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# Heatwaves and cold snaps have complex effects on the relationship between *Daphnia magna* and its microsporidian parasite *Ordospora colligata*

*by*

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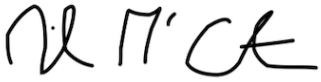

Niamh McCartan

# Summary

Climate change is resulting in far-reaching impacts on nature, the economy, and society. Yet climate change is not just a mean increase in temperature but can involve an increase in anomalous weather events like heatwaves, cold snaps. Indeed, the frequency and intensity of extreme thermal variation is on the rise globally. Climate change is altering disease outcomes, yet little is known about just how attributes of heatwaves and cold snaps (for example, amplitude or duration) alter the host-pathogen dynamic, particularly in real-world systems. In this thesis, I investigate how a water flea (*Daphnia magna*) responds to infection with its microsporidian parasite *Ordospora colligata,* under variable thermal conditions. Over two experiments, 128 unique heatwave and cold snap treatments with varying amplitudes and durations were performed at the individual level across four time points in relation to parasite exposure, while four treatments with the same duration but different amplitudes were performed at the population level. All experiments occurred across an identical, broad range of baseline temperatures (14, 17, 20, and 23°C). My results show that the effects of both cold snaps and heatwaves are highly context-dependent; not all extreme temperature changes produce the same outcome. Indeed, the outcome of parasite fitness can depend on the baseline temperature at which the thermal event is applied, the timing of the thermal event in relation to parasite exposure, and the amplitude and duration of the pulse. Heatwaves and cold snaps have distinct and often contrasting effects: heatwaves tended to reduce parasite fitness at high baseline temperatures, while cold snaps increase fitness. At the population level, heatwaves will have long-lasting effects, while cold snaps will have a short-term, delayed increase in parasite fitness. Finally, parasite fitness can scale from individual to population-level. Overall, this work highlights the complexity of disease outcomes under thermal extremes and underscores the challenges of making ecological predictions in a changing climate. These studies contribute to a growing body of work on anomalous weather and its implications for infectious disease.

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# Chapter 1: Introduction

## 1.1 The impact of climate change

Climate change is resulting in far-reaching impacts on agriculture (Malhi *et al.*, 2021), human health (Rocque *et al.*, 2021), and the economy (Gomez-Zavaglia *et al.*, 2020). Indeed, climate change has increased rates of food, water, and vector-borne diseases globally (Abbass *et al.*, 2022). For instance, a multi-country study found $94.7 billion in global annual economic losses (peaking at $20 billion/year) were attributed to *Aedes*-borne diseases like dengue, Zika, and chikungunya, due to the spread by mosquitoes thriving under warmer, wetter conditions (Roiz *et al.*, 2024). Climate change is also altering biodiversity (Habibullah *et al.*, 2022), ecosystem function (Scholes, 2016) and species interactions (Zhu *et al.*, 2023). For example, during the 2023-24 marine heatwave, over 80% of corals on parts of the Great Barrier Reef bleached, with nearly half dying by mid-year (Byrne *et al.*, 2025). Such events threaten marine biodiversity and could result in global economic losses of up to $69 billion from declining fisheries and reef-related services (Speers *et al.*, 2016). Climate change is altering disease dynamics by changing species interactions due to alterations in range (Kafle *et al.*, 2020), developmental timing (Skendžić *et al.*, 2021), and host and pathogen thermal performance or susceptibility (Byers, 2020, Molnár *et al.*, 2017). By 2070, thousands of new mammal-to-mammal viral sharing events are likely to occur due to overlapping range expansion as a result of climate change (Carlson *et al.*, 2022). Moreover, these changes are contributing to a global rise in disease outbreaks across terrestrial, freshwater, and marine systems (Pfenning-Butterworth *et al.*, 2024, Sanderson and Alexander, 2020). The emergence and re-emergence of infectious diseases, including zoonoses, vector-borne infections, and those affecting

wildlife and crops, are increasingly linked to climatic drivers, combining ecological and public health challenges (El-Sayed and Kamel, 2020). Yet climate change is not merely a gradual increase in temperature but involves considerable variability across time and space.

## 1.2 Anomalous weather on the rise

Climate change is resulting in an increase in the frequency and intensity of anomalous weather events, such as heatwaves, cold snaps, and droughts (Clarke *et al.*, 2022). Cold snaps are occurring with increasing frequency and intensity, mainly due to shifts in large-scale atmospheric dynamics. Persistent high-pressure systems, known as blocking events, and the positive phase of the North Atlantic Oscillation (NAO) can disrupt typical weather patterns and lead to the buildup of cold air masses (Wade *et al.*, 2015, D'Errico *et al.*, 2022). For instance, in January 2022, a combination of atmospheric blocking and a positive NAO phase triggered cold snaps and unusually low temperatures across the Euro-Mediterranean region, illustrating how extreme cold events can still occur under ongoing global warming (Demirtaş, 2023). Moreover, heatwaves have been on the rise in frequency, intensity, and duration since the 1950s (Perkins-Kirkpatrick and Lewis, 2020, Seneviratne *et al.*, 2021). For instance, Europe experienced one of the hottest Julys ever recorded in 2025 due to a strong high-pressure system trapping dry air from Northern Africa (World Meteorological Organization, 2025). Indeed, by 2050, more than 60% of the European population is expected to be exposed to unfamiliar heatwave attributes (intensity and duration) (Dosio *et al.*, 2025). Importantly, it is now widely recognised that fine-grained thermal variation can have distinct biological effects compared to mean temperature changes, highlighting the need to incorporate thermal fluctuations into ecological modelling (Vasseur *et al.*, 2014). These fluctuations can disrupt ecological stability (Polazzo *et al.*, 2022), decouple host-pathogen interactions (Claar and Wood, 2020), and alter coevolutionary trajectories (Schade *et al.*, 2014). As a result, organisms must contend not only with gradual

warming but also with heightened exposure to unpredictable and extreme thermal events that can disrupt key biological processes.

## 1.3 Thermal variation affects organisms

Anomalous weather events are impacting many ecosystems globally. Temperature is an important factor in maintaining homeostasis (Wang *et al*., 2024), influencing behaviour (van de Pol *et al*., 2017) and shaping immunity (Rollins-Smith and Le Sage, 2023). For instance, coral experience decreased fitness during heatwaves due to reduced metabolic outputs (Innis *et al*., 2021). Additionally, cold snaps reduced fledgling success by 36% in Tree Swallows (*Tachycineta bicolor*) (Garrett *et al*., 2022). Indeed, the predator-prey relationship is sensitive to thermal changes (Csik *et al*., 2023, Abrahams *et al*., 2007), particularly extreme thermal fluctuations (Allan *et al*., 2015, Vermaak *et al*., 2025), which can shift population dynamics (Eskuche-Keith *et al*., 2024, Buxton *et al*., 2020). For example, juvenile guppies (*Poecilia reticulata*) exhibited reduced anti-predator behaviours when exposed to a heatwave (Breedveld *et al*., 2025). Moreover, extreme thermal variation can shift the timing of life-history events (Giménez, 2011), causing mismatches between species (Miller-Rushing *et al*., 2010), potentially altering community structure (Lancaster *et al*., 2017), disrupting trophic interactions (Harvey *et al*., 2020), and contributing to biodiversity loss (Sabater *et al*., 2023). Therefore, anomalous weather events can alter co-evolutionary trajectories (Kingsolver and Buckley, 2017) by imposing strong and sudden selection pressures that reshape species interactions and drive rapid evolutionary change (Grant *et al*., 2017). These evolutionary responses, along with ecological disruptions, can have lasting legacy effects, altering population genetic structure (DuBois *et al*., 2020) and reducing ecosystem resilience (De Boeck *et al*., 2018). Yet, the outcomes of such events are highly context dependent, shaped by species' thermal sensitivities, plasticity, and trophic role (Nowicki *et al*., 2019, Regan and Sheldon, 2023). As a result, predicting the consequences of anomalous weather events

remains a major challenge in global change biology, particularly when considering infection dynamics.

## 1.4 Thermal variation affects host and parasite

Both hosts and parasites are also affected by temperature (Samuel *et al*., 2016, Gehman *et al*., 2018, Sun *et al*., 2023, Suh *et al*., 2024); therefore, extreme thermal variation can shift the outcome of the interaction (Moore *et al*., 2021). Indeed, when temperatures fluctuate, the parasite can benefit (Pintanel *et al*., 2022, Rohr and Raffel, 2010). For instance, three-spined sticklebacks infected with the tapeworm *Schistocephalus solidus* experienced increased burden as the parasite grew significantly faster under warmer conditions than its host (Franke *et al*., 2017). However, when temperatures exceed the physiological limits of the host or parasite, fitness is reduced (Paull *et al*., 2015, Tobin *et al*., 2024). For example, in monarch butterflies infected by the protozoan parasite *Ophryocystis elektroscirrha,* extreme heat reduced parasite establishment and host survival (Ragonese *et al*., 2024). The outcome also depends on whether thermal sensitivities differ between host and parasite (Cohen *et al*., 2017, Venesky *et al*., 2022). For instance, a global meta-analysis found that hosts adapted to cooler climates often experienced higher infection risk during heat anomalies, while warm-adapted hosts were more vulnerable during cold snaps (Cohen *et al*., 2020). Additionally, temperature-driven shifts in timing can desynchronise host and parasite development, again altering the advantage depending on which organism tracks environmental cues more effectively (Pardikes *et al*., 2022). For example, in amphibians infected with a chytrid fungus, cold weather events occurring early in parasite development delayed host immune responses without halting parasite progression, increasing infection intensity (Raffel *et al*., 2015). Thus, there is a need to investigate how temperature variation, especially in the form of extreme events, shapes host-parasite dynamics across different thermal environments.

## 1.5 Referenced Hypotheses

The outcome of parasite fitness in response to thermal alterations may be explained by numerous hypotheses. In this thesis, the primary focus is on three main hypotheses and Jensen's Inequality. **The Thermal Stress Hypothesis** posits that organisms experience physiological stress when temperatures deviate from their thermal optimum (Paull et al., 2015). Such deviations can reduce growth, reproduction, and immune function (Morley and Lewis, 2014). For example, in coral reef systems, elevated sea surface temperatures lead to bleaching and immune suppression in corals, while simultaneously promoting faster pathogen proliferation and virulence (Bruno et al., 2007). Importantly, this hypothesis encompasses both heat and cold stress, as low temperatures can also impair metabolic activity and immune competence (Zhou et al., 2025, Vialard and Olivier, 2020). Thermal stress may dictate the success of an antagonist as temperatures shift from their thermal optima (Hector et al., 2023). The **Thermal Mismatch Hypothesis focuses** on differences in the thermal performance curves of hosts and parasites (Cohen et al., 2017). When environmental temperatures shift toward the parasite's optimum but away from that of the host, infection risk can increase; conversely, disease may decline when conditions favour host performance. For instance, amphibians infected with *Batrachochytrium dendrobatidis* experience severe disease when cooler temperatures enhance fungal growth while suppressing host immunity (Raffel et al., 2013). While the Thermal Stress and Thermal Mismatch Hypotheses focus on mean temperature shifts, they overlook how variability itself can alter biological outcomes.

**Jensen's Inequality** provides a mathematical framework for understanding how fluctuations around the mean temperature can nonlinearly affect performance and infection dynamics (Ruel and Ayres, 1999). In host-parasite interactions, this means that temperature fluctuations (such as heatwaves and cold snaps) can push parasite fitness away from the expected

outcome based on constant conditions, depending on the curvature of the underlying thermal performance relationship (Shocket, 2023). For example, due to non-linearity, an amphibian host can have accelerated development while its trematode parasite shows enhanced host penetration but reduced establishment (Paull et al., 2012). While Jensen's Inequality highlights how nonlinearity can alter mean outcomes under temperature fluctuations, the **Thermal Variability Hypothesis** extends this idea to an ecological and evolutionary context. This theory postulates that, since parasites are generally smaller than their hosts, they will exhibit higher metabolic rates, as suggested by the Metabolic Theory of Ecology (Brown et al., 2004). Consequently, parasites can acclimate to changing environmental conditions more rapidly than their hosts, potentially gaining a temporary performance advantage under fluctuating temperatures (Raffel et al., 2015). Collectively, these frameworks illustrate that both the mean and variability of temperature can shape host–parasite outcomes.

These hypotheses are not mutually exclusive; instead, they capture different mechanisms through which temperature can influence disease outcomes. The Thermal Stress and Thermal Mismatch Hypotheses focus on mean temperature effects and thermal extremes, while Jensen's Inequality and the Thermal Variability Hypothesis account for the influence of fluctuations around that mean. Together, they provide a complementary framework for understanding the multiple pathways by which thermal change can affect host-parasite interactions. This thesis directly tests these ideas by comparing parasite fitness across both constant and thermally fluctuating treatments, using *Daphnia magna* and its microsporidian parasite *Ordospora colligata*, allowing assessment of how physiological stress, thermal mismatch, and nonlinear responses to variability jointly shape infection outcomes. Understanding these mechanisms is essential for predicting how host–parasite systems will respond to the increasing frequency and intensity of temperature fluctuations under climate change.

## 1.6 Model System

*Daphnia magna* is a freshwater crustacean widely distributed across the northern hemisphere and commonly found in temperate ponds and lakes that experience substantial seasonal temperature variation (Ebert, 2022). It is a cyclic parthenogen, reproducing clonally under favourable conditions, with sexual reproduction occurring prior to diapause. Under laboratory conditions, individuals reach sexual maturity within 7–10 days after birth and reproduce every 3-4 days, allowing for consistent maintenance of clonal lines and the removal of genetic variability among replicates (Ebert, 2005). Throughout this thesis, a single clonal line (FI-OER-3-3) originating from Tvärminne, Finland, was used. The microsporidian parasite *Ordospora colligata* is an obligate intracellular parasite that infects *D. magna* via horizontal transmission. Spores are released into the water column through faecal pellets and are acquired by hosts through filter feeding. After ingestion, spores infect the epithelial cells of the upper gut, where the parasite replicates within the host's intestinal tissue (Pombert et al., 2015). The infection cycle involves an incubation phase of approximately 9-12 days before mature spore clusters (typically 32-64 spores) become visible (Kirk et al., 2019). These clusters rupture the host cell and spread to adjacent cells, eventually leading to widespread infection throughout the gut epithelium. As the infection progresses, spores are continually released into the environment, completing the transmission cycle. *Ordospora* is typically avirulent, primarily manifesting as reduced fecundity and gut tissue damage rather than acute mortality (Ebert, 2005). Maximum spore loads can reach approximately 4000 clusters per host, and fecundity is typically reduced by about 20% (Ebert et al., 2000). *Ordospora* reproduces by parasitising host metabolism, directly utilising ATP from the cytoplasm of gut epithelial cells to fuel its own growth and replication (Tsaousis et al., 2008). Furthermore, *Daphnia* have a thermal tolerance ranging from 6-33.3ºC with an optimum temperature of 20ºC, while *Ordospora* has a similar, but somewhat narrower thermal tolerance of 11.8-29.7ºC (Kirk et al., 2018). Therefore, given these biological

and thermal characteristics of both host and parasite, the timing of environmental variation becomes especially important in shaping infection outcomes.

Because the full infection cycle from exposure to spore release typically unfolds within 2–3 weeks, temperature events imposed during the experiment can differentially affect distinct stages of the host-parasite interaction (Ebert, 2005). Heatwaves or cold snaps occurring shortly after exposure could influence parasite establishment by altering host feeding rate (Hall et al., 2007) or gut epithelial integrity (Sun et al., 2023), thereby affecting the likelihood of successful invasion. Events occurring midway through infection (roughly 7-14 days post-exposure) coincide with the parasite's replication phase and could affect within-host growth (Krichel et al., 2023) and immune response (Sun et al., 2023), with elevated temperatures potentially accelerating parasite replication but also increasing host metabolic stress. In contrast, late events, after 14 days post-exposure, may influence spore maturation, release, and onward transmission, particularly through effects on host survival, gut shedding rate, or water-column persistence of spores (Manzi et al., 2021). Thus, temperature extremes have the potential to alter virulence both directly, by affecting parasite replication and tissue damage, and indirectly, by modifying host condition and resource allocation between reproduction and immune defence. Thus, this host-parasite system is a popular model for the study of disease ecology and evolution (Ebert, 2022), especially in light of ongoing global change.

## 1.7 Research outline

In this thesis, I examine how extreme thermal variation (that is, cold snaps and heatwaves) alters infection outcomes using the freshwater crustacean host *Daphnia magna* and its microsporidian parasite *Ordospora colligata*. In the first three chapters, I aim to observe how varying thermal pulse attributes (amplitude, duration, and timing in relation to parasite

exposure) across a wide spectrum of baseline temperatures alter the host-parasite relationship at the individual level. The final chapter combines the knowledge from the previous chapters and focuses on the host-parasite infection dynamics at the population level.

***Chapter 2: Cold snaps lead to a 5-fold increase or a 3-fold decrease in disease proliferation, depending on the baseline temperature.***

Climate change is not only resulting in an increase in constant temperatures but is also increasing thermal variability globally. Indeed, the frequency and intensity of cold snaps are on the rise, yet our understanding of how different cold snap attributes influence host-parasite relationships remains limited. Here, I investigated the effect of varying amplitudes and durations of cold snaps across multiple baseline temperatures using the *Daphnia-Ordospora* system. I observed parasite fitness (prevalence and proliferation) in hosts at the individual level using a factorial design. I modelled the data to find important variables, so conclusions could be drawn about discovering important cold snap attributes for infection outcomes.

***Chapter 3: Timing of a cold snap relative to exposure can alter disease proliferation up to 17-fold relative to baseline temperature and treatment.***

Not only are the attributes of cold snaps important, but the timing in relation to parasite exposure can alter these infection dynamics. The focus of this chapter was examining how different timings in relation to exposure, along with varying cold snap attributes, alter the host-parasite relationship, also at the individual level, using the same host-parasite system. Here, I used the same cold snap attributes (amplitude and duration) across three different time points and used the same set of baseline temperatures as in Chapter 2.

***Chapter 4: Impact of heatwave amplitude, duration, and timing on parasite fitness at different baseline temperatures.***

Like cold snaps, heatwaves are increasing in frequency and intensity globally. While there has been some focus on heatwaves and disease interactions, there has been a lack of emphasis on how varying heatwave attributes at different time points in relation to parasite exposure can affect the host-parasite relationship. Here, I used the same baseline temperatures as the first two chapters and used similar heatwave attributes (amplitude and duration), which occurred at four distinct time points in relation to parasite exposure in a factorial design at the individual level.

***Chapter 5: Heatwaves and cold snaps alter host-parasite population dynamics in the Daphnia magna-Ordospora colligata system.***

Climate change is altering not only physiological host-pathogen interactions at the individual level but also shaping disease dynamics across populations. Here, the information collected from previous chapters facilitated a targeted approach on how to explore the effect of both cold snaps and heatwaves on populations of *D. magna* infected with *O. colligata*. A factorial design was created to observe the effects of both pulse regimes across the same baseline temperatures used in previous chapters. All pulses occurred at the same time and had the same intensity (±6ºC). Disease dynamics were observed by looking at parasite fitness and population size across nine weeks to observe any lasting effects of the pulse treatments.

# Chapter 2: Cold snaps lead to a 5-fold increase or a 3-fold decrease in disease proliferation, depending on the baseline temperature.

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**Author Contributions:** NMcC and PL designed the experiment. NMcC conducted the experiment with the assistance of PL and SDiC. NMcC conducted the analysis with the aid of PL and JP. NMcC generated figures. NMcC and PL wrote the first draft of the manuscript, and all authors contributed to revisions. All authors read and approved the final manuscript.

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## 2.1 Abstract

Climate change is driving increased extreme weather events, that can impact ecology by moderating host-pathogen interactions. To date, few studies have explored how cold snaps affect disease prevalence and proliferation. Using the *Daphnia magna* – *Ordospora colligata* host-parasite system, a popular model system for environmentally transmitted diseases, the amplitude and duration of cold snaps were manipulated at four baseline temperatures, 10-days post-exposure, with *O. colligata* fitness recorded at the individual level. Cold snaps induced a 5-fold increase or a 3-fold decrease in parasite burden relative to baseline temperature, with complex nuances and varied outcomes resulting from different treatment combinations. Both amplitude and duration can interact with the baseline temperature, highlighting the complexity and baseline dependence of cold snaps. Furthermore, parasite fitness, i.e., infection prevalence and burden, were simultaneously altered in opposite directions in the same cold snap treatment. We found that cold snaps can yield complicated outcomes that are unique from other types of temperature variation (for example, heatwaves). These results underpin the challenges and complexity in understanding and predicting how climate and extreme weather may alter disease under global change.

## 2.2 Introduction

Global change has far-reaching impacts in natural, agricultural, and human systems. It has been implicated in the deterioration of ecosystem structure and function (Doney *et al*., 2012), food production (Gomez-Zavaglia *et al*., 2020), and human health (IPCC, 2022). For instance, biodiversity is particularly vulnerable to the influence of climate change (for review, see Bellard *et al*. (Bellard *et al*., 2012)). Moreover, climate change is also altering the incidence and distribution of infectious diseases (Altizer *et al*., 2013), due to range shifts and changes in behaviour of hosts, parasites, and vectors (Bouchard *et al*., 2019). The important role temperature plays in disease dynamics and transmission has been well studied empirically

(Kirk *et al*., 2018, Kirk *et al*., 2020, Hector *et al*., 2021a) and theoretically (Ewing *et al*., 2016, Lusekelo *et al*., 2022, Shi *et al*., 2020). For example, temperature can influence the host foraging rate (Shocket *et al*., 2018a) and food availability (McKee and Ebert, 1996), which in turn can impact the encounter rate between the host and parasite. Furthermore, temperature can also lower host immunity when hosts need to expend more energy on maintaining homeostasis due to temperature fluctuations (Seppälä and Jokela, 2011), potentially leading to an increased parasitic burden (Mignatti *et al*., 2016). Conversely, other studies have reported no effect of temperature on host immunity (Manzi *et al*., 2020) or even an increased parasite resistance with higher temperature (Blanford *et al*., 2003). Importantly, to successfully infect a host, the parasite needs to overcome a multitude of host defences (avoidance behaviour, physical barriers, innate and adaptive immunity) (Ebert *et al*., 2016), each of which may be affected directly or indirectly by climate change (Lõhmus and Björklund, 2015). Thus, shifts in disease prevalence and burden due to climate change can be complex (Marcogliese, 2008, Claar and Wood, 2020, Kirk *et al*., 2018). While many studies have highlighted interactions between climate change (that is, rising mean temperatures) and disease (El-Sayed and Kamel, 2020, Fouque and Reeder, 2019, Kurane, 2010), studies that have empirically investigated the role of temperature variation in the context of climate change and disease dynamics (Raffel *et al*., 2015, Raffel *et al*., 2013, Rohr and Cohen, 2020, Rohr and Raffel, 2010, Rohr *et al*., 2013, Krichel *et al*., 2023) have been less common.

Climate change is causing an increased occurrence and magnitude of anomalous weather events, including heatwaves, cold snaps, droughts, and flooding (Leahy *et al*., 2021). Trends in extreme temperature variation, frequency, and duration of heatwaves have accelerated since the 1950s (Perkins-Kirkpatrick and Lewis, 2020), while cold snaps have increased in frequency since 1979 (Kug *et al*., 2015). These climatic changes in variation may lead to shifts in host-parasite dynamics (Rohr *et al*., 2013). For example, natural disasters associated with extreme weather can lead to respiratory (Mirsaeidi *et al*., 2016), waterborne (Cann *et al*., 2013), and

vector-borne diseases (Filho *et al*., 2019). Moreover, shifts in temperature can influence host survival, which can indirectly alter the host-parasite relationship (Claar and Wood, 2020). For example, a heatwave reaching 28°C resulted in increased host immune activity in three-spined sticklebacks (*Gasterosteus aculeatus*) (Dittmar *et al*., 2014). Not only are host traits (e.g., fecundity (Moreno and Møller, 2011), survival (Frederiksen *et al*., 2008), and behaviour (van de Pol *et al*., 2017)) affected by extreme weather, but parasite traits can also be altered. For instance, recent work by Kunze *et al*. (2022) using water fleas (*Daphnia magna*) infected with a microsporidium parasite showed that a heatwave not only altered parasite burden, but that the direction of this effect depended on the mean temperature at which a heatwave occurred (Kunze *et al*., 2022). While recent studies have started exploring the impact of extreme heat events (Raffel *et al*., 2015, Raffel *et al*., 2013, Rohr and Cohen, 2020, Rohr and Raffel, 2010, Rohr *et al*., 2013, Krichel *et al*., 2023), the effect of cold snaps on disease outbreak and spread remains underexplored (Raffel *et al*., 2013, Rohr and Cohen, 2020, Paull *et al*., 2012, Novak, 1978), and not yet studied in the *Daphnia* system.

Surprisingly, few studies have focused on the effects of cold snaps on organisms (Williams *et al*., 2015), ecosystems (Boucek *et al*., 2016, Schlegel *et al*., 2021) and health (Sun *et al*., 2022), while even less is known about the impact of extreme cold events on disease dynamics (Morley and Lewis, 2014). Nonetheless, the effects of cold snaps may become increasingly relevant in spite of rising mean temperatures as, for example, the negative phases of the North Atlantic Oscillations lead to colder, drier winters in Northern Europe (Wade *et al*., 2015) and Southern Europe (D'Errico *et al*., 2022). Like heatwaves, cold snaps can affect host-parasite relationships. For example, the establishment of the pathogenic trematode (*Ribeiroia ondatrae*) in its amphibian host is more successful at lower temperatures (Paull *et al*., 2012). Similarly, a decrease in temperature has led to an increase in infections in frogs due to lowered resistance to chytrid fungus (*Batrachochytrium dendrobatidis*) (Raffel *et al*., 2013). Conversely, mice housed at cold temperatures had a lower metacestode intensity and growth than

individuals housed at room temperature (Novak, 1978). Indeed, how cold snaps alter disease outcomes remains an open question, to our knowledge.

Here, we examine the effect of different cold snap treatments across a broad range of baseline temperatures (the mean constant temperature throughout the experiment) using the *Daphnia magna – Ordospora colligata* host-pathogen system to test how cold snaps may alter disease prevalence and proliferation. This is a popular model system for environmentally transmitted diseases (horizontal transmission, infection by mass action), which are a common concern for humans, livestock and agriculture (Ebert, 2022). As mentioned, in the study by Kunze *et al*. (2022), an experimental heatwave event (characterised by a 6°C increase in temperature occurring 20 days post-exposure and lasting for 3 days) led to an alteration in parasite burden. Building upon this observation, we hypothesised that cold snaps, like heatwaves, may alter parasite fitness in complex ways by increasing or decreasing infection prevalence and/or burden and may be dependent on the baseline temperature. We believed that at extreme temperatures, parasite fitness could decrease as a result of thermal mismatch (Cohen *et al*., 2017), while parasite fitness could also be affected by differences in acclimation speed of host and parasite (Rohr and Raffel, 2010) and Jensen's Inequality (Krichel *et al*., 2023). To test how cold snaps affect *Ordospora* prevalence and burden, we manipulated the amplitude (-3ºC and -6ºC) and duration (3 days and 6 days) of a cold snap occurring 10 days post-exposure at four baseline temperatures (14, 17, 20, and 23ºC) (Figure 2.1). In total, the impact of 16 different cold snaps on individual *Daphnia* (15 replicates per cold snap treatment) were tested. Infection prevalence and burden were observed upon natural death or at the end of the experiment, 27 days post-exposure. We demonstrated that cold snaps can alter parasite fitness in unexpected and nuanced ways depending on baseline temperature and cold snap properties.

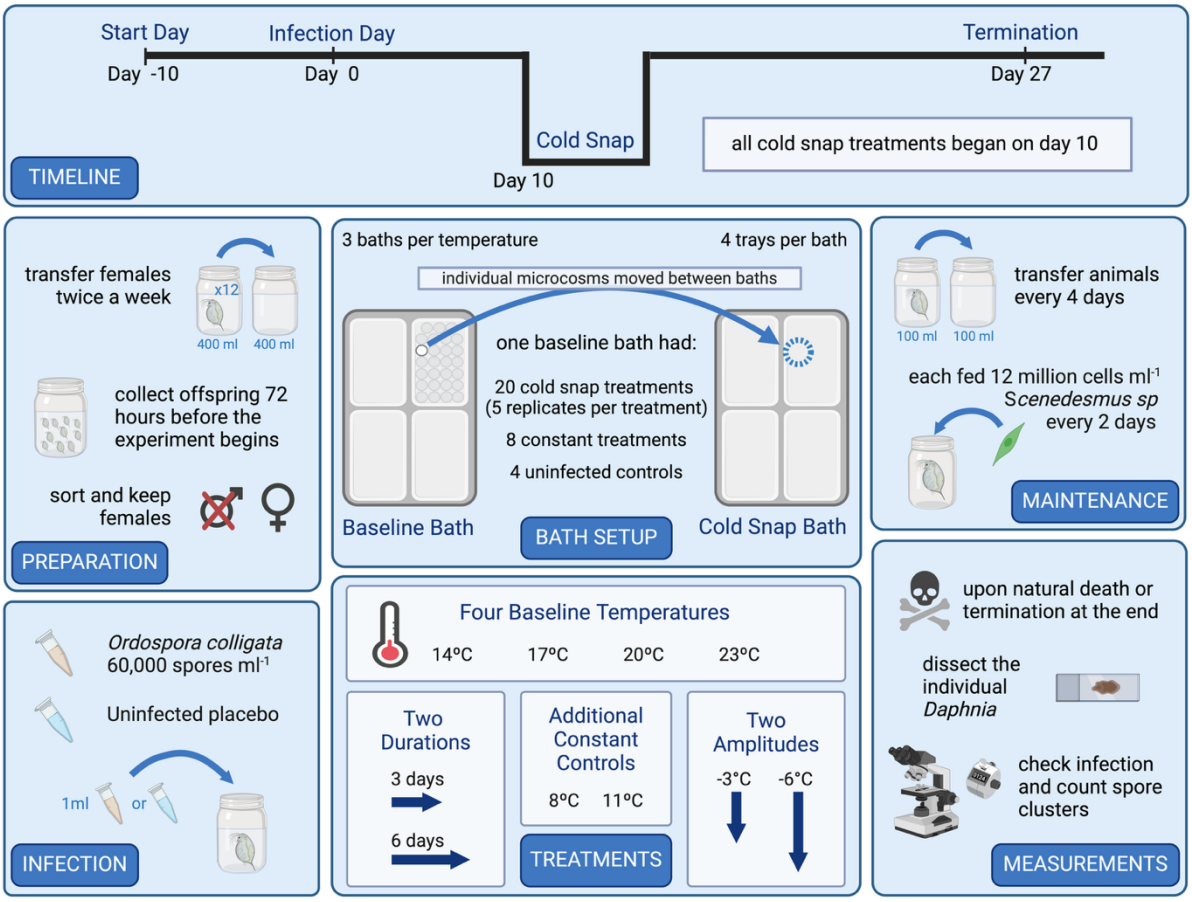


**Figure 2.1: Chapter 2 experimental setup.**
TIMELINE; illustrates the timeline for the experiment starting at day -10 and terminating at day 27, exposure occurred on day 0, and every cold snap began on day 10. PREPARATION; preparation started three weeks prior to exposure when 10-12 mothers were added 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 twenty cold snap treated individuals (five replicates per cold snap treatment), eight constant temperature treatments, and four uninfected controls. To simulate a cold snap, microcosms were moved between baths and then returned to the baseline bath when the cold snap finished. TREATMENTS; sixteen treatments were included in the experiment (four baseline temperatures • two amplitudes • two durations). Additionally, at the cold snap specific temperatures (8ºC and 11ºC), each bath was organised the same as the baseline temperatures, however, no cold snap treatments were included. MAINTAINENCE; maintenance and measurements were carried out between days -4 and day 27. Figure created on Biorender.com.

## 2.3 Results

### *2.3.1 Infection prevalence*

The baseline temperature at which exposure occurred was important for predicting infection prevalence (*quadratic effect, glmnet, effect = -36.52, 95% CI: -46.68 to -27.89, linear effect, glmnet, effect = 21.66, 95% CI: 14.50 to 30.72 and feature importance of the combined polynomial = 58.51,* Table 2.1). Infection prevalence followed a classic temperature response curve with the lowest infection prevalence at the extreme temperatures (~8ºC, ~23ºC) and highest infection prevalence at the optimal range (~17ºC) (Figure 2.2). The strong and long cold snap (6-days of -6ºC) in combination with the baseline temperature (3-way interaction between baseline temperature, duration 6-days, and amplitude -6ºC) behaved differently from the other treatment combinations and was important for predicting infection prevalence (*linear effect, glmnet, effect = -34.08, 95% CI: -61.39 to -2.92 and feature importance of the combined polynomial = 62.63,* Table 2.1). Here, this long and strong treatment behaved differently to other cold snap treatments by decreasing with increasing baseline temperature (Figure 2.1), although no specific treatments in the same baseline temperature were statistically different (Table A.4). The finding of differences between cold snaps and a complex (3-way) interaction shows that baseline temperature, amplitude, and duration of extreme cold weather events modify and complicate the infection prevalence in the *Daphnia-Ordospora* system.

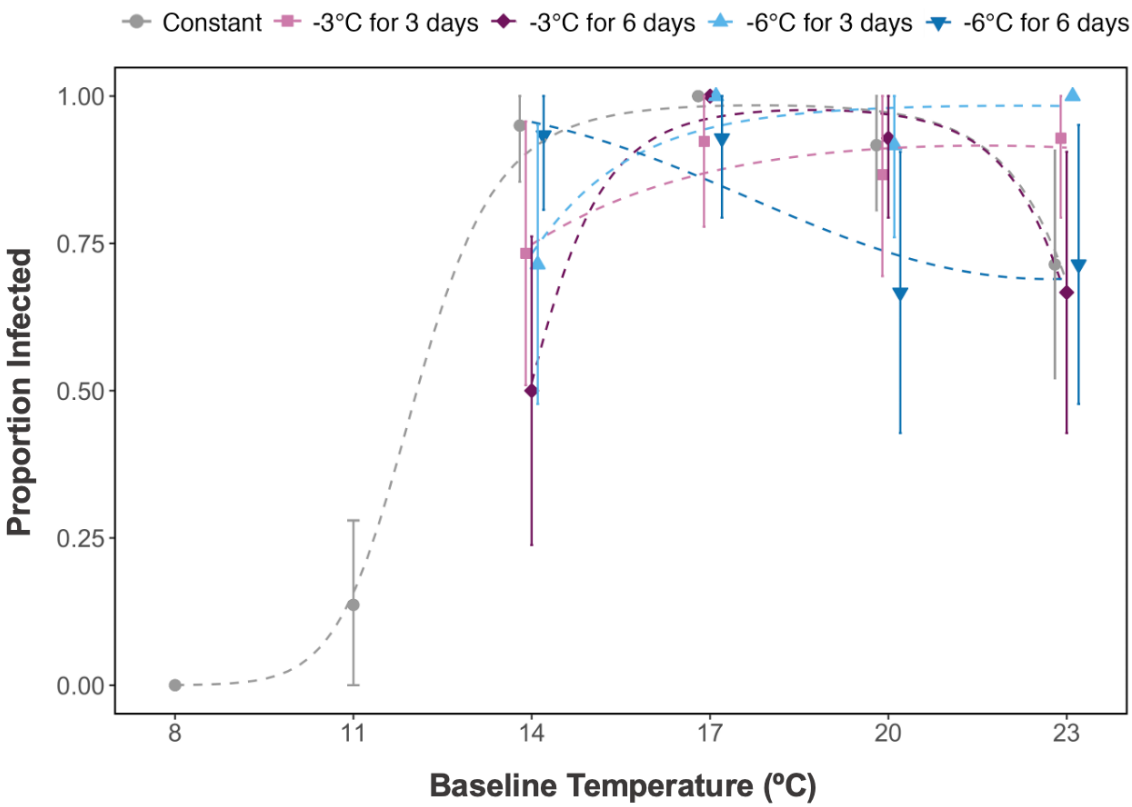


**Figure 2.2: The effect of cold snap treatments on the proportion infected 10 days post-exposure.**
In *Daphnia magna* infected with *Ordospora colligata*. Error bars represent the standard error, and each dashed line is the quadratic fit of the elastic net regression (see Table A.1 for statistical results).

**Table 2.1: Coefficient estimates, and feature importance of the proportion infected.**
A) Coefficient estimates and B) feature importance of proportion infected in *Daphnia magna* infected with *Ordospora colligata*. Coefficient Effect (Eff) represents the estimated coefficient of the elastic net regression model, while Lower (Lower CI) and Upper (Upper CI) Confidence Intervals (95th) indicate the range. Non-significant values have CIs passing 0. Positive and negative effects indicate corresponding relationships. Temperature, modelled as a quadratic polynomial, is delineated by each degree (linear [L], quadratic [Q]). Feature importance is calculated by combining the absolute values of the coefficients' effect for each variable and polynomial degree. For clarity, only the most impactful variables are shown. Refer to Table A.1 for full coefficient estimate output and Table A.4 for a full list of feature importance variables.

| A | Explanatory Variable | Eff | Lower CI | Upper CI |
|---|---|---|---|---|
| | Temperature (L): Amplitude -6: Duration 6 | -38.33 | -71.71 | -7.07 |
| | Temperature (Q) | -36.52 | -46.68 | -27.89 |
| | Temperature (Q): Amplitude -6: Duration 6 | 24.30 | 0 | 51.41 |
| | Temperature (L): Amplitude -3: Duration 6 | 21.99 | 0 | 48.87 |
| | Temperature (L) | 21.66 | 14.50 | 30.72 |

| B | Explanatory Variable | Importance |
|---|---|---|
| | Temperature: Amplitude -6: Duration 6 | 62.63 |
| | Temperature | 58.51 |

### *2.3.2 Burden*

Of the infected individuals, cold snaps occurring 10-days post-exposure resulted in a 5.1-fold increase (485 vs 89 spores clusters, respectively) in disease proliferation or a 3.3-fold decrease (29 vs 98 spores clusters, respectively) depending on the treatment and the baseline temperature at which the cold snap occurred (Figure 2.3 and Figure 2.4). Generally, cold snaps led to similar or lower burden compared to the constant treatment at lower baseline temperatures (14-20ºC) and higher at hotter temperatures (23ºC) (Figure 2.3). At 14ºC all cold snap treatments resulted in a lower burden compared to the constant treatment, e.g., the mean spore cluster count of the treatment where the cold snap was reduced in temperature by -3ºC for 6-days was 69 spore clusters less than that of the 14°C constant treatment (Figure 2.4A) (*emmean, z(inf) = -2.937, p = 0.002*, see Table A7 for significant p-values of all cold snap custom contrasts, Figure 2.3). Moreover, at intermediate temperatures (17°C) the difference in burden between cold snaps and constant treatments was minimal, with only one lower burden for the 6-days at -6°C cold snap when compared to the constant treatment (*emmean, z(inf) = -2.508, p = 0.049*, Table A.7, Figure 2.3). In contrast, all cold snap treatments at 23ºC had increased burden compared to constant temperature treatment; for example, the cold snap for 6-days at -6°C had a burden higher burden (204 spore clusters greater) than the constant temperature, (*emmean, z(inf) = 3.02, p = 0.018,* Table A.7, Figure 2.3). While burden was differentially affected by baseline temperature, cold snap traits (amplitude and duration) also influenced the outcome of both spore burden and infection prevalence.

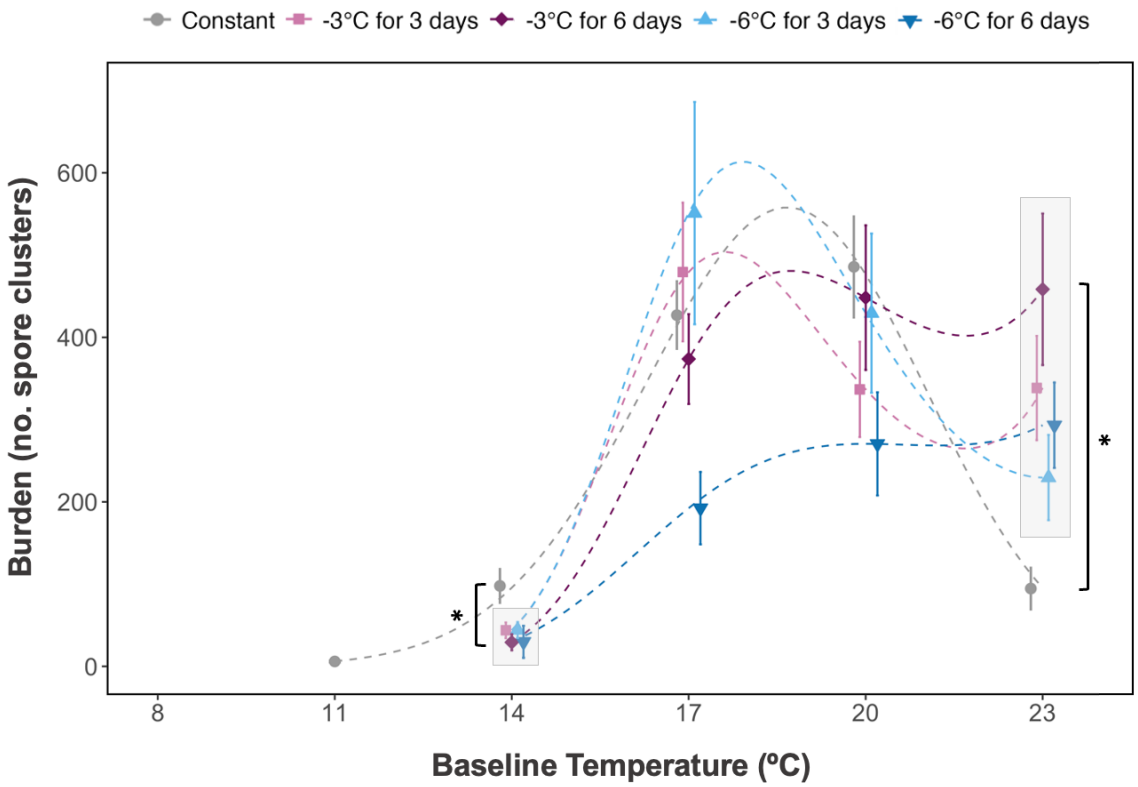


**Figure 2.3: The effect of cold snap treatments on burden 10 days post-exposure.**
In *Daphnia magna* infected with *Ordospora colligata*. Burden was the mean number of spore clusters with each spore cluster containing 32-62 individual *Ordospora*. Error bars represent the standard error, and each dashed line is the cubic fit of the elastic net regression (see Table A.5 for statistical results). The absence of the data point at 8°C, for the constant temperature, is due to the absence of infection at this temperature. The asterisk and bracket ( *[ ) indicate significant contrasts between means of the cold snap treatments when compared to the constant treatment.

**Table 2.2: Coefficient estimates and feature importance of burden.**
A) Coefficient estimates and B) feature importance of burden in *Daphnia magna* infected with *Ordospora colligata*. Burden was the mean number of spore clusters with each spore cluster containing 32-62 individual *Ordospora*. Coefficient Effect (Eff) represents the estimated coefficient of the elastic net regression model, while Lower (Lower CI) and Upper (Upper CI) Confidence Intervals (95th) indicate the range. Non-significant values have CIs passing 0. Positive and negative effects indicate corresponding relationships. Temperature, modelled as a cubic polynomial, is delineated by each degree (linear [L], quadratic [Q], cubic [C]). Feature importance is calculated by combining the absolute values of the coefficients' effect for each variable and polynomial degree. For clarity, only the most impactful variables are shown. Refer to Table A.5 for full coefficient estimate output and Table A.8 for a full list of feature importance variables.

| A | Explanatory Variable | Eff | Lower CI | Upper CI |
|---|---|---|---|---|
| | Temperature (Q) | -12.12 | -14.58 | -10.06 |
| | Temperature (L): Duration 6 | 7.15 | 2.83 | 10.62 |
| | Temperature (L): Amplitude -3 | 6.28 | 2.63 | 8.57 |
| | Temperature (L): Amplitude -6 | 4.49 | 1.03 | 7.58 |

| | | | |
|---|---|---|---|
| Temperature (C): Duration 3 | 3.61 | 0 | 6.59 |
| Temperature (L): Duration 3 | 3.45 | 0.41 | 5.92 |

| B | Explanatory Variable | Importance |
|---|---|---|
| | Temperature | 14.19 |
| | Temperature: Amplitude -3 | 9.27 |
| | Temperature: Duration 6 | 8.76 |
| | Temperature: Duration 3 | 8.54 |
| | Temperature: Amplitude -6 | 6.84 |

Each of the factors in our experiment (baseline temperature, cold snap amplitude, and duration) were statistically important for predicting burden of *O. colligata*. However, baseline temperature was the most important for predicting burden (*quadratic main effect, glmnet, effect = -12.12, 95% CI: -14.58 to -10.06 and feature importance of the combined polynomial = 14.19,* Table 2.2). In general, burden also followed a classical temperature response curve with lower burdens at extreme temperatures (~11ºC, ~23ºC) and optimal performance at intermediate temperatures (~17-20ºC). Yet, the cold snap treatments can alter the outcome. Indeed, both the amplitude and duration of the cold snap influenced burden depending on the baseline temperature (i.e., both separately interacted with temperature). A weak cold snap (-3ºC) had the largest influence on burden, where the burden increased with increasing baseline temperature (*linear main effect, glmnet effect = 6.28, 95% CI: 2.63 to 8.57 and feature importance of the combined polynomial = 9.27,* Table 2.2). For example, at 23ºC a cold snap of -3ºC resulted in a higher burden (SE = ±124) than the constant at 23ºC (SE = ± 29) (Figure 2.4D), and nearly equal to the performance of the constant three degrees lower (SE = ±64) (Figure 2.4C). A strong cold snap with an amplitude of -6ºC showed similar results, that is, increasing burden with increasing temperature (*linear effect, glmnet, effect = 4.49, 95% CI: 1.03 to 7.58, and feature importance of the combined polynomial = 6.84,* Table 2.2). Similar to

amplitude, cold snap duration increased burden with increasing baseline temperature for both the short (3-day) cold snap and long (6-day) cold snap (*linear effect, glmnet, effect = 3.45, 95% CI: 0.41 to 5.92 and feature importance of the combined polynomial = 8.54,* and *linear effect, glmnet, effect = 7.15, 95% CI: 2.83 to 10.62 and feature importance of the combined polynomial = 8.76*, *respectively,* Table 2.2). Higher order interactions between temperature, amplitude, and duration played a minimal role (low importance, see Table A.8, and confidence intervals overlapping zero, see Table A.5). Thus, temperature and its interaction with both the amplitude and duration (independently) are most important in explaining the burden of *O. colligata,* and as a result, several combinations of amplitude and duration led to alternative outcomes.

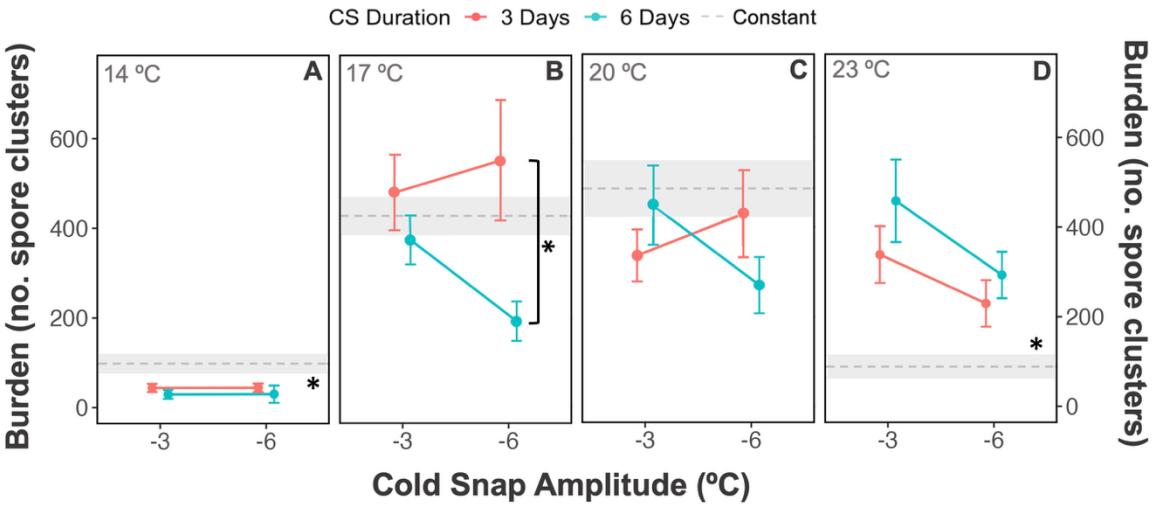


**Figure 2.4: Burden by baseline temperature, amplitude, and duration.**
In *Daphnia magna* infected with *Ordospora colligata*. Burden was the mean number of spore clusters, with each spore cluster containing 32-62 individual *Ordospora*. Error bars represent the standard error; light grey area represents the standard error for the constant treatments. An asterisk (*) near the grey dashed line indicates that all cold snap treatments are different from the constant temperature treatment, and an asterisk next to a solid black bracket ( ]*) indicates a difference between two single treatments.

Certain cold snap treatment combinations led to differential outcomes, and as a result not all cold snaps resulted in the same burden. At 17°C a long and strong cold snap with a 6-day duration and an amplitude of -6°C had a lower burden compared to that of a short cold snap (3-days) with the same amplitude (Figure 2.4B) whereby the difference in burden was 323 spore

clusters (*emmean, z(inf) = -2.983, p = 0.018*, Table A7). Overall, this long and strong cold snap had the lowest mean values at 17ºC and 20ºC and was also among the lowest values at 14ºC and 23ºC (Figure 2.3). Finally, while this long and strong treatment tended to increase burden with increasing baseline temperature (Figure 2.3), the opposite was true for infection prevalence which decreased with increasing baseline temperature (Figure 2.2). Thus, not all cold snaps will lead to the same outcome in disease intensity, and parasite fitness can be simultaneously altered in opposite directions in the same cold snap treatment.

## 2.4 Discussion

### *2.4.1 Effect of baseline temperature*

This experiment demonstrated that cold snaps can alter the performance of *Ordospora colligata;* however, the direction and influence depend on the baseline temperature. At higher baseline temperatures (23ºC), a cold snap may increase the parasite's performance as temperatures near the optimal growth temperature (17-20ºC), resulting in up to a 5-fold increased burden. At lower temperatures (14ºC), burden instead decreased by up to 3-fold in response to a cold snap, likely due to decreased parasite performance as temperatures reached or exceeded the thermal limits of the parasite (Paull *et al*., 2015). As *Ordospora* has a smaller thermal range than its *Daphnia* host (Kirk *et al*., 2018), the decrease in parasite performance at temperatures near the thermal maximum may be attributed to the Thermal Mismatch Hypothesis (Cohen *et al*., 2017). This hypothesis suggests that the performance of either the host or the parasite may be most affected at temperatures where the difference in thermal performance between the two antagonists is the greatest. This is supported by the lower burden and infection prevalence of *Ordospora* at the edge of its thermal tolerance during cold snaps at lower temperatures, compared to the constant treatment. However, to formally test this hypothesis, separate measures of host and parasite thermal performance would be

needed, which is not possible in this system, as *Ordospora* cannot survive outside the host. The fact that thermal variation near the maximum thresholds can lead to decreased or increased performance of diseases has been demonstrated in various study organisms. For example, daily temperature fluctuations are expected to increase malaria transmission at lower baseline temperatures while decreasing transmission at higher baseline temperatures (Paaijmans *et al*., 2010). Furthermore, the effect of a heatwave can also depend on the baseline temperature, as demonstrated in a related *Daphnia-Ordospora* experiment that resulted in a 4-fold increase in burden at 16ºC but a 3-fold decrease at 22ºC (Kunze *et al*., 2022). Indeed, our results show that cold snaps, like heatwaves, may modify parasite proliferation, and the direction of this effect depends on the baseline temperature. While the impact of cold snaps on disease proliferation and outbreak have received less attention, these findings are in line with other studies that have shown that cold snaps can alter host morphology and physiology (Kristan and Hammond, 2000), immunity (van der Lans *et al*., 2015, Huang *et al*., 2023), and behaviour (Hance *et al*., 2007). Thus, the baseline temperature at which the cold snap occurred was the leading factor in explaining the performance of *O. colligata,* yet both the duration and the amplitude of the cold snap also played an important role.

### *2.4.2 The effect of amplitude and duration*

The amplitude and the duration of a cold snap can affect parasite fitness, and the effect of both depends on the baseline temperature (Amplitude • ºC and Duration • ºC interaction). Both amplitude and duration independently decreased burden at low temperatures while increasing proliferation at higher temperatures. Infection prevalence, however, showed a more context-specific response as illustrated by the long and strong cold snap which resulted in a decrease in parasite fitness with increasing temperatures. Additionally, some combinations of amplitude and duration led to alternate patterns, indicating an interaction between amplitude,

duration, and baseline temperature. Similar to burden, if the temperature exceeds the parasite's thermal range, then infection prevalence (i.e., ability to establish an infection in the host) may decrease (Cohen *et al*., 2017), for example, as seen at 14ºC (Figure 2). Moreover, it has previously been noted that short fluctuations can reduce infection prevalence in the same system (Kunze *et al*., 2022). Indeed, previous studies have highlighted the importance of the strength of temperature fluctuations, for example, in *Aedes albopictus,* (Thomas *et al*., 2012) where duration influenced the egg hatching response at the parasite's thermal limits, and in herbivore-parasitoid (Zhang *et al*., 2019a) and plant-endoparasite (Schreven *et al*., 2017) systems, the fitness of the parasite depended on the amplitude of a heatwave. Additionally, both factors (amplitude and duration) can influence human systems, for instance, population dynamics in *Anopheles* infected with *Plasmodium* were affected by the amplitude and type of fluctuation around a baseline temperature (Beck-Johnson *et al*., 2017), heatwave amplitude and duration can influence *Salmonella* serotypes and phage types resulting in increased risk of infection with increasing amplitude and/or duration (Milazzo *et al*., 2016), and heatwave amplitude and duration increased infection risk for children infected with Shiga toxin-producing *Escherichia coli* (Acquaotta *et al*., 2017). While for heatwaves we have some insight into the effect of duration and amplitude on disease proliferation (Kunze *et al*., 2022), for cold snaps such insight is lacking. We show here that in addition to the baseline temperature, both the amplitude and duration of a cold snap can alter parasite fitness, with variable outcomes.

### *2.4.3 Not all cold snaps result in the same outcome*

Different combinations of the duration and amplitude of a cold snap can lead to divergent disease outcomes. The findings for burden at 17ºC demonstrate that cold snaps can result in differential outcomes at the same baseline temperature, with the highest burden found in response to a short, strong cold snap, while a long, strong cold snap led to the lowest burden. Another such example is the decreasing infection prevalence with increasing baseline temperature in the long, strong cold snap, which exhibits a different response compared to the

other cold snap treatments. These context-specific differences may be explained by trade-offs associated with the Temperature Variability Hypothesis (Raffel *et al*., 2013) and Thermal Mismatch Hypothesis (Cohen *et al*., 2017). High parasite fitness as observed in the short, weak cold snap is expected under fluctuating temperatures according to the Temperature Variability Hypothesis (Rohr *et al*., 2013). This theory posits that since parasites are almost always smaller than their hosts and therefore have higher metabolic rates (according to the Metabolic Theory of Ecology (Brown *et al*., 2004)), they should acclimatise faster to shifts in temperature, giving them an advantage over their hosts as the host's thermal acclimation is slower following temperature shifts (Rohr and Raffel, 2010). In line with this hypothesis, parasite fitness is increased in the *Daphnia-Ordospora* system when temperature fluctuates (both in individuals (Kunze *et al*., 2022) and whole populations (Krichel *et al*., 2023)), which is also found in other systems (caribou-nematode (Kutz *et al*., 2009), Cuban tree frog-chytrid fungus (Raffel *et al*., 2013), and protist-bacteria (Duncan *et al*., 2013)). However, thermal mismatch (Cohen *et al*., 2017) or thermal stress (Paull *et al*., 2015) can also play an important role alongside variable temperatures and may decrease or reverse the advantage of the parasite, especially if the parasite has a narrower thermal performance than its host, as is the case for *Ordospora colligata* (Wharton, 1999). Moreover, the reduced burden of *Ordospora* when exposed to the long and strong cold snap over much of the temperature range suggests that the parasite may be experiencing higher levels of stress and a decrease in performance due to temperatures outside the optimal range. Yet, the increased burden at high temperatures suggests that even a small temporary relief from stressful temperatures can help *Ordospora* to establish higher levels of burden (Kirk *et al*., 2020). Furthermore, cold snaps with a short duration and weak amplitude showed the same increase in burden, highlighting that even weak cold snaps may alter disease patterns. Indeed, previous studies have shown the advantage of weak and short cold snaps. For example, short-term fluctuations ±6ºC in the same *Daphnia-Ordospora* system have also been found to increase endemic infection prevalence (Krichel et al., 2023), and short-term cold snaps increased burden in *Alytes obstetricans* tadpoles infected with

*Batrachochytrium* (Fernández-Beaskoetxea *et al*., 2015). Thus, even weak and short cold snaps may have a substantial effect on disease, and this effect can be different depending on the duration and amplitude of the cold snap and the parasite trait.

