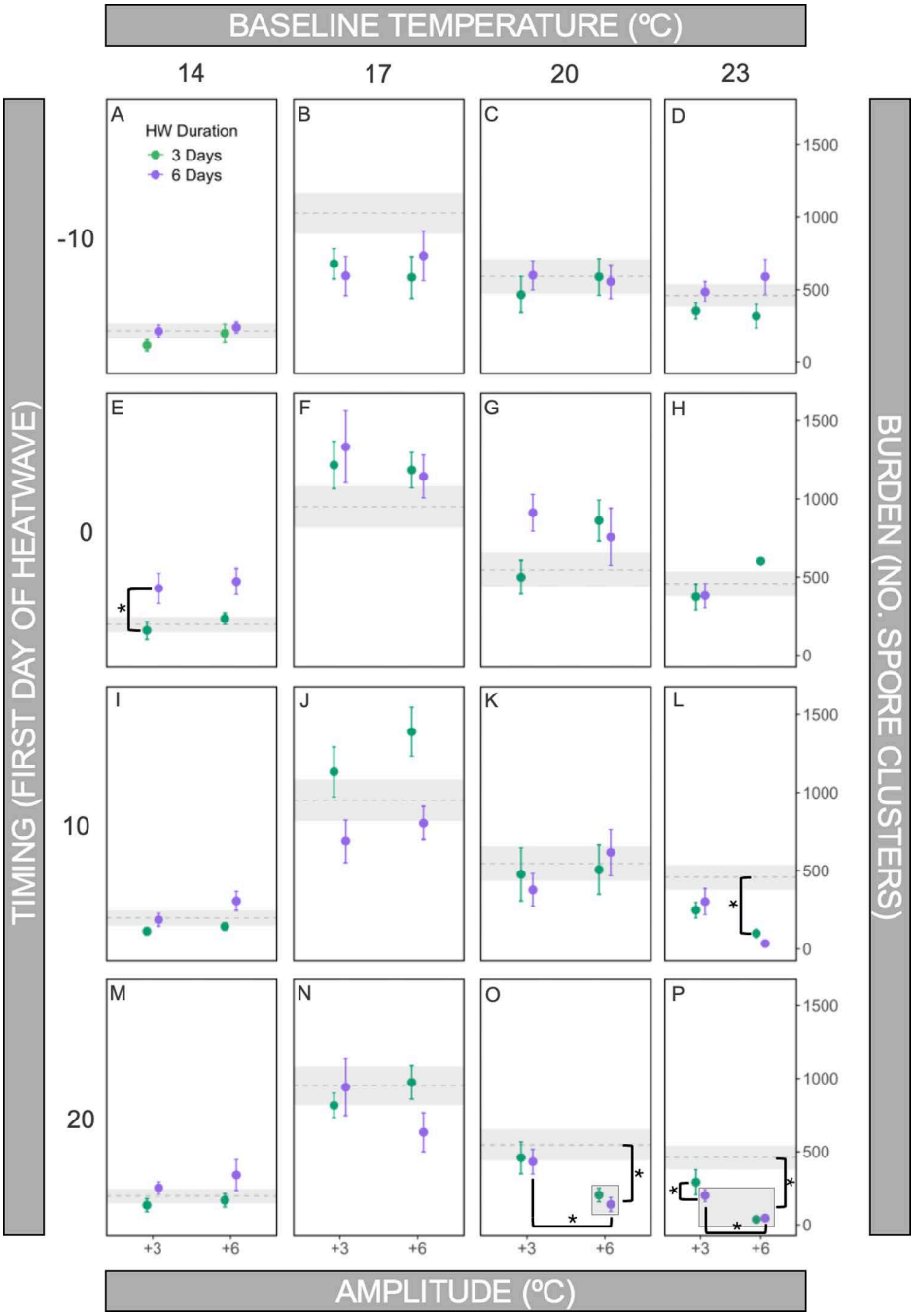

Fig 4. ***Interaction effects on burden (average number of spore clusters) between amplitude and duration at different baseline temperatures and heatwave timing in Daphnia magna infected with Ordospora colligata.*** *Points are grouped by duration. The solid grey line represents the mean for constant treatments. Error bars represent the standard error; the light grey area represents the standard error for the constant treatments. An asterisk next to a solid black bracket (*[) indicates significant contrasts between the means of the heatwave treatments when compared to the constant treatment or between two single treatments. For all custom contrasts using emmeans, see Table H in S1 Text.*

