Warming strengthens priority effects driven by an ontogenetic shift from host to predator

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Abstract

Priority effects arise when species arrival order determines interaction outcomes. In many ecological systems, interacting species undergo ontogenetic shifts that reverse their roles as consumer and resource, making the consequences of arrival order inherently time dependent. Here, we examine how temperature alters priority effects using a host-parasite system where the treehole mosquito *Aedes sierrensis* undergoes an ontogenetic shift from a vulnerable host to a predator of its parasite, *Lambornella clarki*. We hypothesized that the relative timing of mosquito hatching and parasite emergence would determine infection outcomes and that warming could either reduce or increase infection depending on whether mosquito development or parasite proliferation is more temperature-sensitive. In microcosm experiments, we manipulated the mosquito larval stage (1^{st} vs. 4^{th} instar) at parasite introduction across a 6–14°C temperature gradient, mimicking variation in arrival order and temperatures that might occur in their native habitats in California. Our results revealed a stark priority effect mediated by an ontogenetic role reversal: when parasites encountered 4^{th} instar larvae, they were rapidly consumed by the larvae, and infection was completely prevented. Conversely, when parasites encountered 1^{st} instar larvae, infection occurred, and parasitism rates increased significantly with increasing temperature. Warming strengthened the priority effect by disproportionately accelerating parasite proliferation relative to host development and defense, allowing the parasite to establish infection before the host reached a less-susceptible stage. This finding highlights the potential for temperature to fundamentally alter community assembly by shifting the rate at which ontogenetic role reversals occur relative to the timing of species arrival.

Introduction

The order of species arrival can have lasting effects on community structure and function (Chase 2003, Zou and Rudolf 2023). This phenomenon, known as priority effects, has been studied most intensively in competitive communities, where early arriving species can preempt resources and exclude later colonists (Fukami 2015). However, priority effects can also play important roles in consumer-resource interactions such as predation and parasitism, which often unfold in temporally structured ways (Yang and Rudolf 2009, Hoverman et al. 2013, MacDonald and Brisson 2022, Stroud et al. 2024). In predator-prey and host-parasite systems, the relative timing of species' introductions can alter interaction outcomes, including the complete reversal of predator-prey roles (Choh et al. 2012). In a classic marine example, rock lobsters prey on whelks, but where whelks are established first at high densities, they can overwhelm and consume the lobsters, creating alternative stable community states (Barkai and McQuaid 1988, Sánchez-Garduño et al. 2014).

Priority effects can be particularly strong when species undergo distinct ontogenetic transitions that alter their ecological roles, so that the timing of arrival determines the developmental stage at which individuals interact (Rasmussen et al. 2014). Phenological differences in emergence or colonization timing that alter relative body size at first encounter can shift the balance between competitors, cannibals, or predators and prey, and can produce reciprocal intraguild predation or role reversals (Fonseca et al. 2018, Sniegula et al. 2019, Raczyński et al. 2022). Intraguild predation arising from these stage-structured encounters is a context in which priority effects are often especially strong (Holt and Polis 1997). In pond and treehole communities, for example, the identity and arrival timing of intraguild predators are known to strongly influence subsequent assemblage structure of other intraguild predators and prey (Fincke 1994, Fink 1999, Blaustein

and Margalit 1996, Padeffke and Suhling 2003). Host parasite systems may also show similar historical contingencies when infectious stages are vulnerable to predation, which is known to depend on both predator and parasite size (Johnson et al. 2010, Orlofske et al. 2015). To date, however, most empirical work on priority effects in host-parasite systems has focused on the relative arrival timing of multiple parasite species, genotypes, or strains within hosts (Hoverman et al. 2013, MacDonald and Brisson 2022), rather than on the arrival order of hosts and parasites themselves.

The strength of stage-structured priority effects is likely to be temperature-dependent. Temperature governs developmental rates, metabolic demands, and behavior in ectothermic organisms, and can differentially affect species based on their thermal sensitivities (Angilletta 2009, Dell et al. 2014). For interacting competitors, warming is expected to amplify priority effects by accelerating the impact of early colonizers, increasing the likelihood of competitive exclusion when inferior competitors arrive late (Grainger et al. 2018, Vass et al. 2021, Dawson-Glass et al. 2025). In consumer-resource systems, however, predicting the effect of warming can be more complex. The outcome hinges on a thermal performance race between the interacting species and on which species arrives first to establish the priority effect. In a scenario where the consumer arrives first, the priority effect is driven by the consumer's ability to suppress the later-arriving resource (Olito and Fukami 2009). Warming should amplify the priority effect if it disproportionately benefits the consumer, for instance by increasing rates of predation or parasitism more than the resource can compensate for (Öhlund et al. 2015, Cohen et al. 2020). Conversely, warming can dampen or even reverse a priority effect if it disproportionately benefits the resource, for instance by allowing it to accelerate its development or growth to a less-vulnerable stage (Pepi et al. 2023).

Further, when the role of consumers and resources switches between species ontogenetically, temperature can interact with arrival order to affect which species consumes the other. Experimental studies show that warming can alter intraguild predation strength and shift competitive hierarchies in crabs (Rogers et al. 2018) and odonate larvae (Suhling and Suhling 2013, Frances and McCauley 2018, Stahl and Johansson 2024). Theory further predicts that temperature-dependent changes in growth and attack rates in stage-structured intraguild predation can cause abrupt switches between predator dominance, prey dominance, and coexistence (Thunell et al. 2021). However, this work has been conducted with synchronous introductions, and we still lack empirical tests of how warming modifies priority effects in systems with predator-prey role reversals. To understand the effects of warming on consumer-resource priority effects requires measuring how temperature changes the speed at which species undergo predator-prey role reversal, relative to the pace at which local communities assemble.

In this study, we examine temperature-dependent priority effects in a natural host-parasite and predator-prey system composed of the western treehole mosquito (*Aedes sierrensis*) and its facultative ciliate parasite (*Lambornella clarki*). Larval mosquitoes consume microbial resources in treehole water, including ciliates, whereas *Lambornella* can take on a free-living bacterivorous stage or a parasitic stage that infects mosquito larvae (Washburn et al. 1988). Both species exist in dormant stages in the dry season and emerge when treeholes fill with water, but the relative timing of mosquito hatching and *Lambornella* excystment can vary (Washburn et al. 1991a). This, combined with their dual roles as consumers and resources, creates a dynamic where stage-dependent priority effects are likely, as the timing of interaction can determine whether the ciliate becomes a parasite or prey, and the immature mosquito a host or predator, in turn governing which species persists in a treehole.

