

Hot or Not: Temperature-Mediated Parasite Inhibition by Honey Bee Gut Symbionts

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GRAPHICAL ABSTRACT

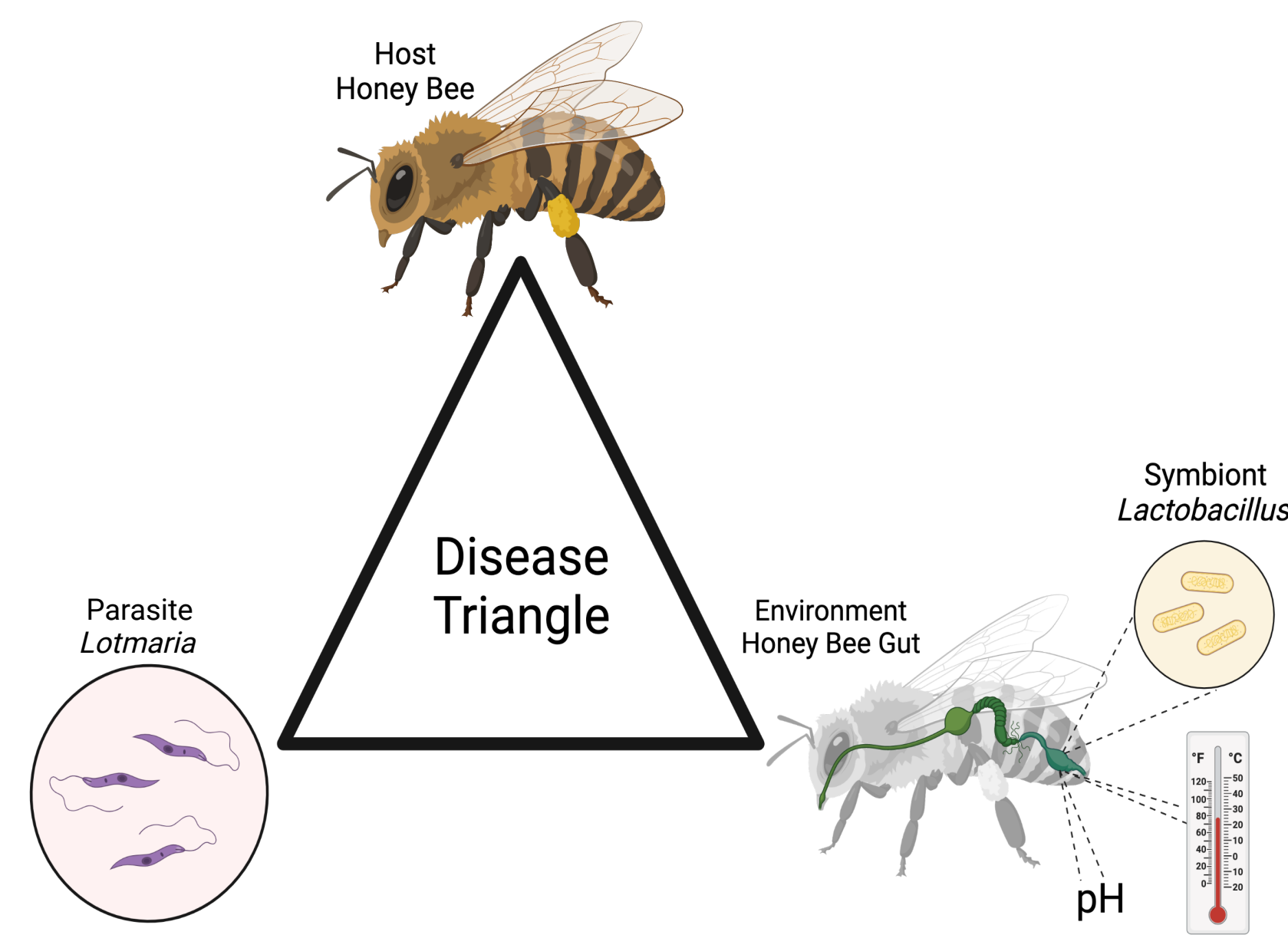


Figure 1: Interspecific Interactions Within Honey Bee Disease System Disease is a manifestation of interactions between a host, parasite, and their environment. Honey bees are subject to infection by a trypanosomatid gut parasite *Lotmaria passim*, and within the honey bee gut environment, factors such as pH, temperature, and the presence of symbiotic *Lactobacilli* further influence disease dynamics.¹