### *2.4.4 The difference between cold snaps and other thermal variations*

Cold snaps are different from other types of temperature variation and can affect parasite traits in opposing directions. Comparisons to previous studies using the same *Daphnia-Ordospora* model system reveal that cold snaps alter within-host proliferation in a different direction than heatwaves or daily fluctuating temperatures. Both Kunze *et al.* (2022), who studied heatwaves and daily fluctuation in the same host-parasite system, and our study (cold snaps) found large differences in burden for different types of temperature variation. However, there are clear differences between heatwaves, daily fluctuations, and cold snaps at higher temperatures, with cold snaps increasing disease burden at 23ºC while daily fluctuations and heatwaves lead to a decrease. Moreover, in line with previous findings, which showed that different host and parasite traits respond in a unique way to changes in baseline temperature (Kirk *et al*., 2019, Kirk *et al*., 2018, Kirk *et al*., 2020), here, we demonstrated that two parasite traits (burden and infection prevalence) can be affected in opposite directions for some but not all combinations of duration and amplitude. This is illustrated by the decrease in infection prevalence with increasing temperature for the long and strong cold snap, while burden increased with increasing temperature for the same treatment. Our findings address an outstanding question in the field (Rohr *et al*., 2013) and show that the direction of the temperature shift, the type of variation and the life history trait studied matters.

### *2.4.5 Conclusion*

In conclusion, we show that in the *Daphnia-Ordospora* system, cold snaps are distinct from other forms of temperature variation, and these can lead to increased or decreased disease

proliferation depending on the prevailing baseline temperature. Moreover, different combinations of amplitude and duration lead to differential outcomes, and that parasite's fitness traits can be simultaneously affected in opposite directions. Overall, the impact of cold snaps on *Ordospora* fitness is therefore complex. Thus, the effect of cold snaps and temperature variation on disease may result in unexpected outcomes in the face of climate change. Given *Daphnia's* key ecological role (Ebert, 2022), this may result in far-reaching ecological impacts as *Daphnia* diseases are known to alter inter- and intra-specific competition (Decaestecker *et al*., 2015), cause trophic cascades (Duffy, 2007), influence host evolution (Capaul and Ebert, 2003), and host stoichiometry (Miner *et al*., 2012). For example, the infection of *Daphnia dentifera* led to a reduction in host density, and consequently, alterations in the food web, which could lead to potential losses in biodiversity (Duffy, 2007). Similarly, in other systems, for example, sea star wasting disease in *Pisaster ochraceus*, infection led to a trophic cascade, and the collapse of a keystone predator with unexpected repercussions (Menge *et al*., 2016). Adding to the complexity of host-parasite dynamics, there is often a disconnect between thermal performance and parasite burden in many systems where no correlation is found between heat tolerance and burden in a parasite (see Hector *et al*. (Hector *et al*., 2023) for a review). Indeed, understanding temperature variation has been a challenge for years (Rodó *et al*., 2013), and although some theoretical studies are highlighting that the addition of temperature shifts to predictive models is key to accurate predictions, (Costas and Yuri, 2019) we still lack the needed empirical data and mechanisms. Thus, there is an urgent need to understand the underlying mechanisms responsible for the impact of temperature variation on diseases, both with this host-parasite genotype combination and with others to facilitate in generalisation of the mechanisms and conclusions. Future research is needed on disease interactions in response to different types of extreme weather (such as heatwaves and cold snaps) to better understand how disease outbreaks will respond to intensifying global change.

## 2.5 Methods

### *2.5.1 Study system*

*Daphnia magna* (genotype FI-OER-3-3), previously isolated from a rockpool at Tvärminne archipelago, Finland, was infected with its microsporidian parasite *Ordospora colligata* (isolate 3), sampled from the same location. *Daphnia magna* is a popular model organism for the study of host-pathogen interactions, given their fast generation time of a new clutch every 3-4 days, clonal reproduction via parthenogenesis, which allows for experiments to be conducted on genetically identical individuals (Ebert, 2005), their well-known ecology and their key role in ecosystems (Sarnelle, 2005, Ebert, 2022). *Ordospora colligata* is a microparasite (that is, an infectious disease) which invades the upper gut epithelium of its host upon ingestion of spores via filter feeding and is then horizontally transmitted (from host to host) via faeces into the water (Pombert *et al*., 2015). This parasite has been well studied with respect to climate change (and especially the specific host genotype and parasite isolate used here (Kirk *et al*., 2020, Krichel *et al*., 2023, Kunze *et al*., 2022, Kirk *et al*., 2019, Capaul and Ebert, 2003) , with multiple papers focused on the effects of baseline temperature and temperature variation (Kirk *et al*., 2020, Kunze *et al*., 2022). *Daphnia* have a temperature tolerance ranging from 6-33.3ºC with an optimum temperature of 20ºC, while *Ordospora* has a similar, but somewhat narrower thermal tolerance of 11.8-29.7ºC (Kirk *et al*., 2018).

### *2.5.2 Experimental setup*

This experiment had a fully factorial design, whereby the amplitude and duration of cold snaps were manipulated and assessed at four baseline temperatures (14, 17, 20, and 23ºC). Here, we define a cold snap as an event which reduced the baseline temperature at a set amplitude for a specific duration. The experiment began 10 days pre-exposure, giving the juvenile animals time to mature, while the cold snap itself was initiated 10 days post-exposure. During the cold

snap, the temperature was lowered by either -3ºC or -6ºC (two amplitudes), and the duration was varied by 3 days or 6 days (two durations) (Figure 1). A change of 6ºC is within the limits of naturally encountered temperature fluctuations in the rockpools these animals inhabit (Jocque *et al.*, 2010). A constant temperature control (amplitude 0°C) was included for each of the four baseline temperatures, with additional temperature constant controls at 8ºC and 11ºC. Additionally, we included uninfected controls for parasite exposure for each of the constant treatments. In total, there were 16 different cold snap treatments (four baseline temperatures • two amplitudes • two durations), each with 15 replicates per treatment that were organised into trays. Trays were submerged into 18 water baths (four trays per bath, three baths per temperature) that regulated the temperature, and trays were repositioned daily to avoid positioning effects. Each baseline temperature bath held one hundred and eight 100 mL microcosms (filled with 80 mL of Artificial *Daphnia* Medium (ADaM (Klüttgen *et al.*, 1994)) of which four microcosms were uninfected controls, eight were constant-temperature replicates, and 20 were parasite exposed cold snap treated microcosms (four treatments • five replicates, per bath) (Figure 1), remaining microcosms only contained water and were present for stability when moving the trays. In total, the experiment contained 456 individual *Daphnia*, each placed in a separate microcosm (i.e., 240 cold snap treatments, 144 constant treatments and 72 uninfected controls).

The eighteen temperature-controlled water baths ranged from 8-23ºC (three baths per temperature). 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 create a constant flow and ensure even water and temperature distribution. The temperature for each bath was regulated using a programmable controller (Inkbird ITC-308 or ITC-310T) and monitored daily using HOBO temperature loggers (HOBO UA-001-08). Baths were kept at a constant water level, and any evaporation was offset by adding tap water. The baths were kept under natural lighting conditions of 16:8, light:dark. To simulate the cold

snaps, individual microcosms were moved between assigned baths, while each bath was maintained at a constant temperature throughout the experiment. The microcosms were added directly to the target temperature bath, upon which the microcosm would immediately start to equilibrate, and the target cold snap temperature was reached within an hour. Once the cold snap treatment was concluded, the microcosm was then returned to its original position in the baseline temperature water bath. For example, five replicates of a cold snap treatment, which decreased by 6ºC for 6 days, would stay in the first 14ºC water bath until day 10. These five treatments would then be transferred to the first 8ºC water bath for 6 days, then after the duration of the cold snap, they were then returned to the original 14ºC water bath.

In preparation for the experiment, *Daphnia* were grown asexually under ideal laboratory conditions for three weeks. Each small population of 10-12 females was held in a 400 mL microcosm containing ~300 mL of ADaM, under continuous light at 20ºC. The populations were transferred twice a week to fresh ADaM and fed *ad libitum* with batch culture algae (*Scenedesmus* species) (Kilham *et al*., 1998). Seventy-two hours prior to the beginning of the experiment, the animals were transferred to fresh medium with no juveniles present. Any juveniles born over the next 72 hours were collected and sexed (using a dissecting microscope at 8x to 12x magnification). Any males were removed, as *Daphnia* have environmental sex determination and are therefore not truly clonal (Ebert, 2005). To start the experiment, one female juvenile was added to each of the 456 100 mL microcosms. Microcosms were filled with 50 mL of ADaM, and a pinch of Cetyl alcohol was also added to break the surface tension (which can entangle young animals). On day -10, the experiment began; *Daphnia* were unexposed until day 0 when the exposed individuals were given 1 mL of ADaM containing ~60,000 *O. colligata* spores, to ensure near 100% infection prevalence. The spore dose was created by crushing (using a mortar and pestle) 1942 infected *D. magna* with a known average burden (quantified by using phase-contrast microscopy at 400x magnification), and the resulting slurry was diluted to 500 mL. Unexposed controls were exposed to a placebo dose

made by crushing uninfected individuals. Individuals were transferred to fresh ADaM every four days (to remove any offspring produced) and fed every two days (from 5 million algae/mL at the start of the experiment to 12 million algae/mL by day 5, which was maintained until the end of the experiment) (Figure 2.1).

### *2.5.3 Measurement of parasite fitness*

Upon natural death or at the end of the experiment (day 27), the infection prevalence (i.e., presence or absence of spores) and burden (i.e., number of spore clusters inside the host) were observed for each individual. At the end of the experiment, animals were terminated within five days of day 27. If infected, spore clusters (each cluster holds up to 64 individual spores) were counted using phase-contrast microscopy with 400x magnification (Figure 2.1). Deaths occurring before day 12 were omitted (n = 6) from the analysis as infections could not be accurately diagnosed prior to this period. Any misidentified male *Daphnia* (n = 4) and inconclusive infections (n = 12) were also omitted.

### *2.5.4 Data analysis*

Analysis was performed using R version 4.0.3 (R Core Team, 2022). Infection prevalence (i.e., presence or absence of infection) and burden (i.e., the number of clusters of *Ordospora* spores) were the response variables, while the explanatory variables were baseline temperature, amplitude, and duration. Infection prevalence data included all exposed individuals, while burden data included confirmed infections only. Baseline temperature was a continuous centred variable to help reduce issues present with collinearity. A polynomial (quadratic for infection prevalence, and cubic for burden) was added to account for non-linearity in the response to temperature. Amplitude and duration were ordered factors with two levels each ("-3" and "-6" for amplitude, and "3" and "6" for duration), both also included the constant treatments with the reference level "0", this allowed for statistical testing on the two variables both independently and combined. Elastic net regression using the 'glmnet' package

(Friedman *et al*., 2010) was used for variable selection and regression to model infection prevalence and burden; this regression fits a generalized linear model (GLM) via penalized maximum likelihood reducing the issues associated with collinearity (Zou and Hastie, 2005) which were present in a regular 'GLM' with a binomial (infection prevalence) or a negative binomial (burden) distribution. Thus, a 'glmnet' can effectively handle multicollinearity by shrinking the coefficients, preventing overfitting, and providing more stable estimates. While a random effect for 'bath' could have accounted for batch-specific variability, 'glmnet' does not support random effects, as it focuses on regularisation of fixed-effects models. Mixed-effects models could address this, but 'glmnet' was chosen for its ability to handle multicollinearity and high-dimensional data. For infection prevalence data, a 'cv.glmnet' function was used for cross validation and to find the optimal lambda (lambda = 0.00533), the regression fit (alpha = 0.5) was chosen based on the lowest cross-validation error value, and a binomial family was used. For burden data, a 'glmnet' function was used for modelling, and the regression fit (alpha = 0.5) was chosen based on the lowest AIC score (Akaike Information Criterion score) to find a good balance between model complexity and data fit. The strength of regularisation in the model (lambda = 0.0711) was selected using the lowest AIC score and post-model assessments, and the family used was negative binomial (theta = 1.2208) to account for overdispersion. Bootstrapping with 25000 iterations was used for both infection prevalence and burden to assess the model stability and significance, resulting in the estimated coefficients (effect), and their 95% confidence intervals. Finally, feature importance was calculated for each factor by finding the absolute value of each coefficient to indicate which features are the most important for predicting the outcome (response variable) based on the elastic net regression model. This was carried out individually per polynomial degree and then added together as a whole per variable. Additionally, custom contrasts were created using the 'emmeans' package (Russell V. Lenth, 2022) to compare the average infection prevalence/burden of treatments. For this, amplitude and duration were combined into one factor and modelled with temperature to avoid issues with collinearity and ensure clarity in

interpretation. The contrast p-values were then adjusted for multiple comparisons using the 'Benjamini-Hochberg' method (Benjamini and Hochberg, 1995). Analysis code and supporting data sets are available in an online repository (McCartan *et al.*, 2024a).

# Chapter 3: Timing of a cold snap relative to exposure drives large shifts in disease proliferation

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**Author Contributions:** NMcC and PL designed the experiment. NMcC conducted the experiment with the assistance of PL and SDiC. NMcC conducted the analysis with the aid of PL and JP. NMcC generated figures. NMcC and PL wrote the first draft of the manuscript, and all authors contributed to revisions. All authors read and approved the final manuscript.

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## 3.1 Abstract

In recent years, the frequency and intensity of anomalous weather events (such as cold snaps) have increased due to climate change. While heatwaves have received substantial attention, the effect of cold snaps, particularly the effects of the timing of the event, has remained underexplored in host-pathogen systems. Here, we investigated the effect of 48 cold snap treatments using *Daphnia magna* infected with its microsporidian parasite *Ordospora colligata*. The amplitude and duration of cold snaps were manipulated across four baseline temperatures and at three distinct time points in relation to parasite exposure. Parasite burden varied up to 17-fold compared to constant baseline temperature conditions. Indeed, cold snaps can either enhance or suppress parasite fitness depending on the interplay between baseline temperature, cold snap treatment (amplitude and duration), and timing. Notably, cold snaps occurring before or after infection produced contrasting outcomes, likely due to mismatches in host and parasite developmental timing. These findings underscore the context-specific nature of extreme thermal variation and its capacity to alter host-parasite dynamics in non-linear ways. To better predict climate change impacts on disease ecology, it is crucial to incorporate timing and thermal variability, not just mean temperature, into forecasting models.

## 3.2 Introduction

Climate change is becoming increasingly prevalent globally, with impacts on human health (Rocque *et al.*, 2021), wildlife (Cohen *et al.*, 2020), and the economy (Gomez-Zavaglia *et al.*, 2020). Among its many consequences, climate change is projected to shift the distribution and seasonality of infectious diseases (Semenza and Paz, 2021) by altering the biology and ecology of hosts, vectors, and parasites (Gallana *et al.*, 2013). For instance, climate-driven range

expansion of *Aedes aegypti* mosquitoes has enabled outbreaks of dengue and Zika virus in parts of southern Europe that were previously unaffected (Liu-Helmersson *et al*., 2016). Indeed, temperature can alter nearly every aspect of the host-pathogen relationship. It affects host traits such as immune function (Catalán *et al*., 2012), behaviour (Augustin *et al*., 2021), and metabolism (O'Niel *et al*., 2024), which in turn influence susceptibility to infection and disease outcomes. Pathogens are equally sensitive, as temperature shapes their development rate (Suh *et al*., 2024), survival (Roncarati *et al*., 2025), and replication (Samuel *et al*., 2016). For example, exposure to warmer temperatures accelerated the growth and reproductive success of the parasite *Schistocephalus solidus* in three-spined stickleback fish, while simultaneously suppressing host immune responses through altered immunometabolism (Scharsack *et al*., 2021). Moreover, temperature can modulate the timing and intensity of interactions between hosts and pathogens (Gehman *et al*., 2018, Reece *et al*., 2017). For instance, in the mosquito *Anopheles gambiae*, warmer temperatures accelerated ageing and shifted the timing of infection, leading to higher *Escherichia coli* loads later in life (Barr *et al*., 2024). Yet, while many studies focus on gradual warming or mean temperature increases (Gehman *et al*., 2018, Shocket *et al*., 2019, Hector *et al*., 2021a, Suh *et al*., 2024), temperature extremes may have especially disruptive effects on host-parasite interactions (Chen *et al*., 2019, Marcus *et al*., 2023, Chen and Lewis, 2023, Tobin *et al*., 2024, Vajedsamiei *et al*., 2024), but remain less understood.

Anomalous weather events (like heatwaves and cold snaps) are increasing in frequency and intensity (Leahy *et al*., 2021). Indeed, rapid transitions from extreme heat to cold and vice versa have affected over 60% of the globe between 1961 and 2023 (Wu *et al*., 2025). Cold snaps are becoming more frequent, driven by large-scale atmospheric patterns like blocking events (persistent high-pressure systems that disrupt normal weather flow and trap cold air) and the positive phase of the North Atlantic Oscillation (NAO) (Wade *et al*., 2015, D'Errico *et al*., 2022). For example, in January 2022, atmospheric blocking and a positive NAO contributed to cold

snaps and unusually low temperatures across the Euro-Mediterranean region, despite ongoing global warming (Demirtaş, 2023). Yet, while heatwaves have received considerable attention due to their immediate impacts on host-parasite relationships (Fischer and Schär, 2010, Cheng *et al*., 2020, Kunze *et al*., 2022, Lian *et al*., 2023, Chen and Lewis, 2023), cold snaps can be equally disruptive (Raffel *et al*., 2015, Cohen *et al*., 2020), particularly for ectothermic organisms whose physiology is tightly coupled to ambient conditions (Roitberg and Mangel, 2016). For example, exposure to low temperatures impaired the ability of southern leopard frogs (*Rana sphenocephala*) to produce antimicrobial peptides crucial for defending against the fungal pathogen *Batrachochytrium dendrobatidis,* resulting in exacerbated susceptibility (Robak *et al*., 2019). Moreover, sudden temperature drops can reduce insect activity levels or shift developmental timing, potentially increasing susceptibility to infection through mismatches in host-parasite phenology or reduced immune investment (Abarca and Spahn, 2021). Furthermore, the timing of the thermal variation in relation to exposure can alter the outcome of host-parasite relationships (Valls *et al*., 2020, Costaz *et al*., 2023, McCartan *et al*., 2025, Morley and Lewis, 2014). Yet, the biological consequences of cold snaps remain relatively understudied (but see (Canning-Clode *et al*., 2011, Roitberg and Mangel, 2016, Cohen *et al*., 2021, Laaidi *et al*., 2013, Morley and Lewis, 2014, Wang *et al*., 2016)) compared to other types of temperature variation (Chen and Lewis, 2023, Lian *et al*., 2023, Tobin *et al*., 2024, Cohen *et al*., 2019, Rohr and Cohen, 2020, Rohr and Raffel, 2010), despite growing evidence of their ecological and epidemiological significance.

The impact of various cold snap treatments was examined here, across a broad range of baseline temperatures, using the *Daphnia magna* infected with its microsporidian parasite *Ordospora colligata*. This is a well-established model system for studying environmentally transmitted diseases (characterised by horizontal transmission mechanisms and infection dynamics governed by mass action principles) (Ebert, 2022). We examined the effect of 48 different cold snap treatments on parasite fitness in the *Daphnia-Ordospora* system. We

manipulate cold snap amplitude (-3ºC and -6ºC) and duration (3 days and 6 days) at three different time points (10 days before exposure, the day of exposure, and 20 days after exposure). This research builds upon earlier studies using the same *Daphnia–Ordospora* system (McCartan *et al*., 2024b, Kunze *et al*., 2022, McCartan *et al*., 2025), which examined how extreme weather events (heatwaves and cold snaps) influence parasite prevalence and proliferation. They demonstrated that temperature extremes can result in considerable changes in the host-parasite relationship. For example, McCartan *et al*. (2024) found that cold snaps occurring 10 days after exposure could increase the burden by 5-fold and decrease it by 3-fold, depending on baseline temperature. This research, however, did not consider the timing of the cold snap event relative to exposure to the pathogen. Whether a cold snap event occurring prior to exposure, during establishment, or after exposure to the pathogen could lead to differential outcomes remains underexplored. For example, earlier thermal conditions experienced by the host's mother can shape infection outcomes, as maternal temperature has been shown to influence offspring susceptibility (Garbutt *et al*., 2014). Moreover, Phenological Mismatch Theory suggests that cold snaps may desynchronise host and parasite life cycles, particularly if environmental cues shift the timing of development or reproduction in only one antagonist (Pardikes *et al*., 2022). Thus, understanding how the timing of cold snap events interacts with the host-parasite relationship is critical for predicting their impacts on infection dynamics under variable environmental conditions. Here, we expected that cold snaps would alter parasite fitness in complex and context-dependent ways by increasing or decreasing infection prevalence and/or burden, depending on the baseline temperature.

## 3.3 Methods

### *3.3.1 Experimental protocol*

This experiment followed a similar factorial design and setup as described in McCartan *et al*. (2024b), with modifications to investigate how the timing of cold snaps affected infection outcomes. As in the previous study, *Daphnia magna* (genotype FI-OER-3-3), infected with *Ordospora colligata* (isolate 3), were exposed to cold snaps that varied in amplitude (-3ºC or -6ºC) and duration (3 or 6 days) across four baseline temperatures (14, 17, 20, and 23ºC). Constant temperature controls were included at each baseline, as well as at 8ºC and 11ºC, with uninfected controls also maintained under constant conditions. While the previous study only applied cold snaps 10 days post-exposure, this experiment assessed the effects of cold snaps occurring at three distinct time points relative to parasite exposure: 10 days before exposure (before), on the day of exposure (during), and 20 days after exposure (after) (Figure 3.1). This resulted in 48 cold snap treatments (four baseline temperatures • three cold snap timings • two amplitudes • two durations), each with 15 replicates per treatment, which were organised into trays.

There were three baths per temperature, each containing one hundred and eight 100 mL microcosms (filled with 80 mL of Artificial *Daphnia* Medium (ADaM (Klüttgen *et al*., 1994)) distributed across four trays. Each bath held 60 parasite-exposed cold-snap-treated replicates, eight constant temperature replicates and four uninfected controls; the remaining microcosms only contained water and were present for stability when moving the trays. In total, the experiment contained 936 individual *Daphnia,* each in an individual microcosm (i.e., 720 cold snap treatments, 144 constant treatments, and 72 uninfected controls). The bath set up used the same equipment as McCartan *et al*. (2024b) (heaters and chillers interfacing with a control unit). When a cold snap occurred, a microcosm was moved to the target baseline bath for the duration of the cold snap, where it reached the target temperature within an hour. When the cold snap concluded, it was returned to its original position in the baseline bath.

All other experimental conditions, including *Daphnia* culturing, parasite dosing (~60,000 spores), feeding regime, and infection assessment (prevalence and burden quantified via 400x microscopy at natural death or day 28), were identical to the previously published setup. In short, juvenile female *Daphnia* were placed into individual microcosms just before the first cold snap occurred, and ten days prior to the exposure to *Ordospora* (Figure 3.1). Individuals were transferred to fresh ADaM every four days (to avoid the accumulation of offspring produced and waste products) and fed *Scenedesmus sp.* algae every two days (from 5 million algae/mL on day -10 to 12 million algae/mL by day 5, which remained constant until the end of the experiment). To make the parasite dose, 3,881 infected *Daphnia* with a known average burden were crushed, and the resulting slurry was diluted to 900 mL. Unexposed controls were treated the same and were exposed to a placebo dose made by crushing uninfected individuals. Deaths occurring before day 12 (n = 32) were omitted from the analysis as infections could not be accurately diagnosed prior to this period. Any misidentified male *Daphnia* (n = 10) and inconclusive infections (n = 35) were also omitted.

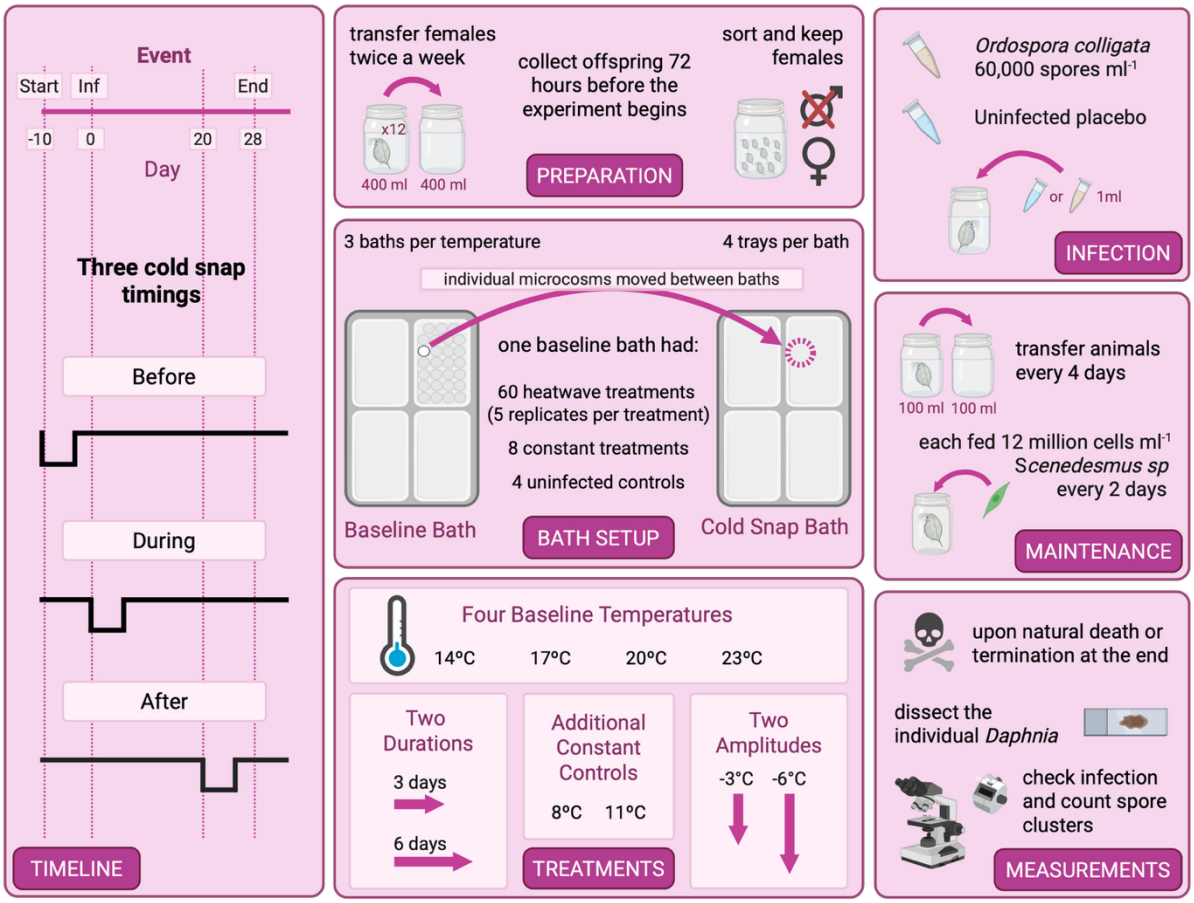


**Figure 3.1 Chapter 3 experimental setup.**
TIMELINE; illustrates the timeline for the experiment starting at day -10 and terminating at day 28, exposure occurred on day 0, and heatwaves began on day -10, 0, and 20. PREPARATION; preparation started three weeks prior to exposure when 10-12 mothers were 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 60 cold-snap-treated individuals (five replicates per heatwave treatment), eight constant temperature treatments, and four uninfected controls. To simulate a cold snap, microcosms were moved between baths and then returned to the baseline bath when the cold snap finished. TREATMENTS; 48 treatments were included in the experiment (four baseline temperatures • three cold snap timings • two amplitudes • two durations). Additionally, at the cold-snap-specific temperatures (8°C and 11°C), a tray held 27 microcosms but only two constant treatments and one uninfected control, the remaining microcosms contained no *Daphnia*. MAINTENANCE; maintenance and measurements were carried out between days -8 and day 28. Figure created on Biorender.com.

### *3.1.1 Data analysis*

Analysis was performed using R version 4.4.1 (R Core Team, 2024). Infection prevalence (i.e., presence or absence of infection) and burden (i.e., the number of clusters of *Ordospora* spores) were the response variables, while the explanatory variables were baseline

temperature, timing, and treatment. ‘Treatment’ was a factor which combined the variables ‘amplitude’ and ‘duration’ to increase model simplicity and reduce collinearity issues. Infection data included all exposed individuals, while burden data included confirmed infections only. Baseline temperature was a continuous, scaled, and centred variable to help reduce issues present with collinearity. A polynomial (quadratic for infection prevalence, and cubic for burden) was added to account for non-linearity in the response to temperature. Elastic net regression was carried out using the ‘glmnet’ package (Engebretsen and Bohlin, 2019) to model infection prevalence and burden. The glmnet package fits Generalised Linear Models (GLMs) via penalised maximum likelihood, helping to reduce collinearity by applying regularisation (lasso, ridge, or elastic net) to shrink or select coefficients. The potential batch effect associated with the 'bath' variable was tested and found to be non-significant for both infection prevalence and burden, so generalised linear mixed models (GLMMs) were not necessary. For infection prevalence data, a ‘cv.glmnet’ function with a binomial family was used for cross-validation and to find the optimal lambda (lambda = 0.01201); the regression fit (alpha = 1) was chosen based on the lowest cross-validation error value. For burden data, the ‘glmnet’ function with a negative binomial family (theta = 1.3020) was used to account for overdispersion, as the ‘cv.glmnet’ function cannot handle this distribution. The regression fit (alpha = 0.87) was selected based on the lowest Akaike Information Criterion (AIC) to balance fit and complexity. The regularisation parameter (lambda = 0.03336) was chosen based on this optimal AIC score. To assess model robustness and estimate significance, bootstrapping with 50,000 iterations was applied for both infection prevalence and burden models, yielding effect estimates and 95% confidence intervals. Feature importance was calculated by summing the absolute values of coefficients across polynomial terms for each variable, reflecting their relative influence in predicting the response. All polynomial terms per treatment were then added together to create a combined feature importance (CFI). Additionally, the ‘emmeans’ package (Russell V. Lenth, 2022) was used to define custom contrasts, allowing comparisons of mean infection prevalence and burden across treatments. Here, baseline temperature was

treated as a factor and treatment was combined with timing into a new factor to avoid collinearity issues and ensure clarity in interpretation within specific treatments. P-values from the contrasts were corrected for multiple testing using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995).

Parasite burden was also assessed using a 'degree-day' proxy. Degree days above the infection threshold (the lowest temperature at which infection was observed) were estimated by subtracting 11.2ºC from each individual's mean exposure temperature (with negative values set to zero) and multiplying this value by the number of days the individual survived. The threshold of 11.2ºC was set as it was the lowest temperature experienced where confirmed infections occurred. This approach incorporates any variation in thermal exposure (e.g., cold snaps) into a single metric of cumulative thermal energy above the infection threshold. This 'degree day' variable was combined with 'treatment' and modelled against burden. 'Degree day' was scaled, and a cubic polynomial was added to avoid collinearity issues and account for non-linearity. Like the first burden model, a 'glmnet' with a negative binomial (theta = 1.2664) distribution was run. The model ($\alpha = 0.7$) and regularisation parameter ($\lambda = 0.04827$) corresponded to the optimal AIC. Then, 50,000 bootstrap iterations were run, and feature importance for the combined total was calculated.

## 3.4 Results

### *3.4.1 Infection prevalence*

All factors (baseline temperature, timing, and treatment) were important for predicting infection prevalence. Baseline temperature was most important for predicting prevalence (*feature importance = 37.98,* Table 3.1) with both linear and quadratic relationships (*linear effect, glmnet = 10.93, 95% CI: 4.94 to 16.11, and quadratic effect, glmnet, effect = -27.05, 95%*

*CI: -32.78 to -23.18,* Table 3.1). The positive linear and negative quadratic coefficients indicate a classic temperature response curve with optimum temperatures at the median ~17°C (Figure 3.2). Moreover, a strong and long cold snap that decreased the temperature by 6°C and lasted 6 days (-6°C for 6 days) occurring after exposure was also important in predicting infection prevalence. Here, as baseline temperatures increased, the proportion of infected individuals increased (*linear effect, glmnet = 25.47, 95% CI: 1.62 to 50.18,* Table 3.1, Figure 3). Additionally, cold snaps occurring before exposure were also associated with an increased infection prevalence (*glmnet = 0.61, 95% CI: 0.13 to 1.02,* Table 3.1, Figure B.1A), although the importance was lower. Thus, both the timing and intensity of thermal variability can interact with baseline temperature to shape infection prevalence, highlighting the complexity in which climate extremes can influence host-parasite dynamics.

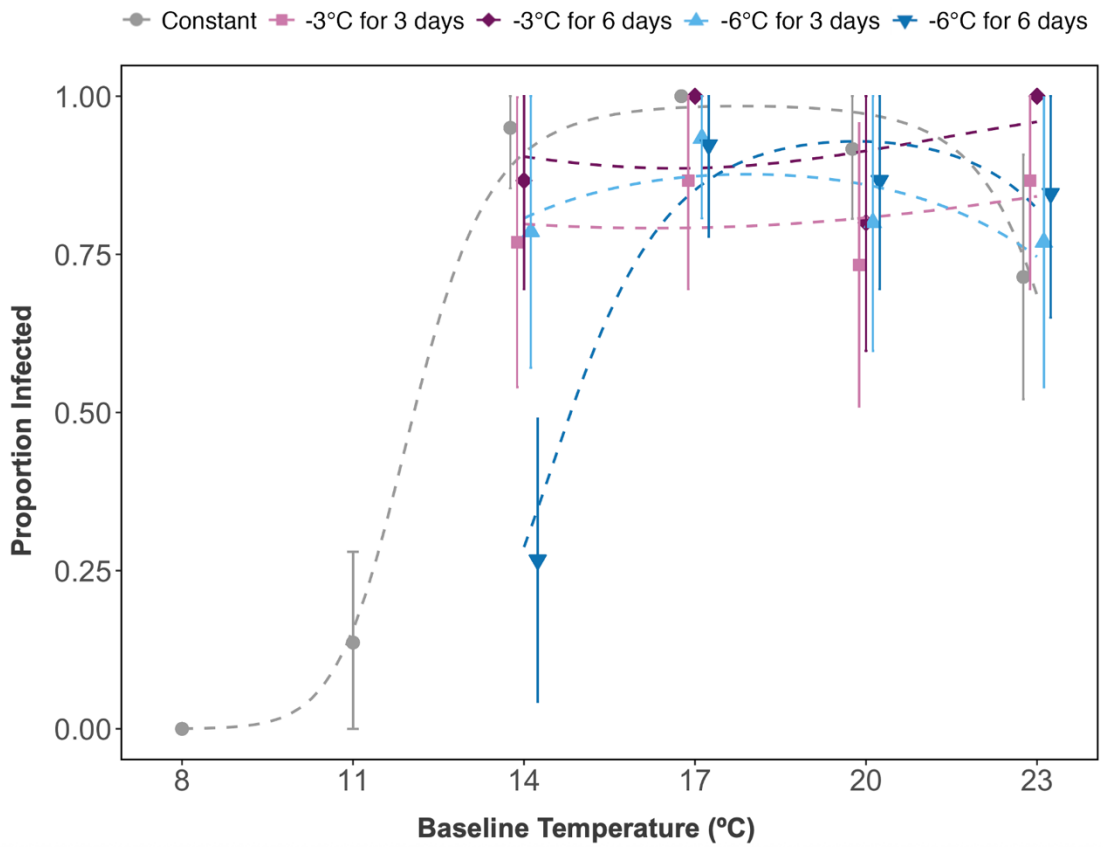


**Figure 3.2: The effect of cold snap treatments occurring after exposure on infection prevalence**
In *Daphnia magna* infected with *Ordospora colligata*. Each spore cluster contains 32-62 individual *Ordospora*. Error bars represent the standard error, and each dashed line is the linear fit of the elastic net regression (see Table B.1 for complete model output). Grey; constant treatment, pink; cold snap lasting 3 days where the temperature was decreased by 3°C,

purple; cold snap lasting 6 days where the temperature was decreased by 3ºC, light blue; cold snap lasting 3 days where the temperature was decreased by 6ºC, dark blue; cold snap lasting 6 days where the temperature was decreased by 6ºC. See Figure B.1 for full visualisation of all treatments.

**Table 3.1: Coefficient estimates and feature importance of infection prevalence.**
A) Coefficient estimates and B) combined feature importance (CFI) 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 increase 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 quadratic polynomial to account for non-linearity and is therefore separated by each degree (linear [L] and quadratic [Q]). 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 B.1 for the full list of coefficient estimate outputs and Table B.2 for combined feature importance variables.

| A | Explanatory Variable | Effect | Lower CI | Upper CI |
|---|---|---|---|---|
| | Temperature (Q) | -27.05 | -32.78 | -23.18 |
| | Temperature (L): Timing (After): Treatment (-6ºC for 6 days) | 25.47 | 1.62 | 50.18 |
| | Temperature (L) | 10.93 | 4.94 | 16.11 |
| | Timing (Before) | 0.61 | 0.13 | 1.02 |

| B | Explanatory Variable | Importance |
|---|---|---|
| | Temperature | 37.98 |
| | Temperature: Timing (After): Treatment (-6ºC for 6 days) | 25.47 |
| | Timing (Before) | 0.61 |

### *3.4.2 Burden*

Cold snaps can substantially alter parasite proliferation with complex interactions between baseline temperature, timing, and treatment (Table 3.2), with effects ranging from a 17.0-fold decrease to a 7.2-fold increase in parasite burden (Figure 3.3 and Figure 3.4). The most important treatments in predicting burden were the strong and long (-6ºC for 6 days) and the weak and long (-3ºC for 6 days) cold snaps occurring after exposure, where the effect depended on the baseline temperature it occurred at (*linear effect, glmnet, effect = 17.54, 95%*

*CI: 5.16 to 26.44, CFI =18.80, and cubic effect, glmnet, effect = 14.20, 95% CI: 6.09 to 19.88, CFI = 20.17, respectively,* Table 3.2, Figure 3.3E). For example, after exposure, the strong and long cold snap (-6°C for 6 days) decreased burden at 14ºC by 17-fold (mean of 6 spore clusters vs constant mean of 98 clusters)(Figure 3.4I), while it increased burden at 23ºC by 7.2-fold (mean of 395 clusters vs constant mean of 95 clusters) (Figure 3.4L) (*emmean, z(inf) = -5.378, p < 0.001* and *emmean, z(inf) = 4.079, p = 0.001*, *respectively*, see Table B.8 for full list of custom contrasts). The degree day analysis gave similar results with the strong and long (-6ºC for 6 days) and the weak and long (-3ºC for 6 days) cold snap occurring after exposure identified as being most influential (*linear effect, glmnet, effect = 17.54, 95% CI: 5.16 to 26.44, CFI = 18.80, and cubic effect, glmnet, effect = 14.20, 95% CI: 6.10 to 19.88, CFI = 20.17, respectively,* Table 3.3, Figure 3.3F). However, in addition to identifying that a cold snap after exposure was important, this analysis also indicated that the strong and long treatment (-6ºC for 6 days) occurring before exposure led to a reduction in burden, especially at higher degree-days, compared to the constant (*linear effect, glmnet, effect = -8.00, 95% CI: -14.45 to -0.20, CFI = 8.00,* Table 3.3, Figure 3.3B). That the timing of a cold snap before exposure occurred can influence burden is further supported by the weak and long cold snap (-3ºC for 6 days) where burden increased compared to the constant when it occurred at 23ºC, before exposure *(emmean, z(inf) = 2.991, p = 0.038,* Table B.8, Figure 3.4D). Therefore, due to the complex interactions, not all cold snaps will result in the same outcome.

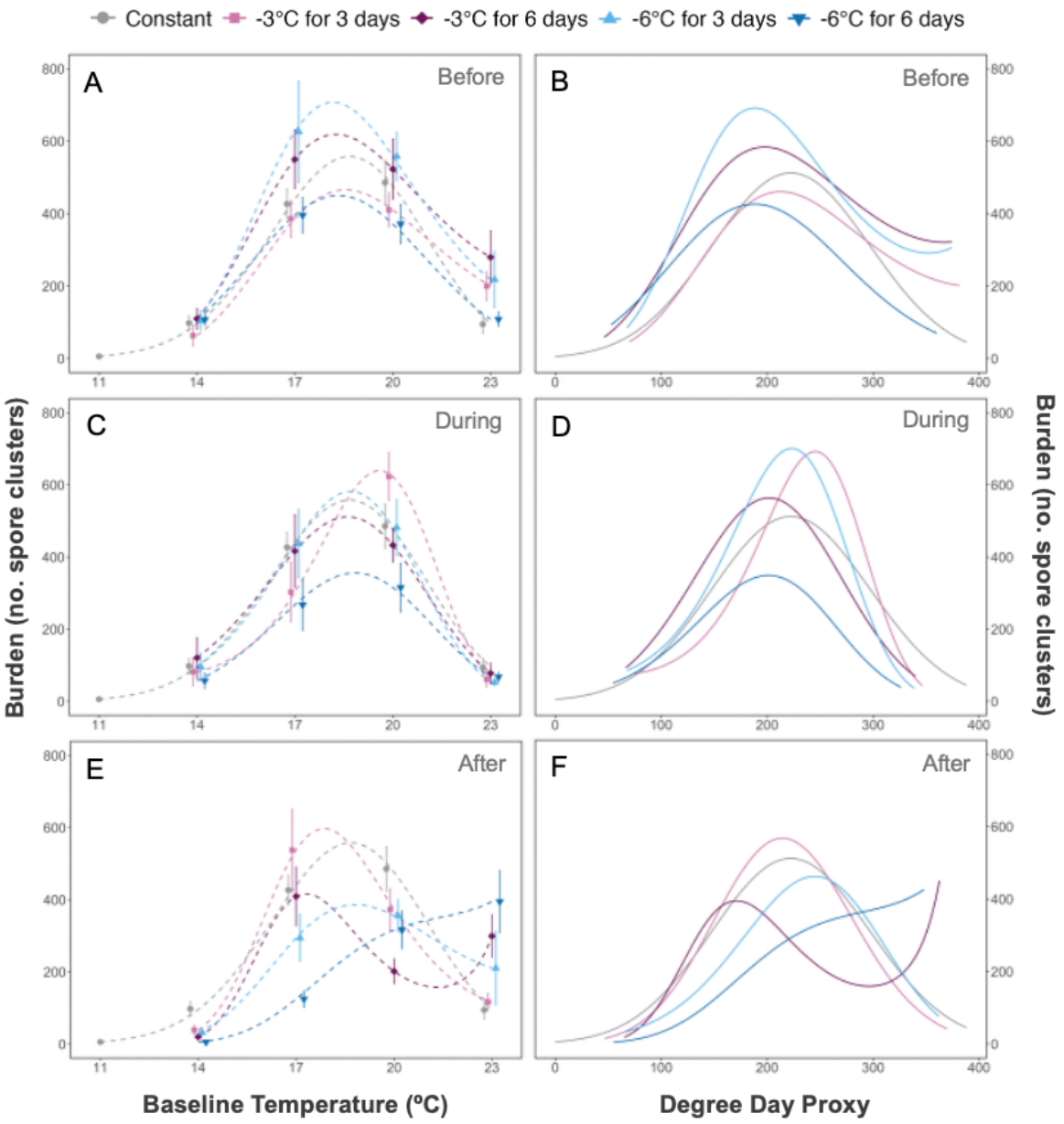


**Figure 3.3: The effect of cold snap treatments on burden.**
In *Daphnia magna* infected with *Ordospora colligata*. Each spore cluster contains 32-62 individual *Ordospora*. Error bars represent the standard error, and each dashed line is the linear fit of the elastic net regression (see Table B.5 for complete model output). Degree days were calculated as the cumulative thermal exposure relative to the 11.2ºC threshold. Left panels; burden according to baseline temperature, Right panels; burden according to degree day proxy. Grey; constant treatment, pink; cold snap lasting 3 days where the temperature was decreased by 3ºC, purple; cold snap lasting 6 days where the temperature was decreased by 3ºC, light blue; cold snap lasting 3 days where the temperature was decreased by 6ºC, dark blue; cold snap lasting 6 days where the temperature was decreased by 6ºC. A and B; cold snap treatments which began before exposure, C and D; cold snap treatments which began on the day of exposure, E and F; cold snap treatments which began after exposure.

**Table 3.2: Coefficient estimates and feature importance of burden.**
In *Daphnia magna* infected with *Ordospora colligata*. A) Coefficient estimates, B) combined feature importance (CFI), C) Coefficient estimates with degree day proxy, and D) combined feature importance (CFI) with degree day proxy. Degree days were calculated as the cumulative thermal exposure relative to the 11.2ºC threshold. 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 increase 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 (95$^{th}$ percentile) from bootstrapping. Non-significant values have CIs encompassing 0, suggesting no important relationship to the response variable. Temperature was a cubic polynomial to account for non-linearity and is therefore separated by each degree (linear [L], quadratic [Q], and cubic [C]). 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 B.5 for the full list of coefficient estimate outputs and Table B.6 for combined feature importance variables with temperature, and Tables B.9 and B.10 for degree day outputs.

| A | Explanatory Variable | Effect | Lower CI | Upper CI |
|---|---|---|---|---|
| | Temperature (L): Timing (After): Treatment (-6ºC for 6 days) | 22.26 | 7.81 | 30.46 |
| | Temperature (Q) | -18.09 | -20.63 | -15.65 |
| | Temperature (C): Timing (After): Treatment (-3ºC for 6 days) | 11.20 | 2.53 | 20.58 |
| | Temperature (Q): Timing (After) | 8.65 | 1.30 | 16.74 |

| B | Explanatory Variable | Importance |
|---|---|---|
| | Temperature: Timing (After): Treatment (-3ºC for 6 days) | 24.42 |
| | Temperature: Timing (After): Treatment (-6ºC for 6 days) | 22.26 |
| | Temperature | 19.15 |
| | Temperature: Timing (After) | 13.08 |

| C | Explanatory Variable | Effect | Lower CI | Upper CI |
|---|---|---|---|---|
| | Degree day (L): Timing (After): Treatment (-6ºC for 6 days) | 17.54 | 5.16 | 26.44 |
| | Degree day (Q) | -16.95 | -20.14 | -14.56 |
| | Degree day (C): Timing (After): Treatment (-3ºC for 6 days) | 14.20 | 6.09 | 19.88 |
| | Degree day (L): Timing (Before): Treatment (-6ºC for 6 days) | -8.00 | -14.45 | -0.20 |
| | Degree day (L) | 6.31 | 1.41 | 8.90 |

| D | Explanatory Variable | Importance |
|---|---|---|
| | Degree day | 23.26 |
| | Degree day: Timing (After): Treatment (-3ºC for 6 days) | 20.17 |

| | |
|---|---|
| Degree day: Timing (After): Treatment (-6ºC for 6 days) | 18.80 |
| Degree day: Timing (Before): Treatment (-6ºC for 6 days) | 8.00 |

Cold snaps can have a unique impact on parasite fitness depending on baseline temperature, timing in relation to exposure, the duration and amplitude. For example, when a cold snap occurred after exposure at 14°C, there was a reduction in burden in all treatments, apart from the short and weak cold snap (-3ºC for 3 days), compared to the constant mean (*emmean, -3°C for 6 days, z(inf) = -4.912 p < 0.001, -6°C for 3 days, z(inf) = -3.282 p = 0.014, and -6°C for 6 days, z(inf) = -5.378 p < 0.001,* Table B.8, Figure 3.4I). Moreover, within these treatments at 14ºC occurring after exposure, there was a difference between treatments with the same amplitude (-6ºC) but different durations. Here, a strong-amplitude short-duration cold snap (-6°C for 3 days) resulted in a greater burden than a strong and long cold snap (-6ºC for 6 days) (*emmean, z(inf) = -3.1, p = 0.023,* Table B.8, Figure 3.4I). Therefore, due to the multifaceted interactions, not all cold snaps will result in the same outcome. Parasite burden is ultimately determined by the combined influence of temperature, timing, and treatment, reinforcing the context-specific nature of thermal impacts on disease interactions.

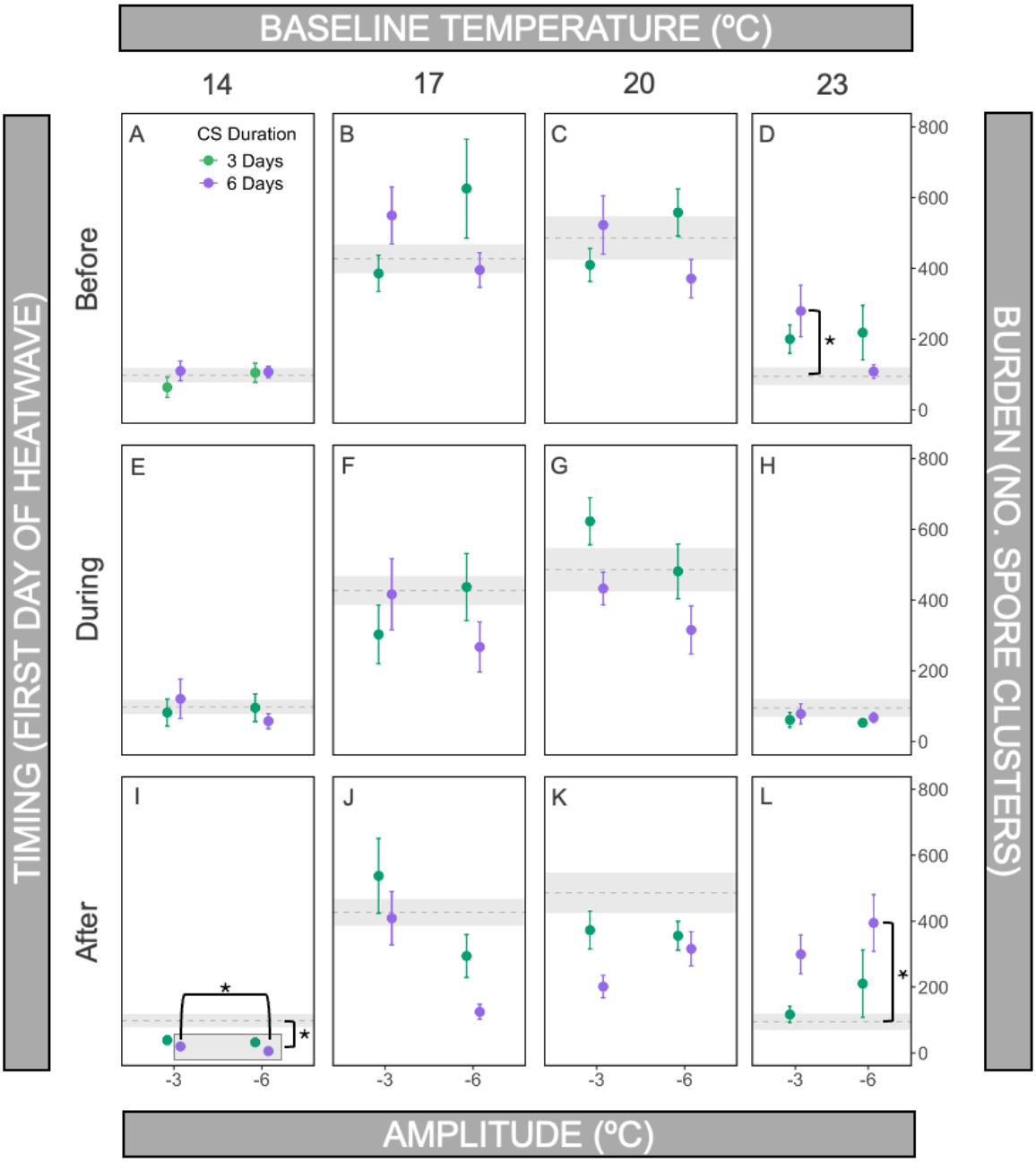


**Figure 3.4: Interaction effects on burden between amplitude and duration at different baseline temperatures and cold snap timing.**
In *Daphnia magna* infected with *Ordospora colligata*. Points are grouped by duration. Burden is the mean number of spore clusters in infected individuals. The solid 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 B.8.

## 3.5 Discussion

Cold snaps influenced infection outcomes in ways consistent with thermal performance theory: parasite fitness increased when temperature fluctuations brought conditions closer to its thermal optimum and declined when extremes pushed the system beyond physiological limits. Therefore, outcomes depended on the interaction between baseline temperature, timing, and treatment type. These interacting factors explain why parasite fitness sometimes reversed direction, enhancing parasite success under warm baselines but reducing it under cooler ones. Overall, fluctuations at thermal extremes amplified contrasts, favouring either host or parasite depending on how far conditions deviated from their respective optima.

### *3.5.1 Effect of baseline temperature*

Baseline temperature can alter the performance of *Ordospora colligata*, with peak performance occurring at intermediate temperatures (~17ºC) and reduced performance at the thermal extremes. This is consistent with non-linear thermal performance theory (Huey and Kingsolver, 1989) and Jensen's Inequality (Denny, 2017). Under Jensen's Inequality, when biological responses to temperature are non-linear, fluctuations can shift the outcomes away from those under constant conditions (Blanford *et al*., 2013). This effect is especially pronounced under thermal stress, when performance curves are often steep, meaning even small temperature deviations can lead to large impacts (Bagni *et al*., 2024). This is also seen in other systems, for example, coral-disease (Aeby *et al*., 2020), fish-parasite (Lõhmus and Björklund, 2015), and plant-fungi (Shelake *et al*., 2024) systems. In mosquito-borne systems, pathogens like *Plasmodium falciparum* show reduced transmission efficiency outside of their thermal optima (Mordecai *et al*., 2013). Given *Ordospora's* narrower thermal range (11.8-29.7ºC) relative to its *Daphnia* host (6-33.3ºC) (Kirk *et al*., 2020), decreased performance at high and low thermal extremes may also reflect predictions from the Thermal Mismatch Hypothesis (Cohen *et al*., 2017). This hypothesis posits that infection outcomes are most

strongly affected when the difference in thermal performance between host and parasite is greatest, resulting in the host or parasite being at a disproportionate advantage (Venesky *et al*., 2022). For example, in the three-spined stickleback (*Gasterosteus aculeatus*) infected with its tapeworm (*Schistocephalus solidus*), the host fitness decreased, and parasite growth increased at intermediate temperatures where the thermal performance of the parasite exceeded that of the host, while the reverse was true at thermal extremes (Franke *et al*., 2019). Moreover, infection can alter thermal limits of hosts, sometimes narrowing their thermal safety margins and shifting performance curves in a way that increases susceptibility at temperature extremes (Hector *et al*., 2021b). Thus, parasitism can amplify the effects of thermal stress (Morley and Lewis, 2014), particularly when host and parasite differ in their thermal optima or breadth (Rohr *et al*., 2018), making infection outcomes increasingly sensitive to even modest shifts in baseline temperature, a phenomenon especially relevant as climate variability continues to increase.

### *3.5.2 Effect of timing*

The impact of a cold snap can depend on its timing relative to parasite exposure. A cold snap occurring after exposure tended to amplify parasite burden at higher temperatures. In contrast, cold snaps before exposure had variable outcomes; infection rates were slightly elevated overall, but a strong and long cold snap reduced parasite development at higher degree days, whereas a weak and long cold snap led to increased burden at 23ºC. These patterns may reflect Phenological Mismatch Theory (Pardikes *et al*., 2022), where disruptions in synchrony between host and parasite development stages could hinder successful establishment or proliferation (Altizer *et al*., 2013). For example, in amphibian-trematode systems, shifts in the timing of host and parasite development due to environmental changes can create phenological mismatches that alter infection severity and host pathology (McDevitt-Galles *et al*., 2020). These shifts can modify the pace and direction of adaptation in both host resistance

and parasite virulence, potentially reshaping host-parasite dynamics under ongoing climate variability (Lafferty and Mordecai, 2016, Blanquart and Gandon, 2013). Here, an increase in burden following a long and strong cold snap (-6ºC for 6 days) that occurred after exposure at high baseline temperatures (23ºC) may reflect a lag in host physiological responses, creating a mismatch that favoured the parasite due to its faster development. In contrast, parasite development was reduced when the same cold snap (-6ºC for 6 days) occurred before exposure, possibly because the cold snap delayed parasite development or altered host physiological conditions at a critical stage, disrupting the precise timing needed for the parasite to successfully establish infection. Therefore, this mismatch favoured the host by desynchronising parasite development from host susceptibility windows. Thus, the variable outcomes associated with cold snap timing highlight the critical role of temporal context in shaping infection, showing that even brief climatic disturbances can either desynchronise or enhance host-parasite alignment, depending on when they occur.

