The role of temperature in the start of seasonal infectious disease epidemics

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Abstract

Many infectious diseases display strong seasonal dynamics. When both hosts and parasites are influenced by seasonal variables, it is unclear if the start of epidemics is limited by host or parasite factors or both. The Daphnia—Pasteuria host—parasite system exhibits seasonal epidemics. We experimentally tested if low Spring temperatures limit the onset of these epidemics. We used sediments from a natural population containing parasite spores at five constant temperatures ranging from 10-20 oC. We added either Daphnia magna resting stages (ephippia), juveniles from largely susceptible clonal cultures or juveniles from largely resistant clonal cultures from the same population. The acceleration of development with increasing temperature was much faster for the parasite than for the host. This finding supports our hypotheses that parasite outbreaks are limited by temperature, and not solely the availability of hosts. These results imply that climate change could lead earlier seasonal epidemics for this host-parasite system.

Introduction

Seasonality is a major driver of ecological dynamics, and its significance only becomes more important in the context of global climate change, as seasonal conditions are predicted to change (for example, warmer/earlier Springs in temperate regions). Many infectious diseases display strong seasonal dynamics which may in part be due to phenology of the host, parasite or vector, as well as changes in abiotic environmental conditions or a combination of these factors (Altizer et al. 2006, Martinez 2018). Many factors—such as light and temperature, host and parasite phenology, and the phenology of other species in the community—fluctuate approximately simultaneously with seasons, making it difficult to attribute epidemic dynamics to specific factors and furthermore, to understand if these factors affect hosts and parasites in the same way. For parasites with a free-living stage, it is often unclear whether their dynamics are driven by the seasonality of the host population, or the response of the parasite itself to environmental changes. To understand how epidemics may be influenced by changes in seasonal conditions, it is therefore necessary to disentangle how hosts and parasites are affected by these changes.

In temperate regions, temperature is a key factor of seasonality, particularly for ectothermal organisms. The seasonality observed in epidemics of parasites with free-living stages, especially those that overwinter independent of their hosts, suggests that both may respond to temperature differently (Gehman et al. 2018, McDevitt-Galles et al. 2020), but observational work does not readily allow us to disentangle these differing relationships to temperature or to predict how changes to temperature may influence epidemic dynamics. Understanding host and parasite response to temperature has therefore become more vital in the context of ongoing climate change, which is expected to cause changes in infectious disease dynamics (Cook 1992, Haines et al. 2006, Patz et al. 2008, Lafferty 2009, Lafferty and Mordecai 2016, Kirk et al. 2020). The objective of this study is to distinguish between host and parasite seasonality by determining to what degree a parasite is limited by temperature versus the availability, development, and susceptibility of its seasonal host. To understand the drivers of seasonal epidemics, and in particular the compare the effect of temperature

on host development to the effect on parasite development, experiments which disentangle these factors through controlled experiments are necessary. Controlling environmental factors such as light and humidity, as well as daily or seasonal fluctuations in temperature, can help to not only disentangle the effects of these factors from each other, but also isolate the response to specific constant temperatures in a way that seasonal observations do not allow. For such work, the use of a biological model system which is well understood and easily maintained in the lab can offer insight into complex natural systems.

The *Daphnia magna —Pasteuria ramosa* system offers the opportunity to further our understanding of infectious disease epidemiology through a combination of well-known epidemic patterns in nature and the ability to manipulate various ecological parameters with controlled experiments. Their trophic position, sensitivity to environmental conditions and the fact that host and parasite are well-understood make this system suitable for disentangling the relative importance of various factors influencing disease dynamics. *P. ramosa* is a common bacterial parasite of the aquatic microcrustacean*D. magna* , causing 100 % mortality of those infected and strong reduction in fecundity, therefore having significant impacts on the host's population dynamics (Ebert et al. 2004, Duncan and Little 2007, Ebert et al. 2016). The parasite transmits horizontally when transmission stages (spores) are picked up from the water column or environmental reservoirs in the pond sediments (Decaestecker et al. 2004), hereafter referred to as the "spore bank," where spores can survive decades (Decaestecker et al. 2007). Previous work has suggested that the rate of infection with spores from the water column increases with temperature from around 15 °C (Vale et al. 2008), indicating that infection may in some way be limited by temperature. However, different steps of the natural infection process may differ in their sensitivity to temperature (Hall et al. 2019, Izhar et al. 2020), making it essential to include all steps of the infection process when assessing temperature effects on the seasonal disease outbreaks in natural populations. For example, activation of the resting spores and attachment to the oesophagus of susceptible *Daphnia* was not different across a range of temperatures from 10 to 25°C (Duneau et al. 2011). However, exposure rate may be reduced at lower temperatures due to generally lowered activity and filtering rates of the *Daphnia* host (Burns 1969). Furthermore, Spring hatchlings of Daphnia from sexual resting stages may be different in various aspects from the later born asexual offspring and this may influence their likelihood of getting infected. Here we aim to understand the role of temperature in determining the onset of an epidemic.

