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

Marta S. Shocket[1*†] (mshocket@stanford.edu), Alexandra Magnante[1], Meghan A. Duffy[2], Carla E. Cáceres[3], and Spencer R. Hall[1]

[1] Department of Biology, Indiana University, Bloomington, IN 47405 USA

[2] Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109 USA

[3] School of Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

* Corresponding Author: Marta S. Shocket, 650-723-5923, mshocket@ucla.edu

† Present Address: Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, USA

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

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

Data Accessibility: Upon acceptance all data and code will be stored in Dryad Data Repository.

ABSTRACT (316 / 350 WORDS)

1. Thermal ecology theory predicts that transmission of infectious diseases should respond unimodally to temperature, i.e., be maximized at intermediate temperatures and constrained at extreme low and high temperatures. However, empirical evidence linking hot temperatures to decreased transmission in nature remains limited.
2. We tested the hypothesis that hot temperatures constrain transmission in a zooplankton-fungus (*Daphnia dentifera-Metschnikowia bicuspidata*) disease system where autumnal epidemics typically start after lakes cool from their peak summer temperatures. This pattern suggested that maximally hot summer temperatures could be inhibiting disease spread.
3. Using a series of lab experiments, we examined the effects of high temperatures on five mechanistic components of transmission. We found that (1) high temperatures increased exposure to parasites by speeding up foraging rate but (2) did not alter infection success post-exposure. (3) High temperatures lowered parasite production (due to faster host death and an inferred delay in parasite growth). (4) Parasites made in hot conditions were less infectious to the next host (instilling a parasite ‘rearing’ or 'trans-host' effect of temperature during the prior infection). (5) High temperatures in the free-living stage also reduce parasite infectivity, either by killing or harming parasites.
4. We then assembled the five mechanisms into an index of disease spread. The resulting unimodal thermal response was most strongly driven by the rearing effect. Transmission peaked at intermediate-hot temperatures (25-26°C) and then decreased at maximally hot temperatures (30-32°C). However, transmission at these maximally hot temperatures only trended slightly lower than the baseline control (20°C), which easily sustains epidemics

in laboratory conditions and in nature. Overall, we conclude that while exposure to hot epilimnetic temperatures does somewhat constrain disease, we lack evidence that this effect fully explains the lack of summer epidemics in this natural system. This work demonstrates the importance of experimentally testing hypothesized mechanisms of thermal constraints on disease transmission. Furthermore, it cautions against drawing conclusions based on field patterns and theory alone.

52 **INTRODUCTION**

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

73 Conceptually, upper thermal constraints act like fever, taking advantage of a common
74 thermal mismatch between hosts and parasites. Because hosts can often endure hotter

75 environments than their parasites, many animals increase their body temperature when infected
76 (see citations below). In ectotherms, fever arises from behavioral thermoregulation (microhabitat
77 selection) and is widespread, occurring in vertebrates (including amphibians, reptiles, and fish:
78 Rakus, Ronsmans, & Vanderplasschen, 2017), snails (Zbikowska, Wrotek, Cichy, & Kozak,
79 2013), and insects (including bees, flies, grasshoppers, mosquitoes, and beetles: Stahlschmidt &
80 Adamo, 2013; Thomas & Blanford, 2003). Behavioral fever can impair parasite performance,
81 enhancing clearance or reducing virulence of infection. An analogous process can occur within
82 ectothermic hosts inhabiting high ambient temperatures (regardless of infection status)—in
83 essence, an environmental fever. High ambient temperatures can also harm parasites with free-
84 living stages outside of hosts. Mechanistically linking high temperatures to reduced disease
85 requires examining thermal effects on components of the transmission process (McCallum et al.
86 2017). We use the term 'transmission (process)' to broadly refer to the full parasite life cycle,
87 including infective propagule production and propagule survival in the environment; we also use
88 'transmission rate' narrowly defined as the rate of new infections (i.e., the parameter 'β'
89 calculated from infection prevalence and densities of hosts and parasites; McCallum et al. 2017).
90 Here, we use a series of experiments to evaluate mechanisms for potential upper thermal
91 constraints on transmission in a planktonic-fungal disease system. Autumnal epidemics start
92 once lake waters cool below summer maxima (Fig. 1A). These delayed starts could reflect hot
93 temperatures inhibiting disease if they push any of five transmission components past their
94 thermal optima (Fig. 1B). First, hot temperatures could slow host feeding and lower
95 consumption-based exposure to parasites. Second, hot temperatures could reduce parasite
96 infectivity inside hosts, lowering the probability of successful infection (via effects on hosts
97 and/or parasites). Third, hot temperatures could decrease the quantity of parasite propagules

98 [spores] produced by an infection. This decrease could stem from slower host growth rate (since
99 parasite production often scales with host growth: Hall, Knight, et al., 2009; Hall, Simonis,
100 Nisbet, Tessier, & Cáceres, 2009), slower parasite growth independent from host growth, or
101 enhanced mortality of infected hosts (truncating production time; Auld, Hall, Housley Ochs,
102 Sebastian, & Duffy, 2014; Civitello, Forys, Johnson, & Hall, 2012). Fourth, hot temperatures
103 could lower the quality of parasite spores released from dead hosts into the environment
104 (Shocket, Vergara, et al., 2018). Finally, these free-living spores could be harmed or killed by
105 hot temperatures. Thus, high temperatures could constrain this fungal disease at multiple stages
106 of the transmission process.
107
108 **STUDY SYSTEM**
109 The hosts (*Daphnia dentifera*) are zooplankton grazers in freshwater temperate lakes
110 across the Midwestern United States; the fungal parasite *Metschnikowia biscupidata* causes
111 epidemics in some host populations, with prevalence reaching up to 60% (Penczykowski, Hall,
112 Civitello, & Duffy, 2014). Hosts become infected when they filter-feed on algae and
113 inadvertently consume fungal spores (Hall et al., 2007). The spores pierce the host's gut wall,
114 entering its body cavity. Inside, fungal conidia replicate in the hemolymph before maturing into
115 new spores (Stewart Merrill & Cáceres, 2018). Following host death, spores are released into the
116 water for new hosts to consume (Ebert, 2005).
117 The seasonality of epidemics motivated a focus on high temperatures. Epidemics
118 typically begin in late summer or early fall (August–October) and wane in late fall or early
119 winter (November–December; Penczykowski, Hall, et al., 2014). During this time, lake water
120 temperature declines (Shocket, Strauss, et al., 2018). Many traits that influence disease spread