We hypothesized that the relative arrival time of the host and parasite would determine infection outcomes. Specifically, we predicted that early parasite arrival (or emergence from their dormant state) while the hosts remain at a vulnerable early developmental stage would lead to high infection rates, whereas later parasite arrival, when hosts are larger, would allow hosts to suppress the parasite via predation, resulting in low infection rates. We next asked how warming alters the strength of these priority effects. Because temperature can differentially affect host developmental rates and parasite proliferation (Ismail et al. 2024), warming could either shorten the period when hosts are vulnerable and hasten the onset of predation on free-living parasites, thereby reducing infection, or it could accelerate parasite growth enough to increase infection despite faster host development. To test these predictions, we conducted a factorial microcosm experiment manipulating mosquito developmental stage at parasite introduction (1^{st} vs. 4^{th} instar) and temperature, and a complementary microcosm experiment to quantify mosquito predation rates on free-living *Lambornella* across larval instars.

Methods

Study system

Aedes sierrensis and *Lambornella clarki* occupy water filled treeholes across western North America from the coast to montane areas (Arnell and Nielson 1972, Corliss and Coats 1976). Both species exist in a dormant state through the long dry summer season and become active when winter rains or melting snow flood their habitat (Washburn et al. 1991a). As a facultative parasite, *Lambornella* can exist in a free living form, which feeds on bacteria, and a parasitic form, which develops only when free-living cells are exposed to mosquito larvae or mosquito-conditioned water (Washburn et al. 1988). In response to these cues, free-living cells divide and

their daughter cells transform into invasive parasitic cells that encyst on the larval cuticle, invade mosquito larvae, proliferate internally, and are released upon host death (Washburn et al. 1991a). This facultative parasite life history strategy has been described as an evolutionary adaptation to escape stressful conditions like predation (Luong and Mathot 2019). *Lambornella* is the only ciliate parasite known to infect this mosquito species (Washburn and Anderson 1986). In coastal California, *Lambornella* and mosquito larvae are active almost exclusively during the wet season and our previous work has shown that the peak of the infection response lies roughly between 10 and 13 °C both in lab observations (Ismail et al. 2024, Farner et al. 2025) and field surveys (Farner et al. 2025). *Lambornella* is thought to disperse when infected adult female mosquitoes introduce cysts or free-living cells into new treeholes during pseudo-oviposition (Egerter et al. 1986).

Mosquito and parasite source

The mosquito population used in the experiment was collected from a single uninfected treehole in Jasper Ridge Biological Preserve ('Ootchamin 'Ooyakma), hereafter JRBP('O'O), located in the Santa Cruz Mountains of California (37.40736, -122.23756). Mosquitoes were collected as larvae, identified to species (Darsie and Ward 2005), reared to adulthood, and blood-fed to produce eggs (detailed methods in Couper et al. 2024). Eggs were incubated at 21°C for four weeks and then 5°C for six weeks to mimic seasonal changes until being used in the experiment. Mosquitoes were hatched sequentially, either one day or 16 days before the experiment started. To hatch the eggs, we submerged them in a tray filled with 500 ml distilled water, 300 ml autoclaved tree hole water, and 12 ml Brewer’s yeast. From hatching onwards, mosquitoes were kept at 10°C to match the average temperature of the wettest quarter—the season in which these species interact—at JRBP('O'O) as measured by WorldClim (Fick and Hijmans 2017).

Mosquitoes were fed 0.1mg of food per larva per day made of a finely-ground mix of Tetramin fish flakes (48% by weight), guinea pig chow (48%), and liver powder (4%) (Maiga 2017).

The *Lambornella* used in the experiment were collected from a nearby treehole at JRBP('O'O). The *Lambornella* culture was isolated from a single infected mosquito in December 2022, which was crushed and put in 2 ml of autoclaved culturing media (made up of 1000 ml distilled water, 1 alfalfa pellet, and 0.38g of Herptivite) and a barley seed (Fukami 2004). The culture was maintained at 21°C in 4ml vials in the dark. Every two or three weeks half of the media was replenished. Four weeks before the experiment began the culture was moved to 10°C to prevent any temperature shock and the media was doubled weekly.

Experiment 1: Priority effects across temperatures

We established microcosms in 6-well Falcon plates to test for priority effects across five constant temperatures: 6, 8, 10, 12, and 14°C, which were selected to reflect the range of seasonal temperatures experienced by mosquitoes and *Lambornella* at JRBP('O'O) during the wettest quarter, when ciliates and larval mosquitoes are active (see Supplementary Text S1 for more details). At each temperature, all microcosms were inoculated with *Lambornella* and assigned to one of three arrival treatment groups (n = 10 replicates per treatment): (1) introduction of 1st instar larvae; (2) introduction of 4th instar larvae; or (3) a no-larvae control with mosquito-conditioned water. Each replicate included five mosquito larvae. For each temperature treatment we used two separate incubators. Replicates 1 to 6 were placed in the first incubator assigned to that temperature, and replicates 7 to 10 were placed in the second incubator assigned to that temperature.

Microcosms contained 3 mL of *Lambornella* culture (100 cells/100 µl), 2 ml of mosquito-conditioned water, and a barley seed as a nutrient source, resulting in an initial concentration of 60 cells/100 µl. Previous studies have shown this is a sufficient dose to cause infection (Ismail et al. 2024, Lyberger et al. 2024), and a pilot experiment confirmed that infection probability increases with dose (Figure S1).

Microcosms were monitored daily for the first week and biweekly thereafter. We recorded (a) the density of free-living *Lambornella* (counted in 100 µl samples under 40x magnification); (b) the number of larvae with parasitic cysts attached to their cuticle; (c) the number of infected larvae (with parasites inside the cuticle); (d) the intensity of these infections (the number of infectious cells inside the cuticle); and (e) larval mortality. The total number of cysts was also quantified but is not presented, as this metric was redundant with the number of larvae with cysts. After removing 100 µl for counting, each sample was replenished with an equal volume of fresh media. Larvae were fed every other day at half the usual amount (0.05 mg/larva/day) to encourage grazing (Maiga 2017). The experiment's duration was temperature-dependent, concluding when larvae in the 1st instar treatment level began to pupate, which ranged from 21 days (14°C) to 42 days (8°C). The 6°C treatment level, in which no pupation occurred, was terminated at 56 days when free-living cells were no longer observed.