METHODS

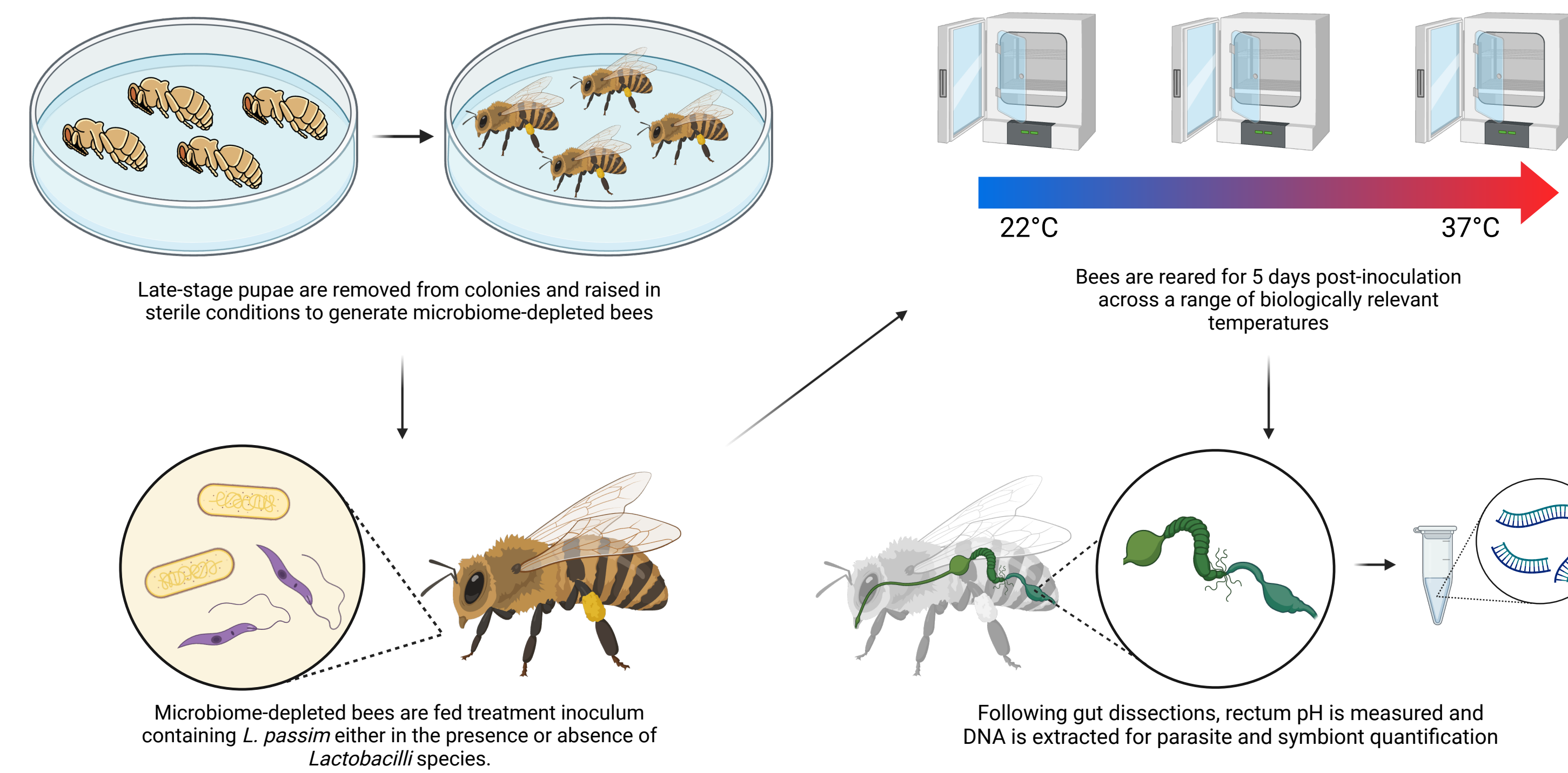