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

135 **METHODS**

136 **Field Survey**

137 Field survey data generated the motivating pattern (Fig. 1A: the relationship between
138 epidemic start date and epilimnetic temperature). We surveyed 10–28 lakes in Indiana (Greene
139 and Sullivan Counties) weekly (2009–2011) or bi-weekly (2013–2014) from August to
140 December. For each visit, we collected a zooplankton sample (13 cm diameter net with 153 µm
141 mesh) and measured lake water temperature data at 0.5–1 meter intervals with a Hydrolab
142 multiprobe (Hach Environmental). For each sample, we visually diagnosed 400+ live hosts with
143 a dissecting scope (20-50X magnification). An epidemic 'started' when infection prevalence first

144 exceeded 1% for two consecutive sampling visits (Shocket, Strauss, et al., 2018). We calculated
145 the epilimnetic temperature by fitting a spline to temperature across water depth, and averaging
146 from the water surface to the depth where the temperature gradient first exceeded 1°C m^{-1} (i.e.,
147 the thermocline; see Hite et al. 2016 Appendix S2).
148
149 **General Approach**
150 We measured how high temperatures influence five components of the transmission
151 process with laboratory assays (Table 1). Then we combined them into a synthetic index of
152 disease spread: 'transmission potential' (Auld et al., 2014). For mechanisms involving the host or
153 host-parasite interaction (mechanisms 1-3: foraging rate [f], spore infectivity from within-host
154 processes [u], and spore yield [σ]), we used fluctuating temperatures to expose hosts to high
155 temperatures for part of the day (they cannot survive constant temperatures >27°C). Hosts were
156 kept on a 16:8 light:dark cycle. All hosts experienced the same 20°C temperature for 8 hours,
157 and then 20, 26, or 32°C for 16 hours ('maximum temperature'). For mechanisms 4-5 (rearing
158 effect on spore quality [ρ] and free-living spore effect [φ]), we conducted common garden
159 infection assays, exposing uniform hosts at constant 20°C to spores from different treatments.
160 Thus, variation in transmission rate can be attributed to differences in spore infectivity.
161 Temperatures varied slightly among experiments (25 or 26°C, 30 or 32°C) based on incubator
162 availability. For calculating transmission potential, we treat temperature categorically and pool
163 these treatments.
164 Due to time and incubator constraints, we were unable to replicate experiments across
165 multiple incubators. Thus, our temperature treatments are 'pseudo-replicated' in that all
166 replicates for a treatment were conducted in the same incubator at the same time. Accordingly,

our results may be influenced by random incubator effects.

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

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

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

190

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

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

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

213 increased linearly over the full time series at 26 and 32ºC. Spore load plateaued after day 19 at
214 20ºC, so we truncated the time series to estimate the linear slope for only that portion. Finally,
215 we calculated death rate (d) of infected hosts (see Appendix), since spore yield can decline with
216 shorter host lifespan (Auld et al., 2014; Civitello et al., 2012). We compared treatments with
217 randomization tests.
218
219 **Mechanisms 4 & 5: rearing (ρ) and free-living spore (φ) effects on infectivity**
220 We measured how high temperatures modify spore infectivity prior to encountering hosts
221 via a rearing effect on baseline spore quality (ρ) and harm to free-living spores (φ). We
222 conducted infection assays on 'common garden' groups of hosts at 20°C using different spore
223 treatments (i.e., from different spore rearing temperatures for ρ and from different spore
224 incubation temperatures for φ). Thus, variation in transmission rate reflects differences in spore
225 infectivity. To measure ρ, we conducted two experiments, one with spores produced in the $\beta + u$
226 measurement assay (20/20 and 32/32°C treatments) and another with spores produced in the
227 within-host parasite growth assay (20, 26, and 32°C treatments). To measure φ, we used spores
228 incubated at three temperatures (20, 25, and 30°C) for two durations (1-day and 7-days) in
229 constant, non-fluctuating temperatures (spores do not migrate between stratified water layers).
230 One-day incubations were stored at 4°C for the first six days (standard procedure for spore
231 storage). We estimated transmission rates (β) from the prevalence data (see Appendix).
232 Both mechanisms influence transmission by modifying spore infectivity (already
233 estimated from within-host processes as u, mechanism 1). Thus, in order to incorporate these
234 mechanisms into a synthetic metric for disease spread (transmission potential, see below), we
235 calculated unit-less rearing (ρ) and free-living (φ) effects standardized to infectivity at 20°C.