The Study Population

In the Swiss Aegelsee pond, *D. magna* and *P. ramosa*dynamics have been monitored for the last 10 years (Andras and Ebert 2013, Ameline et al. 2020, Ameline et al. 2021). *D. magna* hatch from ephippia in early Spring (March or April), when water temperatures are around 10 °C. Strong seasonal epidemics have been observed every year, with parasite prevalence increasing in May, when water temperatures are about 15 °C (Ameline et al. 2020). Prevalence peaks in midsummer and declines in the Fall (as does temperature). Concurrently, as a consequence of natural selection, the proportions of susceptible *D. magna* decline throughout the course of the season, and the proportion of resistant clones increases as the epidemic progresses (Ameline et al. 2020). *D. magna* and *P. ramosa* populations overwinter through resting stages (ephippia) and dormant parasite stages in the spore bank (endospores), resulting in cycles of epidemics and host demographic dynamics each year. The parallel dynamics of *P. ramosa* prevalence, temperature and*D. magna* population size in Spring prevent us from understanding if *P. ramosa* outbreaks are limited by temperature, the availability of the susceptible hosts or other factors. Previous work has demonstrated that warmer temperatures are more favourable for infections from the water column (Vale et al. 2008). However, this work did not include the natural means of spore uptake from the pond sediments, nor did it consider that *D. magna* hatchlings from ephippia resting stages might differ in behaviour, physiology and/or resistance to parasites from *D. magna* bred as clonal lines in preparation of the experiment. It is therefore unknown whether spore bank transmission is triggered or accelerated by temperature increase, or simply by the presence of susceptible *D. magna* , which activate the spores upon contact (Duneau et al. 2011).

The objectives of this study were thus to *1)* disentangle whether the onset of *P. ramosa* epidemics (defined as when infections are first observed in the population) is independently driven by temperature, or simply by the presence of susceptible *D. magna* , and *2)* determine how temperature influences the speed/timing of

initial infections, and to thereby *3)* ascertain how temperature contributes to the seasonality of *P. ramosa* epidemics. We hypothesized the development of both the host and the parasite depends on temperature, and that the parasite is limited by temperature beyond its need for available hosts. We therefore predicted that the acceleration of development of *P. ramosa* (time to visible infection) with increasing temperature is faster than that of the host, such that relative development is faster for hosts at low temperature and faster for the parasite at high temperatures. Support for this hypothesis would indicate that the seasonality of the epidemics, especially the timing of outbreaks in Spring, are driven by temperature effects on the parasite rather than solely the presence of susceptible hosts. It would also indicate that the effects of temperature on the parasite are different from those on the host.

Methods

Study System

We used the well-studied host-parasite model system (Ebert et al. 2016) *D. magna* and its bacterial parasite *P. ramosa* to experimentally investigate the role that temperature may play in driving seasonal host-parasite dynamics. *D. magna* are small planktonic crustaceans broadly distributed throughout the northern hemisphere (Bekker et al. 2018, Fields et al. 2018, Bourgeois et al. 2021) and are keystone species within aquatic food webs (Lampert 2011). *D. magna* reproduces via cyclic parthenogenesis, producing clonal (asexual) daughters, but may reproduce sexually in poor environmental conditions. Sexual reproduction results in resting stages (ephippia) that can survive freezing and drought and will hatch when conditions improve.*Daphnia* spp. Metabolism, grazing, development rates and reproductive output are accelerated by warming temperatures, up to around 25 °C (Burns 1969, Kirk et al. 2018), and this increase in metabolic rate may also increase their encounters with parasites and development of infection (Kirk et al. 2018, Kirk et al. 2019, Kirk et al. 2020). *P. ramosa* infection follows a stepwise process (Ebert et al. 2016) whereby 1) *Daphnia* spp. are exposed to spores by filtering contaminated water or mud; 2) spores become "activated" when in contact with *Daphnia* spp. (Duneau et al. 2011); 3) spores attach to, then 4) penetrate the gut wall; and 5) grow and reproduce within the host. During parasite growth inside the host,*Daphnia* spp. stop reproducing (parasitic castration), grow larger (gigantism) and turn dark red/orange in colour, making it easy to observe whether an individual is infected without destructive sampling and to track changes in population prevalence over time. The interactions of *D. magna* and *P. ramosa* are known to be highly polymorphic within populations and the two antagonists co-evolve (Decaestecker et al. 2007, Auld et al. 2016). A matching-allele-infection matrix has been observed (Bento et al. 2017), which may influence epidemic dynamics as the proportion of individuals susceptible to the dominant strain is likely to change.

