SUPPORTING INFORMATION: Appendix S2

In this Supplementary Appendix, we describe detailed methods from the field survey and all experiments discussed in the main text. Next, we present the mechanistic model used to estimate exposure rate, $E(L,Z)$, and resistance, $\beta(L,Z)$, for each host stage (equs. 1,2). Then, we present additional results, including time series from the low nutrient portion of the lake mesocosm experiment (Fig. S1). We also present data from the exposure (foraging) rate assay and mortality data used to parameterize the model. Finally, we explain why foraging-based exposure rate (equ. 1) declines with increasing levels of spores, Z (at least for adult females).

A MOTIVATING FIELD PATTERN AND EXPERIMENTAL CONFIRMATION

Additional Methods: Lake mesocosms

We suspended polyethylene enclosures (depth: 6 m, diameter: 1 m; 1 mm mesh screen lids; eight replicates per treatment; randomized block design) from wooden rafts in University Lake (Monroe County, Bloomington, Indiana) during the epidemic season (early September–late October 2011). We stocked enclosures with sieved (80 μm) lake water and added lake-collected hosts (initial density of *D. dentifera*: ~ 5000 *Daphnia* m^{-2}) on 6 September. Two days later (8 September), we began the nutrient treatments by initiating low- (*in situ* lake conditions: 10 μg P L^{-1}, 400 μg N L^{-1}) and high- (30 μg P L^{-1}, 750 μg N L^{-1}) nutrient levels. Five days later (13 September), we inoculated half of the enclosures with a single fungal isolate (3.6 spores mL^{-1}). Each productivity x parasite treatment was replicated 7 times for a total of 28 enclosures and maintained for 40 days post spore inoculation (~ 7 *Daphnia* generations). One mesocosm from the low nutrient treatment/+ parasite treatment was accidentally damaged during the experiment and subsequently removed. We maintained nutrient levels with bi-weekly additions of $NaNO_3$

and K_2HPO_4 (assuming a 5% instantaneous daily loss/settling rate; Civitello *et al*. 2013). We sampled each mesocosm twice per week (at night) for 40 days post-spore inoculation (~ 7 host generations). On each sampling date, we collected hosts with three vertical tows of a Wisconsin net (13 cm diameter, 153μm mesh; towed bottom to surface) and nutrient samples (with an integrated tube sampler). We then subsampled ~ 400 *Daphnia* per sample and visually diagnosed infection status, host stage, and ephippia production with a dissecting scope at 20 – 50X magnification to estimate: stage-specific infection prevalence, host density, and investment in males (as in the field survey). No ephippia were produced (likely because we ended the experiment too early in the season). To rule out stress from crowding as a driver of male frequency, we also quantified the mean total density of hosts (integrated area divided by duration of the experiment post inoculation).

Additional Results: Lake mesocosms

Additional temporal dynamics in the low nutrient treatment illustrate changes in the frequency of males and ephippial-females throughout the season, with and without parasites. These dynamics mirror those in the high nutrient treatment (Fig. 3*c, d*). Across all low nutrient treatments, the onset of male production occurred on ordinal date 278 (5 October 2011; Fig. S1*a-b*). Without parasites, peak male production reached c. 42%, occurred on Ordinal date 292 (19 October; dashed line, both figures), and then declined on ordinal date 295 (22 October). With parasites, however, both peak (c. 66%) and overall male frequency was higher relative to the parasite-free control (Fig. S1*b*). Hence, in the parasite-addition treatments (Fig. S1*b*), sexual reproduction was higher relative to the parasite-free treatments (which therefore explains the field pattern presented in the text; Fig. 2*a*).