236 Specifically, we calculated the parameters by dividing the estimates for transmission rate (β) at
237 26 and 32°C by that at 20°C. Accordingly, values of $\rho<1$ or $\varphi<1$ mean spores are less infectious
238 due to rearing or free-living effects than at 20°C, respectively; conversely, values >1 mean
239 spores are more infectious than at 20°C. To calculate confidence intervals at 20°C, we divided a
240 bootstrapped distribution of transmission rates by a randomly-shuffled version of itself.
241 Additionally, because harm to free-living spores occurs over time as spores are removed by
242 hosts, we used a simple model to estimate time-weighted transmission rates for φ. We assumed
243 that spore infectivity declined linearly over the 7-day assay, and that hosts consume spores at a
244 constant foraging rate (resulting in an exponential decay in spores remaining over time). Thus,
245 we weighted the estimated transmission rate on each day by the proportion of spores consumed
246 by hosts on that day (see Appendix for detailed methods and a sensitivity analysis of the model).
247

248 **Transmission potential**

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

257 **Additional Statistical Analyses**

258 For all parameters, we bootstrapped 95% confidence intervals (data sampled within

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

269 **RESULTS**

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

271 Contrary to our predictions, high temperature did not lower transmission rate (β) during
272 either step (exposure or infection establishment; Fig. 2A). Instead, transmission rate was higher
273 when hosts were exposed at 32°C than at 20°C (20°C infection establishment: p=0.0013; 32°C
274 infection establishment: p<0.0001). Temperature during infection establishment exerted no effect
275 on transmission rate (20°C exposure: p=0.10; 32°C exposure: p=0.31). When exposure and
276 establishment temperatures were equal (as in nature; the 20/20 and 32/32°C treatments here),
277 transmission rate was higher at 32°C than at 20°C (p=0.0068). Thus, even at maximal
278 epilimnetic temperatures, the impacts of higher temperatures on transmission rate promoted
279 rather than inhibited disease.

280 The thermal response of transmission rate was mechanistically driven by foraging rate of
281 hosts (f), not spore infectivity from within-host processes (u). Foraging rate increased from 20 to

282 25°C (*PD*=0; see *Methods* and Appendix for a description of PD values, which are analogous but
283 not identical to *p*-values) and then plateaued at 30°C (*PD*=0.11; Fig. 2B). Thus, hosts encounter
284 more spores at 25 and 30°C than at 20°C. After we accounted for predicted host-parasite contact,
285 spore infectivity was fairly insensitive to high temperatures (Fig. 2C). Temperature during
286 infection establishment did not impact spore infectivity (20°C exposure: p=0.10; 32°C exposure:
287 p=0.31). Exposure temperature increased spore infectivity (20°C infection establishment:
288 p=0.034; 32°C infection establishment: p=0.0052), but in the opposite direction of the
289 hypothesized mechanism (hotter temperature increased infectivity). When exposure and infection
290 establishment temperatures were equal (as in nature), spore infectivity did not differ (p=0.37).
291 Thus, high temperatures increased the foraging rate of hosts, elevating host contact with spores,
292 while spore infectivity barely changed. These changes in parasite exposure led to more
293 transmission at high temperatures.
294
295 **Mechanism 3: Spore yield (σ) and other measures of host and parasite growth**
296 Final spore yield (σ) in hosts that died from infection was lower at 32°C than at 20 and
297 26°C (Fig. 3A; best-fitting model had two means, see Table S5 for model AIC scores and Akaike
298 weights). This pattern was not explained by host condition estimated via growth rate. Host
299 growth rate (g_h, Fig. 3B) always increased with temperature (20 versus 26°C: p=4.7x10^{-6}; 26 vs.
300 32°C: p=0.00038). Instead, the pattern was explained by a combination of host death rates and
301 delays in spore maturation. Infected hosts died more quickly at 26°C than 20°C (p<0.0001), and
302 death rate trended higher from 26 to 32°C (p=0.063; Fig. 3C). Meanwhile, growth rate of mature
303 parasite spores (g_p, time series in Fig. 3D, linear slopes [growth rate] in Fig. 3E) did not change
304 with temperature (*PD*>0.15). However, temperature did affect the timing of initial spore

305 production within hosts (i.e., intercepts of linear model). At the earliest point in the sacrifice
306 series (day 8), spore load was highest at 26°C, intermediate at 32°C, and nearly zero at 20°C
307 (Fig. 3D). Given thermally insensitive daily growth rates of parasites (g_P; Fig. 3E), these head-
308 starts were maintained over time (Fig. 3D). This effect on early spore production, coupled with
309 host death rate (Fig 3C), explains the spore yield pattern. Final spore yield was lower at 32 than
310 26°C because there were fewer spores initially (on day 8) and hosts died more quickly (less time
311 to produce spores). At 20°C, spore production started even later, but the delay was compensated
312 for by much longer lifespans of infected hosts (lower death rate, d; Fig. 3C).
313
314 **Mechanisms 4 & 5: rearing (ρ) and free-living spore (φ) effects on infectivity**
315 Spore infectivity (measured as transmission rate) responded unimodally to temperature in
316 the previous infection (rearing effect on spore quality, ρ; Fig. 4A). Infectivity increased
317 significantly for spores made at 20 versus 26°C for one of two spore sources (p=0.0083 for
318 spores from $\beta + u$ measurement assay [square, Fig 4A]; p=0.092 for spores from within-host
319 growth assay [diamond]). Infectivity then declined for spores made at 26 versus 32°C (p=0.0001
320 for both spore sources). Infectivity was significantly lower for spores made at 32 versus 20°C for
321 one of two spore sources (p=0.16 for spores from $\beta + u$ measurement assay [square]; p=0.026 for
322 spores from within-host growth assay [diamond]). The parameter ρ (Fig. 4C) shows the rearing
323 effect pooled for both spore sources and normalized by transmission rate at 20°C (used for
324 calculating transmission potential).
325 The thermal environment of free-living spores also impacted their infectivity (φ; Fig
326 4B,D). Spore infectivity decreased with higher incubation temperatures after 7 days (20 versus
327 25°C: p=0.0031; 25 versus 30°C: p<0.0001; diamonds on Fig. 4B). However, spore infectivity