The interaction of timing with thermal intensity and duration produced non-linear effects, with the same cold snap treatment having different impacts depending on its timing relative to parasite exposure. This pattern reinforces that timing modifies not only the magnitude but also the direction of a thermal event's effect, highlighting how similar treatments can yield opposing outcomes. That the timing of thermal variation relative to host or parasite life stages plays a critical role has also been established in other systems (Barber *et al.*, 2016, Costaz *et al.*, 2023). For example, in honey bees infected with *Nosema ceranae*, heat stress applied shortly after spore ingestion enhanced parasite establishment, whereas heat stress occurring before exposure reduced infection success (Doublet *et al.*, 2015). Once infection is established, extreme thermal variation may impair host defences (Seppälä and Jokela, 2011, Dittmar *et al.*, 2014) or prolong parasite development windows (Studer *et al.*, 2010). In contrast, thermal variation prior to parasite exposure may trigger maternal and epigenetic effects that enhance stress tolerance and resistance to parasites (Abdolmaleky *et al.*, 2025, Shocket *et al.*, 2018b).

For instance, Garbutt *et al*. (2014) demonstrated that parental exposure to stressors can induce transgenerational immune priming, enhancing offspring resilience against infection. Furthermore, the timing of environmental stressors, such as cold snaps or heatwaves, may not only influence immediate infection outcomes but could also alter co-evolutionary trajectories by shifting the selective pressures on hosts and parasites over time (Paplauskas *et al*., 2021). For example, in host-parasitoid systems, heatwaves at different developmental stages of the interaction have contrasting effects on survival and infection success of the parasitoid, reflecting differences in thermal tolerance between species and life stages (Moore *et al*., 2022). Moreover, integrating degree day models with event-based thermal stressors like cold snaps offers a nuanced understanding of the cumulative effects of thermal stress under fluctuating temperatures (Jeffrey *et al*., 2025, Mitchell *et al*., 2005). Thus, the timing of thermal stress relative to exposure to a parasite can determine whether thermal fluctuations desynchronise host-parasite interactions to the host's advantage or amplify infection by exploiting periods of host vulnerability.

### *3.5.3 Effect of treatment*

Beyond baseline temperature and timing effects, the nature of the cold snap treatment itself (i.e., cold snap attributes) also played a crucial role. Cold snap treatments often resulted in increased infection or burden, particularly when they occurred after exposure and at higher baseline temperatures. This aligns with predictions that parasites, due to their smaller size and faster acclimation (Brown *et al*., 2004), may better exploit fluctuating environments, as explained by the Thermal Variability Hypothesis (Paull *et al*., 2015). At 23ºC, the parasite would have experienced a cold snap of 17 or 20ºC (close to their thermal optima), which could have resulted in the increased parasite fitness. In the same host-parasite system, short-term fluctuations can increase infection prevalence and intensity (Krichel *et al*., 2023, McCartan *et al*., 2025). In other systems, for example, amphibians infected with the chytrid fungus

*Batrachochytrium dendrobatidis,* temperature shifts increased susceptibility to infection as the fungal pathogen acclimated more rapidly than its frog host, leading to greater pathogen growth following a temperature drop (Raffel *et al*., 2015). One possible mechanism is that rapid temperature change may suppress or delay host immune responses, creating a window of vulnerability that parasites can exploit (Huang *et al*., 2023, Tobin *et al*., 2024). However, not all treatments had uniform effects; cold snaps produced the opposite outcome under certain conditions, revealing important physiological thresholds and context dependencies.

The effects of cold snaps on parasite performance are not uniform but instead hinge on the interplay between cold snap characteristics and host-parasite thermal tolerances. Certain treatments, for example, a strong and long cold snap (-6ºC for 6 days) at low baseline temperatures, resulted in decreased parasite burden. This suggests that physiological limits may have been exceeded, limiting parasite success as explained by the Thermal Stress Hypothesis (Tobin *et al*., 2024). For example, in *Daphnia magna* infected with the bacteria *Pasteuria ramosa*, prolonged exposure at low temperatures reduced parasite fitness and development, likely due to thermal constraints on replication and transmission (Vale *et al*., 2008). Moreover, even treatments with the same duration but different amplitudes occurring at the same baseline temperature can have alternative outcomes, highlighting this context-specific nature. Similar patterns were seen before for cold snaps in this system, with the same amplitude but different durations at the same baseline temperature, resulting in differential effects (McCartan *et al*., 2024b). Comparable patterns have also been observed in other systems (Leathers *et al*., 2024, Leicht *et al*., 2013); for instance, in a parasitoid-host interaction, Zhang *et al*. (2019a) found that heatwaves of varying intensity and duration altered key life history traits of the parasitoid, affecting development and survival. Thus, the outcome of cold snaps on infection dynamics is not only dependent on the presence of thermal stress, but also on the specific combination of timing, amplitude, duration, and baseline temperature. Thus, cold snap treatments can act as amplifiers of thermal sensitivity, exerting the greatest

influence near the edges of host and parasite thermal performance curves, where even brief deviations can shift the outcome of infection

### *3.5.4 Conclusion*

In conclusion, the effect of cold snaps on parasite fitness is multifaceted in the *Daphnia-Ordospora* system. Cold snaps shaped infection outcomes in ways consistent with thermal performance theory, enhancing parasite fitness when temperatures shifted toward its optimum and reducing it when they moved beyond physiological limits. Thus, the combined effects of baseline temperature, timing, and treatment type account for the contrasting responses observed across thermal environments. Consequently, cold snaps may have far-reaching ecological impacts, particularly in systems like *Daphnia-Ordospora*, as *Daphnia* are a key species in their ecosystem (Ebert, 2022). For example, infection in *Daphnia dentifera* reduced host density, which led to a trophic cascade and losses in biodiversity (Duffy, 2007). Moreover, in an era of increasing climate variability, understanding how transient thermal stressors like cold snaps shape host-parasite dynamics is crucial (Rohr and Cohen, 2020). As these dynamics can affect biodiversity (Martinez and Merino, 2011), ecosystem functioning (Shocket *et al.*, 2018a), and the stability of ecological communities (Friesen *et al.*, 2021), with potential consequences for freshwater resource management and conservation in a changing climate. Therefore, the addition of extreme thermal variation to disease models is crucial to gain a more accurate predictive insight into how the host-parasite relationship will be altered under climate change (Costas and Yuri, 2019). Moreover, identification of mechanisms which underlie these responses to thermal variation will be essential for gaining a more in-depth understanding of how the host-parasite relationship will change in the face of climate change (Kirk *et al.*, 2020, Gehman *et al.*, 2018). Advancing our understanding of host-parasite dynamics in response to thermal variation is essential for predicting ecological responses to

climate change and developing more effective strategies for biodiversity conservation and ecosystem resilience.

# Chapter 4: Impact of heatwave amplitude, duration, and timing on parasite fitness at different baseline temperatures.

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**Author Contributions:** NMcC and PL designed the experiment. NMcC conducted the experiment with the assistance of PL, FO'K, and GZ. NMcC conducted the analysis with the aid of PL. NMcC generated figures. NMcC and PL wrote the first draft of the manuscript, and all authors contributed to revisions. All authors read and approved the final manuscript.

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## 4.1 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.

## 4.2 Introduction

Climate change is increasingly recognised as a pivotal driver of alterations in human health (McMichael *et al*., 2006), biodiversity (Nunez *et al*., 2019), and ecosystem changes (Malhi *et al*., 2020). The rapid anthropogenic change in climate can disrupt host-parasite dynamics (Brunner and Eizaguirre, 2016, Paull and Johnson, 2014, Hance *et al*., 2007), as it has been well established that the host-parasite relationship can be modified by temperature (Kirk *et al*., 2018, Kirk *et al*., 2020, Rohr and Raffel, 2010, Raffel *et al*., 2015, Rohr and Cohen, 2020). Indeed, a recent study reported that 58% of human pathogenic diseases have been aggravated by climate change (Mora *et al*., 2022). Temperature can influence host susceptibility by altering host homeostasis (Seppälä and Jokela, 2011), immunity (Dittmar *et al*., 2014), and behaviour (Labaude *et al*., 2015). For example, in marine ecosystems, elevated sea temperatures can increase the prevalence of coral diseases by impairing coral immune functions and promoting pathogen growth (Harvell *et al*., 2007). Moreover, temperature changes can directly affect the parasite by modifying its infectivity (Shocket *et al*., 2018b), proliferation (Claar and Wood, 2020), and survival in the environment (Dias and dos Santos Lima, 2012). 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 (Paull *et al*., 2012). Thus, the outcome of host-parasite relationships in response to temperature can be complex (Wolinska and King, 2009, Raffel *et al*., 2013, Claar and Wood, 2020). Yet, climate change is not only a linear increase in mean temperature (Denny, 2017), but it also modifies temperature variability, leading to altered temperature fluctuations and more frequent extreme weather (van der Wiel and Bintanja, 2021).

Climate change is expected to increase the frequency and magnitude of anomalous weather events, such as heatwaves, cold snaps, and droughts (Leahy *et al*., 2021). Indeed, the frequency, intensity, and duration of heatwaves have accelerated globally since the 1950s

(Perkins-Kirkpatrick and Lewis, 2020, Seneviratne *et al*., 2021). Like rising mean temperatures, these temperature shifts caused by extreme and fluctuating weather can influence the survival of both the host and the parasite (Claar and Wood, 2020). For example, thermal fluctuations at the lower thermal limits of malaria will increase infection rates, while decreasing infection rates at the upper thermal limits (Paaijmans *et al*., 2013). Moreover, climatic extremes are expected to result in biodiversity loss (Kohl *et al*., 2016), changes in coevolutionary trajectories (Wolinska and King, 2009), and shifts in ecosystem structure (Byers, 2020) 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 (Menge *et al*., 2016). Regardless of parasite exposure, thermal extremes are expected to influence life history traits (Ma *et al*., 2021), including development (Stahlschmidt, 2024), ageing (Burraco *et al*., 2020), and reproductive output (Sales *et al*., 2018). Furthermore, temperature variability can disrupt transmission pathways, alter host susceptibility, and shift parasite life cycles in multi-host parasite systems (Barber *et al*., 2016, Cohen *et al*., 2019). 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 (Krichel *et al*., 2023, Dallas and Drake, 2016, Duncan *et al*., 2013, Kunze *et al*., 2022, Lambrechts *et al*., 2011, McCartan *et al*., 2024b). 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 (Rollins-Smith and Le Sage, 2023, Ferguson *et al*., 2018). Extreme

temperature events such as heatwaves can also modulate immune function, either by suppressing immune defences or altering energetic trade-offs (Leicht *et al*., 2013, Catalán *et al*., 2012). 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 (Jackson *et al*., 2021). 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 (Hector *et al*., 2023). 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 (Molnár *et al*., 2017, Paull and Johnson, 2014). 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 (Kunze *et al*., 2022, Milazzo *et al*., 2016, Schreven *et al*., 2017) 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 (Krichel *et al*., 2023, Kunze *et al*., 2022).

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) (Ebert, 2022). 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 (Paull *et al*., 2015)), while shifts in temperatures may lead to increased parasite performance according to the Thermal Variability Hypothesis (Rohr and Raffel, 2010). 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 (Brown *et al*., 2004)). 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 (Krichel *et al*., 2023). In addition, as shifts in temperature are known to distinctly affect various host and parasite life history traits (Rohr and Cohen, 2020, Paull *et al*., 2012, Kunze *et al*., 2022, Paull *et al*., 2015, Cohen *et al*., 2017), 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 (Bernhardt *et al*., 2018b). 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.

## 4.3 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.

### *4.3.1 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 (Ebert, 2005). They are a popular model system for the study of host-pathogen interactions (Ebert, 2022) due to their fast generation time, their well-known ecology, and their key role in aquatic ecosystems (Sarnelle, 2005). *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 (Ebert, 2005). 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 6-33.3ºC compared to *Ordospora's* narrower range of 11.8-29.7ºC (Kirk *et al*., 2018).

### *4.3.2 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.

### *4.3.3 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 (Morit, 2022), 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) (Figure 4.1). 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 0ºC) was included for each baseline temperature with additional constant controls at 26ºC and 29ºC. 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 (Klüttgen *et al*., 1994)). 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) (Figure 4.1). 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).

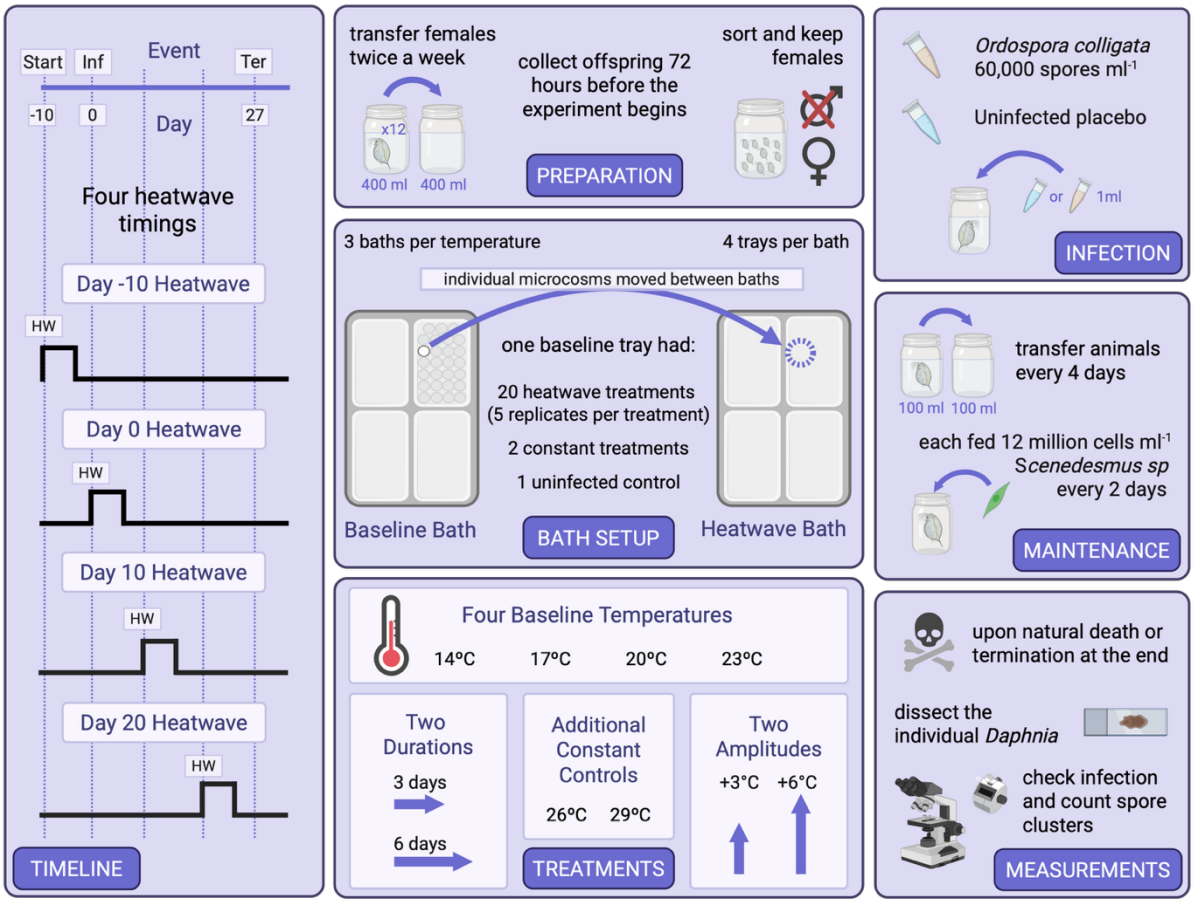


**Figure 4.1: Chapter 4 experimental setup.**
TIMELINE; illustrates the timeline for the experiment starting at day -10 and terminating at day 27, exposure occurred on day 0, and heatwaves began on day -10, 0, 10, and 20. PREPARATION; preparation started three weeks prior to exposure when 10-12 mothers 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.

### *4.3.4 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 23ºC (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 (17ºC) and two heatwave baths (23ºC). 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.

### *4.3.5 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.

### *4.3.6 Data analysis for both experiments*

Analysis was performed using R version 4.0.3 (RStudio Team, 2022). 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 (Table C.4 and Table C.5) 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 (Friedman *et al.*, 2010) 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 (Russell V. Lenth, 2022) 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 (Benjamini and Hochberg, 1995).

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 ($\chi^2$) 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 C.11). Like with experiment 1, contrast p-values were adjusted for multiple comparisons using the 'Benjamini-Hochberg' method.

## 4.4 Results

### *4.4.1 Infection prevalence*

All factors (i.e., the timing, amplitude, and duration of the heatwave, and baseline temperature) influenced infection prevalence. The timing of the heatwave (before, during, or after infection) led to variable outcomes (Figure 4.2). Infection prevalence was reduced in heatwaves beginning on the day of exposure (day 0) that had a strong amplitude of +6ºC above the baseline 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 4.1). These heatwaves only had a single infection at 23ºC (Figure 4.2B) when the heatwave co-occurred during parasite exposure. Indeed, here, the strong and short heatwave treatment (a heatwave of +6ºC for 3 days) was different from the constant treatment (*emmean, z(inf) = -3.848, p <0.008,* see Table C.3 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 (Figure 4.2A). Furthermore, only a small reduction (not significant) was observed when a heatwave occurred 10- and 20-days post-exposure for the short 3-day heatwave with a strong amplitude of +6ºC (Figure 4.2C and 4.2D). 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 +6ºC 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 4.1). For example, at 23ºC, a strong and long heatwave (heatwave of +6ºC for 6 days) occurring 10 days post-exposure was different from the constant temperature treatment (*emmean, z(inf) = -3.904, p <0.008,* Table C.3, Figure 4.2C). 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 4.1*).* Infection prevalence declined as baseline temperatures increased across all heatwave timings (Figure 4.2). 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.

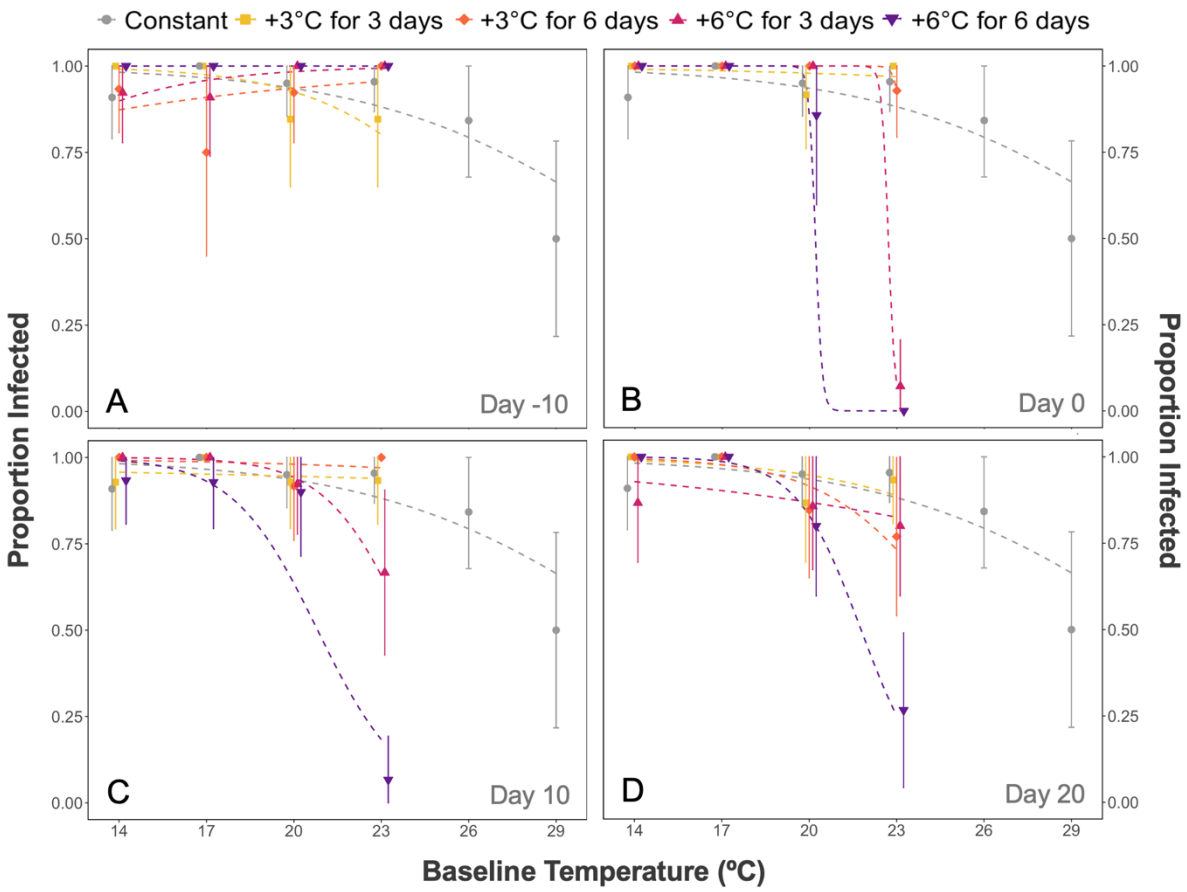


**Figure 4.2: The effect of heatwave treatments on infection prevalence.**
In *Daphnia magna* infected with *Ordospora colligata*. Each spore cluster contains 32-62 individual *Ordospora*. Error bars represent the standard error, and each dashed line is the linear fit of the elastic net regression (see Table C.1 for statistical results). Grey; constant treatment, yellow; heatwave lasting 3 days where the temperature was increased by 3ºC, orange; heatwave lasting 6 days where the temperature was increased by 3ºC, pink; heatwave lasting 3 days where the temperature was increased by 6ºC, purple; heatwave lasting 6 days where the temperature was increased by 6ºC. 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 6ºC) occurring on the day of exposure influenced infection prevalence depending on baseline temperature. Furthermore, the heatwave treatment, which increased temperature by 6ºC 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.

**Table 4.1: 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 increase 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 C.1 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 |

### *4.4.2 Burden*

For parasite proliferation, the effects of a heatwave ranged from a 13.5-fold decrease in burden to a 2.4-fold increase (Figure 4.3, Figure 4.4). 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) (Figure 4.4L), 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 14ºC resulted in a 2.4-fold increase in burden compared to the baseline constant (mean of 472 vs 197 spore clusters, respectively) (Figure 4.4E). 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 (Figure 4.3). This is supported by the high feature importance of the complex 3-way and 2-way interactions (Table 4.2), which highlight the intricacy and context-specific nature of the outcomes in burden.

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 4.2). 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 (Figure 4.3). However, in heatwave treatments occurring after exposure (day 10 and 20), proliferation generally decreased compared to the constant treatment (Figure 4.3C and 4.3D). 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 (+6ºC for 6 days) and the weak and long (+3ºC for 6 days) heatwaves at 14ºC and 20ºC led to differential outcomes (Figure 4.3D). At 14ºC, 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 C.8, Figure 4.4M). However, at 20ºC, the opposite was true, whereby the weak and long heatwave had the greater burden (*emmean, z(inf) = -3.01, p = 0.048,* see Table C.8, Figure 4.4O). Moreover, while baseline temperature can influence the proliferation of *Ordospora*, the timing of the heatwave can lead to alternative outcomes.

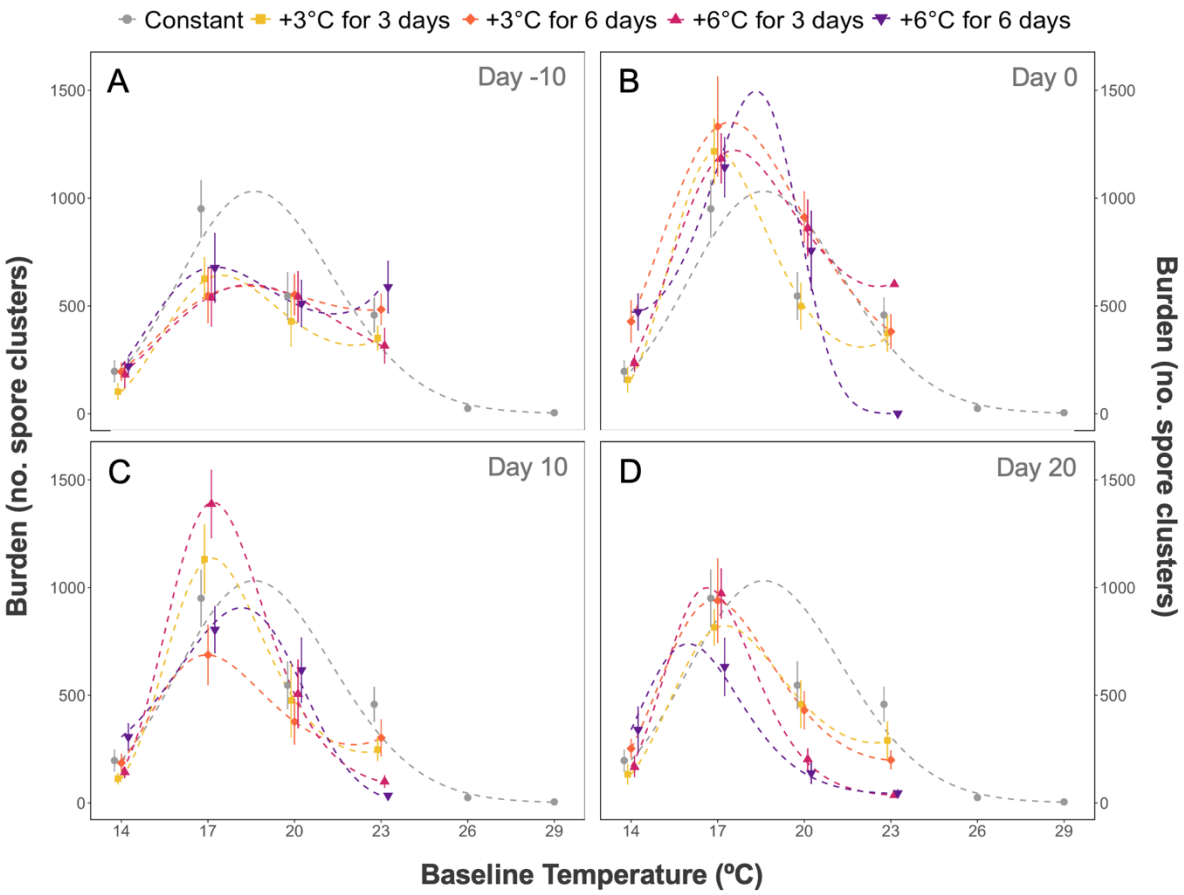


**Figure 4.3: The effect of heatwave treatments on burden.**
In *Daphnia magna* infected with *Ordospora colligata*. Burden was the mean number of spore clusters, with each spore cluster containing 32-62 individual *Ordospora*. Error bars represent the standard error, and each dashed line is the cubic fit of the elastic net regression (see Table C.6 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 3ºC, orange; heatwave lasting 6 days where the temperature was increased by 3ºC, pink; heatwave lasting 3 days where the temperature was increased by 6ºC, purple; heatwave lasting 6 days where the temperature was increased by 6ºC. 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 6ºC) 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 the baseline temperature. Specifically, a heatwave treatment, which increased temperature by 3ºC for 3 days, had distinct effects on burden depending on baseline temperature.

**Table 4.2: Coefficient estimates and feature importance of burden.**
A) Coefficient estimates and B) feature importance of infection prevalence in *Daphnia magna* infected with *Ordospora colligata*. Burden was the mean number of spore clusters, with each spore cluster containing 32-62 individual *Ordospora*. 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 increase 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 (95$^{th}$ 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 C.6 for full coefficient estimate output and Table C.9 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 |

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 (Figure 4.3A, Table C.6). 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 4.2, Figure 4.3D). Timing also interacted with baseline temperature and amplitude to influence proliferation. Specifically, at 20 days post-exposure, a strong amplitude (+6ºC) heatwave resulted in a lower burden compared to the constant treatments at and above 20ºC (*linear effect, glmnet, effect = -14.65, 95% CI: -21.91 to -0.75 and feature importance of the combined polynomial = 25.52,* Table 4.2, Figure 4.3D). For example, at 23ºC, both the strong and short (+6ºC for 3 days) and the strong and long (+6ºC 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 C.8). 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 4.2). 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 (Figure 4.4J). 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 (+3ºC 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 4.2, Figure 4.3). For example, at 14ºC, there was a 1.6-fold decrease in burden in the heatwave treatment (+3ºC 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 (+3ºC for 6 days) resulted in a 2.7 times higher spore burden than a weak and short heatwave (+3ºC for 3 days) (*emmean, z(inf) = 2.970, p = 0.048,* see Table C.8, Figure 4.4E). 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.

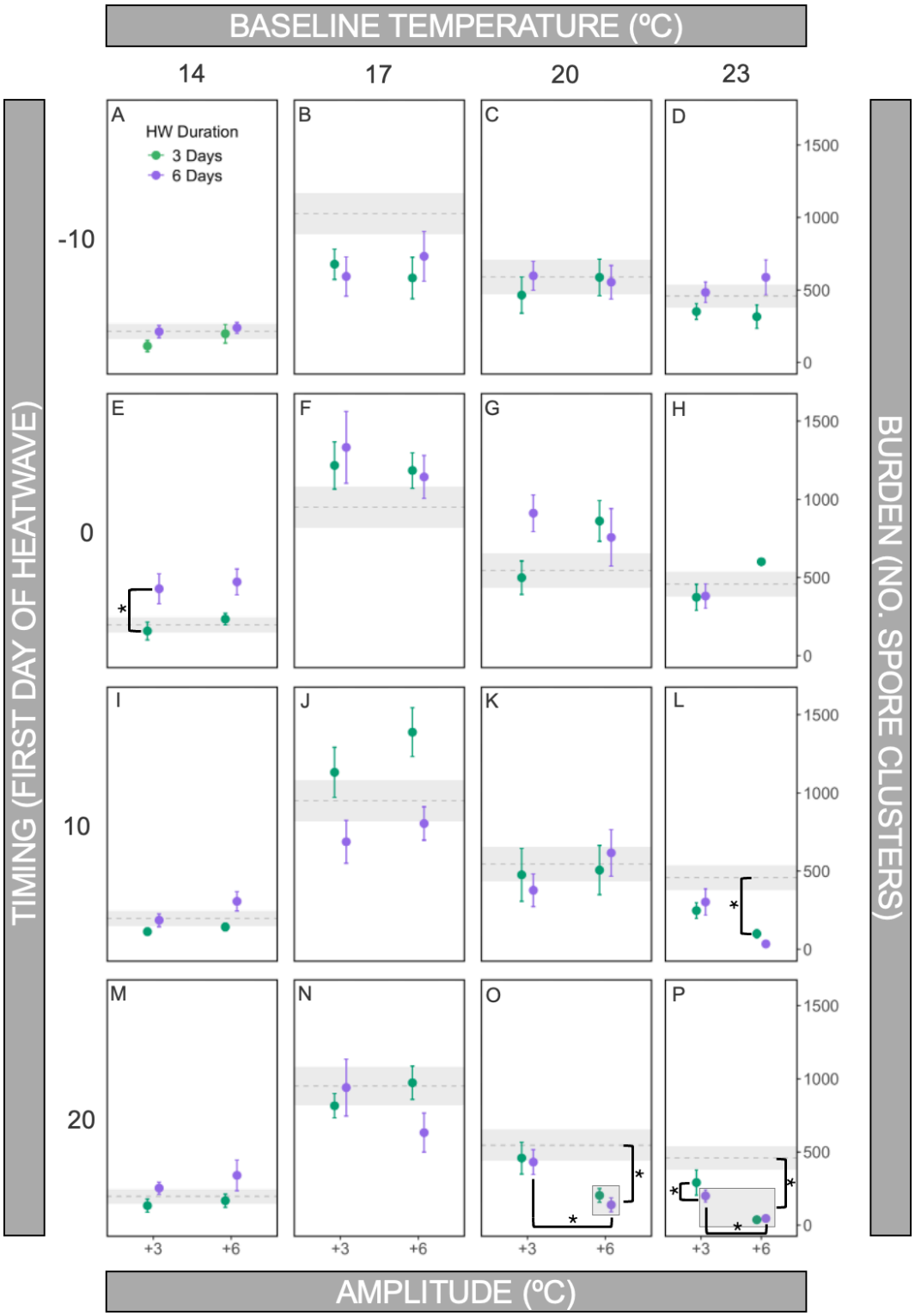


**Figure 4.4: Interaction effects on burden between amplitude and duration at different baseline temperatures and heatwave timing.**
In *Daphnia magna* infected with *Ordospora colligata*. Burden was the mean number of spore clusters, with each spore cluster containing 32-62 individual *Ordospora*. Points 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 C.8.

### *4.4.3 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 (Figure 4.5, Table 4.3). Duration also influenced burden (*GLM, 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) (Figure 4.5). Finally, timing also influenced burden (*GLM, analysis of deviance* $X^2_{(1,273)} p = 0.013$), whereby no timing resulted in the same burden (Figure 4.5). Thus, both timing and duration were consistently important in influencing the outcome of spore burden.

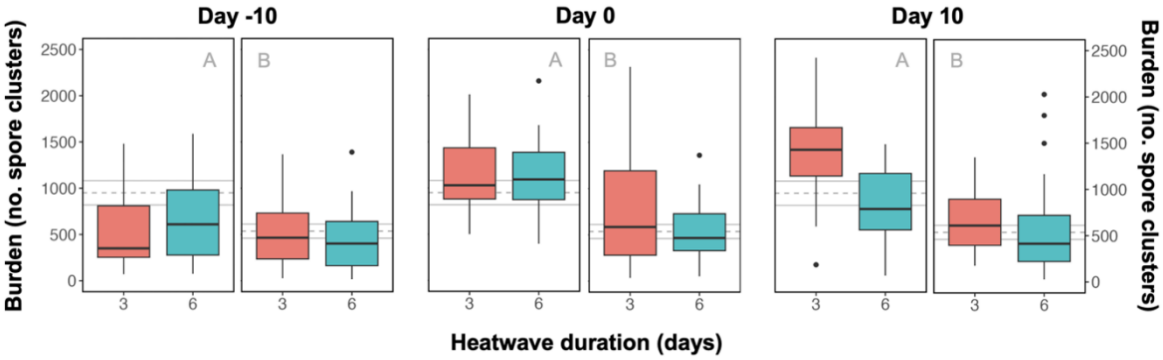


**Figure 4.5: Boxplots of burden for experiments 1 and 2 comparing heatwave durations.** In *Daphnia magna* infected with *Ordospora colligata*. Burden was the mean number of spore clusters, with each spore cluster containing 32-62 individual *Ordospora*. Red boxplots indicate a 3-day heatwave, and blue boxplots indicate a 6-day heatwave. The grey dashed line shows the mean burden for the constant baseline treatment at 17ºC, and light grey solid lines represent the upper and lower standard errors for the constant. All heatwaves had an amplitude of +6ºC. 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.

**Table 4.3: GLM Analysis of deviance $X_2$ comparing the burden in experiments 1 and 2.**
In *Daphnia magna* infected with *Ordospora colligata*. Burden was the mean number of spore clusters, with each spore cluster containing 32-62 individual *Ordospora*. 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** |

## 4.5 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.

### *4.5.1The 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 (Paull *et al.*, 2015). Since *Ordospora* has a narrower thermal tolerance (11.8-29.7ºC) compared to its host (6-33.3ºC) (Kirk *et al.*, 2018), 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 (Gehman *et al.*, 2018, Aleuy and Kutz, 2020). For example, malaria and trematodes can adjust their development to temperature variation (Paaijmans *et al.*, 2010). 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 (Studer and Poulin, 2013). 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 (Ragonese *et al.*, 2024, Blanford *et al.*, 2013, Greenspan *et al.*, 2017)). 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 (Ragonese *et al.*, 2024). 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 (Cohen *et al.*, 2017) 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 (Rohr and Cohen, 2020). 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 (Gehman *et al*., 2018, Nowakowski *et al*., 2016). For instance, the parasitoid *Cotesia congregata* will suffer more under increasing temperatures than its lepidopteran larval host, *Manduca sexta,* due to a difference in thermal tolerances (Moore *et al*., 2022). Over evolutionary timescales, such thermal mismatches may alter host-parasite co-evolutionary trajectories (Hector *et al*., 2023). Hosts under recurrent heat stress may evolve enhanced thermal resilience, potentially reducing parasite transmission over time (Blanford *et al*., 2003). 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 (Cable *et al*., 2017). 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 (Ruel and Ayres, 1999). 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 (Duncan *et al*., 2013, Greenspan *et al*., 2017, Beck-Johnson *et al*., 2017), 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 (Bagni *et al*., 2024). 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.

### *4.5.2 The 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 (Zhang *et al*., 2019b). 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* (Shocket *et al*., 2018b). Other systems have shown that thermal extreme events can alter host heat tolerance (Hector *et al*., 2023), parasite survival (Marcus *et al*., 2023) and affect host susceptibility (Tobin *et al*., 2024, Porras *et al*., 2023). 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 (Paaijmans *et al*., 2010). 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 (Paull *et al*., 2015) or thermal mismatch (Cohen *et al*., 2017) 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 (Tobin *et al*., 2024). 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 (Catalán *et al*., 2012, Leicht *et al*., 2013).

Heat stress can temporally shape immune function in invertebrates, with effects varying depending on the severity and duration of exposure (Murdock *et al*., 2012b). In fish-parasite interactions, increased temperatures can either enhance or suppress immune responses, depending on the severity and duration of the thermal stress (Macnab and Barber, 2012). 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*) (Dittmar *et al*., 2014). Moreover, heat-induced immune priming can also enhance resistance (Kurtz and Franz, 2003, Browne *et al*., 2014). For example, in the mealworm beetle (*Tenebrio beetle*), brief exposure to elevated temperatures enhances resistance to bacterial infections through upregulated immune defences (Herren *et al*., 2023). 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$^{o}$C) 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 (Xiao *et al*., 2024). 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.

### *4.5.3 The 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 to 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 (Krichel *et al*., 2023). Furthermore, an increase in disease following thermal fluctuations has been observed in several study systems (e.g., human-parasite (Paaijmans *et al*., 2010), animal-parasite (Raffel *et al*., 2013), and plant-parasite (Velásquez *et al*., 2018)). For instance, short-term temperature fluctuations are believed to increase dengue virus infections in *Aedes aegypti* compared to large temperature fluctuations (Lambrechts *et al*., 2011). Here, the increase in burden following temperature fluctuations could be explained by the Thermal Variability Hypothesis (Raffel *et al*., 2015). 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 (Brown *et al*., 2004)). Overall, both amplitude and duration are important in many systems for predicting the fitness of a parasite (Vajedsamiei *et al*., 2021, Bruno *et al*., 2007, Cook *et al*., 1998). For instance, the amplitude of the heatwave was important in the host-feeding parasitoid *Eretmocerus hayati* (Zhang *et al*., 2019a), while heatwave duration affected the life history and immunity of *Lymnaea stagnalis* snails when exposed to trematodes, as prolonged duration heatwaves decreased host performance (Leicht, 2014).

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 (Greenspan *et al*., 2017). Thus, both amplitude and duration are important to consider when predicting the fitness of *Ordospora,* particularly under the influence of climate change.

### *4.5.4 Conclusion*

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 (McCartan *et al*., 2024b). 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 (Ebert, 2022), 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 (Duffy, 2007). 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 (Lian *et al*., 2023). 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 (Seneviratne *et al*., 2021). A greater insight into how temperature variation affects host-parasite dynamics will aid in predicting ecological impacts, as parasites can affect host population dynamics (Tompkins *et al*., 2002), biodiversity (Duffy, 2007), and ecosystem functioning (Frainer *et al*., 2018). Since heatwaves can either suppress or enhance parasite fitness depending on their characteristics (Altizer *et al*., 2013, Costas and Yuri, 2019), 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 (Harvell *et al*., 2002), 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.

# Chapter 5: Heatwaves and cold snaps alter host-parasite population dynamics in the *Daphnia magna-Ordospora colligata* system

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**Author Contributions:** NMcC and PL designed the experiment. NMcC and LB conducted the experiment with the assistance of JS, FO'K, and SDiC. NMcC conducted the analysis with the aid of PL. NMcC generated figures. NMcC and PL wrote the first draft of the manuscript, and all authors contributed to revisions. All authors read and approved the final manuscript.

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## 5.1 Abstract

Climate change is driving more frequent and severe temperature extremes, including heatwaves and cold snaps, with growing implications for ecology and disease. Yet, our understanding of how heatwaves and cold snaps influence disease dynamics remains underexplored. Using the host *Daphnia magna* infected with its microsporidian microparasite *Ordospora colligata,* pathogen fitness and host population size were measured in experimental populations using a factorial design at four baseline temperatures (14, 17, 20 and 23ºC). A heatwave or cold snap treatment with an amplitude of ±6ºC was administered four weeks after measurements began and lasted for ten days. The effect of heatwaves is dependent on baseline temperature but can induce long-lasting increases in burden (>4 weeks). The impact of cold snaps are also temperature-dependent, leading to short-term increases in parasite fitness at higher temperatures. Host population size also varies in response to temperature and treatment. Importantly, burden and host density are interdependent, jointly shaping infection patterns. At lower temperatures, parasite burden and host population size are positively correlated, whereas at higher temperatures, increased host population size corresponds with reduced burden. These patterns were consistent at both individual and population levels, underscoring how individual physiological responses can scale up to impact disease dynamics across populations. Thus, extreme temperature variation can have complex, context-specific outcomes on disease dynamics. As climate extremes become more frequent, understanding these nuanced responses is critical for predicting and managing disease risk in natural populations.

## 5.2 Introduction

Climate change is transforming ecological and epidemiological systems by altering the environment and influencing interactions among organisms (Malhi *et al*., 2020) with far-reaching consequences for human health (Rocque *et al*., 2021), ecosystem functioning (Parmesan, 2006), and the global economy (Malhi *et al*., 2021). Changing global temperatures have shifted the geographic ranges of both free-living organisms (Dube *et al*., 2023) and pathogens (Baker *et al*., 2022), affected the frequency and severity of disease outbreaks (Anikeeva *et al*., 2024), influenced host immunity (Catalán *et al*., 2012) and resulted in higher disease prevalence in keystone species, leading to declines in biodiversity (Habibullah *et al*., 2022). For example, a recent study projected that rising global temperatures will expand the geographical and seasonal range of mosquito-borne diseases like malaria and dengue, potentially placing an additional 4.7 billion people at risk of infection by 2070 (Colón-González *et al*., 2021). Indeed, host-parasite relationships may be particularly vulnerable to temperature changes (Gehman *et al*., 2018) as temperature can influence host-pathogen interactions in numerous complex ways. For example, the effect of changing mean temperature can be direct by altering parasite development (Thomas *et al*., 2018), transmission (Shocket *et al*., 2019), and survival in the environment (Goedknegt *et al*., 2015). Temperature can also act indirectly by influencing host behaviour (Batres and Perrett, 2020), immunity (Catalán *et al*., 2012), or modification of physical barriers in the host (Fedorka *et al*., 2013). Moreover, temperature-driven shifts in host mortality (Sauer *et al*., 2020), fecundity (Hector *et al*., 2021a), and feeding strategies (Shocket *et al*., 2018a) can reshape host and parasite population trajectories over time, potentially altering outbreak dynamics (Auld and Brand, 2017), population stability (Bruijning *et al*., 2022), and long-term persistence (Gehman *et al*., 2018). Not only do rising global temperatures affect many aspects of host-pathogen interactions simultaneously, but they can also have opposing effects on different traits. For example, McCartan *et al.* (2024) found that after a cold snap, infectivity decreased with increasing temperature while within-

host growth of the pathogen accelerated. While the relationship between rising global mean temperature and disease is complex, extreme weather events and altered temperature variation associated with global warming may further complicate disease outcomes.

Climate change not only raises mean global temperatures but also increases the frequency, intensity, and unpredictability of extreme temperature events such as heatwaves and cold snaps (Leahy *et al*., 2021). These anomalous weather events are becoming more common and prolonged, with heatwaves accelerating in frequency and duration since the 1950s (Perkins-Kirkpatrick and Gibson, 2017) and cold snap frequency accelerating since 1979 (Kug *et al*., 2015). Such extreme temperature events can have pronounced effects on host-pathogen systems, as both heatwaves and cold snaps may challenge the physiological limits of hosts and parasites (Kunze *et al*., 2022, Roitberg and Mangel, 2016, Novak, 1978). For example, simulated heatwaves facilitated the invasion of a lowland *Drosophila* species into high-elevation habitats, leading to cascading effects on resident parasitoid species and their host interactions (Chen and Lewis, 2023). Similarly, cold snaps can increase disease burden up to five-fold or decrease it by three-fold, depending on the baseline temperature (McCartan *et al*., 2024b). Moreover, these temperature anomalies can destabilise population dynamics (Ragonese *et al*., 2024), alter epidemic cycles (Cheng *et al*., 2020), or, in some cases, cause population crashes or regime shifts (Devkota, 2021). Yet, despite growing evidence of their ecological significance, the role of anomalous temperature events in shaping disease dynamics at the population level remains underexplored (Landis *et al*., 2012, Ragonese *et al*., 2024), especially in systems where temperature variability interacts non-linearly with parasite transmission and host resilience.

Here, we examined the effect of heatwaves and cold snaps on disease dynamics in experimental populations of *Daphnia magna* infected with its microsporidium micro-parasite *Ordospora colligata* (a unicellular gut pathogen) across a broad temperature range. This study

was based on previous work using the same host-pathogen system (Kunze *et al*., 2022, McCartan *et al*., 2024b, McCartan *et al*., 2025), which studied the effects of extreme weather events at an individual level. These studies found that even minor shifts in temperature lead to increased parasite performance, as suggested by the Thermal Variability Hypothesis (Rohr and Raffel, 2010). Declines in performance under extreme thermal stress were also observed when temperatures exceeded their physiological limits, a pattern described by the Thermal Stress Hypothesis (Paull *et al*., 2015). Moreover, these studies also showed differential thermal sensitivity of *Daphnia* and *Ordospora*, such that, mismatches in performance optima favoured either the host or the parasite depending on environmental conditions, which conforms to the Thermal Mismatch Hypothesis (Cohen *et al*., 2017). These results indicate that temperature can profoundly and unpredictably alter infection outcomes at the individual level. These physiological and interaction-level responses can scale up to influence epidemic trajectories (Auld and Brand, 2017), drive changes in host density (Ebert *et al*., 2000a), and shift host-parasite distribution (Gsell *et al*., 2023). Yet, whether the previous observations at the individual level in the *Daphnia-Ordospora* model system scale up to the population level remains an open question.

Here, we tested whether the effects observed at the individual level could inform population-level outcomes using populations of *Daphnia magna* infected with *Ordospora colligata* and measured disease proliferation and population size. We compared exposed populations maintained at constant baseline temperatures to those which experienced either a heatwave or a cold snap. Both heatwave and cold snap treatments lasted the same duration and started at the same time. However, the heatwave increased the baseline temperature by +6ºC, and the cold snap decreased the temperature by -6ºC. We expected that extreme temperature variation would generate complex, context-dependent outcomes in both parasite burden and population size, depending on the baseline temperature, with potential consequences for long-term population stability and disease dynamics under climate change.

## 5.3 Methods

### *5.3.1 Study system*

*Daphnia magna* is a freshwater crustacean found across the northern hemisphere and is a key species in many aquatic ecosystems (Sarnelle, 2005). This is a popular model system for environmentally transmitted diseases (characterised by horizontal transmission mechanisms and infection dynamics governed by mass action principles) (Kirk *et al*., 2019); for example, due to their fast generation times, well-known ecology (Ebert, 2022), and parthenogenic life cycle (Ebert, 2005). *Ordospora colligata* is a microsporidian microparasite infecting many genotypes of *D. magna* and is horizontally transmitted to the host via filter feeding (Pombert *et al*., 2015). The parasite invades the upper gut epithelium, forming intracellular clusters of 32-64 spores, which burst and shed into the water via faeces (Ebert, 2005). Moreover, *Daphnia* have a wider thermal range of 6-33.3ºC compared to *Ordospora's* narrower range of 11.8-29.7ºC (Kirk *et al*., 2018), which may cause temperature shifts that disproportionally affect one of the antagonists. Here we used *D. magna* genotype Fi-OER-3-3 and *O. colligata* isolate 3, originally sampled from Tvärminne, Finland, and both have been maintained in the laboratory cultures for at least 10 years.

### *5.3.2 Experimental design*

To determine whether extreme weather can alter disease dynamics in the *Daphnia-Ordospora* system, we used a factorial experimental design, testing two temperature regimes (constant and pulse) at four baseline temperatures (14, 17, 20, and 23ºC). All pulse treatments occurred eight weeks after the experiment began (that is, four weeks after measurements started) (Figure 5.1). Temperature pulses either consisted of a heatwave or a cold snap, depending on the baseline temperature, with lower temperatures experiencing a heatwave (14 and 17ºC) and higher temperatures a cold snap treatment (20 and 23ºC). A heatwave treatment increased the

temperature by +6ºC for 10 days, while a cold snap decreased by -6ºC for 10 days relative to the baseline temperature. Once the pulse concluded, the treatment was returned to its original baseline temperature. In total, there were eight treatments (two temperature regimes • four baseline temperatures), each with five replicates, for a total of 40 populations (Figure 5.1). Every replicate started with a population of 300 *Daphnia,* kept in a 5 L mesocosm (filled with 2 L of Artificial *Daphnia* Medium (ADaM (Klüttgen *et al*., 1994)), in which infection was well established. Temperature was manipulated by placing each of the mesocosms into temperature-controlled water baths. There were three baths per baseline temperature, and treatments were spread between baths. Of these, two baths per baseline temperature contained two constant and two pulse treatments, and the remaining bath per baseline temperature contained one constant and one pulse treatment.

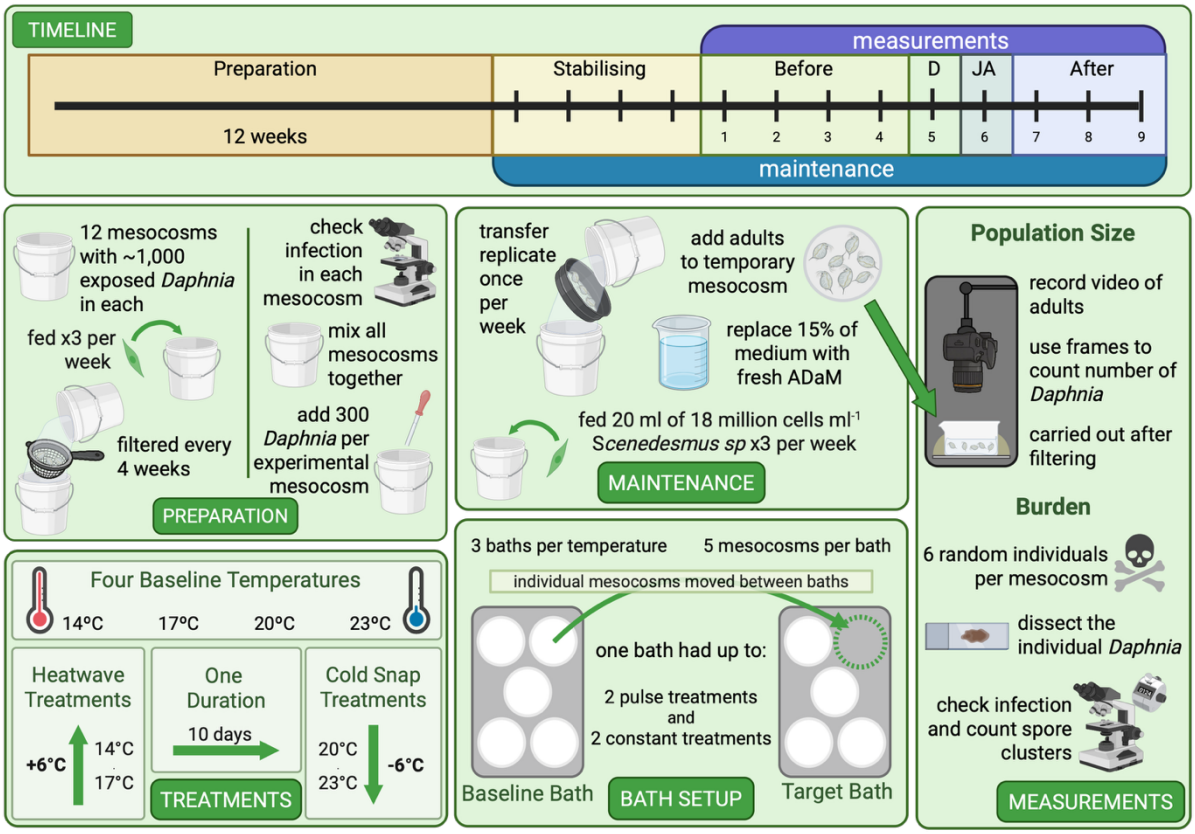


**Figure 5.1: Chapter 5 experimental setup.**
TIMELINE; illustrates the timeline with twelve weeks of preparation, four weeks of stabilising, and nine weeks of measurements. The pulse treatment began on week four, and measurements during the heatwave (D) occurred in week five. All pulse treatments ended after 10 days, so the counts occurring in week six were just after the pulse treatment (JA). PREPARATION; preparation started twelve weeks prior to exposure when ~1,000 exposed

*Daphnia* were added to 2 L mesocosms. The week before the experiment began, all mesocosms were checked for infections and then mixed. From this mix, 300 *Daphnia* of varying sizes were added to an experimental mesocosm. Replicate one began on day one, and then the next replicate began the day after to stagger maintenance and measurements. TREATMENTS; eight treatments were included in the experiment (four baseline temperatures • two pulse treatments [either heatwave or cold snap]), and all baseline temperatures also had constant controls. Each treatment had five replicates. BATH SETUP; each temperature had three baths, and each bath held five mesocosms. A bath contained up to two pulse treatments and two constant treatments. To simulate a pulse treatment, mesocosms were moved between baths and then returned to the baseline bath when the pulse treatment was finished. MAINTENANCE; maintenance was carried out once a week when the *Daphnia* were filtered to remove debris, and 15% of the medium was changed for fresh medium. MEASUREMENTS; adult *Daphnia* were separated from the rest of the population by sieving into a temporary mesocosm, and two videos were taken for population size analysis. On the same day, six *Daphnia* from the mesocosm were dissected to check for infection. Figure created on Biorender.com.

### *5.3.3 Temperature-controlled water baths*

In total, there were twelve temperature-controlled water baths (three baths per baseline temperature). Each bath was equipped with an aquarium chiller (Hailea HC-150A, DC300, or DC750), a heater (EHEIM JÄGER 300W), pumps (Micro-Jet Oxy and Oase Optimax 500) and a programmable controller (Inkbird ITC-308 or ITC-310T) which regulated the temperature. Temperatures in each bath were continuously monitored in an extra mesocosm filled with only ADaM using a HOBO logger (HOBO UA-001-08) and checked daily. While pumps ensure a uniform temperature distribution, mesocosms were also rotated clockwise daily to negate any temperature variation within the baths. To simulate temperature pulses (i.e., heatwaves or cold snaps), population mesocosms were moved between designated baths, while the baths were maintained at a constant baseline temperature for the duration of the experiment. After moving mesocosms, they reached the target temperatures within an hour. After ten days, the pulse period ended, and the mesocosms were returned to their original positions.

### *5.3.4 Daphnia preparation*

To initiate the experiment, twelve large populations of *Daphnia* (~12,000 animals) exposed to *Ordospora* were set up twelve weeks prior to the beginning of the experiment using the following method. These populations were generated from existing stock populations that

were kept in the laboratory for over five years. The existing stock populations were filtered using a coarse (1 mm) mesh filter, and animals were added to a fresh 5 L mesocosm with ~2 L of fresh Artificial *Daphnia* Medium (ADaM) (Klüttgen *et al.*, 1994) (Figure 5.1). To set up twelve mesocosms, small amounts from each stock population were added to the empty mesocosms until all housed equal volumes of ADaM and animals. Each of the mesocosms was fed *ad libitum* with batch culture algae (*Scenedesmus* sp.) (Kilham *et al.*, 1998). These populations were maintained by separating accumulated debris from the adult animals every four weeks using a coarse (1 mm) mesh filter. Animals caught by the filter were transferred to a clean mesocosm with 500 mL of the original ADaM (to retain juveniles and *Ordospora* spores), and 1500 mL of fresh ADaM was added to replenish the medium.

After the final maintenance, right before the beginning of the experiment, five animals in each mesocosm were checked for infection, and the presence of the pathogen was verified for all twelve mesocosms. Then, all animals from the twelve mesocosms were pooled and mixed to ensure uniform exposure across populations before redistribution. To set up each of the 40 experimental populations, 300 *Daphnia* of different developmental stages (e.g. juveniles and adults) were added to a 5 L mesocosm with 2 L of ADaM and fed 20 mL of 18 million algae/mL. The replicates were staggered, so all replicate-one populations began on day 1 (i.e., eight populations), then all replicate-two populations began on day 2, and so on. Populations were maintained for four weeks after initialisation to stabilise before measurements of burden and population size were taken.

### *5.3.5 Population maintenance*

Populations were maintained every seven days. Before filtering a population, any large debris was removed from the mesocosm manually by using a pipette. The population was sifted twice through a 1320 µm mesh to catch all adults and larger debris; the remaining medium was then sifted through a fine mesh (200 µm) to catch the juveniles. Fifteen per cent of the original

medium was replaced with fresh ADaM (i.e., 300 mL of fresh ADaM, 1700 mL of original ADaM) (Figure 5.1). Around half of this medium was then used to gently wash the adult *Daphnia* away from the mesh into a temporary mesocosm while keeping the remaining debris caught in the mesh. The other half of the medium was used to wash the fine mesh filter into a separate mesocosm. This enabled independent recording of the adult population size. When measurements were completed, the adult *Daphnia* were added back in with the remaining population. Feeding occurred three times a week when mesocosms received 20 mL of 18 million algae/mL.