Experiment 2: Stage-structured feeding trial

To determine the developmental stage at which mosquito larvae are capable of consuming *Lambornella*, we conducted a separate feeding trial using all larval stages. The rearing procedures for this experiment followed those described for Experiment 1. To generate larvae at each of the four instar stages simultaneously, we staggered hatching times. Individuals were

starved for at least 24 hours prior to the experiment to standardize hunger levels and encourage feeding. For each instar (n = 5 per stage), we placed a single larva in a well of a Falcon plate containing 2 ml of *Lambornella* culture and 1 ml of treehole water (total volume = 3 ml). The *Lambornella* culture used in this trial had an initial concentration of 85 cells per 100 µl. Both the larvae and cultures were kept at 10°C both prior to and during this trial.

We checked each well at 3, 6, 12, 24, and 48 hours post-exposure for evidence of feeding (i.e., reduction in *Lambornella* cell density). Each larva was housed in an individual well (total = 25 units), and no food was added during the course of the trial. This short-term assay allowed us to assess whether the ability to graze on the free-living parasite varied by developmental stage.

Statistical analysis

For experiment 1, we separately analyzed each response variable including free-living cell density, number of larvae with cysts, number and intensity of larval infections, and larval survival. Free-living cell densities across time were analyzed using a generalized linear mixed-effects model (GLMM) with a generalized Poisson distribution, including fixed effects of temperature, instar, and their interaction. All models included a random intercept for tray nested within incubator to account for the experimental design unless otherwise noted. The proportion of larvae with cysts was analyzed at the time of peak cyst formation using a binomial GLMM with fixed effects of temperature (modeled as a quadratic polynomial to account for potentially nonlinear effects of temperature observed in previous work (Lyberger et al. 2024)) and instar treatment (1^{st} or 4^{th}). The proportion of larvae infected at the end of the experiment was analyzed using a binomial GLMM with temperature as a linear fixed effect, as the relationship appeared approximately linear. Infection intensity, measured as the total number of cells across infected

larvae, was analyzed using a zero-inflated negative binomial model. In the analyses of infection and intensity, we only analyzed the 1st instar treatment level because we observed zero infections in the 4th instar treatment level. Larval survival was analyzed at the end of the experiment using a binomial GLMM including instar and temperature as fixed effects. Because mortality was nearly absent in the 4th instar treatment, we additionally evaluated temperature-dependent mortality within the 1st instar treatment using a binomial GLMM with linear and quadratic temperature terms. To better account for the temporal dynamics of larval mortality, we also analyzed survival using a discrete time proportional hazards model. We constructed Kaplan–Meier style survival curves and reported the resulting hazard ratios for temperature. For experiment 2, to measure grazing intensity we analyzed the effects of larval instar stage and time on log transformed free-living *Lambornella* cell counts using a linear mixed-effects model with a random intercept to account for repeated measures. All analyses were conducted in R version 4.1.1 (R Core Team, 2021), using the packages "lme4" (Bates et al. 2015) for GLMMs and "glmmTMB" (Brooks et al. 2017) for the zero-inflated model and tested fixed effects using Type III Wald chi-square tests in the "car" package (Fox and Weisberg 2012). Model assumptions were evaluated using simulation-based residual diagnostics from the "DHARMa" package, including checks for overdispersion and zero inflation (Hartig and Hartig 2017). Statistical significance was assessed using likelihood ratio tests, with Bonferroni correction applied within predictor families to control for multiple testing.

Results

Experiment 1: Free-living cells

In the late arrival treatment level, where *Lambornella* were introduced to 4th instar larvae, free-living *Lambornella* densities declined rapidly across all temperatures (Figure 1). Densities dropped by more than 50% within one day, demonstrating strong suppression by larger larvae. In contrast, 1st instar larvae suppressed *Lambornella* more gradually, with cell densities reaching half of the initial values only after roughly 10–20 days. This suppression occurred earlier at higher temperatures, likely due to accelerated larval development to predatory stages. Control treatment levels without larvae showed that *Lambornella* populations increased to peak densities at warmer temperatures (10, 12, and 14°C), whereas at colder temperatures (6 and 8°C) densities declined slightly from initial concentrations. Our statistical model indicated significant effects of time and larval stage on free-living *Lambornella* density, with these effects varying across temperatures as evidenced by significant time by temperature ($\chi^2(1) = 33.94$, $p < 0.001$), time by larval stage ($\chi^2(2) = 26.01$, $p < 0.001$), and three-way time by temperature by larval stage interactions ($\chi^2(2) = 151.43$, $p < 0.001$).

Experiment 1: Parasitic cysts and infection

Both temperature and arrival treatment significantly affected the proportion of larvae with parasitic cysts. The number of larvae with parasitic cysts was significantly higher in the 1st instar treatment level compared to the 4th instar treatment level ($\chi^2(1) = 81.88$, $p < 0.001$). Quadratic effects of temperature indicated a nonlinear relationship, with intermediate temperatures yielding the highest proportion of larvae with cysts ($\chi^2(2) = 18.22$, $p < 0.001$; Figure 2A). The interaction between temperature and instar treatment was not significant and was dropped from our model.

No infections were observed in the 4th instar treatment level, likely a result of the strong suppression of free-living *Lambornella*. For the 1st instar treatment level, the infection rate

averaged across all temperature treatments was 27%. Within the 1st instar treatment level, we found a significant positive effect of temperature on infection rates ($\chi^2(1) = 14.12$, $p = 0.002$, Figure 2B) and infection intensities ($\chi^2(1) = 12.73$, $p < 0.001$, Figure S2).