TEST OF THE ALLOCATION TO SEX MECHANISM

Additional Methods: Life-table Assay

This life table assay used similar general methods as the joint exposure-infection assay. More specifically, we used the same host clone, fungal isolate, water source, food, light/temperature combination, etc. We filled six replicate 1L flasks with filtered lake water [PALL A/E: 1.0μm) and stocked them with hosts at high-density levels (initial density: 75 animals/L). We maintained hosts at 15 °C and 8:16 light: dark cycle and fed them (1.0 mg dw L^{-1} of *A. falcatus*) every other day. To create epidemics within the '+ parasite environments', we inoculated three flasks with two spore doses two days apart: (20 spores/mL and 7.5 spores/mL, respectively). Eighteen days post-inoculation, we collected 15 adult females from each flask and placed them individually in centrifuge tubes containing 15mL of 'culture water' (a mixture of food [1.0 mg dw L^{-1}], filtered lake water [PALL A/E: 1.0μm) and 10% water from their original flask). Every other day, we transferred these hosts to fresh culture water. Thus, we provided cues of high population density and background infection dynamics to hosts. On change days, we collected and sexed offspring for up to three clutches. We calculated sex investment (the number of males out of the total offspring produced) by each female; no ephippia were produced. We estimated final densities (log normal GLM) and infection prevalence (binomial GLM) in all flasks. Here, flask environment was nested within parasite treatment.

QUANTITATIVE EVALUATION OF THE MALE RESISTANCE MECHANISM

Additional Methods: Field Survey and Mesocosms vs. Lab Assay

To estimate mean stage-specific infection prevalence (e.g., # infected males/total # males)

from the field survey and field experiment, we used data from a subset of observations. Specifically, we only used sample time points where a minimum number of ten males were counted. Then, we calculated the averages (i.e., across all time points) for each stage and each lake. This criterion helped eliminate inflated outliers of male infection prevalence arising from low sample sizes of male hosts. The results are qualitatively the same with and without this restriction. However, we feel that the subset provides a more conservative estimate.

Additional Methods: A Size-Based Model of Resistance

Lab assay: stage-specific exposure and infection: To test the male resistance mechanism, we conducted an experiment where we jointly measured stage-specific exposure and infection. We used a single fungal isolate (cultured *in vivo*) and a single host clone that demonstrates a high degree of sexual reproduction. Prior to conducting both experiments, we maintained cultures for at least three generations to minimize any potential maternal effects and under temperature and light conditions reflecting the end of the epidemic season: 15 °C and 8:16 light: dark cycle (Tessier and Cáceres 2004)

We exposed individual hosts in 14 mL of media consisting of algal food (initially 1.0 mg dw L^{-1} of *Ankistrodesmus falcatus*), a dose of fungal spores, and filtered lake water (PALL A/E: 1.0μm pore size) in 15-mL centrifuge tubes. We conducted the entire foraging-rate assay in the dark to prevent algal growth. We factorially crossed host stage (juvenile female, adult female, and male) with parasite density (0, 150, and 350 spores per mL^{-1}) and replicated each treatment 15 times. To ensure that spores and food remained suspended throughout the assay, we gently rotated tubes every 10-hours. Hosts grazed for 48 h (in 15 °C incubators). Then, we transferred hosts to fresh, parasite-free water and estimated food remaining in the tubes. Specifically, we

93 used *in vivo* fluorimetry to calculate the fluorescence of ungrazed and grazed algae (using a
94 Turner Trilogy Laboratory Fluorometer Sunnyvale, CA, USA; Sarnelle and Wilson 2008).
95 Finally, we measured all individual hosts (middle of the eye to base of the tail spine). We then
96 maintained hosts at 15 °C and 8:16 light: dark cycle (i.e., changed to fresh media with water and
97 food: 1.0 mg dw L^{-1} of *A. falcatus*) every other day for 19 days. Afterwards, we visually
98 diagnosed infection status of all remaining individuals (as in the field survey).
99 *A size-based model of resistance:*
100 We used the exposure and infection prevalence data from the experiment to estimate sex- and
101 stage-specific differences in exposure E, per parasite susceptibility, u, and resistance ($\beta = u \times E$;
102 low β means high resistance). Here, exposure rate, $E(L,Z)$ is a function of length (L) and spores
103 (Z):

