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Article type : Research Article

Section: Community Ecology

Editor: Dr. Dana Hawley

Can hot temperatures limit disease transmission? A test of mechanisms in a zooplankton-fungus system

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Keywords: *Daphnia dentifera*, fungal pathogen, host-parasite interaction, infectious disease, *Metschnikowia bicuspidata*, temperature, thermal ecology, transmission rate, upper thermal constraints, upper thermal limits

ABSTRACT (316 / 350 WORDS)

1. Thermal ecology theory predicts that transmission of infectious diseases should respond

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/1365-2435.13403](https://doi.org/10.1111/1365-2435.13403)

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29 unimodally to temperature, i.e., be maximized at intermediate temperatures and
30 constrained at extreme low and high temperatures. However, empirical evidence linking
31 hot temperatures to decreased transmission in nature remains limited.

- 32 2. We tested the hypothesis that hot temperatures constrain transmission in a zooplankton-
33 fungus (*Daphnia dentifera*-*Metschnikowia bicuspidata*) disease system where autumnal
34 epidemics typically start after lakes cool from their peak summer temperatures. This
35 pattern suggested that maximally hot summer temperatures could be inhibiting disease
36 spread.
- 37 3. Using a series of lab experiments, we examined the effects of high temperatures on five
38 mechanistic components of transmission. We found that (1) high temperatures increased
39 exposure to parasites by speeding up foraging rate but (2) did not alter infection success
40 post-exposure. (3) High temperatures lowered parasite production (due to faster host
41 death and an inferred delay in parasite growth). (4) Parasites made in hot conditions were
42 less infectious to the next host (instilling a parasite 'rearing' or 'trans-host' effect of
43 temperature during the prior infection). (5) High temperatures in the free-living stage also
44 reduce parasite infectivity, either by killing or harming parasites.
- 45 4. We then assembled the five mechanisms into an index of disease spread. The resulting
46 unimodal thermal response was most strongly driven by the rearing effect. Transmission
47 peaked at intermediate-hot temperatures (25-26°C) and then decreased at maximally hot
48 temperatures (30-32°C). However, transmission at these maximally hot temperatures only
49 trended slightly lower than the baseline control (20°C), which easily sustains epidemics
50 in laboratory conditions and in nature. Overall, we conclude that while exposure to hot
51 epilimnetic temperatures does somewhat constrain disease, we lack evidence that this
52 effect fully explains the lack of summer epidemics in this natural system. This work
53 demonstrates the importance of experimentally testing hypothesized mechanisms of
54 thermal constraints on disease transmission. Furthermore, it cautions against drawing
55 conclusions based on field patterns and theory alone.

56 INTRODUCTION

57 How do high temperatures affect the spread of infectious diseases? In the current
58 prevailing view, warming from climate change will shift the geographic range of diseases: some
59 new areas will become warm enough to support disease, whereas others that previously sustained

60 disease will become too hot (Altizer, Ostfeld, Johnson, Kutz, & Harvell, 2013; Lafferty, 2009;
61 Lafferty & Mordecai, 2016). This hypothesis stems from a principle of thermal biology: most
62 biological traits have unimodal reaction norms, where performance peaks at intermediate
63 temperatures and declines to zero at cooler and warmer temperatures (Dell, Pawar, & Savage,
64 2011). Thus, once temperatures exceed the thermal optima of traits driving transmission, disease
65 should decline. Many models predict upper thermal constraints on diseases, e.g., helminthic
66 ungulate parasites (Molnár, Kutz, Hoar, & Dobson, 2013), a rhizocephalan crab parasite
67 (Gehman, Hall, & Byers, 2018), a microsporidian *Daphnia* parasite (Kirk et al., 2018),
68 schistosomiasis (Mangal, Paterson, & Fenton, 2008), and mosquito-borne pathogens (Mordecai
69 et al., *in press*). Additionally, there is evidence for upper thermal constraints on disease in natural
70 populations of the crab parasite (Gehman et al., 2018), mosquito-borne pathogens (e.g., Shocket
71 et al., *in review*) and fungi infecting grasshoppers (Carruthers, Larkin, & Firstencel, 1992),
72 amphibians (Berger et al., 2004; Raffel, Michel, Sites, & Rohr, 2010), and bats (Langwig et al.,
73 2015). However, temperature often co-varies with other seasonal environmental factors, so
74 causally linking temperature to observed patterns of disease is challenging (Altizer et al., 2006;
75 Pascual & Dobson, 2005). Thus, the generality of upper thermal constraints excluding disease
76 remains unclear.

77 Conceptually, upper thermal constraints act like fever, taking advantage of a common
78 thermal mismatch between hosts and parasites. Because hosts can often endure hotter
79 environments than their parasites, many animals increase their body temperature when infected
80 (see citations below). In ectotherms, fever arises from behavioral thermoregulation (microhabitat
81 selection) and is widespread, occurring in vertebrates (including amphibians, reptiles, and fish:
82 Rakus, Ronsmans, & Vanderplasschen, 2017), snails (Zbikowska, Wrotek, Cichy, & Kozak,
83 2013), and insects (including bees, flies, grasshoppers, mosquitoes, and beetles: Stahlschmidt &
84 Adamo, 2013; Thomas & Blanford, 2003). Behavioral fever can impair parasite performance,
85 enhancing clearance or reducing virulence of infection. An analogous process can occur within
86 ectothermic hosts inhabiting high ambient temperatures (regardless of infection status)—in
87 essence, an environmental fever. High ambient temperatures can also harm parasites with free-
88 living stages outside of hosts. Mechanistically linking high temperatures to reduced disease
89 requires examining thermal effects on components of the transmission process (McCallum et al.
90 2017). We use the term ‘transmission (process)’ to broadly refer to the full parasite life cycle,

91 including infective propagule production and propagule survival in the environment; we also use
92 ‘transmission rate’ narrowly defined as the rate of new infections (i.e., the parameter ‘ β ’
93 calculated from infection prevalence and densities of hosts and parasites; McCallum et al. 2017).

94 Here, we use a series of experiments to evaluate mechanisms for potential upper thermal
95 constraints on transmission in a planktonic-fungal disease system. Autumnal epidemics start
96 once lake waters cool below summer maxima (Fig. 1A). These delayed starts could reflect hot
97 temperatures inhibiting disease if they push any of five transmission components past their
98 thermal optima (Fig. 1B). First, hot temperatures could slow host feeding and lower
99 consumption-based exposure to parasites. Second, hot temperatures could reduce parasite
100 infectivity inside hosts, lowering the probability of successful infection (via effects on hosts
101 and/or parasites). Third, hot temperatures could decrease the quantity of parasite propagules
102 [spores] produced by an infection. This decrease could stem from slower host growth rate (since
103 parasite production often scales with host growth: Hall, Knight, et al., 2009; Hall, Simonis,
104 Nisbet, Tessier, & Cáceres, 2009), slower parasite growth independent from host growth, or
105 enhanced mortality of infected hosts (truncating production time; Auld, Hall, Housley Ochs,
106 Sebastian, & Duffy, 2014; Civitello, Forys, Johnson, & Hall, 2012). Fourth, hot temperatures
107 could lower the quality of parasite spores released from dead hosts into the environment
108 (Shocket, Vergara, et al., 2018). Finally, these free-living spores could be harmed or killed by
109 hot temperatures. Thus, high temperatures could constrain this fungal disease at multiple stages
110 of the transmission process.

111

112

STUDY SYSTEM

113 The hosts (*Daphnia dentifera*) are zooplankton grazers in freshwater temperate lakes
114 across the Midwestern United States; the fungal parasite *Metschnikowia biscupidata* causes
115 epidemics in some host populations, with prevalence reaching up to 60% (Penczykowski, Hall,
116 Civitello, & Duffy, 2014). Hosts become infected when they filter-feed on algae and
117 inadvertently consume fungal spores (Hall et al., 2007). The spores pierce the host’s gut wall,
118 entering its body cavity. Inside, fungal conidia replicate in the hemolymph before maturing into
119 new spores (Stewart Merrill & Cáceres, 2018). Following host death, spores are released into the
120 water for new hosts to consume (Ebert, 2005).

121 The seasonality of epidemics motivated a focus on high temperatures. Epidemics

122 typically begin in late summer or early fall (August–October) and wane in late fall or early
123 winter (November–December; Penczykowski, Hall, et al., 2014). During this time, lake water
124 temperature declines (Shocket, Strauss, et al., 2018). Many traits that influence disease spread
125 (host demographic traits, transmission rate, and spore production) change plastically with
126 temperature (Hall, Tessier, Duffy, Huebner, & Cáceres, 2006; Shocket, Strauss, et al., 2018).
127 Transmission increases with constant temperatures up to 26°C, and hosts cannot be cultured in
128 constant temperatures above 27°C (Shocket, Strauss, et al., 2018). However, organisms can
129 withstand otherwise lethal temperatures in fluctuating environments (Niehaus, Angilletta, Sears,
130 Franklin, & Wilson, 2012). For instance, in our stratified lakes, hosts experience temperatures
131 exceeding 27°C in summer (typical maxima 29–32°C; Fig. 1A): they migrate between the colder,
132 deeper hypolimnion during day (to avoid mortality from visually-oriented fish predators) and the
133 warmer, upper epilimnion at night (to take advantage of greater algal resources and faster growth
134 in warmer temperatures) (Hall, Duffy, Tessier, & Cáceres, 2005; Lampert, 1989). Epidemics
135 often begin as lakes start cooling from maximum summer temperatures (Fig. 1A). This pattern
136 suggested that high temperatures could constrain disease spread, as predicted by theory (Lafferty,
137 2009; Lafferty & Mordecai, 2016).