Larval survival in this experiment was generally high (Figure 2C). Consistent with the lack of infection observed, nearly all larvae survived in the 4th instar treatment level with the exception of 3 individuals in the 14°C treatment, equal to a 1% mortality rate. In contrast, we saw an average mortality rate of 11% in the 1st instar treatment level. In the binomial GLMM including both instar treatments, mortality was significantly higher in the 1st instar treatment than in the 4th instar treatment ($\chi^2(1) = 13.26$, $p < 0.001$), whereas temperature was not significant ($\chi^2(1) = 0.28$, $p = 0.59$). Because mortality was nearly absent in the 4th instar treatment, we then evaluated the shape of the temperature-mortality relationship within the 1st instar treatment only. In this model, mortality was highest at intermediate temperatures, but the quadratic temperature effect was not statistically significant ($\chi^2(1) = 2.74$, $p = 0.10$). Consistent with these patterns, a Cox proportional hazards analysis of first instar larvae showed no significant temperature effect on mortality risk (hazard ratios 1.5–4.8, all 95% CIs overlapping; Figure S3). All statistically significant effects reported here remained significant after correction for multiple comparisons.

Experiment 2: Stage-structured feeding

We found that larval instar stage had a significant effect on grazing of free-living *Lambornella* ($\chi^2(4) = 34.98$, $p < 0.001$, Figure 3). Later instars consumed *Lambornella* more rapidly than earlier instars. Specifically, 4th instars reduced *Lambornella* density by an average of 71% after 24 hours. Second and third instars reduced densities by 29% and 34%, respectively. In contrast,

1st instars had no measurable effect on *Lambornella* density, with a 1% increase from initial densities, comparable to the no-larvae control (12% increase).

Discussion

Our study demonstrates that stage-specific priority effects, mediated by an ontogenetic shift from vulnerable host to effective predator, can be a strong determinant of infection outcomes in the *Aedes sierrensis–Lambornella clarki* system. The relative arrival (or emergence) timing of the mosquito larvae and their parasites was the primary driver of the interaction's outcome, shaping whether the ciliate population was suppressed or successfully established infections and, conversely, whether the mosquito population had high survival or infection-induced mortality. As we predicted, when the host had a significant developmental priority (4th instar larvae), they acted as predators, rapidly driving the free-living ciliates to low population densities and completely preventing infection (Figure 1). In contrast, when the parasite had the temporal advantage of encountering the early host stage (1st instar larvae), they were able to establish infections, escaping predation and eventually killing some infected larvae (Figure 2). Experiment 2 confirmed that at 10°C only instars 2-4 are capable grazers of free-living *Lambornella*, a finding consistent with a previous observation (Washburn et al. 1988), which suggests a sharp developmental threshold in predation on the parasite (Figure 3). In pilot experiments we have found that at 10°C larvae transition from 1st to 2nd instars at around 6 days, which roughly corresponds to the start of a decline in free-living cells in Experiment 1 (see Figure S4 for all transition times between instars). This dynamic provides a clear example that demonstrates how priority effects can be powerful drivers in consumer-resource interactions.

We also found that temperature mediated the strength of these priority effects, in which warmer temperatures significantly amplified the priority effect of the parasite. While infection was only possible when parasites arrived before hosts developed into later instars, the rate of successful infection of larvae exposed as 1st instars rose substantially as temperatures increased (Figure 2B). This result occurred despite several key host advantages being enhanced at warmer temperatures. Specifically, higher temperatures accelerate the host's developmental rate toward larger, less-susceptible instars (Couper et al. 2024, Washburn et al. 1988), which have higher predation rates on the parasite's free-living stage (Figure 3). Future studies testing the temperature sensitivity of this stage-dependent predation are needed to confirm the generality of this result. In addition, the host's immune capacity increases with temperature across the range tested in this experiment (Murdock et al. 2012, Farner et al. 2025). Ultimately, the thermal acceleration of the parasite's replication and invasion rates was enough to outweigh increases in the host developmental, immunological, and predatory defenses. However, our previous work shows that infection rates decline at temperatures above 14°C (Ismail et al. 2024, Lyberger et al. 2024), suggesting that the effect of temperature is nonlinear. As a result, at temperatures higher than tested here, the effect of warming may be reduced or even reversed.

Prior work on temperature-dependent intraguild predation suggests that the strengthening of priority effects we observed with warming may occur broadly in aquatic communities. In these communities, ontogenetic role-reversals are widespread, where the young of many species begin as vulnerable planktivores but grow to become predators, sometimes preying on the young of their own former predators (Nilsson et al. 2019, Minto and Worm 2012). Warming can disproportionately benefit predators by increasing foraging efficiency more steeply than their prey's growth toward a size refuge (e.g., Maszczyk et al. 2023, Twardochleb et al. 2020), which

would strengthen priority effects in intraguild predation systems. In one example, warming was found to shift the balance from invasive green crabs towards native blue crabs by increasing attack rates and favoring the intraguild predator at higher temperatures, which in a system with alternative states creates the potential for amplified priority effects (Rogers et al. 2018). In freshwater odonates, warming can create asymmetric predation when one species develops much faster at higher temperatures (Suhling et al. 2013) and can intensify intraguild predation overall (Frances and McCauley 2018). Across these examples, warming tends to increase predation by the species whose foraging and growth rates respond to temperature most strongly, which tightens the temporal window in which later arriving, vulnerable stages can escape suppression. This temporal window is a critical feature determining how size-mediated priority effects structure communities (Rasmussen et al. 2014).

By contrast, warming can have weak, neutral, or negative effects when temperature does not favor the process that generates a given priority effect. Under conditions tested here, warming accelerated parasite proliferation and invasion more than host development, predation, and immune defense. This produced higher infection rates, thus strengthening the parasite-favored priority effect. At higher temperatures above 14°C, however, faster host development or improved defense could weaken the parasite-favored priority effect. In other cases, temperature may have little influence on priority effects, as reported in other consumer-resource contexts (e.g., Sniegula et al. 2019, Raczyński et al. 2022). For example, in odonate assemblages from Neotropical treeholes, temperature was not a strong predictor of species occupancy, despite strong priority effects among predators (Fincke 1999). Hatching phenology itself might be temperature dependent, but shifts in emergence do not always translate into size-mediated priority effects (Stahl and Johansson 2024). Theoretical work points to similar contingencies; in

a stage-structured intraguild predation model, Thunell et al. (2021) found that warming can increase interspecific competition while reducing effective predation on their resource, moving the system away from parameter space in which priority effects occur. More broadly, this highlights that the strength and direction of priority effects emerge from the relative thermal sensitivities of interacting traits, not from temperature alone.