104 $$E(L,Z) = \hat{E}\, L^2 \exp(-\alpha\, \hat{E} L^2\, Z) \qquad \text{(S1)}$$

105 where $\hat{E}$ is the size-corrected (size-specific) feeding rate (assuming a linear functional response),
106 L^2 is proportional to surface area, exp(...) is the exponential function, and α governs how
107 sensitively feeding rate declines with exposure to spores ($\hat{E} L^2\, Z$, part of the relevant biology
108 here). In this function (equ. 1), exposure (foraging) rate increases with surface area but decreases
109 with higher exposure to spores. To estimate parameters $\hat{E}_J$, u_j, and α (where j denotes stage
110 [males, juvenile females, adult females]), we inserted $E(L,Z)$ into a model of the feeding and
111 infection process (equ. S2):

112 $$dS/dt = S\, [\, -u_j\, E_j(L,Z) Z - \phi E_j(L,Z)\, Z\,] \qquad \text{(S2)}$$

113 $$dI/dt = u_j\, E_j\, (L,Z)\, S\, Z \qquad \text{(S3)}$$

114 $$dD/dt = \phi E_j(L,Z)\, S\, Z \qquad \text{(S4)}$$

115 $$dZ/dt = -\, E_j(L,Z)\, (S+I+D)\, Z \qquad \text{(S5)}$$

$$dA/dt = - E_j(L,Z)\,(S+I+D)\,A \qquad \text{(S6)}$$

An individual host at stage j leaves the susceptible class (equ. S2) as they become infected (first term, moving into the I class in equ. S3) or die, after this assay, without producing spores (second term, moving into D class in equ. S4). Again, these 'dead' individuals were exposed, ate food and spores, but died too quickly to be diagnosed many days later – yet while alive, they produced valuable data from this short-term assay. Parameter ϕ is a per spore death coefficient, so per capita death rate becomes $d = \phi E_j(L,Z)\,Z$. Additionally, spores, Z (equ. S5) and algae, A (equ. S6) decrease as all host classes (S+I+D) consume them at common rate $E_j(L,Z)$.

We fit the model to the data with numerical integration and maximum likelihood. We numerically integrated the model (equ. 2), given initial algal food (A_0 = 1 mg dw/L), spores (Z_0 = 0, 150, or 350 spores/mL), and host density (S = 1 host per 14 mL), for the duration of the 2-day exposure (t_E = 2 days). Length of each host, L, was independently measured. We then compared predictions of the integrated model to data collected in each tube. First, we calculated the negative log-likelihood of the observed food in each tube after the two-day incubation, $\boldsymbol{\ell_A}$. This likelihood uses the normal distribution where residuals, ε, are the difference between observed food, A_E, and predicted food remaining, A_P, on a log scale, i.e., $\varepsilon = \ln(A_E) - \ln(A_P)$, and where the subscript P denotes 'predicted from the model'. Then, we compared the status of the individual host (uninfected, infected, or dead) at the end of the experiment to the predictions of the model (equ. 2), where, e.g., the predicted proportion of infected hosts was $p_I = I_P / (S_P + I_P + D_P)$. We used the multinomial function to calculate this negative log likelihood for host status, $\boldsymbol{\ell_H}$**.** We then summed these two negative log likelihood values ($\boldsymbol{\ell_A} + \boldsymbol{\ell_H}$) to produce one value for each tube. We minimized this sum among all individuals by finding the best fitting parameter values for the eight parameters of this model (u_j and $\hat{E}_j$ for each stage, and common ϕ and α shared

among stages). We then calculated size-corrected resistance, $\hat{\beta}_j$, for each class, j, as $\hat{\beta}_j = u\hat{E}_j$

Once the model was fit, we compared stage-specific parameters and made predictions for exposure and resistance with length. We bootstrapped 95% confidence intervals (CI) around these point estimates (see Table S1 below), using 1,000 stratified, non-parametric bootstraps. We also directly compared differences between parameter estimates for each host stage with pair-wise randomization tests based on 2,500 iterations (see Table S1). Additionally, we calculated exposure rate, $E(L,Z)$, and resistance, $\beta(L,Z)$, along a gradient of host length observed for each stage in the experiment. We presented calculations using the high dose of spores (350 sp/mL) in the text but include those from the zero dose and low spore dose (Z = 150 sp/mL) below. We calculated point-wise, 95% bootstrapped confidence envelopes around these exposure and resistance functions over a gradient of relevant length (L).

