SUPPORTING INFORMATION

TITLE: Experimental evidence of warming-induced disease emergence and its prediction by a trait-based mechanistic model

RUNNING HEAD: Warming-induced disease emergence

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Model parameterization and assumptions

The population-level model is listed in the main text in Eq. 1-4, with the expression for R_0 listed in Eq. 5 and the thirteen parameters listed in Table 2. Seven parameters were modeled as temperature independent. The input rate of susceptibles (ϕ_S) and infecteds (ϕ_I) was determined by experimental conditions and the prevalence of infections in the stocks from which exposed individuals were introduced into the experiment (ϕ_S = 3.535 d^{-1} ϕ_I = 0.465 d^{-1}). Twelve of the ~170 individuals were harvested every three days; therefore, harvesting rate (h) was set to 0.0235 d^{-1}. Environmental spore mortality was set to equal

39 the rate at which we removed medium (3 of the 35L / 3 days): $\gamma = 0.0286\ d^{-1}$. Infected
40 corpse degradation rate (θ), which does not affect R_0 but affects the timing of how
41 quickly spores are released into the environment, was set to 0.1 d^{-1}. This is the average
42 degradation rate in an independent experiment that we conducted, where we visually
43 assessed the time point at which the *Daphnia* gut was completely degraded (mean
44 degradation rate = 0.108 d^{-1}, standard deviation = 0.0659; Kirk et al. unpublished data).
45 We note that this value (0.108 d^{-1}) was averaged across nine experimental temperatures
46 (5°C - 32°C) and that the experiment used *Daphnia* of varying sizes, and while
47 degradation did increase with temperature, we did not use MTE to model it, as the
48 parameter does not appear in the R_0 equation (Eq. 5) and therefore does not affect the
49 critical transition temperature to an epidemic. Maximum recruitment rate (ψ) was set to
50 1.33 d^{-1} to allow for population abundances to remain around the carrying capacity K,
51 which was set to 170 to mirror the approximate abundances in the experiment (Fig. S1).
52 Finally, since we did not measure *Daphnia* length (which can be used to infer *Daphnia*
53 size and mass) in the experimental populations, we assumed a length of 2.7mm for the
54 large individuals that were sampled based on previous observations of our stock
55 populations. We explored the effects of this assumption using further simulations and did
56 not observe any large changes in model predictions (Fig. S2).
57 To capture parameters that scaled with temperature we used five different
58 parameterizations of MTE functions. Contact rate (χ) was modeled using a Sharpe-
59 Schoolfield function (Schoolfield et al. 1981) with only an upper temperature threshold
60 (Kirk et al. 2019, Fig. 1), and standardized to the volume of the container (35L) with
61 *Daphnia* size set to 2700μm. The probability of infection after contact (σ) arises from a

Sharpe-Schoolfield model with upper and lower temperature thresholds that predicts the infection rate within the host, as well as how long the parasite remains in contact with the host (residence time of the parasite in the gut), which in turn is determined by *Daphnia* filtration rate (i.e. χ), algae concentration, and the size of the *Daphnia* (Kirk et al. 2019, Fig. 1). Previously, we modeled natural mortality using a two-parameter Weibull distribution in which the hazard can change through time depending on the shape parameter (Kirk et al. 2018). Since we did not track individuals through time in this model, we here used a constant hazard rate (μ). To obtain this value for each temperature, we simulated our MTE Weibull model, using Sharpe-Schoolfield functions for both parameters, to predict the natural survival curve for an uninfected individual at each temperature. From this curve, we found the time point at which survival probability is 0.5 (i.e. the median survival) and used this as the expected lifespan. Finally, we set natural mortality rate (μ) in our model to be 1 / predicted lifespan.