The local food web in the Aegelsee is relatively simple, including*Daphnia pulex* and *D. curvirostris* in addition to *D. magna* and *P. ramosa* , and small insect predators such as*Chaoborus* larvae and chorixid water-bugs. Only *D. magna*becomes infected with *P. ramosa* in this pond. The seasonal monitoring includes assessment of prevalence of *P. ramosa* and*D. magna* population dynamics and estimates of the proportions of*Daphnia* "resistotypes", i.e. the genetically determined ability of the *D. magna* to resist specific genotypes of the parasite. Finally, this pond undergoes a massive disturbance in late September/early October when hot, ammonium-rich, condensation water from a nearby sugar refinery is released into it, killing off all planktonic *D. magna* and other invertebrates in the pond, but not the resting stages of the host or parasite in the sediment.

Source of Organisms

This experiment used *P. ramosa* spores and *D. magna*ephippia from natural pond sediments, and *D. magna* genotypes collected earlier from the field site and propagated clonally in the laboratory. Sediment from the Aegelsee was collected in September 2021 and stored in darkness at 10 °C. Sediment was filtered using distilled water and 80-µm mesh to remove large particles,*Daphnia* ephippia and rotifer resting eggs from the sediment, but not the much smaller (about 5-6 µm diameter) *P. ramosa* spores, and was mixed homogeneously. *D. magna* ephippia were collected under a dissecting microscope from a sediment sample collected in 2019 and stored at 4 °C in a refrigerator. This prolonged resting period increases hatching success and synchrony (Stross 1966). For the live *Daphnia* experiment, *D. magna* clones were isolated from the Aegelsee

from previous field seasons (2019 and 2014) and maintained in isogenic lines under laboratory conditions (ADaM media (Klüttgen et al. 1994) at 20 °C, 16 hour light:8 hour dark cycle, and 80 % humidity and fed with a suspension of*Tetradesmus obliquus*). Prior to this experiment, each clone was assessed for its resistance/susceptibility to a panel of*P. ramosa* isolates maintained in the lab, two of which are present in the Aegelsee, using an fluorescence based "attachment test" (Duneau et al. 2011). For the current experiment, genotypes that were susceptible to all *P. ramosa* isolates of the test-panel (type SSSSS, hereafter referred to as "susceptible"), and clones that were resistant to most tested isolates (type RRSRS hereafter referred to as "resistant") (clones from (Ameline et al. 2020)) were propagated by transferring and multiplying cultures twice weekly. These two resistotypes are very common in the Aegelsee population (Ameline et al. 2020), but are not "susceptible" or "resistant" to all *P. ramosa* isolates present in the study pond. Clones were kept in population cultures in 360-mL jars.

Once population sizes were large enough, adult females were transferred to isolated jars in an incubator at 15 °C to acclimate them to a temperature in the centre of the experimental treatment range (10 to 20 °C). To reduce maternal effects of temperature on resistance to *P. ramosa* (Garbutt et al. 2014), these females were bred two generations under these conditions by removing adult females once they had given birth to offspring, which were then isolated upon reaching maturity. Third- or subsequent-brood juveniles from the second generation were used for the actual experiment, to account for the fact that the first two broods are often smaller in numbers and size of offspring than subsequent broods (Lampert 1993). Prior to the start of the experiment, these juveniles were moved to experimental incubators and allowed to acclimate for 48 hours to their experimental temperatures.

We aimed to run the experiment at each temperature for the same length of biological time, since *D. magna* physiology and life cycles are accelerated by higher temperature. A pilot experiment was conducted to calibrate the biological scale for *D. magna* and estimate the hatching rate and time to reach maturity (first eggs) at 10 and 20°C. This scale was used to estimate how long it would be necessary to run the experiment for all temperatures to achieve an approximately equal physiological age.

Experimental Design

This study featured a 5x3 factorial design of temperature and host availability to assess the role of temperature on the start of seasonal epidemics. Five incubators at 10, 12.5, 15, 17.5 and 20°C were used for temperature treatments, into which 360-mL jars with media and a thin layer of filtered sediment were placed. These temperatures were chosen to determine infection dynamics in a range around 15 °C, the temperature at which infections are first observed in the pond (Ameline et al. 2020). Infections in the pond are typically observed when *D. magna* of different age classes are already present (Ameline et al. 2020), but further hatchlings from resting eggs may still arise. Therefore, three treatments of host availability were used: broadly susceptible or broadly resistant juvenile *D. magna* from asexually reproducing cultures, and ephippia from which genetically diverse hatchlings could arise. We placed either 15 juvenile *D. magna* of a broadly susceptible or broadly resistant clone, or 10 ephippia (each containing two eggs, but with an average hatch rate of ˜30-50 % as determined in a pilot experiment) into each jar. These treatments were chosen to disentangle the effects of the presence of susceptible hosts on temperature, as behaviour or physiology of hatched ephippia may differ from asexual juveniles. To increase hatching rate, ephippia were treated with a 50:50 solution of commercial bleach (4 %) and distilled water for three minutes (Catur and Ebert 2016), before being rinsed and added to jars. A total of 20 replicates of the full experimental design were performed, with 20 jars of ephippia, 20 jars of broadly susceptible juveniles and 20 jars of broadly resistant juveniles each at each temperature. Within the live *D. magna* treatments, five different clones were used for each resistotype, with four jars of each clone. All treatments and replicates were run concurrently. Within incubators, trays of 12 jars were arranged randomly and rotated each day to reduce potential position effects. All jars were also covered with a transparent lid to reduce temperature fluctuations due to evaporation. Live *D. magna* jars were fed with *T . obliquus* at a rate adjusted to account for differences in *D. magna*metabolic/filtering rates at different temperatures (Burns 1969) to avoid build-up of algae, and to avoid interactive effects of food availability with temperature on *D. magna* development (McKee and Ebert 1996, Giebelhausen and