138 139 MATERIALS AND METHODS

140 Field Survey

141 Field survey data generated the motivating pattern (Fig. 1A: the relationship between
142 epidemic start date and epilimnetic temperature). We surveyed 10–28 lakes in Indiana (Greene
143 and Sullivan Counties) weekly (2009–2011) or bi-weekly (2013–2014) from August to
144 December. For each visit, we collected a zooplankton sample (13 cm diameter net with 153 µm
145 mesh) and measured lake water temperature data at 0.5–1 meter intervals with a Hydrolab
146 multiprobe (Hach Environmental). For each sample, we visually diagnosed 400+ live hosts with
147 a dissecting scope (20-50X magnification). An epidemic ‘started’ when infection prevalence first
148 exceeded 1% for two consecutive sampling visits (Shocket, Strauss, et al., 2018). We calculated
149 the epilimnetic temperature by fitting a spline to temperature across water depth, and averaging
150 from the water surface to the depth where the temperature gradient first exceeded 1°C m⁻¹ (i.e.,
151 the thermocline; see Hite et al. 2016 Appendix S2).

152

153 **General Approach**

154 We measured how high temperatures influence five components of the transmission
155 process with laboratory assays (Table 1). Then we combined them into a synthetic index of
156 disease spread: ‘transmission potential’ (Auld et al., 2014). For mechanisms involving the host or
157 host-parasite interaction (mechanisms 1-3: foraging rate [f], spore infectivity from within-host
158 processes [u], and spore yield [σ]), we used fluctuating temperatures to expose hosts to high
159 temperatures for part of the day (they cannot survive constant temperatures $>27^{\circ}\text{C}$). Hosts were
160 kept on a 16:8 light:dark cycle. All hosts experienced the same 20°C temperature for 8 hours,
161 and then 20, 26, or 32°C for 16 hours (‘maximum temperature’). For mechanisms 4-5 (rearing
162 effect on spore quality [ρ] and free-living spore effect [ϕ]), we conducted common garden
163 infection assays, exposing uniform hosts at constant 20°C to spores from different treatments.
164 Thus, variation in transmission rate can be attributed to differences in spore infectivity.
165 Temperatures varied slightly among experiments (25 or 26°C , 30 or 32°C) based on incubator
166 availability. For calculating transmission potential, we treat temperature categorically and pool
167 these treatments.

168 Due to time and incubator constraints, we were unable to replicate experiments across
169 multiple incubators. Thus, our temperature treatments are ‘pseudo-replicated’ in that all
170 replicates for a treatment were conducted in the same incubator at the same time. Accordingly,
171 our results may be influenced by random incubator effects.

172

173 **Mechanisms 1 & 2: Foraging rate (f) and spore infectivity from within-host processes (u)**

174 We measured foraging rate of hosts by comparing the fluorescence of ungrazed and
175 grazed algae (Penczykowski, Lemanski, et al., 2014; Sarnelle & Wilson, 2008). We added
176 estimates of foraging rate at 30°C to those at 20 and 25°C presented elsewhere using the same
177 methods (Shocket, Vergara, et al., 2018). In both experiments, we measured foraging rate across
178 a gradient of host body size (Kooijman, 2009) to index foraging at a common size among
179 experiments (1.5 mm). We used maximum likelihood estimation (MLE) to fit size-dependent
180 functions of foraging with the ‘bbmle’ package (Bolker & R Development Core Team, 2017) in
181 R (R Core Team, 2017). See Appendix for details.

182 We measured how high temperature impacts transmission rate (β) and spore infectivity
183 from within-host processes (u) with an infection assay (‘ $\beta + u$ measurement assay’). For

184 successful infection, the fungus must break through the host gut barrier and then replicate and
185 develop within the host hemolymph. High temperatures could inhibit the parasite during either
186 process. Thus, we factorially manipulated the maximum temperature (20 and 32°C) during
187 parasite exposure and infection establishment (for four exposure/establishment treatments: 20/20,
188 20/32, 32/20, and 32/32°C) to reveal if high temperatures interfere at either step (similar to Allen
189 & Little, 2011). Hosts were exposed individually in their 'exposure temperature' for 24 hours,
190 then moved to their 'infection establishment temperature.' Later, hosts were visually diagnosed
191 for infection. Transmission rate was calculated from proportion infected (see Appendix). We
192 calculated spore infectivity from within-host processes (u) for each treatment by dividing
193 transmission rate (β) by foraging rate (f) at the exposure temperature ($u=\beta/f$).

194

195 **Mechanism 3: Spore yield (σ) and related host and parasite traits**

196 We measured how high temperatures impact final spore yield (σ) of infected hosts that
197 died from their infection. This trait estimates spore release into the environment. We pooled
198 spore yields from the $\beta + u$ measurement assay (above; treatments = 20/20 and 32/32°C) and the
199 within-host parasite growth assay (below; treatments = 20, 26, and 32°C) since they did not
200 differ statistically (20°C: $p=0.65$; 32°C $p=0.93$). We tested for differences between temperatures
201 by fitting a suite of models via MLE: in each model spore yield was normally distributed and
202 temperature treatments could exhibit the same or different means and standard deviations. We
203 compared models using AIC and calculated p -values with likelihood ratio tests.

204 To distinguish between three possible mechanisms driving the thermal response of spore
205 yield, we quantified related host and parasite traits. First, we measured host growth rate (g_h) with
206 a juvenile growth rate assay (Lampert & Trubetskova, 1996)(see Appendix), since spore yield
207 often scales with g_h (e.g., with different host food resources: Hall, Knight, et al., 2009; Hall,
208 Simonis, et al., 2009). We compared treatments with t-tests. Second, we measured parasite
209 growth (i.e., number of mature spores within hosts over time) using a sacrifice series ('within-
210 host parasite growth assay;' see Appendix), since spore yield could decline if the number of
211 parasites increases more slowly, independently of host condition (Thomas & Blanford, 2003).
212 We fit and bootstrapped linear models of 'spore load' over time to estimate parasite growth rate
213 (g_p , the model slope). 'Spore load' estimates included spores in living (i.e., sacrificed) hosts,
214 unlike 'spore yield,' which was calculated only from dead hosts that were killed by the parasite.

215 Spore yield is directly relevant for the epidemiology of the system, while spore load measures an
216 underlying process (parasite growth rate per day, g_p) that contributes to spore yield. Spore load
217 increased linearly over the full time series at 26 and 32°C. Spore load plateaued after day 19 at
218 20°C, so we truncated the time series to estimate the linear slope for only that portion. Finally,
219 we calculated death rate (d) of infected hosts (see Appendix), since spore yield can decline with
220 shorter host lifespan (Auld et al., 2014; Civitello et al., 2012). We compared treatments with
221 randomization tests.

222

223 **Mechanisms 4 & 5: rearing (ρ) and free-living spore (φ) effects on infectivity**

224 We measured how high temperatures modify spore infectivity prior to encountering hosts
225 via a rearing effect on baseline spore quality (ρ) and harm to free-living spores (φ). We
226 conducted infection assays on ‘common garden’ groups of hosts at 20°C using different spore
227 treatments (i.e., from different spore rearing temperatures for ρ and from different spore
228 incubation temperatures for φ). Thus, variation in transmission rate reflects differences in spore
229 infectivity. To measure ρ , we conducted two experiments, one with spores produced in the $\beta + u$
230 measurement assay (20/20 and 32/32°C treatments) and another with spores produced in the
231 within-host parasite growth assay (20, 26, and 32°C treatments). To measure φ , we used spores
232 incubated at three temperatures (20, 25, and 30°C) for two durations (1-day and 7-days) in
233 constant, non-fluctuating temperatures (spores do not migrate between stratified water layers).
234 One-day incubations were stored at 4°C for the first six days (standard procedure for spore
235 storage). We estimated transmission rates (β) from the prevalence data (see Appendix).