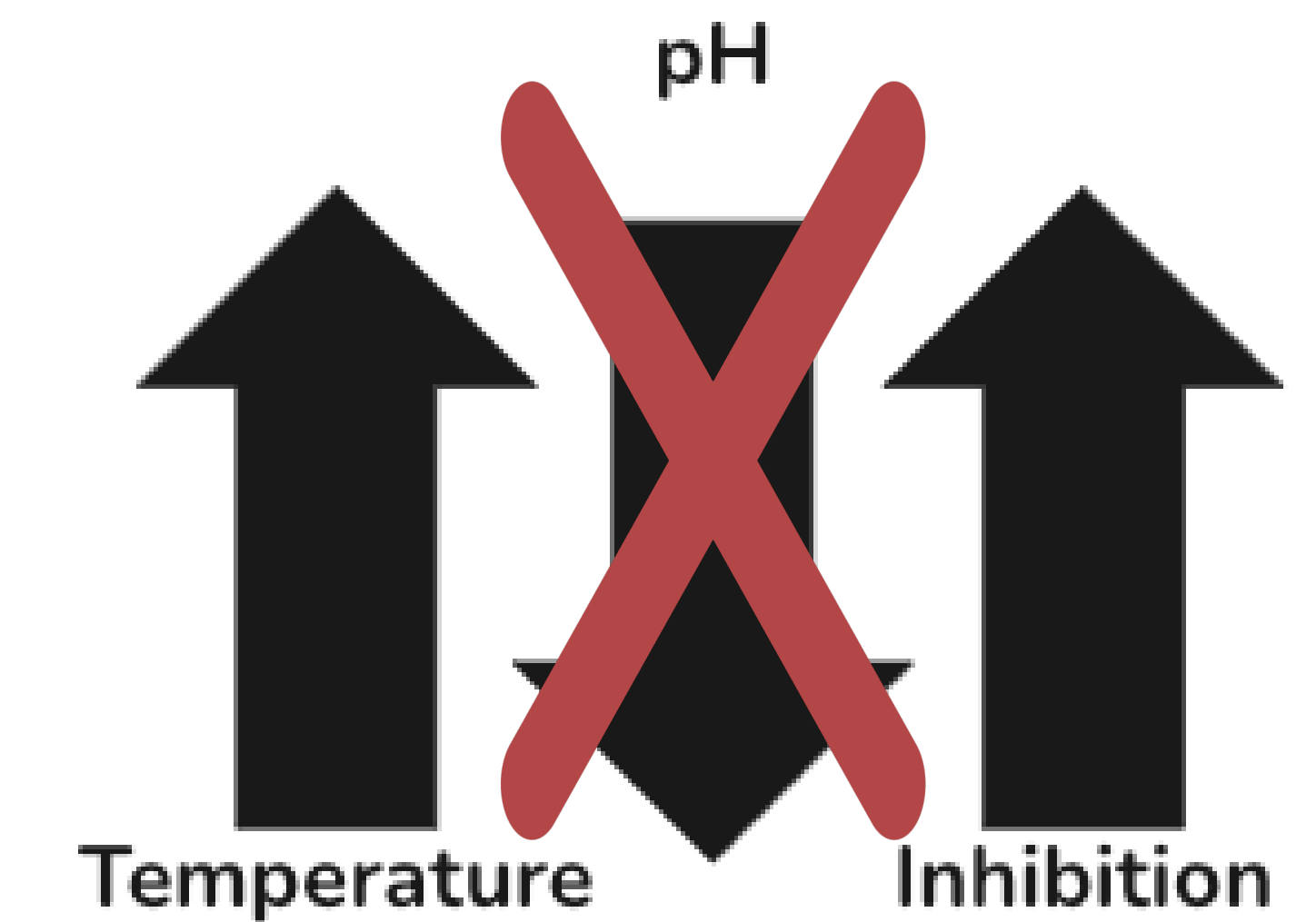