328 did not change after 1-day incubations (flat line in Fig. 4B [squares]; 20 versus 25°C: p=0.65, 25
329 versus 30°C: p=0.64). All 1-day incubations used stored (refrigerated) spores. They had lower
330 infectivity than the 7-day incubation at 20°C, likely because storage at 4°C also lowers spore
331 infectivity (1 versus 7-day incubations at 20°C: p<0.0001) (Duffy & Hunsberger, 2019). The
332 parameter φ (Fig. 4D) shows the free-living spore effect assuming that spores lose infectivity
333 gradually over seven days as they are consumed by hosts (see *Methods* and Appendix) and
334 normalized by transmission rate at 20°C (used for calculating transmission potential).
335
336 **Transmission potential ($f\ u\ \sigma\ \rho\ \varphi$)**
337 Transmission potential, the product of all five mechanisms ($f\ u\ \sigma\ \rho\ \varphi$), responded
338 unimodally to high temperatures. This metric first increased from 20 to 25/26°C (PD=0.017);
339 then, it decreased from 25/26 to 30/32°C (PD=0.0001; Fig. 5A, 'full transmission potential').
340 Transmission potential at 30/32°C trended (non-significantly) lower than at 20°C (PD=0.11).
341 Thus, high temperatures do not constrain disease enough via these five mechanisms to explain
342 the absence of summer epidemics.
343 The initial increase in transmission potential from 20 to 25/26°C was driven most
344 strongly by host foraging (f, mechanism 1) and the rearing effect on spore quality (ρ, mechanism
345 4): holding either trait constant removes the significant difference between temperatures (Fig. 5B
346 and 5E, respectively). The subsequent drop in transmission potential from 25/26 to 30/32°C was
347 driven most strongly by the rearing effect (ρ): holding it constant again removes the significant
348 difference (Fig. 5E). Harm to free-living spores (φ, mechanism 5) also contributes somewhat
349 (Fig. 5F vs. Fig. 5A), though not enough to affect the statistical significance. Additionally, the
350 thermal response of host foraging (f) is key for maintaining transmission at high temperatures:

351 without increased exposure to spores, the remaining mechanisms would significantly reduce
352 transmission at 30/32°C compared to 20°C (Fig 5B). Spore infectivity from within-host
353 processes (u, mechanism 2) and spore yield (σ, mechanism 3) had no effect (Fig. 5C vs. Fig. 5A)
354 and very little effect (Fig. 5D vs. Fig. 5A) on transmission potential, respectively.
355

356 **DISCUSSION**

357 We investigated upper thermal constraints on fungal epidemics in a *Daphnia* zooplankton
358 host. The seasonality of the autumnal epidemics suggested that hot conditions might constrain
359 disease: epidemics usually start after lakes cool from maximal summer temperatures in the
360 epilimnion (29-32°C). We tested five potential thermal constraints on transmission. First,
361 foraging (exposure) rate of hosts (f) increased at high temperatures (Fig 2B), while, second, high
362 temperatures did not affect the infectivity of spores from within-host processes (u; Fig 2C). Thus,
363 high temperatures increased transmission rate, β (where $\beta=uf$; Fig 2A). Third, spore yield (σ)
364 declined slightly at 32°C (Fig 3A). Fourth, a rearing effect on spore quality driven by
365 temperature during the previous infection (ρ) emerged: spores made at 32°C were less infectious
366 than those made at 26°C and sometimes 20°C (for one of two spore sources, Fig 4A). Finally,
367 harm to free-living spores (φ) lowered infectivity as temperature increased (Fig 4B). Overall,
368 transmission potential is much lower at 32°C than 26°C, but still similar to at 20°C (Fig 5A), a
369 temperature that easily supports epidemics in both nature (Shocket, Strauss, et al., 2018) and
370 laboratory environments (Civitello et al., 2012; Shocket, Strauss, et al., 2018). Thus, maximally
371 high temperatures do constrain disease, but not sufficiently to explain the absence of summer
372 epidemics on their own.

373 Contrary to our initial hypothesis, high temperatures *increased* transmission rate (Fig

374 2A). In principle, high temperatures can lower infection success if pathogens tolerate heat less
375 well than hosts (Thomas & Blanford, 2003). For instance, many ectothermic hosts behaviorally
376 induce fever to reduce the negative costs of infection (Rakus et al., 2017; Stahlschmidt &
377 Adamo, 2013). Further, fungi are particularly sensitive to high temperatures compared to other
378 pathogen taxa (Robert & Casadevall, 2009) and fungal pathogens are often limited by high
379 temperatures (Berger et al., 2004; Carruthers et al., 1992; Langwig et al., 2015; Raffel et al.,
380 2010; Thomas & Blanford, 2003). However, high temperatures did not interfere with this
381 fungus's success at either stage of transmission: the day of exposure, when most spores penetrate
382 the host's gut, or infection establishment, when the fungus replicates and develops within the
383 host (Stewart Merrill & Cáceres, 2018). Instead, high temperatures elevated host foraging rate
384 (Fig 2B), which increases exposure to parasites, thereby increasing transmission rate (Hall et al.,
385 2007). In lakes, the thermal response of foraging (exposure) drives variation in the size of
386 epidemics, which occur in autumn: epidemics that start earlier in warmer conditions grow larger
387 than those starting later and colder (Shocket, Strauss, et al., 2018). This foraging-controlled
388 exposure to parasites is a potentially general mechanism: higher temperatures also increase
389 outbreak size for armyworms that consume virus particles on leaves (Elderd & Reilly, 2014).
390 However, transmission plateaued with temperature for another ingested *Daphnia* pathogen (Vale,
391 Stjernman, & Little, 2008).
392 Spore yield (σ) declined at the highest temperature (32°C; Fig. 3). Although the effect on
393 transmission potential was minimal (Fig 5D), the results for related traits provide mechanistic
394 insights into host-parasite interactions. Parasite burdens often decline at temperatures near the
395 thermal maxima of the host and/or parasite, e.g., for nematodes in slugs (Wilson, Digweed,
396 Brown, Ivanonva, & Hapca, 2015), trematodes in snails (Paull, Lafonte, & Johnson, 2012),