Additional Results: A Size-Based Model of Resistance

The joint experiment of exposure and infection: Exposure rate data from the laboratory experiment demonstrate why, in the model, exposure (E) is a function of both host length (L) and spore dose (Z). These data, combined with model fits of exposure rate (Fig. S1*a*), illustrate two key points: (1) Across all host stages, exposure rate (f) increases with length. (2) At higher spore levels, exposure rates decrease, especially for adult females (darkest triangles, dotted line). Additionally, mortality was very low over the course of the experiment. Using the controlled laboratory assay of resistance, there was a significant effect of spore dose on the proportion of animals that died (logistic regression, quasibinomial likelihood): Dose effect (D): $\widehat{b_1}$ = 0.007, t = 2.44, $\hat{c}$ = 13.0, p = 0.017, Fig. S1*b*). However, there was no difference between host stages (St: t = -1.34, $\widehat{b_2}$ = -1.02, p = 0.184) or between spore dose and host stage (D x St: t = -0.829, $\widehat{b_3}$ = -

0.005, $p = 0.410$). The controlled laboratory assay also indicated a significant effect of spore dose on infection prevalence (logistic regression [quasibinomial likelihood]: dose effect [D]: $\widehat{b_1}$ = 0.014, $t = 5.65$, $\hat{c} = 5.0$, $p < 0.001$, Fig. 5*c*). However, there was no difference between host stages (St: $z = -0.61$, $\widehat{b_2} = -0.399$, $p = 0.54$) or between spore dose and host stage (D x St: $z = 0.52$, $\widehat{b_3} = 0.003$, $p = 0.601$).

The model of exposure and susceptibility: The foraging rate function (equ. 1) demonstrates that per-spore exposure rate, $E(L,Z)$ is a function of length (L) and spores (Z) where α governs how sensitively foraging declines with total exposure to spores, $E(L,Z)\,Z$.

A comparison between changes in exposure rate, $E(L,Z)$, resistance, $\beta(L,Z)$, death rate, $d(L,Z)$ as a function of host size, L, at no spores and low vs. high spore levels (Fig. S3) illustrates this crucial point. Across both spore levels and all host stages, all three functions increased with size. However, this size-dependent increase is much less pronounced at high spore levels (350 spores/mL). More specifically, adult females had considerably higher realized exposure (E^*Z) rates at low spore levels (Fig. S3*b*) relative to high spore levels (Fig. S3*c*). This drop in realized exposure (E^*Z) at higher spore levels likely arises because at high spore doses, large adults decrease their foraging (i.e., exposure) rates to resemble that of smaller juveniles (Fig. S3*c*). Hence, adult females have similar per-spore resistance (which accounts for both exposure, E and susceptibility, u) and only slightly elevated death rates in the two spore levels, despite the density of spores in the water column being twice as high. However, relative to adult females, small juveniles and males only marginally decrease their foraging (i.e., exposure) in response to higher spore levels.

Extended Discussion: No Ingredients for Red Queen

In the Discussion, we posit that this *Daphnia*-fungus system lacks key ingredients for the Red Queen hypothesis to work. For instance, no evidence exists for genetic specificity of infection (Auld et al. 2012; Duffy and Sivars-Becker 2007; Searle et al. 2015) — unlike in other *Daphnia*-parasite systems (e.g., *Pasteuria ramosa;* Auld et al. 2012; Duncan and Little 2007; Ebert 2008). Additionally, the focal parasite exhibits no apparent genetic variation in infectiousness (Duffy and Sivars-Becker 2007; Searle et al. 2015) and no response to artificial selection (Auld et al. 2014; Duffy and Sivars-Becker 2007). With such low-variation, hosts and parasites likely cannot co-evolve, despite that hosts themselves evolve rapidly during epidemics (e.g., Auld et al. 2013; Duffy et al. 2008; Duffy and Hall 2008). Finally, we see no evidence for local adaptation of parasites (Searle et al. 2015), a common corollary of the RQH (Lively et al. 2004).