236 Both mechanisms influence transmission by modifying spore infectivity (already
237 estimated from within-host processes as u , mechanism 1). Thus, in order to incorporate these
238 mechanisms into a synthetic metric for disease spread (transmission potential, see below), we
239 calculated unit-less rearing (ρ) and free-living (φ) effects standardized to infectivity at 20°C.
240 Specifically, we calculated the parameters by dividing the estimates for transmission rate (β) at
241 26 and 32°C by that at 20°C. Accordingly, values of $\rho < 1$ or $\varphi < 1$ mean spores are less infectious
242 due to rearing or free-living effects than at 20°C, respectively; conversely, values > 1 mean
243 spores are more infectious than at 20°C. To calculate confidence intervals at 20°C, we divided a
244 bootstrapped distribution of transmission rates by a randomly-shuffled version of itself.
245 Additionally, because harm to free-living spores occurs over time as spores are removed by

246 hosts, we used a simple model to estimate time-weighted transmission rates for φ . We assumed
247 that spore infectivity declined linearly over the 7-day assay, and that hosts consume spores at a
248 constant foraging rate (resulting in an exponential decay in spores remaining over time). Thus,
249 we weighted the estimated transmission rate on each day by the proportion of spores consumed
250 by hosts on that day (see Appendix for detailed methods and a sensitivity analysis of the model).

251

252 **Transmission potential**

253 We calculated an index of disease spread to synthesize the effects of all five mechanisms.
254 We defined transmission potential as the product of all five parameters ($f u \sigma \rho \varphi$). We generated
255 confidence intervals using bootstrapped parameter distributions. To visualize the contribution of
256 each parameter, we calculated transmission potential for each of the five possible four-parameter
257 combinations, holding the fifth parameter constant at its 20°C point estimate. These values reveal
258 how each parameter affects the magnitude and uncertainty of transmission potential (i.e., a type
259 of sensitivity analysis).

260

261 **Additional Statistical Analyses**

262 For all parameters, we bootstrapped 95% confidence intervals (data sampled within
263 groups, with replacement; 10,000 samples). For parameters derived from transmission rates (β , u ,
264 ρ , and φ), we used randomization tests to compare temperature treatments, since a single value is
265 calculated from all individuals (treatment labels shuffled among host individuals, without
266 replacement; 10,000 samples). For f and transmission potential (for which traditional statistical
267 tests were not available), we used the bootstrapped distributions to compare treatments.
268 Specifically, we calculated the cumulative probability density of the best estimate from one
269 treatment according to the bootstrapped distribution of the other. These ‘PD-values’ are
270 analogous to p -values. We considered treatments significantly different if $PD < 0.025$. See
271 Appendix for details and a complete list of statistical tests and results.

272

273

RESULTS

274 **Mechanisms 1 & 2: Foraging rate (f) and spore infectivity from within-host processes (u)**

275 Contrary to our predictions, high temperature did not lower transmission rate (β) during
276 either step (exposure or infection establishment; Fig. 2A). Instead, transmission rate was higher

277 when hosts were exposed at 32°C than at 20°C (20°C infection establishment: $p=0.0013$; 32°C
278 infection establishment: $p<0.0001$). Temperature during infection establishment exerted no effect
279 on transmission rate (20°C exposure: $p=0.10$; 32°C exposure: $p=0.31$). When exposure and
280 establishment temperatures were equal (as in nature; the 20/20 and 32/32°C treatments here),
281 transmission rate was higher at 32°C than at 20°C ($p=0.0068$). Thus, even at maximal
282 epilimnetic temperatures, the impacts of higher temperatures on transmission rate promoted
283 rather than inhibited disease.

284 The thermal response of transmission rate was mechanistically driven by foraging rate of
285 hosts (f), not spore infectivity from within-host processes (u). Foraging rate increased from 20 to
286 25°C ($PD=0$; see *Methods* and Appendix for a description of PD values, which are analogous but
287 not identical to p -values) and then plateaued at 30°C ($PD=0.11$; Fig. 2B). Thus, hosts encounter
288 more spores at 25 and 30°C than at 20°C. After we accounted for predicted host-parasite contact,
289 spore infectivity was fairly insensitive to high temperatures (Fig. 2C). Temperature during
290 infection establishment did not impact spore infectivity (20°C exposure: $p=0.10$; 32°C exposure:
291 $p=0.31$). Exposure temperature increased spore infectivity (20°C infection establishment:
292 $p=0.034$; 32°C infection establishment: $p=0.0052$), but in the opposite direction of the
293 hypothesized mechanism (hotter temperature increased infectivity). When exposure and infection
294 establishment temperatures were equal (as in nature), spore infectivity did not differ ($p=0.37$).
295 Thus, high temperatures increased the foraging rate of hosts, elevating host contact with spores,
296 while spore infectivity barely changed. These changes in parasite exposure led to more
297 transmission at high temperatures.

298

299 **Mechanism 3: Spore yield (σ) and other measures of host and parasite growth**

300 Final spore yield (σ) in hosts that died from infection was lower at 32°C than at 20 and
301 26°C (Fig. 3A; best-fitting model had two means, see Table S5 for model AIC scores and Akaike
302 weights). This pattern was not explained by host condition estimated via growth rate. Host
303 growth rate (g_h , Fig. 3B) always increased with temperature (20 versus 26°C: $p=4.7\times 10^{-6}$; 26 vs.
304 32°C: $p=0.00038$). Instead, the pattern was explained by a combination of host death rates and
305 delays in spore maturation. Infected hosts died more quickly at 26°C than 20°C ($p<0.0001$), and
306 death rate trended higher from 26 to 32°C ($p=0.063$; Fig. 3C). Meanwhile, growth rate of mature
307 parasite spores (g_p , time series in Fig. 3D, linear slopes [growth rate] in Fig. 3E) did not change

308 with temperature ($PD>0.15$). However, temperature did affect the timing of initial spore
309 production within hosts (i.e., intercepts of linear model). At the earliest point in the sacrifice
310 series (day 8), spore load was highest at 26°C, intermediate at 32°C, and nearly zero at 20°C
311 (Fig. 3D). Given thermally insensitive daily growth rates of parasites (g_P ; Fig. 3E), these head-
312 starts were maintained over time (Fig. 3D). This effect on early spore production, coupled with
313 host death rate (Fig 3C), explains the spore yield pattern. Final spore yield was lower at 32 than
314 26°C because there were fewer spores initially (on day 8) and hosts died more quickly (less time
315 to produce spores). At 20°C, spore production started even later, but the delay was compensated
316 for by much longer lifespans of infected hosts (lower death rate, d ; Fig. 3C).

317

318 **Mechanisms 4 & 5: rearing (ρ) and free-living spore (ϕ) effects on infectivity**

319 Spore infectivity (measured as transmission rate) responded unimodally to temperature in
320 the previous infection (rearing effect on spore quality, ρ ; Fig. 4A). Infectivity increased
321 significantly for spores made at 20 versus 26°C for one of two spore sources ($p=0.0083$ for
322 spores from $\beta + u$ measurement assay [square, Fig 4A]; $p=0.092$ for spores from within-host
323 growth assay [diamond]). Infectivity then declined for spores made at 26 versus 32°C ($p=0.0001$
324 for both spore sources). Infectivity was significantly lower for spores made at 32 versus 20°C for
325 one of two spore sources ($p=0.16$ for spores from $\beta + u$ measurement assay [square]; $p=0.026$ for
326 spores from within-host growth assay [diamond]). The parameter ρ (Fig. 4C) shows the rearing
327 effect pooled for both spore sources and normalized by transmission rate at 20°C (used for
328 calculating transmission potential).

329 The thermal environment of free-living spores also impacted their infectivity (ϕ ; Fig
330 4B,D). Spore infectivity decreased with higher incubation temperatures after 7 days (20 versus
331 25°C: $p=0.0031$; 25 versus 30°C: $p<0.0001$; diamonds on Fig. 4B). However, spore infectivity
332 did not change after 1-day incubations (flat line in Fig. 4B [squares]; 20 versus 25°C: $p=0.65$, 25
333 versus 30°C: $p=0.64$). All 1-day incubations used stored (refrigerated) spores. They had lower
334 infectivity than the 7-day incubation at 20°C, likely because storage at 4°C also lowers spore
335 infectivity (1 versus 7-day incubations at 20°C: $p<0.0001$) (Duffy & Hunsberger, 2019). The
336 parameter ϕ (Fig. 4D) shows the free-living spore effect assuming that spores lose infectivity
337 gradually over seven days as they are consumed by hosts (see *Methods* and Appendix) and
338 normalized by transmission rate at 20°C (used for calculating transmission potential).

339

340 **Transmission potential ($f u \sigma \rho \phi$)**

341 Transmission potential, the product of all five mechanisms ($f u \sigma \rho \phi$), responded
342 unimodally to high temperatures. This metric first increased from 20 to 25/26°C ($PD=0.017$);
343 then, it decreased from 25/26 to 30/32°C ($PD=0.0001$; Fig. 5A, ‘full transmission potential’).
344 Transmission potential at 30/32°C trended (non-significantly) lower than at 20°C ($PD=0.11$).
345 Thus, high temperatures do not constrain disease enough via these five mechanisms to explain
346 the absence of summer epidemics.