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Table 2. Coefficient estimates and feature importance of burden. A) Coefficient estimates and B) feature importance of infection prevalence in Daphnia magna infected with Ordospora colligata. Coefficient Effect (Effect) represents the estimated coefficient of the 'glmnet' elastic net regression model. Positive values indicate a positive relationship between the variable and infection prevalence, while negative values indicate a negative relationship (i.e., a positive value means the explanatory variables increased prevalence). Effect sizes represent the strength of these relationships, with larger absolute values indicating stronger effects. Lower (Lower CI) and Upper (Upper CI) Confidence Intervals indicate the range (95th percentile) from bootstrapping; non-significant values have CIs encompassing 0, suggesting no important relationship to the response variable. Temperature, modelled as a cubic polynomial, is delineated by each degree (linear [L], quadratic [Q], cubic [C]). Feature importance is calculated by combining all polynomial degrees per variable and reporting the absolute values of coefficient effects. For clarity, only the most impactful variables are shown here. Refer to Table F in S1 Text for full coefficient estimate output and Table I in S1 Text for a full list of combined feature importance variables.

A	Explanatory Variable	Effect	Lower CI	Upper CI
	Temperature (Q)	-19.40	-22.36	-16.81
	Temperature (L): Timing 20: Amplitude +6	-14.65	-21.91	-0.75
	Temperature (C): Duration 3	9.44	3.66	14.78
	Temperature (L): Amplitude +3: Duration 3	6.77	0.13	12.38
	Timing 20: Amplitude +6	-0.54	-0.76	-0.20
B	**Explanatory Variable**	**Importance**		
	Temperature: Timing 20: Amplitude +6	25.52		
	Temperature	19.40		
	Temperature: Duration 3	9.82		
	Temperature: Amplitude +3: Duration 3	6.77		

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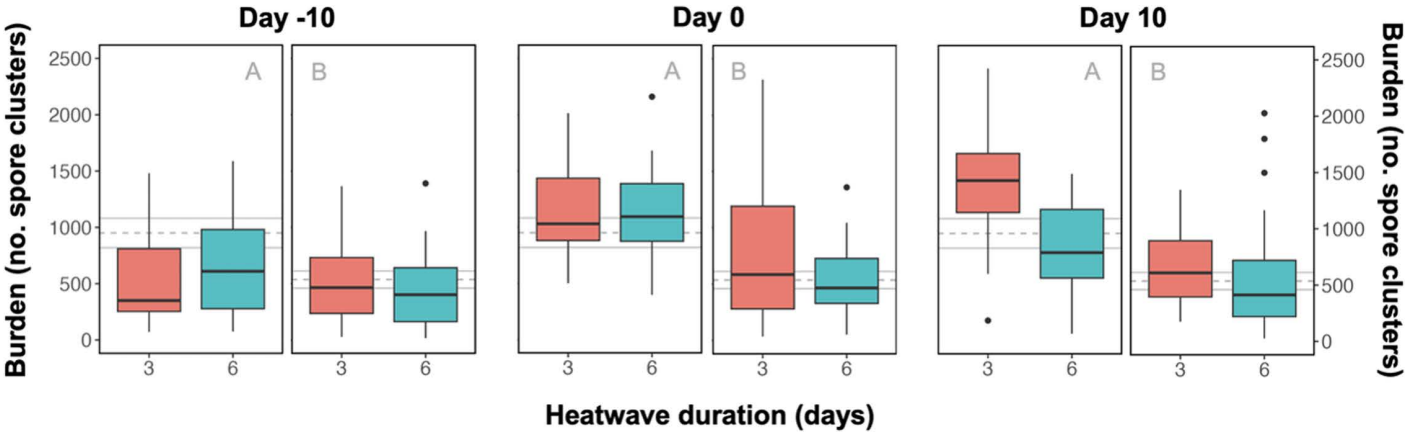