These experimentally demonstrated priority effects provide a potential explanation for the high degree of temporal and spatial variability in infection rates seen in natural treehole communities (Egerter and Anderson 1985, Washburn and Anderson 1986, Farner et al. 2025). Several patterns observed in the field—where some treeholes harbor infected mosquitoes one year but not the next, or where infection appears late in a season—could be the result of these priority effects. Even, subtle year-to-year or within-season variation in rainfall, flooding, and temperature may generate large enough phenological shifts in mosquito hatching, larval development, and *Lambornella* emergence to change whether ciliates encounter larvae during the vulnerable early-instar window or after larvae have developed into predators of the free-living ciliate. Many *Aedes* mosquitoes characteristically lay their eggs in successive bands above the waterline, meaning that different rainfall events can trigger different cohorts of mosquito eggs to hatch (Edgerly et al. 1993). Although little is known about the specific triggers of *Lambornella* transformation from desiccated resting cysts, it is likely similar to other ciliates and driven by environmental factors like flooding, temperature, and metabolites (Washburn et al. 1991b, Bracht et al. 2016). Such cue-driven shifts can alter trophic interactions when species differ in their sensitivity to temperature or precipitation (Thackeray et al. 2016), particularly when altered timing changes the stage or abundance of prey, predators, hosts, or parasites at encounter (Damien and Tougeron 2019).

The interplay between phenology and stage-dependent predation may be part of what generates the dynamic mosaic of infection prevalence seen across the landscape, in addition to dispersal events within the meta-population (Egerter and Anderson 1986). Under climate change, warmer winters may lead to larger outbreaks within infected treeholes in colder parts of the range and a potential shift northward in peak prevalence (Farner et al. 2025). Beyond these two main interacting species, early arrival or emergence of *Lambornella* could also have impacts on other microorganisms within the aquatic treehole community. There is often a strong successional pattern among inhabiting microbes and predation by mosquito larvae can alter this sequence (Norman and Walker 2019, Pascual-Garcia and Bell 2020). In more complex treehole systems, temperature-mediated priority effects may determine the outcome of competition between multiple invertebrate predators (Fincke 1994, 1999) or dictate infection dynamics across multiple co-occurring host species (Copeland and Craig 1992).

Understanding these temperature-dependent priority effects is relevant for other host-parasite systems with complex life cycles where infectious stages are vulnerable to predation (Johnson et al. 2010), such as trematode cercariae (Goedknegt et al. 2015) and the free-swimming zoospores of amphibian fungal pathogens (Kagami et al. 2007). In these systems, the net outcome of warming depends on a "thermal race" between trophic levels: if infective stages respond more strongly to warming than their predators, increasing temperature should widen the range of conditions that lead to successful infection. By contrast, if warming disproportionately accelerates the development, feeding rates, or immune responses of predators, this may greatly suppress these parasites. Because the free-living stages of many parasites occupy open water where they are exposed to diverse grazers, even small differences in phenology and thermal responses among hosts, parasites, and predators may tip these systems between states of high and

low infection. More generally, as climate change continues to alter the phenology of interacting species, we can expect that even small shifts in arrival time may be amplified by warming whenever the biological mechanism required to secure a priority effect, such as infectious proliferation in a parasite or resource preemption in a predator, disproportionately benefits the early-arriving species before the opponent can reach a less-vulnerable stage.

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Statement of Authorship

All authors conceived of the idea, KL ran the experiment and conducted the analysis, wrote the first draft, and all authors contributed to the writing and revisions. TL and EAM provided supervision.

Data and Code Availability

All data and code are available on Dryad at https://doi.org/10.5061/dryad.q573n5twk (Lyberger et al. 2026).

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Figure Legends

Figure 1. The plots show the density of free-living *Lambornella* over time (means ±1 SE) in Experiment 1. Colors represent temperature treatments and panels show arrival timing treatments. 4th instar *Aedes sierrensis* larvae reduced free-living *Lambornella* density immediately and more dramatically than 1st instars, which eventually reduced *Lambornella* density more strongly at higher temperatures.

Alt text: Across the three panels, free-living *Lambornella* densities remain highest in the no-larvae control, decline gradually when exposed to 1st instar larvae, and drop sharply soon after exposure to 4th instar larvae.

Figure 2. The plots show means ±1 SE for A) proportion of larvae with parasitic cysts, B) proportion of larvae infected (1st instar treatment level only, since no larvae were infected in the 4th instar treatment level), and C) proportion of larvae that died from Experiment 1. Colors represent temperature treatments and point shapes represent arrival timing treatments. Parasite cysts, infection, and resulting larval mortality of the mosquito *Aedes sierrensis* increased at intermediate and warm temperatures for larvae exposed to *Lambornella* as 1st instars, while no such effects were observed for 4th instars.

Alt text: The figure compares cyst formation, infection, and mortality across temperatures and larval-stage treatments. Points for 1st instar larvae are consistently higher than points for 4th instar larvae, especially for cysts and mortality, where the 4th instar values cluster near zero.

Figure 3. The plot shows the density of free-living *Lambornella* over time (means ± 1 SE) in the four larval instar treatments from Experiment 2, which was conducted at 10°C. Colors represent

instar stage (1st through 4th) of the mosquito *Aedes sierrensis* or *Lambornella* density in the absence of larval mosquitoes.

Alt text: The lines separate over time by larval stage, with the lowest *Lambornella* densities in wells containing 4th instar larvae and the highest densities in wells with no larvae or 1st instar larvae. The 2nd and 3rd instar treatments fall between these extremes.