347 The initial increase in transmission potential from 20 to 25/26°C was driven most
348 strongly by host foraging (f , mechanism 1) and the rearing effect on spore quality (ρ , mechanism
349 4): holding either trait constant removes the significant difference between temperatures (Fig. 5B
350 and 5E, respectively). The subsequent drop in transmission potential from 25/26 to 30/32°C was
351 driven most strongly by the rearing effect (ρ): holding it constant again removes the significant
352 difference (Fig. 5E). Harm to free-living spores (ϕ , mechanism 5) also contributes somewhat
353 (Fig. 5F vs. Fig. 5A), though not enough to affect the statistical significance. Additionally, the
354 thermal response of host foraging (f) is key for maintaining transmission at high temperatures:
355 without increased exposure to spores, the remaining mechanisms would significantly reduce
356 transmission at 30/32°C compared to 20°C (Fig 5B). Spore infectivity from within-host
357 processes (u , mechanism 2) and spore yield (σ , mechanism 3) had no effect (Fig. 5C vs. Fig. 5A)
358 and very little effect (Fig. 5D vs. Fig. 5A) on transmission potential, respectively.

359

360

DISCUSSION

361 We investigated upper thermal constraints on fungal epidemics in a *Daphnia* zooplankton
362 host. The seasonality of the autumnal epidemics suggested that hot conditions might constrain
363 disease: epidemics usually start after lakes cool from maximal summer temperatures in the
364 epilimnion (29-32°C). We tested five potential thermal constraints on transmission. First,
365 foraging (exposure) rate of hosts (f) increased at high temperatures (Fig 2B), while, second, high
366 temperatures did not affect the infectivity of spores from within-host processes (u ; Fig 2C). Thus,
367 high temperatures increased transmission rate, β (where $\beta=uf$; Fig 2A). Third, spore yield (σ)
368 declined slightly at 32°C (Fig 3A). Fourth, a rearing effect on spore quality driven by
369 temperature during the previous infection (ρ) emerged: spores made at 32°C were less infectious

370 than those made at 26°C and sometimes 20°C (for one of two spore sources, Fig 4A). Finally,
371 harm to free-living spores (ϕ) lowered infectivity as temperature increased (Fig 4B). Overall,
372 transmission potential is much lower at 32°C than 26°C, but still similar to at 20°C (Fig 5A), a
373 temperature that easily supports epidemics in both nature (Shocket, Strauss, et al., 2018) and
374 laboratory environments (Civitello et al., 2012; Shocket, Strauss, et al., 2018). Thus, maximally
375 high temperatures do constrain disease, but not sufficiently to explain the absence of summer
376 epidemics on their own.

377 Contrary to our initial hypothesis, high temperatures *increased* transmission rate (Fig
378 2A). In principle, high temperatures can lower infection success if pathogens tolerate heat less
379 well than hosts (Thomas & Blanford, 2003). For instance, many ectothermic hosts behaviorally
380 induce fever to reduce the negative costs of infection (Rakus et al., 2017; Stahlschmidt &
381 Adamo, 2013). Further, fungi are particularly sensitive to high temperatures compared to other
382 pathogen taxa (Robert & Casadevall, 2009) and fungal pathogens are often limited by high
383 temperatures (Berger et al., 2004; Carruthers et al., 1992; Langwig et al., 2015; Raffel et al.,
384 2010; Thomas & Blanford, 2003). However, high temperatures did not interfere with this
385 fungus's success at either stage of transmission: the day of exposure, when most spores penetrate
386 the host's gut, or infection establishment, when the fungus replicates and develops within the
387 host (Stewart Merrill & Cáceres, 2018). Instead, high temperatures elevated host foraging rate
388 (Fig 2B), which increases exposure to parasites, thereby increasing transmission rate (Hall et al.,
389 2007). In lakes, the thermal response of foraging (exposure) drives variation in the size of
390 epidemics, which occur in autumn: epidemics that start earlier in warmer conditions grow larger
391 than those starting later and colder (Shocket, Strauss, et al., 2018). This foraging-controlled
392 exposure to parasites is a potentially general mechanism: higher temperatures also increase
393 outbreak size for armyworms that consume virus particles on leaves (Elder & Reilly, 2014).
394 However, transmission plateaued with temperature for another ingested *Daphnia* pathogen (Vale,
395 Stjernman, & Little, 2008).

396 Spore yield (σ) declined at the highest temperature (32°C; Fig. 3). Although the effect on
397 transmission potential was minimal (Fig 5D), the results for related traits provide mechanistic
398 insights into host-parasite interactions. Parasite burdens often decline at temperatures near the
399 thermal maxima of the host and/or parasite, e.g., for nematodes in slugs (Wilson, Digweed,
400 Brown, Ivanonva, & Hapca, 2015), trematodes in snails (Paull, Lafonte, & Johnson, 2012),

401 bacteria in *Daphnia* (Vale et al., 2008) and fruit flies (Lazzaro, Flores, Lorigan, & Yourth, 2008),
402 and powdery mildew in plants (Laine, 2007). In theory, reduced parasite production at hot
403 temperatures could arise from several mechanisms. First, parasite production could decline if
404 host growth slows, since spore yield often scales with host growth, at least along resource
405 gradients (Hall, Knight, et al., 2009; Hall, Simonis, et al., 2009). However, here host growth rate
406 (g_h) increased with temperature while spore yield was flat and then decreased (Fig 3B).
407 Therefore, spore production was decoupled from host growth rate (i.e., the link between host
408 growth and parasite production that occurs for resources did not occur for temperature). Second,
409 the parasite itself could grow more slowly at high temperatures. For example, high temperatures
410 slow bacterial growth inside fruit flies (Lazzaro et al., 2008), fungal growth in grasshoppers
411 (Springate & Thomas, 2005), and fungal growth on warm-adapted (but not cold-adapted)
412 amphibians (Cohen et al., 2017). In contrast, here parasite growth rate (g_p) did not respond to
413 temperature (slope in Fig 3D; Fig 3E).

414 Instead, the decline in spore production at high temperatures arose from a combination of
415 host death rate and the timing of initial spore production. Temperature determined spore load on
416 day 8 (the earliest sampling time in the assay; Fig. 3D). Based on that information (and the
417 constant parasite growth rates, Fig 3E), we infer that spore production began earliest at 26°C,
418 followed by 32°C, and then 20°C. These head starts were maintained over time and explain the
419 spore yield pattern when combined with death rate of infected hosts (Fig. 3C). In general, shorter
420 lifespan of infected hosts decreases time for spore production, thereby depressing spore yield
421 (Auld et al., 2014; Civitello et al., 2012). Here, spore yield was lower at 32 than 26°C because
422 spore production started later and hosts died more quickly. At 20°C, spore production started
423 even later, but longer host lifespan compensated for this delay (i.e., the fungus had longer to
424 grow within hosts). Do similar patterns exist in other systems? Unfortunately, few studies focus
425 on traits underlying thermal responses of parasite load. Hence questions remain: How often does
426 temperature change the timing versus the rate of parasite production? How often does
427 temperature decouple positive relationships between host growth and parasite production? The
428 answers matter because spore yield can influence epidemic size for obligate killer parasites (like
429 the fungus here: Civitello et al., 2015). Thus, developing a general framework from data across
430 host-parasite systems remains a fruitful area for future research.

431 High temperatures reduced transmission potential via two effects on spore infectivity that

432 act outside the focal host. First, a rearing effect on spore quality (ρ) driven by temperature of
433 spore production in the previous host elevated (26°C) and then lowered (32°C) spore infectivity
434 (compared to 20°C). Rearing effects on parasite performance can arise with variation in
435 resources consumed by hosts (Cornet, Bichet, Larcombe, Faivre, & Sorci, 2014; Little, Birch,
436 Vale, & Tseng, 2007; Tseng, 2006), temperature experienced by hosts (Shocket, Vergara, et al.,
437 2018), or host genotype (Searle et al., 2015). These understudied rearing effects may drive
438 performance of parasites to an unappreciated extent (Shocket, Vergara, et al., 2018). Second,
439 harm to free-living spores (ϕ , including spore mortality) also inhibited infection at high
440 temperatures. After seven days in 30°C, spores lost 92% of their initial infectivity. This
441 constraint may arise in other systems: for example, high temperatures elevate mortality in free-
442 living helminths of Arctic ungulates (Molnár et al., 2013). However, in the planktonic system
443 here, the 7-day result likely exaggerates the thermal constraint. While difficult to quantify,
444 physical sinking, consumption (Civitello, Pearsall, Duffy, & Hall, 2013; Penczykowski, Hall, et
445 al., 2014; Shocket, Vergara, et al., 2018; Strauss, Civitello, Cáceres, & Hall, 2015) and damage
446 from radiation (Overholt et al., 2012) likely remove most spores before 7 days. To acknowledge
447 this mortality, we weighted this component of infectivity (ϕ) using a model of spore longevity.
448 Assuming this modeled weighting reflects reality in lakes, the free-living effect lacks enough
449 strength to inhibit epidemics during summer, even when combined with the other mechanisms
450 (see Appendix for sensitivity analysis of the time-weighting model). However, more realistic
451 dynamical models and better resolved trait data for the free-living spore effect could change the
452 estimates for how high temperatures affect transmission.