Fig 5. ***Boxplots of burden for experiments 1 and 2 comparing heatwave durations in Daphnia magna infected with Ordospora colligata.*** *Red boxplots indicate a 3-day heatwave, and blue boxplots indicate a 6-day heatwave. The grey dashed line shows the mean burden for the constant baseline treatment at 17°C, and light grey solid lines represent the upper and lower standard errors for the constant. All heatwaves had an amplitude of +6°C. Boxplots were separated by timing of heatwave (10 days pre-exposure, the day of exposure (day 0), and 10 days post-exposure). Within each timing, plots were separated by experiments: A; experiment 1 burden, and B; experiment 2 burden.*

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Table 3. GLM Analysis of deviance X^2 comparing the burden (number of spore clusters) in experiments 1 and 2, in Daphnia magna infected with Ordospora colligata. A negative binomial generalised linear model with no interacting variables was the best model fit. Significant values are bolded.

Explanatory Variable	Df	Deviance	Residual Df	Residual Deviance	P value
NULL			277	335.23	
Experiment	1	35.719	276	319.51	**<0.001**
Timing	3	10.792	273	308.72	**0.013**
Duration	1	4.740	272	303.98	**0.029**

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analysis of deviance $X^2_{(1,272)}$ $p = 0.029$), and although no individual treatments were different (no significant custom contrasts), a long heatwave (6 days) tended to have a lower burden compared to a short heatwave (3 days) (Fig 5). Finally, timing also influenced burden (*GLM, analysis of deviance* $X^2_{(1,273)}$ $p = 0.013$), whereby no timing resulted in the same burden (Fig 5). Thus, both timing and duration were consistently important in influencing the outcome of spore burden.

Discussion

The effects of heatwaves on host-parasite dynamics are shaped by multiple complex interactions involving baseline temperature, heatwave timing, amplitude, and duration. Our findings demonstrate that these factors can interact in intricate and often unpredictable ways, leading to context-dependent outcomes for both infection prevalence and parasite proliferation. This highlights the inherent complexity in understanding and predicting the impacts of heatwaves on disease dynamics and a need for deeper exploration into heatwave attributes and potential underlying mechanisms.

Effect of baseline temperature

Heatwaves can influence the performance of *Ordospora colligata,* but the pattern and direction of the effects are dependent on the baseline temperature at which the heatwave occurs. A strong and long heatwave (i.e., an increase in the amplitude of +6ºC above the baseline temperature for 6 days) occurring on the day of exposure (day 0), 10-, and 20-days post-exposure at high baseline temperatures (23ºC) can strongly reduce infection prevalence. Therefore, baseline temperature can greatly influence parasite fitness. Indeed, lower parasite performance at thermal extremes may be caused by thermal stress as the parasite is unable to survive outside of its thermal tolerance, as suggested by the Thermal Stress Hypothesis [54]. Since *Ordospora* has a narrower thermal tolerance (11.8-29.7°C) compared to its host (6-33.3°C) [7], the heatwaves approached the parasite's upper thermal tolerance while the *Daphnia* were less affected. However, parasite thermal performance limits may differ, with some species exhibiting adaptive plasticity to alterations in temperature [67,68]. For example, malaria and trematodes can adjust their development to temperature variation [69]. Similarly, in marine trematode-amphipod interactions, temperature fluctuations have been shown to alter parasite transmission success, likely due to changes in host susceptibility and parasite survival [70]. If *Ordospora* lacks similar plasticity, it may be disproportionately affected by temperature extremes, leading to reductions in infection success during heatwaves. Thermal stress may also explain the reduction in burden for heatwaves after exposure (day 10 and day 20) with a strong amplitude treatment (+6ºC) (compared to the constant baseline temperature). A decrease in parasite performance near the thermal extremes was previously demonstrated by McCartan et al. (2024) and in other systems (e.g., butterfly-protozoa, mosquito-malaria, and frog-chytrid fungus systems [71–73]). For instance, in monarch butterflies (*Danaus plexippus*) infected with their protozoan parasite *Ophryocystis elektroscirrha*, infections were reduced at extreme temperatures due to the inability of the parasite to invade the host and replicate [71]. Yet, thermal stress may not account for all variations in response to baseline temperature.