397 bacteria in *Daphnia* (Vale et al., 2008) and fruit flies (Lazzaro, Flores, Lorigan, & Yourth, 2008),
398 and powdery mildew in plants (Laine, 2007). In theory, reduced parasite production at hot
399 temperatures could arise from several mechanisms. First, parasite production could decline if
400 host growth slows, since spore yield often scales with host growth, at least along resource
401 gradients (Hall, Knight, et al., 2009; Hall, Simonis, et al., 2009). However, here host growth rate
402 (g_h) increased with temperature while spore yield was flat and then decreased (Fig 3B).
403 Therefore, spore production was decoupled from host growth rate (i.e., the link between host
404 growth and parasite production that occurs for resources did not occur for temperature). Second,
405 the parasite itself could grow more slowly at high temperatures. For example, high temperatures
406 slow bacterial growth inside fruit flies (Lazzaro et al., 2008), fungal growth in grasshoppers
407 (Springate & Thomas, 2005), and fungal growth on warm-adapted (but not cold-adapted)
408 amphibians (Cohen et al., 2017). In contrast, here parasite growth rate (g_p) did not respond to
409 temperature (slope in Fig 3D; Fig 3E).
410 Instead, the decline in spore production at high temperatures arose from a combination of
411 host death rate and the timing of initial spore production. Temperature determined spore load on
412 day 8 (the earliest sampling time in the assay; Fig. 3D). Based on that information (and the
413 constant parasite growth rates, Fig 3E), we infer that spore production began earliest at 26°C,
414 followed by 32°C, and then 20°C. These head starts were maintained over time and explain the
415 spore yield pattern when combined with death rate of infected hosts (Fig. 3C). In general, shorter
416 lifespan of infected hosts decreases time for spore production, thereby depressing spore yield
417 (Auld et al., 2014; Civitello et al., 2012). Here, spore yield was lower at 32 than 26°C because
418 spore production started later and hosts died more quickly. At 20°C, spore production started
419 even later, but longer host lifespan compensated for this delay (i.e., the fungus had longer to

420 grow within hosts). Do similar patterns exist in other systems? Unfortunately, few studies focus
421 on traits underlying thermal responses of parasite load. Hence questions remain: How often does
422 temperature change the timing versus the rate of parasite production? How often does
423 temperature decouple positive relationships between host growth and parasite production? The
424 answers matter because spore yield can influence epidemic size for obligate killer parasites (like
425 the fungus here: Civitello et al., 2015). Thus, developing a general framework from data across
426 host-parasite systems remains a fruitful area for future research.

427 High temperatures reduced transmission potential via two effects on spore infectivity that
428 act outside the focal host. First, a rearing effect on spore quality (ρ) driven by temperature of
429 spore production in the previous host elevated (26°C) and then lowered (32°C) spore infectivity
430 (compared to 20°C). Rearing effects on parasite performance can arise with variation in
431 resources consumed by hosts (Cornet, Bichet, Larcombe, Faivre, & Sorci, 2014; Little, Birch,
432 Vale, & Tseng, 2007; Tseng, 2006), temperature experienced by hosts (Shocket, Vergara, et al.,
433 2018), or host genotype (Searle et al., 2015). These understudied rearing effects may drive
434 performance of parasites to an unappreciated extent (Shocket, Vergara, et al., 2018). Second,
435 harm to free-living spores (φ, including spore mortality) also inhibited infection at high
436 temperatures. After seven days in 30°C, spores lost 92% of their initial infectivity. This
437 constraint may arise in other systems: for example, high temperatures elevate mortality in free-
438 living helminths of Arctic ungulates (Molnár et al., 2013). However, in the planktonic system
439 here, the 7-day result likely exaggerates the thermal constraint. While difficult to quantify,
440 physical sinking, consumption (Civitello, Pearsall, Duffy, & Hall, 2013; Penczykowski, Hall, et
441 al., 2014; Shocket, Vergara, et al., 2018; Strauss, Civitello, Cáceres, & Hall, 2015) and damage
442 from radiation (Overholt et al., 2012) likely remove most spores before 7 days. To acknowledge

443 this mortality, we weighted this component of infectivity (φ) using a model of spore longevity.
444 Assuming this modeled weighting reflects reality in lakes, the free-living effect lacks enough
445 strength to inhibit epidemics during summer, even when combined with the other mechanisms
446 (see Appendix for sensitivity analysis of the time-weighting model). However, more realistic
447 dynamical models and better resolved trait data for the free-living spore effect could change the
448 estimates for how high temperatures affect transmission.

449 Although the impact of temperature on these five mechanisms does not explain the lack
450 of epidemics during summer, other co-varying environmental factors could combine with
451 thermal effects to sufficiently inhibit transmission. Such factors include damage to free-living
452 spores by solar radiation (Overholt et al., 2012), consumption of spores by resistant zooplankton
453 species that are more abundant earlier in the year (Penczykowski, Hall, et al., 2014), and low
454 spore production due to poor quality of host food resources (Hall, Knight, et al., 2009). These
455 mechanisms could contribute to the observed field pattern, and interact with the thermal effects
456 examined here. Furthermore, climate change could disrupt covariation among drivers. For
457 example, high temperatures may persist later in the year when damaging solar radiation is less
458 intense. Incorporating these other factors may help explain the current field pattern and improve
459 predictions for how climate change will impact epidemics. These predictions should also
460 explicitly account for the effects of temperature variation and extremes, which have distinct
461 impacts on organismal performance (Dowd, King, & Denny, 2015). Here, we employed a
462 relevant form of thermal variation, mimicking migratory behavior of hosts in stratified lakes, but
463 did not isolate effects of thermal variation. Future efforts could estimate this effect to better
464 predict how climate change will impact the host, the parasite, and their interaction.