453 Although the impact of temperature on these five mechanisms does not explain the lack
454 of epidemics during summer, other co-varying environmental factors could combine with
455 thermal effects to sufficiently inhibit transmission. Such factors include damage to free-living
456 spores by solar radiation (Overholt et al., 2012), consumption of spores by resistant zooplankton
457 species that are more abundant earlier in the year (Penczykowski, Hall, et al., 2014), and low
458 spore production due to poor quality of host food resources (Hall, Knight, et al., 2009). These
459 mechanisms could contribute to the observed field pattern, and interact with the thermal effects
460 examined here. Furthermore, climate change could disrupt covariation among drivers. For
461 example, high temperatures may persist later in the year when damaging solar radiation is less
462 intense. Incorporating these other factors may help explain the current field pattern and improve

463 predictions for how climate change will impact epidemics. These predictions should also
464 explicitly account for the effects of temperature variation and extremes, which have distinct
465 impacts on organismal performance (Dowd, King, & Denny, 2015). Here, we employed a
466 relevant form of thermal variation, mimicking migratory behavior of hosts in stratified lakes, but
467 did not isolate effects of thermal variation. Future efforts could estimate this effect to better
468 predict how climate change will impact the host, the parasite, and their interaction.

469 The current prevailing view argues that hot temperatures should constrain disease
470 transmission in nature (Altizer et al., 2013; Lafferty, 2009; Lafferty & Mordecai, 2016). This
471 constraint arises when unimodal thermal reaction norms depress key traits that drive disease
472 spread. However, such constraints have been rigorously tested in only a handful of systems.
473 Here, we hypothesized that high summer temperatures limit transmission of a zooplankton-
474 fungus disease system with autumnal epidemics (i.e., during cooler conditions). High
475 temperatures constrained disease transmission enough to produce a unimodal thermal response.
476 This response arose primarily through a rearing effect on spore quality and due to harm to free-
477 living spores. However, the thermal mechanisms estimated here were not sufficient explain the
478 lack of summer epidemics. Hence, we draw two major lessons. First, we need to continue to
479 rigorously evaluate multiple mechanisms of thermal constraints on components of disease
480 transmission. Second, our example cautions against drawing conclusions about constraints on
481 disease from warming based on field patterns and theory alone.

482
483 **Acknowledgements:** K. Boatman (2009 and 2010), Z. Brown and K. Malins (2011), O. Schmidt
484 (2013), A. Bowling (2014), and P. Orlando and J. Walsman (2015) conducted field sampling.
485 MSS was supported by NSF GRFP. This work was supported by NSF DEB 0841679, 0841817,
486 1120316, 1120804, 1353749, and 1354407.

487 **Author Contributions:** SRH, CEC, and MAD designed and obtained funding for the field
488 survey. SRH and MSS collected field data. MSS and SRH designed the laboratory studies; MSS
489 and AM conducted them. MSS wrote the first draft of the manuscript, and all authors revised and
490 approved the final version.

491 **Data Accessibility:** Data and code are deposited in the Dryad repository:
492 <http://doi.org/10.5061/dryad.6mf734p>. (Shocket, Magnante, Duffy, Cáceres, & Hall,
493 2019). **Table 1:** The experiments (and spore sources) used to test the five mechanistic

494 components of disease transmission.

495

Mechanism	Experiment(s)	Spore source(s)
1. Foraging rate (f , Fig. 2B)	Foraging rate assay	NA
2. Within-host spore infectivity (u , Fig. 2C)	$\beta + u$ measurement assay Foraging rate assay	General lab stock
3. Spore yield (σ , Fig. 3A)	$\beta + u$ measurement assay Within-host parasite growth assay	General lab stock
4. Rearing effect on infectivity (ρ , Fig 4A,C)	Common garden infection assay #1	$\beta + u$ measurement assay Within-host parasite growth assay
5. Free-living spore effect on infectivity (φ , Fig 4B,D)	Common garden infection assay #2	General lab stock incubated at different temperatures

496

497 **Figure 1: Motivating field pattern and mechanistic components of transmission.** (A) Fungal
498 epidemics usually start (dark grey bars) after lakes have cooled from the maximum summer
499 temperature (light grey bars). Epidemics never started when the epilimnion (upper, warmer
500 layer) was hotter than 30°C, suggesting an upper thermal constraint. Data summarize 74
501 epidemics from 20 lakes in Indiana (USA) sampled from 2009-2015. (B) High temperature could
502 limit transmission via five mechanisms. 1-2) Hosts become infected at transmission rate β , which
503 can be divided into 1) host foraging rate (f), i.e., exposure to spores, and 2) spore infectivity, as
504 determined by within-host processes (u). 3) Parasite spores are produced at spore yield (σ). 4) A
505 rearing effect from temperature during the previous infection (ρ) determines initial spore
506 infectivity. 5) Harm to free-living spores (φ) might also impact their infectivity. The product of
507 all five components ($f u \sigma \rho \varphi$) determines ‘transmission potential’.

508

509 **Figure 2: High temperature impacts on transmission rate (β), foraging rate (f , mechanism**
510 **1) and spore infectivity from current within-host processes (u , mechanism 2).** In A and C,
511 the effect of high temperature during parasite exposure and infection establishment (20°C
512 infection establishment = white circles, solid line; 32°C infection establishment = dark grey
513 circles, dotted line). (A) Transmission rate (β) increased when hosts were exposed at 32°C and
514 did not change with infection establishment temperature. For constant temperatures, transmission
515 is higher at 32°C than at 20°C. (B) Foraging (exposure) rate of hosts (f) is higher at 26°C (light
516 grey) and 32°C (dark grey) than at 20°C (white). (C) Spore infectivity ($u=\beta/f$) increased when
517 hosts were exposed at 32°C for both infection establishment temperatures. However, for constant
518 temperatures, infectivity did not differ between 20 and 32°C. Error bars show 95% CIs. Letters
519 indicate significant differences.

520

521 **Figure 3: High temperature impacts on spore yield (σ , mechanism 3) and possible**
522 **underlying traits.** (A) Final spore yield at host death (σ) was lower at 32°C (dark grey) than at
523 20°C (white) or 26°C (light grey). (B) Host growth rate (g_h) increased with temperature for all
524 treatments. (C) Death rate of infected hosts (d) increased from 20 to 26°C and trended higher
525 from 26 to 32°C. (D) Spore load within hosts through time at 32°C (dotted line), 26°C (dashed
526 line), and 20°C (solid line), fit with linear models. 26°C points are shifted over for visual clarity
527 of error bars. (E) Parasite growth rate (g_p , slopes of lines in panel D) did not change with
528 temperature. Hence, declining σ stems from higher death rate of infected hosts and low initial
529 parasite growth, not slower growth rates of hosts or parasites. (A-C,E) Error bars show 95% CIs.
530 (D) Error bars show SE; square points are single hosts. Letters indicate significant differences.

531

532 **Figure 4: High temperature impacts on a rearing effect (ρ , mechanism 4) and harm to free-**
533 **living spores (ϕ , mechanism 5).** Variation in transmission rate from common garden infection
534 assays reflects differences in spore infectivity. (A) Spores came from the $\beta + u$ measurement
535 assay (Fig. 2; squares) and the within host parasite growth assay ('WHPG'; Fig. 3; diamonds).
536 Spore infectivity increased with rearing temperature from 20°C (white) to 26°C (light grey; $\beta + u$
537 only) and decreased with rearing temperatures from 26°C to 32°C (dark grey, both spore
538 sources). Spore infectivity was lower at 32°C than at 20°C (WHPG spores only). (B) Spore
539 infectivity decreased when free-living spores were incubated in high temperatures for 7 days but

540 not for 1 day. Storage at 4°C for 6 days (for all 1-day incubations) also lowered spore infectivity
541 relative to the 7-day incubation at 20°C. (C-D) Parameter values (transmission rate scaled by
542 values at 20°C) for (C) rearing effect, ρ , and (D) free-living effect, φ . Phi values also based on
543 time-weighted model (see text for details). Error bars show 95% CIs. Letters indicate significant
544 differences.

545

546 **Figure 5: High temperature impacts on transmission potential.** (A) Transmission potential
547 ($fu\sigma\rho\varphi$) responds unimodally, increasing from 20°C (white) to 25/26°C (light grey) and
548 decreasing from 25/26°C to 30/32°C (dark grey). (B-F) Transmission potential with each
549 mechanism held constant to show sensitivity to each parameter: (B) foraging rate (f), (C) spore
550 infectivity from within-host effects (u), (D) spore yield (σ), (E) rearing effect (ρ), and (F) harm
551 free-living spores (φ). The rearing effect (E) has the largest impact on transmission potential
552 (hence, without it, the response of transmission potential is flat with temperature). Error bars
553 show 95% CIs. Letters indicate significant differences. Y-axis is ln-transformed.