### *5.3.6 Measurement of population size and parasite fitness*

Measurements of population size and parasite fitness were conducted weekly after populations were stabilised. Immediately after filtration over the 1320 µm mesh during maintenance, the population sizes of the adults were recorded to assess the number of large females in a population. A Canon 2000D camera was attached to a tripod and was hung with the lens 15 cm above a 2 L semi-transparent container. A flat light box was placed below the semi-transparent container to illuminate the individuals from below to increase contrast. The adult *Daphnia* in the temporary mesocosm were added to this semi-transparent container, and the camera zoom was altered so it filled the frame of the camera (Figure 5.1). Two videos were recorded for each set of adult *Daphnia*, all lasting four seconds. Once the videos were taken, both the adults and the remaining *Daphnia* were added to a single mesocosm and placed back into the water bath. All videos were analysed for population counts using the R package 'trackdem' (Bruijning *et al*., 2018). This package works by subtracting moving particles from the background, which can then be counted when appropriate thresholds are set. The package 'trackdem' uses images captured from a video, which were extracted using the application 'ffmpeg' in the computer terminal. Two frames per second were captured from the four-second videos. The channel used was blue, and the average threshold used was -0.12. Any remaining

*Daphnia* that the software did not detect were manually counted to ensure the most accurate population count was recorded.

On the same day population size was quantified, six adult *Daphnia* of varying sizes per mesocosm were sampled to measure the infection prevalence (i.e., presence or absence of spores) and burden (i.e., the number of spore clusters in the host) of *O. colligata*. If infected, spore clusters (each cluster holding up to 64 individual spores) were counted using bright field or phase-contrast microscopy (with 400x magnification). Any males (n = 1) or inconclusive infections (n = 6) were omitted from the analysis. After nine timepoints (weeks) of data collection, the experiment concluded.

### *5.3.7 Data analysis*

Analysis was performed using R version 4.4.1 (R Core Team, 2024). The response variables were burden (i.e., the number of *Ordospora* spore clusters, including zeros for uninfected exposed individuals) and population size (i.e., the mean count of adults from population videos). For both models, 'period' was a factor which included the different time points in the experiment: constant treatment, before pulse (weeks 1-4), during pulse (week 5), just after pulse (week 6), and after pulse (weeks 7-9). This structure allowed for an extended Before-After-Control-Impact (BACI) design to compare temporal responses between pulse and constant treatments (Underwood, 1994). 'Mesocosm' was included as a mixed effect variable to account for batch-specific variability; the remaining variables were fixed effects. Therefore, when analysing burden, the predictor variables were 'baseline temperature', 'period', 'population size', and 'mesocosm'. When analysing population size, the predictor variables were 'baseline temperature', 'period', 'burden', and 'mesocosm'. For the burden model, 'baseline temperature' had a quadratic polynomial to accommodate the non-linear temperature response, while in the population size model, it had a cubic relationship. To avoid issues with convergence and collinearity in both models, 'baseline temperature' was centred.

Moreover, in the burden model, 'population size' was centred, and in the population size model, 'burden' was scaled. A Generalised Linear Mixed Model (GLMM) with a negative binomial link function was fitted using the 'glmmTMB' package (Brooks *et al.*, 2017) to account for overdispersion in count data and batch-specific effects. An analysis of deviance (type III Wald chi-square test) was then used to investigate the relationship between variables and assess the overall significance of the predictor variables. Custom contrasts were created with the "emmeans" package (Russell V. Lenth, 2022) to compare treatments in both burden and population size. For this, 'baseline temperature' was treated as a factor and modelled with 'period' as an interaction. The 'constant' period was split up according to the pulse treatments for discrete comparisons, (i.e., before constant (weeks 1-4), during constant (week 5), just after constant (week 6), and after constant (week 7-9)), and treatments were compared accordingly. The contrast p-values were adjusted using the 'Benjamini-Hochberg' method for multiple comparisons (Benjamini and Hochberg, 1995).

The population-level results were then compared to individual-level findings from previous papers by McCartan *et al.* (2024) and McCartan *et al.* (2025). Data which was comparable to the population-level results was extrapolated from both data sets. Specifically, for heatwave data, the data taken was from the baseline temperatures 14 and 17ºC, where constant temperature data and heatwave treatments occurred on day 0 (day of exposure), and lasted for 6 days with an amplitude of +6ºC. For cold snaps, the data taken was from the baseline temperatures of 20 and 23ºC. Here, constant temperature data was also used, and the cold snaps that lasted for 6 days with an amplitude of -6ºC. A custom contrast was then created, where pulse and constant treatments were compared to each other within the same experiment. The contrast p-values were adjusted using the 'Benjamini-Hochberg' method for multiple comparisons.

## 5.4 Results

### *5.4.1 Parasite fitness*

The prevalence of infection was nearly 100% in all *Daphnia* populations, irrespective of the treatment or temperature regime (p-value not significant), although burden differed between treatments. Extreme temperature variation (that is, a pulse treatment) altered parasite proliferation up to 2.9-fold depending on the baseline temperature and timing in relation to the event (Figure 5.2). Specifically, the mean burden of the heatwave treatment was higher during a heatwave and remained higher for one week after, at a baseline temperature of 14ºC (Figure 5.2A) *(518 vs 256 and 574 vs 198 spore clusters, respectively, emmean, z(inf) = -2.625, p = 0.041, and emmean, z(inf) = -3.989, p < 0.001*, see Table D.5 for p-values of all custom contrasts). Moreover, although no discrete 'after' treatments were significant (i.e., no significant custom contrasts), thermal pulses resulted in long-term effects by shifting the trajectory of burden over time (*GLMM, Estimate = 0.173, p = 0.035,* Table 5.1A); specifically at 14ºC, long-term effects were present after a heatwave occurred (*14ºC GLMM, Estimate = 0.567, p = 0.007,* Table D.3A). Therefore, temperature modulated the persistence of pulse treatment effects, with diminished increases in burden at higher temperatures (*GLMM, Estimate = –8.22, p = 0.042,* Table 5.1A). That extreme temperature can influence the outcome of parasite fitness is also relevant to cold snaps, as the burden increased just after the cold snap occurred (week 6) at a baseline temperature of 23ºC (409 vs 207 spore clusters) (Figure 5.2D) (*emmean, z(inf) = -2.562, p = 0.042,* see Table D.5). However, at baseline temperatures of 17 and 20ºC, extreme temperature events did not alter the disease burden in the populations during any period (*no contrasts were significant;* see Table D.5 for a full list of contrasts). Thus, not all extreme temperature events will lead to the same changes in disease outcomes, and the outcome is dependent on the baseline temperature and time in relation to the pulse event (*GLMM, Type III Wald* $\chi^2_{(8)} = 40.63, p < 0.001,$ Table 5.1B, Figure 5.2). Together, these results

demonstrate that the effects of thermal extremes on parasite burden are highly context-dependent and temporally dynamic, varying with both baseline temperature and the timing of the event, factors which may also interact with other ecological drivers such as host population dynamics.

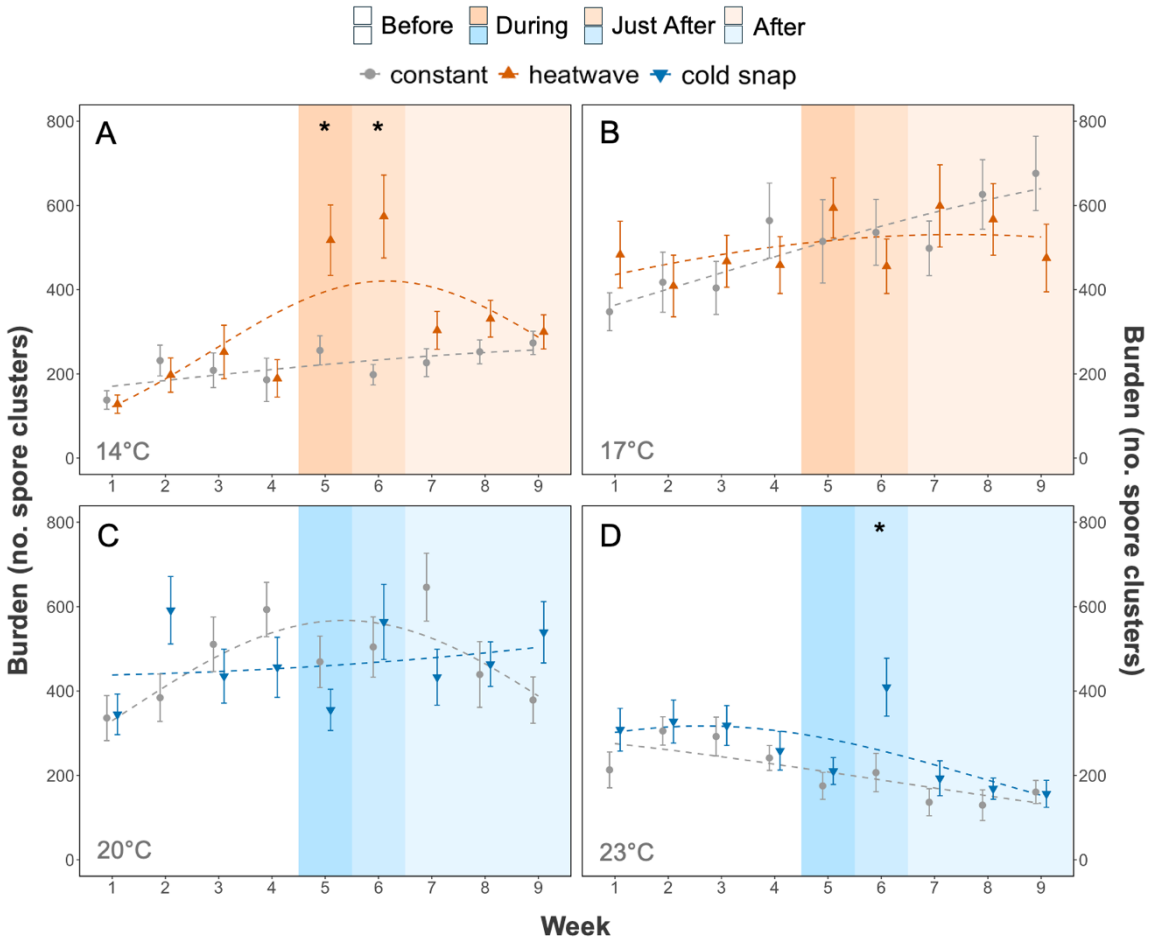


**Figure 5.2: The temporal effects of pulse temperature treatments on burden.**
In *Daphnia magna* infected with *Ordospora colligata*. Burden (mean number of spore clusters, including zeros for uninfected but exposed hosts) is shown over the nine recorded weeks at four baseline temperatures: A; 14ºC, B; 17ºC, C; 20ºC, and D; 23ºC. Each spore cluster contains 32-62 individual *Ordospora*. Error bars represent the standard error, and each dashed line is the quadratic fit of the generalised linear mixed model (see Table 5.1A for statistical results). Grey; constant treatment, orange; heatwave pulse treatment, blue; cold snap pulse treatment. Shaded regions mark experimental periods: Before (white), During (orange/blue), Just After (lighter orange/blue), and After (lightest orange/blue). Asterisks (*) indicate significant differences in burden between pulse and constant treatments in specific time periods (emmeans contrasts, Table D.5).

**Table 5.1: Type III Wald chi-square test results for the generalised linear mixed model (GLMM) analysing burden.**

In *Daphnia magna* infected with *Ordospora colligata*. Burden is the mean number of spore clusters, including zeros for uninfected but exposed hosts. A; GLMM, B; Type III Wald $\chi^2$ test. The GLMM included fixed effects of temperature (modelled as a quadratic polynomial and separated by each degree, linear [L] and quadratic [Q]), time period (relative to a heatwave or cold snap), and population size, with a random effect of mesocosm. The Type III Wald $\chi^2$ tests the significance of each predictor in the context of the full model. Significant values are bolded.

| A | Explanatory Variable | Estimate | SE | z value | p value |
|---|---|---|---|---|---|
| | **Intercept** | 5.880 | 0.083 | 71.200 | <0.001 |
| | Temperature (L) | 0.472 | 2.223 | 0.210 | 0.832 |
| | **Temperature (Q)** | **-18.790** | **2.356** | **-7.970** | **<0.001** |
| | Period before | -0.080 | 0.077 | -1.040 | 0.300 |
| | **Period during** | **0.431** | **0.128** | **3.370** | **<0.001** |
| | **Period just after** | **0.660** | **0.119** | **5.530** | **0.000** |
| | **Period after** | **0.173** | **0.082** | **2.100** | **0.035** |
| | **Population size** | **0.001** | **0.000** | **4.220** | **<0.001** |
| | **Temperature (L): Period before** | **9.637** | **3.846** | **2.510** | **0.012** |
| | Temperature (Q): Period before | 5.250 | 3.658 | 1.440 | 0.151 |
| | **Temperature (L): Period during** | **-17.830** | **6.078** | **-2.930** | **0.003** |
| | **Temperature (Q): Period during** | **13.800** | **5.958** | **2.320** | **0.021** |
| | Temperature (L): Period just after | -3.625 | 5.455 | -0.660 | 0.506 |
| | **Temperature (Q): Period just after** | **19.460** | **5.508** | **3.530** | **<0.001** |
| | **Temperature (L): Period after** | **-8.220** | **4.049** | **-2.030** | **0.042** |
| | Temperature (Q): Period after | 4.189 | 3.778 | 1.110 | 0.268 |
| | **Temperature (L): Population size** | **-0.032** | **0.014** | **-2.300** | **0.021** |
| | **Temperature (Q): Population size** | **-0.043** | **0.012** | **-3.480** | **0.001** |

| B | Explanatory Variable | Chisq | Df | P value |
|---|---|---|---|---|
| | **(Intercept)** | **5069.743** | **1** | **<0.001** |
| | **Temperature** | **53.612** | **2** | **<0.001** |
| | **Time Period** | **39.977** | **4** | **<0.001** |
| | **Population** | **33.267** | **1** | **<0.001** |
| | **Temperature: Time Period** | **40.634** | **8** | **<0.001** |
| | **Temperature: Population** | **16.765** | **2** | **<0.001** |

Population size (i.e., the number of large reproducing adult females) was also important in predicting parasite fitness, both in relation to baseline temperature and independently of other influences (*GLMM, Type III Wald* $\chi^2_{(2)} = 16.77, p < 0.001$, *and GLMM, Type III Wald* $\chi^2_{(1)} = 39.98, p < 0.001$, *respectively,* Table 5.1B). While larger populations were generally associated with higher parasite burden (*17ºC GLMM, Estimate = 0.002, p = 0.003,* Table D.3B, Figure 5.3A*, and 20ºC GLMM, Estimate = 0.001, p = 0.035,* Table D.3C, Figure 5.3C), this effect weakened at thermal extremes. This suggests that temperature modulates the relationship between host density and infection intensity (Figure 5.3). For example, at 23ºC, as population size increased, burden decreased (*23ºC GLMM, Estimate = -0.002, p = 0.024,* Table D.3D, Figure 5.3D). Thus, the baseline temperature, timing in relation to the pulse event, and host population influence parasite proliferation. Yet, while population size influences parasite burden, it is also shaped by the combined effects of baseline temperature, timing relative to pulse events, treatment type, and parasite burden itself.

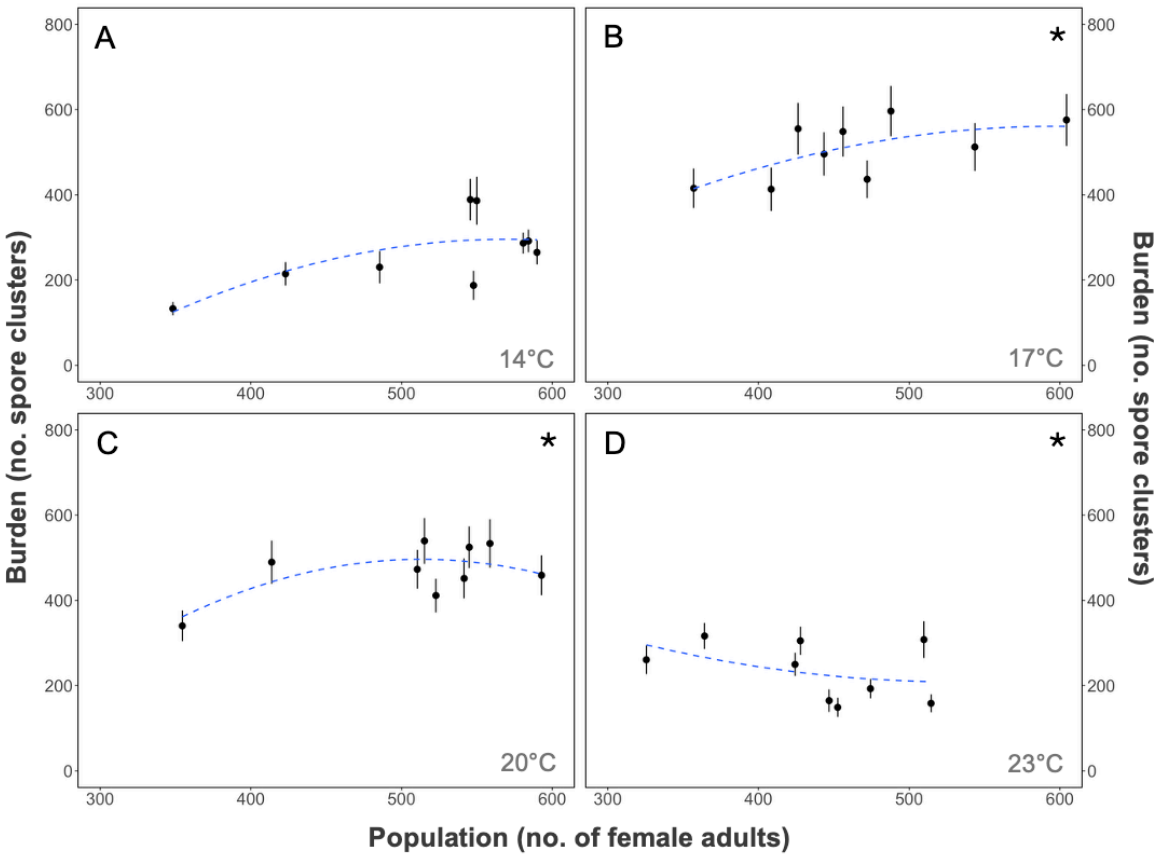


**Figure 5.3: The effect of population size on burden.**
In *Daphnia magna* infected with *Ordospora colligata*. Parasite burden (mean number of spore clusters, including zeros for uninfected but exposed hosts) and population size were averaged across nine weekly observations at four baseline temperatures: A; 14°C, B; 17°C, C; 20°C, and D; 23°C. Each spore cluster contains 32-62 individual *Ordospora*. Error bars represent the standard error, and each dashed line is the fit of the generalised linear mixed model (see Table D.3 for statistical results). The presence of an asterisk (*) in a panel represents a significant relation between population size and burden.

## *5.4.2 Population size*

Like burden, host population size was influenced by baseline temperature, time in relation to the pulse event, treatment type (pulse or constant), and parasite burden. Population size, defined as the number of large reproducing adult females, was altered by up to 1.5-fold depending on the timing in relation to the pulse event and baseline temperature (Figure 5.4). For example, at 17°C the population size in the pulse treatment during the period 'just after' a heatwave occurred (week 6) was reduced by 182 *Daphnia* compared to constant temperature populations (Figure 5.4B) (*emmean, z(inf) = 4.829, p < 0.001,* see Table D.9 for p-values of all custom contrasts). Moreover, at 17°C, all timings in relation to the pulse events resulted in

differential outcomes in population size (*emmean, constant before vs pulse before, z(inf) = -4.548, p < 0.001, constant during vs pulse during, z(inf) = 4.179, p < 0.001, and constant after vs pulse after, z(inf) = 3.804, p < 0.001,* see Table D.9). While the burden increased with a heatwave at 14ºC, the population size decreased in the periods 'just after' and 'after' the heatwave (*emmean, z(inf) = 2.763, p = 0.012, and z(inf) = 3.406, p = 0.002, respectively,* Table D.9). Although by the end of the experiment, the population size at 14ºC had gradually increased to levels similar to those in the constant treatment (Figure 5.4A). Cold snaps had a lesser effect on population size, with a decrease in population size at 20ºC but an increase in size at 23ºC (Figure 5.4C and 5.4D) (*emmeans, constant during vs pulse during at 20ºC, z(inf) = 2.379, p = 0.030, constant during vs pulse during at 23ºC, z(inf) = -2.36, p = 0.030,* Table D.9). Overall, the timing in relation to the pulse event can influence population size mediated by baseline temperature (*GLMM, Type III Wald* $\chi^2_{(12)} = 88.41$, $p < 0.001$, Table 5.2). Similarly, the effect of burden on population size varied with temperature, with marginal significance for an interaction (*GLMM, Type III Wald* $\chi^2_{(3)} = 7.84$, $p = 0.051$; Table 5.2). However, the negative association between population size and burden became stronger at higher temperatures (*GLMM, Estimate = –1.19, p = 0.023;* Table D.6). Thus, all factors can influence the outcome of host population size.

**Table 5.2: Type III Wald chi-square test results for the generalised linear mixed model (GLMM) analysing population size.**
In *Daphnia magna* infected with *Ordospora colligata*. The Type III Wald $\chi^2$ tests the significance of predictor variables, including temperature (modelled as a cubic polynomial), period, and population size. Significant values are bolded (See Table D.7 for GLMM).

| Explanatory Variable | Chisq | Df | P value |
|---|---|---|---|
| **(Intercept)** | **117,340** | **1** | **<0.001** |
| **Temperature** | **59.747** | **3** | **<0.001** |
| **Time Period** | **27.219** | **4** | **<0.001** |
| Burden | 9.472 | 1 | 0.059 |
| **Temperature: Time Period** | **88.408** | **12** | **<0.001** |
| **Temperature: Burden** | **7.839** | **3** | **0.051** |

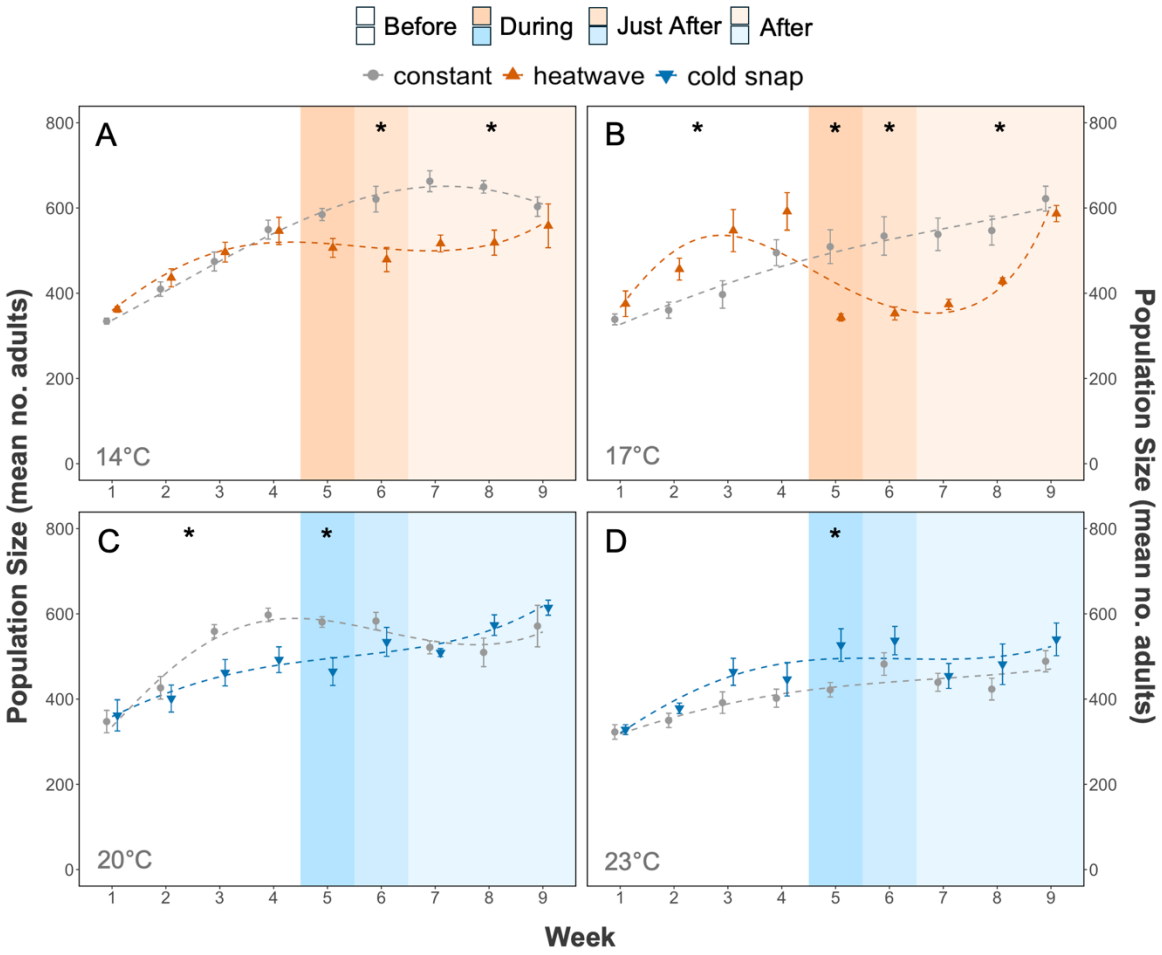


**Figure 5.4: The temporal effects of pulse temperature treatments on population size.** In *Daphnia magna* infected with *Ordospora colligata*. Population size (mean number of adults) is shown over the nine recorded weeks at four baseline temperatures: A; 14ºC, B; 17ºC, C; 20ºC, and D; 23ºC. Each spore cluster contains 32-62 individual *Ordospora*. Error bars represent the standard error, and each dashed line is the cubic fit of the generalised linear mixed model (see Table D.6 for statistical results). Grey; constant treatment, orange; heatwave pulse treatment, blue; cold snap pulse treatment. Shaded regions mark experimental periods: Before (white), During (orange/blue), Just After (lighter orange/blue), and After (lightest orange/blue). Asterisks (*) indicate significant differences in burden between pulse and constant treatments in specific time periods (emmeans contrasts, Table D.9).

### *5.4.3 Comparison of burden in populations and individuals*

The effect of a pulse treatment is dependent on baseline temperature at both the population and individual level (Figure 5.5). For example, at 14ºC, both population and individual burdens increase when a heatwave occurs (*emmean, z(inf) = 4.383, p < 0.001, and z(inf) = 2.527, p = 0.046, respectively,* Table 5.3). Trends were also similar, although less pronounced, at 17ºC, whereby the burden increased in the heatwave treatment at both the population and individual levels (Figure 5.5). Similarly, a cold snap can reflect patterns seen at both the population and

individual levels and is dependent on baseline temperature, but the relationship was less consistent. For example, at 20ºC, both the population and individual burden decreased when a cold snap occurred (Figure 5.5). Therefore, individual-level exposure patterns may be reflected at the population level, particularly at lower temperatures, though the strength of this relationship varies across temperature treatments.

**Table 5.3: Custom contrasts for burden comparison between periods at both population and individual levels.**
In *Daphnia magna* infected with *Ordospora colligata*. Each pulse treatment period was compared to its constant equivalent at the same temperature or to the 'before' pulse period. The response variable was burden, with uninfected but exposed hosts coded as zero. The 'Inf' degrees of freedom reflect the model's complexity and the estimation process. Exp; experiment, CS; cold snap individual experiment, HW; heatwave individual experiment, POP; population experiment. Significant contrasts are bolded.

| Exp | Temp | Contrast | Estimate | SE | Df | z ratio | p value |
|---|---|---|---|---|---|---|---|
| **POP** | **14** | **during HW vs constant** | **0.827** | **0.189** | **Inf** | **4.383** | **0.0001** |
| **HW** | **14** | **during HW vs constant** | **0.875** | **0.346** | **Inf** | **2.527** | **0.046** |
| POP | 17 | during HW vs constant | 0.117 | 0.189 | Inf | 0.621 | 0.5544 |
| HW | 17 | during HW vs constant | 0.185 | 0.312 | Inf | 0.591 | 0.5544 |
| POP | 20 | during CS vs constant | -0.431 | 0.189 | Inf | -2.282 | 0.0599 |
| CS | 20 | during CS vs constant | -0.431 | 0.321 | Inf | -1.345 | 0.3571 |
| POP | 23 | during CS vs constant | 0.174 | 0.189 | Inf | 0.919 | 0.4773 |
| CS | 23 | during CS vs constant | -0.337 | 0.357 | Inf | -0.944 | 0.4773 |

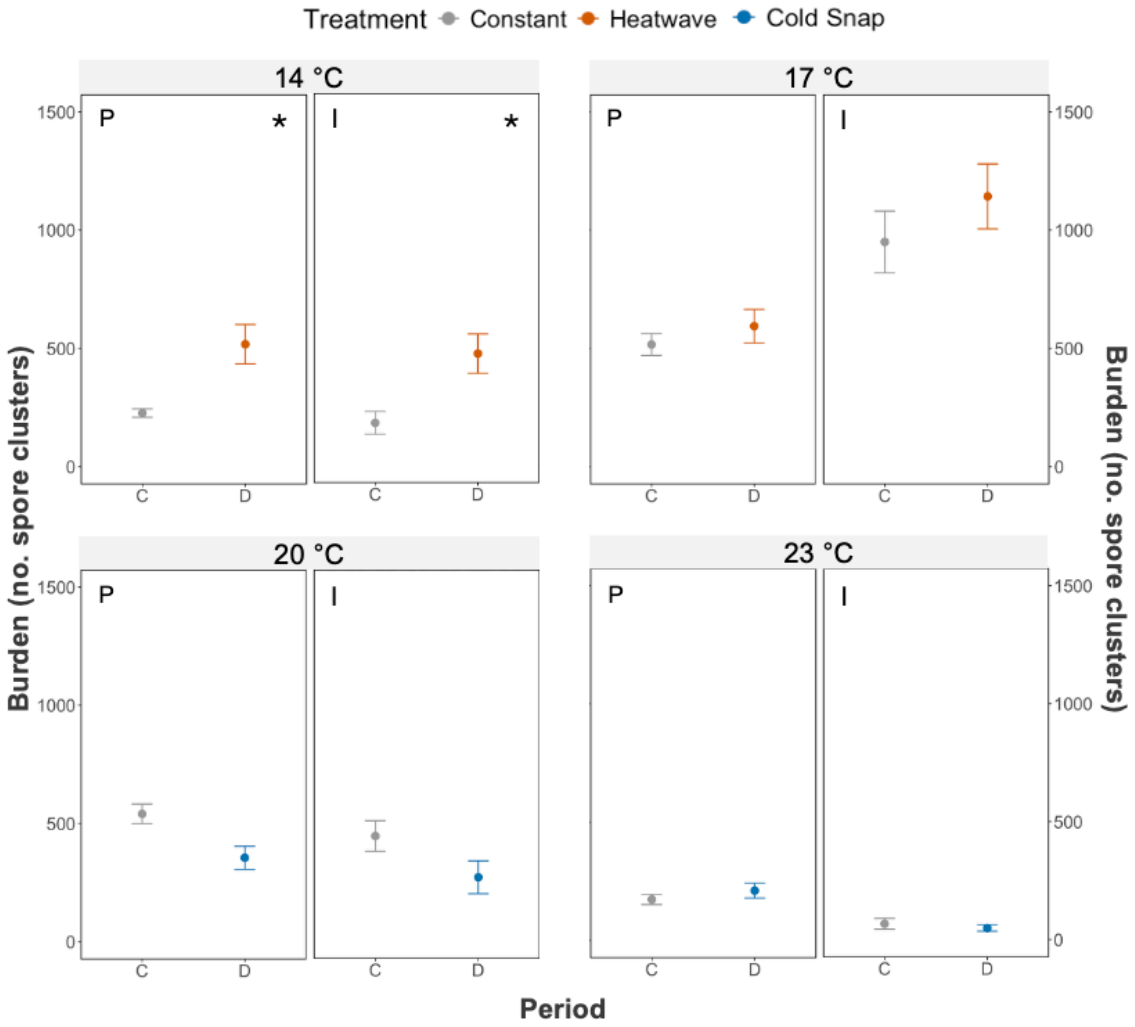


**Figure 5.5: Comparison between the effects of pulse and constant treatments on burden at both individual and population scales.**
In *Daphnia magna* infected with *Ordospora colligata*. For the population level, burden (mean number of spore clusters) is the data from week 5 (during the heatwaves) at four baseline temperatures: 14°C, 17°C, 20°C, and 23°C. For individual-level data, the data is taken from heatwaves/cold snaps occurring on day 0 (day of exposure). Each spore cluster contains 32-62 individual *Ordospora*. Error bars represent the standard error. Grey; constant treatment, orange; heatwave pulse treatment, blue; cold snap pulse treatment. C; constant (no pulse treatment), D; during pulse period. An asterisk (*) indicates a significant relationship between the constant and pulse treatment at a given temperature and in the same experiment.

## 5.5 Discussion

The effect of heatwaves and cold snaps on disease dynamics depended on both the baseline temperature and the nature of the fluctuation. Heatwaves produced lasting increases in parasite burden, while cold snaps had more variable outcomes. Outcomes reflected how far temperature pulses moved the host-parasite system toward or away from optimal

performance. Host population size also changed with temperature, showing that environmental variation can shape demographic as well as infection dynamics. Parasite burden and host density were closely linked; at cooler temperatures, both increased together, whereas at warmer temperatures, higher host densities were associated with lower burden. Understanding these dynamics is crucial, as temperature-induced changes in infection levels and host population size can have cascading effects on ecological processes and disease transmission (Gsell *et al*., 2023).

### *5.5.1 Burden and population size*

Parasite burden and population size were not independent; rather, their relationship varied with baseline temperature, consistent with interactive effects of ecological context on disease dynamics. Larger host populations generally supported higher numbers of pathogens, consistent with density-dependent transmission dynamics (Hu *et al*., 2013), where increased host density facilitates more frequent contact between infected and susceptible individuals. This pattern has been observed across many systems, for example, amphibian-chytrid fungus (Brannelly *et al*., 2015), guppy-platyhelminth (Johnson *et al*., 2011), and moth-fungus (Kyle *et al*., 2020) interactions. However, at higher temperatures (23ºC), this positive relationship reversed, with larger populations linked to lower burdens. This decoupling suggests that high temperatures may disrupt transmission dynamics, resulting in decreased burdens at higher densities. Such reversals are consistent with negative density dependence, where increased host density can suppress, rather than amplify, infection under certain ecological conditions (Albery *et al*., 2020). Several mechanisms could contribute to this reversal, for example, reduced parasite performance (Shocket *et al*., 2019) or decreased parasite viability (Chen *et al*., 2019). Moreover, crowding at high temperatures could intensify competition for food or space, leading to behavioural or physiological changes (Ban *et al*., 2009, Burns, 2000), and enhanced host immunity (Dang *et al*., 2012, Silva and Elliot, 2016), resulting in reduced transmission efficiency. Additionally, high-density conditions may promote avoidance

behaviours, like reduced aggregation or increased movement, further limiting spore encounter rates (Dahlin *et al*., 2024). Similar temperature-driven interference with transmission has been observed in *Daphnia dentifera* infected with *Metschnikowia bicuspidata,* where foraging interference and high host density at high temperatures decreased parasite invasion and prevalence (Civitello *et al*., 2013). Moreover, the negative relation between density and burden at 23ºC may reflect that animals experiencing both thermal and crowding stress are unable to withstand higher parasite burdens, resulting in higher host mortality rates (Ragonese *et al*., 2024). For example, in three-spined sticklebacks (*Gasterosteus aculeatus*), fish with higher parasite loads experienced increased mortality rates under elevated temperatures (Wegner *et al*., 2008). Together, these findings suggest that the relationship between host population size and burden is modulated by ecological context and can shift non-linearly, with extreme temperatures potentially disrupting density-dependent transmission and amplifying disease-induced population decline.

### *5.5.2 Effect of cold snaps*

Cold snaps can increase the burden depending on the baseline temperature. For example, at 23ºC, a cold snap was followed by a delayed increase in burden, without a corresponding change in population size. Indeed, a cold snap in warmer climates can sharply increase fungal disease risk in wildlife populations (Cohen *et al*., 2020). When the thermal optima of the host and parasite differ, fluctuations in temperature can shift the balance in their interaction, resulting in either host or parasite having an advantage over the other, as stated by the Thermal Mismatch Hypothesis (Cohen *et al*., 2017). In other systems, for example, the Red-backed Salamander (*Plethodon cinereus*) and the chytrid fungus (*Batrachochytrium dendrobatidis*), warm-adapted hosts had a higher probability of infection at cool temperatures compared to cold-adapted hosts (Venesky *et al*., 2022). Furthermore, temperature changes may compromise host physiology by suppressing immune responses or slowing metabolic activity,

temporarily reducing the host's ability to resist infection (Catalán *et al*., 2012). For example, cold stress compromised the immune function of the Madagascar hissing cockroach (*Gromphadorhina coquereliana*) (Lubawy and Słocińska, 2020). Here, the suppression of *Daphnia* immunity during the cold snap and subsequent rewarming could have contributed to the delayed increase in burden at higher temperatures (Murdock *et al*., 2012a). An increase in burden was also previously noted at the individual level by McCartan *et al*. (2024), where burden was increased after a cold snap occurred at 23ºC in the same host-parasite system. Thus, cold snaps can shift the outcome of the *Daphnia–Ordospora* interaction in a temperature-dependent manner, highlighting the close relationship between infection intensity and host population dynamics.

### *5.5.3 Effect of heatwaves*

Heatwaves, like cold snaps, can alter disease in the *Daphnia-Ordospora* system in a temperature-dependent way. Indeed, an increase in burden following a heatwave was observed at 14ºC. Here, the rapid temperature rise may have exceeded the Daphnia's physiological tolerance or capacity to acclimate, resulting in heightened stress and a reduction in population size. In contrast, *Ordospora* may have acclimatised faster to the heatwave, increasing replication and spore shedding, thereby raising exposure risk and leading to a higher burden during and after the heatwave occurred. Similar increases in burden near the lower end of the thermal extreme are also present in other systems (Jolma *et al*., 2025, Thomas and Blanford, 2003). For example, daily temperature fluctuations are expected to increase malaria transmission at lower baseline temperatures while decreasing transmission at higher temperatures (Paaijmans *et al*., 2010). Increases in burden following thermal fluctuations can be explained by the Thermal Variability Hypothesis (Paull *et al*., 2015). This theory posits that, as parasites are generally smaller than their hosts and have higher metabolic rates (according to the Metabolic Theory of Ecology (Brown *et al*., 2004)), they can physiologically adjust more quickly to temperature changes, giving them a temporary advantage. This is supported by other

studies using the same host-parasite system, which found that short-term fluctuations led to an increase in burden (Krichel *et al*., 2023, McCartan *et al*., 2024b). Our results furthermore show that such increases can persist up to four weeks after a heatwave (at 14ºC), indicating that parasites may derive long-term fitness benefits from short-term extreme weather events.

Long-term increases in disease burden following extreme weather conditions may arise from sustained changes in host physiology (Rodríguez-Trelles *et al*., 2013), immune function (Rollins-Smith and Le Sage, 2023), or shifts in the host-parasite dynamics that are not quickly reversed once temperatures stabilise (Kirk *et al*., 2020). For example, increased air temperatures following heatwaves elevated *Perkinsus marinus* (a protozoan parasite) infection intensity in oyster populations and suppressed immune defences, leading to prolonged infection levels even after temperatures normalised (Malek and Byers, 2018). These findings raise important questions about the broader consequences of more frequent extreme weather events. For instance, if parasites continue to benefit disproportionately from transient temperature spikes, this could extend the duration of outbreaks, shift their seasonal timing, or amplify their severity (Fox *et al*., 2015, Kazmierczak *et al*., 2022). Thus, brief thermal events can trigger prolonged shifts in disease dynamics, potentially altering the trajectory of epidemics and influencing long-term host population health. As climate change increases the frequency and intensity of heatwaves, understanding these lasting effects is essential for predicting parasite persistence and outbreak potential under future environmental scenarios, especially as heatwaves at 17ºC did not cause the same effect.

There was no observed increase in spore burden at 17ºC, despite reductions in population size both during and after the event. While we observed no response, potential opposing effects of heatwaves on both host and parasite life history traits may have masked any changes in burden. Given the narrower thermal tolerance for *Ordospora* (11.8-29.7ºC) compared to its *Daphnia* host (6-33.3ºC) (Kunze et al., 2022), the heatwave could have imposed physiological

stress on the parasite (Thermal Stress Hypothesis (Paull *et al*., 2015)). However, this may be offset by increased contact rates with higher temperatures due to increased filtration rates of the host (Müller *et al*., 2018). Other systems have shown that different effects on host and parasite traits can cancel each other out (Rohr *et al*., 2011). A similar lack of response to temperature variation has been observed in other systems, such as the monarch butterfly and its protozoan parasite *Ophryocystis elektroscirrha*, where both host and parasite show reduced performance under heat stress (Ragonese *et al*., 2024). Thus, while long-term effects of a heatwave on parasite burden were observed at 14ºC, no effects were present at 17ºC, highlighting that the effect of a heatwave, similar to cold snaps, can depend on the mean temperature at which the extreme weather event occurs. Together, both heatwaves and cold snaps demonstrate that opposing extremes act as mirror images along the same thermal performance axis; cold snaps enhance infection when they relieve heat stress, whereas heatwaves enhance infection when they alleviate cold limitation. Therefore, both fit within a unifying framework based on deviation from thermal optima.

### *5.5.4 Population versus individual level*

When pulse treatment effects were compared across levels, specifically, individual-level experiments where parasites were exposed to long, strong pulses during infection, and population-level experiments where burden was measured during the pulse itself, general concordance in responses was evident, particularly at lower temperatures. For example, the observed increase in burden during a heatwave at 14ºC was consistent across both scales. Although these correspondences were not uniform across all temperatures, they suggest that temperature-driven effects on parasite fitness can, in some cases, scale from individuals to populations. Thus, individual-level physiological responses, such as temperature-induced changes in host immunity (Catalán *et al*., 2012) or parasite replication (Porras *et al*., 2023), may translate into population-level infection dynamics. For instance, temperature dependence

around a thermal optimum can be scaled from the individual to the population level, although not always proportionally (Kirk *et al*., 2022). That is, while rising mean temperatures may consistently affect burden at the individual level, the population-level outcomes (such as prevalence or outbreak size) may also be shaped by ecological complexity (Brehm *et al*., 2025). For example, in a snail-schistosome system, individual-level traits like body size influenced host exposure and susceptibility, leading to predictable patterns of infection prevalence at the population level (Shaw *et al*., 2024). Host density (Bruno *et al*., 2007), resource competition (Amarasekare and Coutinho, 2014), and environmental carrying capacity (Bernhardt *et al*., 2018a) may modulate the strength and direction of temperature effects at different biological levels. These parallels across biological scales suggest that the direction of temperature deviation from the parasite's optimum can be used to predict both individual and population outcomes, providing a mechanistic link between physiology and demography under climate extremes

### *5.5.5 Conclusion*

In conclusion, the effect of extreme temperature variation on parasite fitness depends on both baseline temperature and the type of fluctuation, with a heatwave having long-lasting effects on burden. Host population size also exhibited temperature-dependent patterns. Importantly, parasite burden and host density were interdependent; at lower temperatures, burden and population size had a positive relationship; however, at higher temperatures, higher host densities resulted in lower burdens. Trends in burden showed partial correspondence between the population- and individual-level experiments, suggesting that individual physiological responses may, under certain conditions, scale up to population-level outcomes. These findings underscore the importance of considering both environmental context and temporal dynamics when assessing host-parasite interactions under climate change. As climate change is resulting in increased frequency and intensity of anomalous weather events (Seneviratne *et al*., 2021), understanding the influence of these on host-parasite dynamics will become all the

more important. Given the key role of *Daphnia* in their ecosystem, the effect can have far-reaching impacts (Sarnelle, 2005). For example, a reduction in *Daphnia* density due to infection by another parasite led to alterations in the food web, causing a trophic cascade (Duffy, 2007). Moreover, accounting for temperature variation in predictive models will be essential for more robust predictions (Wolinska and King, 2009), as parasites can alter co-evolutionary dynamics (Marcogliese, 2008), reshape ecological networks (Harvell *et al*., 2002), and drive population-level changes in host species (Cohen *et al*., 2019). Failing to consider temperature fluctuations may lead to overestimates in parasite fitness traits at high temperatures (Bagni *et al*., 2024). Thus, extreme temperature events alter host-parasite interactions in predictable ways; infection increases when fluctuations bring temperatures closer to the parasite's optimum and declines when extremes exceed physiological limits. Accounting for these mechanisms in predictive models will improve projections of parasite persistence, outbreak timing, and ecosystem impact under ongoing climate change.

# Chapter 6: Discussion

Across both individual and population scales, extreme thermal variation (heatwaves and cold snaps) altered host-parasite dynamics in predictable directions relative to organismal thermal optima. Parasite fitness generally increased when fluctuations brought conditions closer to the parasite's optimum and declined when extremes pushed temperatures beyond either host or parasite limits. These shifts depended on baseline temperature, timing relative to exposure, and the amplitude and duration of the event. Cold snaps tended to enhance infection at high baselines by temporarily relieving parasite heat stress, whereas heatwaves often reduced infection at similar temperatures by exceeding tolerance limits. Furthermore, patterns at the individual level sometimes reflected those seen at the population level, with parasite burden and host density influencing each other in temperature-dependent ways. These findings underscore the need to consider thermal variability, not just mean temperatures, when predicting disease outcomes under climate change.

## 6.1 Effect of cold snaps

Cold snaps will result in multifaceted effects on the host-parasite relationship, with outcomes shaped by baseline temperature, timing in relation to exposure, cold snap amplitude and duration. Cold snaps resulted in greater parasite burden at higher baseline temperatures, potentially due to a temporary relief from thermal stress near *Ordospora's* upper limits (Kirk *et al*., 2018). In contrast, burden was reduced at lower temperatures, likely reflecting the effects of thermal mismatch (Cohen *et al*., 2017) or thermal stress (Paull *et al*., 2015). According to the Thermal Mismatch Hypothesis (Cohen *et al*., 2019), mismatches in the thermal sensitivity of host and parasite performance can shift the competitive balance, giving an advantage to one

over the other. Such imbalances may become more frequent as climate change drives increasingly variable and unpredictable temperature regimes (Wade *et al.*, 2015). In this context, cold snaps have the potential to reshape infection outcomes by pushing organisms beyond physiological thresholds (Cohen *et al.*, 2020). Timing also played a critical role; cold snaps that occurred after exposure generally increased burden, whereas those occurring before exposure often reduced parasite proliferation, suggesting phenological mismatches (disruptions in the alignment of host and parasite developmental stages) can hinder successful establishment (Abarca and Spahn, 2021, Pardikes *et al.*, 2022). These mismatches may arise when environmental cues shift one species' phenology more than the others, altering infection windows or host susceptibility (Reece *et al.*, 2017). Moreover, when mismatches reduce parasite establishment early in life or allow better parasite performance once hosts are immunologically compromised (Cohen *et al.*, 2017), they may influence epidemic timing and severity (McDevitt-Galles *et al.*, 2020), with downstream effects on population stability and community structure. Notably, the same cold snap treatment could produce opposite outcomes depending on its timing relative to exposure, highlighting the non-linear and context-specific nature of extreme weather events. These contrasting outcomes may reflect physiological trade-offs, where cold snaps suppress the initial establishment of infection but subsequently favour within-host proliferation once infection is established (Paull *et al.*, 2015, Studer *et al.*, 2010, Seppälä and Jokela, 2011). This decoupling of infection traits underscores the importance of examining multiple fitness components when assessing thermal effects on the parasite. Taken together, these findings highlight that cold snaps increased infection when they brought conditions closer to the parasite's optimum but reduced it when they intensified thermal stress, demonstrating that the direction of deviation from optimal temperature determines infection outcomes.

## 6.2 Effect of heatwaves

Like cold snaps, heatwaves also resulted in nuanced outcomes in the host-parasite relationship, shaped by the baseline temperature, timing in relation to exposure, heatwave amplitude, and duration. Yet, unlike cold snaps, parasite fitness generally decreased as baseline temperature increased. Here, thermal stress exceeded the parasite's upper thermal tolerance, resulting in a reduced infection prevalence and burden when heatwaves occurred on the day of exposure or after exposure. According to the Thermal Stress Hypothesis (Paull *et al*., 2015), such reductions occur when organisms are pushed beyond their physiological limits. Given that *Ordospora colligata* has a narrower thermal range than its *Daphnia* host (Kirk *et al*., 2018), it is more vulnerable to decreased performance under extreme heat. Consequently, intense or prolonged heatwaves may inhibit parasite establishment or suppress proliferation within the host (Hector *et al*., 2023, Moore *et al*., 2022, Porras *et al*., 2023). Moreover, short-term fluctuations occurring before or during the early stages of infection sometimes increase burden, particularly at lower baseline temperatures. These patterns may reflect the Thermal Variability Hypothesis (Raffel *et al*., 2015), which proposes that parasites (due to their smaller size and higher metabolic rates) can acclimate more rapidly than their hosts to changing temperatures (according to the Metabolic Theory of Ecology (Brown *et al*., 2004). As a result, short-term warming events may temporarily give the parasite an advantage, thus enhancing proliferation if the host's immune response is not yet fully activated or is compromised by the temperature shift (Dittmar *et al*., 2014, Herren *et al*., 2023, Paaijmans *et al*., 2013). This could explain why, in some instances, burden was elevated despite reduced infection prevalence**,** suggesting that once infection is established, brief periods of favourable conditions can amplify within-host replication. Overall, heatwaves reduced infection when they pushed temperatures beyond parasite tolerance but occasionally enhanced burden at cooler baselines where they restored near-optimal conditions. These findings, alongside the cold snap treatments, underscore how individual-level traits, such as host immunity (Seppälä

and Jokela, 2011), behaviour (Teemer and Hawley, 2024), and metabolic processes (Li *et al*., 2023), can be shaped by anomalous weather events.

## 6.3 Broader implications at the individual level

Extreme temperature variation can influence life history traits of both host and parasite, ultimately shaping the outcome of their interaction. A thermal pulse occurring during or after infection may impair host immunometabolism (Scharsack *et al*., 2021), leading to increased susceptibility to infection (Dang *et al*., 2012, Lenihan *et al*., 1999, Seppälä and Jokela, 2011). In contrast, thermal variation occurring before exposure may enhance host immune function through immune priming, improving resistance to subsequent infection (Browne *et al*., 2014, Contreras-Garduño *et al*., 2016). Moreover, short-term thermal events can disrupt phenotypic synchrony between hosts and parasites, creating mismatches in developmental timing, metabolic rates, or thermal performance (Cohen *et al*., 2017, Hector *et al*., 2023). These mismatches can result in transient advantages for either host or parasite, which will alter transmission dynamics and disease outcomes (Vazquez *et al*., 2017). In particular, whether parasite fitness increases or decreases depends on how a thermal pulse shifts the system relative to each species' thermal performance curve, with near-optimal conditions favouring infection and extreme deviations reducing it. Over longer timescales, repeated exposure to these thermal extremes could drive co-evolutionary shifts**,** favouring thermal tolerance adaptations or life-history changes that alter host resistance or parasite virulence (Gehman *et al*., 2018, Rohr *et al*., 2011). As a result, fluctuating temperature regimes may not only influence immediate infection outcomes but also reshape the evolutionary trajectory of host-parasite systems under climate change (Aleuy and Kutz, 2020, Vazquez *et al*., 2017). These findings are particularly relevant to short-lived organisms, like *Daphnia,* where rapid generational turnover may accelerate such eco-evolutionary responses (Strauss *et al*., 2017, Yoshida *et al*., 2003).

Additionally, hosts may face energetic trade-offs under thermal stress, redirecting resources from defence to maintenance or growth (Ganeshan *et al*., 2019), while behavioural thermoregulation (e.g. movement to thermally optimal microhabitats) can further modulate host-parasite contact and infection outcomes (de Roode and Lefèvre, 2012, Hector *et al*., 2023). Ultimately, these findings show that individual-level infection responses to temperature extremes can be mechanistically understood through how far organisms are driven from their thermal optima.

## 6.4 Effect on populations

Like at the individual level, cold snaps and heatwaves will result in intricate consequences in whole populations, depending on the baseline temperature and treatment type. Heatwaves, in particular, can have long-lasting effects on parasite burden. At lower baseline temperatures, an increase in temperature may facilitate elevated parasite growth if the parasite acclimatises faster while causing thermal stress for the host (Paull *et al*., 2015), resulting in a decline in population size. Long-lasting effects due to thermal variation highlight concerns about cumulative stress and recovery windows in systems subject to repeated or increasingly frequent extremes (Cohen *et al*., 2020, Gehman *et al*., 2018). Moreover, parasite fitness and population size were codependent, with each influencing the other. Generally, parasite fitness was consistent with density-dependent transmission dynamics (Hu *et al*., 2013, Ebert *et al*., 2000b), a well-established ecological process in which parasite burden often increases with host density. This relationship has long been recognised in host-parasite systems, showing that temperature modifies density-dependent parasitism (Burnett, 1949, Nealis *et al*., 1984). However, this positive correlation reversed at higher baseline temperatures, resulting in negative density dependence. Such a reversal suggests that higher temperatures may shift systems away from the parasite's performance optimum, disrupt transmission dynamics by

intensifying competition for food or space, impairing host condition and immune function, or triggering avoidance behaviours that reduce contact rates (Ban *et al*., 2009, Burns, 2000, Albery *et al*., 2020). It may also reflect threshold effects, where cumulative thermal and density-related stress pushes host populations past a tipping point, limiting parasite replication or reducing transmission opportunities (Shang *et al*., 2022, de Sassi *et al*., 2012). This highlights that density-dependent factors mirror individual level trends; parasite fitness increases when pulses restore near optimal conditions for the parasite while fitness declines when pulses exceed physiological limits. Notably, burden outcomes at the individual level showed partial correspondence with those observed at the population level. This suggests that individual physiological responses, such as temperature-induced changes in host immunity or parasite replication, may scale to population-level infection dynamics (Mideo *et al*., 2008, Hall *et al*., 2010, Clay *et al*., 2020), although not always proportionally (Kirk *et al*., 2022). Thus, integrating both individual and population scale perspectives reveals that demographic consequences of climate extremes can be predicted from the underlying thermal biology of hosts and parasites.

## 6.5 Broader implications at the population level

Beyond immediate infection outcomes, temperature-driven changes in parasite burden and population size can have broader implications for population dynamics and ecosystem functioning. Shifts in population growth rates as a result of thermal fluctuations may cascade through a food web, leading to changes in ecosystem structure (Duffy, 2007). If *Daphnia* populations decline due to high infection burdens under thermal stress, this may reduce grazing pressure on phytoplankton, promoting algal blooms and altering nutrient cycling (Wojtal-Frankiewicz, 2012, Huber *et al*., 2008). In turn, such shifts may feed back into host-parasite interactions by changing resource availability or host condition (Civitello *et al*., 2015,

Hall *et al*., 2009). As thermal extremes can have long-lasting effects on the host and parasite, making it harder for the host to recover, the timing, duration, or intensity of outbreaks could be altered, disrupting seasonal dynamics and making epidemics more difficult to predict (Altizer *et al*., 2013, Marcogliese, 2008). In systems where disease regulates host populations, even temporary increases in burden could shift demographic thresholds or increase extinction risk, particularly in small or isolated populations (Gehman *et al*., 2018, Rohr *et al*., 2011). Furthermore, temperature-driven changes in parasite performance may apply selective pressures on host traits such as thermal tolerance, reproductive timing, or immune function, potentially reshaping life history strategies and co-evolutionary trajectories (Scharsack *et al*., 2021, Aleuy and Kutz, 2020). These broader effects underscore the potential for climate extremes to act as eco-evolutionary drivers, influencing not only infection outcomes but also population persistence, food web stability, and ecosystem resilience.