We also used the Sharpe-Schoolfield function to model equilibrium parasite abundance within the host, which rises quickly from zero to ~160 parasite clusters near 10°C, and then slowly decreases as temperature increases before approaching zero clusters near 30°C (Kirk et al. 2018, Fig. 1). Since equilibrium abundance of the parasite can take months to approach in the 10 - 13.5°C temperature range (Kirk et al. 2018), we modeled infection load in our experiment as a proportion of the equilibrium abundance. We used observed infection loads from our experiment to find the average proportion of equilibrium abundance in this temperature range: 0.182. This infection load temperature function was then used to predict the parasite-induced mortality rate (α), parasite shedding rate (λ) and the number of spores in the host when it dies (ω) for each

temperature. Parasite-induced mortality rate (α) was set to the product of infection load and 5.12×10^{-6}, the per-parasite added mortality previously estimated (Kirk et al. 2018). We note that because we did not track individuals through time, per-parasite added mortality is constant for an infected individual and cannot change through time, though it can change with the shape parameter in Kirk et al. (2018). For λ and ω, which both relate to parasite spores rather than parasite clusters, we assume that a spore cluster has twenty-four spores, which is generally the average that we observe in the lab (between 16 and 32 spores per clusters). For λ, we assumed that when a parasite cluster bursts, half of the twenty-four spores are released into the environment while the other twelve remain in the host (to either re-infect or die), and that this bursting process occurs every seven days. We note that Refardt and Ebert (2006) estimate that the parasite may burst approximately every three days at room temperature, but we assume here that this takes significantly longer in our 10 – 13.5°C range since within-host parasite growth rate is depressed (Kirk et al. 2018). We refer readers to the main text for implications of modeling these rates as functions of temperature.

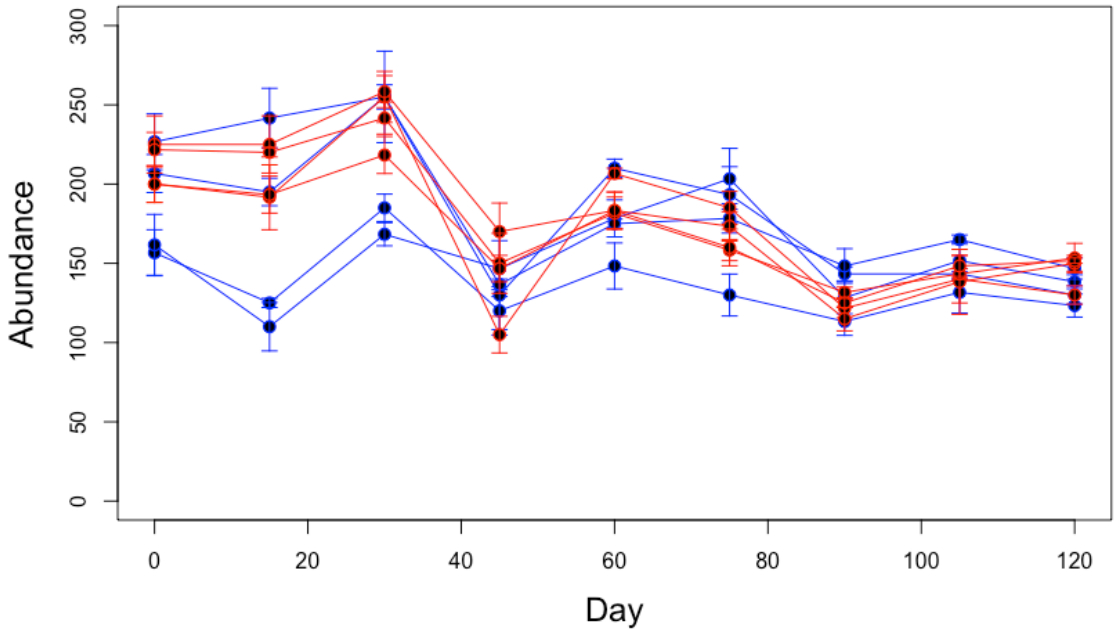

Fig. S1. Daphnia magna abundances in experimental populations. Blue and red points and lines represent populations in constant 10.0°C and warming conditions respectively. Points represent the mean of three counts for each population, and error bars represent the maximum and minimum value from these three counts.