554

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735

736 SUPPORTING INFORMATION

737 Additional supporting information may be found in the online version of this article.

738 Appendix S1: Methods, Figures, and Tables

739 Figure S1: Components of a simple model used to estimate parameter φ .

740 Figure S2: Sensitivity analysis for spore consumption model parameter (c) affecting damage to
741 free-living spores (φ) and transmission potential.

742 Table S1: Raw results from the $\beta + u$ measurement assay.

743 Table S2: Sample sizes for estimating spore yield (σ) and related traits.

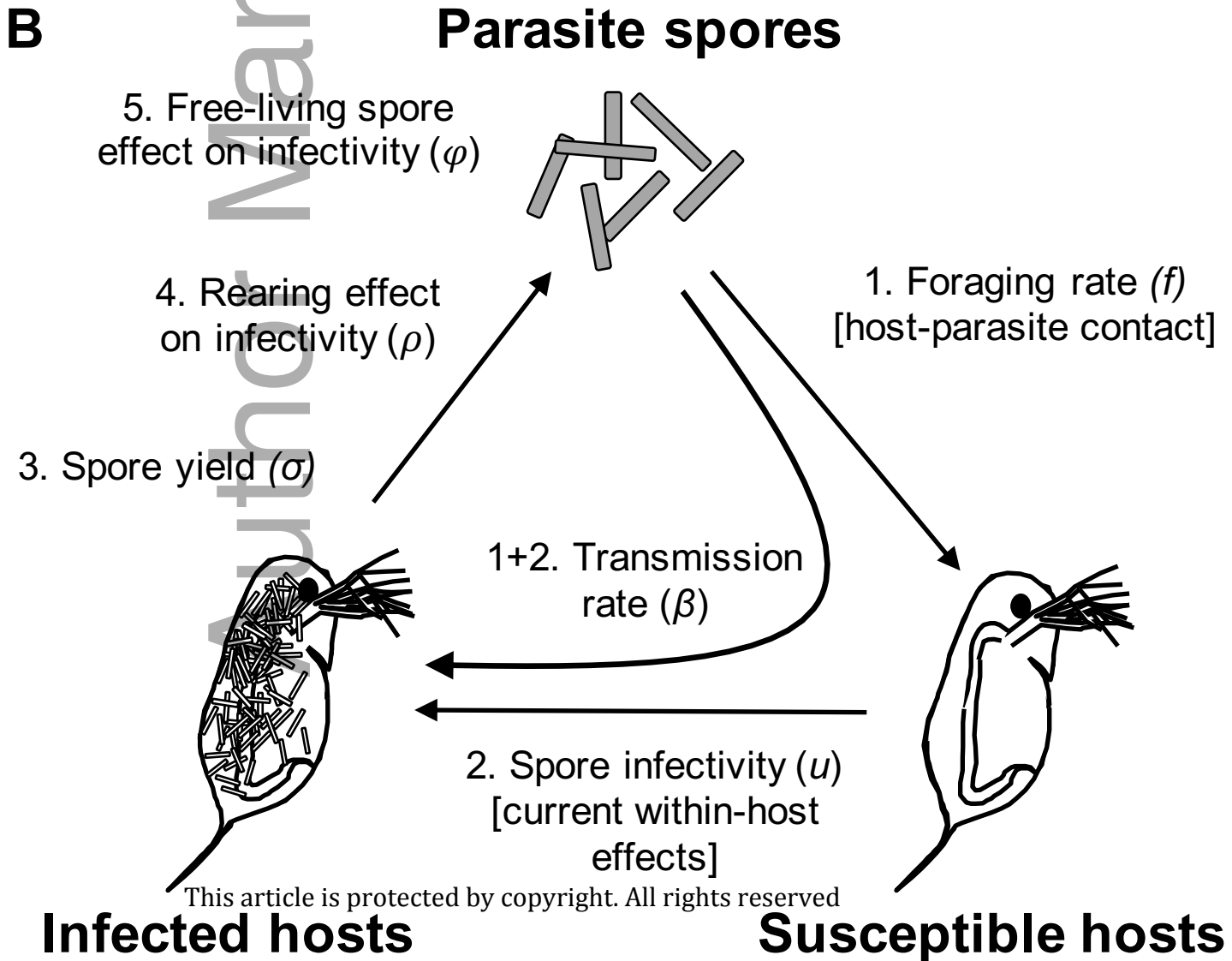
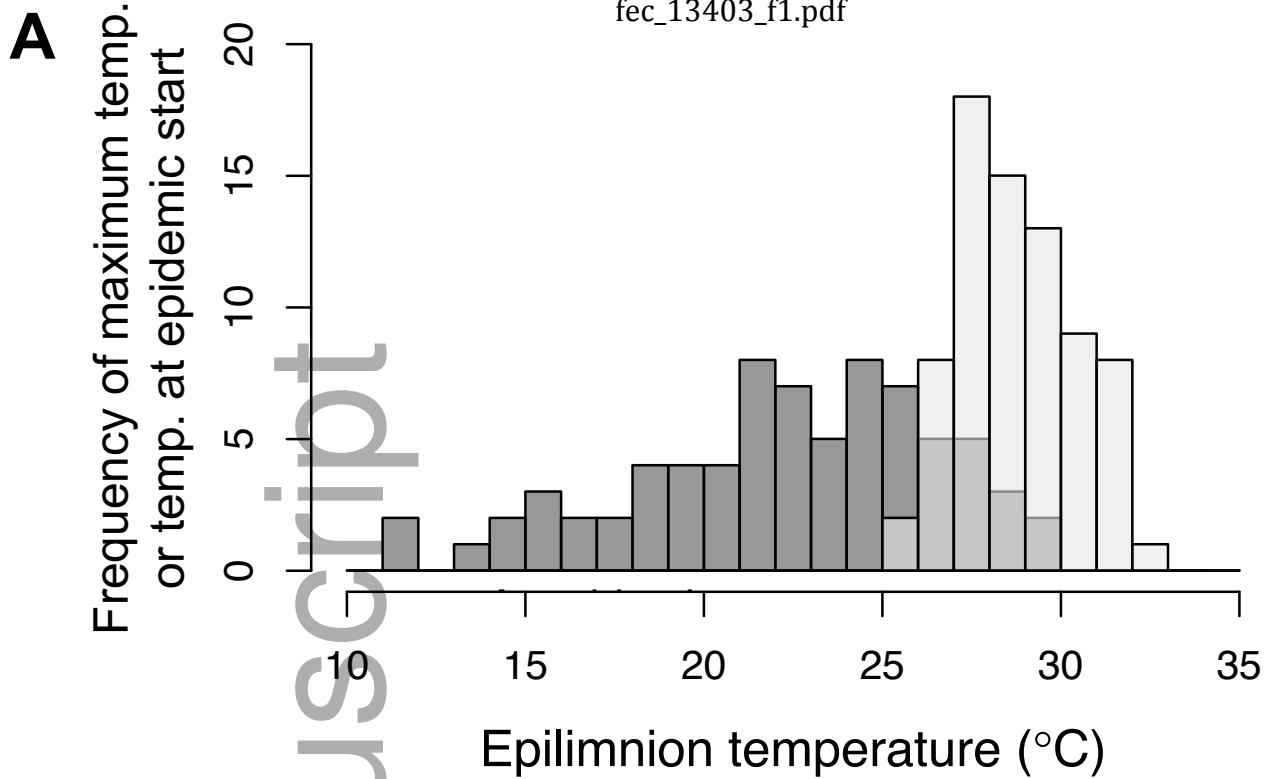
744 Table S3: Raw results from the rearing effect (ρ) and free-living spore effect (φ) assays.