## 6.6 Shortcomings and future directions

While this work offers valuable insights into how thermal variability shapes host-parasite dynamics, it is not without limitations. Although *Daphnia* are a useful model system for the study of ecology and evolution (Ebert, 2022), this thesis focused on a single clonal line of both host and parasite. This overlooks natural genetic variation, which can be important in altering outcomes for population dynamics and epidemic size (Altermatt and Ebert, 2008, Laine, 2008). For example, higher temperatures increased epidemic size in *Daphnia,* but population mixing reduced it (Auld and Brand, 2017). Moreover, the use of a single, uniform feeding regime throughout the experiments does not capture the natural variability in food availability experienced by *Daphnia* in the wild. Indeed, nutritional status is a key factor influencing host condition, immune function, and resistance to infection (Vale *et al*., 2013, Hardison and Eliason, 2024). Variable or limited food resources could interact with thermal stress to

exacerbate or mitigate infection outcomes, affecting both host survival and parasite replication rates (Garbutt *et al*., 2014). Thus, the constant feeding conditions in this study may limit the ecological realism of our findings and warrant further investigation under fluctuating resource scenarios. Additionally, host-parasite interactions occur within complex ecological communities where multiple species, including predators, competitors, and other parasites, can influence infection dynamics (Johnson *et al*., 2015, Civitello *et al*., 2015). Thermal variability may alter these community interactions, thereby modulating disease outcomes in ways not captured by single host-parasite studies. Investigating how temperature extremes affect community-level processes and multispecies interactions will be essential for fully understanding and predicting disease risk under climate change (Govaert *et al*., 2019). While this thesis included 128 individual-level and four population-level thermal pulse treatments, it underscores the need for broader exploration of the complex and diverse responses of host-parasite systems to temperature variation. Future research should expand to include a wider range of host genotypes, parasite strains, and species to capture the full breadth of ecological and evolutionary variation. Moreover, exploration of other thermal regimes (like recurring extreme weather events and climate reddening) would further enhance our understanding of how different forms of thermal variation impact host-parasite interactions. Such efforts will be crucial to aid in generalising findings and to better predict disease dynamics across diverse natural systems facing increasingly variable climates.

## 6.7 Concluding remarks

This thesis demonstrates that the direction of the host-parasite relationship is altered by extreme thermal variation, mediated by the organismal thermal optima. Parasite fitness is shaped by the baseline temperature, pulse timing in relation to exposure, and the pulse amplitude and duration. Cold snaps and heatwaves, therefore, represent opposite but

mechanistically linked drivers of infection dynamics, each capable of shifting the balance between host resistance and parasite success. Notably, individual-level physiological responses can sometimes scale up to influence population-level dynamics, where parasite burden and host population size are interconnected, jointly shaping transmission potential and epidemic timing. Recognising how short-term temperature extremes move systems along their thermal performance curves provides a unifying framework for predicting disease risk under climate change. Overall, this work contributes to the growing recognition that climate extremes act as important eco-evolutionary drivers of infectious disease at the physiological level, with cascading effects on population persistence, ecosystem function, and biodiversity. Incorporating these complexities into disease models and conservation strategies will improve our ability to mitigate the impacts of climate change on host-parasite systems. As climate change continues to increase the frequency and intensity of temperature extremes globally, understanding these complex host-parasite interactions under thermal variability is crucial for anticipating disease outbreaks, preserving biodiversity, and maintaining ecosystem health.

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# Appendix A- Supplementary information for Chapter 2

**Table A.1: Coefficient estimates and feature importance of infection prevalence.**
Coefficient estimates and feature importance of infection prevalence (proportion infected), from elastic net regression, bootstrapping and feature importance in *Daphnia magna* infected with *Ordospora colligata*. effect; estimated coefficient of the model, Lower CI; lower confidence interval (95$^{th}$), Upper CI; upper confidence interval (95$^{th}$), Importance; feature importance for the polynomial degree. Significant values are bolded, any values with confidence intervals on or passing 0 are not significant. A positive effect value indicates a positive relationship between the predictor and response variable, a negative value indicates and negative relationship. Temperature was a cubic polynomial, therefore, to account for non-linearity and is therefore separated by each degree (linear [L], and quadratic [Q]).

| Explanatory Variable | Effect | Lower CI | Upper CI | Importance |
|---|---|---|---|---|
| X Intercept | 1.45 | 1.02 | 1.97 | 0 |
| **Temperature (L)** | **20.75** | **13.64** | **28.96** | 18.92 |
| **Temperature (Q)** | **-34.20** | **-43.44** | **-26.44** | 34.08 |
| Amplitude -3 | -0.06 | -0.83 | 0 | 0.06 |
| Amplitude -6 | 0.24 | 0 | 0.96 | 0.24 |
| Duration 3 | 0 | 0 | 0.74 | 0 |
| Duration 6 | 0 | -0.56 | 0 | 0 |
| Temperature (L): Amplitude -3 | 3.52 | 0 | 22.94 | 3.52 |
| Temperature (Q): Amplitude -3 | 0 | -10.76 | 2.04 | 0 |
| Temperature (L): Amplitude -6 | 0 | -13.25 | 0 | 0 |
| Temperature (Q): Amplitude -6 | 11.25 | 0 | 25.44 | 11.25 |
| Temperature (L): Duration 3 | 1.52 | 0 | 20.20 | 0.91 |
| Temperature (Q): Duration 3 | 5.73 | 0 | 18.58 | 5.73 |
| Temperature (L): Duration 6 | 0 | -0.81 | 0 | 0 |
| Temperature (Q): Duration 6 | 0 | 0 | 5.77 | 0 |
| Amplitude -3: Duration 3 | 0 | -0.14 | 0.57 | 0 |
| Amplitude -6: Duration 3 | 0.23 | -0.02 | 1.10 | 0.23 |
| Amplitude -3: Duration 6 | -0.91 | -1.60 | 0 | 1.52 |
| Amplitude -6: Duration 6 | 0 | 0 | 0.71 | 0 |
| Temperature (L): Amplitude -3: Duration 3 | 0 | -20.98 | 7.03 | 0 |
| Temperature (Q): Amplitude -3: Duration 3 | 10.30 | 0 | 33.54 | 10.30 |

| | | | | |
|---|---|---|---|---|
| Temperature (L): Amplitude -6: Duration 3 | 13.97 | 0 | 42.72 | 13.97 |
| Temperature (Q): Amplitude -6: Duration 3 | 0 | 0 | 6.31 | 0 |
| Temperature (L): Amplitude -3: Duration 6 | 18.92 | 0 | 44.18 | 20.75 |
| Temperature (Q): Amplitude -3: Duration 6 | -12.30 | -36.38 | 0 | 12.30 |
| **Temperature (L): Amplitude -6: Duration 6** | **-34.08** | **-61.39** | **-2.92** | 34.20 |
| Temperature (Q): Amplitude -6: Duration 6 | 20.80 | 0 | 44.91 | 20.80 |

**Table A.2: estimated marginal means (emmean) of infection prevalence.**
Estimated marginal means (emmean) of infection prevalence, in *Daphnia magna* infected with *Ordospora colligata*. The estimates were derived using a Generalised Linear Model (GLM) with baseline temperature as a factor and a treatment variable combining amplitude and duration, 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 binomial distribution and the model itself.

| Temp | Treatment | emmean | SE | df | Lower CI 95% | Upper CI 95% |
|---|---|---|---|---|---|---|
| 14 | 0 0 | 2.944 | 1.026 | Inf | 0.934 | 4.96 |
| 17 | 0 0 | 18.566 | 1360.064 | Inf | -2647.111 | 2684.24 |
| 20 | 0 0 | 2.398 | 0.739 | Inf | 0.95 | 3.85 |
| 23 | 0 0 | 0.916 | 0.483 | Inf | -0.03 | 1.86 |
| 14 | -3 3 | 1.012 | 0.584 | Inf | -0.133 | 2.16 |
| 17 | -3 3 | 2.485 | 1.041 | Inf | 0.445 | 4.53 |
| 20 | -3 3 | 1.872 | 0.76 | Inf | 0.383 | 3.36 |
| 23 | -3 3 | 2.565 | 1.038 | Inf | 0.531 | 4.6 |
| 14 | -3 6 | 0 | 0.534 | Inf | -1.048 | 1.05 |
| 17 | -3 6 | 18.566 | 1684.138 | Inf | -3282.284 | 3319.42 |
| 20 | -3 6 | 2.565 | 1.038 | Inf | 0.531 | 4.6 |
| 23 | -3 6 | 0.693 | 0.548 | Inf | -0.38 | 1.77 |
| 14 | -6 3 | 0.916 | 0.592 | Inf | -0.243 | 2.08 |
| 17 | -6 3 | 18.566 | 1743.248 | Inf | -3398.138 | 3435.27 |
| 20 | -6 3 | 2.398 | 1.044 | Inf | 0.351 | 4.45 |
| 23 | -6 3 | 18.566 | 1684.138 | Inf | -3282.284 | 3319.42 |
| 14 | -6 6 | 2.639 | 1.035 | Inf | 0.61 | 4.67 |
| 17 | -6 6 | 2.565 | 1.038 | Inf | 0.531 | 4.6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 20 | -6 6 | 0.693 | 0.548 | Inf | -0.38 | 1.77 |
| 23 | -6 6 | 0.916 | 0.592 | Inf | -0.243 | 2.08 |

**Table A.3: Custom contrasts of infection prevalence 'emmeans' between treatments at different baseline temperatures.**

Custom contrasts of infection 'emmeans' between 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. 'Inf' degrees of freedom indicate the model's complexity due to the negative binomial distribution, making quantification challenging for estimated marginal means. 'Adjusted p' shows the adjusted p values for multiple comparisons using the 'Benjamini-Hochberg' method.

| Temp | Contrast | Estimate | SE | df | z ratio | p value | Adjusted p |
|---|---|---|---|---|---|---|---|
| 14 | -33 v 00 | -1.9328 | 1.181 | Inf | -1.637 | 0.1016 | 0.4793 |
| 14 | -36 v 00 | -2.9444 | 1.157 | Inf | -2.545 | 0.0109 | 0.3495 |
| 14 | -63 v 00 | -2.0281 | 1.184 | Inf | -1.712 | 0.0868 | 0.4793 |
| 14 | -66 v 00 | -0.3054 | 1.457 | Inf | -0.21 | 0.834 | 1 |
| 14 | -36 v -33 | -1.0116 | 0.792 | Inf | -1.278 | 0.2013 | 0.5855 |
| 14 | -66 v -63 | 1.7228 | 1.192 | Inf | 1.445 | 0.1485 | 0.4793 |
| 14 | -63 v -33 | -0.0953 | 0.831 | Inf | -0.115 | 0.9087 | 1 |
| 14 | -66 v -36 | 2.6391 | 1.165 | Inf | 2.265 | 0.0235 | 0.3759 |
| 17 | -33 v 00 | -16.0812 | 1360 | Inf | -0.012 | 0.9906 | 1 |
| 17 | -36 v 00 | 0 | 2165 | Inf | 0 | 1 | 1 |
| 17 | -63 v 00 | 0 | 2211 | Inf | 0 | 1 | 1 |
| 17 | -66 v 00 | -16.0011 | 1360 | Inf | -0.012 | 0.9906 | 1 |
| 17 | -36 v -33 | 16.0812 | 1684 | Inf | 0.01 | 0.9924 | 1 |
| 17 | -66 v -63 | -16.0011 | 1743 | Inf | -0.009 | 0.9927 | 1 |
| 17 | -63 v -33 | 16.0812 | 1743 | Inf | 0.009 | 0.9926 | 1 |
| 17 | -66 v -36 | -16.0011 | 1684 | Inf | -0.01 | 0.9924 | 1 |
| 20 | -33 v 00 | -0.5261 | 1.059 | Inf | -0.497 | 0.6195 | 1 |
| 20 | -36 v 00 | 0.1671 | 1.274 | Inf | 0.131 | 0.8957 | 1 |
| 20 | -63 v 00 | 0 | 1.279 | Inf | 0 | 1 | 1 |
| 20 | -66 v 00 | -1.7047 | 0.919 | Inf | -1.854 | 0.0637 | 0.4793 |
| 20 | -36 v -33 | 0.6931 | 1.286 | Inf | 0.539 | 0.5899 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 20 | -66 v -63 | -1.7047 | 1.179 | Inf | -1.445 | 0.1483 | 0.4793 |
| 20 | -63 v -33 | 0.5261 | 1.291 | Inf | 0.407 | 0.6837 | 1 |
| 20 | -66 v -36 | -1.8718 | 1.173 | Inf | -1.595 | 0.1107 | 0.4793 |
| 23 | -33 v 00 | 1.6487 | 1.145 | Inf | 1.44 | 0.1498 | 0.4793 |
| 23 | -36 v 00 | -0.2231 | 0.73 | Inf | -0.306 | 0.7599 | 1 |
| 23 | -63 v 00 | 17.6498 | 1684 | Inf | 0.01 | 0.9916 | 1 |
| 23 | -66 v 00 | 0 | 0.764 | Inf | 0 | 1 | 1 |
| 23 | -36 v -33 | -1.8718 | 1.173 | Inf | -1.595 | 0.1107 | 0.4793 |
| 23 | -66 v -63 | -17.6498 | 1684 | Inf | -0.01 | 0.9916 | 1 |
| 23 | -63 v -33 | 16.0011 | 1684 | Inf | 0.01 | 0.9924 | 1 |
| 23 | -66 v -36 | 0.2231 | 0.806 | Inf | 0.277 | 0.782 | 1 |

**Table A.4: Combined feature importance for infection prevalence with importance.** Combined feature importance for infection 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 and quadratic) were combined.

| Explanatory Variable | Importance |
|---|---|
| Temperature: Amplitude -6: Duration 6 | 55.01 |
| Temperature | 52.99 |
| Temperature: Amplitude -3: Duration 6 | 33.05 |
| Temperature: Amplitude -6: Duration 3 | 13.97 |
| Temperature: Amplitude -6 | 11.25 |
| Temperature: Amplitude -3: Duration 3 | 10.30 |
| Temperature: Duration 3 | 6.63 |
| Temperature: Amplitude -3 | 3.52 |
| Amplitude -3: Duration 6 | 1.52 |
| Amplitude -6 | 0.24 |
| Amplitude -6: Duration 3 | 0.23 |
| Amplitude -3 | 0.06 |
| X intercept | 0 |
| Duration 3 | 0 |

| | |
|---|---|
| Duration 6 | 0 |
| Temperature: Duration 6 | 0 |
| Amplitude -3: Duration 3 | 0 |
| Amplitude -6: Duration 6 | 0 |

**Table A.5: Coefficient estimates and feature importance of burden.**
Coefficient estimates and feature importance of burden (number of spore clusters), from elastic net regression, bootstrapping and feature importance in *Daphnia magna* infected with *Ordospora colligata*. effect; estimated coefficient of the model, Lower CI; lower confidence interval (95$^{th}$), Upper CI; upper confidence interval (95$^{th}$), Importance; feature importance for the polynomial degree. Significant values are bolded. A positive effect value indicates a positive relationship between the predictor and response variable, a negative value indicates and negative relationship. Temperature was a cubic polynomial to account for non-linearity and is separated by each degree (linear [L], quadratic [Q], and cubic [C]).

| Explanatory Variable | Effect | Lower CI | Upper CI | Importance |
|---|---|---|---|---|
| X Intercept | 5.37 | 5.22 | 5.49 | 0 |
| Temperature (L) | 2.08 | 0.00 | 5.22 | 2.08 |
| **Temperature (Q)** | **-12.12** | **-14.51** | **-9.97** | 12.12 |
| Temperature (C) | 0 | -0.52 | 1.82 | 0 |
| Amplitude -3 | 0 | -0.03 | 0.09 | 0 |
| Amplitude -6 | 0 | -0.22 | 0 | 0 |
| Duration 3 | 0 | -0.03 | 0.10 | 0 |
| Duration 6 | 0 | -0.23 | 0 | 0 |
| **Temperature (L): Amplitude -3** | **6.28** | **2.83** | **8.77** | 6.28 |
| Temperature (Q): Amplitude -3 | 0 | -2.54 | 0 | 0 |
| Temperature (C): Amplitude -3 | 2.99 | 0 | 5.85 | 2.99 |
| **Temperature (L): Amplitude -6** | **4.49** | **1.19** | **7.34** | 4.49 |
| Temperature (Q): Amplitude -6 | 0 | -3.90 | 0 | 0 |
| Temperature (C): Amplitude -6 | 2.35 | 0 | 5.16 | 2.35 |
| **Temperature (L): Duration 3** | **3.45** | **0.58** | **5.89** | 3.45 |
| Temperature (Q): Duration 3 | -1.48 | -4.67 | 0 | 1.48 |
| Temperature (C): Duration 3 | 3.61 | 0 | 6.74 | 3.61 |
| **Temperature (L): Duration 6** | **7.15** | **3.01** | **10.76** | 7.15 |
| Temperature (Q): Duration 6 | 0 | -1.89 | 1.06 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (C): Duration 6 | 1.61 | 0 | 4.28 | 1.61 |
| Amplitude -3: Duration 3 | 0 | -0.16 | 0.07 | 0.00 |
| Amplitude -6: Duration 3 | 0 | 0 | 0.18 | 0 |
| Amplitude -3: Duration 6 | 0 | 0 | 0.16 | 0 |
| Amplitude -6: Duration 6 | -0.36 | -0.75 | 0 | 0.36 |
| Temperature (L): Amplitude -3: Duration 3 | 0 | 0 | 3.99 | 0 |
| Temperature (Q): Amplitude -3: Duration 3 | 0.00 | -1.77 | 0.22 | 0 |
| Temperature (C): Amplitude -3: Duration 3 | 0.31 | 0 | 5.42 | 0.31 |
| Temperature (L): Amplitude -6: Duration 3 | 0 | 0 | 1.80 | 0 |
| Temperature (Q): Amplitude -6: Duration 3 | -2.29 | -6.86 | 0 | 2.29 |
| Temperature (C): Amplitude -6: Duration 3 | 0 | 0 | 4.47 | 0 |
| Temperature (L): Amplitude -3: Duration 6 | 0 | 0 | 5.81 | 0 |
| Temperature (Q): Amplitude -3: Duration 6 | 0 | -4.09 | 0.52 | 0 |
| Temperature (C): Amplitude -3: Duration 6 | 0 | 0 | 3.22 | 0 |
| Temperature (L): Amplitude -6: Duration 6 | 0.55 | 0 | 7.41 | 0.55 |
| Temperature (Q): Amplitude -6: Duration 6 | 0 | -1.46 | 6.09 | 0 |
| Temperature (C): Amplitude -6: Duration 6 | 0 | 0.00 | 5.53 | 0 |

**Table A.6: estimated marginal means (emmean) of burden.**
Estimated marginal means (emmean) of burden, in *Daphnia magna* infected with *Ordospora colligata*. The estimates were derived using a Generalised Linear Model (GLM) with baseline temperature as a factor and a treatment variable combining amplitude and duration, and a binomial family. The presentation on the log scale is used to provide a more appropriate representation of the data. 'Inf' degrees of freedom suggests that the model is highly flexible, and they cannot be easily quantified due to the nature of the negative binomial distribution and the model's complexity.

| Temp | Treatment | emmean | SE | df | Lower CI 95% | Upper CI 95% |
|---|---|---|---|---|---|---|
| 14 | 0 0 | 4.58 | 0.211 | Inf | 4.17 | 5 |
| 17 | 0 0 | 6.06 | 0.191 | Inf | 5.68 | 6.43 |
| 20 | 0 0 | 6.19 | 0.195 | Inf | 5.8 | 6.57 |
| 23 | 0 0 | 4.55 | 0.237 | Inf | 4.08 | 5.02 |
| 14 | -3 3 | 3.78 | 0.279 | Inf | 3.23 | 4.33 |
| 17 | -3 3 | 6.17 | 0.264 | Inf | 5.65 | 6.69 |
| 20 | -3 3 | 5.82 | 0.254 | Inf | 5.32 | 6.32 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 23 | -3 3 | 5.82 | 0.254 | Inf | 5.33 | 6.32 |
| 14 | -3 6 | 3.38 | 0.352 | Inf | 2.69 | 4.07 |
| 17 | -3 6 | 5.92 | 0.236 | Inf | 5.46 | 6.39 |
| 20 | -3 6 | 6.11 | 0.254 | Inf | 5.61 | 6.6 |
| 23 | -3 6 | 6.13 | 0.289 | Inf | 5.56 | 6.69 |
| 14 | -6 3 | 3.79 | 0.293 | Inf | 3.21 | 4.36 |
| 17 | -6 3 | 6.31 | 0.244 | Inf | 5.83 | 6.79 |
| 20 | -6 3 | 6.06 | 0.276 | Inf | 5.52 | 6.6 |
| 23 | -6 3 | 5.44 | 0.237 | Inf | 4.97 | 5.9 |
| 14 | -6 6 | 3.4 | 0.249 | Inf | 2.91 | 3.89 |
| 17 | -6 6 | 5.26 | 0.254 | Inf | 4.76 | 5.76 |
| 20 | -6 6 | 5.6 | 0.29 | Inf | 5.03 | 6.17 |
| 23 | -6 6 | 5.68 | 0.29 | Inf | 5.11 | 6.25 |

**Table A.7: Custom contrasts of burden 'emmeans' between treatments at different baseline temperatures.**

Custom contrasts of burden 'emmeans' between 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. 'Inf' degrees of freedom indicate the model's complexity due to the negative binomial distribution, making quantification challenging for estimated marginal means. 'Adjusted p' shows the adjusted p values for multiple comparisons using the 'Benjamini-Hochberg' method. Significant contrasts are presented in bold.

| Temp | Contrast | Estimate | SE | df | z ratio | p value | Adjusted p |
|---|---|---|---|---|---|---|---|
| **14** | **-33 v 00** | **-0.8012** | **0.35** | **Inf** | **-2.29** | **0.022** | **0.0784** |
| **14** | **-36 v 00** | **-1.2063** | **0.411** | **Inf** | **-2.937** | **0.0033** | **0.0177** |
| **14** | **-63 v 00** | **-0.7946** | **0.361** | **Inf** | **-2.202** | **0.0277** | **0.0886** |
| **14** | **-66 v 00** | **-1.1845** | **0.326** | **Inf** | **-3.629** | **0.0003** | **0.0030** |
| 14 | -36 v -33 | -0.405 | 0.45 | Inf | -0.901 | 0.3677 | 0.6192 |
| 14 | -66 v -63 | -0.3899 | 0.384 | Inf | -1.014 | 0.3105 | 0.5519 |
| 14 | -63 v -33 | 0.0066 | 0.405 | Inf | 0.016 | 0.987 | 0.9870 |
| 14 | -66 v -36 | 0.0217 | 0.432 | Inf | 0.05 | 0.9599 | 0.9870 |
| 17 | -33 v 00 | 0.1159 | 0.326 | Inf | 0.356 | 0.7221 | 0.7968 |
| 17 | -36 v 00 | -0.1333 | 0.304 | Inf | -0.439 | 0.6607 | 0.7968 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 17 | -63 v 00 | 0.2551 | 0.31 | Inf | 0.822 | 0.4108 | 0.6265 |
| **17** | **-66 v 00** | **-0.7972** | **0.318** | **Inf** | **-2.508** | **0.0121** | **0.0486** |
| 17 | -36 v -33 | -0.2492 | 0.354 | Inf | -0.703 | 0.482 | 0.6614 |
| **17** | **-66 v -63** | **-1.0522** | **0.353** | **Inf** | **-2.983** | **0.0029** | **0.0177** |
| 17 | -63 v -33 | 0.1392 | 0.36 | Inf | 0.387 | 0.699 | 0.7968 |
| 17 | -66 v -36 | -0.6639 | 0.347 | Inf | -1.913 | 0.0558 | 0.1623 |
| 20 | -33 v 00 | -0.3664 | 0.32 | Inf | -1.144 | 0.2525 | 0.5169 |
| 20 | -36 v 00 | -0.0802 | 0.32 | Inf | -0.251 | 0.802 | 0.8555 |
| 20 | -63 v 00 | -0.1232 | 0.338 | Inf | -0.365 | 0.7153 | 0.7968 |
| 20 | -66 v 00 | -0.5853 | 0.349 | Inf | -1.676 | 0.0937 | 0.2499 |
| 20 | -36 v -33 | 0.2861 | 0.359 | Inf | 0.797 | 0.4254 | 0.6265 |
| 20 | -66 v -63 | -0.462 | 0.4 | Inf | -1.155 | 0.248 | 0.5169 |
| 20 | -63 v -33 | 0.2431 | 0.375 | Inf | 0.648 | 0.5167 | 0.6614 |
| 20 | -66 v -36 | -0.505 | 0.385 | Inf | -1.312 | 0.1897 | 0.4669 |
| **23** | **-33 v 00** | **1.2748** | **0.348** | **Inf** | **3.667** | **0.0002** | **0.0030** |
| **23** | **-36 v 00** | **1.5781** | **0.374** | **Inf** | **4.216** | **<.0001** | **0.0008** |
| **23** | **-63 v 00** | **0.8864** | **0.335** | **Inf** | **2.645** | **0.0082** | **0.0374** |
| **23** | **-66 v 00** | **1.1309** | **0.374** | **Inf** | **3.02** | **0.0025** | **0.0177** |
| 23 | -36 v -33 | 0.3033 | 0.385 | Inf | 0.788 | 0.4307 | 0.6265 |
| 23 | -66 v -63 | 0.2445 | 0.374 | Inf | 0.654 | 0.5132 | 0.6614 |
| 23 | -63 v -33 | -0.3884 | 0.347 | Inf | -1.119 | 0.2631 | 0.5169 |
| 23 | -66 v -36 | -0.4472 | 0.409 | Inf | -1.093 | 0.2746 | 0.5169 |

**Table A.8: Combined feature importance for burden with importance.**
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.

| Explanatory Variable | Importance |
|---|---|
| Temperature | 14.19 |
| Temperature: Amplitude -3 | 9.27 |
| Temperature: Duration 6 | 8.76 |

| | |
|---|---|
| Temperature: Duration 3 | 8.54 |
| Temperature: Amplitude -6 | 6.84 |
| Temperature Amplitude -6: Duration 3 | 2.29 |
| Temperature: Amplitude -6: Duration 6 | 0.55 |
| Amplitude -6: Duration 6 | 0.36 |
| Temperature: Amplitude -3: Duration 3 | 0.31 |
| X intercept | 0 |
| Amplitude -3 | 0 |
| Amplitude -6 | 0 |
| Duration 3 | 0 |
| Duration 6 | 0 |
| Amplitude -3: Duration 3 | 0 |
| Amplitude -6: Duration 3 | 0 |
| Amplitude -3: Duration 6 | 0 |
| Temperature: Amplitude -3: Duration 6 | 0 |

# Appendix B - Supplementary information for Chapter 3

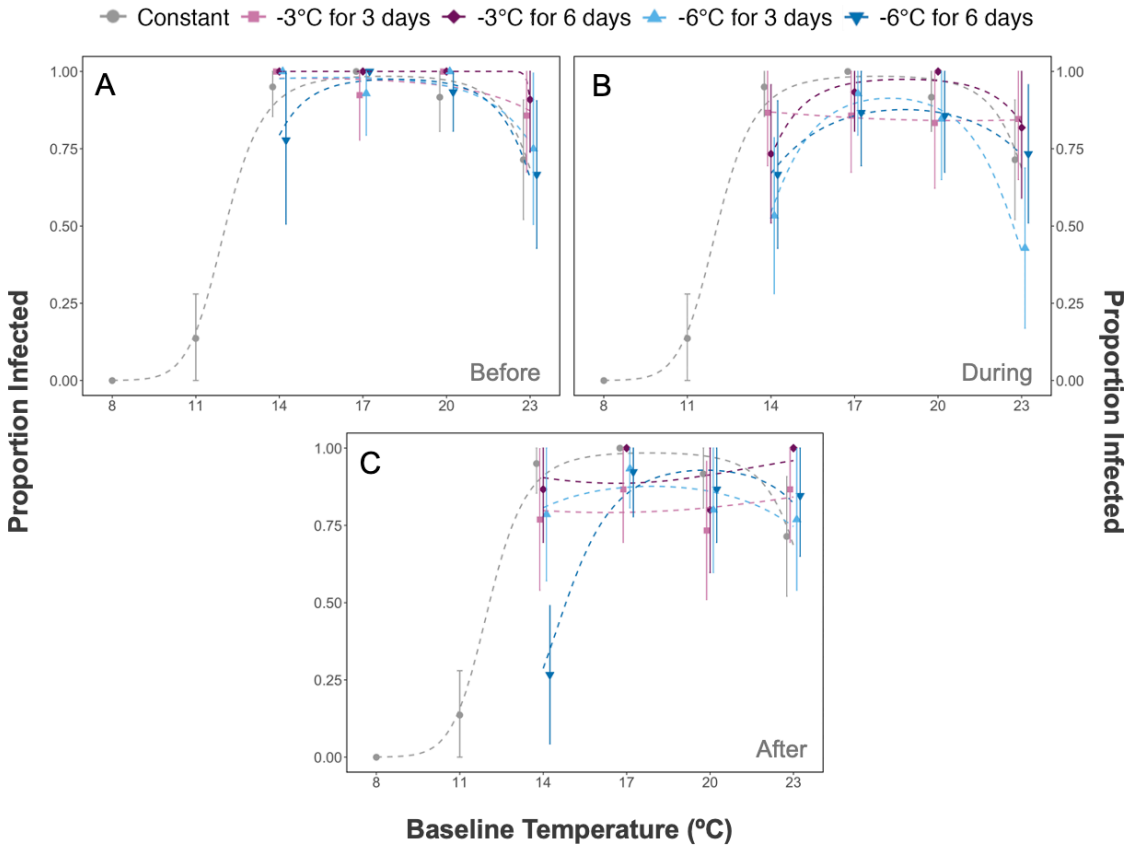


**Figure B.1 The effect of cold snap treatments on infection prevalence**
In *Daphnia magna* infected with *Ordospora colligata*. Each spore cluster contains 32-62 individual *Ordospora*. Error bars represent the standard error, and each dashed line is the linear fit of the elastic net regression (see Table B.1 for complete model output). Grey; constant treatment, pink; cold snap lasting 3 days where the temperature was decreased by 3°C, purple; cold snap lasting 6 days where the temperature was decreased by 3°C, light blue; cold snap lasting 3 days where the temperature was decreased by 6°C, dark blue; cold snap lasting 6 days where the temperature was decreased by 6°C. A; cold snap treatments which began before exposure, B; cold snap treatments which began on the day of exposure, C; cold snap treatments which began after exposure.

**Table B.1 Coefficient estimates and 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 increase 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 quadratic polynomial to account for non-linearity and is therefore separated by each degree (linear [L] and quadratic [Q]). Feature importance (Importance) is calculated by reporting the absolute values of coefficient effects for each variable.

| Explanatory Variable | Effect | Lower CI | Upper CI | Imp |
|---|---|---|---|---|
| X Intercept | 1.48 | 1.26 | 1.81 | 1.48 |
| X Intercept (1) | 0 | 0 | 0 | 0 |
| **Temperature (L)** | **10.93** | **4.94** | **16.11** | 10.93 |
| **Temperature (Q)** | **-27.05** | **-32.78** | **-23.18** | 27.05 |
| **Timing Before** | **0.61** | **0.13** | **1.02** | 0.61 |
| Timing During | 0 | -0.33 | 0 | 0 |
| Timing After | 0 | -0.21 | 0 | 0 |
| Treatment -3ºC for 3 days | 0 | 0 | 0.34 | 0 |
| Treatment -3ºC for 6 days | 0.38 | 0 | 0.87 | 0.38 |
| Treatment -6ºC for 3 days | 0 | -0.22 | 0 | 0 |
| Treatment -6ºC for 6 days | -0.29 | -0.65 | 0 | 0.29 |
| Temperature (L): Timing Before | 0 | -6.19 | 0 | 0 |
| Temperature (Q): Timing Before | -6.28 | -15.51 | 0 | 6.28 |
| Temperature (L): Timing During | 1.28 | 0 | 9.87 | 1.28 |
| Temperature (Q): Timing During | 0 | -3.76 | 0 | 0 |
| Temperature (L): Timing After | 0 | 0 | 9.33 | 0 |
| Temperature (Q): Timing After | 2.70 | 0 | 17.26 | 2.70 |
| Temperature (L): Treatment -3ºC for 3 days | 0 | -7.67 | 0 | 0 |
| Temperature (Q): Treatment -3ºC for 3 days | 5.59 | 0 | 20.46 | 5.59 |
| Temperature (L): Treatment -3ºC for 6 days | 0 | 0 | 9.61 | 0 |
| Temperature (Q): Treatment -3ºC for 6 days | 0 | 0 | 2.16 | 0 |
| Temperature (L): Treatment -6ºC for 3 days | 0 | -6.60 | 0 | 0 |
| Temperature (Q): Treatment -6ºC for 3 days | 0 | -12.35 | 0 | 0 |
| Temperature (L): Treatment -6ºC for 6 days | 1.37 | 0 | 15.77 | 1.37 |
| Temperature (Q): Treatment -6ºC for 6 days | 0 | -12.50 | 0 | 0 |
| Timing Before: Treatment -3ºC for 3 days | 0.09 | 0 | 0.85 | 0.9 |
| Timing During: Treatment -3ºC for 3 days | 0 | -0.12 | 0.57 | 0 |
| Timing After: Treatment -3ºC for 3 days | 0 | -0.53 | 0.11 | 0 |
| Timing Before: Treatment -3ºC for 6 days | 0.23 | 0 | 0.92 | 0.23 |
| Timing During: Treatment -3ºC for 6 days | 0 | -0.06 | 0.21 | 0 |
| Timing After: Treatment -3ºC for 6 days | 0.05 | 0 | 0.81 | 0.05 |

| | | | | |
|---|---|---|---|---|
| Timing Before: Treatment -6ºC for 3 days | 0 | 0 | 0.64 | 0 |
| Timing During: Treatment -6ºC for 3 days | -0.68 | -1.27 | 0 | 0.68 |
| Timing After: Treatment -6ºC for 3 days | 0 | -0.42 | 0.32 | 0 |
| Timing Before: Treatment -6ºC for 6 days | 0 | -0.36 | 0 | 0 |
| Timing During: Treatment -6ºC for 6 days | 0 | -0.45 | 0.05 | 0 |
| Timing After: Treatment -6ºC for 6 days | -0.20 | -0.94 | 0 | 0.20 |
| Temperature (L): Timing Before: Treatment -3ºC for 3 days | 0 | -4.91 | 0 | 0 |
| Temperature (Q): Timing Before: Treatment -3ºC for 3 days | 0 | -7.00 | 7.65 | 0 |
| Temperature (L): Timing During: Treatment -3ºC for 3 days | 0 | -13.42 | 5.53 | 0 |
| Temperature (Q): Timing During: Treatment -3ºC for 3 days | 0 | 0 | 28.86 | 0 |
| Temperature (L): Timing After: Treatment -3ºC for 3 days | 0 | -15.28 | 8.15 | 0 |
| Temperature (Q): Timing After: Treatment -3ºC for 3 days | 6.72 | 0 | 33.62 | 6.72 |
| Temperature (L): Timing Before: Treatment -3ºC for 6 days | 0 | -2.02 | 0 | 0 |
| Temperature (Q): Timing Before: Treatment -3ºC for 6 days | 0 | -11.01 | 0 | 0 |
| Temperature (L): Timing During: Treatment -3ºC for 6 days | 3.92 | 0 | 27.78 | 9.32 |
| Temperature (Q): Timing During: Treatment -3ºC for 6 days | 0 | -16.11 | 3.54 | 0 |
| Temperature (L): Timing After: Treatment -3ºC for 6 days | 0 | -6.86 | 13.46 | 0 |
| Temperature (Q): Timing After: Treatment -3ºC for 6 days | 6.68 | 0 | 23.52 | 6.68 |
| Temperature (L): Timing Before: Treatment -6ºC for 3 days | 0 | -11.96 | 0 | 0 |
| Temperature (Q): Timing Before: Treatment -6ºC for 3 days | 0 | -20.03 | 4.55 | 0 |
| Temperature (L): Timing During: Treatment -6ºC for 3 days | 0 | -6.05 | 17.17 | 0 |
| Temperature (Q): Timing During: Treatment -6ºC for 3 days | -9.98 | -34.48 | 0 | 9.98 |
| Temperature (L): Timing After: Treatment -6ºC for 3 days | 0 | -15.53 | 7.34 | 0 |
| Temperature (Q): Timing After: Treatment -6ºC for 3 days | 0 | -0.52 | 16.84 | 0 |
| Temperature (L): Timing Before: Treatment -6ºC for 6 days | 0 | -15.21 | 6.10 | 0 |
| Temperature (Q): Timing Before: Treatment -6ºC for 6 days | -5.24 | -30.34 | 0 | 5.24 |
| Temperature (L): Timing During: Treatment -6ºC for 6 days | 0 | -3.40 | 15.63 | |
| Temperature (Q): Timing During: Treatment -6ºC for 6 days | 0 | -5.87 | 17.78 | 0 |
| Temperature (L): Timing After: Treatment -6ºC for 6 days | **25.47** | **1.62** | **50.18** | 25.47 |
| Temperature (Q): Timing After: Treatment -6ºC for 6 days | 0 | -16.22 | 3.52 | 0 |

**Table B.2 Combined feature importance of infection prevalence.**
In *Daphnia magna* exposed to *Ordospora colligata*. Importance values were derived by taking the absolute values of the model coefficients for each variable. For variables included as polynomials, the contributions from the linear and quadratic terms were summed to obtain the overall importance.

| Explanatory Variable | Importance |
|---|---|
| Temperature | 37.98 |
| Temperature: Timing After: Treatment -6ºC for 6 days | 25.47 |
| Temperature: Timing During: Treatment -6ºC for 3 days | 9.98 |
| Temperature: Timing After: Treatment -3ºC for 3 days | 6.72 |
| Temperature: Timing After: Treatment -3ºC for 6 days | 6.68 |
| Temperature: Timing Before | 6.28 |
| Temperature: Treatment -3ºC for 3 days | 5.59 |
| Temperature: Timing Before: Treatment -6ºC for 6 days | 5.24 |
| Temperature: Timing During: Treatment -3ºC for 6 days | 3.92 |
| Temperature: Timing After | 2.70 |
| Temperature: Treatment -6ºC for 6 days | 1.37 |
| Temperature: Timing During | 1.28 |
| Timing During: Treatment -6ºC for 3 days | 0.68 |
| Timing Before | 0.61 |
| Treatment -3ºC for 6 days | 0.38 |
| Treatment -6ºC for 6 days | 0.29 |
| Timing Before: Treatment -3ºC for 6 days | 0.23 |
| Timing After: Treatment -6ºC for 6 days | 0.20 |
| Timing Before: Treatment -3ºC for 3 days | 0.09 |
| Timing After: Treatment -3ºC for 6 days | 0.05 |
| (Intercept) | 0 |
| Timing During | 0 |
| Timing After | 0 |
| Treatment -3ºC for 3 days | 0 |
| Treatment -6ºC for 3 days | 0 |
| Temperature: Treatment -3ºC for 6 days | 0 |
| Temperature: Treatment -6ºC for 3 days | 0 |
| Timing During: Treatment -3ºC for 3 days | 0 |

| | |
|---|---|
| Timing After: Treatment -3ºC for 3 days | 0 |
| Timing During: Treatment -3ºC for 6 days | 0 |
| Timing Before: Treatment -6ºC for 3 days | 0 |
| Timing After: Treatment -6ºC for 3 days | 0 |
| Timing Before: Treatment -6ºC for 6 days | 0 |
| Timing During: Treatment -6ºC for 6 days | 0 |
| Temperature: Timing Before: Treatment -3ºC for 3 days | 0 |
| Temperature: Timing During: Treatment -3ºC for 3 days | 0 |
| Temperature: Timing Before: Treatment -3ºC for 6 days | 0 |
| Temperature: Timing Before: Treatment -6ºC for 3 days | 0 |
| Temperature: Timing After: Treatment -6ºC for 3 days | 0 |
| Temperature: Timing During: Treatment -6ºC for 6 days | 0 |

**Table B.3 Estimated marginal means (emmeans) for infection prevalence.**
In *Daphnia magna* exposed to *Ordospora colligata*. Estimates were obtained from a Generalised Linear Model (GLM) that included baseline temperature as a categorical variable and a composite treatment variable (incorporating timing, amplitude, and duration), using a binomial error distribution. Timing is shortened in the table for clarity; C; Constant, B; Before, D; During, A; After. Results are presented on the log scale to more accurately reflect the distribution of the data. The infinite degrees of freedom ('Inf') reflect the model's high flexibility, stemming from its complexity.

| Temp | Treatment | emmean | SE | df | Lower CI 95% | Upper CI 95% |
|---|---|---|---|---|---|---|
| 14 | C 0 0 | 2.944 | 1.026 | Inf | 0.934 | 4.955 |
| 17 | C 0 0 | 18.566 | 1360.064 | Inf | -2647.111 | 2684.243 |
| 20 | C 0 0 | 2.398 | 0.739 | Inf | 0.95 | 3.845 |
| 23 | C 0 0 | 0.916 | 0.483 | Inf | -0.03 | 1.863 |
| 14 | B -3 3 | 18.566 | 2062.639 | Inf | -4024.133 | 4061.265 |
| 17 | B -3 3 | 2.485 | 1.041 | Inf | 0.445 | 4.525 |
| 20 | B -3 3 | 18.566 | 1743.248 | Inf | -3398.138 | 3435.27 |
| 23 | B -3 3 | 1.792 | 0.764 | Inf | 0.295 | 3.289 |
| 14 | B -3 6 | 18.566 | 2062.639 | Inf | -4024.133 | 4061.265 |
| 17 | B -3 6 | 18.566 | 1684.138 | Inf | -3282.284 | 3319.416 |
| 20 | B -3 6 | 18.566 | 1684.138 | Inf | -3282.284 | 3319.416 |
| 23 | B -3 6 | 2.303 | 1.049 | Inf | 0.247 | 4.358 |
| 14 | B -6 3 | 18.566 | 2062.639 | Inf | -4024.133 | 4061.265 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | B -6 3 | 2.565 | 1.038 | Inf | 0.531 | 4.599 |
| 20 | B -6 3 | 18.566 | 1684.138 | Inf | -3282.284 | 3319.416 |
| 23 | B -6 3 | 1.099 | 0.667 | Inf | -0.208 | 2.405 |
| 14 | B -6 6 | 1.253 | 0.802 | Inf | -0.319 | 2.824 |
| 17 | B -6 6 | 18.566 | 1743.248 | Inf | -3398.138 | 3435.27 |
| 20 | B -6 6 | 2.639 | 1.035 | Inf | 0.61 | 4.668 |
| 23 | B -6 6 | 0.693 | 0.548 | Inf | -0.38 | 1.767 |
| 14 | D -3 3 | 1.872 | 0.76 | Inf | 0.383 | 3.361 |
| 17 | D -3 3 | 1.792 | 0.764 | Inf | 0.295 | 3.289 |
| 20 | D -3 3 | 1.609 | 0.775 | Inf | 0.091 | 3.128 |
| 23 | D -3 3 | 1.705 | 0.769 | Inf | 0.198 | 3.211 |
| 14 | D -3 6 | 1.012 | 0.584 | Inf | -0.133 | 2.156 |
| 17 | D -3 6 | 2.639 | 1.035 | Inf | 0.61 | 4.668 |
| 20 | D -3 6 | 18.566 | 1684.138 | Inf | -3282.284 | 3319.416 |
| 23 | D -3 6 | 1.504 | 0.782 | Inf | -0.028 | 3.036 |
| 14 | D -6 3 | 0.134 | 0.517 | Inf | -0.881 | 1.148 |
| 17 | D -6 3 | 2.565 | 1.038 | Inf | 0.531 | 4.599 |
| 20 | D -6 3 | 1.705 | 0.769 | Inf | 0.198 | 3.211 |
| 23 | D -6 3 | -0.288 | 0.54 | Inf | -1.346 | 0.771 |
| 14 | D -6 6 | 0.693 | 0.548 | Inf | -0.38 | 1.767 |
| 17 | D -6 6 | 1.872 | 0.76 | Inf | 0.383 | 3.361 |
| 20 | D -6 6 | 1.792 | 0.764 | Inf | 0.295 | 3.289 |
| 23 | D -6 6 | 1.012 | 0.584 | Inf | -0.133 | 2.156 |
| 14 | A -3 3 | 1.204 | 0.658 | Inf | -0.086 | 2.494 |
| 17 | A -3 3 | 1.872 | 0.76 | Inf | 0.383 | 3.361 |
| 20 | A -3 3 | 1.012 | 0.584 | Inf | -0.133 | 2.156 |
| 23 | A -3 3 | 1.872 | 0.76 | Inf | 0.383 | 3.361 |
| 14 | A -3 6 | 1.872 | 0.76 | Inf | 0.383 | 3.361 |
| 17 | A -3 6 | 18.566 | 1684.138 | Inf | -3282.284 | 3319.416 |
| 20 | A -3 6 | 1.386 | 0.645 | Inf | 0.121 | 2.651 |
| 23 | A -3 6 | 18.566 | 1743.248 | Inf | -3398.138 | 3435.27 |
| 14 | A -6 3 | 1.299 | 0.651 | Inf | 0.023 | 2.576 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | A -6 3 | 2.639 | 1.035 | Inf | 0.61 | 4.668 |
| 20 | A -6 3 | 1.386 | 0.645 | Inf | 0.121 | 2.651 |
| 23 | A -6 3 | 1.204 | 0.658 | Inf | -0.086 | 2.494 |
| 14 | A -6 6 | -1.012 | 0.584 | Inf | -2.156 | 0.133 |
| 17 | A -6 6 | 2.485 | 1.041 | Inf | 0.445 | 4.525 |
| 20 | A -6 6 | 1.872 | 0.76 | Inf | 0.383 | 3.361 |
| 23 | A -6 6 | 1.705 | 0.769 | Inf | 0.198 | 3.211 |

**Table B.4 Custom contrasts of estimated marginal means (emmeans) for infection prevalence.**

In *Daphnia magna* infected with *Ordospora colligata*, comparing treatments across different baseline temperatures. Each treatment's emmean was contrasted either with its constant counterpart at the same temperature or with alternate amplitudes or durations within the same heatwave timing. Infinite degrees of freedom ('Inf') reflect the model's complexity, which limits precise estimation of uncertainty. Statistically significant contrasts are shown in bold. P-values were adjusted using the Benjamini-Hochberg method.

| Day | Temp | Contrast | Estimate | SE | df | z ratio | p value |
|---|---|---|---|---|---|---|---|
| Before | 14 | -33 v 00 | -0.42917 | 0.346 | Inf | -1.239 | 1 |
| Before | 14 | -36 v 00 | 0.11622 | 0.345 | Inf | 0.336 | 1 |
| Before | 14 | -63 v 00 | 0.06965 | 0.346 | Inf | 0.202 | 1 |
| Before | 14 | -66 v 00 | 0.09214 | 0.391 | Inf | 0.236 | 1 |
| Before | 14 | -36 v -33 | 0.54539 | 0.396 | Inf | 1.377 | 1 |
| Before | 14 | -66 v -63 | 0.02249 | 0.436 | Inf | 0.052 | 1 |
| Before | 14 | -63 v -33 | 0.49882 | 0.396 | Inf | 1.259 | 1 |
| Before | 14 | -66 v -36 | -0.02408 | 0.436 | Inf | -0.055 | 1 |
| Before | 17 | -33 v 00 | -0.10155 | 0.323 | Inf | -0.315 | 1 |
| Before | 17 | -36 v 00 | 0.25275 | 0.292 | Inf | 0.865 | 1 |
| Before | 17 | -63 v 00 | 0.38255 | 0.305 | Inf | 1.253 | 1 |
| Before | 17 | -66 v 00 | -0.07653 | 0.298 | Inf | -0.256 | 1 |
| Before | 17 | -36 v -33 | 0.3543 | 0.349 | Inf | 1.014 | 1 |
| Before | 17 | -66 v -63 | -0.45908 | 0.339 | Inf | -1.354 | 1 |
| Before | 17 | -63 v -33 | 0.4841 | 0.361 | Inf | 1.343 | 1 |
| Before | 17 | -66 v -36 | -0.32928 | 0.327 | Inf | -1.007 | 1 |
| Before | 20 | -33 v 00 | -0.16957 | 0.301 | Inf | -0.564 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Before | 20 | -36 v 00 | 0.0739 | 0.295 | Inf | 0.251 | 1 |
| Before | 20 | -63 v 00 | 0.13904 | 0.295 | Inf | 0.472 | 1 |
| Before | 20 | -66 v 00 | -0.26897 | 0.301 | Inf | -0.894 | 1 |
| Before | 20 | -36 v -33 | 0.24347 | 0.327 | Inf | 0.744 | 1 |
| Before | 20 | -66 v -63 | -0.40801 | 0.327 | Inf | -1.248 | 1 |
| Before | 20 | -63 v -33 | 0.30861 | 0.327 | Inf | 0.944 | 1 |
| Before | 20 | -66 v -36 | -0.34287 | 0.327 | Inf | -1.048 | 1 |
| Before | 23 | -33 v 00 | 0.74866 | 0.351 | Inf | 2.136 | 1 |
| Before | 23 | -36 v 00 | 1.08315 | 0.372 | Inf | 2.911 | 1 |
| Before | 23 | -63 v 00 | 0.83586 | 0.372 | Inf | 2.246 | 1 |
| Before | 23 | -66 v 00 | 0.13453 | 0.373 | Inf | 0.361 | 1 |
| Before | 23 | -36 v -33 | 0.33449 | 0.396 | Inf | 0.844 | 1 |
| Before | 23 | -66 v -63 | -0.70133 | 0.416 | Inf | -1.685 | 1 |
| Before | 23 | -63 v -33 | 0.0872 | 0.396 | Inf | 0.22 | 1 |
| Before | 23 | -66 v -36 | -0.94862 | 0.416 | Inf | -2.28 | 1 |
| During | 14 | -33 v 00 | -0.17945 | 0.319 | Inf | -0.563 | 1 |
| During | 14 | -36 v 00 | 0.20867 | 0.335 | Inf | 0.623 | 0.9825 |
| During | 14 | -63 v 00 | -0.02292 | 0.373 | Inf | -0.061 | 0.3466 |
| During | 14 | -66 v 00 | -0.53157 | 0.347 | Inf | -1.533 | 0.8036 |
| During | 14 | -36 v -33 | 0.38813 | 0.362 | Inf | 1.071 | 1 |
| During | 14 | -66 v -63 | -0.50865 | 0.421 | Inf | -1.21 | 1 |
| During | 14 | -63 v -33 | 0.15653 | 0.398 | Inf | 0.394 | 0.8036 |
| During | 14 | -66 v -36 | -0.74024 | 0.387 | Inf | -1.912 | 1 |
| During | 17 | -33 v 00 | -0.34371 | 0.306 | Inf | -1.125 | 1 |
| During | 17 | -36 v 00 | -0.02462 | 0.298 | Inf | -0.083 | 1 |
| During | 17 | -63 v 00 | 0.0236 | 0.305 | Inf | 0.077 | 1 |
| During | 17 | -66 v 00 | -0.46742 | 0.306 | Inf | -1.53 | 1 |
| During | 17 | -36 v -33 | 0.31909 | 0.339 | Inf | 0.941 | 1 |
| During | 17 | -66 v -63 | -0.49102 | 0.345 | Inf | -1.422 | 1 |
| During | 17 | -63 v -33 | 0.36731 | 0.345 | Inf | 1.064 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| During | 17 | -66 v -36 | -0.44279 | 0.339 | Inf | -1.306 | 1 |
| During | 20 | -33 v 00 | 0.24883 | 0.336 | Inf | 0.742 | 1 |
| During | 20 | -36 v 00 | -0.11528 | 0.295 | Inf | -0.391 | 1 |
| During | 20 | -63 v 00 | -0.00874 | 0.325 | Inf | -0.027 | 1 |
| During | 20 | -66 v 00 | -0.43139 | 0.316 | Inf | -1.365 | 1 |
| During | 20 | -36 v -33 | -0.36411 | 0.359 | Inf | -1.014 | 1 |
| During | 20 | -66 v -63 | -0.42265 | 0.367 | Inf | -1.15 | 1 |
| During | 20 | -63 v -33 | -0.25757 | 0.384 | Inf | -0.67 | 1 |
| During | 20 | -66 v -36 | -0.31612 | 0.341 | Inf | -0.927 | 1 |
| During | 23 | -33 v 00 | -0.44028 | 0.352 | Inf | -1.251 | 1 |
| During | 23 | -36 v 00 | -0.19295 | 0.388 | Inf | -0.498 | 1 |
| During | 23 | -63 v 00 | -0.58252 | 0.429 | Inf | -1.358 | 0.9825 |
| During | 23 | -66 v 00 | -0.33686 | 0.352 | Inf | -0.958 | 1 |
| During | 23 | -36 v -33 | 0.24733 | 0.412 | Inf | 0.6 | 1 |
| During | 23 | -66 v -63 | 0.24566 | 0.451 | Inf | 0.545 | 0.9825 |
| During | 23 | -63 v -33 | -0.14224 | 0.451 | Inf | -0.315 | 0.6516 |
| During | 23 | -66 v -36 | -0.14391 | 0.412 | Inf | -0.349 | 1 |
| After | 14 | -33 v 00 | -0.92236 | 0.348 | Inf | -2.652 | 1 |
| After | 14 | -36 v 00 | -1.58762 | 0.323 | Inf | -4.912 | 1 |
| After | 14 | -63 v 00 | -1.10913 | 0.338 | Inf | -3.282 | 1 |
| After | 14 | -66 v 00 | -2.83416 | 0.527 | Inf | -5.378 | 0.0772 |
| After | 14 | -36 v -33 | -0.66526 | 0.378 | Inf | -1.759 | 1 |
| After | 14 | -66 v -63 | -1.72502 | 0.556 | Inf | -3.1 | 0.2639 |
| After | 14 | -63 v -33 | -0.18677 | 0.391 | Inf | -0.478 | 1 |
| After | 14 | -66 v -36 | -1.24653 | 0.548 | Inf | -2.277 | 0.1255 |
| After | 17 | -33 v 00 | 0.23032 | 0.305 | Inf | 0.754 | 1 |
| After | 17 | -36 v 00 | -0.0428 | 0.292 | Inf | -0.147 | 1 |
| After | 17 | -63 v 00 | -0.37213 | 0.298 | Inf | -1.247 | 1 |
| After | 17 | -66 v 00 | -1.22904 | 0.314 | Inf | -3.912 | 1 |
| After | 17 | -36 v -33 | -0.27312 | 0.333 | Inf | -0.819 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| After | 17 | -66 v -63 | -0.8569 | 0.347 | Inf | -2.469 | 1 |
| After | 17 | -63 v -33 | -0.60245 | 0.339 | Inf | -1.777 | 1 |
| After | 17 | -66 v -36 | -1.18623 | 0.342 | Inf | -3.473 | 1 |
| After | 20 | -33 v 00 | -0.26446 | 0.325 | Inf | -0.814 | 1 |
| After | 20 | -36 v 00 | -0.87894 | 0.316 | Inf | -2.78 | 1 |
| After | 20 | -63 v 00 | -0.31109 | 0.316 | Inf | -0.985 | 1 |
| After | 20 | -66 v 00 | -0.42932 | 0.308 | Inf | -1.394 | 1 |
| After | 20 | -36 v -33 | -0.61447 | 0.368 | Inf | -1.671 | 1 |
| After | 20 | -66 v -63 | -0.11823 | 0.352 | Inf | -0.335 | 1 |
| After | 20 | -63 v -33 | -0.04663 | 0.367 | Inf | -0.127 | 1 |
| After | 20 | -66 v -36 | 0.44961 | 0.353 | Inf | 1.275 | 1 |
| After | 23 | -33 v 00 | 0.2118 | 0.342 | Inf | 0.619 | 1 |
| After | 23 | -36 v 00 | 1.15233 | 0.334 | Inf | 3.445 | 1 |
| After | 23 | -63 v 00 | 0.79983 | 0.36 | Inf | 2.219 | 1 |
| After | 23 | -66 v 00 | 1.42854 | 0.35 | Inf | 4.079 | 1 |
| After | 23 | -36 v -33 | 0.94052 | 0.353 | Inf | 2.663 | 1 |
| After | 23 | -66 v -63 | 0.62871 | 0.385 | Inf | 1.633 | 1 |
| After | 23 | -63 v -33 | 0.58802 | 0.378 | Inf | 1.556 | 1 |
| After | 23 | -66 v -36 | 0.27621 | 0.361 | Inf | 0.766 | 1 |

**Table B.5 Coefficient estimates and feature importance of burden.**
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 increase 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 to account for non-linearity and is therefore separated by each degree (linear [L], quadratic [Q], and cubic [C]). Feature importance (Importance) is calculated by reporting the absolute values of coefficient effects for each variable.