Model sensitivity to assumptions

We explored how sensitive our model results are to five different assumptions: 1) infection load proportion of equilibrium abundance, 2) *Daphnia* size, 3) spores per cluster, 4) cluster burst time, and 5) the number of spores released out of the host per cluster. Assumptions were tested by simulating our model 250 times (without demographic stochasticity) in which we allowed parameters to take a value from along uniform distributions (with replacement) in which the median is the value used for the main analysis, and upper and lower range limits are our best estimates at realistic ranges for the parameter values (see Table S1). While changing the assumptions incorporated more variation, our results were generally robust across the entire parameter space (Fig. S2).

We also investigated the effects of sampling noise on our results, as we sampled a subset of the population (twelve individuals) on each sampling day. We simulated the model in a deterministic framework 250 times, and then used a binominal sampling process to randomly select twelve individuals every three days. This process captures the sampling noise observed in the warming samples well, but somewhat overestimates the sampled prevalence in the constant 10°C populations (Fig. S3).

Table S1. Range of parameter values used in simulations to test model sensitivity to assumptions.

	Lower range limit	Main text value	Upper range limit
Parasite load as proportion of equilibrium parasite abundance	0.132	0.182	0.232
Parasite cluster burst time	4.5 d^{-1}	7 d^{-1}	9.5 d^{-1}
Spores per cluster	16	24	32
Proportion of spores released into environment per cluster	0.25	0.50	0.75
Daphnia length	2500 μm	2700 μm	2900 μm