Lampert 2001). Specifically, live *D. magna* jars were fed three times per week with $100x10^6$cells at 20 °C, $70x10^6$cells at 17.5°C, $50x10^6$ cells at 15°C, $20x10^6$ cells at 12.5°C and $10x10^6$ cells 10°C based on our pilot. Ephippia treatment jars were fed at these same rates once hatchlings were observed.

Sampling and Experimental Procedure

All jars were monitored using a dissecting microscope at a frequency according to their temperature, based on the approximate biological time scale from our pilot (20 and 17.5 °C thrice weekly, 15 and 12.5 °C twice weekly, and 10 °C weekly). Each time, the number of *D. magna* at each age class (juvenile, adult, adult with eggs) and number of *D. magna* with visible signs of infection (reddish colouration, no eggs in the brood chamber, gigantism) were recorded. For the 10 °C treatment, a walk-in chamber was used so that monitoring could be done without removing the jars from their temperature. For 12.5, 15 and 17.5 and 20 °C, trays of jars were moved to an insulated cooler box one by one to minimize the amount of time any jar was outside its experimental temperature for monitoring at room temperature.

After the first generations of new-borns were released into the media, experimental *D. magna* were transferred to jars of fresh medium without sediment at the appropriate temperature to continue observation of infection development without their offspring. Observations continued for 30 days at 20 °C, 35 days at 17.5°C, 44 days at 15 °C, 56 days at 12.5 °C and 81 days at 10 °C, according to the biological time scale determined by the pilot.

Statistical Analysis

All analyses were conducted in R Language and Environment for Statistical Version 4.0 (R Core Team 2020) and α was set to p=0.05. All reported values are means ± standard error unless otherwise stated. Our dependent variables of interest were: prevalence (defined as proportion of jars with at least one infected animal in observed by the end of the experiment), and the average time to visible infection for individuals in each jar (calculated by the number of new infections on each observation day). For *D. magna* development rates, the time to emergence of hatchlings in days was calculated for ephippia-only treatments, and the average time to maturity (develop first egg clutch) for all treatments.

The data for the live *D. magna* treatment and the data from the ephippia treatment were analysed separately. For the ephippia treatment, average time to hatch, time to maturation, and time to infection (all in days) were compared among ephippia treatments using linear regressions with temperature as the independent variable. The effect of temperature on prevalence was analysed using a generalized linear model with a binomial distribution (package *MASS*). For the live *D. magna* treatments, the dependent variables time to maturation, time to infection and prevalence at the end of the experiment were compared among host resistotype and temperature treatments. Here, we used generalized linear mixed effects models (package *lme4*) with different error distributions depending on the nature of the data (binomial for prevalence, normal for all others) using temperature and host resistotype and their interactions as fixed variables, with clone nested within resistotype as a random effect (DV˜Temp*Resistotype+(1|Clone:Resistotype)). When interactions were not significant, they were removed from the model and only main effects were assessed.

Results

A total of 200 jars with live *Daphnia* and 100 jars with ephippia were used, at the same time in the same incubators. Of the 100 ephippia jars all but three (one each at 10, 12.5 and 20 °C) had at least one *D. magna* from the 10 ephippia hatch, with an average number of hatchlings of 6.4 ± 0.33 (32 %). All jars experienced mortality (unexplained and parasite-mediated) over the course of the experiment (74.3 ± 1.9 % for ephippia and 69.4 ± 1.4 % for live treatments).

D. magna *Development*

Time to hatch (slope estimate = -0.76 ± 0.06, p<0.0001) (Figure 1) and time to maturation (slope estimate= -0.76 ± 0.06, p<0.001) (Figure 2b) decreased with increased temperature for ephippia treatments. Time to

maturation also decreased with increased temperature for the live *D. magna* experiment (slope estimate= -0.86 ± 0.82, p<0.001), with no effect of resistotype detected (Figure 2a).