| Explanatory Variable | Effect | Lower CI | Upper CI | Imp |
|---|---|---|---|---|
| X Intercept | 5.36 | 5.23 | 5.51 | 5.36 |
| X Intercept (1) | 0 | 0 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (L) | 1.05 | 0 | 4.69 | 1.05 |
| **Temperature (Q)** | **-18.09** | **-20.63** | **-15.65** | 18.09 |
| Temperature (C) | 0 | -0.27 | 1.45 | 0 |
| Timing Before | 0.12 | 0 | 0.31 | 0.12 |
| Timing During | -0.01 | -0.28 | 0 | -0.01 |
| Timing After | -0.13 | -0.42 | 0 | -0.13 |
| Treatment -3ºC for 3 days | 0 | -0.11 | 0 | 0 |
| Treatment -3ºC for 6 days | 0 | 0 | 0.01 | 0 |
| Treatment -6ºC for 3 days | 0 | 0 | 0.11 | 0 |
| Treatment -6ºC for 6 days | -0.14 | -0.34 | 0 | 0.14 |
| Temperature (L): Timing Before | 3.43 | 0 | 5.98 | 3.43 |
| Temperature (Q): Timing Before | 0 | 0 | 2.33 | 0 |
| Temperature (C): Timing Before | 2.13 | 0 | 4.00 | 2.13 |
| Temperature (L): Timing During | 0 | -2.46 | 0.79 | 0 |
| Temperature (Q): Timing During | -2.63 | -7.43 | 0 | 2.63 |
| Temperature (C): Timing During | 0 | -4.09 | 0 | 0 |
| **Temperature (L): Timing After** | **8.65** | **1.30** | **16.74** | 8.65 |
| Temperature (Q): Timing After | 0 | -4.06 | 0 | 0 |
| Temperature (C): Timing After | 4.43 | 0 | 7.73 | 4.43 |
| Temperature (L): Treatment -3ºC for 3 days | 0 | 0 | 3.17 | 0 |
| Temperature (Q): Treatment -3ºC for 3 days | 0 | -5.16 | 0 | 0 |
| Temperature (C): Treatment -3ºC for 3 days | 0 | 0 | 0 | 0 |
| Temperature (L): Treatment -3ºC for 6 days | 0 | 0 | 7.88 | 0 |
| Temperature (Q): Treatment -3ºC for 6 days | 0 | -2.17 | 0 | 0 |
| Temperature (C): Treatment -3ºC for 6 days | 0.63 | 0 | 5.62 | 0.63 |
| Temperature (L): Treatment -6ºC for 3 days | 0 | 0 | 5.55 | 0 |
| Temperature (Q): Treatment -6ºC for 3 days | 0 | -4.45 | 0 | 0 |
| Temperature (C): Treatment -6ºC for 3 days | 0 | -0.38 | 2.11 | 0 |
| Temperature (L): Treatment -6ºC for 6 days | 0 | 0 | 2.84 | 0 |
| Temperature (Q): Treatment -6ºC for 6 days | 0 | -0.31 | 1.93 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (C): Treatment -6ºC for 6 days | 0 | -0.33 | 1.81 | 0 |
| Timing Before: Treatment -3ºC for 3 days | 0 | -0.16 | 0 | 0 |
| Timing During: Treatment -3ºC for 3 days | 0 | -0.33 | 0.11 | 0 |
| Timing After: Treatment -3ºC for 3 days | 0 | 0 | 0.28 | 0 |
| Timing Before: Treatment -3ºC for 6 days | 0.23 | 0 | 0.47 | 0.23 |
| Timing During: Treatment -3ºC for 6 days | 0 | -0.31 | 0.25 | 0 |
| Timing After: Treatment -3ºC for 6 days | -0.13 | -0.39 | 0 | 0.13 |
| Timing Before: Treatment -6ºC for 3 days | 0.21 | 0 | 0.46 | 0.21 |
| Timing During: Treatment -6ºC for 3 days | 0 | -0.35 | 0.14 | 0 |
| Timing After: Treatment -6ºC for 3 days | 0 | -0.25 | 0.04 | 0 |
| Timing Before: Treatment -6ºC for 6 days | 0 | 0 | 0 | 0 |
| Timing During: Treatment -6ºC for 6 days | -0.15 | -0.56 | 0 | 0.15 |
| Timing After: Treatment -6ºC for 6 days | -0.28 | -0.57 | 0 | 0.28 |
| Temperature (L): Timing Before: Treatment -3ºC for 3 days | 3.74 | 0 | 14.17 | 3.74 |
| Temperature (Q): Timing Before: Treatment -3ºC for 3 days | 0 | -2.08 | 4.15 | 0 |
| Temperature (C): Timing Before: Treatment -3ºC for 3 days | 0.01 | 0 | 6.22 | 0.01 |
| Temperature (L): Timing During: Treatment -3ºC for 3 days | 0 | -7.01 | 7.48 | 0 |
| Temperature (Q): Timing During: Treatment -3ºC for 3 days | 0 | -9.60 | 1.74 | 0 |
| Temperature (C): Timing During: Treatment -3ºC for 3 days | -6.16 | -13.88 | 0 | 6.16 |
| Temperature (L): Timing After: Treatment -3ºC for 3 days | 0 | -9.31 | 0 | 0 |
| Temperature (Q): Timing After: Treatment -3ºC for 3 days | -4.81 | -12.88 | 0 | 4.81 |
| Temperature (C): Timing After: Treatment -3ºC for 3 days | 0.17 | 0 | 9.62 | 0.17 |
| Temperature (L): Timing Before: Treatment -3ºC for 6 days | 1.03 | 0 | 8.55 | 1.03 |
| Temperature (Q): Timing Before: Treatment -3ºC for 6 days | 1.16 | 0 | 6.06 | 1.16 |
| Temperature (C): Timing Before: Treatment -3ºC for 6 days | 0 | 0 | 4.12 | 0 |
| Temperature (L): Timing During: Treatment -3ºC for 6 days | -0.01 | -11.53 | 5.84 | 0.01 |
| Temperature (Q): Timing During: Treatment -3ºC for 6 days | 0 | -12.42 | 3.68 | 0 |
| Temperature (C): Timing During: Treatment -3ºC for 6 days | 0 | -3.68 | 2.98 | 0 |
| Temperature (L): Timing After: Treatment -3ºC for 6 days | 10.75 | 0 | 18.68 | 10.75 |
| Temperature (Q): Timing After: Treatment -3ºC for 6 days | -2.46 | -8.66 | 0 | 2.46 |

| | | | | |
|---|---|---|---|---|
| **Temperature (C): Timing After: Treatment -3ºC for 6 days** | **11.20** | **2.53** | **20.58** | 11.20 |
| Temperature (L): Timing Before: Treatment -6ºC for 3 days | 0 | -1.16 | 7.55 | 0 |
| Temperature (Q): Timing Before: Treatment -6ºC for 3 days | 0 | -6.22 | 2.33 | 0 |
| Temperature (C): Timing Before: Treatment -6ºC for 3 days | 0 | -0.21 | 6.46 | 0 |
| Temperature (L): Timing During: Treatment -6ºC for 3 days | 0 | -9.24 | 6.03 | 0 |
| Temperature (Q): Timing During: Treatment -6ºC for 3 days | -1.48 | -14.82 | 0 | 1.48 |
| Temperature (C): Timing During: Treatment -6ºC for 3 days | 0 | -5.35 | 2.28 | 0 |
| Temperature (L): Timing After: Treatment -6ºC for 3 days | 3.72 | 0 | 11.97 | 3.72 |
| Temperature (Q): Timing After: Treatment -6ºC for 3 days | 0 | -5.06 | 5.01 | 0 |
| Temperature (C): Timing After: Treatment -6ºC for 3 days | 0 | -4.07 | 3.21 | 0 |
| Temperature (L): Timing Before: Treatment -6ºC for 6 days | -1.14 | -5.67 | 0 | 1.14 |
| Temperature (Q): Timing Before: Treatment -6ºC for 6 days | 0 | -1.80 | 2.03 | 0 |
| Temperature (C): Timing Before: Treatment -6ºC for 6 days | 0 | -1.34 | 1.63 | 0 |
| Temperature (L): Timing During: Treatment -6ºC for 6 days | 0.28 | -2.03 | 10.01 | 0.28 |
| Temperature (Q): Timing During: Treatment -6ºC for 6 days | 0 | -8.36 | 2.32 | 0 |
| Temperature (C): Timing During: Treatment -6ºC for 6 days | 0 | -5.09 | 5.34 | 0 |
| **Temperature (L): Timing After: Treatment -6ºC for 6 days** | **22.26** | **7.81** | **30.46** | 22.26 |
| Temperature (Q): Timing After: Treatment -6ºC for 6 days | 0 | 0 | 9.49 | 0 |
| Temperature (C): Timing After: Treatment -6ºC for 6 days | 0 | -0.22 | 4.33 | 0 |

**Table B.6 Combined feature importance of burden.**
In *Daphnia magna* exposed to *Ordospora colligata*. Importance values were derived by taking the absolute values of the model coefficients for each variable. For variables included as polynomials, the contributions from the linear and quadratic terms were summed to obtain the overall importance.

| Explanatory Variable | Importance |
|---|---|
| Temperature: Timing After: Treatment -3ºC for 6 days | 24.42 |
| Temperature: Timing After: Treatment -6ºC for 6 days | 22.26 |
| Temperature | 19.15 |
| Temperature: Timing After | 13.08 |
| Temperature: Timing During: Treatment -3ºC for 3 days | 6.16 |
| (Intercept) | 5.36 |
| Temperature: Treatment -3ºC for 3 days | 5.59 |

| | |
|---|---|
| Temperature: Timing Before | 5.56 |
| Temperature: Timing After: Treatment -3ºC for 3 days | 4.98 |
| Temperature: Timing Before: Treatment -3ºC for 3 days | 3.75 |
| Temperature: Timing After: Treatment -6ºC for 3 days | 3.72 |
| Temperature: Timing During | 2.63 |
| Temperature: Timing Before: Treatment -3ºC for 6 days | 2.19 |
| Temperature: Timing During: Treatment -6ºC for 3 days | 1.48 |
| Temperature: Timing Before: Treatment -6ºC for 6 days | 1.14 |
| Temperature: Treatment -3ºC for 6 days | 0.63 |
| Temperature: Timing During: Treatment -6ºC for 6 days | 0.28 |
| Timing After: Treatment -6ºC for 6 days | 0.28 |
| Timing Before: Treatment -3ºC for 6 days | 0.23 |
| Timing Before: Treatment -6ºC for 3 days | 0.21 |
| Timing During: Treatment -6ºC for 6 days | 0.15 |
| Treatment -6ºC for 6 days | 0.14 |
| Timing After: Treatment -3ºC for 6 days | 0.13 |
| Timing After | 0.13 |
| Timing Before | 0.12 |
| Timing During | 0.01 |
| Temperature: Timing During: Treatment -3ºC for 6 days | 0.01 |
| Treatment -3ºC for 3 days | 0 |
| Treatment -3ºC for 6 days | 0 |
| Treatment -6ºC for 3 days | 0 |
| Temperature: Treatment -3ºC for 3 days | 0 |
| Temperature: Treatment -6ºC for 3 days | 0 |
| Temperature: Treatment -6ºC for 6 days | 0 |
| Timing Before: Treatment -3ºC for 3 days | 0 |
| Timing During: Treatment -3ºC for 3 days | 0 |
| Timing After: Treatment -3ºC for 3 days | 0 |
| Timing During: Treatment -3ºC for 6 days | 0 |
| Timing During: Treatment -6ºC for 3 days | 0 |
| Timing After: Treatment -6ºC for 3 days | 0 |

| | |
|---|---|
| Timing Before: Treatment -6ºC for 6 days | 0 |
| Temperature: Timing Before: Treatment -6ºC for 3 days | 0 |

**Table B.7 Estimated marginal means (emmeans) for burden.**
In *Daphnia magna* exposed to *Ordospora colligata*. Estimates were obtained from a Generalised Linear Model (GLM) that included baseline temperature as a categorical variable and a composite treatment variable (incorporating timing, amplitude, and duration), using a binomial error distribution. Timing is shortened in the table for clarity; C; Constant, B; Before, D; During, A; After. Results are presented on the log scale to more accurately reflect the distribution of the data. The infinite degrees of freedom ('Inf') reflect the model's high flexibility, stemming from its complexity.

| Temp | Treatment | emmean | SE | df | Lower CI 95% | Upper CI 95% |
|---|---|---|---|---|---|---|
| 14 | C 0 0 | 4.58 | 0.203 | Inf | 4.186 | 4.98 |
| 17 | C 0 0 | 6.06 | 0.184 | Inf | 5.697 | 6.42 |
| 20 | C 0 0 | 6.19 | 0.188 | Inf | 5.818 | 6.55 |
| 23 | C 0 0 | 4.55 | 0.228 | Inf | 4.102 | 5 |
| 14 | B -3 3 | 4.15 | 0.281 | Inf | 3.604 | 4.7 |
| 17 | B -3 3 | 5.96 | 0.265 | Inf | 5.435 | 6.48 |
| 20 | B -3 3 | 6.02 | 0.235 | Inf | 5.555 | 6.48 |
| 23 | B -3 3 | 5.3 | 0.266 | Inf | 4.777 | 5.82 |
| 14 | B -3 6 | 4.7 | 0.28 | Inf | 4.152 | 5.25 |
| 17 | B -3 6 | 6.31 | 0.227 | Inf | 5.864 | 6.75 |
| 20 | B -3 6 | 6.26 | 0.227 | Inf | 5.814 | 6.7 |
| 23 | B -3 6 | 5.63 | 0.294 | Inf | 5.057 | 6.21 |
| 14 | B -6 3 | 4.65 | 0.28 | Inf | 4.105 | 5.2 |
| 17 | B -6 3 | 6.44 | 0.244 | Inf | 5.961 | 6.92 |
| 20 | B -6 3 | 6.32 | 0.227 | Inf | 5.879 | 6.77 |
| 23 | B -6 3 | 5.39 | 0.294 | Inf | 4.81 | 5.96 |
| 14 | B -6 6 | 4.68 | 0.334 | Inf | 4.021 | 5.33 |
| 17 | B -6 6 | 5.98 | 0.235 | Inf | 5.519 | 6.44 |
| 20 | B -6 6 | 5.92 | 0.235 | Inf | 5.455 | 6.38 |
| 23 | B -6 6 | 4.68 | 0.295 | Inf | 4.107 | 5.26 |
| 14 | D -3 3 | 4.4 | 0.246 | Inf | 3.922 | 4.89 |
| 17 | D -3 3 | 5.71 | 0.244 | Inf | 5.234 | 6.19 |
| 20 | D -3 3 | 6.43 | 0.278 | Inf | 5.889 | 6.98 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 23 | D -3 3 | 4.11 | 0.268 | Inf | 3.585 | 4.63 |
| 14 | D -3 6 | 4.79 | 0.266 | Inf | 4.27 | 5.31 |
| 17 | D -3 6 | 6.03 | 0.235 | Inf | 5.571 | 6.49 |
| 20 | D -3 6 | 6.07 | 0.227 | Inf | 5.625 | 6.52 |
| 23 | D -3 6 | 4.36 | 0.313 | Inf | 3.743 | 4.97 |
| 14 | D -6 3 | 4.56 | 0.313 | Inf | 3.947 | 5.17 |
| 17 | D -6 3 | 6.08 | 0.244 | Inf | 5.602 | 6.56 |
| 20 | D -6 3 | 6.18 | 0.265 | Inf | 5.657 | 6.7 |
| 23 | D -6 3 | 3.97 | 0.363 | Inf | 3.255 | 4.68 |
| 14 | D -6 6 | 4.05 | 0.281 | Inf | 3.501 | 4.6 |
| 17 | D -6 6 | 5.59 | 0.244 | Inf | 5.11 | 6.07 |
| 20 | D -6 6 | 5.75 | 0.254 | Inf | 5.256 | 6.25 |
| 23 | D -6 6 | 4.21 | 0.268 | Inf | 3.688 | 4.74 |
| 14 | A -3 3 | 3.66 | 0.282 | Inf | 3.107 | 4.21 |
| 17 | A -3 3 | 6.29 | 0.244 | Inf | 5.809 | 6.77 |
| 20 | A -3 3 | 5.92 | 0.265 | Inf | 5.401 | 6.44 |
| 23 | A -3 3 | 4.76 | 0.255 | Inf | 4.261 | 5.26 |
| 14 | A -3 6 | 3 | 0.252 | Inf | 2.503 | 3.49 |
| 17 | A -3 6 | 6.01 | 0.227 | Inf | 5.568 | 6.46 |
| 20 | A -3 6 | 5.31 | 0.255 | Inf | 4.808 | 5.81 |
| 23 | A -3 6 | 5.7 | 0.244 | Inf | 5.223 | 6.18 |
| 14 | A -6 3 | 3.47 | 0.27 | Inf | 2.945 | 4 |
| 17 | A -6 3 | 5.68 | 0.235 | Inf | 5.223 | 6.15 |
| 20 | A -6 3 | 5.87 | 0.254 | Inf | 5.376 | 6.37 |
| 23 | A -6 3 | 5.35 | 0.279 | Inf | 4.803 | 5.9 |
| 14 | A -6 6 | 1.75 | 0.486 | Inf | 0.796 | 2.7 |
| 17 | A -6 6 | 4.83 | 0.255 | Inf | 4.328 | 5.33 |
| 20 | A -6 6 | 5.76 | 0.244 | Inf | 5.278 | 6.23 |
| 23 | A -6 6 | 5.98 | 0.265 | Inf | 5.458 | 6.5 |

**Table B.8 Custom contrasts of estimated marginal means (emmeans) for burden.**
In *Daphnia magna* infected with *Ordospora colligata*, comparing treatments across different baseline temperatures. Each treatment's emmean was contrasted either with its constant

counterpart at the same temperature or with alternate amplitudes or durations within the same heatwave timing. Infinite degrees of freedom ('Inf') reflect the model's complexity, which limits precise estimation of uncertainty. Statistically significant contrasts are shown in bold. P-values were adjusted using the Benjamini-Hochberg method.

| Day | Temp | Contrast | Estimate | SE | df | z ratio | p value |
|---|---|---|---|---|---|---|---|
| Before | 14 | -33 v 00 | -0.42917 | 0.346 | Inf | -1.239 | 0.4923 |
| Before | 14 | -36 v 00 | 0.11622 | 0.345 | Inf | 0.336 | 0.8709 |
| Before | 14 | -63 v 00 | 0.06965 | 0.346 | Inf | 0.202 | 0.9166 |
| Before | 14 | -66 v 00 | 0.09214 | 0.391 | Inf | 0.236 | 0.9083 |
| Before | 14 | -36 v -33 | 0.54539 | 0.396 | Inf | 1.377 | 0.4923 |
| Before | 14 | -66 v -63 | 0.02249 | 0.436 | Inf | 0.052 | 0.9689 |
| Before | 14 | -63 v -33 | 0.49882 | 0.396 | Inf | 1.259 | 0.4923 |
| Before | 14 | -66 v -36 | -0.02408 | 0.436 | Inf | -0.055 | 0.9689 |
| Before | 17 | -33 v 00 | -0.10155 | 0.323 | Inf | -0.315 | 0.8709 |
| Before | 17 | -36 v 00 | 0.25275 | 0.292 | Inf | 0.865 | 0.6402 |
| Before | 17 | -63 v 00 | 0.38255 | 0.305 | Inf | 1.253 | 0.4923 |
| Before | 17 | -66 v 00 | -0.07653 | 0.298 | Inf | -0.256 | 0.9057 |
| Before | 17 | -36 v -33 | 0.3543 | 0.349 | Inf | 1.014 | 0.5911 |
| Before | 17 | -66 v -63 | -0.45908 | 0.339 | Inf | -1.354 | 0.4923 |
| Before | 17 | -63 v -33 | 0.4841 | 0.361 | Inf | 1.343 | 0.4923 |
| Before | 17 | -66 v -36 | -0.32928 | 0.327 | Inf | -1.007 | 0.5911 |
| Before | 20 | -33 v 00 | -0.16957 | 0.301 | Inf | -0.564 | 0.7752 |
| Before | 20 | -36 v 00 | 0.0739 | 0.295 | Inf | 0.251 | 0.9057 |
| Before | 20 | -63 v 00 | 0.13904 | 0.295 | Inf | 0.472 | 0.8153 |
| Before | 20 | -66 v 00 | -0.26897 | 0.301 | Inf | -0.894 | 0.6256 |
| Before | 20 | -36 v -33 | 0.24347 | 0.327 | Inf | 0.744 | 0.677 |
| Before | 20 | -66 v -63 | -0.40801 | 0.327 | Inf | -1.248 | 0.4923 |
| Before | 20 | -63 v -33 | 0.30861 | 0.327 | Inf | 0.944 | 0.6052 |
| Before | 20 | -66 v -36 | -0.34287 | 0.327 | Inf | -1.048 | 0.589 |
| Before | 23 | -33 v 00 | 0.74866 | 0.351 | Inf | 2.136 | 0.1743 |
| **Before** | **23** | **-36 v 00** | **1.08315** | **0.372** | **Inf** | **2.911** | **0.0384** |
| Before | 23 | -63 v 00 | 0.83586 | 0.372 | Inf | 2.246 | 0.1483 |
| Before | 23 | -66 v 00 | 0.13453 | 0.373 | Inf | 0.361 | 0.8709 |
| Before | 23 | -36 v -33 | 0.33449 | 0.396 | Inf | 0.844 | 0.6482 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Before | 23 | -66 v -63 | -0.70133 | 0.416 | Inf | -1.685 | 0.3954 |
| Before | 23 | -63 v -33 | 0.0872 | 0.396 | Inf | 0.22 | 0.9112 |
| Before | 23 | -66 v -36 | -0.94862 | 0.416 | Inf | -2.28 | 0.146 |
| During | 14 | -33 v 00 | -0.17945 | 0.319 | Inf | -0.563 | 0.7752 |
| During | 14 | -36 v 00 | 0.20867 | 0.335 | Inf | 0.623 | 0.7571 |
| During | 14 | -63 v 00 | -0.02292 | 0.373 | Inf | -0.061 | 0.9689 |
| During | 14 | -66 v 00 | -0.53157 | 0.347 | Inf | -1.533 | 0.4484 |
| During | 14 | -36 v -33 | 0.38813 | 0.362 | Inf | 1.071 | 0.5872 |
| During | 14 | -66 v -63 | -0.50865 | 0.421 | Inf | -1.21 | 0.5055 |
| During | 14 | -63 v -33 | 0.15653 | 0.398 | Inf | 0.394 | 0.8673 |
| During | 14 | -66 v -36 | -0.74024 | 0.387 | Inf | -1.912 | 0.2825 |
| During | 17 | -33 v 00 | -0.34371 | 0.306 | Inf | -1.125 | 0.556 |
| During | 17 | -36 v 00 | -0.02462 | 0.298 | Inf | -0.083 | 0.9689 |
| During | 17 | -63 v 00 | 0.0236 | 0.305 | Inf | 0.077 | 0.9689 |
| During | 17 | -66 v 00 | -0.46742 | 0.306 | Inf | -1.53 | 0.4484 |
| During | 17 | -36 v -33 | 0.31909 | 0.339 | Inf | 0.941 | 0.6052 |
| During | 17 | -66 v -63 | -0.49102 | 0.345 | Inf | -1.422 | 0.4923 |
| During | 17 | -63 v -33 | 0.36731 | 0.345 | Inf | 1.064 | 0.5872 |
| During | 17 | -66 v -36 | -0.44279 | 0.339 | Inf | -1.306 | 0.4923 |
| During | 20 | -33 v 00 | 0.24883 | 0.336 | Inf | 0.742 | 0.677 |
| During | 20 | -36 v 00 | -0.11528 | 0.295 | Inf | -0.391 | 0.8673 |
| During | 20 | -63 v 00 | -0.00874 | 0.325 | Inf | -0.027 | 0.9785 |
| During | 20 | -66 v 00 | -0.43139 | 0.316 | Inf | -1.365 | 0.4923 |
| During | 20 | -36 v -33 | -0.36411 | 0.359 | Inf | -1.014 | 0.5911 |
| During | 20 | -66 v -63 | -0.42265 | 0.367 | Inf | -1.15 | 0.5456 |
| During | 20 | -63 v -33 | -0.25757 | 0.384 | Inf | -0.67 | 0.7314 |
| During | 20 | -66 v -36 | -0.31612 | 0.341 | Inf | -0.927 | 0.6067 |
| During | 23 | -33 v 00 | -0.44028 | 0.352 | Inf | -1.251 | 0.4923 |
| During | 23 | -36 v 00 | -0.19295 | 0.388 | Inf | -0.498 | 0.8137 |
| During | 23 | -63 v 00 | -0.58252 | 0.429 | Inf | -1.358 | 0.4923 |
| During | 23 | -66 v 00 | -0.33686 | 0.352 | Inf | -0.958 | 0.6052 |
| During | 23 | -36 v -33 | 0.24733 | 0.412 | Inf | 0.6 | 0.763 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| During | 23 | -66 v -63 | 0.24566 | 0.451 | Inf | 0.545 | 0.7813 |
| During | 23 | -63 v -33 | -0.14224 | 0.451 | Inf | -0.315 | 0.8709 |
| During | 23 | -66 v -36 | -0.14391 | 0.412 | Inf | -0.349 | 0.8709 |
| After | 14 | -33 v 00 | -0.92236 | 0.348 | Inf | -2.652 | 0.0641 |
| **After** | **14** | **-36 v 00** | **-1.58762** | **0.323** | **Inf** | **-4.912** | **<.0001** |
| **After** | **14** | **-63 v 00** | **-1.10913** | **0.338** | **Inf** | **-3.282** | **0.0141** |
| **After** | **14** | **-66 v 00** | **-2.83416** | **0.527** | **Inf** | **-5.378** | **<.0001** |
| After | 14 | -36 v -33 | -0.66526 | 0.378 | Inf | -1.759 | 0.3593 |
| **After** | **14** | **-66 v -63** | **-1.72502** | **0.556** | **Inf** | **-3.1** | **0.0232** |
| After | 14 | -63 v -33 | -0.18677 | 0.391 | Inf | -0.478 | 0.8153 |
| After | 14 | -66 v -36 | -1.24653 | 0.548 | Inf | -2.277 | 0.146 |
| After | 17 | -33 v 00 | 0.23032 | 0.305 | Inf | 0.754 | 0.677 |
| After | 17 | -36 v 00 | -0.0428 | 0.292 | Inf | -0.147 | 0.953 |
| After | 17 | -63 v 00 | -0.37213 | 0.298 | Inf | -1.247 | 0.4923 |
| After | 17 | -66 v 00 | -1.22904 | 0.314 | Inf | -3.912 | 0.0022 |
| After | 17 | -36 v -33 | -0.27312 | 0.333 | Inf | -0.819 | 0.6545 |
| After | 17 | -66 v -63 | -0.8569 | 0.347 | Inf | -2.469 | 0.1 |
| After | 17 | -63 v -33 | -0.60245 | 0.339 | Inf | -1.777 | 0.3593 |
| After | 17 | -66 v -36 | -1.18623 | 0.342 | Inf | -3.473 | 0.0091 |
| After | 20 | -33 v 00 | -0.26446 | 0.325 | Inf | -0.814 | 0.6545 |
| After | 20 | -36 v 00 | -0.87894 | 0.316 | Inf | -2.78 | 0.0522 |
| After | 20 | -63 v 00 | -0.31109 | 0.316 | Inf | -0.985 | 0.5995 |
| After | 20 | -66 v 00 | -0.42932 | 0.308 | Inf | -1.394 | 0.4923 |
| After | 20 | -36 v -33 | -0.61447 | 0.368 | Inf | -1.671 | 0.3954 |
| After | 20 | -66 v -63 | -0.11823 | 0.352 | Inf | -0.335 | 0.8709 |
| After | 20 | -63 v -33 | -0.04663 | 0.367 | Inf | -0.127 | 0.959 |
| After | 20 | -66 v -36 | 0.44961 | 0.353 | Inf | 1.275 | 0.4923 |
| After | 23 | -33 v 00 | 0.2118 | 0.342 | Inf | 0.619 | 0.7571 |
| After | 23 | -36 v 00 | 1.15233 | 0.334 | Inf | 3.445 | 0.0091 |
| After | 23 | -63 v 00 | 0.79983 | 0.36 | Inf | 2.219 | 0.1495 |
| **After** | **23** | **-66 v 00** | **1.42854** | **0.35** | **Inf** | **4.079** | **0.0014** |
| After | 23 | -36 v -33 | 0.94052 | 0.353 | Inf | 2.663 | 0.0641 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| After | 23 | -66 v -63 | 0.62871 | 0.385 | Inf | 1.633 | 0.4095 |
| After | 23 | -63 v -33 | 0.58802 | 0.378 | Inf | 1.556 | 0.4484 |
| After | 23 | -66 v -36 | 0.27621 | 0.361 | Inf | 0.766 | 0.677 |

**Table B.9 Coefficient estimates and feature importance of burden in relation to degree day accumulation.**

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 increase 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 (95$^{th}$ percentile) from bootstrapping. Non-significant values have CIs encompassing 0, suggesting no important relationship to the response variable. Degree day was a cubic polynomial to account for non-linearity and is therefore separated by each degree (linear [L], quadratic [Q], and cubic [C]). Feature importance (Importance) is calculated by reporting the absolute values of coefficient effects for each variable.

| **Explanatory Variable** | **Effect** | **Lower CI** | **Upper CI** | **Imp** |
|---|---|---|---|---|
| X Intercept | 5.36 | 5.22 | 5.49 | 5.36 |
| X Intercept (1) | 0 | 0 | 0 | 0 |
| Degree Day (L) | **6.31** | **1.41** | **8.90** | 6.31 |
| **Degree Day (Q)** | **-16.95** | **-20.14** | **-14.56** | 16.95 |
| Degree Day (C) | 0 | -0.02 | 1.98 | 0 |
| Timing Before | 0.12 | 0 | 0.34 | 0.12 |
| Timing During | 0 | -0.27 | 0 | 0 |
| Timing After | -0.16 | -0.38 | 0 | 0.16 |
| Treatment -3ºC for 3 days | 0 | -0.13 | 0 | 0 |
| Treatment -3ºC for 6 days | 0 | 0 | 0.08 | 0 |
| Treatment -6ºC for 3 days | 0 | 0 | 0.12 | 0 |
| Treatment -6ºC for 6 days | -0.10 | -0.33 | 0 | 0.10 |
| Degree Day (L): Timing Before | 0.87 | 0 | 4.95 | 0.87 |
| Degree Day (Q): Timing Before | 1.18 | 0 | 4.64 | 1.18 |
| Degree Day (C): Timing Before | 2.79 | 0 | 4.68 | 2.79 |
| Degree Day (L): Timing During | -6.20 | -10.42 | 0 | 6.20 |
| Degree Day (Q): Timing During | -4.21 | -9.82 | 0 | 4.21 |
| Degree Day (C): Timing During | 0 | -6.20 | 0 | 0 |
| Degree Day (L): Timing After | 3.86 | 0 | 11.48 | 3.86 |

| | | | | |
|---|---|---|---|---|
| Degree Day (Q): Timing After | 0 | -3.57 | 0 | 0 |
| Degree Day (C): Timing After | 0 | 0 | 3.11 | 0 |
| Degree Day (L): Treatment -3ºC for 3 days | 0 | 0 | 3.69 | 0 |
| Degree Day (Q): Treatment -3ºC for 3 days | 0 | -5.10 | 0 | 0 |
| Degree Day (C): Treatment -3ºC for 3 days | 0 | 0 | 0 | 0 |
| Degree Day (L): Treatment -3ºC for 6 days | 0 | 0 | 5.10 | 0 |
| Degree Day (Q): Treatment -3ºC for 6 days | 0 | 0 | 2.77 | 0 |
| Degree Day (C): Treatment -3ºC for 6 days | 1.28 | 0 | 6.19 | 1.28 |
| Degree Day (L): Treatment -6ºC for 3 days | 0 | 0 | 4.69 | 0 |
| Degree Day (Q): Treatment -6ºC for 3 days | 0 | -4.53 | 0 | 0 |
| Degree Day (C): Treatment -6ºC for 3 days | 0 | -1.19 | 0 | 0 |
| Degree Day (L): Treatment -6ºC for 6 days | 0 | 0 | 0 | 0 |
| Degree Day (Q): Treatment -6ºC for 6 days | 0 | -0.52 | 2.73 | 0 |
| Degree Day (C): Treatment -6ºC for 6 days | 0 | -0.52 | 1.83 | 0 |
| Timing Before: Treatment -3ºC for 3 days | 0 | -0.18 | 0 | 0 |
| Timing During: Treatment -3ºC for 3 days | -0.01 | -0.34 | 0.15 | 0.01 |
| Timing After: Treatment -3ºC for 3 days | 0 | -0.05 | 0.23 | 0 |
| Timing Before: Treatment -3ºC for 6 days | 0.31 | 0 | 0.52 | 0.31 |
| Timing During: Treatment -3ºC for 6 days | 0 | -0.32 | 0.30 | 0 |
| Timing After: Treatment -3ºC for 6 days | -0.04 | -0.34 | 0 | 0.04 |
| Timing Before: Treatment -6ºC for 3 days | 0.28 | 0 | 0.50 | 0.28 |
| Timing During: Treatment -6ºC for 3 days | 0 | -0.39 | 0.20 | 0 |
| Timing After: Treatment -6ºC for 3 days | 0 | -0.32 | 0.03 | 0 |
| Timing Before: Treatment -6ºC for 6 days | 0 | 0 | 0 | 0 |
| Timing During: Treatment -6ºC for 6 days | -0.30 | -0.71 | 0 | 0.30 |
| Timing After: Treatment -6ºC for 6 days | -0.13 | -0.44 | 0 | 0.13 |
| Degree Day (L): Timing Before: Treatment -3ºC for 3 days | 4.82 | 0 | 14.94 | 4.82 |
| Degree Day (Q): Timing Before: Treatment -3ºC for 3 days | 0 | 0 | 4.04 | 0 |
| Degree Day (C): Timing Before: Treatment -3ºC for 3 days | 0 | 0 | 5.58 | 0 |
| Degree Day (L): Timing During: Treatment -3ºC for 3 days | 0 | -6.47 | 5.69 | 0 |
| Degree Day (Q): Timing During: Treatment -3ºC for 3 days | -0.58 | -11.71 | 0.72 | 0.58 |
| Degree Day (C): Timing During: Treatment -3ºC for 3 days | -11.77 | -19.87 | 0 | 11.77 |

| | | | | |
|---|---|---|---|---|
| Degree Day (L): Timing After: Treatment -3ºC for 3 days | 0 | -6.39 | 0.20 | 0 |
| Degree Day (Q): Timing After: Treatment -3ºC for 3 days | -7.04 | -13.22 | 0 | 7.04 |
| Degree Day (C): Timing After: Treatment -3ºC for 3 days | 0 | -2.86 | 3.51 | 0 |
| Degree Day (L): Timing Before: Treatment -3ºC for 6 days | 0 | -1.45 | 6.07 | 0 |
| Degree Day (Q): Timing Before: Treatment -3ºC for 6 days | 2.38 | 0 | 7.31 | 2.38 |
| Degree Day (C): Timing Before: Treatment -3ºC for 6 days | 0 | -0.89 | 4.87 | 0 |
| Degree Day (L): Timing During: Treatment -3ºC for 6 days | -0.03 | -15.35 | 5.26 | 0.03 |
| Degree Day (Q): Timing During: Treatment -3ºC for 6 days | 0 | -15.30 | 1.87 | 0 |
| Degree Day (C): Timing During: Treatment -3ºC for 6 days | 0 | -6.40 | 3.95 | 0 |
| Degree Day (L): Timing After: Treatment -3ºC for 6 days | 5.97 | 0 | 14.93 | 5.97 |
| Degree Day (Q): Timing After: Treatment -3ºC for 6 days | 0 | -3.26 | 3.91 | 0 |
| **Degree Day (C): Timing After: Treatment -3ºC for 6 days** | **14.20** | **6.09** | **19.88** | 14.20 |
| Degree Day (L): Timing Before: Treatment -6ºC for 3 days | 0.80 | -0.80 | 7.76 | 0.80 |
| Degree Day (Q): Timing Before: Treatment -6ºC for 3 days | 0 | -3.11 | 4.61 | 0 |
| Degree Day (C): Timing Before: Treatment -6ºC for 3 days | 3.18 | 0 | 9.77 | 3.18 |
| Degree Day (L): Timing During: Treatment -6ºC for 3 days | 0 | -13.37 | 4.85 | 0 |
| Degree Day (Q): Timing During: Treatment -6ºC for 3 days | -3.05 | -20.63 | 0 | 3.05 |
| Degree Day (C): Timing During: Treatment -6ºC for 3 days | -2.37 | -12.11 | 0 | 2.37 |
| Degree Day (L): Timing After: Treatment -6ºC for 3 days | 1.70 | -1.57 | 10.54 | 1.70 |
| Degree Day (Q): Timing After: Treatment -6ºC for 3 days | 0 | -7.78 | 3.75 | 0 |
| Degree Day (C): Timing After: Treatment -6ºC for 3 days | 0 | -8.14 | 0.76 | 0 |
| **Degree Day (L): Timing Before: Treatment -6ºC for 6 days** | **-8.00** | **-14.45** | **-0.20** | 8.00 |
| Degree Day (Q): Timing Before: Treatment -6ºC for 6 days | 0 | -2.58 | 3.43 | 0 |
| Degree Day (C): Timing Before: Treatment -6ºC for 6 days | 0 | -4.16 | 0.17 | 0 |
| Degree Day (L): Timing During: Treatment -6ºC for 6 days | -3.27 | -15.81 | 0 | 3.27 |
| Degree Day (Q): Timing During: Treatment -6ºC for 6 days | 0 | -9.26 | 2.04 | 0 |
| Degree Day (C): Timing During: Treatment -6ºC for 6 days | 0 | -4.01 | 7.26 | 0 |
| **Degree Day (L): Timing After: Treatment -6ºC for 6 days** | **17.54** | **5.16** | **26.44** | 17.54 |
| Degree Day (Q): Timing After: Treatment -6ºC for 6 days | 0 | -2.37 | 8.38 | 0 |
| Degree Day (C): Timing After: Treatment -6ºC for 6 days | 1.26 | -3.30 | 7.09 | 1.26 |

**Table B.10 Combined feature importance of burden in relation to degree day accumulation.**

In *Daphnia magna* exposed to *Ordospora colligata*. Importance values were derived by taking the absolute values of the model coefficients for each variable. For variables included as polynomials, the contributions from the linear and quadratic terms were summed to obtain the overall importance.

| Explanatory Variable | Importance |
|---|---|
| Degree Day | 23.26 |
| Degree Day: Timing After: Treatment -3ºC for 6 days | 20.17 |
| Degree Day: Timing After: Treatment -6ºC for 6 days | 18.8 |
| Degree Day: Timing During: Treatment -3ºC for 3 days | 12.35 |
| Degree Day: Timing During | 10.41 |
| Degree Day: Timing Before: Treatment -6ºC for 6 days | 8.00 |
| Degree Day: Timing After: Treatment -3ºC for 3 days | 7.04 |
| Degree Day: Timing During: Treatment -6ºC for 3 days | 5.42 |
| X Intercept | 5.36 |
| Degree Day: Timing Before | 4.84 |
| Degree Day: Timing Before: Treatment -3ºC for 3 days | 4.82 |
| Degree Day: Timing Before: Treatment -6ºC for 3 days | 3.98 |
| Degree Day: Timing After | 3.86 |
| Degree Day: Timing During: Treatment -6ºC for 6 days | 3.27 |
| Degree Day: Timing Before: Treatment -3ºC for 6 days | 2.38 |
| Degree Day: Timing After: Treatment -6ºC for 3 days | 1.7 |
| Degree Day: Treatment -3ºC for 6 days | 1.28 |
| Timing Before: Treatment -3ºC for 6 days | 0.31 |
| Timing During: Treatment -6ºC for 6 days | 0.3 |
| Timing Before: Treatment -6ºC for 3 days | 0.28 |
| Timing After | 0.16 |
| Timing After: Treatment -6ºC for 6 days | 0.13 |
| Timing Before | 0.12 |
| Treatment -6ºC for 6 days | 0.1 |
| Timing After: Treatment -3ºC for 6 days | 0.04 |
| Degree Day: Timing During: Treatment -3ºC for 6 days | 0.03 |
| Timing During: Treatment -3ºC for 3 days | 0.01 |

| | |
|---|---|
| Timing During | 0 |
| Treatment -3ºC for 3 days | 0 |
| Treatment -3ºC for 6 days | 0 |
| Treatment -6ºC for 3 days | 0 |
| Degree Day: Treatment -3ºC for 3 days | 0 |
| Degree Day: Treatment -6ºC for 3 days | 0 |
| Degree Day: Treatment -6ºC for 6 days | 0 |
| Timing Before: Treatment -3ºC for 3 days | 0 |
| Timing After: Treatment -3ºC for 3 days | 0 |
| Timing During: Treatment -3ºC for 6 days | 0 |
| Timing During: Treatment -6ºC for 3 days | 0 |
| Timing After: Treatment -6ºC for 3 days | 0 |
| Timing Before: Treatment -6ºC for 6 days | 0 |

# Appendix C - Supplementary information for Chapter 4

**Table C.1: 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. Effect sizes represent the strength of these relationships, with larger absolute values indicating stronger effects. Lower CI; lower confidence interval (95th), Upper CI; upper confidence interval (95th). 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.

| Explanatory Variable | Effect | Lower CI | Upper CI | Imp |
|---|---|---|---|---|
| X Intercept | 2.41 | 2.18 | 2.72 | 0 |
| **Temperature (L)** | **-0.08** | **-0.14** | **-0.02** | 0.08 |
| Timing -10 | 0 | 0 | 0.14 | 0.06 |
| Timing 0 | 0 | 0 | 0 | 0.24 |
| Timing 10 | 0 | 0 | 0 | 0 |
| Timing 20 | 0 | 0 | 0 | 0 |
| Amplitude +3 | 0.12 | 0 | 0.39 | 0.12 |
| Amplitude +6 | 0 | -0.23 | 0 | 0 |
| Duration 3 | 0 | 0 | 0 | 0 |
| Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing -10 | 0 | 0 | 0.02 | 3.52 |
| Temperature (L): Timing 0 | -0.01 | -0.09 | 0 | 0.01 |
| Temperature (L): Timing 10 | 0 | -0.02 | 0 | 0 |
| Temperature (L): Timing 20 | 0 | -0.08 | 0 | 0 |
| Temperature (L): Amplitude +3 | 0 | 0 | 0 | 0 |
| Temperature (L): Amplitude +6 | -0.08 | -0.17 | 0 | 0.08 |
| Timing -10: Amplitude +3 | 0 | 0 | 0 | 0 |
| Timing 0: Amplitude +3 | 0 | 0 | 0.22 | 0 |
| Timing 10: Amplitude +3 | 0 | 0 | 0.12 | 0 |
| Timing 20: Amplitude +3 | 0 | 0 | 0 | 0 |
| Timing -10: Amplitude +6 | 0.08 | 0 | 0.47 | 0.08 |

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|---|---|---|---|---|
| Timing 0: Amplitude +6 | 0 | -0.12 | 0 | 0 |
| Timing 10: Amplitude +6 | 0 | -0.23 | 0 | 0 |
| Timing 20: Amplitude +6 | 0 | -0.32 | 0 | 0 |
| Temperature (L): Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Duration 6 | 0 | -0.04 | 0 | 0 |
| Timing -10: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 0: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 10: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 20: Duration 3 | 0 | 0 | 0 | 0 |
| Timing -10: Duration 6 | 0 | 0 | 0.13 | 0 |
| Timing 0: Duration 6 | 0 | 0 | 0 | 0 |
| Timing 10: Duration 6 | 0 | 0 | 0 | 0 |
| Timing 20: Duration 6 | 0 | -0.10 | 0 | 0 |
| Amplitude +3: Duration 3 | 0 | 0 | 0.08 | 0 |
| Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Amplitude +3: Duration 6 | 0 | 0 | 0.04 | 0 |
| Amplitude +6: Duration 6 | 0 | -0.20 | 0 | 0 |
| Temperature (L): Timing -10: Amplitude +3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 0: Amplitude +3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 10: Amplitude +3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 20: Amplitude +3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing -10: Amplitude +6 | 0.02 | 0 | 0.12 | 0.02 |
| **Temperature (L): Timing 0: Amplitude +6** | **-0.44** | **-0.49** | **-0.31** | 0.44 |
| Temperature (L): Timing 10: Amplitude +6 | -0.11 | -0.26 | 0 | 0.11 |
| Temperature (L): Timing 20: Amplitude +6 | 0 | -0.06 | 0 | 0 |
| Temperature (L): Timing -10: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 0: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 10: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 20: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing -10: Duration 6 | 0 | 0 | 0.09 | 0 |
| Temperature (L): Timing 0: Duration 6 | 0 | -0.01 | 0 | 0 |
| Temperature (L): Timing 10: Duration 6 | 0 | 0 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (L): Timing 20: Duration 6 | -0.12 | -0.25 | 0 | 0.12 |
| Temperature (L): Amplitude +3: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| **Temperature (L): Amplitude +6: Duration 6** | **-0.17** | **-0.25** | **-0.06** | 0.17 |
| Timing -10: Amplitude +3: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 0: Amplitude +3: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 10: Amplitude +3: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 20: Amplitude +3: Duration 3 | 0 | 0 | 0 | 0 |
| Timing -10: Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 0: Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 10: Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 20: Amplitude +6: Duration 3 | 0 | -0.02 | 0 | 0 |
| Timing -10: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Timing 0: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Timing 10: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Timing 20: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Timing -10: Amplitude +6: Duration 6 | 0.08 | 0 | 0.25 | 0.08 |
| Timing 0: Amplitude +6: Duration 6 | 0 | 0 | 0 | 0 |
| Timing 10: Amplitude +6: Duration 6 | -0.52 | -1.21 | 0 | 0.52 |
| Timing 20: Amplitude +6: Duration 6 | 0 | -0.22 | 0 | 0 |
| Temperature (L): Timing -10: Amplitude +3: Duration 3 | 0 | -0.09 | 0 | 0 |
| Temperature (L): Timing 0: Amplitude +3: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 10: Amplitude +3: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 20: Amplitude +3: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing -10: Amplitude +6: Duration 3 | 0 | 0 | 0.05 | 0 |
| Temperature (L): Timing 0: Amplitude +6: Duration 3 | -0.07 | -0.21 | 0 | 0.07 |
| Temperature (L): Timing 10: Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 20: Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing -10: Amplitude +3: Duration 6 | 0 | 0 | 0.02 | 0 |
| Temperature (L): Timing 0: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 10: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|
| Temperature (L): Timing 20: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing -10: Amplitude +6: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 0: Amplitude +6: Duration 6 | -0.01 | -0.13 | 0 | 0.01 |
| Temperature (L): Timing 10: Amplitude +6: Duration 6 | -0.07 | -0.30 | 0 | 0.07 |
| Temperature (L): Timing 20: Amplitude +6: Duration 6 | -0.11 | -0.27 | 0 | 0.11 |

**Table C.2: estimated marginal means (emmeans) for infection prevalence.**
In *Daphnia magna* infected with *Ordospora colligata*. The estimates were derived using a Generalised Linear Model (GLM) with baseline temperature as a factor, 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.

| Temp | Treatment | emmean | SE | df | Lower CI 95% | Upper CI 95% |
|---|---|---|---|---|---|---|
| 14 | C 0 0 | 2.303 | 0.742 | Inf | 0.849 | 3.756 |
| 17 | C 0 0 | 20.566 | 3780.128 | Inf | -7390 | 7429.48 |
| 20 | C 0 0 | 2.944 | 1.026 | Inf | 0.934 | 4.955 |
| 23 | C 0 0 | 3.045 | 1.024 | Inf | 1.040 | 5.051 |
| 14 | -10 +3 3 | 20.566 | 4738.641 | Inf | -9270 | 9308.131 |
| 17 | -10 +3 3 | 20.566 | 4917.52 | Inf | -9620 | 9658.728 |
| 20 | -10 +3 3 | 1.705 | 0.769 | Inf | 0.198 | 3.211 |
| 23 | -10 +3 3 | 1.705 | 0.769 | Inf | 0.198 | 3.211 |
| 14 | -10 +3 6 | 2.639 | 1.035 | Inf | 0.610 | 4.668 |
| 17 | -10 +3 6 | 1.099 | 0.816 | Inf | -0.502 | 2.699 |
| 20 | -10 +3 6 | 2.485 | 1.041 | Inf | 0.445 | 4.525 |
| 23 | -10 +3 6 | 20.566 | 5118.317 | Inf | -10000 | 10052.28 |
| 14 | -10 +6 3 | 2.485 | 1.041 | Inf | 0.445 | 4.525 |
| 17 | -10 +6 3 | 2.303 | 1.049 | Inf | 0.247 | 4.358 |
| 20 | -10 +6 3 | 20.566 | 5910.123 | Inf | -11600 | 11604.2 |
| 23 | -10 +6 3 | 20.566 | 4917.52 | Inf | -9620 | 9658.728 |
| 14 | -10 +6 6 | 20.566 | 5118.317 | Inf | -10000 | 10052.28 |
| 17 | -10 +6 6 | 20.566 | 5606.835 | Inf | -11000 | 11009.76 |
| 20 | -10 +6 6 | 20.566 | 5345.908 | Inf | -10500 | 10498.35 |
| 23 | -10 +6 6 | 20.566 | 5118.317 | Inf | -10000 | 10052.28 |
| 14 | 0 +3 3 | 20.566 | 4917.52 | Inf | -9620 | 9658.728 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | 0 +3 3 | 20.566 | 4738.641 | Inf | -9270 | 9308.131 |
| 20 | 0 +3 3 | 2.398 | 1.044 | Inf | 0.351 | 4.445 |
| 23 | 0 +3 3 | 20.566 | 4738.641 | Inf | -9270 | 9308.131 |
| 14 | 0 +3 6 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 17 | 0 +3 6 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 20 | 0 +3 6 | 20.566 | 5910.123 | Inf | -11600 | 11604.2 |
| 23 | 0 +3 6 | 2.565 | 1.038 | Inf | 0.531 | 4.599 |
| 14 | 0 +6 3 | 20.566 | 4738.641 | Inf | -9270 | 9308.131 |
| 17 | 0 +6 3 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 20 | 0 +6 3 | 20.566 | 5606.835 | Inf | -11000 | 11009.76 |
| 23 | 0 +6 3 | -2.565 | 1.038 | Inf | -4.600 | -0.531 |
| 14 | 0 +6 6 | 20.566 | 5606.835 | Inf | -11000 | 11009.76 |
| 17 | 0 +6 6 | 20.566 | 4917.52 | Inf | -9620 | 9658.728 |
| 20 | 0 +6 6 | 1.792 | 1.08 | Inf | -0.325 | 3.909 |
| 23 | 0 +6 6 | -20.566 | 5118.317 | Inf | -10100 | 10011.15 |
| 14 | 10 +3 3 | 2.565 | 1.038 | Inf | 0.531 | 4.599 |
| 17 | 10 +3 3 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 20 | 10 +3 3 | 2.565 | 1.038 | Inf | 0.531 | 4.599 |
| 23 | 10 +3 3 | 2.639 | 1.035 | Inf | 0.610 | 4.668 |
| 14 | 10 +3 6 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 17 | 10 +3 6 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 20 | 10 +3 6 | 2.398 | 1.044 | Inf | 0.351 | 4.445 |
| 23 | 10 +3 6 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 14 | 10 +6 3 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 17 | 10 +6 3 | 20.566 | 4738.641 | Inf | -9270 | 9308.131 |
| 20 | 10 +6 3 | 2.485 | 1.041 | Inf | 0.445 | 4.525 |
| 23 | 10 +6 3 | 0.693 | 0.548 | Inf | -0.380 | 1.767 |
| 14 | 10 +6 6 | 2.639 | 1.035 | Inf | 0.610 | 4.668 |
| 17 | 10 +6 6 | 2.565 | 1.038 | Inf | 0.531 | 4.599 |
| 20 | 10 +6 6 | 2.197 | 1.054 | Inf | 0.131 | 4.263 |
| 23 | 10 +6 6 | -2.639 | 1.035 | Inf | -4.670 | -0.61 |
| 14 | 20 +3 3 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | 20 +3 3 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 20 | 20 +3 3 | 1.872 | 0.76 | Inf | 0.383 | 3.361 |
| 23 | 20 +3 3 | 2.639 | 1.035 | Inf | 0.610 | 4.668 |
| 14 | 20 +3 6 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 17 | 20 +3 6 | 20.566 | 4738.641 | Inf | -9270 | 9308.131 |
| 20 | 20 +3 6 | 1.705 | 0.769 | Inf | 0.198 | 3.211 |
| 23 | 20 +3 6 | 1.204 | 0.658 | Inf | -0.086 | 2.494 |
| 14 | 20 +6 3 | 1.872 | 0.76 | Inf | 0.383 | 3.361 |
| 17 | 20 +6 3 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 20 | 20 +6 3 | 1.792 | 0.764 | Inf | 0.295 | 3.289 |
| 23 | 20 +6 3 | 1.386 | 0.645 | Inf | 0.121 | 2.651 |
| 14 | 20 +6 6 | 20.566 | 4738.641 | Inf | -9270 | 9308.131 |
| 17 | 20 +6 6 | 20.566 | 4577.962 | Inf | -8950 | 8993.206 |
| 20 | 20 +6 6 | 1.386 | 0.645 | Inf | 0.121 | 2.651 |
| 23 | 20 +6 6 | -1.012 | 0.584 | Inf | -2.160 | 0.133 |