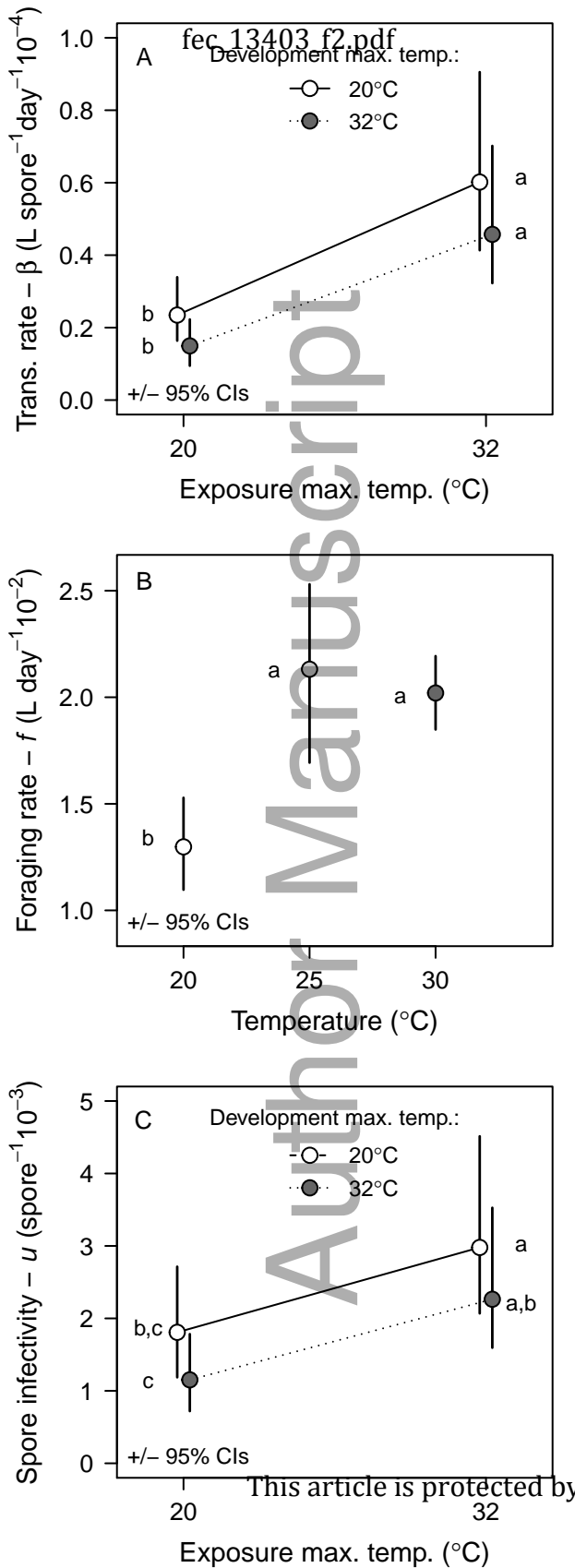
745 Table S4: p -values from randomization tests.

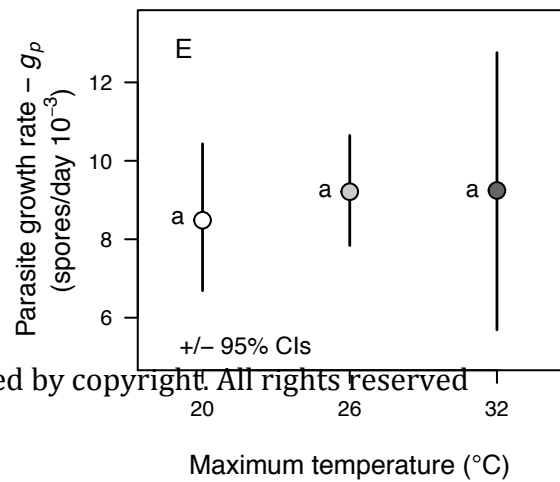
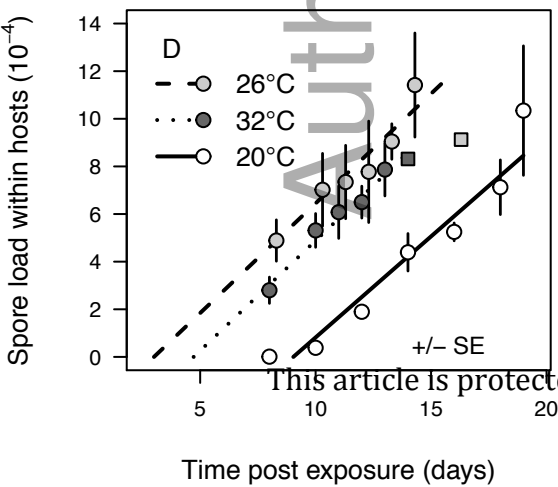
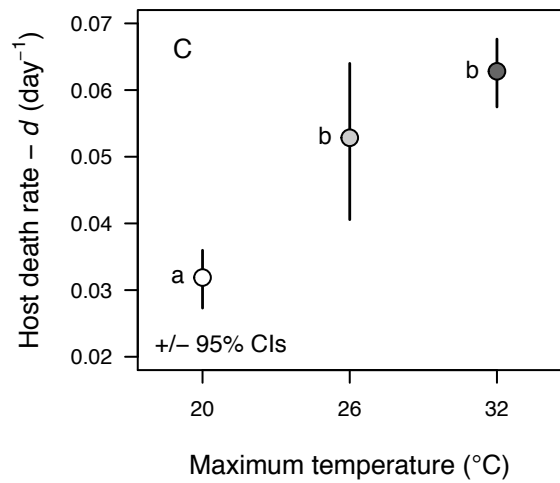
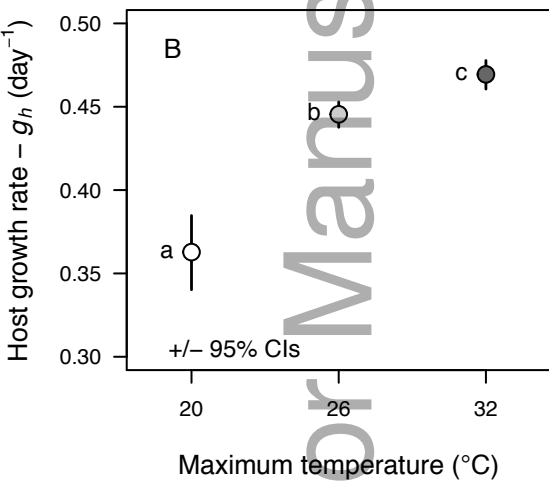
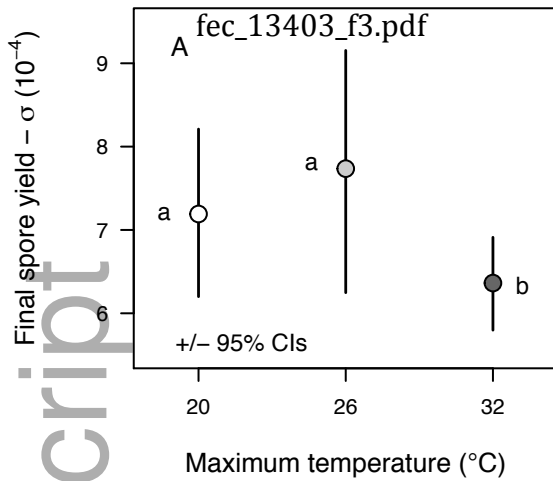
746 Table S5: p -values and Δ AIC from model selection.