Figure 3: Gnotobiotic Live Bee Experiments Pupae aged 17 to 18 days post-egg laying were extracted from brood frames using aseptic technique and raised in sterile conditions until 2 days post-emergence. Bees were randomly assigned to treatment groups, and were inoculated with *L. passim* either in the presence or absence of Firm-5 *Lactobacilli*. They were reared in sterile dishes for 5 days across 6 temperatures, and at the conclusion of the study the bees were frozen at -80°C. Individual bee guts were dissected, hindgut pH was measured, and DNA was extracted from each gut. Single-bee parasite and symbiont loads were measured via qPCR detection.

CONCLUSIONS

- In the presence of Firm-5 *Lactobacilli*, *Lotmaria passim* levels were significantly lower than in the absence of Firm-5 *Lactobacilli*.
- As temperature rises, the inhibitory effect of Firm-5 *Lactobacilli* increased.
- It is unlikely that these interactions were pH-mediated, as was observed previously *in vitro*.



INTRODUCTION

- European honey bees (*Apis mellifera*) are the most important pollinators for crop monocultures worldwide.²
- Honey bee populations in Europe and the US have decreased by 25% and 50-60%, respectively, since the 1980s.³ This is partially attributable to pathogens and parasites.
- *Lotmaria passim* is a trypanosomatid gut parasite that increases mortality in honey bee hosts.⁴
- Honey bees have beneficial bacterial gut symbionts, such as *Lactobacillus* spp. that are known to provide resistance to infection.⁵
- *In vitro*, *L. passim* growth is limited by temperature and acidification of the environment by Firm-5 *Lactobacilli*.⁶