Parasite Development

A total of 192 out of the 300 jars had at least one *D. magna* that showed signs of visible infection by the end of the experiment. In the experiment with live *D. magna* , we observed a general increase in the proportion of jars with infected hosts with increasing temperature, with between 90-100 % being infected from 15 °C upwards for both resistotypes. At 10 and 12.5 °C the more-resistant clones had much lower prevalence than the more-susceptible ones (15 vs 55 % at 10 °C, 20 vs. 55 % at 12.5 °C), resulting in a significant interaction between resistotype and temperature (estimate= -0.61 ± 0.25, p=0.017) (Figure 2c). For animals that became infected, main effects of temperature and resistotype on time to visible infection were detected, with time to infection decreasing with increased temperature (estimate= -4.12 ± 0.19; p<0.0001), and more-susceptible resistotypes becoming visibly infected faster than more-resistant ones (estimate= -4.17 ± 1.5; p=0.005) within each temperature (Figure 2e).

In the ephippia treatment, prevalence by the end of the experiment was also positively associated with temperature (estimate 1.38 ± 0.4; p=0.0005) (Figure 2d). However, in contrast to the live *D. magna*treatment, below 15 °C no infections were observed and the overall proportion of jars with infected animals at higher temperature were lower than for live *D. magna* treatments (30 % at 15 °C, 65% at 17.5 and 20 °C). Among ephippia hatchlings that became infected, time to infection shortened with increasing temperature (estimate= -1.87 ± 0.36; p<0.0001) (Figure 2f). The time to visible infections for these animals was comparable to those born from asexual reproduction at these temperatures.

Comparing Host and Parasite Development

Development of both hosts (as determined by hatching and maturation) and parasites (as determined by the presence of visible infections and the speed at which it was observed) were accelerated by higher temperatures. However, this effect was much stronger for the parasite (Figure 3). While the time to visible infection was more than four times longer at 10 °C than the time to maturity, these estimates differed only by factor two at 20 °C.

Discussion

The objective of this study was to experimentally disentangle the role of warming temperatures in Spring from the emergence of susceptible hosts on the timing of seasonal parasite outbreaks. Using a range of constant temperatures and availability of susceptible hosts, we found that temperature influenced both host and parasite development, but that–consistent with our main hypothesis–parasite development was more strongly limited by low temperatures than host development. Variation in host susceptibility modifies this pattern, with more susceptible clones becoming infected faster than more resistant ones and with hatchlings borne from ephippia less likely to become infected then those produced asexually.

Effects of Temperature Different for Host and Parasite

Consistent with previous work (Burns 1969, Kirk et al. 2018), we found that *D. magna* time to hatching and time to maturation were slowed, though not prevented, at lower temperatures and decreased with higher temperatures. However, we also found that *P. ramosa*infections are slowed and/or limited at low temperatures and accelerated at higher temperatures to a greater degree than the host's relationship to temperature. For both ephippia and live *D. magna* treatments, final prevalence increased with increasing temperature, indicating that the onset of an epidemic in Spring is significantly influenced by the effect of temperature on the parasite, and not only the presence of hosts. Additionally, time to visible infection decreased with increasing temperature for both ephippia-hatched and live *D. magna* . Importantly, differing reaction norms obtained for our host and parasite suggests that increases in temperature due to climate change and earlier Springs may accelerate infection rates more severely than host growth is accelerated, which could have implications for epidemic and long-term population dynamics. Since *P. ramosa* is a castrating parasite, our findings imply that as temperature increases, it is possible that infected hosts might lose their ability to

reproduce shortly after or even before reaching maturity. Overall, this finding supports our hypothesis that outbreak timing is driven by temperature rather than other seasonal forces, and highlights the importance of considering the impacts of environmental factors on host and parasite separately, especially for those that spend part of their life cycles apart (Gethings et al. 2015, Gehman et al. 2018, McDevitt-Galles et al. 2020).

The finding that live *D. magna* became visibly infected at 10 and 12.5 °C (confirmed by dissection at the end of the experiment) suggests that warming temperatures may not "trigger" outbreaks in the manner previously assumed, but rather accelerate them. To our knowledge infections at these temperatures had not been observed before, and previous experiments suggested that infection cannot occur below about 13 °C (Vale et al. 2008, Vale et al. 2011), despite *D. magna* being able to hatch and reproduce at these temperatures. This contrast may be explained by the fact that we ran our experiment for much longer than previous ones to allow for different exposure rates due to slower *D. magna* metabolism at lower temperatures. These results may also clarify why infection at these low temperatures has not been observed in the wild at our field site, as our findings indicate that the time it would take for infection to occur at these low temperatures is much longer than the time the pond typically spends at these temperatures in Spring (Ameline et al. 2020).