465 The current prevailing view argues that hot temperatures should constrain disease

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

ACKNOWLEDGMENTS

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

484 **Table 1**: The experiments (and spore sources) used to test the five mechanistic components of

485 disease transmission.

486

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

487

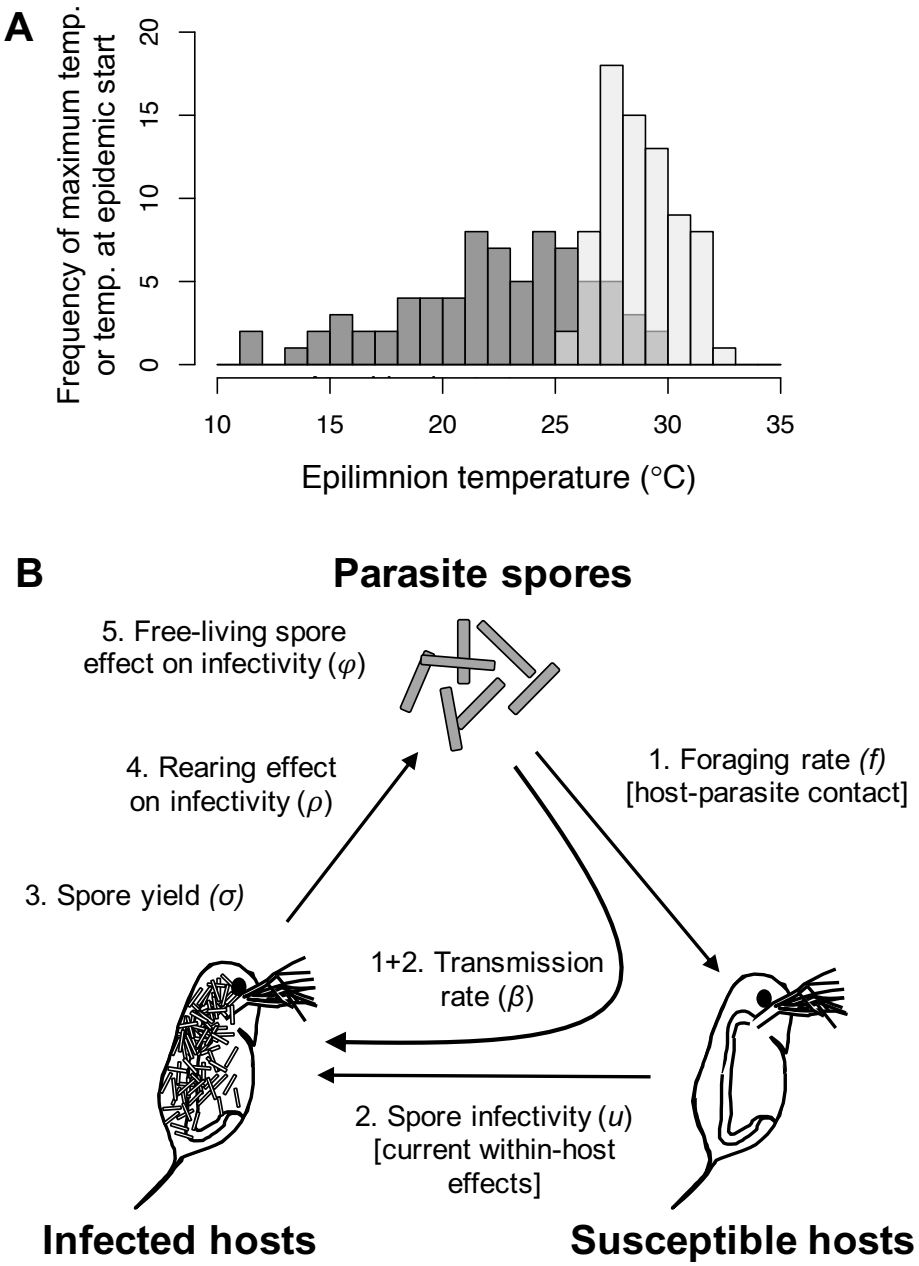

Figure 1: Motivating field pattern and mechanistic components of transmission. (A) Fungal epidemics usually start (dark grey bars) after lakes have cooled from the maximum summer temperature (light grey bars). Epidemics never started when the epilimnion (upper, warmer layer) was hotter than 30°C, suggesting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