The Thermal Mismatch hypothesis and Jensen's Inequality could explain some of the variability in parasite fitness when *Ordospora* is exposed to heatwaves. The Thermal Mismatch Hypothesis [56] posits that differences in the optimal temperature ranges of the host and parasite, driven by temperature fluctuations, can shape their interactions, potentially giving an advantage to either the host or the parasite, resulting in context-specific outcomes that are complex [11]. For example, at the thermal extremes where the *Daphnia's* thermal performance exceeds that of *Ordospora*, the parasite is at a disadvantage. Other studies that have measured host and parasite thermal performance found evidence for the Thermal Mismatch Hypothesis [67, 74]. For instance, the parasitoid *Cotesia congregate* will suffer more under increasing temperatures than its lepidopteran larval host, *Manduca sexta,* due to a difference in thermal tolerances [75]. Over evolutionary timescales, such thermal mismatches may alter host-parasite co-evolutionary trajectories [49]. Hosts under recurrent heat stress may evolve enhanced thermal resilience, potentially reducing parasite transmission over time [76]. Conversely, parasites that can rapidly adapt to warming conditions may gain a selective advantage, particularly in multi-host systems where thermal stress disrupts transmission pathways [77]. However, given that it is not possible to measure the thermal performance of *Ordospora* independently of its *Daphnia* host (as it cannot be grown in culture media), formally testing this hypothesis in the *Daphnia*-*Ordospora* system is currently not possible. Moreover, Jensen's Inequality, which states that averaging over a nonlinear curve can lead to deviations and thus disproportionately large alterations to the host-parasite relationship, may also explain some of the observed differences [78]. The presence of multiple quadratic and cubic terms when *Ordospora* burden is modelled across temperature indicates that the parasite's response to temperature is non-linear and heatwaves can thus lead to larger than expected increases or decreases in parasite performance. Overall, that heatwaves can result in a shift in thermal performance depending on the baseline temperature at which the heatwave occurred has been shown in other systems [40, 73, 79], highlighting the importance of including fluctuations in disease models. For example, the fitness of the moth *Spodoptera littoralis* is overestimated at higher temperatures when thermal fluctuations are ignored [80]. Thus, heatwaves can affect the parasite's success depending on the baseline temperature; however, timing, duration, and amplitude also influence parasite fitness when temperature is varied.

Effect of Timing

No heatwave timings resulted in the same outcome for either infection prevalence or burden. For example, while no infections occurred in a strong amplitude (+6ºC) at 23ºC on the day of exposure (day 0), the same treatments had a 100% infection rate when the heatwaves occurred 10 days prior to exposure. Moreover, the timing of the heatwave played a role in both experiments, with similar impacts, but different overall burdens. Other systems have highlighted the importance of heatwave timing, such as the parasitoid (*Eretmocerus hayati*), where performance decreased depending on the heatwave timing and duration [81]. Additionally, a long duration (6 days) heatwave occurring on the day of exposure (day 0) led to an increased burden at lower temperatures (14ºC and 17ºC) as *Ordospora* may have had enough time in the optimal temperature to successfully establish as higher temperatures lead to increased feeding rates and greater contact rate between *Daphnia* and *Ordospora* [17]. Other systems have shown that thermal extreme events can alter host heat tolerance [49], parasite survival [82] and affect host susceptibility [83,84]. For example, short-term heatwaves can disrupt parasite development within *Anopheles* mosquitoes, where transient high temperatures delay or block oocyst maturation, ultimately reducing transmission potential [69]. However, while we see a reduction in burden in *Ordospora* when exposure occurred during a heatwave (day 0), this was only the case in heatwaves with a high baseline temperature (23ºC) and larger amplitude (+6ºC). Here, increased mortality associated with thermal stress [54] or thermal mismatch [56] between host and parasite may make it harder for the parasite to establish. In other systems, like *Crithidia bombi* in bumblebees, no differences in infection were found when a heatwave occurred after exposure, potentially because any negative effects resulting from infection were counteracted and masked by the host's susceptibility [83]. Yet, not all differential effects of the timing of a heatwave relative to parasite exposure may be explained by heat stress and another aspect to consider is how temperature may interact with host immunity [46,47].