Interactive Effects of Temperature and Available Hosts on Infection

Among live *D. magna* treatments, the more resistant clones were less likely to become infected and had a longer time to infection than the more susceptible ones. Moreover, host resistotype interacted with temperature to influence infection prevalence, with most observed infections at low temperature being among the more susceptible clones. As our broadly resistant clones are susceptible to at least one of the known *P. ramosa* strains in the spore bank, it is not surprising that they still became infected. However, that fewer individuals did so compared to the more susceptible clones (especially at low temperatures) and that they also took longer to become infected could be due either to the frequency of *P. ramosa* strains within the mud that are able to infect them, or the possibility that these strains indeed take longer to cause visible infection (i.e. reproduce inside the host slower than other strains). Although we are unable to elucidate which of these two hypotheses is more likely with these data, these findings nevertheless suggest an important interactive effect of host resistance with temperature on outbreak timing and dynamics. Similar temperature-by-genotype interactions have been found in *D. magna*resistance to other parasites (Bruijning et al. 2022, Santos and Ebert 2022) and in many other host-parasite systems (Judelson and Michelmore 1992, Gsell et al. 2013). Our results imply that the host-parasite relationships in this system are strongly linked to temperature, and that changes in temperature may alter the infection and host demographic cycles we have repeatedly observed.

Prevalence in the ephippia treatments at the end of the experiment was at all temperatures considerably lower than in the treatments with live*D. magna* (compare Figure 2c,d). Moreover, within ephippia treatments no infection was observed below 15 °C a finding consistent with dynamics observed at the field site (Ameline et al. 2020). Although we do not know the resistotypes of animals used in this treatment, it is unlikely that this result is due to resistotype composition, as the known resistotypes used in the live treatment are also known to hatch from ephippia (Ameline et al. 2020, Ameline et al. 2021). It is possible that females hatching from resting stages behave differently than asexually produced females and that this reduces the likelihood of encountering the parasite. Sediment-borne parasite stages, like the spores of *P. ramosa* , are picked up when the host browses over the sediment surface, to enrich its food (Arbore et al. 2016). We do not know if this behaviour differs among the two types of females, but if so, it might partially explain these observed differences and possibly why Spring epidemics of *P. ramosa* are delayed, because all *D. magna* in early Spring hatch from ephippia. This hypothesis could further explain why infections are not observed in nature at lower temperatures, since the time for the first generation of asexual females to be born compounded with the impacts of temperature on development would further prolong the time until outbreak.

Implications and Avenues for Future Work

Our findings provide several avenues for future work that may further clarify the onset of epidemics in this system. While the constant temperatures of our study are a strength in that they allow us to disentangle the role of temperature on this system from other seasonal dynamics, it remains unclear how temperature

changes (i.e. the speed of warming in the Spring or daily/seasonal fluctuations) may impact these dynamics. As climate change is predicted to cause more extreme temperature fluctuations in addition to warming in most of the areas*D. magna* and *P. ramosa* inhabit, and previous work has shown that daily fluctuations in temperature and heatwaves differentially impact the performance of *D. magna* and another of its parasites, the microsporidium *Ordospora colligata* (Kunze et al. 2022), this question remains important for future work to address. Identifying which steps of *P. ramosa* infection (Ebert et al. 2016) are limited by temperature could also further clarify this parasite's relationship to temperature. *D. magna* exposure to*P. ramosa* is slower at lower temperature due to slower *D. magna* filtering/metabolism, however we have controlled for this effect by running the experiment longer at lower temperatures, yet differences in infection by temperature persist. Previous work shows spore activation and attachment are possible at all our tested temperatures (Duneau et al. 2011). Penetration of the host cuticle and reproduction within the host are remaining candidates for which step(s) are limited by infection and remain an interesting avenue for future work. Furthermore, identifying if sexual offspring have a different behaviour and/or physiology that makes them less likely to be infected than asexual offspring would also be important. Future work may focus on how temperature affects later stages of the epidemic. Additionally, a longer-term study that examines selection for resistance in a population could determine whether rapid warming would alter the diversity of phenotypes and genotypes in the population.

Conclusions

Through controlled quantitative experiments, we were able to disentangle the impacts of temperature, host phenology, host resistance and host offspring type (sexual vs asexual) on the timing of seasonal disease outbreaks that are repeatedly observed in nature in a well-studied host-parasite model system where host and parasite overwinter separately. We provide several lines of evidence that parasite infection is strongly driven by temperature, beyond the effects of temperature on host development, and that this relationship is modified by host resistance and offspring type. This work highlights the importance of understanding how hosts and parasites may respond differently to environmental changes and provides insight into the seasonality of epidemics, particularly for parasites with free-living stages. It also allows us to improve our predictions regarding the response of infectious disease outbreaks to climate change.