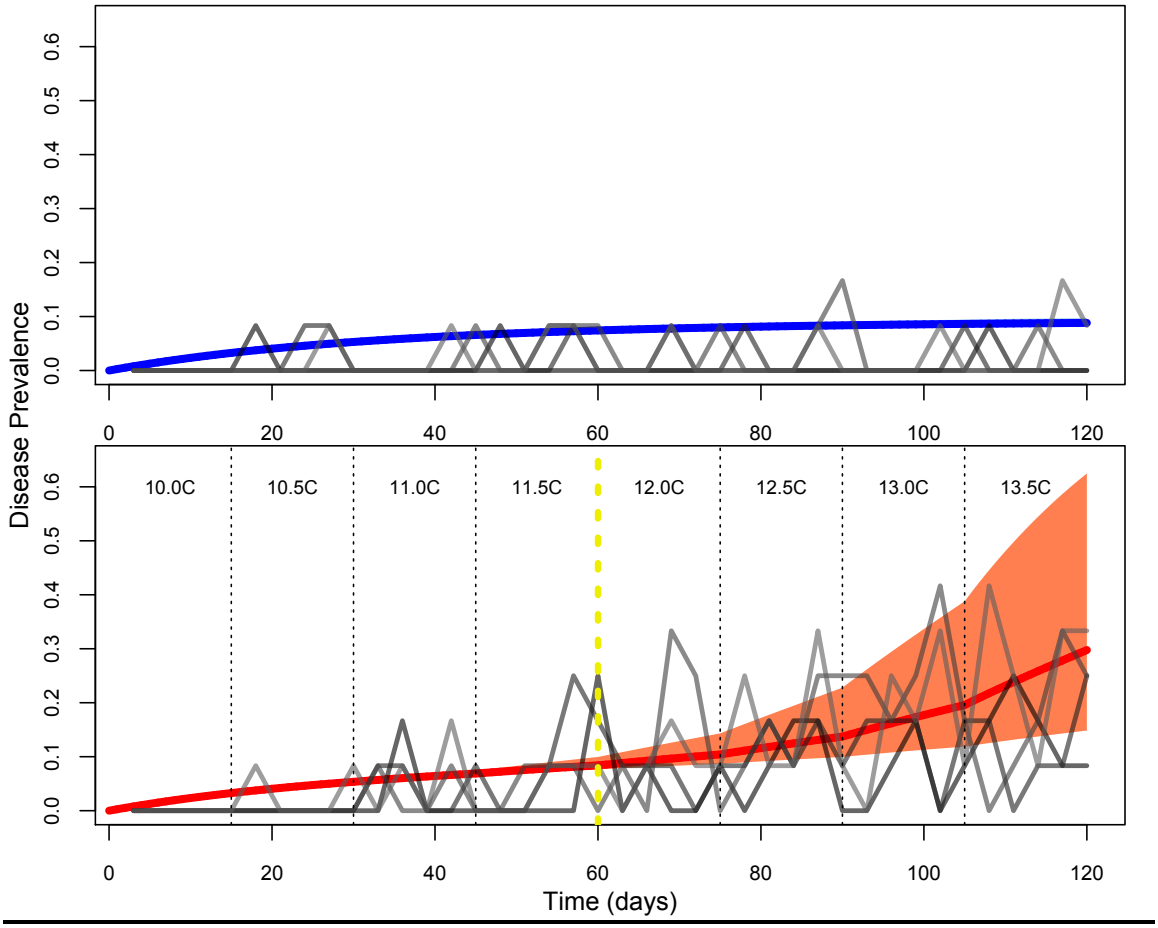

Fig. S2. Sensitivity to model assumptions. Blue (top panel) and red lines (bottom panel) represent mean disease prevalence for constant 10.0°C and warming conditions, respectively, across 250 simulations that sample from the parameter space. The shaded red region in the bottom panel represents the 95% confidence interval under warming conditions. The small shaded blue region in the top panel represents the 95% confidence interval, but is not visible due to the parameter assumptions having negligible effects on disease prevalence at 10°C. The yellow, dashed vertical line represents the temperature/time point at which the MTE model predicts $R_0 > 1$ for warming conditions.