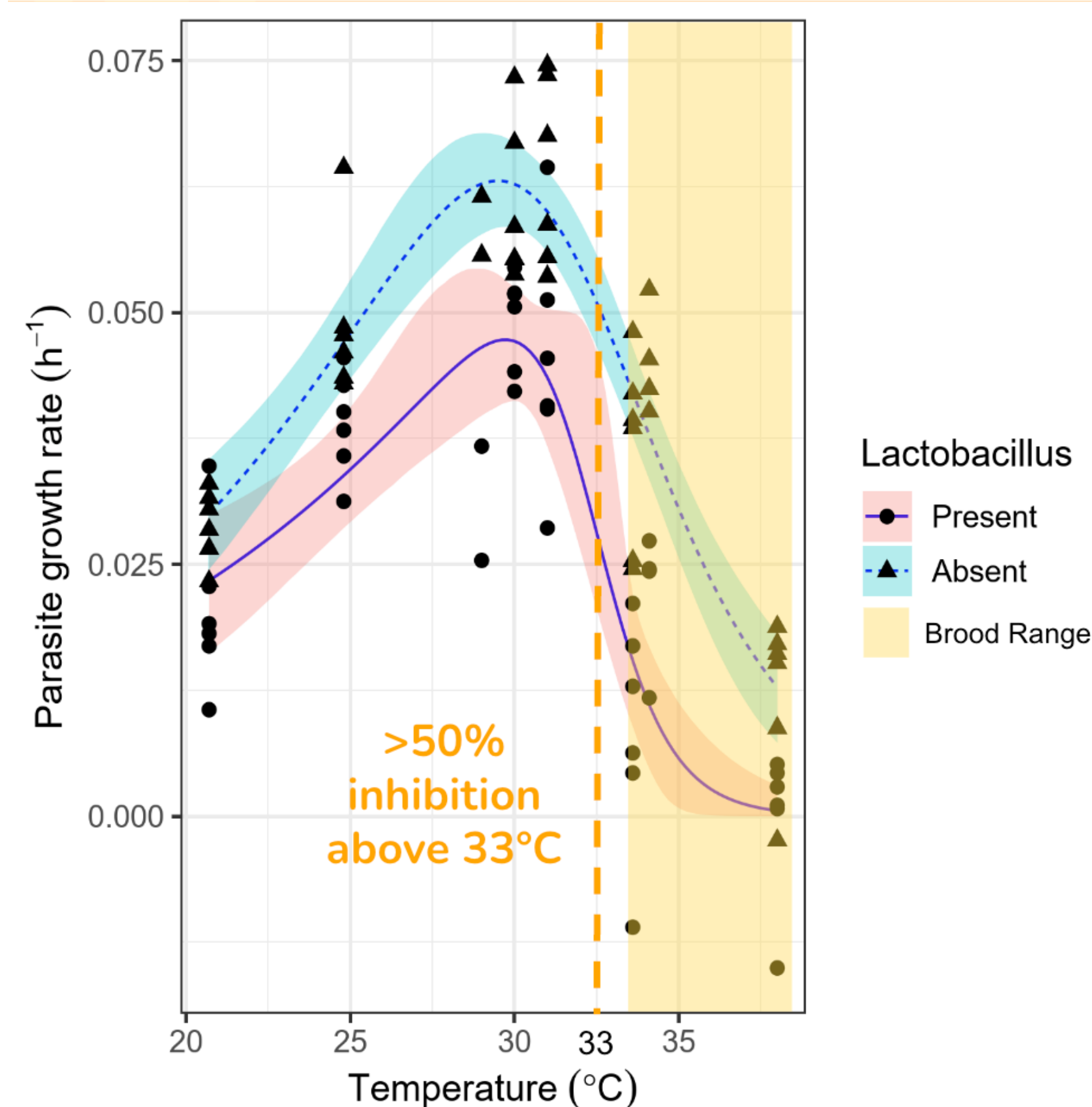


Figure 2: Symbiotic *Lactobacillus* Limits the Thermal Niche of *L. passim* In cocultures of *Lactobacillus* and *L. passim*, parasite growth is limited in a temperature-dependent manner, with inhibition over 50% at temperatures above 33°C. Increasing inhibition is observed in temperatures within the brood range of about 34°C to 38°C.⁶