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ONLINE APPENDIX S2: TABLE

Table S1. Best-fit parameter estimates from the mechanistic, size-based model of resistance (equs. 1,2). Estimates are accompanied by lower and upper 95% confidence intervals (CI) generated with 1,000 stratified random bootstraps.

Par.[a]	Explanation	Units[b]	Male	Juvenile	Adult female
α^c	Sensitivity coefficient	host·day·sp^{-1}×10^{-4}	1.50 (1.07, 2.00)	1.50 (1.07, 2.00)	1.50 (1.07, 2.00)
$\widehat{\beta_J}^d$	Size-corrected resistance	L·sp^{-1}·day^{-1}· mm^{-2} ×10^{-3}	1.70 (0.9, 3.0)	3.57 (2.0, 7.0)	1.88 (1.0, 3.0)
ϕ^{c*}	Per-spore mortality coefficient	host·sp^{-1}×10^{-5}	0.57 (0.13, 1.18)	0.57 (0.13, 1.18)	0.57 (0.13, 1.18)
$\widehat{E_J}$	Size-corrected foraging (exposure) rate	L·host^{-1}·day^{-1}·mm^{-2} ×10^{-3}	2.65 (2.06, 3.26)	4.83 (4.29, 5.30)	5.71 (5.22, 6.12)
u_j	Per-spore susceptibility	host·sp^{-1}×10^{-4}	6.39 (3.52,11.91)	7.42 (4.61, 13.11)	3.33 (1.91, 5.34)

[a] Parameter of the model. [b] Explanation of units: L = liter, sp = spore. [c] Parameter estimates for α and ϕ do not depend on stage j. [d] Size-corrected resistance is the product of per-spore susceptibility times foraging rate, $\widehat{\beta_J} = u\widehat{E_J}$. *Calculated over (2 days of exposure/19 days observation).