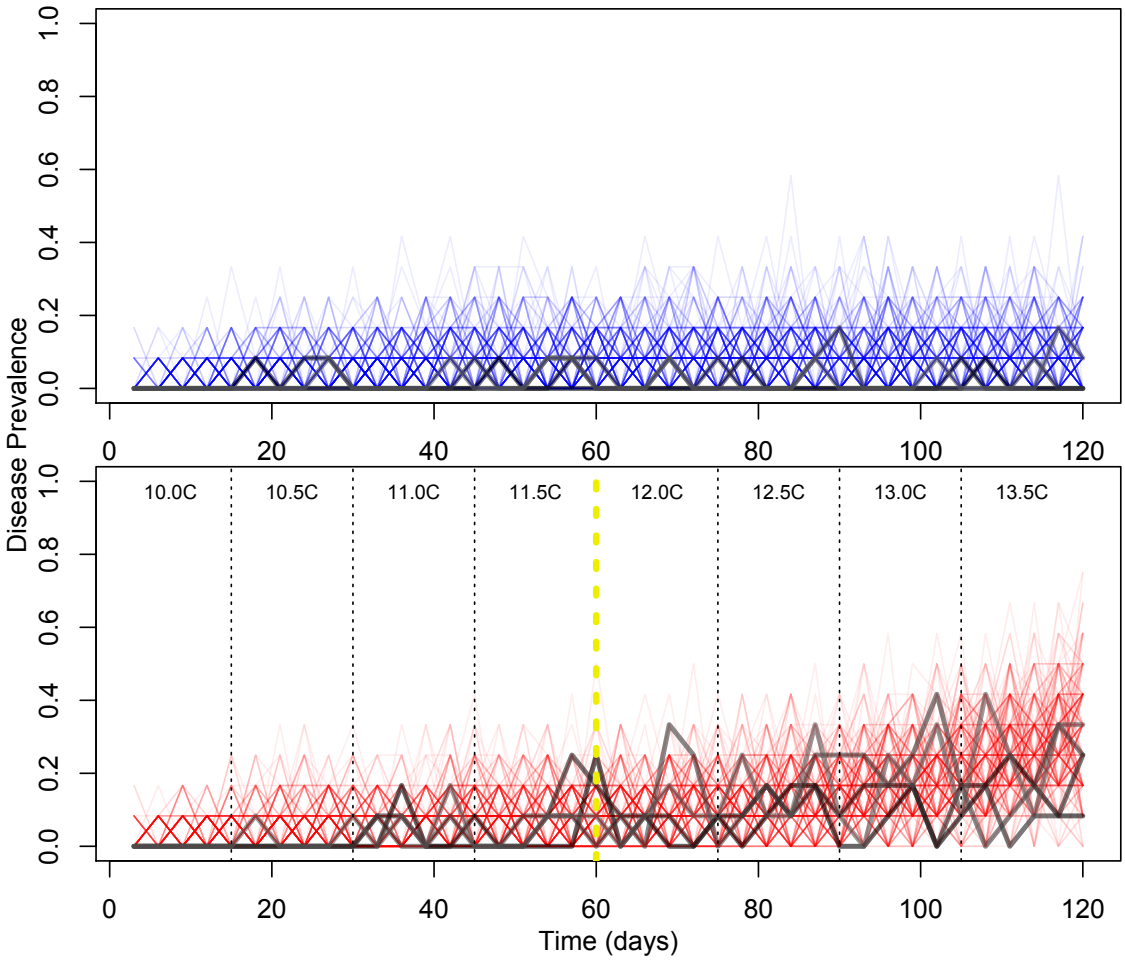

Figure S3. Effects of sampling noise. Blue and red lines represent 250 random samples from deterministic simulation of the model. Grey lines represent experimental data for constant 10.0°C (top panel) and warming populations (bottom panel) respectively.

Observed versus predicted prevalence

The goal of this work was not to specifically quantify model performance, but rather to leverage an experimental system to provide a proof of principle that the MTE approach can be used to predict warming-induced disease emergence. Nevertheless, below we provide observed versus model predicted values of disease prevalence for our four warming populations. We note that observed prevalence values are discretized at only six

different levels due to sampling (i.e. 0/12 infected, 1/12 infected… up to 5/12 infected), while the model predicts prevalence continuously as it is averaged across our 250 stochastic simulations. R^2 values ranged from 0.297 – 0.533 across the four warming populations.