**Table C.3: 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.

| Day | Temp | Contrast | Estimate | SE | df | z ratio | p value | Adjusted p |
|---|---|---|---|---|---|---|---|---|
| Day -10 | 14 | +33 v 00 | 18.263 | 4738.64 | Inf | 0.004 | 0.9969 | 1.000 |
| Day -10 | 14 | +36 v 00 | 0.336 | 1.27 | Inf | 0.264 | 0.7916 | 1.000 |
| Day -10 | 14 | +63 v 00 | 0.182 | 1.28 | Inf | 0.143 | 0.8866 | 1.000 |
| Day -10 | 14 | +66 v 00 | 18.263 | 5118.32 | Inf | 0.004 | 0.9972 | 1.000 |
| Day -10 | 14 | +36 v +33 | -17.927 | 4738.64 | Inf | -0.004 | 0.997 | 1.000 |
| Day -10 | 14 | +66 v +63 | 18.081 | 5118.32 | Inf | 0.004 | 0.9972 | 1.000 |
| Day -10 | 14 | +63 v +33 | -18.081 | 4738.64 | Inf | -0.004 | 0.997 | 1.000 |
| Day -10 | 14 | +66 v +36 | 17.927 | 5118.32 | Inf | 0.004 | 0.9972 | 1.000 |
| Day -10 | 17 | +33 v 00 | 0 | 6202.53 | Inf | 0 | 1 | 1.000 |
| Day -10 | 17 | +36 v 00 | -19.467 | 3780.13 | Inf | -0.005 | 0.9959 | 1.000 |
| Day -10 | 17 | +63 v 00 | -18.263 | 3780.13 | Inf | -0.005 | 0.9961 | 1.000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day -10 | 17 | +66 v 00 | 0 | 6762.1 | Inf | 0 | 1 | 1.000 |
| Day -10 | 17 | +36 v +33 | -19.467 | 4917.52 | Inf | -0.004 | 0.9968 | 1.000 |
| Day -10 | 17 | +66 v +63 | 18.263 | 5606.84 | Inf | 0.003 | 0.9974 | 1.000 |
| Day -10 | 17 | +63 v +33 | -18.263 | 4917.52 | Inf | -0.004 | 0.997 | 1.000 |
| Day -10 | 17 | +66 v +36 | 19.467 | 5606.84 | Inf | 0.003 | 0.9972 | 1.000 |
| Day -10 | 20 | +33 v 00 | -1.24 | 1.28 | Inf | -0.967 | 0.3335 | 1.000 |
| Day -10 | 20 | +36 v 00 | -0.46 | 1.46 | Inf | -0.314 | 0.7532 | 1.000 |
| Day -10 | 20 | +63 v 00 | 17.622 | 5910.12 | Inf | 0.003 | 0.9976 | 1.000 |
| Day -10 | 20 | +66 v 00 | 17.622 | 5345.91 | Inf | 0.003 | 0.9974 | 1.000 |
| Day -10 | 20 | +36 v +33 | 0.78 | 1.29 | Inf | 0.603 | 0.5465 | 1.000 |
| Day -10 | 20 | +66 v +63 | 0 | 7969.21 | Inf | 0 | 1 | 1.000 |
| Day -10 | 20 | +63 v +33 | 18.861 | 5910.12 | Inf | 0.003 | 0.9975 | 1.000 |
| Day -10 | 20 | +66 v +36 | 18.081 | 5345.91 | Inf | 0.003 | 0.9973 | 1.000 |
| Day -10 | 23 | +33 v 00 | -1.34 | 1.28 | Inf | -1.047 | 0.2953 | 1.000 |
| Day -10 | 23 | +36 v 00 | 17.522 | 5118.32 | Inf | 0.003 | 0.9973 | 1.000 |
| Day -10 | 23 | +63 v 00 | 17.522 | 4917.52 | Inf | 0.004 | 0.9972 | 1.000 |
| Day -10 | 23 | +66 v 00 | 17.522 | 5118.32 | Inf | 0.003 | 0.9973 | 1.000 |
| Day -10 | 23 | +36 v +33 | 18.861 | 5118.32 | Inf | 0.004 | 0.9971 | 1.000 |
| Day -10 | 23 | +66 v +63 | 0 | 7097.83 | Inf | 0 | 1 | 1.000 |
| Day -10 | 23 | +63 v +33 | 18.861 | 4917.52 | Inf | 0.004 | 0.9969 | 1.000 |
| Day -10 | 23 | +66 v +36 | 0 | 7238.39 | Inf | 0 | 1 | 1.000 |
| Day 0 | 14 | +33 v 00 | 18.263 | 4917.52 | Inf | 0.004 | 0.997 | 1.000 |
| Day 0 | 14 | +36 v 00 | 18.263 | 4577.96 | Inf | 0.004 | 0.9968 | 1.000 |
| Day 0 | 14 | +63 v 00 | 18.263 | 4738.64 | Inf | 0.004 | 0.9969 | 1.000 |
| Day 0 | 14 | +66 v 00 | 18.263 | 5606.84 | Inf | 0.003 | 0.9974 | 1.000 |
| Day 0 | 14 | +36 v +33 | 0 | 6718.61 | Inf | 0 | 1 | 1.000 |
| Day 0 | 14 | +66 v +63 | 0 | 7341.07 | Inf | 0 | 1 | 1.000 |
| Day 0 | 14 | +63 v +33 | 0 | 6829.11 | Inf | 0 | 1 | 1.000 |
| Day 0 | 14 | +66 v +36 | 0 | 7238.39 | Inf | 0 | 1 | 1.000 |
| Day 0 | 17 | +33 v 00 | 0 | 6061.69 | Inf | 0 | 1 | 1.000 |
| Day 0 | 17 | +36 v 00 | 0 | 5936.93 | Inf | 0 | 1 | 1.000 |
| Day 0 | 17 | +63 v 00 | 0 | 5936.93 | Inf | 0 | 1 | 1.000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day 0 | 17 | +66 v 00 | 0 | 6202.53 | Inf | 0 | 1 | 1.000 |
| Day 0 | 17 | +36 v +33 | 0 | 6588.81 | Inf | 0 | 1 | 1.000 |
| Day 0 | 17 | +66 v +63 | 0 | 6718.61 | Inf | 0 | 1 | 1.000 |
| Day 0 | 17 | +63 v +33 | 0 | 6588.81 | Inf | 0 | 1 | 1.000 |
| Day 0 | 17 | +66 v +36 | 0 | 6718.61 | Inf | 0 | 1 | 1.000 |
| Day 0 | 20 | +33 v 00 | -0.547 | 1.46 | Inf | -0.373 | 0.7089 | 1.000 |
| Day 0 | 20 | +36 v 00 | 17.622 | 5910.12 | Inf | 0.003 | 0.9976 | 1.000 |
| Day 0 | 20 | +63 v 00 | 17.622 | 5606.84 | Inf | 0.003 | 0.9975 | 1.000 |
| Day 0 | 20 | +66 v 00 | -1.153 | 1.49 | Inf | -0.774 | 0.4391 | 1.000 |
| Day 0 | 20 | +36 v +33 | 18.168 | 5910.12 | Inf | 0.003 | 0.9975 | 1.000 |
| Day 0 | 20 | +66 v +63 | -18.774 | 5606.84 | Inf | -0.003 | 0.9973 | 1.000 |
| Day 0 | 20 | +63 v +33 | 18.168 | 5606.84 | Inf | 0.003 | 0.9974 | 1.000 |
| Day 0 | 20 | +66 v +36 | -18.774 | 5910.12 | Inf | -0.003 | 0.9975 | 1.000 |
| Day 0 | 23 | +33 v 00 | 17.522 | 4738.64 | Inf | 0.004 | 0.997 | 1.000 |
| Day 0 | 23 | +36 v 00 | -0.48 | 1.46 | Inf | -0.329 | 0.7421 | 1.000 |
| **Day 0** | **23** | **+63 v 00** | **-5.609** | **1.46** | **Inf** | **-3.848** | **0.0001** | **0.0076** |
| Day 0 | 23 | +66 v 00 | -23.611 | 5118.32 | Inf | -0.005 | 0.9963 | 1.000 |
| Day 0 | 23 | +36 v +33 | -18.001 | 4738.64 | Inf | -0.004 | 0.997 | 1.000 |
| Day 0 | 23 | +66 v +63 | -18.001 | 5118.32 | Inf | -0.004 | 0.9972 | 1.000 |
| Day 0 | 23 | +63 v +33 | -23.131 | 4738.64 | Inf | -0.005 | 0.9961 | 1.000 |
| Day 0 | 23 | +66 v +36 | -23.131 | 5118.32 | Inf | -0.005 | 0.9964 | 1.000 |
| Day 10 | 14 | +33 v 00 | 0.262 | 1.28 | Inf | 0.206 | 0.837 | 1.000 |
| Day 10 | 14 | +36 v 00 | 18.263 | 4577.96 | Inf | 0.004 | 0.9968 | 1.000 |
| Day 10 | 14 | +63 v 00 | 18.263 | 4577.96 | Inf | 0.004 | 0.9968 | 1.000 |
| Day 10 | 14 | +66 v 00 | 0.336 | 1.27 | Inf | 0.264 | 0.7916 | 1.000 |
| Day 10 | 14 | +36 v +33 | 18.001 | 4577.96 | Inf | 0.004 | 0.9969 | 1.000 |
| Day 10 | 14 | +66 v +63 | -17.927 | 4577.96 | Inf | -0.004 | 0.9969 | 1.000 |
| Day 10 | 14 | +63 v +33 | 18.001 | 4577.96 | Inf | 0.004 | 0.9969 | 1.000 |
| Day 10 | 14 | +66 v +36 | -17.927 | 4577.96 | Inf | -0.004 | 0.9969 | 1.000 |
| Day 10 | 17 | +33 v 00 | 0 | 5936.93 | Inf | 0 | 1 | 1.000 |
| Day 10 | 17 | +36 v 00 | 0 | 5936.93 | Inf | 0 | 1 | 1.000 |
| Day 10 | 17 | +63 v 00 | 0 | 6061.69 | Inf | 0 | 1 | 1.000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day 10 | 17 | +66 v 00 | -18.001 | 3780.13 | Inf | -0.005 | 0.9962 | 1.000 |
| Day 10 | 17 | +36 v +33 | 0 | 6474.22 | Inf | 0 | 1 | 1.000 |
| Day 10 | 17 | +66 v +63 | -18.001 | 4738.64 | Inf | -0.004 | 0.997 | 1.000 |
| Day 10 | 17 | +63 v +33 | 0 | 6588.81 | Inf | 0 | 1 | 1.000 |
| Day 10 | 17 | +66 v +36 | -18.001 | 4577.96 | Inf | -0.004 | 0.9969 | 1.000 |
| Day 10 | 20 | +33 v 00 | -0.379 | 1.46 | Inf | -0.26 | 0.7948 | 1.000 |
| Day 10 | 20 | +36 v 00 | -0.547 | 1.46 | Inf | -0.373 | 0.7089 | 1.000 |
| Day 10 | 20 | +63 v 00 | -0.46 | 1.46 | Inf | -0.314 | 0.7532 | 1.000 |
| Day 10 | 20 | +66 v 00 | -0.747 | 1.47 | Inf | -0.508 | 0.6115 | 1.000 |
| Day 10 | 20 | +36 v +33 | -0.167 | 1.47 | Inf | -0.113 | 0.9097 | 1.000 |
| Day 10 | 20 | +66 v +63 | -0.288 | 1.48 | Inf | -0.194 | 0.846 | 1.000 |
| Day 10 | 20 | +63 v +33 | -0.08 | 1.47 | Inf | -0.054 | 0.9566 | 1.000 |
| Day 10 | 20 | +66 v +36 | -0.201 | 1.48 | Inf | -0.135 | 0.8924 | 1.000 |
| Day 10 | 23 | +33 v 00 | -0.405 | 1.46 | Inf | -0.279 | 0.7806 | 1.000 |
| Day 10 | 23 | +36 v 00 | 17.522 | 4577.96 | Inf | 0.004 | 0.9969 | 1.000 |
| Day 10 | 23 | +63 v 00 | -2.351 | 1.16 | Inf | -2.026 | 0.0428 | 0.7829 |
| **Day 10** | **23** | **+66 v 00** | **-5.684** | **1.46** | **Inf** | **-3.904** | **0.0001** | **0.0076** |
| Day 10 | 23 | +36 v +33 | 17.927 | 4577.96 | Inf | 0.004 | 0.9969 | 1.000 |
| Day 10 | 23 | +66 v +63 | -3.332 | 1.17 | Inf | -2.845 | 0.0044 | 0.1419 |
| Day 10 | 23 | +63 v +33 | -1.946 | 1.17 | Inf | -1.662 | 0.0966 | 1.000 |
| Day 10 | 23 | +66 v +36 | -23.205 | 4577.96 | Inf | -0.005 | 0.996 | 1.000 |
| Day 20 | 14 | +33 v 00 | 18.263 | 4577.96 | Inf | 0.004 | 0.9968 | 1.000 |
| Day 20 | 14 | +36 v 00 | 18.263 | 4577.96 | Inf | 0.004 | 0.9968 | 1.000 |
| Day 20 | 14 | +63 v 00 | -0.431 | 1.06 | Inf | -0.406 | 0.6849 | 1.000 |
| Day 20 | 14 | +66 v 00 | 18.263 | 4738.64 | Inf | 0.004 | 0.9969 | 1.000 |
| Day 20 | 14 | +36 v +33 | 0 | 6474.22 | Inf | 0 | 1 | 1.000 |
| Day 20 | 14 | +66 v +63 | 18.694 | 4738.64 | Inf | 0.004 | 0.9969 | 1.000 |
| Day 20 | 14 | +63 v +33 | -18.694 | 4577.96 | Inf | -0.004 | 0.9967 | 1.000 |
| Day 20 | 14 | +66 v +36 | 0 | 6588.81 | Inf | 0 | 1 | 1.000 |
| Day 20 | 17 | +33 v 00 | 0 | 5936.93 | Inf | 0 | 1 | 1.000 |
| Day 20 | 17 | +36 v 00 | 0 | 6061.69 | Inf | 0 | 1 | 1.000 |
| Day 20 | 17 | +63 v 00 | 0 | 5936.93 | Inf | 0 | 1 | 1.000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day 20 | 17 | +66 v 00 | 0 | 5936.93 | Inf | 0 | 1 | 1.000 |
| Day 20 | 17 | +36 v +33 | 0 | 6588.81 | Inf | 0 | 1 | 1.000 |
| Day 20 | 17 | +66 v +63 | 0 | 6474.22 | Inf | 0 | 1 | 1.000 |
| Day 20 | 17 | +63 v +33 | 0 | 6474.22 | Inf | 0 | 1 | 1.000 |
| Day 20 | 17 | +66 v +36 | 0 | 6588.81 | Inf | 0 | 1 | 1.000 |
| Day 20 | 20 | +33 v 00 | -1.073 | 1.28 | Inf | -0.84 | 0.4008 | 1.000 |
| Day 20 | 20 | +36 v 00 | -1.24 | 1.28 | Inf | -0.967 | 0.3335 | 1.000 |
| Day 20 | 20 | +63 v 00 | -1.153 | 1.28 | Inf | -0.901 | 0.3675 | 1.000 |
| Day 20 | 20 | +66 v 00 | -1.558 | 1.21 | Inf | -1.285 | 0.1986 | 1.000 |
| Day 20 | 20 | +36 v +33 | -0.167 | 1.08 | Inf | -0.155 | 0.8771 | 1.000 |
| Day 20 | 20 | +66 v +63 | -0.405 | 1 | Inf | -0.405 | 0.6851 | 1.000 |
| Day 20 | 20 | +63 v +33 | -0.08 | 1.08 | Inf | -0.074 | 0.9408 | 1.000 |
| Day 20 | 20 | +66 v +36 | -0.318 | 1 | Inf | -0.317 | 0.7511 | 1.000 |
| Day 20 | 23 | +33 v 00 | -0.405 | 1.46 | Inf | -0.279 | 0.7806 | 1.000 |
| Day 20 | 23 | +36 v 00 | -1.841 | 1.22 | Inf | -1.512 | 0.1304 | 1.000 |
| Day 20 | 23 | +63 v 00 | -1.658 | 1.21 | Inf | -1.37 | 0.1706 | 1.000 |
| Day 20 | 23 | +66 v 00 | -4.056 | 1.18 | Inf | -3.442 | 0.0006 | 0.246 |
| Day 20 | 23 | +36 v +33 | -1.435 | 1.23 | Inf | -1.17 | 0.242 | 1.000 |
| Day 20 | 23 | +66 v +63 | -2.398 | 0.87 | Inf | -2.755 | 0.0059 | 0.1503 |
| Day 20 | 23 | +63 v +33 | -1.253 | 1.22 | Inf | -1.027 | 0.3044 | 1.000 |

**Table C.4: Coefficient estimates and feature importance of exposed.**
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 (Eff) 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 CI; lower confidence interval (95th percentile), Upper CI; upper confidence interval (95th percentile), Non-significant values have CIs encompassing 0, suggesting no important relationship to the response variable. Temperature was a cubic polynomial to account for non-linearity and is therefore separated by each degree (linear [L], quadratic [Q], and cubic [C]). Feature importance (Imp) was calculated by taking the absolute value of the effect.

| Explanatory Variable | Effect | Lower CI | Upper CI | Imp |
|---|---|---|---|---|
| X Intercept | 5.91 | 5.71 | 6.07 | 0 |
| Temperature (L) | -3.32 | -9.42 | 0.99 | 3.32 |

| | | | | |
|---|---|---|---|---|
| **Temperature (Q)** | **-27.79** | **-33.63** | **-23.72** | 27.79 |
| Temperature (C) | 0.65 | -2.29 | 5.44 | 0.65 |
| Timing -10 | 0 | 0 | 0.03 | 0 |
| Timing 0 | 0 | 0 | 0 | 0 |
| Timing 10 | 0 | -0.15 | 0 | 0 |
| Timing 20 | 0 | -0.24 | 0 | 0 |
| Amplitude +3 | 0 | 0 | 0.15 | 0 |
| Amplitude +6 | -0.37 | -0.70 | -0.01 | 0.37 |
| Duration 3 | 0 | -0.08 | 0 | 0 |
| Duration 6 | 0 | -0.09 | 0 | 0 |
| Temperature (L): Timing -10 | 7.07 | 0 | 19.77 | 7.07 |
| Temperature (Q): Timing -10 | 7.64 | 0 | 18.91 | 7.64 |
| Temperature (C): Timing -10 | 0 | 0 | 3.94 | 0 |
| Temperature (L): Timing 0 | 0 | -4.25 | 0 | 0 |
| Temperature (Q): Timing 0 | 0 | -2.08 | 0 | 0 |
| Temperature (C): Timing 0 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 10 | 0 | 0 | 0 | 0 |
| Temperature (Q): Timing 10 | 0 | 0 | 0 | 0 |
| Temperature (C): Timing 10 | 0 | 0 | 4.31 | 0 |
| Temperature (L): Timing 20 | 0 | -4.46 | 0 | 0 |
| Temperature (Q): Timing 20 | 7.01 | 0 | 22.95 | 7.01 |
| Temperature (C): Timing 20 | 5.10 | 0 | 20.17 | 5.10 |
| Temperature (L): Amplitude +3 | 0 | 0 | 11.32 | 0 |
| **Temperature (Q): Amplitude +3** | **20.71** | **4.37** | **30.67** | 20.71 |
| Temperature (C): Amplitude +3 | 11.49 | 0 | 20.85 | 11.49 |
| Temperature (L): Amplitude +6 | -10 | -20.14 | 0 | 10 |
| Temperature (Q): Amplitude +6 | 0 | 0 | 0 | 0 |
| Temperature (C): Amplitude +6 | 0 | 0 | 0 | 0 |
| Timing -10: Amplitude +3 | 0 | -0.23 | 0 | 0 |
| Timing 0: Amplitude +3 | 0.22 | 0 | 0.47 | 0.22 |
| Timing 10: Amplitude +3 | 0 | 0 | 0.09 | 0 |
| Timing 20: Amplitude +3 | 0 | 0 | 0.06 | 0 |

| | | | | |
|---|---|---|---|---|
| Timing -10: Amplitude +6 | 0.28 | 0 | 0.62 | 0.28 |
| Timing 0: Amplitude +6 | -0.12 | -2.63 | 0 | 0.12 |
| Timing 10: Amplitude +6 | 0 | -0.42 | 0 | 0 |
| Timing 20: Amplitude +6 | -0.42 | -0.81 | 0 | 0.42 |
| Temperature (L): Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (Q): Duration 3 | 7.43 | 0 | 19.18 | 7.43 |
| Temperature (C): Duration 3 | 13.09 | 0 | 23.06 | 13.09 |
| Temperature (L): Duration 6 | 0 | -2.09 | 0 | 0 |
| Temperature (Q): Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (C): Duration 3 | 0 | 0 | 0 | 0 |
| Timing -10: Duration 3 | 0 | -0.08 | 0 | 0 |
| Timing 0: Duration 3 | 0 | 0 | 0.07 | 0 |
| Timing 10: Duration 3 | 0 | 0 | 0.12 | 0 |
| Timing 20: Duration 3 | 0 | -0.16 | 0 | 0 |
| Timing -10: Duration 6 | 0.05 | 0 | 0.24 | 0.05 |
| Timing 0: Duration 6 | 0 | 0 | 0 | 0 |
| Timing 10: Duration 6 | -0.03 | -0.33 | 0 | 0.03 |
| Timing 20: Duration 6 | -0.03 | -0.31 | 0 | 0.03 |
| Amplitude +3: Duration 3 | 0 | -0.03 | 0.02 | 0 |
| Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Amplitude +3: Duration 6 | 0 | 0 | 0.13 | 0 |
| Amplitude +6: Duration 6 | -0.31 | -1.18 | 0 | 0.31 |
| Temperature (L): Timing -10: Amplitude +3 | 0 | 0 | 2.57 | 0 |
| Temperature (Q): Timing -10: Amplitude +3 | 0 | 0 | 4.89 | 0 |
| Temperature (C): Timing -10: Amplitude +3 | 0 | 0 | 6.66 | 0 |
| Temperature (L): Timing 0: Amplitude +3 | 0 | 0 | 1.55 | 0 |
| Temperature (Q): Timing 0: Amplitude +3 | 0 | 0 | 4.27 | 0 |
| Temperature (C): Timing 0: Amplitude +3 | 0 | 0 | 8.37 | 0 |
| Temperature (L): Timing 10: Amplitude +3 | 0.55 | 0 | 15.73 | 0.55 |
| Temperature (Q): Timing 10: Amplitude +3 | 1.54 | 0 | 24.87 | 1.54 |
| Temperature (C): Timing 10: Amplitude +3 | 0 | 0 | 16.80 | 0 |
| Temperature (L): Timing 20: Amplitude +3 | 0 | 0 | 3.28 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (Q): Timing 20: Amplitude +3 | 0 | 0 | 8.77 | 0 |
| Temperature (C): Timing 20: Amplitude +3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing -10: Amplitude +6 | 15.28 | 0 | 25.94 | 15.28 |
| Temperature (Q): Timing -10: Amplitude +6 | 5.60 | 0 | 20.83 | 5.60 |
| Temperature (C): Timing -10: Amplitude +6 | 0 | -4.10 | 1.06 | 0 |
| **Temperature (L): Timing 0: Amplitude +6** | **-25.36** | **-158.67** | **-1.97** | 25.36 |
| Temperature (Q): Timing 0: Amplitude +6 | -32.19 | -164.06 | 0 | 32.19 |
| Temperature (C): Timing 0: Amplitude +6 | -11.72 | -69.68 | 0 | 11.72 |
| Temperature (L): Timing 10: Amplitude +6 | 0 | -25.73 | 0 | 0 |
| Temperature (Q): Timing 10: Amplitude +6 | 0 | -22.74 | 0 | 0 |
| Temperature (C): Timing 10: Amplitude +6 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 20: Amplitude +6 | -0.27 | -20.56 | 0 | 0.27 |
| Temperature (Q): Timing 20: Amplitude +6 | 12.74 | 0 | 37.12 | 12.74 |
| Temperature (C): Timing 20: Amplitude +6 | 13.91 | 0 | 38.39 | 13.91 |
| Temperature (L): Timing -10: Duration 3 | 0 | 0 | 2.52 | 0 |
| Temperature (Q): Timing -10: Duration 3 | 0 | 0 | 10.70 | 0 |
| Temperature (C): Timing -10: Duration 3 | 0 | 0 | 2.62 | 0 |
| Temperature (L): Timing 0: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (Q): Timing 0: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (C): Timing 0: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 10: Duration 3 | 0 | 0 | 4.21 | 0 |
| Temperature (Q): Timing 10: Duration 3 | 0 | 0 | 18.77 | 0 |
| Temperature (C): Timing 10: Duration 3 | 10.59 | 0 | 27.50 | 10.59 |
| Temperature (L): Timing 20: Duration 3 | 0 | 0 | 3.73 | 0 |
| Temperature (Q): Timing 20: Duration 3 | 0 | 0 | 18.97 | 0 |
| Temperature (C): Timing 20: Duration 3 | 0 | 0 | 6.69 | 0 |
| Temperature (L): Timing -10: Duration 6 | 10.97 | 0 | 18.86 | 10.97 |
| Temperature (Q): Timing -10: Duration 6 | 0 | 0 | 14.60 | 0 |
| Temperature (C): Timing -10: Duration 6 | 0 | -5.66 | 2.83 | 0 |
| Temperature (L): Timing 0: Duration 6 | -1.43 | -16.00 | 0 | 1.43 |
| Temperature (Q): Timing 0: Duration 6 | -3.30 | -13.70 | 0 | 3.30 |
| Temperature (C): Timing 0: Duration 6 | 0 | -9.65 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (L): Timing 10: Duration 6 | 0 | -3.51 | 0 | 0 |
| Temperature (Q): Timing 10: Duration 6 | 0 | -1.84 | 0 | 0 |
| Temperature (C): Timing 10: Duration 6 | 0 | -5.72 | 0 | 0 |
| Temperature (L): Timing 20: Duration 6 | 0 | -15.15 | 0 | 0 |
| Temperature (Q): Timing 20: Duration 6 | 0 | 0 | 17.55 | 0 |
| Temperature (C): Timing 20: Duration 6 | 5.37 | 0 | 17.89 | 5.37 |
| **Temperature (L): Amplitude +3: Duration 3** | **18.36** | **1.84** | **25.46** | 18.36 |
| Temperature (Q): Amplitude +3: Duration 3 | 1.36 | 0 | 25.38 | 1.36 |
| Temperature (C): Amplitude +3: Duration 3 | 1.53 | 0 | 23.78 | 1.53 |
| Temperature (L): Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (Q): Amplitude +6: Duration 3 | 0 | 0 | 1.01 | 0 |
| Temperature (C): Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (Q): Amplitude +3: Duration 6 | 0 | 0 | 4.83 | 0 |
| Temperature (C): Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (L): Amplitude +6: Duration 6 | -27.17 | -54.65 | 0 | 27.17 |
| Temperature (Q): Amplitude +6: Duration 6 | 0 | -28.11 | 0 | 0 |
| Temperature (C): Amplitude +6: Duration 6 | 0 | -9.67 | 0 | 0 |
| Timing -10: Amplitude +3: Duration 3 | -0.10 | -0.39 | 0 | 0.10 |
| Timing 0: Amplitude +3: Duration 3 | 0 | 0 | 0.02 | 0 |
| Timing 10: Amplitude +3: Duration 3 | 0 | -0.18 | 0.15 | 0 |
| Timing 20: Amplitude +3: Duration 3 | 0 | -0.06 | 0.22 | 0 |
| Timing -10: Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 0: Amplitude +6: Duration 3 | 0 | -0.31 | 0.08 | 0 |
| Timing 10: Amplitude +6: Duration 3 | 0 | 0 | 0.08 | 0 |
| Timing 20: Amplitude +6: Duration 3 | 0 | -0.25 | 0 | 0 |
| Timing -10: Amplitude +3: Duration 6 | 0 | -0.14 | 0 | 0 |
| Timing 0: Amplitude +3: Duration 6 | 0.29 | 0 | 0.58 | 0.29 |
| Timing 10: Amplitude +3: Duration 6 | 0 | 0 | 0.14 | 0 |
| Timing 20: Amplitude +3: Duration 6 | 0 | -0.08 | 0.07 | 0 |
| Timing -10: Amplitude +6: Duration 6 | 0.51 | 0 | 1.47 | 0.51 |
| Timing 0: Amplitude +6: Duration 6 | -1.86 | -2.45 | 0 | 1.86 |

| | | | | |
|---|---|---|---|---|
| Timing 10: Amplitude +6: Duration 6 | -0.93 | -2.54 | 0 | 0.93 |
| Timing 20: Amplitude +6: Duration 6 | 0 | -1.02 | 0 | 0 |
| Temperature (L): Timing -10: Amplitude +3: Duration 3 | 0 | 0 | 6.01 | 0 |
| Temperature (Q): Timing -10: Amplitude +3: Duration 3 | 0 | 0 | 20.22 | 0 |
| Temperature (C): Timing -10: Amplitude +3: Duration 3 | 5.34 | 0 | 20.05 | 5.34 |
| Temperature (L): Timing 0: Amplitude +3: Duration 3 | 0 | 0 | 21.24 | 0 |
| Temperature (Q): Timing 0: Amplitude +3: Duration 3 | 0 | 0 | 31.49 | 0 |
| Temperature (C): Timing 0: Amplitude +3: Duration 3 | 3.64 | 0 | 24.84 | 3.64 |
| Temperature (L): Timing 10: Amplitude +3: Duration 3 | 0 | 0 | 15.16 | 0 |
| Temperature (Q): Timing 10: Amplitude +3: Duration 3 | 0 | -0.34 | 28.72 | 0 |
| Temperature (C): Timing 10: Amplitude +3: Duration 3 | 0 | 0 | 14.72 | 0 |
| Temperature (L): Timing 20: Amplitude +3: Duration 3 | 0 | 0 | 18.04 | 0 |
| Temperature (Q): Timing 20: Amplitude +3: Duration 3 | 0 | 0 | 8.86 | 0 |
| Temperature (C): Timing 20: Amplitude +3: Duration 3 | 0 | -1.39 | 0 | 0 |
| Temperature (L): Timing -10: Amplitude +6: Duration 3 | 0 | 0 | 0.92 | 0 |
| Temperature (Q): Timing -10: Amplitude +6: Duration 3 | 0 | 0 | 7.57 | 0 |
| Temperature (C): Timing -10: Amplitude +6: Duration 3 | 0 | -12.58 | 0 | 0 |
| Temperature (L): Timing 0: Amplitude +6: Duration 3 | 0 | -18.90 | 0 | 0 |
| Temperature (Q): Timing 0: Amplitude +6: Duration 3 | 0 | -32.75 | 0 | 0 |
| Temperature (C): Timing 0: Amplitude +6: Duration 3 | 0 | -20.09 | 0 | 0 |
| Temperature (L): Timing 10: Amplitude +6: Duration 3 | 0 | 0 | 1.49 | 0 |
| Temperature (Q): Timing 10: Amplitude +6: Duration 3 | 0 | 0 | 24.36 | 0 |
| Temperature (C): Timing 10: Amplitude +6: Duration 3 | 2.91 | 0 | 25.42 | 2.91 |
| Temperature (L): Timing 20: Amplitude +6: Duration 3 | 0 | -6.69 | 1.31 | 0 |
| Temperature (Q): Timing 20: Amplitude +6: Duration 3 | 0 | 0 | 36.20 | 0 |
| Temperature (C): Timing 20: Amplitude +6: Duration 3 | 8.58 | 0 | 35.68 | 8.58 |
| Temperature (L): Timing -10: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (Q): Timing -10: Amplitude +3: Duration 6 | 0 | 0 | 1.44 | 0 |
| Temperature (C): Timing -10: Amplitude +3: Duration 6 | 0 | -17.99 | 0 | 0 |
| Temperature (L): Timing 0: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (Q): Timing 0: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (C): Timing 0: Amplitude +3: Duration 6 | 0 | 0 | 0.23 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (L): Timing 10: Amplitude +3: Duration 6 | 5.50 | 0 | 16.17 | 5.50 |
| Temperature (Q): Timing 10: Amplitude +3: Duration 6 | 0 | 0 | 28.96 | 0 |
| Temperature (C): Timing 10: Amplitude +3: Duration 6 | 0 | 0 | 14.55 | 0 |
| Temperature (L): Timing 20: Amplitude +3: Duration 6 | 0 | -3.39 | 0 | 0 |
| Temperature (Q): Timing 20: Amplitude +3: Duration 6 | 0 | 0 | 15.55 | 0 |
| Temperature (C): Timing 20: Amplitude +3: Duration 6 | 0.97 | 0 | 16.49 | 0.97 |
| Temperature (L): Timing -10: Amplitude +6: Duration 6 | 12.12 | 0 | 48.09 | 12.12 |
| Temperature (Q): Timing -10: Amplitude +6: Duration 6 | 0.78 | 0 | 32.25 | 0.78 |
| Temperature (C): Timing -10: Amplitude +6: Duration 6 | 0 | -0.83 | 15.25 | 0 |
| Temperature (L): Timing 0: Amplitude +6: Duration 6 | -94.64 | -130.03 | 0 | 94.64 |
| Temperature (Q): Timing 0: Amplitude +6: Duration 6 | -98.07 | -135.65 | 0 | 98.07 |
| Temperature (C): Timing 0: Amplitude +6: Duration 6 | -37.17 | -59.46 | 0 | 37.17 |
| **Temperature (L): Timing 10: Amplitude +6: Duration 6** | **-49.58** | **-140.53** | **-11.36** | 49.58 |
| **Temperature (Q): Timing 10: Amplitude +6: Duration 6** | **-64.29** | **-154.04** | **-11.42** | 64.29 |
| Temperature (C): Timing 10: Amplitude +6: Duration 6 | -16.81 | -66.26 | 0 | 16.81 |
| Temperature (L): Timing 20: Amplitude +6: Duration 6 | 0 | -55.88 | 0 | 0 |
| Temperature (Q): Timing 20: Amplitude +6: Duration 6 | 0 | -26.36 | 35.66 | 0 |
| Temperature (C): Timing 20: Amplitude +6: Duration 6 | 0 | 0 | 8.58 | 0 |

**Table C.5: Combined feature importance for exposed.**
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.

| Explanatory Variable | Importance |
|---|---|
| Temperature: Timing 0: Amplitude +6: Duration 6 | 226.74 |
| Temperature: Timing 10: Amplitude +6: Duration 6 | 131.23 |
| Temperature: Timing 0: Amplitude +6 | 70.87 |
| Temperature: Amplitude +3 | 33.90 |
| Temperature | 31.73 |
| Temperature: Timing 20: Amplitude +6 | 28.47 |
| Temperature: Amplitude +6: Duration 6 | 27.36 |
| Temperature: Timing -10: Amplitude +6 | 22.38 |
| Temperature: Duration 3 | 21.38 |

| | |
|---|---|
| Temperature: Amplitude +3: Duration 3 | 18.79 |
| Temperature: Timing -10 | 12.96 |
| Temperature: Timing -10: Amplitude +6: Duration 6 | 12.69 |
| Temperature: Timing 20 | 11.58 |
| Temperature: Timing 10: Duration 3 | 11.48 |
| Temperature: Timing -10: Duration 6 | 11.27 |
| Temperature: Amplitude +6 | 9.68 |
| Temperature: Timing 20: Amplitude +6: Duration 3 | 7.52 |
| Temperature: Timing 10: Amplitude +6 | 7 |
| Temperature: Timing 0: Duration 6 | 6.22 |
| Temperature: Timing -10: Amplitude +3: Duration 3 | 6.15 |
| Temperature: Timing 0: Amplitude +3: Duration 3 | 6.02 |
| Temperature: Timing 10: Amplitude +3: Duration 6 | 5.75 |
| Temperature: Timing 20: Duration 6 | 4.29 |
| Temperature: Timing 10: Amplitude +6: Duration 3 | 2.10 |
| Timing 0: Amplitude +6: Duration 6 | 1.86 |
| Temperature: Timing 20: Amplitude +3: Duration 6 | 1.12 |
| Timing 10: Amplitude +6: Duration 6 | 0.94 |
| Timing -10: Amplitude +6: Duration 6 | 0.51 |
| Timing 20: Amplitude +6 | 0.42 |
| Amplitude +6 | 0.36 |
| Amplitude +6: Duration 6 | 0.31 |
| Timing 0: Amplitude +3: Duration 6 | 0.29 |
| Timing -10: Amplitude +6 | 0.27 |
| Timing 0: Amplitude +3 | 0.22 |
| Timing 0: Amplitude +6 | 0.13 |
| Timing -10: Amplitude +3: Duration 3 | 0.11 |
| Timing -10: Duration 6 | 0.05 |
| Timing 10: Duration 6 | 0.03 |
| Timing 20: Duration 6 | 0.03 |
| X Intercept | 0 |
| Timing -10 | 0 |

| | |
|---|---|
| Timing 0 | 0 |
| Timing 10 | 0 |
| Timing 20 | 0 |
| Amplitude +3 | 0 |
| Duration 3 | 0 |
| Duration 6 | 0 |
| Temperature: Timing 0 | 0 |
| Temperature: Timing 10 | 0 |
| Timing -10: Amplitude +3 | 0 |
| Timing 10: Amplitude +3 | 0 |
| Timing 20: Amplitude +3 | 0 |
| Timing 10: Amplitude +6 | 0 |
| Temperature: Duration 6 | 0 |
| Timing -10: Duration 3 | 0 |
| Timing 0: Duration 3 | 0 |
| Timing 10: Duration 3 | 0 |
| Timing 20: Duration 3 | 0 |
| Timing 0: Duration 6 | 0 |
| Amplitude +3: Duration 3 | 0 |
| Amplitude +6: Duration 3 | 0 |
| Amplitude +3: Duration 6 | 0 |
| Temperature: Timing -10: Amplitude +3 | 0 |
| Temperature: Timing 0: Amplitude +3 | 0 |
| Temperature: Timing 10: Amplitude +3 | 0 |
| Temperature: Timing 20: Amplitude +3 | 0 |
| Temperature: Timing -10: Duration 3 | 0 |
| Temperature: Timing 0: Duration 3 | 0 |
| Temperature: Timing 20: Duration 3 | 0 |
| Temperature: Timing 10: Duration 6 | 0 |
| Temperature: Amplitude +6: Duration 3 | 0 |
| Temperature: Amplitude +3: Duration 6 | 0 |
| Timing 0: Amplitude +3: Duration 3 | 0 |

| | |
|---|---|
| Timing 10: Amplitude +3: Duration 3 | 0 |
| Timing 20: Amplitude +3: Duration 3 | 0 |
| Timing -10: Amplitude +6: Duration 3 | 0 |
| Timing 0: Amplitude +6: Duration 3 | 0 |
| Timing 10: Amplitude +6: Duration 3 | 0 |
| Timing 20: Amplitude +6: Duration 3 | 0 |
| Timing -10: Amplitude +3: Duration 6 | 0 |
| Timing 10: Amplitude +3: Duration 6 | 0 |
| Timing 20: Amplitude +3: Duration 6 | 0 |
| Timing 20: Amplitude +6: Duration 6 | 0 |
| Temperature: Timing 10: Amplitude +3: Duration 3 | 0 |
| Temperature: Timing 20: Amplitude +3: Duration 3 | 0 |
| Temperature: Timing -10: Amplitude +6: Duration 3 | 0 |
| Temperature: Timing 0: Amplitude +6: Duration 3 | 0 |
| Temperature: Timing -10: Amplitude +3: Duration 6 | 0 |
| Temperature: Timing 0: Amplitude +3: Duration 6 | 0 |
| Temperature: Timing 20: Amplitude +6: Duration 6 | 0 |

**Table C.6: 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 (Eff) 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 CI; lower confidence interval (95th percentile), Upper CI; upper confidence interval (95th percentile), Imp; feature importance for the polynomial degree. Non-significant values have CIs encompassing 0, suggesting no important relationship to the response variable. Temperature was a cubic polynomial to account for non-linearity and is therefore separated by each degree (linear [L], quadratic [Q], and cubic [C]). Feature importance (Imp) was calculated by taking the absolute value of the effect.

| **Explanatory Variable** | **Effect** | **Lower CI** | **Upper CI** | **Imp** |
|---|---|---|---|---|
| X Intercept | 5.97 | 5.84 | 6.10 | 0 |
| Temperature (L) | 0 | -3.73 | 0 | 0 |
| **Temperature (Q)** | **-19.40** | **-22.36** | **-16.81** | 19.40 |
| Temperature (C) | 0 | -0.36 | 2.54 | 0 |

| | | | | |
|---|---|---|---|---|
| Timing -10 | 0 | -0.06 | 0 | 0 |
| Timing 0 | 0.24 | 0 | 0.37 | 0.24 |
| Timing 10 | 0 | -0.14 | 0 | 0 |
| Timing 20 | 0 | -0.20 | 0 | 0 |
| Amplitude +3 | 0 | -0.04 | 0 | 0 |
| Amplitude +6 | 0 | -0.03 | 0 | 0 |
| Duration 3 | -0.10 | -0.20 | 0 | 0.10 |
| Duration 6 | 0 | 0 | 0.08 | 0 |
| Temperature (L): Timing -10 | 6.36 | 0 | 10.52 | 6.36 |
| Temperature (Q): Timing -10 | 4.79 | 0 | 8.88 | 4.79 |
| Temperature (C): Timing -10 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 0 | 0 | -0.28 | 0 | 0 |
| Temperature (Q): Timing 0 | 0 | 0 | 0 | 0 |
| Temperature (C): Timing 0 | 0.14 | 0 | 4.52 | 0.14 |
| Temperature (L): Timing 10 | 0 | -0.03 | 0 | 0 |
| Temperature (Q): Timing 10 | 0 | 0 | 3.01 | 0 |
| Temperature (C): Timing 10 | 0 | 0 | 9.51 | 0 |
| Temperature (L): Timing 20 | 0 | -5.09 | 0 | 0 |
| Temperature (Q): Timing 20 | 4.38 | 0 | 11.43 | 4.38 |
| Temperature (C): Timing 20 | 1.86 | 0 | 12.89 | 1.86 |
| Temperature (L): Amplitude +3 | 0 | 0 | 2.98 | 0 |
| Temperature (Q): Amplitude +3 | 3.17 | 0 | 8.11 | 3.17 |
| Temperature (C): Amplitude +3 | 5.03 | 0 | 8.92 | 5.03 |
| Temperature (L): Amplitude +6 | 0 | -2.88 | 0 | 0 |
| Temperature (Q): Amplitude +6 | 1.00 | 0 | 4.65 | 1.00 |
| Temperature (C): Amplitude +6 | 1.23 | 0 | 5.65 | 1.23 |
| Timing -10: Amplitude +3 | 0 | -0.17 | 0 | 0 |
| Timing 0: Amplitude +3 | 0 | 0 | 0.09 | 0 |
| Timing 10: Amplitude +3 | 0 | -0.11 | 0 | 0 |
| Timing 20: Amplitude +3 | 0 | 0 | 0.03 | 0 |
| Timing -10: Amplitude +6 | 0 | 0 | 0.09 | 0 |
| Timing 0: Amplitude +6 | 0.01 | 0 | 0.20 | 0.01 |

| | | | | |
|---|---|---|---|---|
| Timing 10: Amplitude +6 | 0 | -0.12 | 0 | 0 |
| **Timing 20: Amplitude +6** | **-0.54** | **-0.76** | **-0.20** | 0.54 |
| Temperature (L): Duration 3 | 0 | 0 | 1.42 | 0 |
| Temperature (Q): Duration 3 | 0.38 | 0 | 4.81 | 0.38 |
| **Temperature (C): Duration 3** | **9.44** | **3.66** | **14.78** | 9.44 |
| Temperature (L): Duration 6 | 0 | -1.14 | 0 | 0 |
| Temperature (Q): Duration 3 | 1.42 | 0 | 7.21 | 1.42 |
| Temperature (C): Duration 3 | 0 | 0 | 2.49 | 0 |
| Timing -10: Duration 3 | 0 | -0.25 | 0 | 0 |
| Timing 0: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 10: Duration 3 | 0 | -0.11 | 0 | 0 |
| Timing 20: Duration 3 | 0 | -0.20 | 0 | 0 |
| Timing -10: Duration 6 | 0 | 0 | 0.11 | 0 |
| Timing 0: Duration 6 | 0.18 | 0 | 0.39 | 0.18 |
| Timing 10: Duration 6 | 0 | -0.12 | 0 | 0 |
| Timing 20: Duration 6 | 0 | -0.15 | 0 | 0 |
| Amplitude +3: Duration 3 | 0 | -0.09 | 0 | 0 |
| Amplitude +6: Duration 3 | 0 | -0.09 | 0 | 0 |
| Amplitude +3: Duration 6 | 0 | 0 | 0.04 | 0 |
| Amplitude +6: Duration 6 | 0 | 0 | 0.02 | 0 |
| Temperature (L): Timing -10: Amplitude +3 | 0 | 0 | 5.71 | 0 |
| Temperature (Q): Timing -10: Amplitude +3 | 0 | 0 | 3.59 | 0 |
| Temperature (C): Timing -10: Amplitude +3 | 0 | 0 | 1.52 | 0 |
| Temperature (L): Timing 0: Amplitude +3 | 0 | 0 | 0 | 0 |
| Temperature (Q): Timing 0: Amplitude +3 | 0 | 0 | 1.04 | 0 |
| Temperature (C): Timing 0: Amplitude +3 | 0.61 | 0 | 7.07 | 0.61 |
| Temperature (L): Timing 10: Amplitude +3 | 0 | 0 | 5.95 | 0 |
| Temperature (Q): Timing 10: Amplitude +3 | 0 | 0 | 9.07 | 0 |
| Temperature (C): Timing 10: Amplitude +3 | 0 | 0 | 1.52 | 0 |
| Temperature (L): Timing 20: Amplitude +3 | 0 | 0 | 0 | 0 |
| Temperature (Q): Timing 20: Amplitude +3 | 0 | 0 | 2.09 | 0 |
| Temperature (C): Timing 20: Amplitude +3 | 0 | 0 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (L): Timing -10: Amplitude +6 | 0 | 0 | 4.94 | 0 |
| Temperature (Q): Timing -10: Amplitude +6 | 0.91 | 0 | 8.07 | 0.91 |
| Temperature (C): Timing -10: Amplitude +6 | 0 | -2.77 | 0 | 0 |
| Temperature (L): Timing 0: Amplitude +6 | 0 | -3.58 | 0 | 0 |
| Temperature (Q): Timing 0: Amplitude +6 | 0 | 0 | 0 | 0 |
| Temperature (C): Timing 0: Amplitude +6 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 10: Amplitude +6 | -3.31 | -9.61 | 0 | 3.31 |
| Temperature (Q): Timing 10: Amplitude +6 | 0 | -1.22 | 0 | 0 |
| Temperature (C): Timing 10: Amplitude +6 | 4.28 | 0 | 9.59 | 4.28 |
| **Temperature (L): Timing 20: Amplitude +6** | **-14.65** | **-21.91** | **-0.75** | 14.65 |
| Temperature (Q): Timing 20: Amplitude +6 | 0 | 0 | 16.57 | 0 |
| Temperature (C): Timing 20: Amplitude +6 | 10.87 | 0 | 23.80 | 10.87 |
| Temperature (L): Timing -10: Duration 3 | 0 | 0 | 4.24 | 0 |
| Temperature (Q): Timing -10: Duration 3 | 0 | 0 | 6.44 | 0 |
| Temperature (C): Timing -10: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 0: Duration 3 | 0 | 0 | 5.20 | 0 |
| Temperature (Q): Timing 0: Duration 3 | 0 | 0 | 0.99 | 0 |
| Temperature (C): Timing 0: Duration 3 | 0 | 0 | 3.20 | 0 |
| Temperature (L): Timing 10: Duration 3 | 0 | 0 | 0.31 | 0 |
| Temperature (Q): Timing 10: Duration 3 | 0 | -0.20 | 4.19 | 0 |
| Temperature (C): Timing 10: Duration 3 | 5.50 | 0 | 15.33 | 5.50 |
| Temperature (L): Timing 20: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (Q): Timing 20: Duration 3 | 0 | 0 | 5.39 | 0 |
| Temperature (C): Timing 20: Duration 3 | 0 | 0 | 0.81 | 0 |
| Temperature (L): Timing -10: Duration 6 | 0.87 | 0 | 6.94 | 0.87 |
| Temperature (Q): Timing -10: Duration 6 | 0 | 0 | 5.92 | 0 |
| Temperature (C): Timing -10: Duration 6 | 0 | -0.70 | 0 | 0 |
| Temperature (L): Timing 0: Duration 6 | -3.73 | -9.31 | 0 | 3.73 |
| Temperature (Q): Timing 0: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (C): Timing 0: Duration 6 | 0 | 0 | 3.28 | 0 |
| Temperature (L): Timing 10: Duration 6 | 0 | -2.89 | 0 | 0 |
| Temperature (Q): Timing 10: Duration 6 | 0 | 0 | 2.08 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (C): Timing 10: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing 20: Duration 6 | -2.90 | -9.55 | 0 | 2.90 |
| Temperature (Q): Timing 20: Duration 6 | 0 | 0 | 8.31 | 0 |
| Temperature (C): Timing 20: Duration 6 | 5.91 | 0 | 12.38 | 5.91 |
| **Temperature (L): Amplitude +3: Duration 3** | **6.77** | **0.13** | **12.38** | 6.77 |
| Temperature (Q): Amplitude +3: Duration 3 | 0 | 0 | 7.07 | 0 |
| Temperature (C): Amplitude +3: Duration 3 | 0 | 0 | 6.06 | 0 |
| Temperature (L): Amplitude +6: Duration 3 | 0 | -0.29 | 0 | 0 |
| Temperature (Q): Amplitude +6: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (C): Amplitude +6: Duration 3 | 0 | 0 | 3.38 | 0 |
| Temperature (L): Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (Q): Amplitude +3: Duration 6 | 0 | 0 | 0.69 | 0 |
| Temperature (C): Amplitude +3: Duration 6 | 0 | 0 | 0.02 | 0 |
| Temperature (L): Amplitude +6: Duration 6 | -0.97 | -4.77 | 0 | 0.97 |
| Temperature (Q): Amplitude +6: Duration 6 | 0 | 0 | 5.53 | 0 |
| Temperature (C): Amplitude +6: Duration 6 | 0 | 0 | 1.92 | 0 |
| Timing -10: Amplitude +3: Duration 3 | -0.08 | -0.34 | 0 | 0.08 |
| Timing 0: Amplitude +3: Duration 3 | 0 | 0 | 0 | 0 |
| Timing 10: Amplitude +3: Duration 3 | 0 | -0.04 | 0.08 | 0 |
| Timing 20: Amplitude +3: Duration 3 | 0 | 0 | 0.07 | 0 |
| Timing -10: Amplitude +6: Duration 3 | 0 | -0.17 | 0.01 | 0 |
| Timing 0: Amplitude +6: Duration 3 | 0.05 | 0 | 0.25 | 0.05 |
| Timing 10: Amplitude +6: Duration 3 | -0.02 | -0.28 | 0 | 0.02 |
| Timing 20: Amplitude +6: Duration 3 | 0 | -0.32 | 0 | 0 |
| Timing -10: Amplitude +3: Duration 6 | 0 | -0.06 | 0.03 | 0 |
| Timing 0: Amplitude +3: Duration 6 | 0.04 | 0 | 0.37 | 0 |
| Timing 10: Amplitude +3: Duration 6 | -0.04 | -0.29 | 0 | 0.04 |
| Timing 20: Amplitude +3: Duration 6 | 0 | 0 | 0.09 | 0 |
| Timing -10: Amplitude +6: Duration 6 | 0.06 | 0 | 0.24 | 0.06 |
| Timing 0: Amplitude +6: Duration 6 | 0 | 0 | 0.03 | 0 |
| Timing 10: Amplitude +6: Duration 6 | 0 | -0.05 | 0.17 | 0 |
| Timing 20: Amplitude +6: Duration 6 | 0 | -0.52 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (L): Timing -10: Amplitude +3: Duration 3 | 0 | 0 | 6.31 | 0 |
| Temperature (Q): Timing -10: Amplitude +3: Duration 3 | 0 | 0 | 5.65 | 0 |
| Temperature (C): Timing -10: Amplitude +3: Duration 3 | 0 | 0 | 6.88 | 0 |
| Temperature (L): Timing 0: Amplitude +3: Duration 3 | 0 | 0 | 9.20 | 0 |
| Temperature (Q): Timing 0: Amplitude +3: Duration 3 | 0 | 0 | 7.83 | 0 |
| Temperature (C): Timing 0: Amplitude +3: Duration 3 | 0 | 0 | 9.02 | 0 |
| Temperature (L): Timing 10: Amplitude +3: Duration 3 | 0 | 0 | 6.67 | 0 |
| Temperature (Q): Timing 10: Amplitude +3: Duration 3 | 0 | -0.76 | 11.25 | 0 |
| Temperature (C): Timing 10: Amplitude +3: Duration 3 | 0 | 0 | 2.59 | 0 |
| Temperature (L): Timing 20: Amplitude +3: Duration 3 | 0 | 0 | 11.72 | 0 |
| Temperature (Q): Timing 20: Amplitude +3: Duration 3 | 0 | 0 | 9.19 | 0 |
| Temperature (C): Timing 20: Amplitude +3: Duration 3 | 0 | 0 | 0 | 0 |
| Temperature (L): Timing -10: Amplitude +6: Duration 3 | 0 | 0 | 6.24 | 0 |
| Temperature (Q): Timing -10: Amplitude +6: Duration 3 | 0 | 0 | 9.52 | 0 |
| Temperature (C): Timing -10: Amplitude +6: Duration 3 | 0 | -8.03 | 0 | 0 |
| Temperature (L): Timing 0: Amplitude +6: Duration 3 | 0 | 0 | 4.48 | 0 |
| Temperature (Q): Timing 0: Amplitude +6: Duration 3 | 0 | -1.05 | 0 | 0 |
| Temperature (C): Timing 0: Amplitude +6: Duration 3 | 0 | -1.27 | 0 | 0 |
| Temperature (L): Timing 10: Amplitude +6: Duration 3 | 0 | -5.40 | 0 | 0 |
| Temperature (Q): Timing 10: Amplitude +6: Duration 3 | 0 | -5.07 | 3.81 | 0 |
| Temperature (C): Timing 10: Amplitude +6: Duration 3 | 3.50 | 0 | 14.34 | 3.50 |
| Temperature (L): Timing 20: Amplitude +6: Duration 3 | 0 | -8.56 | 0 | 0 |
| Temperature (Q): Timing 20: Amplitude +6: Duration 3 | 0 | 0 | 1.26 | 0 |
| Temperature (C): Timing 20: Amplitude +6: Duration 3 | 3.26 | 0 | 16.18 | 3.26 |
| Temperature (L): Timing -10: Amplitude +3: Duration 6 | 0 | 0 | 4.92 | 0 |
| Temperature (Q): Timing -10: Amplitude +3: Duration 6 | 0 | 0 | 4.84 | 0 |
| Temperature (C): Timing -10: Amplitude +3: Duration 6 | 0 | -4.16 | 0 | 0 |
| Temperature (L): Timing 0: Amplitude +3: Duration 6 | 0 | -4.65 | 0 | 0 |
| Temperature (Q): Timing 0: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (C): Timing 0: Amplitude +3: Duration 6 | 0 | 0 | 3.91 | 0 |
| Temperature (L): Timing 10: Amplitude +3: Duration 6 | 0 | 0 | 6.27 | 0 |
| Temperature (Q): Timing 10: Amplitude +3: Duration 6 | 0 | 0 | 9.47 | 0 |

| | | | | |
|---|---|---|---|---|
| Temperature (C): Timing 10: Amplitude +3: Duration 6 | 0 | 0 | 0.52 | 0 |
| Temperature (L): Timing 20: Amplitude +3: Duration 6 | 0 | -1.78 | 0 | 0 |
| Temperature (Q): Timing 20: Amplitude +3: Duration 6 | 0 | 0 | 0 | 0 |
| Temperature (C): Timing 20: Amplitude +3: Duration 6 | 0 | 0 | 9.38 | 0 |
| Temperature (L): Timing -10: Amplitude +6: Duration 6 | 0 | 0 | 5.62 | 0 |
| Temperature (Q): Timing -10: Amplitude +6: Duration 6 | 0 | 0 | 6.11 | 0 |
| Temperature (C): Timing -10: Amplitude +6: Duration 6 | 0 | -1.30 | 1.92 | 0 |
| Temperature (L): Timing 0: Amplitude +6: Duration 6 | -1.10 | -12.82 | 0 | 1.10 |
| Temperature (Q): Timing 0: Amplitude +6: Duration 6 | 0 | 0 | 0.64 | 0 |
| Temperature (C): Timing 0: Amplitude +6: Duration 6 | 0 | 0 | 5.52 | 0 |
| Temperature (L): Timing 10: Amplitude +6: Duration 6 | -2.58 | -12.84 | 0 | 2.58 |
| Temperature (Q): Timing 10: Amplitude +6: Duration 6 | 0 | -0.21 | 0 | 0 |
| Temperature (C): Timing 10: Amplitude +6: Duration 6 | 0 | 0 | 1.77 | 0 |
| Temperature (L): Timing 20: Amplitude +6: Duration 6 | 0 | -21.21 | 0 | 0 |
| Temperature (Q): Timing 20: Amplitude +6: Duration 6 | 9.29 | 0 | 24.23 | 9.29 |
| Temperature (C): Timing 20: Amplitude +6: Duration 6 | 0 | 0 | 5.92 | 0 |

**Table C.7: estimated marginal means (emmean) for burden.**
In *Daphnia magna* infected with *Ordospora colligata*. The estimates were derived using a Generalised Linear Model (GLM) with baseline temperature as a factor, 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.

| Temp | Treatment | emmean | SE | df | Lower CI 95% | Upper CI 95% |
|---|---|---|---|---|---|---|
| 14 | C 0 0 | 5.28 | 0.198 | Inf | 4.89 | 5.67 |
| 17 | C 0 0 | 6.86 | 0.188 | Inf | 6.49 | 7.23 |
| 20 | C 0 0 | 6.3 | 0.202 | Inf | 5.91 | 6.7 |
| 23 | C 0 0 | 6.13 | 0.193 | Inf | 5.75 | 6.5 |
| 14 | -10 +3 3 | 4.63 | 0.237 | Inf | 4.17 | 5.1 |
| 17 | -10 +3 3 | 6.44 | 0.266 | Inf | 5.92 | 6.96 |
| 20 | -10 +3 3 | 6.06 | 0.266 | Inf | 5.54 | 6.58 |
| 23 | -10 +3 3 | 5.86 | 0.266 | Inf | 5.34 | 6.38 |
| 14 | -10 +3 6 | 5.27 | 0.236 | Inf | 4.81 | 5.74 |
| 17 | -10 +3 6 | 6.31 | 0.36 | Inf | 5.6 | 7.01 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 20 | -10 +3 6 | 6.31 | 0.255 | Inf | 5.81 | 6.81 |
| 23 | -10 +3 6 | 6.18 | 0.255 | Inf | 5.68 | 6.68 |
| 14 | -10 +6 3 | 5.2 | 0.267 | Inf | 4.68 | 5.72 |
| 17 | -10 +6 3 | 6.29 | 0.294 | Inf | 5.71 | 6.87 |
| 20 | -10 +6 3 | 6.3 | 0.294 | Inf | 5.72 | 6.87 |
| 23 | -10 +6 3 | 5.76 | 0.245 | Inf | 5.27 | 6.24 |
| 14 | -10 +6 6 | 5.39 | 0.255 | Inf | 4.89 | 5.89 |
| 17 | -10 +6 6 | 6.52 | 0.279 | Inf | 5.97 | 7.06 |
| 20 | -10 +6 6 | 6.24 | 0.266 | Inf | 5.72 | 6.76 |
| 23 | -10 +6 6 | 6.38 | 0.255 | Inf | 5.88 | 6.88 |
| 14 | 0 +3 3 | 5.06 | 0.245 | Inf | 4.58 | 5.55 |
| 17 | 0 +3 3 | 7.1 | 0.236 | Inf | 6.64 | 7.57 |
| 20 | 0 +3 3 | 6.21 | 0.266 | Inf | 5.69 | 6.73 |
| 23 | 0 +3 3 | 5.92 | 0.236 | Inf | 5.46 | 6.39 |
| 14 | 0 +3 6 | 6.06 | 0.228 | Inf | 5.61 | 6.51 |
| 17 | 0 +3 6 | 7.19 | 0.228 | Inf | 6.75 | 7.64 |
| 20 | 0 +3 6 | 6.82 | 0.312 | Inf | 6.2 | 7.43 |
| 23 | 0 +3 6 | 5.94 | 0.255 | Inf | 5.44 | 6.44 |
| 14 | 0 +6 3 | 5.46 | 0.236 | Inf | 4.99 | 5.92 |
| 17 | 0 +6 3 | 7.08 | 0.228 | Inf | 6.63 | 7.52 |
| 20 | 0 +6 3 | 6.76 | 0.279 | Inf | 6.21 | 7.31 |
| 23 | 0 +6 3 | 6.4 | 0.882 | Inf | 4.67 | 8.13 |
| 14 | 0 +6 6 | 6.16 | 0.279 | Inf | 5.61 | 6.7 |
| 17 | 0 +6 6 | 7.04 | 0.245 | Inf | 6.56 | 7.52 |
| 20 | 0 +6 6 | 6.63 | 0.36 | Inf | 5.92 | 7.33 |
| 23 | 0 +6 6 | nonEst | NA | NA | NA | NA |
| 14 | 10 +3 3 | 4.72 | 0.246 | Inf | 4.24 | 5.21 |
| 17 | 10 +3 3 | 7.03 | 0.228 | Inf | 6.59 | 7.48 |
| 20 | 10 +3 3 | 6.17 | 0.255 | Inf | 5.67 | 6.66 |
| 23 | 10 +3 3 | 5.51 | 0.236 | Inf | 5.05 | 5.97 |
| 14 | 10 +3 6 | 5.22 | 0.228 | Inf | 4.78 | 5.67 |
| 17 | 10 +3 6 | 6.53 | 0.228 | Inf | 6.09 | 6.98 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 20 | 10 +3 6 | 5.93 | 0.279 | Inf | 5.39 | 6.48 |
| 23 | 10 +3 6 | 5.71 | 0.228 | Inf | 5.26 | 6.16 |
| 14 | 10 +6 3 | 4.96 | 0.229 | Inf | 4.51 | 5.41 |
| 17 | 10 +6 3 | 7.24 | 0.236 | Inf | 6.77 | 7.7 |
| 20 | 10 +6 3 | 6.23 | 0.266 | Inf | 5.71 | 6.75 |
| 23 | 10 +6 3 | 4.6 | 0.281 | Inf | 4.05 | 5.15 |
| 14 | 10 +6 6 | 5.72 | 0.236 | Inf | 5.26 | 6.19 |
| 17 | 10 +6 6 | 6.69 | 0.245 | Inf | 6.21 | 7.17 |
| 20 | 10 +6 6 | 6.42 | 0.294 | Inf | 5.85 | 7 |
| 23 | 10 +6 6 | 3.53 | 0.898 | Inf | 1.77 | 5.29 |
| 14 | 20 +3 3 | 4.89 | 0.229 | Inf | 4.44 | 5.34 |
| 17 | 20 +3 3 | 6.7 | 0.228 | Inf | 6.26 | 7.15 |
| 20 | 20 +3 3 | 6.13 | 0.245 | Inf | 5.65 | 6.61 |
| 23 | 20 +3 3 | 5.67 | 0.236 | Inf | 5.21 | 6.13 |
| 14 | 20 +3 6 | 5.53 | 0.228 | Inf | 5.08 | 5.98 |
| 17 | 20 +3 6 | 6.85 | 0.236 | Inf | 6.38 | 7.31 |
| 20 | 20 +3 6 | 6.07 | 0.266 | Inf | 5.54 | 6.59 |
| 23 | 20 +3 6 | 5.3 | 0.28 | Inf | 4.75 | 5.84 |
| 14 | 20 +6 3 | 5.12 | 0.245 | Inf | 4.64 | 5.6 |
| 17 | 20 +6 3 | 6.88 | 0.228 | Inf | 6.43 | 7.33 |
| 20 | 20 +6 3 | 5.31 | 0.255 | Inf | 4.81 | 5.81 |
| 23 | 20 +6 3 | 3.59 | 0.259 | Inf | 3.09 | 4.1 |
| 14 | 20 +6 6 | 5.83 | 0.236 | Inf | 5.37 | 6.29 |
| 17 | 20 +6 6 | 6.45 | 0.228 | Inf | 6 | 6.9 |
| 20 | 20 +6 6 | 4.93 | 0.267 | Inf | 4.41 | 5.45 |
| 23 | 20 +6 6 | 3.81 | 0.447 | Inf | 2.94 | 4.69 |