Heat stress can temporally shape immune function in invertebrates, with effects varying depending on the severity and duration of exposure [85]. In fish-parasite interactions, increased temperatures can either enhance or suppress immune responses, depending on the severity and duration of the thermal stress [86]. Indeed, elevated temperatures can suppress immune defences, leading to increased susceptibility, for example, heatwaves induced long-lasting immune disorders in three-spined sticklebacks (*Gasterosteus aculeatus*) [14]. Moreover, heat-induced immune priming can also enhance resistance [87,88]. For example, in the mealworm beetle (*Tenebrbrio beetle*) brief exposure to elevated temperatures enhances resistance to bacterial infections through upregulated immune defences [89]. Here, a heatwave occurring 20 days post-exposure could have increased *Daphnia* immune function, which would aid in the clearance of *Ordospora,* leading to decreased spore burdens, especially with a strong amplitude (+6 ºC) heatwave. This is seen in other systems, for example, the Pacific white shrimp (*Litopenaeus vannamei*) where elevated temperatures post-infection activated Heat Shock Factor 1, leading to the upregulation of antimicrobial peptides that enhanced antiviral defences [90]. These temperature-driven immune modulations across taxa may explain why the timing of heatwaves relative to infection is critical in determining infection success, as seen in other host-parasite systems. Thus, the timing of a heatwave relative to infection can result in context-dependent outcomes, with varying effects for the same heatwave treatment depending on its timing relative to exposure.

Effect of Amplitude and Duration

Alternate amplitudes and durations of a heatwave can result in differential outcomes for parasite prevalence and proliferation, indicating that the impact of heatwaves on parasite fitness is context-dependent. Moreover, that the duration of a heatwave is key, is highlighted by the replicability of its importance in both experiments. While strong heatwaves (+6ºC) can reduce both infection and burden with increasing baseline temperatures (depending on the heatwave timing), a short-duration heatwave (3 days) can increase burden (at specific baseline temperatures). For example, when a heatwave occurred 10 days post-exposure at 17ºC, the burden increased from a mean of 950–1260 spore clusters. A 4-fold increase in the burden of *Ordospora* following a heatwave at 16ºC was also observed by Kunze et al (2022). Short-term fluctuations have also been known to increase infection in the *Daphnia-Ordospora* system. For example, endemic infection prevalence increased in the *Daphnia-Ordospora* system with short fluctuations of ±6ºC [38]. Furthermore, an increase in disease following thermal fluctuations has been observed in several study systems (e.g., human-parasite [69], animal-parasite [22], and plant-parasite [91]). For instance, short-term temperature fluctuations are believed to increase dengue virus infections in *Aedes aegypti* compared to large temperature fluctuations [42]. Here, the increase in burden following temperature fluctuations could be explained by the Thermal Variability Hypothesis [10]. This hypothesis posits that parasites should acclimatise faster to changing environments due to their mass-specific differences in metabolic rates, giving them an advantage over their larger host (as stated by the Metabolic Theory of Ecology [55]). Overall, both amplitude and duration are important in many systems for predicting the fitness of a parasite [92–94]. For instance, the amplitude of the heatwave was important in the host-feeding parasitoid *Eretmocerus hayati* [95], while heatwave duration affected the life history and immunity of *Lymnaea stagnalis* snails when exposed to trematodes, as prolonged duration heatwaves decreased host performance [96]. Conversely, heatwaves have also been known to reduce infection. For example, the chytrid fungus (*Batrachochytrium dendrobatidis*) reduced infection in frogs when subjected to pulse heat treatments [73]. Thus, both amplitude and duration are important to consider when predicting the fitness of *Ordospora,* particularly under the influence of climate change.