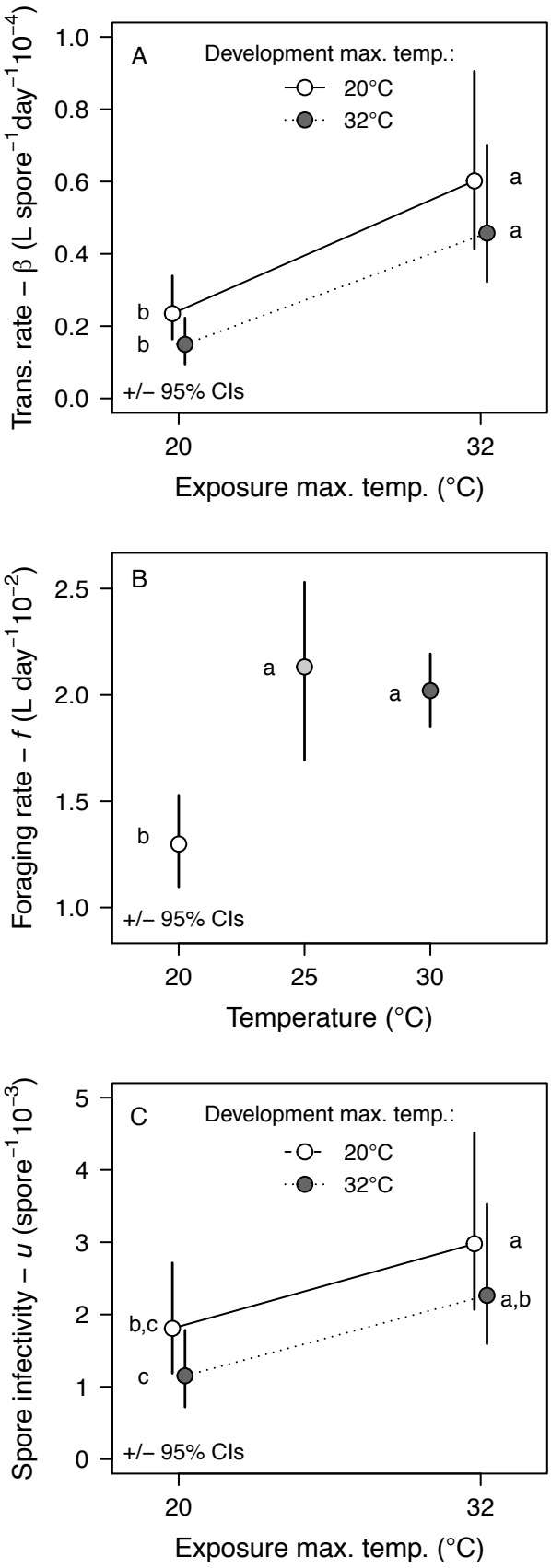

Figure 2: High temperature impacts on transmission rate (*β*), foraging rate (*f*, mechanism 1) and spore infectivity from current within-host processes (*u*, mechanism 2). In A and C, the effect of high temperature during parasite exposure and infection establishment (20°C infection establishment =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

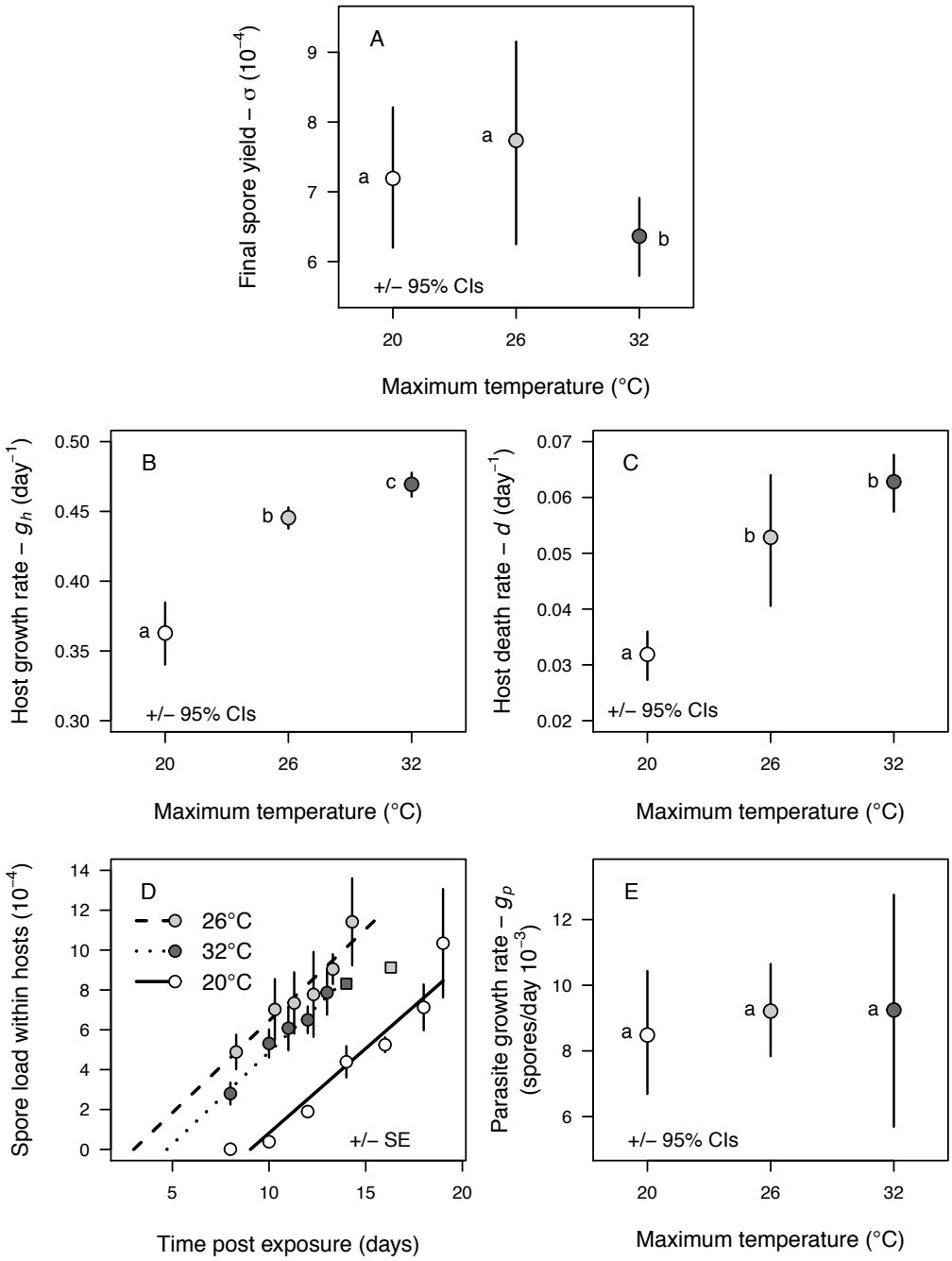

Figure 3: High temperature impacts on spore yield (σ, mechanism 3) and possible underlying traits. (A) Final spore yield at host death (σ) was lower at 32°C (dark grey) than at 20°C (white) or 26°C (light grey). (B) Host growth rate (g_h) increased with temperature for all treatments. (C) Death rate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