**Table C.8: Custom contrasts for burden ‘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.

| Day | Temp | Contrast | Estimate | SE | df | z ratio | p value | Adjusted p |
|---|---|---|---|---|---|---|---|---|

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day -10 | 14 | +33 v 00 | -0.64841 | 0.309 | Inf | -2.101 | 0.0357 | 0.2464 |
| Day -10 | 14 | +36 v 00 | -0.00908 | 0.308 | Inf | -0.029 | 0.9765 | 1.000 |
| Day -10 | 14 | +63 v 00 | -0.08044 | 0.332 | Inf | -0.242 | 0.8086 | 0.9495 |
| Day -10 | 14 | +66 v 00 | 0.11194 | 0.323 | Inf | 0.347 | 0.7288 | 0.9315 |
| Day -10 | 14 | +36 v +33 | 0.63933 | 0.335 | Inf | 1.91 | 0.0561 | 0.3123 |
| Day -10 | 14 | +66 v +63 | 0.19238 | 0.369 | Inf | 0.521 | 0.6022 | 0.8686 |
| Day -10 | 14 | +63 v +33 | 0.56797 | 0.357 | Inf | 1.592 | 0.1114 | 0.4918 |
| Day -10 | 14 | +66 v +36 | 0.12102 | 0.348 | Inf | 0.348 | 0.7279 | 0.9315 |
| Day -10 | 17 | +33 v 00 | -0.41725 | 0.326 | Inf | -1.281 | 0.2003 | 0.5826 |
| Day -10 | 17 | +36 v 00 | -0.55062 | 0.406 | Inf | -1.355 | 0.1754 | 0.5614 |
| Day -10 | 17 | +63 v 00 | -0.56821 | 0.349 | Inf | -1.628 | 0.1036 | 0.4912 |
| Day -10 | 17 | +66 v 00 | -0.33952 | 0.336 | Inf | -1.009 | 0.3129 | 0.7026 |
| Day -10 | 17 | +36 v +33 | -0.13337 | 0.448 | Inf | -0.298 | 0.7658 | 0.9315 |
| Day -10 | 17 | +66 v +63 | 0.22869 | 0.405 | Inf | 0.564 | 0.5727 | 0.8686 |
| Day -10 | 17 | +63 v +33 | -0.15096 | 0.397 | Inf | -0.381 | 0.7035 | 0.9188 |
| Day -10 | 17 | +66 v +36 | 0.2111 | 0.456 | Inf | 0.463 | 0.6431 | 0.8904 |
| Day -10 | 20 | +33 v 00 | -0.24257 | 0.334 | Inf | -0.725 | 0.4682 | 0.8686 |
| Day -10 | 20 | +36 v 00 | 0.01061 | 0.325 | Inf | 0.033 | 0.974 | 1.000 |
| Day -10 | 20 | +63 v 00 | -0.00751 | 0.357 | Inf | -0.021 | 0.9832 | 1.000 |
| Day -10 | 20 | +66 v 00 | -0.06631 | 0.334 | Inf | -0.198 | 0.8428 | 0.9576 |
| Day -10 | 20 | +36 v +33 | 0.25318 | 0.368 | Inf | 0.687 | 0.4919 | 0.8686 |
| Day -10 | 20 | +66 v +63 | -0.0588 | 0.397 | Inf | -0.148 | 0.8821 | 0.9651 |
| Day -10 | 20 | +63 v +33 | 0.23506 | 0.397 | Inf | 0.593 | 0.5535 | 0.8686 |
| Day -10 | 20 | +66 v +36 | -0.07692 | 0.368 | Inf | -0.209 | 0.8346 | 0.9576 |
| Day -10 | 23 | +33 v 00 | -0.26613 | 0.329 | Inf | -0.81 | 0.418 | 0.8629 |
| Day -10 | 23 | +36 v 00 | 0.05456 | 0.319 | Inf | 0.171 | 0.8644 | 0.9576 |
| Day -10 | 23 | +63 v 00 | -0.37165 | 0.312 | Inf | -1.193 | 0.233 | 0.6213 |
| Day -10 | 23 | +66 v 00 | 0.2495 | 0.319 | Inf | 0.781 | 0.4346 | 0.8686 |
| Day -10 | 23 | +36 v +33 | 0.3207 | 0.368 | Inf | 0.87 | 0.3841 | 0.8334 |
| Day -10 | 23 | +66 v +63 | 0.62115 | 0.353 | Inf | 1.758 | 0.0788 | 0.3879 |
| Day -10 | 23 | +63 v +33 | -0.10552 | 0.362 | Inf | -0.292 | 0.7705 | 0.9315 |
| Day -10 | 23 | +66 v +36 | 0.19494 | 0.36 | Inf | 0.541 | 0.5884 | 0.8686 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day 0 | 14 | +33 v 00 | -0.21839 | 0.315 | Inf | -0.693 | 0.4884 | 0.8686 |
| Day 0 | 14 | +36 v 00 | 0.77653 | 0.302 | Inf | 2.574 | 0.0101 | 0.1125 |
| Day 0 | 14 | +63 v 00 | 0.17318 | 0.308 | Inf | 0.562 | 0.574 | 0.8686 |
| Day 0 | 14 | +66 v 00 | 0.87475 | 0.342 | Inf | 2.557 | 0.0105 | 0.1125 |
| **Day 0** | **14** | **+36 v +33** | **0.99491** | **0.335** | **Inf** | **2.97** | **0.003** | **0.0476** |
| Day 0 | 14 | +66 v +63 | 0.70156 | 0.366 | Inf | 1.919 | 0.055 | 0.3123 |
| Day 0 | 14 | +63 v +33 | 0.39157 | 0.341 | Inf | 1.15 | 0.2503 | 0.6389 |
| Day 0 | 14 | +66 v +36 | 0.09822 | 0.36 | Inf | 0.273 | 0.7852 | 0.9315 |
| Day 0 | 17 | +33 v 00 | 0.24708 | 0.302 | Inf | 0.819 | 0.4125 | 0.8629 |
| Day 0 | 17 | +36 v 00 | 0.33764 | 0.295 | Inf | 1.143 | 0.2529 | 0.6389 |
| Day 0 | 17 | +63 v 00 | 0.22016 | 0.295 | Inf | 0.746 | 0.456 | 0.8686 |
| Day 0 | 17 | +66 v 00 | 0.18459 | 0.309 | Inf | 0.598 | 0.5496 | 0.8686 |
| Day 0 | 17 | +36 v +33 | 0.09057 | 0.328 | Inf | 0.276 | 0.7823 | 0.9315 |
| Day 0 | 17 | +66 v +63 | -0.03557 | 0.334 | Inf | -0.106 | 0.9152 | 0.9928 |
| Day 0 | 17 | +63 v +33 | -0.02692 | 0.328 | Inf | -0.082 | 0.9345 | 1.000 |
| Day 0 | 17 | +66 v +36 | -0.15306 | 0.334 | Inf | -0.458 | 0.6469 | 0.8904 |
| Day 0 | 20 | +33 v 00 | -0.09078 | 0.334 | Inf | -0.272 | 0.786 | 0.9315 |
| Day 0 | 20 | +36 v 00 | 0.51184 | 0.372 | Inf | 1.377 | 0.1686 | 0.5534 |
| Day 0 | 20 | +63 v 00 | 0.45505 | 0.345 | Inf | 1.32 | 0.1867 | 0.569 |
| Day 0 | 20 | +66 v 00 | 0.32553 | 0.413 | Inf | 0.788 | 0.4307 | 0.8686 |
| Day 0 | 20 | +36 v +33 | 0.60262 | 0.41 | Inf | 1.47 | 0.1415 | 0.5329 |
| Day 0 | 20 | +66 v +63 | -0.12952 | 0.456 | Inf | -0.284 | 0.7761 | 0.9315 |
| Day 0 | 20 | +63 v +33 | 0.54584 | 0.385 | Inf | 1.416 | 0.1568 | 0.5534 |
| Day 0 | 20 | +66 v +36 | -0.18631 | 0.476 | Inf | -0.391 | 0.6957 | 0.9181 |
| Day 0 | 23 | +33 v 00 | -0.20298 | 0.305 | Inf | -0.666 | 0.5052 | 0.8686 |
| Day 0 | 23 | +36 v 00 | -0.18277 | 0.319 | Inf | -0.572 | 0.5672 | 0.8686 |
| Day 0 | 23 | +63 v 00 | 0.27193 | 0.903 | Inf | 0.301 | 0.7633 | 0.9315 |
| Day 0 | 23 | +66 v 00 | nonEst | NA | NA | NA | NA | NA |
| Day 0 | 23 | +36 v +33 | 0.02021 | 0.347 | Inf | 0.058 | 0.9536 | 1.000 |
| Day 0 | 23 | +66 v +63 | nonEst | NA | NA | NA | NA | NA |
| Day 0 | 23 | +63 v +33 | 0.47491 | 0.913 | Inf | 0.52 | 0.6031 | 0.8686 |
| Day 0 | 23 | +66 v +36 | nonEst | NA | NA | NA | NA | NA |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day 10 | 14 | +33 v 00 | -0.55846 | 0.315 | Inf | -1.77 | 0.0767 | 0.3879 |
| Day 10 | 14 | +36 v 00 | -0.05813 | 0.302 | Inf | -0.192 | 0.8474 | 0.9576 |
| Day 10 | 14 | +63 v 00 | -0.32333 | 0.302 | Inf | -1.07 | 0.2847 | 0.6749 |
| Day 10 | 14 | +66 v 00 | 0.44184 | 0.308 | Inf | 1.435 | 0.1513 | 0.5534 |
| Day 10 | 14 | +36 v +33 | 0.50033 | 0.336 | Inf | 1.491 | 0.1359 | 0.5272 |
| Day 10 | 14 | +66 v +63 | 0.76518 | 0.329 | Inf | 2.329 | 0.0199 | 0.159 |
| Day 10 | 14 | +63 v +33 | 0.23513 | 0.336 | Inf | 0.7 | 0.4837 | 0.8686 |
| Day 10 | 14 | +66 v +36 | 0.49997 | 0.328 | Inf | 1.522 | 0.1279 | 0.5272 |
| Day 10 | 17 | +33 v 00 | 0.1745 | 0.295 | Inf | 0.591 | 0.5546 | 0.8686 |
| Day 10 | 17 | +36 v 00 | -0.32407 | 0.295 | Inf | -1.097 | 0.2726 | 0.6709 |
| Day 10 | 17 | +63 v 00 | 0.37878 | 0.301 | Inf | 1.256 | 0.209 | 0.5945 |
| Day 10 | 17 | +66 v 00 | -0.1672 | 0.309 | Inf | -0.542 | 0.5879 | 0.8686 |
| Day 10 | 17 | +36 v +33 | -0.49857 | 0.322 | Inf | -1.548 | 0.1216 | 0.5189 |
| Day 10 | 17 | +66 v +63 | -0.54598 | 0.34 | Inf | -1.607 | 0.108 | 0.4918 |
| Day 10 | 17 | +63 v +33 | 0.20428 | 0.328 | Inf | 0.623 | 0.533 | 0.8686 |
| Day 10 | 17 | +66 v +36 | 0.15688 | 0.334 | Inf | 0.469 | 0.6388 | 0.8904 |
| Day 10 | 20 | +33 v 00 | -0.13797 | 0.325 | Inf | -0.424 | 0.6716 | 0.8954 |
| Day 10 | 20 | +36 v 00 | -0.37061 | 0.345 | Inf | -1.075 | 0.2825 | 0.6749 |
| Day 10 | 20 | +63 v 00 | -0.07667 | 0.334 | Inf | -0.229 | 0.8186 | 0.9526 |
| Day 10 | 20 | +66 v 00 | 0.12058 | 0.357 | Inf | 0.338 | 0.7356 | 0.9315 |
| Day 10 | 20 | +36 v +33 | -0.23264 | 0.378 | Inf | -0.616 | 0.5382 | 0.8686 |
| Day 10 | 20 | +66 v +63 | 0.19725 | 0.397 | Inf | 0.497 | 0.6189 | 0.8803 |
| Day 10 | 20 | +63 v +33 | 0.0613 | 0.368 | Inf | 0.166 | 0.8678 | 0.9576 |
| Day 10 | 20 | +66 v +36 | 0.49119 | 0.406 | Inf | 1.211 | 0.2258 | 0.6164 |
| Day 10 | 23 | +33 v 00 | -0.61554 | 0.305 | Inf | -2.02 | 0.0434 | 0.2778 |
| Day 10 | 23 | +36 v 00 | -0.41557 | 0.299 | Inf | -1.392 | 0.1639 | 0.5534 |
| **Day 10** | **23** | **+63 v 00** | **-1.52852** | **0.34** | **Inf** | **-4.492** | **<.0001** | **0.0002** |
| Day 10 | 23 | +66 v 00 | -2.6003 | 0.918 | Inf | -2.832 | 0.0046 | 0.0628 |
| Day 10 | 23 | +36 v +33 | 0.19997 | 0.328 | Inf | 0.609 | 0.5425 | 0.8686 |
| Day 10 | 23 | +66 v +63 | -1.07178 | 0.941 | Inf | -1.139 | 0.2546 | 0.6389 |
| Day 10 | 23 | +63 v +33 | -0.91298 | 0.367 | Inf | -2.49 | 0.0128 | 0.1259 |
| Day 10 | 23 | +66 v +36 | -2.18473 | 0.926 | Inf | -2.358 | 0.0184 | 0.1567 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day 20 | 14 | +33 v 00 | -0.3941 | 0.302 | Inf | -1.304 | 0.1923 | 0.5726 |
| Day 20 | 14 | +36 v 00 | 0.2491 | 0.302 | Inf | 0.825 | 0.4093 | 0.8629 |
| Day 20 | 14 | +63 v 00 | -0.16583 | 0.315 | Inf | -0.526 | 0.5987 | 0.8686 |
| Day 20 | 14 | +66 v 00 | 0.54587 | 0.308 | Inf | 1.773 | 0.0762 | 0.3879 |
| Day 20 | 14 | +36 v +33 | 0.6432 | 0.323 | Inf | 1.991 | 0.0465 | 0.2832 |
| Day 20 | 14 | +66 v +63 | 0.7117 | 0.34 | Inf | 2.09 | 0.0366 | 0.2464 |
| Day 20 | 14 | +63 v +33 | 0.22827 | 0.335 | Inf | 0.681 | 0.4962 | 0.8686 |
| Day 20 | 14 | +66 v +36 | 0.29677 | 0.328 | Inf | 0.904 | 0.3659 | 0.8076 |
| Day 20 | 17 | +33 v 00 | -0.15321 | 0.295 | Inf | -0.519 | 0.6039 | 0.8686 |
| Day 20 | 17 | +36 v 00 | -0.01139 | 0.302 | Inf | -0.038 | 0.9699 | 1.000 |
| Day 20 | 17 | +63 v 00 | 0.02315 | 0.295 | Inf | 0.078 | 0.9375 | 1.000 |
| Day 20 | 17 | +66 v 00 | -0.408 | 0.295 | Inf | -1.381 | 0.1672 | 0.5534 |
| Day 20 | 17 | +36 v +33 | 0.14182 | 0.328 | Inf | 0.433 | 0.6652 | 0.8954 |
| Day 20 | 17 | +66 v +63 | -0.43115 | 0.322 | Inf | -1.339 | 0.1807 | 0.5642 |
| Day 20 | 17 | +63 v +33 | 0.17636 | 0.322 | Inf | 0.548 | 0.584 | 0.8686 |
| Day 20 | 17 | +66 v +36 | -0.39661 | 0.328 | Inf | -1.21 | 0.2263 | 0.6164 |
| Day 20 | 20 | +33 v 00 | -0.17618 | 0.318 | Inf | -0.555 | 0.5791 | 0.8686 |
| Day 20 | 20 | +36 v 00 | -0.23834 | 0.334 | Inf | -0.713 | 0.476 | 0.8686 |
| **Day 20** | **20** | **+63 v 00** | **-0.99142** | **0.326** | **Inf** | **-3.043** | **0.0023** | **0.0476** |
| **Day 20** | **20** | **+66 v 00** | **-1.3735** | **0.335** | **Inf** | **-4.099** | **<.0001** | **0.0011** |
| Day 20 | 20 | +36 v +33 | -0.06215 | 0.362 | Inf | -0.172 | 0.8635 | 0.9576 |
| Day 20 | 20 | +66 v +63 | -0.38209 | 0.369 | Inf | -1.034 | 0.3009 | 0.6952 |
| Day 20 | 20 | +63 v +33 | -0.81523 | 0.354 | Inf | -2.305 | 0.0212 | 0.1593 |
| **Day 20** | **20** | **+66 v +36** | **-1.13517** | **0.377** | **Inf** | **-3.011** | **0.0026** | **0.0476** |
| Day 20 | 23 | +33 v 00 | -0.4553 | 0.305 | Inf | -1.494 | 0.1351 | 0.5272 |
| **Day 20** | **23** | **+36 v 00** | **-0.83135** | **0.34** | **Inf** | **-2.449** | **0.0143** | 0.1311 |
| **Day 20** | **23** | **+63 v 00** | **-2.53393** | **0.323** | **Inf** | **-7.853** | **<.0001** | **<.0001** |
| **Day 20** | **23** | **+66 v 00** | **-2.31446** | **0.487** | **Inf** | **-4.756** | **<.0001** | **0.0001** |
| Day 20 | 23 | +36 v +33 | -0.37604 | 0.366 | Inf | -1.028 | 0.3041 | 0.6952 |
| Day 20 | 23 | +66 v +63 | 0.21947 | 0.516 | Inf | 0.425 | 0.6709 | 0.8954 |
| **Day 20** | **23** | **+63 v +33** | **-2.07862** | **0.35** | **Inf** | **-5.933** | **<.0001** | **<.0001** |
| **Day 20** | **23** | **+66 v 36** | **-1.48311** | **0.527** | **Inf** | **-2.813** | **0.0049** | **0.0628** |

**Table C.9: Combined feature importance for burden.**
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.

| Explanatory Variable | Importance |
|---|---|
| Temperature: Timing 20: Amplitude +6 | 25.52 |
| Temperature | 19.10 |
| Temperature: Timing -10 | 11.15 |
| Temperature: Duration 3 | 9.83 |
| Temperature: Timing 20: Amplitude +6: Duration 6 | 9.29 |
| Temperature: Timing 20: Duration 6 | 8.81 |
| Temperature: Amplitude +3 | 8.20 |
| Temperature: Timing 10: Amplitude +6 | 7.58 |
| Temperature: Amplitude +3: Duration 3 | 6.77 |
| Temperature: Timing 20 | 6.24 |
| Temperature: Timing 10: Duration 3 | 5.50 |
| Temperature: Timing 0: Duration 6 | 3.73 |
| Temperature: Timing 10: Amplitude +6: Duration 3 | 3.26 |
| Temperature: Timing 10: Amplitude +6: Duration 6 | 2.58 |
| Temperature: Amplitude +6 | 2.33 |
| Temperature: Duration 6 | 1.42 |
| Temperature: Timing 0: Amplitude +6: Duration 6 | 1.10 |
| Temperature: Amplitude +6: Duration 6 | 0.97 |
| Temperature: Timing -10: Amplitude +6 | 0.91 |
| Temperature: Timing -10: Duration 6 | 0.87 |
| Temperature: Timing 0: Amplitude +3 | 0.61 |
| Timing 20: Amplitude +6 | 0.54 |
| Timing 0 | 0.24 |
| Timing 0: Duration 6 | 0.18 |
| Temperature: Timing 0 | 0.14 |
| Duration 3 | 0.10 |
| Timing -10: Amplitude +3: Duration 3 | 0.08 |

| | |
|---|---|
| Timing -10: Amplitude +6: Duration 6 | 0.06 |
| Timing 0: Amplitude +6: Duration 3 | 0.05 |
| Timing 0: Amplitude +3: Duration 6 | 0.04 |
| Timing 10: Amplitude +3: Duration 6 | 0.04 |
| Timing 10: Amplitude +6: Duration 3 | 0.02 |
| Timing 0: Amplitude +6 | 0.01 |
| X Intercept | 0 |
| Timing -10 | 0 |
| Timing 10 | 0 |
| Timing 20 | 0 |
| Amplitude +3 | 0 |
| Amplitude +6 | 0 |
| Duration 6 | 0 |
| Temperature: Timing 10 | 0 |
| Timing -10: Amplitude +3 | 0 |
| Timing 0: Amplitude +3 | 0 |
| Timing 10: Amplitude +3 | 0 |
| Timing 20: Amplitude +3 | 0 |
| Timing -10: Amplitude +6 | 0 |
| Timing 10: Amplitude +6 | 0 |
| Timing -10: Duration 3 | 0 |
| Timing 0: Duration 3 | 0 |
| Timing 10: Duration 3 | 0 |
| Timing 20: Duration 3 | 0 |
| Timing -10: Duration 6 | 0 |
| Timing 10: Duration 6 | 0 |
| Timing 20: Duration 6 | 0 |
| Amplitude +3: Duration 3 | 0 |
| Amplitude +6: Duration 3 | 0 |
| Amplitude +3: Duration 6 | 0 |
| Amplitude +6: Duration 6 | 0 |
| Temperature: Timing -10: Amplitude +3 | 0 |

| | |
|---|---|
| Temperature: Timing 10: Amplitude +3 | 0 |
| Temperature: Timing 20: Amplitude +3 | 0 |
| Temperature: Timing 0: Amplitude +6 | 0 |
| Temperature: Timing -10: Duration 3 | 0 |
| Temperature: Timing 0: Duration 3 | 0 |
| Temperature: Timing 20: Duration 3 | 0 |
| Temperature: Timing 10: Duration 6 | 0 |
| Temperature: Amplitude +6: Duration 3 | 0 |
| Temperature: Amplitude +3: Duration 6 | 0 |
| Timing 0: Amplitude +3: Duration 3 | 0 |
| Timing 10: Amplitude +3: Duration 3 | 0 |
| Timing 20: Amplitude +3: Duration 3 | 0 |
| Timing -10: Amplitude +6: Duration 3 | 0 |
| Timing 20: Amplitude +6: Duration 3 | 0 |
| Timing -10: Amplitude +3: Duration 6 | 0 |
| Timing 20: Amplitude +3: Duration 6 | 0 |
| Timing 0: Amplitude +6: Duration 6 | 0 |
| Timing 10: Amplitude +6: Duration 6 | 0 |
| Timing 20: Amplitude +6: Duration 6 | 0 |
| Temperature: Timing -10: Amplitude +3: Duration 3 | 0 |
| Temperature: Timing 0: Amplitude +3: Duration 3 | 0 |
| Temperature: Timing 10: Amplitude +3: Duration 3 | 0 |
| Temperature: Timing 20: Amplitude +3: Duration 3 | 0 |
| Temperature: Timing -10: Amplitude +6: Duration 3 | 0 |
| Temperature: Timing 0: Amplitude +6: Duration 3 | 0 |
| Temperature: Timing 20: Amplitude +6: Duration 3 | 0 |
| Temperature: Timing -10: Amplitude +3: Duration 6 | 0 |
| Temperature: Timing 0: Amplitude +3: Duration 6 | 0 |
| Temperature: Timing 10: Amplitude +3: Duration 6 | 0 |
| Temperature: Timing 20: Amplitude +3: Duration 6 | 0 |
| Temperature: Timing -10: Amplitude +6: Duration 6 | 0 |

**Table C.10: estimated marginal means (emmean) for burden.**
In *Daphnia magna* infected with *Ordospora colligata* combining two experiments. The estimates were derived using a Generalised 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).

| Exp | Treatment | emmean | SE | df | Lower CI 95% | Upper CI 95% |
|---|---|---|---|---|---|---|
| A | -10 for 3days | 6.29 | 0.249 | Inf | 5.8 | 6.78 |
| A | -10 for 6 days | 6.52 | 0.236 | Inf | 6.05 | 6.98 |
| A | 0 for 3 days | 7.08 | 0.193 | Inf | 6.7 | 7.45 |
| A | 0 for 6 days | 7.04 | 0.207 | Inf | 6.64 | 7.45 |
| A | 10 for 3 days | 7.24 | 0.199 | Inf | 6.84 | 7.63 |
| A | 10 for 6 days | 6.69 | 0.207 | Inf | 6.28 | 7.1 |
| A | Constant | 6.86 | 0.159 | Inf | 6.54 | 7.17 |
| B | -10 for 3days | 6.27 | 0.147 | Inf | 5.98 | 6.55 |
| B | -10 for 6 days | 6.07 | 0.141 | Inf | 5.8 | 6.35 |
| B | 0 for 3 days | 6.62 | 0.141 | Inf | 6.34 | 6.9 |
| B | 0 for 6 days | 6.24 | 0.147 | Inf | 5.95 | 6.52 |
| B | 10 for 3 days | 6.47 | 0.152 | Inf | 6.17 | 6.77 |
| B | 10 for 6 days | 6.35 | 0.144 | Inf | 6.07 | 6.63 |
| B | Constant | 6.28 | 0.156 | Inf | 5.98 | 6.59 |

# Appendix D - Supplementary information for Chapter 5

**Table D.1: Generalised Linear Mixed Model (GLMM) output for burden.**
In *Daphnia magna* infected with *Ordospora colligata*. Temperature was modelled as a quadratic polynomial to account for non-linearity and is presented separately by degree (linear [L] and quadratic [Q] The GLMM included fixed effects of temperature (modelled as a quadratic polynomial), time period (relative to a heatwave or cold snap), and population size, with a random effect of bucket. The response variable was spore burden, with uninfected but exposed hosts coded as zero. Significant values are bolded.

| Explanatory Variable | Estimate | SE | z value | p value |
|---|---|---|---|---|
| **Intercept** | **5.88E+00** | **8.26E-02** | **71.2** | **< 2e-16** |
| Temperature (L) | 4.72E-01 | 2.22E+00 | 0.21 | 0.831797 |
| **Temperature (Q)** | **-1.88E+01** | **2.36E+00** | **-7.97** | **1.53E-15** |
| Period before | -8.01E-02 | 7.73E-02 | -1.04 | 0.299821 |
| **Period during** | **4.31E-01** | **1.28E-01** | **3.37** | **0.000742** |
| **Period just after** | **6.60E-01** | **1.19E-01** | **5.53** | **3.26E-08** |
| **Period after** | **1.73E-01** | **8.23E-02** | **2.1** | **0.035464** |
| **Population size** | **1.12E-03** | **2.65E-04** | **4.22** | **2.47E-05** |
| **Temperature (L): Period before** | **9.64E+00** | **3.85E+00** | **2.51** | **0.01222** |
| Temperature (Q): Period before | 5.25E+00 | 3.66E+00 | 1.44 | 0.151188 |
| **Temperature (L): Period during** | **-1.78E+01** | **6.08E+00** | **-2.93** | **0.00335** |
| **Temperature (Q): Period during** | **1.38E+01** | **5.96E+00** | **2.32** | **0.020545** |
| Temperature (L): Period just after | -3.63E+00 | 5.46E+00 | -0.66 | 0.50635 |
| **Temperature (Q): Period just after** | **1.95E+01** | **5.51E+00** | **3.53** | **0.000412** |
| **Temperature (L): Period after** | **-8.22E+00** | **4.05E+00** | **-2.03** | **0.042344** |
| Temperature (Q): Period after | 4.19E+00 | 3.78E+00 | 1.11 | 0.267579 |
| **Temperature (L): Population size** | **-3.20E-02** | **1.39E-02** | **-2.3** | **0.0214** |
| **Temperature (Q): Population size** | **-4.34E-02** | **1.25E-02** | **-3.48** | **0.000503** |

**Table D.2: Type III Wald chi-square test results for the Generalised Linear Mixed Model (GLMM) analysing burden.**
In *Daphnia magna* infected with *Ordospora colligata*. The analysis of deviance tests the significance of predictor variables, including temperature (modelled as a quadratic polynomial), period, and population size. The response variable was spore burden, with uninfected but exposed hosts coded as zero. Significant values are bolded.

| Explanatory Variable | Chisq | df | p value |
|---|---|---|---|

| | | | |
|---|---|---|---|
| **(Intercept)** | **5069.743** | **1** | **<2.2e-16** |
| **Temperature** | **63.660** | **2** | **1.501e-14** |
| **Period** | **43.733** | **4** | **7.291e-09** |
| **Population** | **17.788** | **1** | **2.470e-05** |
| **Temperature: Time Period** | **49.634** | **8** | **2.440e-06** |
| **Temperature: Population** | **16.765** | **2** | **0.0002289** |

**Table D.3: Temperature-specific Generalised Linear Mixed Model (GLMM) outputs for burden.**
In *Daphnia magna* infected with *Ordospora colligata*. A; For 14ºC, B; for 17ºC, C; for 20ºC, D; for 23ºC. The GLMM included fixed effects of time period (relative to a heatwave or cold snap), and population size, with a random effect of bucket. The response variable was spore burden, with uninfected but exposed hosts coded as zero. Significant values are bolded.

| A | Explanatory Variable | Estimate | SE | z value | p value |
|---|---|---|---|---|---|
| | **Intercept** | **5.3468302** | **0.1297381** | **41.21** | **< 2e-16** |
| | Period before | 0.0323354 | 0.1970243 | 0.16 | 0.86964 |
| | **Period during** | **1.3173233** | **0.3320625** | **3.97** | **7.28E-05** |
| | **Period just after** | **1.3037497** | **0.3340307** | **3.9** | **9.50E-05** |
| | **Period after** | **0.5672415** | **0.211233** | **2.69** | **0.00724** |
| | Population size | 0.0012458 | 0.0006853 | 1.82 | 0.06909 |

| B | Explanatory Variable | Estimate | SE | z value | p value |
|---|---|---|---|---|---|
| | **Intercept** | **6.2350207** | **0.2323581** | **26.834** | **< 2e-16** |
| | Period before | -0.126104 | 0.1750547 | -0.72 | 0.4713 |
| | Period during | 0.5889966 | 0.3249171 | 1.813 | 0.06987 |
| | Period just after | -0.1452004 | 0.3178615 | -0.457 | 0.64781 |
| | Period after | 0.2984897 | 0.2011913 | 1.484 | 0.13791 |
| | **Population size** | **0.0018756** | **0.0006401** | **2.93** | **0.00339** |

| C | Explanatory Variable | Estimate | SE | z value | p value |
|---|---|---|---|---|---|
| | **Intercept** | **6.3621473** | **0.0828645** | **76.78** | **<2e-16** |
| | Period before | -0.2634292 | 0.1500391 | -1.76 | 0.0791 |
| | Period during | -0.4218696 | 0.2544664 | -1.66 | 0.0973 |
| | Period just after | 0.3180347 | 0.2506788 | 1.27 | 0.2046 |
| | Period after | -0.0097109 | 0.1594903 | -0.06 | 0.9514 |

| | | | | | |
|---|---|---|---|---|---|
| | Population size | 0.0012288 | 0.0005833 | 2.11 | 0.0352 |

| C | Explanatory Variable | Estimate | SE | z value | p value |
|---|---|---|---|---|---|
| | **Intercept** | **5.2660067** | **0.1287328** | **40.91** | **< 2e-16** |
| | **Period before** | **0.4534382** | **0.1718182** | **2.64** | **0.00831** |
| | Period during | 0.4506579 | 0.3173837 | 1.42 | 0.15563 |
| | **Period just after** | **1.2496773** | **0.3202429** | **3.9** | **9.53E-05** |
| | Period after | 0.0731139 | 0.199369 | 0.37 | 0.71382 |
| | **Population size** | **-0.0016701** | **0.0007391** | **-2.26** | **0.02384** |

**Table D.4: estimated marginal means (emmean) for burden.**
In *Daphnia magna* infected with *Ordospora colligata*. The estimates were derived using a generalised linear model (GLM) with baseline temperature and treatment period as factors, using a negative binomial family. The response variable was spore burden, with uninfected but exposed hosts coded as zero. Values are presented on the log scale for appropriate interpretation of the data. The 'Inf' degrees of freedom reflect the large sample size and the flexibility of the model.

| Temp | Period | emmean | SE | df | Lower CI 95% | Upper CI 95% |
|---|---|---|---|---|---|---|
| 14 | Constant before | 5.25 | 0.0943 | Inf | 5.07 | 5.44 |
| 17 | Constant before | 6.07 | 0.0946 | Inf | 5.89 | 6.26 |
| 20 | Constant before | 6.12 | 0.0946 | Inf | 5.94 | 6.31 |
| 23 | Constant before | 5.57 | 0.0943 | Inf | 5.39 | 5.76 |
| 14 | Constant during | 5.54 | 0.1918 | Inf | 5.17 | 5.92 |
| 17 | Constant during | 6.24 | 0.1916 | Inf | 5.87 | 6.62 |
| 20 | Constant during | 6.15 | 0.1916 | Inf | 5.78 | 6.53 |
| 23 | Constant during | 5.17 | 0.1887 | Inf | 4.8 | 5.54 |
| 14 | Constant just after | 5.29 | 0.1886 | Inf | 4.92 | 5.66 |
| 17 | Constant just after | 6.28 | 0.1884 | Inf | 5.92 | 6.65 |
| 20 | Constant just after | 6.22 | 0.1884 | Inf | 5.85 | 6.59 |
| 23 | Constant just after | 5.33 | 0.1886 | Inf | 4.96 | 5.7 |
| 14 | Constant after | 5.52 | 0.1089 | Inf | 5.31 | 5.74 |
| 17 | Constant after | 6.4 | 0.1087 | Inf | 6.18 | 6.61 |
| 20 | Constant after | 6.19 | 0.1088 | Inf | 5.98 | 6.4 |
| 23 | Constant after | 4.96 | 0.109 | Inf | 4.74 | 5.17 |
| 14 | Pulse before | 5.26 | 0.0943 | Inf | 5.07 | 5.44 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | Pulse before | 6.12 | 0.0946 | Inf | 5.93 | 6.3 |
| 20 | Pulse before | 6.12 | 0.0942 | Inf | 5.94 | 6.31 |
| 23 | Pulse before | 5.71 | 0.0942 | Inf | 5.53 | 5.9 |
| 14 | Pulse during | 6.25 | 0.1884 | Inf | 5.88 | 6.62 |
| 17 | Pulse during | 6.39 | 0.1883 | Inf | 6.02 | 6.76 |
| 20 | Pulse during | 5.87 | 0.1884 | Inf | 5.5 | 6.24 |
| 23 | Pulse during | 5.35 | 0.1886 | Inf | 4.98 | 5.72 |
| 14 | Pulse just after | 6.35 | 0.1883 | Inf | 5.98 | 6.72 |
| 17 | Pulse just after | 6.12 | 0.1884 | Inf | 5.75 | 6.49 |
| 20 | Pulse just after | 6.33 | 0.1916 | Inf | 5.96 | 6.71 |
| 23 | Pulse just after | 6.01 | 0.1884 | Inf | 5.64 | 6.38 |
| 14 | Pulse after | 5.74 | 0.1088 | Inf | 5.53 | 5.95 |
| 17 | Pulse after | 6.3 | 0.1087 | Inf | 6.09 | 6.52 |
| 20 | Pulse after | 6.17 | 0.1088 | Inf | 5.96 | 6.38 |
| 23 | Pulse after | 5.15 | 0.1089 | Inf | 4.94 | 5.36 |

**Table D.5: Custom contrasts for burden 'emmeans' comparing treatments across baseline temperatures and periods.**
In *Daphnia magna* infected with *Ordospora colligata*. Each pulse treatment period was compared to its constant equivalent at the same temperature or to the 'before' pulse period. The response variable was spore burden, with uninfected but exposed hosts coded as zero. The 'Inf' degrees of freedom reflect the model's complexity and the estimation process. C; constant treatment, P; pulse treatment. Significant contrasts are bolded.

| Temp | Contrast | Estimate | SE | df | z ratio | p value |
|---|---|---|---|---|---|---|
| 14 | C before vs P before | -0.00335 | 0.133 | Inf | -0.025 | 0.9964 |
| **14** | **C during vs P during** | **-0.70551** | **0.269** | **Inf** | **-2.625** | **0.0405** |
| **14** | **C just after vs P just after** | **-1.06324** | **0.267** | **Inf** | **-3.989** | **0.0006** |
| 14 | C after vs P after | -0.21609 | 0.154 | Inf | -1.404 | 0.4457 |
| **14** | **P before vs P during** | **-0.99366** | **0.211** | **Inf** | **-4.717** | **<.0001** |
| **14** | **P before vs P just after** | **-1.09697** | **0.211** | **Inf** | **-5.208** | **<.0001** |
| **14** | **B before vs P after** | **-0.48543** | **0.144** | **Inf** | **-3.371** | **0.0042** |
| 17 | C before vs P before | -0.04708 | 0.134 | Inf | -0.352 | 0.871 |
| 17 | C during vs P during | -0.14332 | 0.269 | Inf | -0.533 | 0.7916 |
| 17 | C just after vs P just after | 0.16263 | 0.266 | Inf | 0.61 | 0.7627 |
| 17 | C after vs P after | 0.09313 | 0.154 | Inf | 0.606 | 0.7627 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | P before vs P during | -0.26775 | 0.211 | Inf | -1.27 | 0.4457 |
| 17 | P before vs P just after | -0.00256 | 0.211 | Inf | -0.012 | 0.9964 |
| 17 | B before vs P after | -0.18509 | 0.144 | Inf | -1.284 | 0.4457 |
| 20 | C before vs P before | -0.00061 | 0.133 | Inf | -0.005 | 0.9964 |
| 20 | C during vs P during | 0.27823 | 0.269 | Inf | 1.035 | 0.5259 |
| 20 | C just after vs P just after | -0.11157 | 0.269 | Inf | -0.415 | 0.8628 |
| 20 | C after vs P after | 0.01929 | 0.154 | Inf | 0.125 | 0.9964 |
| 20 | P before vs P during | 0.25141 | 0.211 | Inf | 1.193 | 0.4654 |
| 20 | P before vs P just after | -0.21044 | 0.213 | Inf | -0.986 | 0.534 |
| 20 | B before vs P after | -0.04649 | 0.144 | Inf | -0.323 | 0.871 |
| 23 | C before vs P before | -0.1424 | 0.133 | Inf | -1.068 | 0.5259 |
| 23 | C during vs P during | -0.18194 | 0.267 | Inf | -0.682 | 0.7627 |
| **23** | **C just after vs P just after** | **-0.68293** | **0.267** | **Inf** | **-2.562** | **0.0417** |
| 23 | C after vs P after | -0.19451 | 0.154 | Inf | -1.262 | 0.4457 |
| 23 | P before vs P during | 0.36609 | 0.211 | Inf | 1.736 | 0.2888 |
| 23 | P before vs P just after | -0.29973 | 0.211 | Inf | -1.423 | 0.4457 |
| **23** | **B before vs P after** | **0.5631** | **0.144** | **Inf** | **3.909** | **0.0006** |

**Table D.6: Generalised Linear Mixed Model (GLMM) output for population size.**
In *Daphnia magna* infected with *Ordospora colligata*. Temperature was modelled as a cubic polynomial to account for non-linearity and is presented separately by degree (linear [L] and quadratic [Q], and cubic [C]). A random intercept for bucket was included to account for repeated measures. Significant values are bolded.

| Explanatory Variable | Estimate | SE | z value | p value |
|---|---|---|---|---|
| **Intercept** | **6.175158** | **0.018028** | **342.5** | **< 2e-16** |
| **Temperature (L)** | **-4.259503** | **0.595854** | **-7.1** | **8.77E-13** |
| Temperature (Q) | -0.691695 | 0.600454 | -1.2 | 0.24934 |
| **Temperature (C)** | **-2.834766** | **0.605939** | **-4.7** | **2.89E-06** |
| **Period before** | **-0.080116** | **0.021731** | **-3.7** | **0.000227** |
| **Period during** | **-0.079866** | **0.038858** | **-2.1** | **0.039849** |
| Period just after | -0.04132 | 0.03944 | -1 | 0.294796 |
| Period after | 0.045188 | 0.023918 | 1.9 | 0.058853 |
| Burden | 0.018618 | 0.009855 | 1.9 | 0.058863 |
| Temperature (L): Period before | 1.355771 | 1.009656 | 1.3 | 0.179335 |

| | | | | |
|---|---|---|---|---|
| Temperature (Q): Period before | -0.308379 | 1.008675 | -0.3 | 0.759813 |
| **Temperature (C): Period before** | **4.125078** | **1.006704** | **4.1** | **4.17E-05** |
| **Temperature (L): Period during** | **7.207802** | **1.833254** | **3.9** | **8.43E-05** |
| **Temperature (Q): Period during** | **6.351635** | **1.805293** | **3.5** | **0.000434** |
| Temperature (C): Period during | -2.119249 | 1.773516 | -1.2 | 0.23211 |
| Temperature (L): Period just after | 9.11574 | 1.881191 | 4.8 | 1.26E-06 |
| **Temperature (Q): Period just after** | **4.494843** | **1.82964** | **2.5** | **0.014023** |
| Temperature (C): Period just after | -2.059117 | 1.776238 | -1.2 | 0.246351 |
| **Temperature (L): Period after** | **3.75332** | **1.115479** | **3.4** | **0.000766** |
| Temperature (Q): Period after | 1.037393 | 1.109648 | 0.9 | 0.349848 |
| Temperature (C): Period after | -0.890302 | 1.105118 | -0.8 | 0.420464 |
| **Temperature (L): Burden** | **-1.185942** | **0.523006** | **-2.3** | **0.023357** |
| Temperature (Q): Burden | -0.859871 | 0.456881 | -1.9 | 0.05983 |
| Temperature (C): Burden | -0.273834 | 0.384537 | -0.7 | 0.476394 |

**Table D.7: Type III Wald chi-square test results for the Generalised Linear Mixed Model (GLMM) analysing population size.**
In *Daphnia magna* infected with *Ordospora colligata*. The analysis of deviance tests the significance of predictor variables, including temperature (modelled as a cubic polynomial), period, and population size. Significant values are bolded.

| Explanatory Variable | Chisq | df | p value |
|---|---|---|---|
| **(Intercept)** | **11,340** | **1** | **<2.2e-16** |
| **Temperature** | **59.747** | **3** | **4.534e-16** |
| **Time Period** | **27.219** | **4** | **2.658e-05** |
| Burden | 9.472 | 1 | 0.5949 |
| **Temperature: Time Period** | **88.408** | **12** | **9.944e-14** |
| **Temperature: Burden** | **7.839** | **3** | **0.05147** |

**Table D.8: estimated marginal means (emmean) for population size.**
In *Daphnia magna* infected with *Ordospora colligata*. The estimates were derived using a Generalised Linear Model (GLM) with baseline temperature and treatment period as factors, using a negative binomial family. Values are presented on the log scale for appropriate interpretation of the data. The 'Inf' degrees of freedom reflect the large sample size and the flexibility of the model.

| Temp | Period | emmean | SE | df | Lower CI 95% | Upper CI 95% |
|---|---|---|---|---|---|---|
| 14 | Constant before | 6.09 | 0.0333 | Inf | 6.03 | 6.16 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | Constant before | 5.99 | 0.0334 | Inf | 5.92 | 6.05 |
| 20 | Constant before | 6.18 | 0.0332 | Inf | 6.11 | 6.24 |
| 23 | Constant before | 5.9 | 0.0335 | Inf | 5.84 | 5.97 |
| 14 | Constant during | 6.37 | 0.0662 | Inf | 6.24 | 6.5 |
| 17 | Constant during | 6.23 | 0.0664 | Inf | 6.1 | 6.36 |
| 20 | Constant during | 6.36 | 0.0662 | Inf | 6.23 | 6.49 |
| 23 | Constant during | 6.04 | 0.0667 | Inf | 5.91 | 6.18 |
| 14 | Constant just after | 6.43 | 0.0661 | Inf | 6.3 | 6.56 |
| 17 | Constant just after | 6.28 | 0.0663 | Inf | 6.15 | 6.41 |
| 20 | Constant just after | 6.37 | 0.0662 | Inf | 6.24 | 6.5 |
| 23 | Constant just after | 6.18 | 0.0664 | Inf | 6.05 | 6.31 |
| 14 | Constant after | 6.46 | 0.0381 | Inf | 6.38 | 6.53 |
| 17 | Constant after | 6.34 | 0.0382 | Inf | 6.27 | 6.42 |
| 20 | Constant after | 6.28 | 0.0383 | Inf | 6.21 | 6.36 |
| 23 | Constant after | 6.11 | 0.0384 | Inf | 6.04 | 6.19 |
| 14 | Pulse before | 6.13 | 0.0333 | Inf | 6.07 | 6.2 |
| 17 | Pulse before | 6.2 | 0.0332 | Inf | 6.13 | 6.27 |
| 20 | Pulse before | 6.06 | 0.0333 | Inf | 6 | 6.13 |
| 23 | Pulse before | 6 | 0.0334 | Inf | 5.94 | 6.07 |
| 14 | Pulse during | 6.23 | 0.0664 | Inf | 6.1 | 6.36 |
| 17 | Pulse during | 5.84 | 0.0671 | Inf | 5.71 | 5.97 |
| 20 | Pulse during | 6.14 | 0.0665 | Inf | 6.01 | 6.27 |
| 23 | Pulse during | 6.27 | 0.0663 | Inf | 6.14 | 6.4 |
| 14 | Pulse just after | 6.17 | 0.0664 | Inf | 6.04 | 6.3 |
| 17 | Pulse just after | 5.87 | 0.067 | Inf | 5.73 | 6 |
| 20 | Pulse just after | 6.28 | 0.0663 | Inf | 6.15 | 6.41 |
| 23 | Pulse just after | 6.29 | 0.0663 | Inf | 6.16 | 6.42 |
| 14 | Pulse after | 6.28 | 0.0383 | Inf | 6.2 | 6.35 |
| 17 | Pulse after | 6.14 | 0.0384 | Inf | 6.06 | 6.21 |
| 20 | Pulse after | 6.34 | 0.0382 | Inf | 6.26 | 6.41 |
| 23 | Pulse after | 6.2 | 0.0383 | Inf | 6.12 | 6.27 |

**Table D.9: Custom contrasts for population size 'emmean' comparing treatments across baseline temperatures and periods.**

In *Daphnia magna* infected with *Ordospora colligata*. Each pulse treatment period was compared to its constant equivalent at the same temperature or to the 'before' pulse period. The response variable was spore burden, with uninfected but exposed hosts coded as zero. The 'Inf' degrees of freedom reflect the model's complexity and the estimation process. C; constant treatment, P; pulse treatment. Significant contrasts are bolded.

| Temp | Contrast | Estimate | SE | Df | z ratio | p value |
|---|---|---|---|---|---|---|
| 14 | C before vs P before | -0.0406 | 0.0471 | Inf | -0.862 | 0.403 |
| 14 | C during vs P during | 0.1436 | 0.0937 | Inf | 1.532 | 0.1757 |
| **14** | **C just after vs P just after** | **0.2589** | **0.0937** | **Inf** | **2.763** | **0.0115** |
| **14** | **C after vs P after** | **0.184** | **0.054** | **Inf** | **3.406** | **0.0017** |
| 14 | P before vs P during | -0.0957 | 0.0742 | Inf | -1.289 | 0.2631 |
| 14 | P before vs P just after | -0.0401 | 0.0743 | Inf | -0.539 | 0.5896 |
| **14** | **B before vs P after** | **-0.1434** | **0.0507** | **Inf** | **-2.829** | **0.0101** |
| **17** | **C before vs P before** | **-0.2141** | **0.0471** | **Inf** | **-4.548** | **0.0001** |
| **17** | **C during vs P during** | **0.3942** | **0.0943** | **Inf** | **4.179** | **0.0001** |
| **17** | **C just after vs P just after** | **0.4158** | **0.0943** | **Inf** | **4.412** | **0.0001** |
| **17** | **C after vs P after** | **0.2061** | **0.0542** | **Inf** | **3.804** | **0.0004** |
| **17** | **P before vs P during** | **0.3614** | **0.0748** | **Inf** | **4.829** | **<.0001** |
| **17** | **P before vs P just after** | **0.3347** | **0.0748** | **Inf** | **4.475** | **0.0001** |
| 17 | B before vs P after | 0.0621 | 0.0508 | Inf | 1.223 | 0.2819 |
| **20** | **C before vs P before** | **0.1168** | **0.047** | **Inf** | **2.482** | **0.0244** |
| **20** | **C during vs P during** | **0.2232** | **0.0938** | **Inf** | **2.379** | **0.0301** |
| 20 | C just after vs P just after | 0.0879 | 0.0937 | Inf | 0.939 | 0.3745 |
| 20 | C after vs P after | -0.0575 | 0.0541 | Inf | -1.064 | 0.3219 |
| 20 | P before vs P during | -0.0791 | 0.0744 | Inf | -1.064 | 0.3219 |
| **20** | **P before vs P just after** | **-0.2183** | **0.0742** | **Inf** | **-2.943** | **0.0076** |
| **20** | **B before vs P after** | **-0.2757** | **0.0507** | **Inf** | **-5.438** | **<.0001** |
| 23 | C before vs P before | -0.0979 | 0.0473 | Inf | -2.073 | 0.0594 |
| **23** | **C during vs P during** | **-0.2219** | **0.094** | **Inf** | **-2.36** | **0.0301** |
| 23 | C just after vs P just after | -0.1082 | 0.0938 | Inf | -1.153 | 0.303 |
| 23 | C after vs P after | -0.0882 | 0.0543 | Inf | -1.625 | 0.1536 |
| **23** | **P before vs P during** | **-0.2644** | **0.0742** | **Inf** | **-3.562** | **0.001** |
| **23** | **P before vs P just after** | **-0.2843** | **0.0742** | **Inf** | **-3.832** | **0.0004** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **23** | **B before vs P after** | **-0.1964** | **0.0508** | **Inf** | **-3.864** | **0.0004** |

**Table D.10: estimated marginal means (emmean) for burden comparisons between periods in both population and individual experiments.**
In *Daphnia magna* infected with *Ordospora colligata*. The estimates were derived using a Generalised Linear Model (GLM) with treatment period as a factor, using a negative binomial family. Treatment combined the factors: experiment, temperature, treatment, and period. The response variable was spore burden, with uninfected but exposed hosts coded as zero. Exp; experiment, CS; cold snap individual experiment, HW; heatwave individual experiment, POP; population experiment. Period; constant; continuous baseline with no pulse, during; the week when the pulse occurred. Values are presented on the log scale for appropriate interpretation of the data. The 'Inf' degrees of freedom reflect the large sample size and the flexibility of the model.

| Exp | Temp | Treatment | Period | emmean | SE | df | Lower CI 95% | Upper CI 95% |
|---|---|---|---|---|---|---|---|---|
| CS | 20 | constant | constant | 6.19 | 0.1904 | Inf | 5.81 | 6.56 |
| CS | 20 | cold snap | during | 5.75 | 0.258 | Inf | 5.25 | 6.26 |
| CS | 23 | constant | constant | 4.55 | 0.2319 | Inf | 4.1 | 5 |
| CS | 23 | cold snap | during | 4.21 | 0.2715 | Inf | 3.68 | 4.74 |
| HW | 14 | constant | constant | 5.28 | 0.2001 | Inf | 4.89 | 5.67 |
| HW | 14 | heatwave | during | 6.16 | 0.2825 | Inf | 5.6 | 6.71 |
| HW | 17 | constant | constant | 6.86 | 0.1903 | Inf | 6.48 | 7.23 |
| HW | 17 | heatwave | during | 7.04 | 0.2475 | Inf | 6.56 | 7.53 |
| POP | 14 | constant | constant | 5.42 | 0.0948 | Inf | 5.24 | 5.61 |
| POP | 14 | heatwave | during | 6.25 | 0.1631 | Inf | 5.93 | 6.57 |
| POP | 17 | constant | constant | 6.27 | 0.0958 | Inf | 6.08 | 6.46 |
| POP | 17 | heatwave | during | 6.39 | 0.163 | Inf | 6.07 | 6.71 |
| POP | 20 | constant | constant | 6.3 | 0.0952 | Inf | 6.12 | 6.49 |
| POP | 20 | heatwave | during | 5.87 | 0.1632 | Inf | 5.55 | 6.19 |
| POP | 23 | constant | constant | 5.17 | 0.0954 | Inf | 4.99 | 5.36 |
| POP | 23 | heatwave | during | 5.35 | 0.1634 | Inf | 5.03 | 5.67 |

**Table D.11: Custom contrasts for burden comparisons between periods in both population and individual experiments.**
In *Daphnia magna* infected with *Ordospora colligata*. Each pulse treatment period was compared to its constant equivalent at the same temperature or to the 'before' pulse period. The response variable was spore burden, with uninfected but exposed hosts coded as zero. The 'Inf' degrees of freedom reflect the model's complexity and the estimation process. Exp; experiment, CS; cold snap individual experiment, HW; heatwave individual experiment, POP; population experiment.

| Exp | Temp | Contrast | Estimate | SE | Df | z ratio | p value |
|---|---|---|---|---|---|---|---|
| POP | 14 | during HW vs constant | 0.827 | 0.189 | Inf | 4.383 | 0.0001 |
| HW | 14 | during HW vs constant | 0.875 | 0.346 | Inf | 2.527 | 0.046 |
| POP | 17 | during HW vs constant | 0.117 | 0.189 | Inf | 0.621 | 0.5544 |
| HW | 17 | during HW vs constant | 0.185 | 0.312 | Inf | 0.591 | 0.5544 |
| POP | 20 | during CS vs constant | -0.431 | 0.189 | Inf | -2.282 | 0.0599 |
| CS | 20 | during CS vs constant | -0.431 | 0.321 | Inf | -1.345 | 0.3571 |
| POP | 23 | during CS vs constant | 0.174 | 0.189 | Inf | 0.919 | 0.4773 |
| CS | 23 | during CS vs constant | -0.337 | 0.357 | Inf | -0.944 | 0.4773 |