Conclusions

In conclusion, all factors (baseline temperature, timing of the heatwave, amplitude, and duration) influenced parasite fitness, leading to variable outcomes in the *Daphnia-Ordospora* system. Moreover, heatwaves behave differently compared to other forms of temperature variation. For example, cold snaps increase burden with increasing temperatures in the *Daphnia-Ordospora* system, whereas heatwaves decrease burden [43]. Overall, at high baseline temperatures, different

combinations of amplitude and duration can decrease fitness, while at lower temperatures, only smaller fluctuations can increase burden, highlighting the complexity with added temperature variation. Our findings highlight the complex and context-dependent nature of heatwave impacts on parasite fitness.

Considering the complexity of the impact of heatwaves on *Ordospora*, and given *Daphnia's* role as a key organism in their ecosystem [53], our results may have widespread impacts on ecology. For example, another parasite of *Daphnia magna* has been suggested to induce trophic cascades by reducing host densities, leading to increased algae densities and decreased water clarity [97]. Moreover, our study highlights the importance of including temperature variation in future models and its impact on disease dynamics. For example, it is believed that 69.3% of COVID-19 cases in the summer of 2022 could have been avoided if there had been no heatwaves [98]. Therefore, future research should be conducted to explore the dynamics between temperature variation and parasite fitness as climate change continues to result in increased frequency and intensity of heatwaves and other anomalous weather events [27]. A greater insight into how temperature variation affects host-parasite dynamics will aid in predicting ecological impacts, as parasites can affect host population dynamics [99], biodiversity [97], and ecosystem functioning [100]. Since heatwaves can either suppress or enhance parasite fitness depending on their characteristics [101, 102], identifying and incorporating the mechanisms underlying these effects in epidemiological models is essential for accurately assessing risks to wildlife and human health. Conservation efforts should also account for these effects, as parasite burdens may shift unpredictably under changing climates [103], further underscoring the need for refined predictive models and targeted mitigation strategies to assess the broader ecological consequences of climate-driven disease dynamics. Future studies should also consider the potential for adaptive plasticity and co-evolutionary dynamics across a wider range of host-parasite systems to better understand the broader implications of heatwaves on disease spread.

Supporting information

S1 Text: ***Table A: coefficient estimates and feature importance of infection prevalence in Daphnia magna infected with Ordospora colligata.*** *Calculated using elastic net regression, bootstrapping and feature importance. Coefficient Effect (Effect) represents the estimated coefficient of the 'glmnet' elastic net regression model. Positive values indicate a positive relationship between the variable and infection prevalence, while negative values indicate a negative relationship (i.e., a positive value means the explanatory variables increased prevalence). Effect sizes represent the strength of these relationships, with larger absolute values indicating stronger effects. Lower (Lower CI) and Upper (Upper CI) Confidence Intervals indicate the range (95th percentile) from bootstrapping; non-significant values have CIs encompassing 0, suggesting no important relationship to the response variable. Temperature was modelled as a linear (L) relationship. Feature importance (Imp) was calculated by taking the absolute value of the effect.* ***Table B: estimated marginal means (emmeans) for infection prevalence*** *in Daphnia magna infected with Ordospora colligata. The estimates were derived using a Generalized Linear Model (GLM) with baseline temperature as a factor and a treatment variable (timing, amplitude, and duration combined), and a binomial family. The presentation on the log scale is used to provide a more appropriate representation of the data. The 'Inf' degrees of freedom indicate the model's high flexibility, which arises from the complex nature of the model itself.* ***Table C: custom contrasts for infection prevalence 'emmeans' comparing treatments at different baseline temperatures*** *in Daphnia magna Infected with Ordospora colligata. The 'emmeans' for each treatment were compared to the constant equivalent at the same temperature or to the alternate amplitude or duration at the same heatwave timing. 'Inf' degrees of freedom indicate the model's complexity, making quantification challenging for estimated marginal means. Significant contrasts are bolded.* ***Table D: coefficient estimates, and feature importance of exposed*** *Daphnia magna infected with Ordospora colligata. Exposed included all individuals who received an infective dose. Calculated using elastic net regression, bootstrapping and feature importance. Coefficient Effect (Effect) represents the estimated coefficient of the 'glmnet' elastic net regression model. Positive values indicate a positive relationship between the variable and infection prevalence, while negative values indicate a negative relationship (i.e., a positive value*