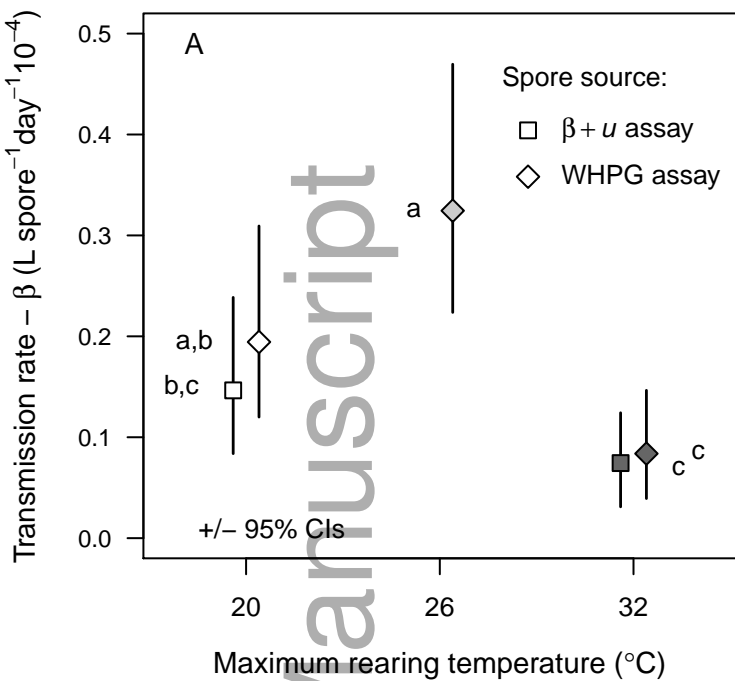
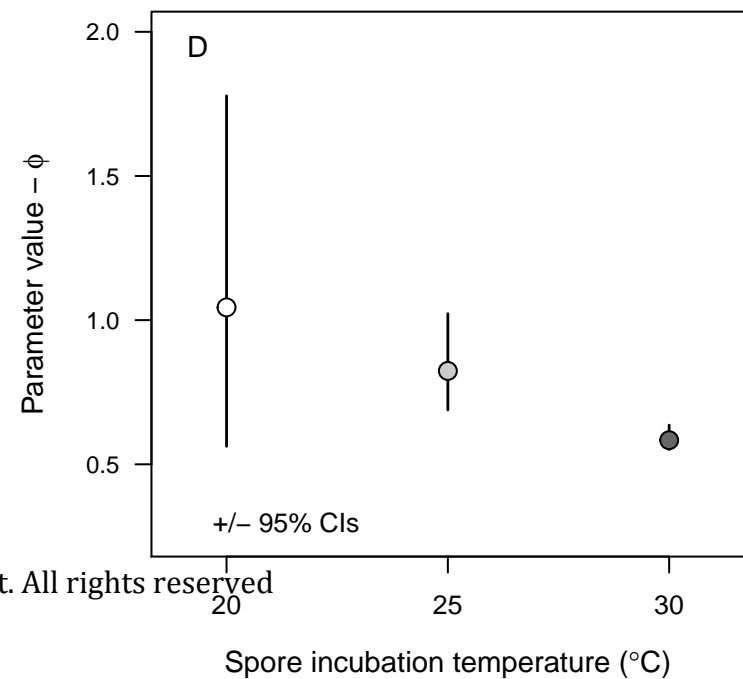
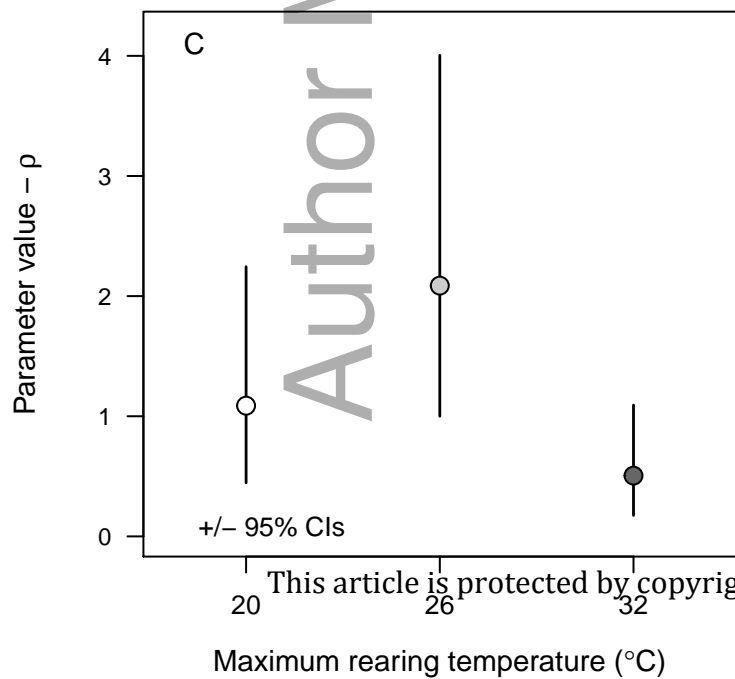
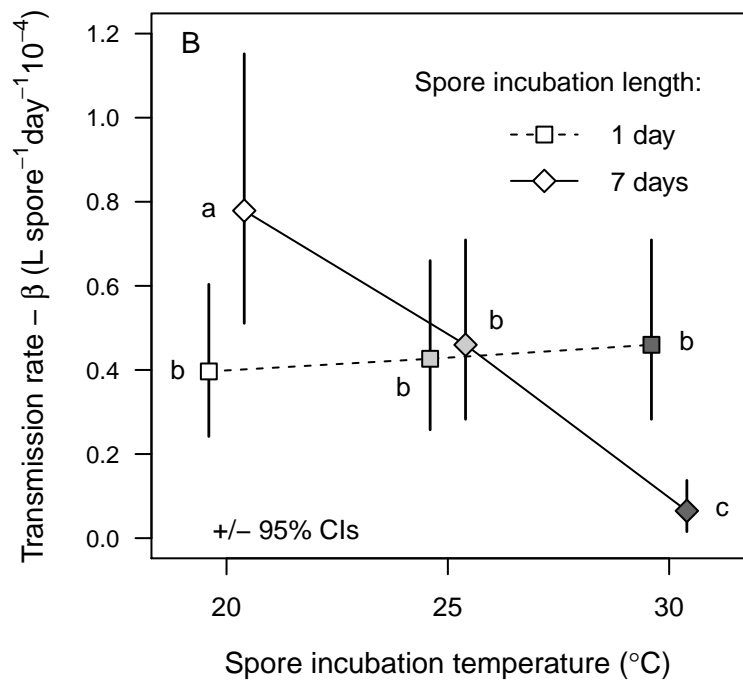
747 Table S6: PD (probability density) values for traits.

748 Table S7: PD (probability density) values for transmission potential ‘sensitivity analysis’
749 calculations.



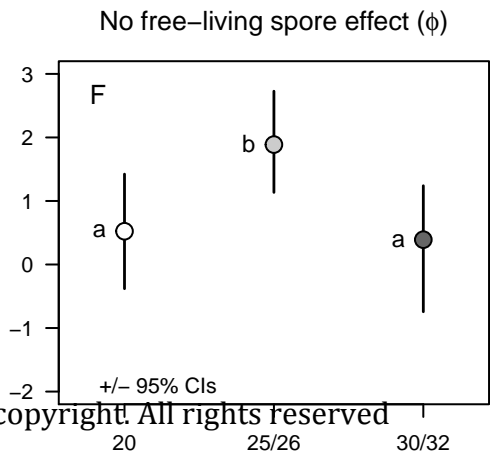
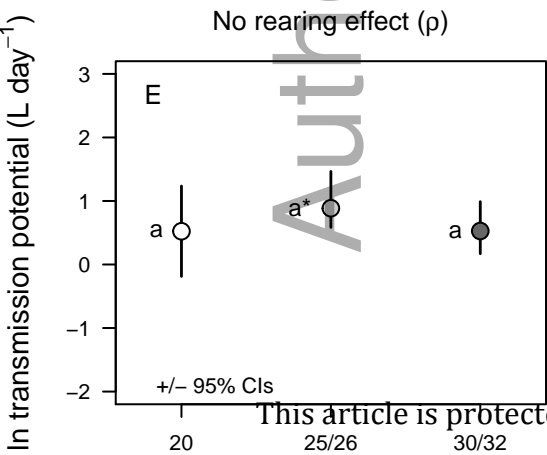
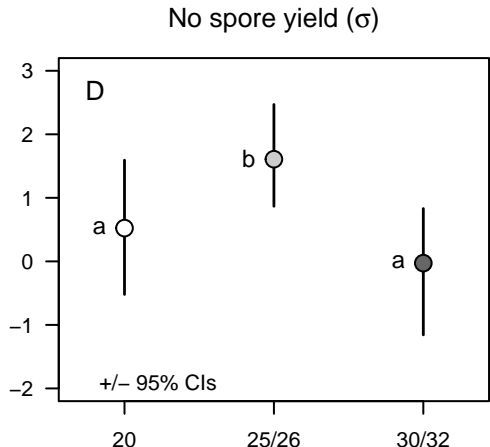
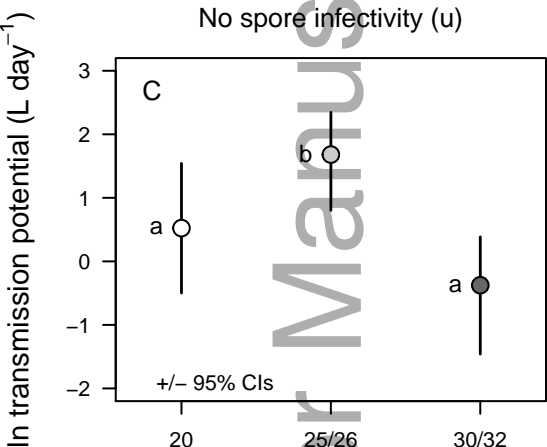
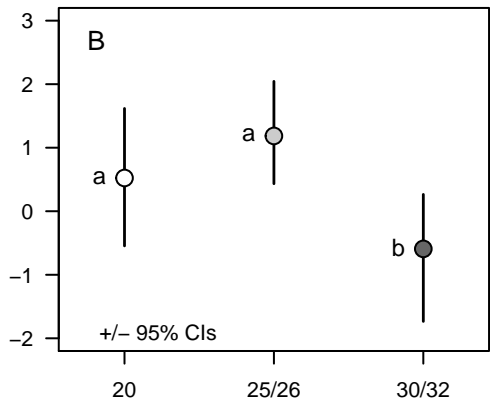
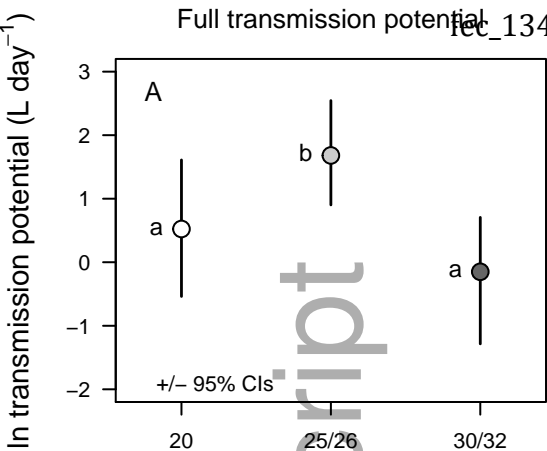




Rearing effect – ρ Free-living spore effect – ϕ 

Full transmission potential

No foraging rate (f)



Maximum temperature (°C)

Maximum temperature (°C)