Figure 1

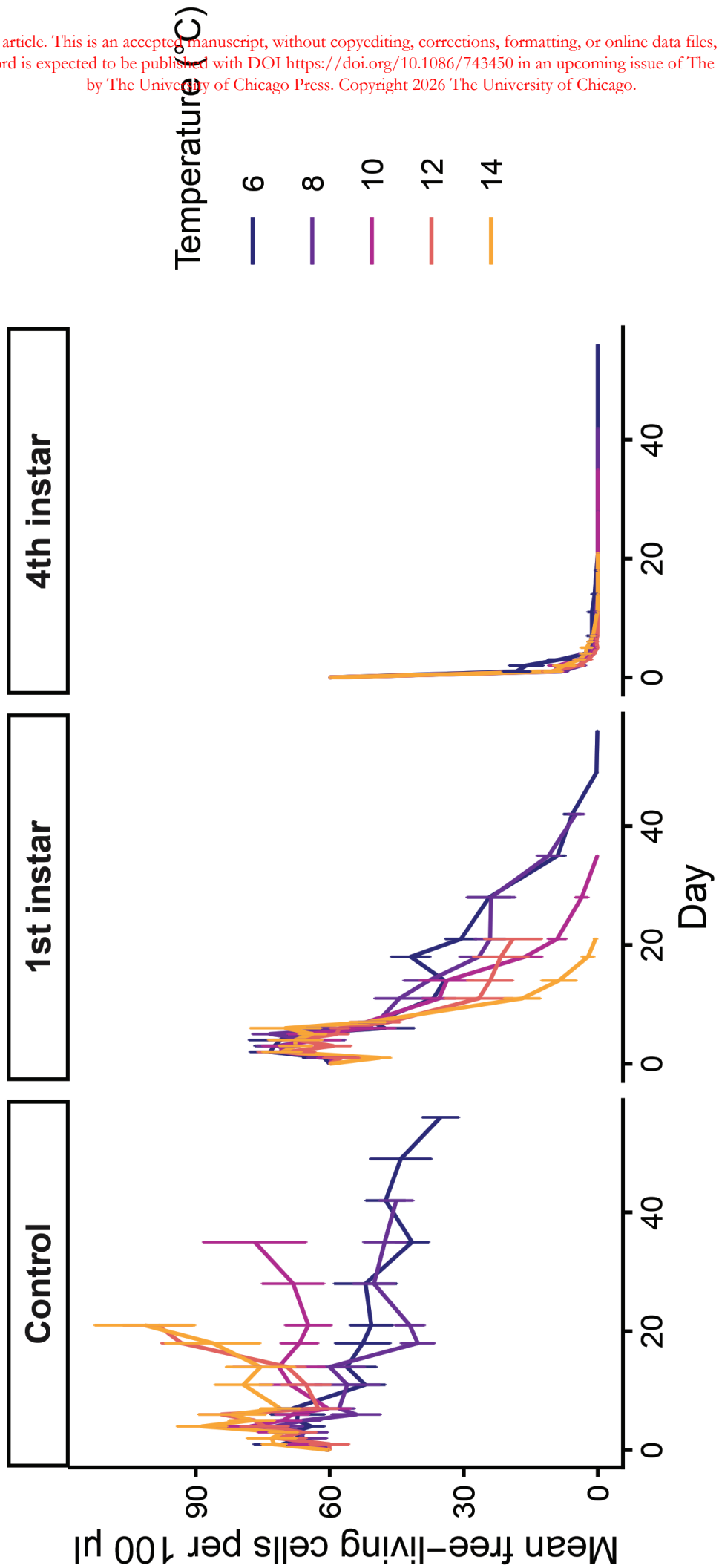


Figure 2

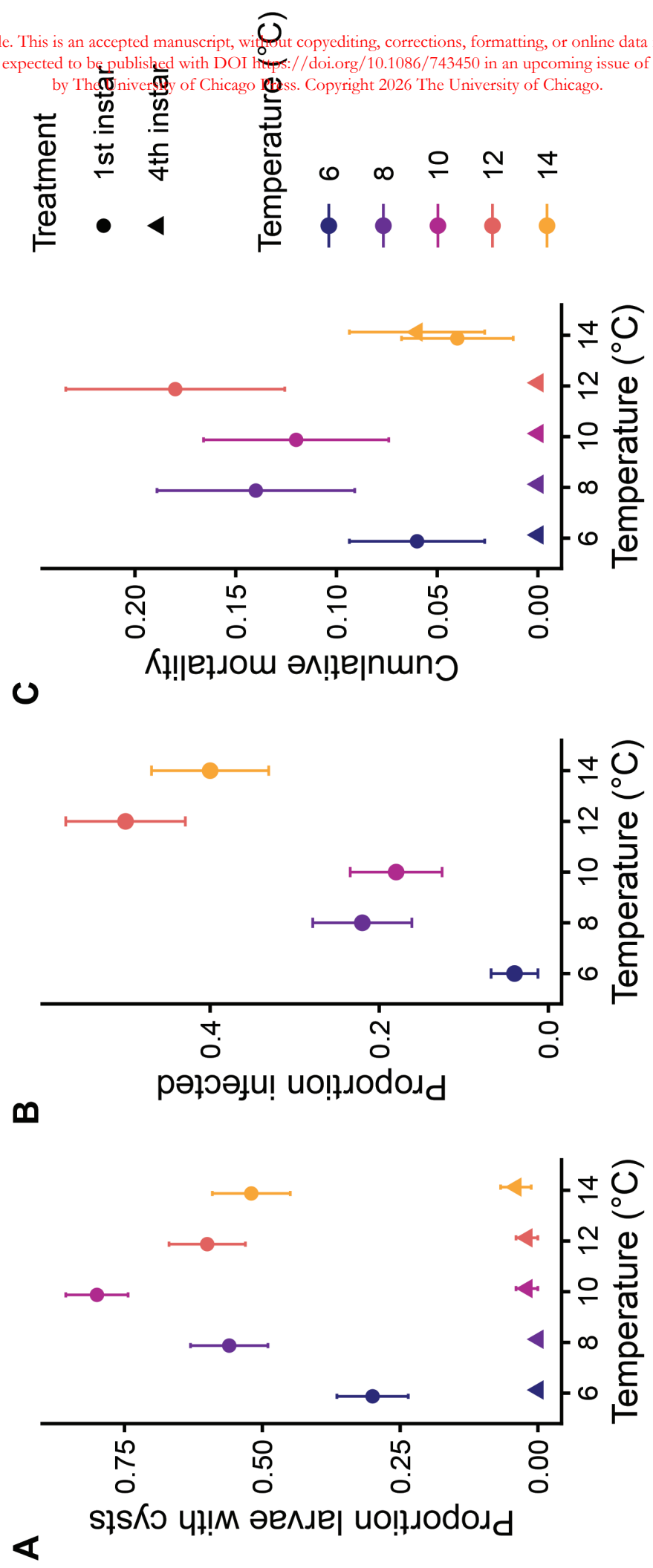
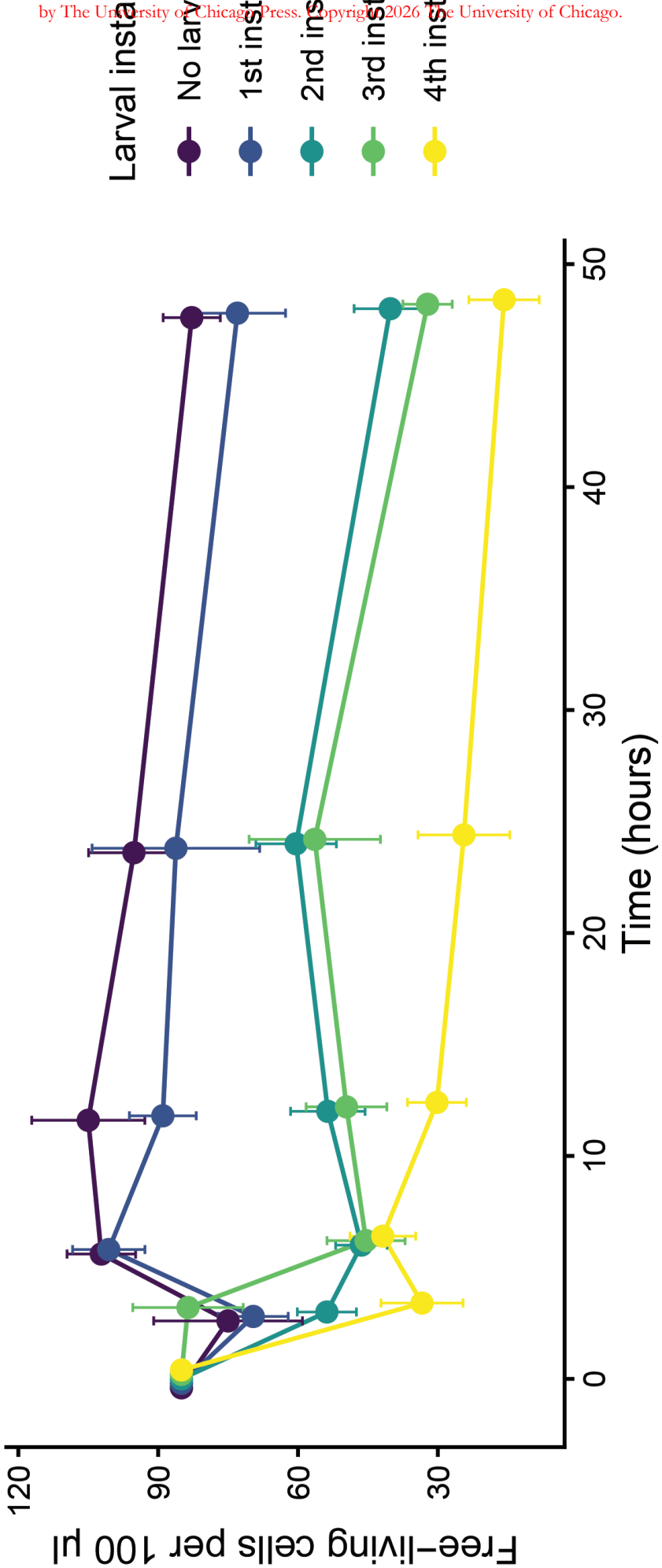


Figure 3



Supplementary Information for "Warming strengthens priority effects driven by an ontogenetic shift from host to predator" in *The American Naturalist*

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Supplementary Text S1. The experimental temperatures (6, 8, 10, 12, and 14°C) were chosen to reflect the range of daily average temperatures at JRBP('O'O) during the wet season. Our selection is justified by two analyses of historical temperature data (2013-2023) from the weather station at the preserve. First, an analysis of two-week periods following significant rainfall (>150 mm) identified a temperature range