means the explanatory variables increased prevalence). Effect sizes represent the strength of these relationships, with larger absolute values indicating stronger effects. Lower (Lower CI) and Upper (Upper CI) Confidence Intervals indicate the range (95th percentile) from bootstrapping; non-significant values have CIs encompassing 0, suggesting no important relationship to the response variable. Temperature was a cubic polynomial, therefore, to account for non-linearity and is therefore separated by each degree (linear [L], and quadratic [Q]). Feature importance (Imp) was calculated by taking the absolute value of the effect. ***Table E: combined feature importance for exposed*** *Daphnia magna infected with Ordospora colligata. The feature importance was calculated by getting the absolute value of the coefficient for each variable from the coefficient estimates and then the three degrees per polynomial (linear, quadratic, and cubic) were combined.* ***Table F: coefficient estimates, and feature importance of burden*** *in Daphnia magna infected with Ordospora colligata. Exposed included all individuals who received an infective dose. Calculated using elastic net regression, bootstrapping and feature importance. Coefficient Effect (Effect) represents the estimated coefficient of the 'glmnet' elastic net regression model. Positive values indicate a positive relationship between the variable and infection prevalence, while negative values indicate a negative relationship (i.e., a positive value means the explanatory variables increased prevalence). Effect sizes represent the strength of these relationships, with larger absolute values indicating stronger effects. Lower (Lower CI) and Upper (Upper CI) Confidence Intervals indicate the range (95th percentile) from bootstrapping; non-significant values have CIs encompassing 0, suggesting no important relationship to the response variable. Temperature was a cubic polynomial, therefore, to account for non-linearity and is therefore separated by each degree (linear [L], and quadratic [Q]). Feature importance (Imp) was calculated by taking the absolute value of the effect.* ***Table G: estimated marginal means (emmeans) for burden (number of spore clusters)*** *in Daphnia magna infected with Ordospora colligata. The estimates were derived using a Generalized Linear Model (GLM) with baseline temperature as a factor and a treatment variable (timing, amplitude, and duration combined), and a negative binomial family. The presentation on the log scale is used to provide a more appropriate representation of the data. The 'Inf' degrees of freedom indicate the model's high flexibility, which arises from the complex nature of the model itself. NA indicates lack of infected individuals in a treatment.* ***Table H: custom contrasts for burden (number of spore clusters) 'emmeans' comparing treatments at different baseline temperatures*** *in Daphnia magna Infected with Ordospora colligata. The 'emmeans' for each treatment were compared to the constant equivalent at the same temperature or to the alternate amplitude or duration at the same heatwave timing. 'Inf' degrees of freedom indicate the model's complexity, making quantification challenging for estimated marginal means. Significant contrasts are bolded. NA indicates a lack of infected individuals in a treatment.* ***Table I: combined feature importance for burden with importance*** *in Daphnia magna infected with Ordospora colligata. The feature importance was calculated by getting the absolute value of the coefficient for each variable from the coefficient estimates and then the three degrees per polynomial (linear, quadratic, and cubic) were combined.* ***Table J: estimated marginal means (emmeans) for burden (number of spore clusters),*** *in Daphnia magna infected with Ordospora colligata combining two experiments. The estimates were derived using a Generalized Linear Model (GLM) with baseline temperature as a factor and a treatment variable (timing, amplitude, and duration combined), and a negative binomial family. The presentation on the log scale is used to provide a more appropriate representation of the data. The 'Inf' degrees of freedom indicate the model's high flexibility, which arises from the complex nature of the model itself. NA indicates a lack of infected individuals in a treatment. Exp: experiment 1 (A) or experiment 2 (B).* ***Table K: custom contrasts for burden (number of spore clusters) 'emmeans' comparing treatments at different baseline temperatures*** *in Daphnia magna Infected with Ordospora colligata. The 'emmeans' for each treatment were compared to the constant equivalent at the same temperature or to the alternate amplitude or duration at the same heatwave timing. 'Inf' degrees of freedom indicate the model's complexity, making quantification challenging for estimated marginal means. Exp: experiment 1 (A) or experiment 2 (B).*

(DOCX)

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