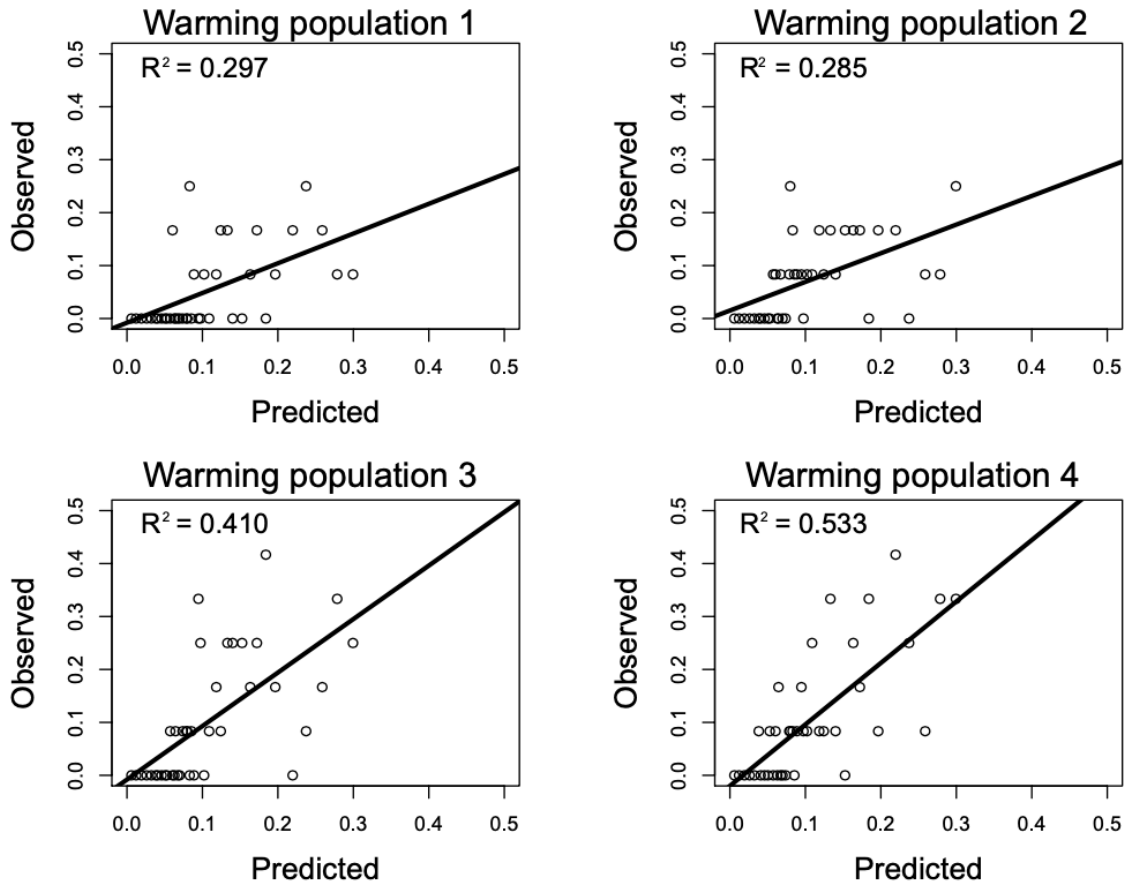

Figure S4. Experimentally observed versus model predicted values of prevalence for the four warming populations. R^2 values are provided for each population.

R_0 formulation

In our R_0 formulation (equation 5 of the main text, reproduced as Eq. S1 here), the first term in parentheses represents the total number of spores produced per infected individual, on average.

$$R_0(T) = \left(\frac{\lambda(T)}{\mu(T)+\alpha(T)+h} + \omega(T) * \frac{(\mu(T)+\alpha(T))}{(\mu(T)+\alpha(T)+h)}\right)\left(\frac{\chi(T) * \sigma(T) * S_{eq}}{\gamma}\right) \quad \text{Eq. S1}$$

In this first term, $\frac{\lambda(T)}{\mu(T)+\alpha(T)+h}$ represents the number of spores shed by an infected individual during their lifetime, and $\omega(T) * \frac{(\mu(T)+\alpha(T))}{(\mu(T)+\alpha(T)+h)}$ represents the number of spores released by an infected individual after they die, weighted by the fraction of infected hosts that remain in the system until death and are not harvested prior.

The second term in parentheses represents the probability a spore infects a new host as opposed to being lost via medium removal, and has two key assumptions:

1) Spores that are ingested but do not infect the host are expelled, re-enter the water column, and remain viable;
2) The rate of spore loss from the water column due to ingestion and subsequent infection is very small compared to spore loss via media removal; i.e., $\sigma\chi S << \gamma$.

Regarding assumption 1, to our knowledge, there have been no studies in this host – parasite system that investigate the proportion of spores that remain viable after passing through the host gut. However, evidence from a similar system with this same host and a bacterial parasite, *Daphnia magna – Pasteuria ramosa*, shows that the parasite is not killed if it fails to infect the host (King et al. 2013). Moreover, based on our observations working with this system, we believe that at least a large proportion of spores must

187 remain viable after passing through the host gut. This is because an average sized
188 *Daphnia* filters ~1ml of medium per hour (Kirk et al. 2019), meaning that the dense
189 populations we maintain under lab conditions (~200 hosts/2L at 20C) should filter
190 through all of their medium in their mesocosm every 10 hours. If spores not causing
191 infection were destroyed upon ingestion, this scenario would lead to very low levels of
192 spores in the medium resulting in little or no infection in the population, which is not
193 concordant with the high levels of infection prevalence we regularly observe in our stock
194 populations (47% prevalence, ref: this study). Moreover, we know that new viable spores
195 are released after host cell lysis within the anterior of the *Daphnia* gut. These spores then
196 must pass through the remainder of the gut before entering the environment, implying
197 that passage through the gut does not kill spores. Finally, microsporidian spores are
198 generally durable, and have been shown to survive months of winter in other *Daphnia* –
199 microsporidian systems (Ebert 2005).
200 With the assumption that ingested spores that do not cause infection remain viable
201 after passing through the gut, the probability that a spore causes an infection is
202 $$[\sigma\chi S/(\chi S+\gamma)] * \sum_{i=0}^{\infty}[(1-\sigma)\chi S/(\chi S+\gamma)]^{i} \qquad \text{Eq. S2}$$
203 where χ is the filtrate rate, σ is the probability that an ingested spore causes infection, S is
204 the abundance of susceptible hosts, and γ is the rate of medium exchange. This equation
205 represents the sum of the probabilities that a spore infects a host upon its first ingestion
206 ($i = 1$), or that it passes through the gut on its first ingestion and infects a host the
207 second time it is ingested ($i = 2$), or that it passes through the host gut for the first two
208 ingestions and infects a host on its third ingestion ($i = 3$), and so on. Via the formula for
209 the sum for a geometric power series the equation becomes