from 6.5°C (coldest period: Dec 10–23, 2022) to 14.1°C (warmest period: Oct 25–Nov 7, 2021). Second, an analysis of the entire wet season (November to April) found the 25th, 50th, and 75th percentile for daily average temperatures to be 6.5°C, 10.5°C, and 14.3°C, respectively. Together, these data establish a biologically relevant thermal range of approximately 6 to 14°C, which is well-represented by our chosen experimental conditions.

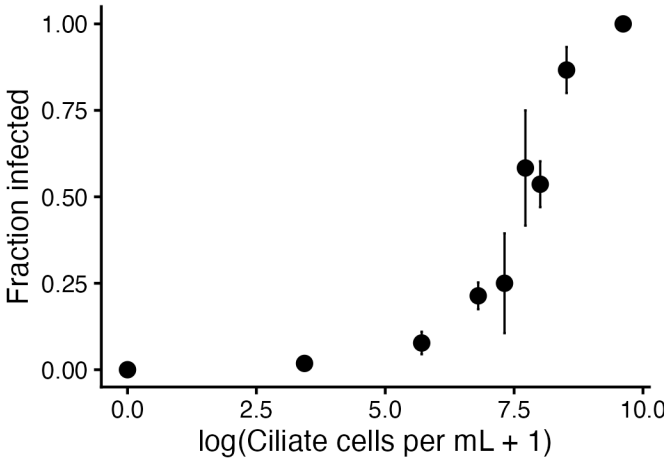

Figure S1. Dose-response relationship at 10°C. Parasite density ranged from 3 to 1500 parasites per mL. Each treatment consisted of three replicate populations, with five mosquito hosts per replicate. Points show the mean fraction of hosts infected by week 4, with error bars indicating ±1 SE.