RESULTS

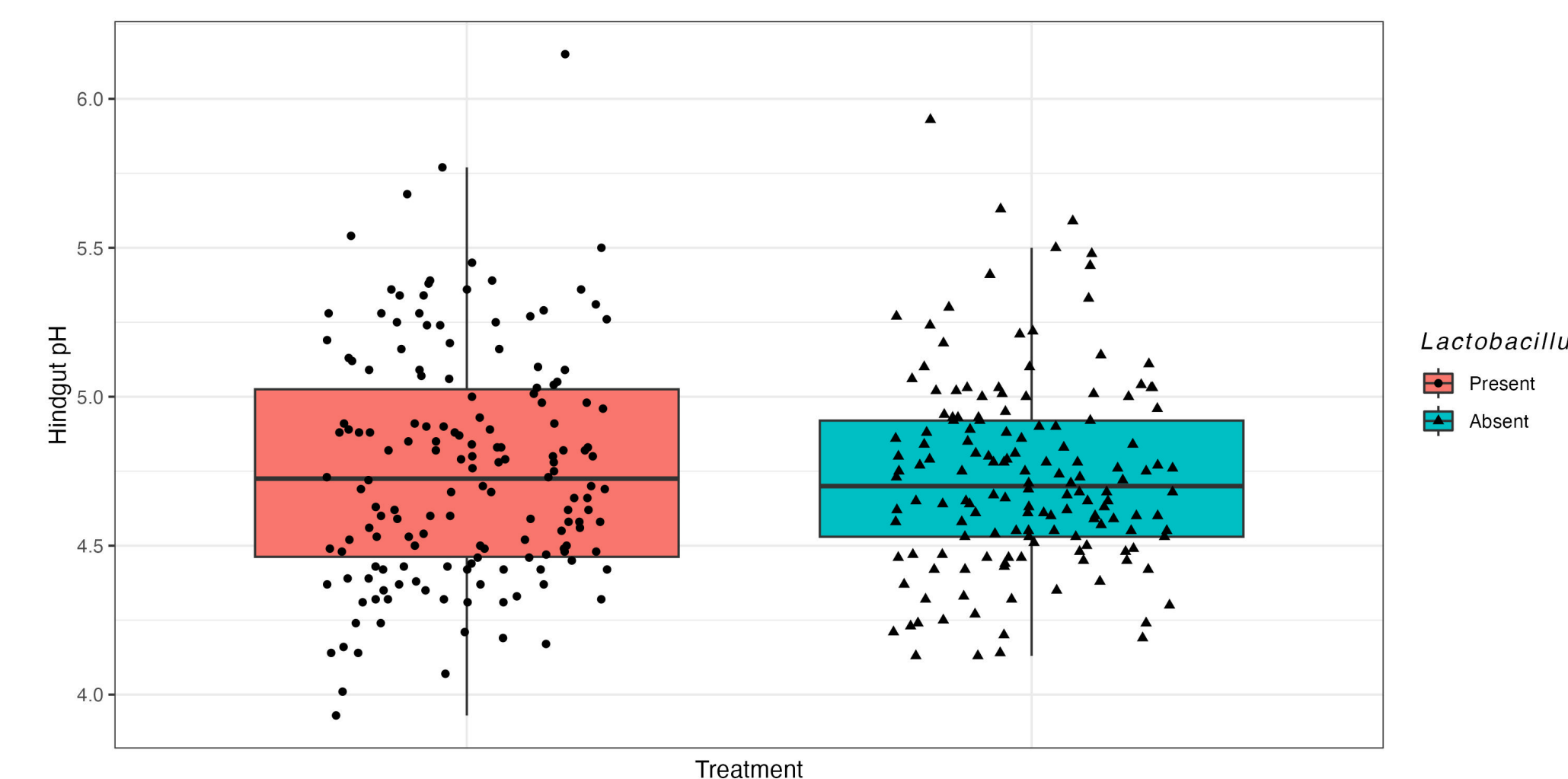


Figure 4: Hindgut pH Does Not Differ Between Treatment Groups There are no significant differences in hindgut pH in the presence (mean = 4.76) and absence (mean = 4.73) of *Lactobacilli* according to a Welch's T-test ($t = 0.614$, $p = 0.5394$, 95% CI: -0.055, 0.105).