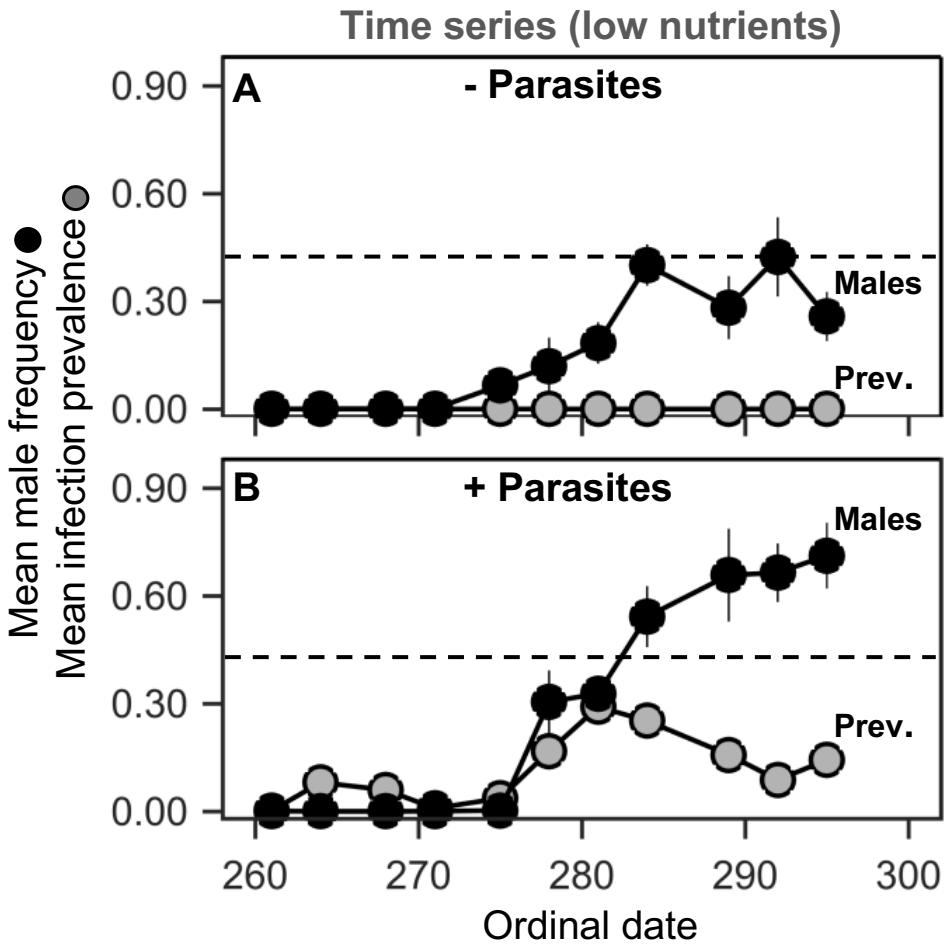

Figure S1. Data from the low nutrient treatments in the lake mesocosms illustrate changes in the frequency of males (black) through the season in treatments (A) without (—, top) and (A) with (+, bottom) parasites (grey). The dashed line denotes maximum frequency of males in the parasite-free treatment at low nutrients (from panel *A* - see text). Symbols represent means ± SE.

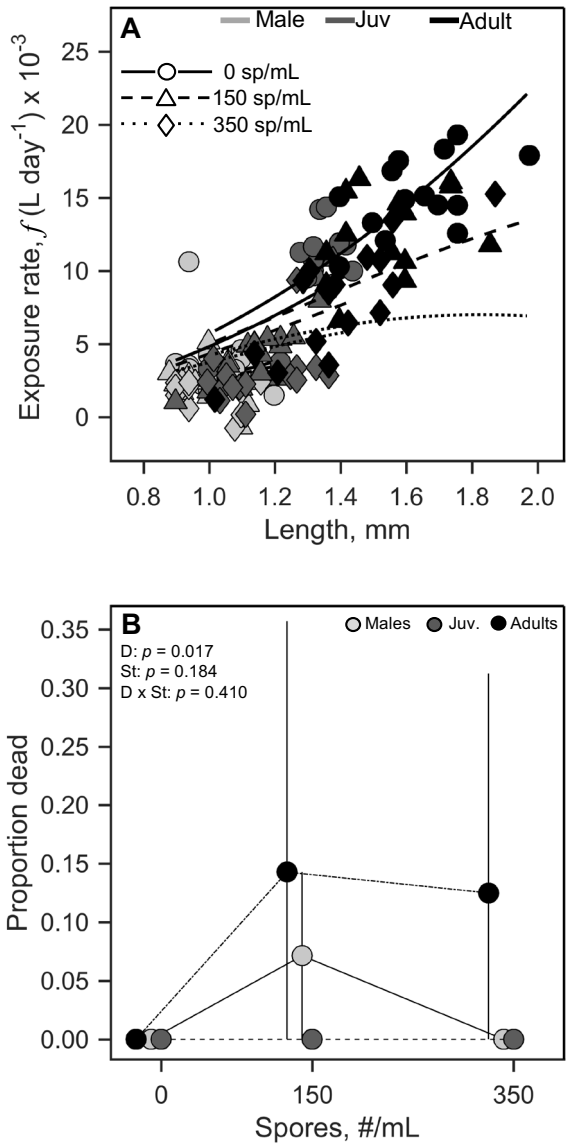

Figure S2. Data from the foraging assay and proportion dead data used to parameterize the model of resistance. (*A*) Across all spore treatments (0, 150, and 350 spores/mL), exposure rate (*f*) increases with host length, (*L*). Adult females (darkest symbols) drastically decrease foraging rate as a function of spore dose. (B) The proportion of hosts that died tended to increase with spore dose for adult females. However, there were no significant differences between stages or spore doses. The p-values presented are from a logistic regression model with "D" representing parasite-dose effects, "St" representing stage effects, and "D x St" representing their interaction.