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Tables and Figures

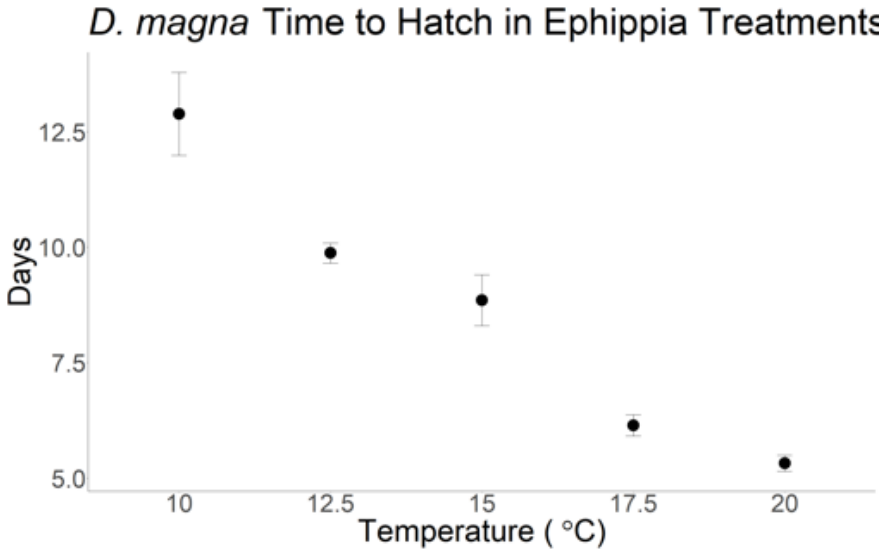

Figure 1: Average (+/- standard error) time (in days) until hatching (b) among ephippia treatments by temperature.