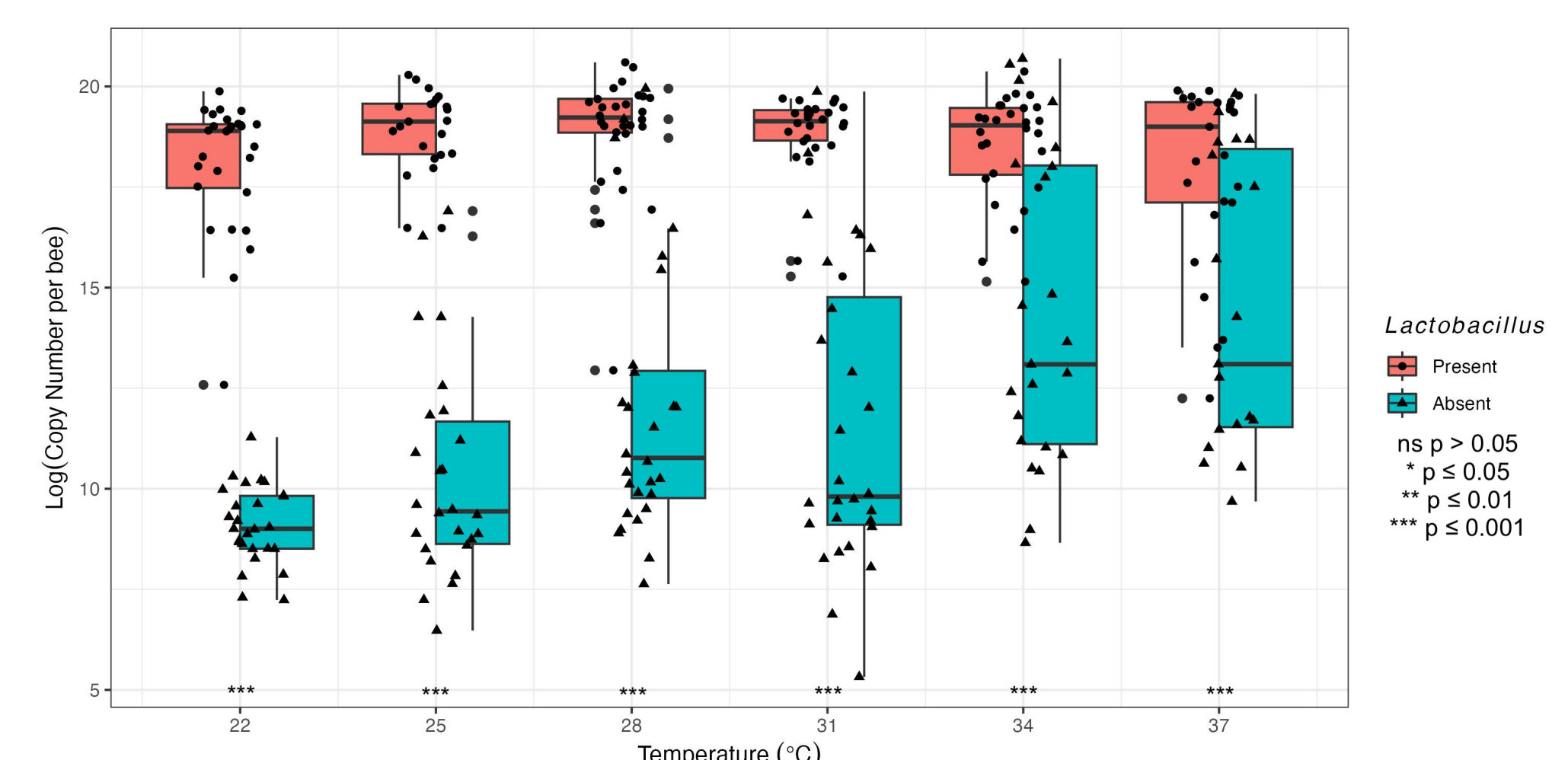


Figure 5: *Lactobacillus* Absent Bees Established Symbiont Gut Communities 7-Days Post-Emergence At the time of inoculation, there were no detectable symbiont levels in the microbiome-depleted bee guts, although bees developed *Lactobacilli* communities by the end of the trial period. These communities were significantly lower in the absence of inoculated *Lactobacilli* according to Welch's T-tests.