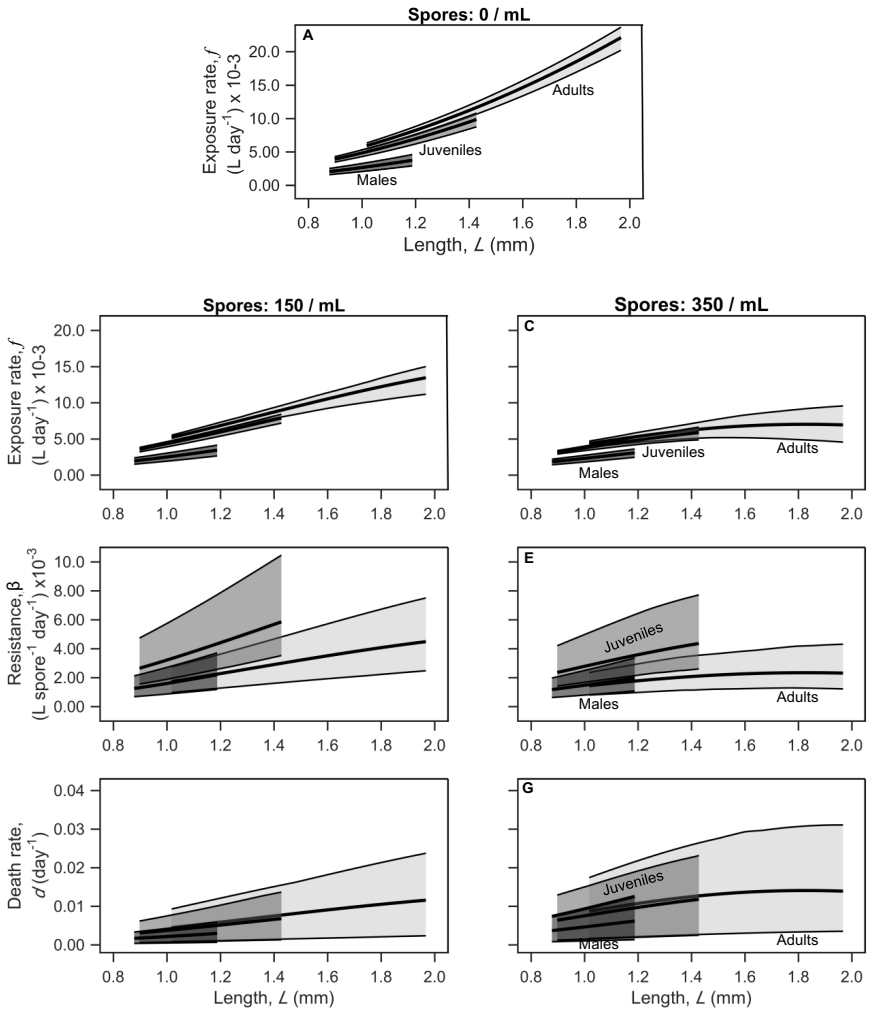

Figure S3. Predictions of the best-fit model of exposure rate, resistance, and death rate for each host stage from both no (0 sp/mL), lower (150 sp/mL), and higher (350 sp/mL) spore treatments (means ± 95% bootstrapped, point-wise confidence envelopes). (Infection risk and death rate were zero in the controls). The values for exposure and infection risk for the higher spore treatment from the main text (Fig. 6*D*,*E*) are represented here for comparison. (*A*-*F*) Across both spore treatments, exposure rate, $E(L,Z)$, resistance, $\beta(L,Z)$, and death rate of hosts, $d(L,Z)$, all tended to increase with host size but decrease as large hosts become exposed to more spores. Overall, however, larger adult females and smaller males had similar infection risks and death rates.