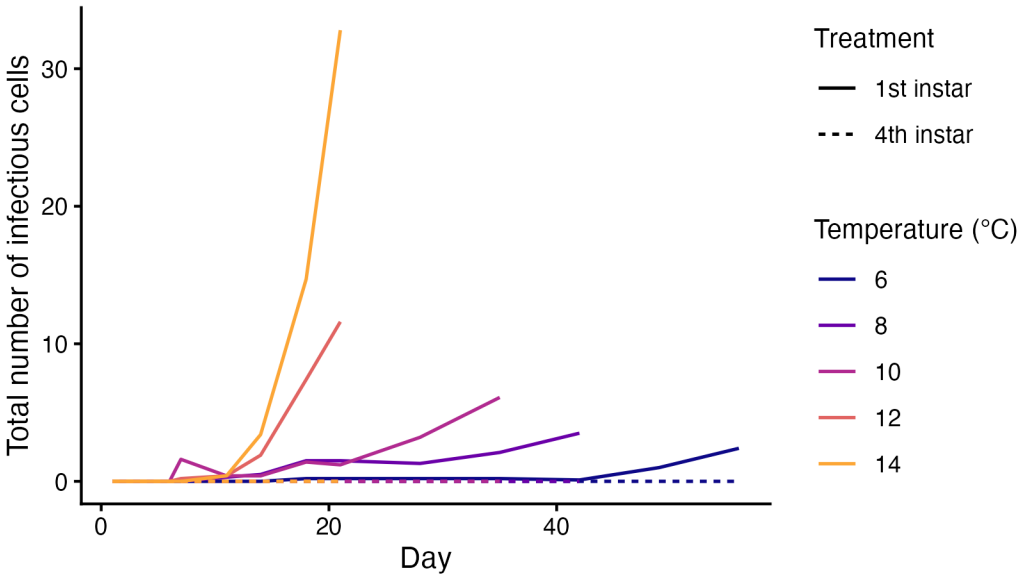

Figure S2. Intensity of infections as measured by the number of infectious cells within larvae, colored by temperature treatment.

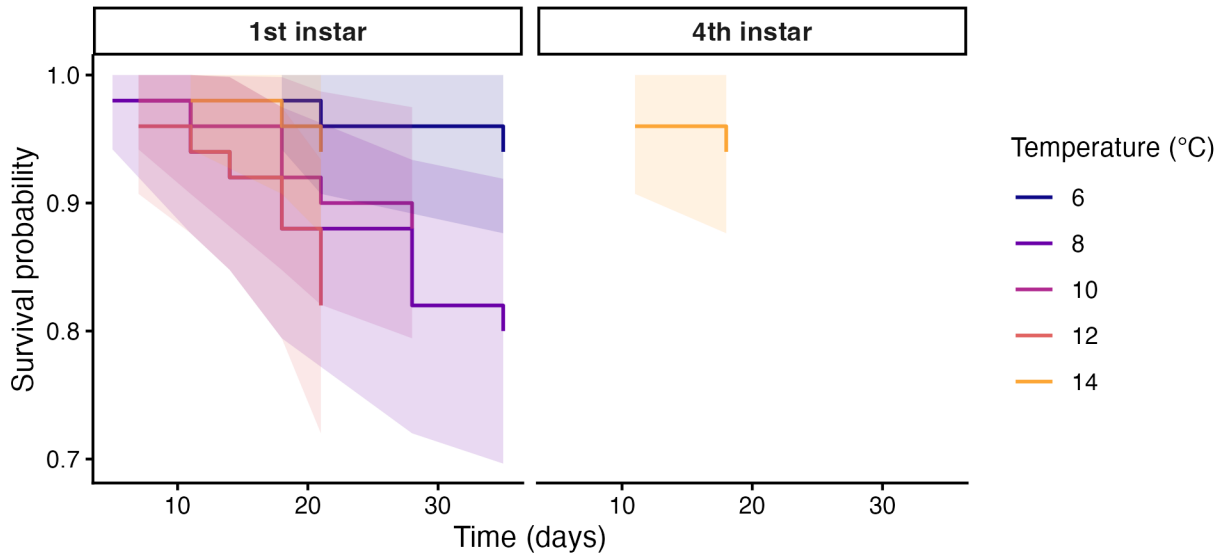

Figure S3. Kaplan–Meier survival curves for larvae colored by temperature with separate panels for each treatment group (first or fourth instars). Lines show estimated survival probability over time (days), and shaded bands indicate 95% confidence intervals.

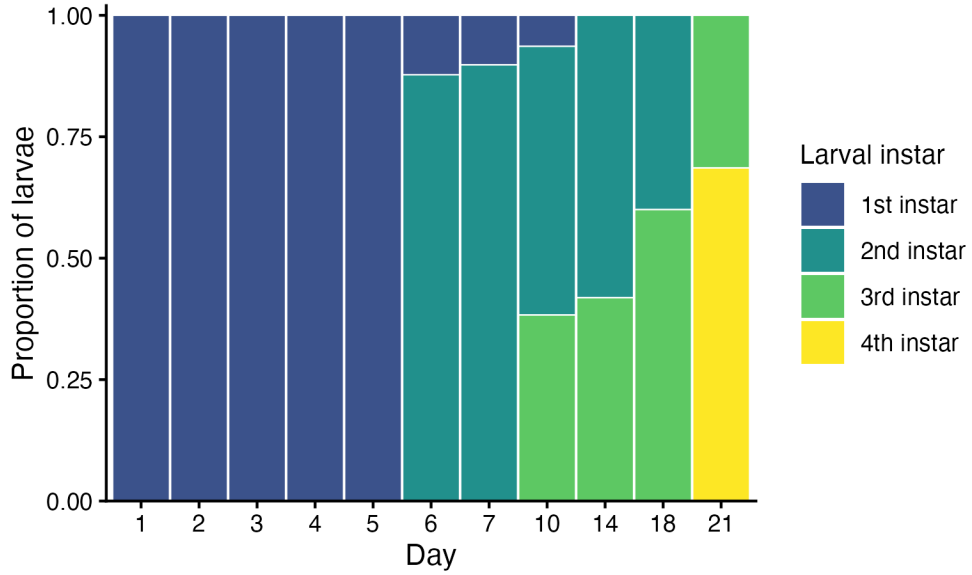

Figure S4. Larval stage distribution over time for the first-instar treatment in a pilot experiment conducted using the same methods as experiment 1, but only at 10°C. Bars show the proportion of larvae in each instar stage on each observation day, with colors indicating larval instar.