$$[\sigma\chi S/(\chi S+\gamma)] * [1-(1-\sigma)\chi S/(\chi S+\gamma)]^{-1} \qquad \text{Eq. S3}$$

which simplifies to

$$\sigma\chi S/(\sigma\chi S+\gamma) \qquad \text{Eq. S4}$$

We therefore assume there are only two ultimate fates for a spore: either it is ingested and infects (at rate $\sigma\chi S$) or it is removed from the system via medium exchange (at rate γ). Additionally, spores could of course also die in the environment, but microsporidian spores are highly durable and we assume their death rate is negligible over the timescale of the experiment as we have noted in the main text.

Under the approximation that $\sigma\chi S \ll \gamma$, we arrive at the expression for the second parenthesis for R_0: $\sigma\chi S/\gamma$, but as an approximation rather than exactly correct. This assumption that $\sigma\chi S \ll \gamma$ is strongly supported for our system, as we know that σ is very small (Kirk et al. 2019). For example, at 12°C, $\sigma\chi S$=0.000018 d^{-1}, while γ is constant across temperature and equals 0.0286 d^{-1}. This means that spore loss from medium exchange (γ) is nearly 1600x larger than spore loss from infection ($\sigma\chi S$) at this temperature, and the assumption that $\sigma\chi S \ll \gamma$ is valid. Because of this, if we look at the temperature range of the experiment (10°C -13.5°C), there is no discernible difference between predictions from the simpler R_0 expression (Eq. 5) that assumes spore loss only from medium removal (black line; Fig. S2) compared to a more complicated expression that explicitly accounts for removal of spores from infection (dashed blue line; Fig. S5).

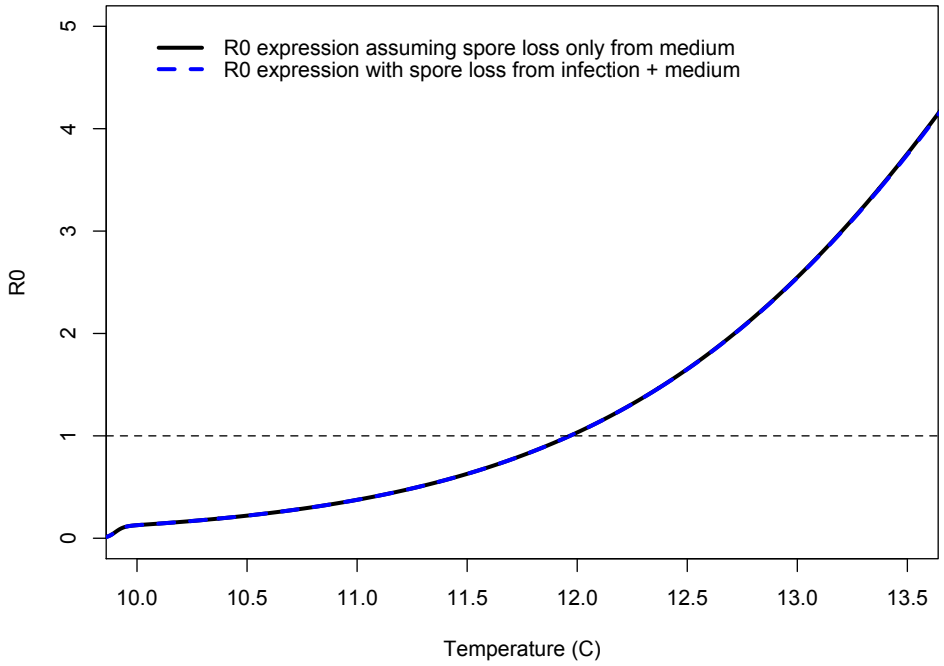

Fig. S5. Comparing R_0 expressions in relation to temperature, with (black line) and without (dashed blue line) the assumption that spore loss from infection is negligible compared to spore loss from medium removal. The temperature range of our experiment was 10.0°C – 13.5,

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