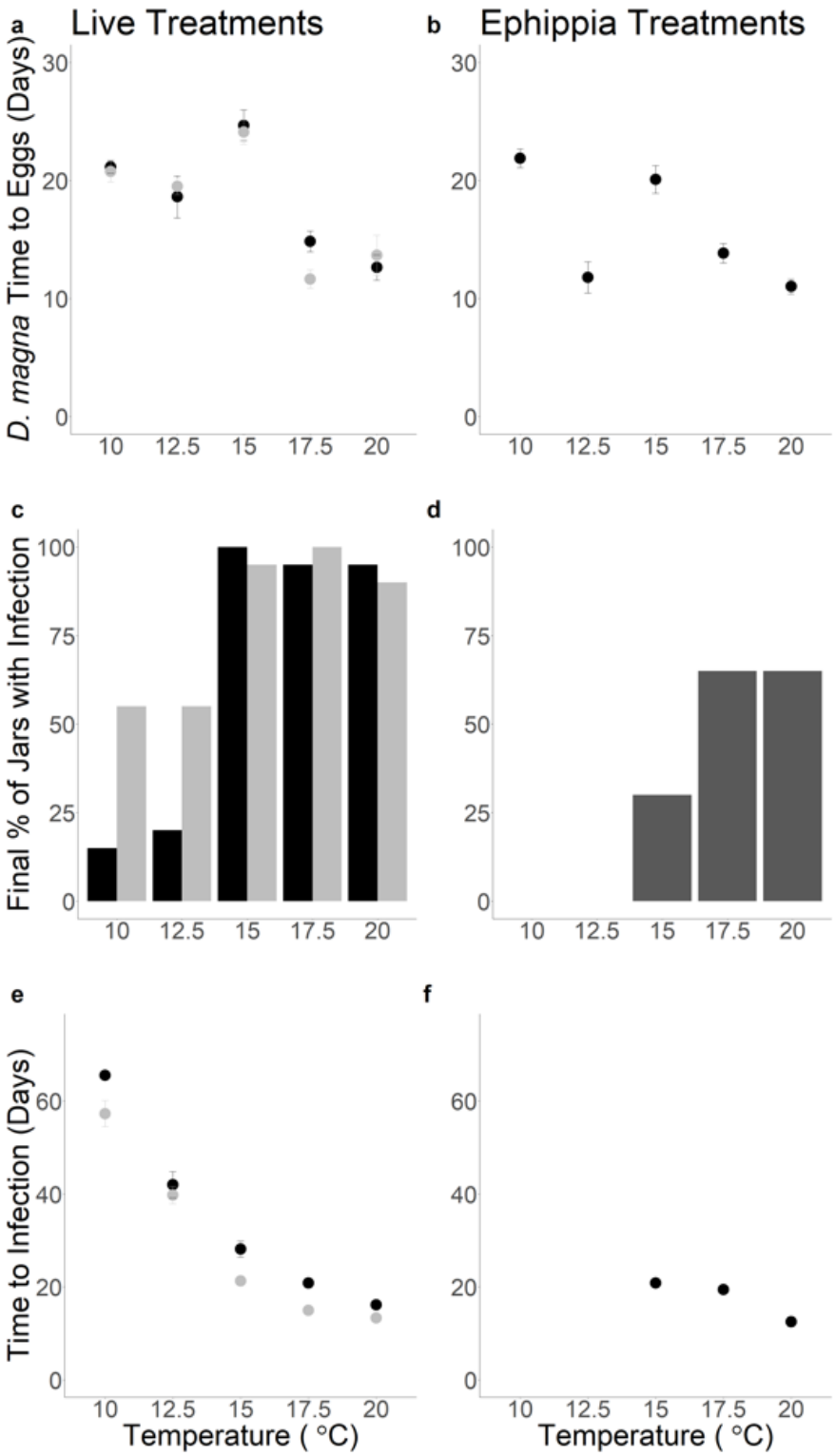

Figure 2: Host and parasite development in live D. magna (left) and ephippia (right) treatments by temperature. For live treatments, broadly resistant clones are in black and broadly susceptible clones are in

grey. D. magna development is measured in the time to maturation based on the average day (+/- standard error) in a jar when an individual first developed eggs (a-b). P. ramosa development is shown by the final (at experiment end) prevalence with the number of jars (out of 20) visibly infected for each temperature and resistotype (c-d), and by the average number (+/- standard error) in days until an individual looked infected for each jar (e-f). For both ephippia and live D. magna treatments, time to maturation and time to infection were negatively associated with temperature. Within live treatments, an interaction between resistotype and temperature was detected for prevalence, with the slope of the temperature-prevalence relationship being steeper for broadly resistant clones. An additional main effect of resistotype was detected for time to infection, with susceptible clones becoming infected faster than broadly resistant ones.

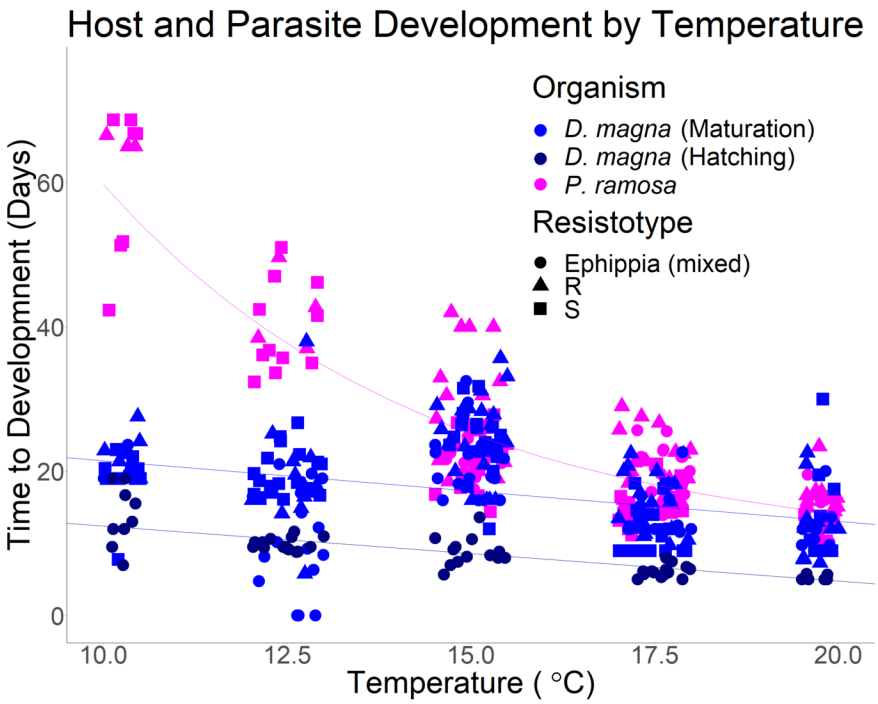

Figure 3: Relationship between temperature and development for P. ramosa (pink) and D. magna (blue), with D. magna resistotype indicated by shape (square for susceptible, triangle for resistant and circles for ephippia). Points are jittered horizontally to display individual points at the same temperature. D. magna development is represented both by the average number of days until hatching (navy; for ephippia treatments only) and by maturation (royal blue), the average number of days until the first clutch (minus the average number of days until hatching for ephippia treatment) and P. ramosa development is measured by the time from exposure to signs of visible infection in the host. Development times for both species are shortened by increasing temperatures, however the slope is much steeper for parasite than for the host indicating that as temepratures increase, the time between when the host reaches reproductive maturity and when they are castrated by infection is shortened. Line of best fit for P. ramosa was added using the SSasymp function (packgage=stats) with $f(x)= 8.815+(498.043-8.815)^{-1.486(x)}$ and for D. magna the linear model y= —0.838 (x)+ 29.8539 was used for time to eggs and y= —0.7608(x)+20.018.