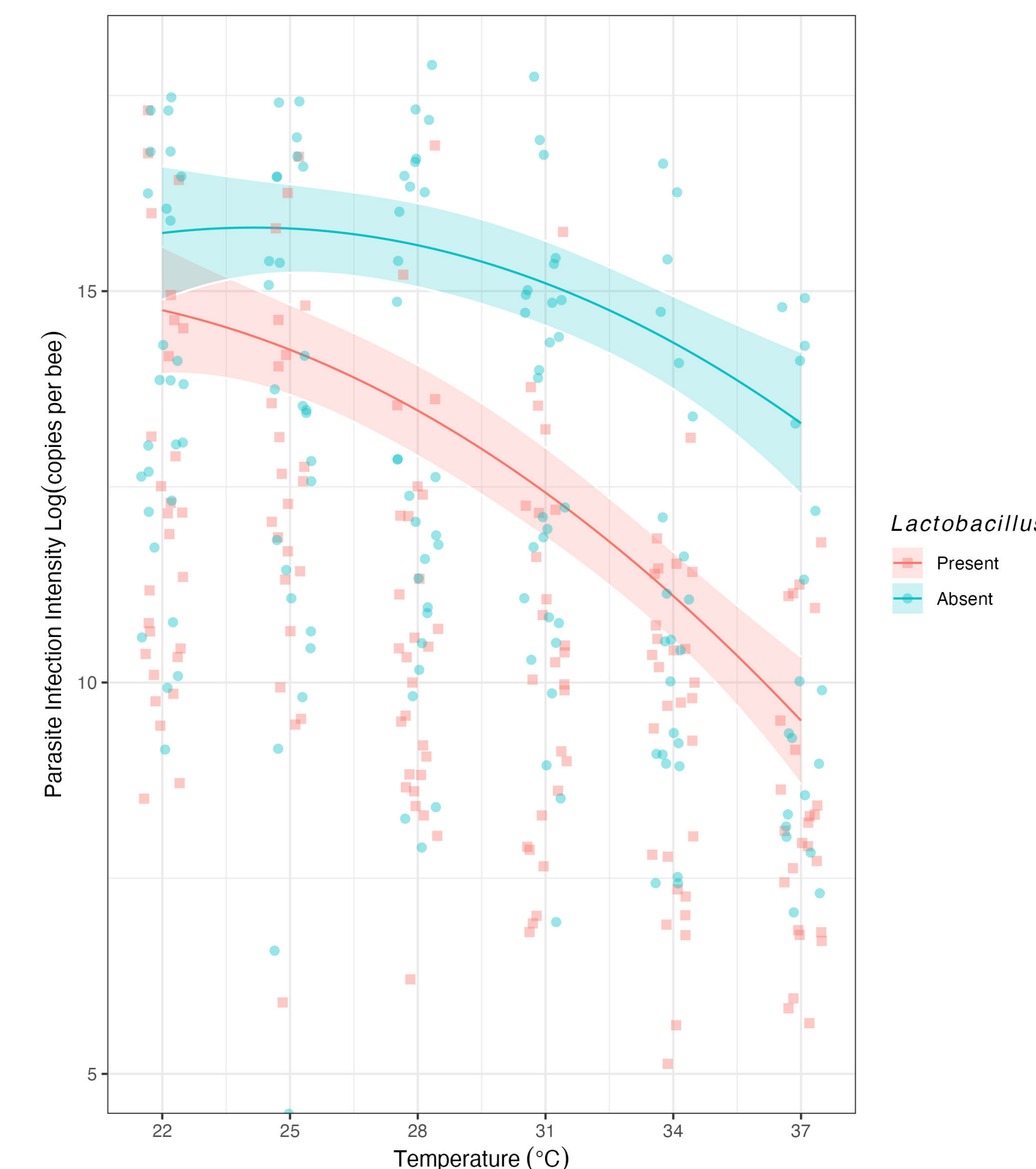


Figure 6: Infection Intensity Decreases as Temperature Increases in the Presence of *Lactobacilli* In the presence of *Lactobacilli*, *L. passim* infection intensity decreased by an estimated 10.5 fold \pm 3.19 SE ($p < .001$) across all temperatures. This effect increased with temperature, with the greatest inhibition seen within brood range temperatures (34°C to 38°C). According to model predictions, Treatment (Coefficient = -3.14 ± 5.85 SE, $\chi^2 = 59.82$, $p < .001$), Temperature² (Coefficient = -0.015 ± 0.007 SE, $\chi^2 = 4.88$, $p = 0.027$), and the interaction between Treatment and Temperature (Coefficient = 0.19 ± 0.06 SE, $\chi^2 = 9.91$, $p = .002$) all had significant effects on *L. passim* infection intensity.

FUTURE WORK

- Further research is needed to elucidate the mechanistic relationship between rising Firm-5 *Lactobacilli* levels and *L. passim* mediation.
- Many aspects of infection remain unknown, including:
 - The timing and processes underlying host immune responses to *L. passim* invasion.
 - Transmission modes of *L. passim* that facilitate disease spread.

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