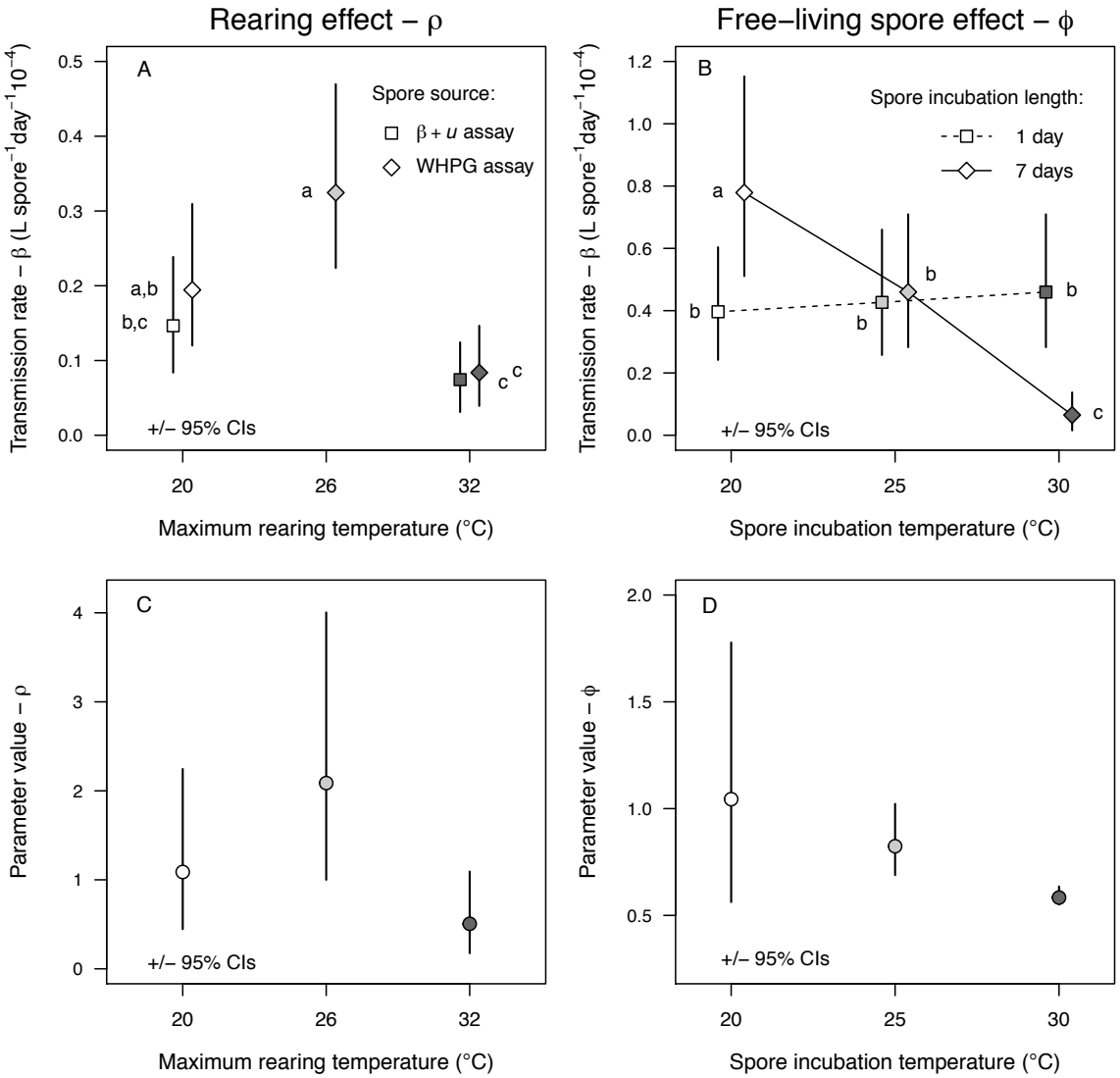

Figure 4: High temperature impacts on a rearing effect (*ρ*, mechanism 4) and harm to free-living spores (*φ*, mechanism 5). Variation in transmission rate from common garden infection assays reflects differences in spore infectivity. (A) Spores came from the β + *u* measurement assay (Fig. 2; squares) and the within host parasite growth assay ('WHPG'; Fig. 3; diamonds). Spore infectivity increased with rearing temperature from 20°C (white) to 26°C (light grey; β + *u* only) and decreased with rearing temperatures from 26°C to 32°C (dark grey, both spore sources). Spore infectivity was lower at 32°C than at 20°C (WHPG spores only). (B) Spore infectivity decreased when free-living spores were incubated in high temperatures for 7 days but not for 1 day. Storage at 4°C for 6 days (for all 1-day incubations) also lowered spore infectivity relative to the 7-day incubation at 20°C. (C-D) Parameter values (transmission rate scaled by values at 20°C) for (C) rearing effect, *ρ,* and (D) free-living effect, *φ*. Phi values also based on time-weighted model (see text for details). Error bars show 95% CIs. Letters indicate significant differences.

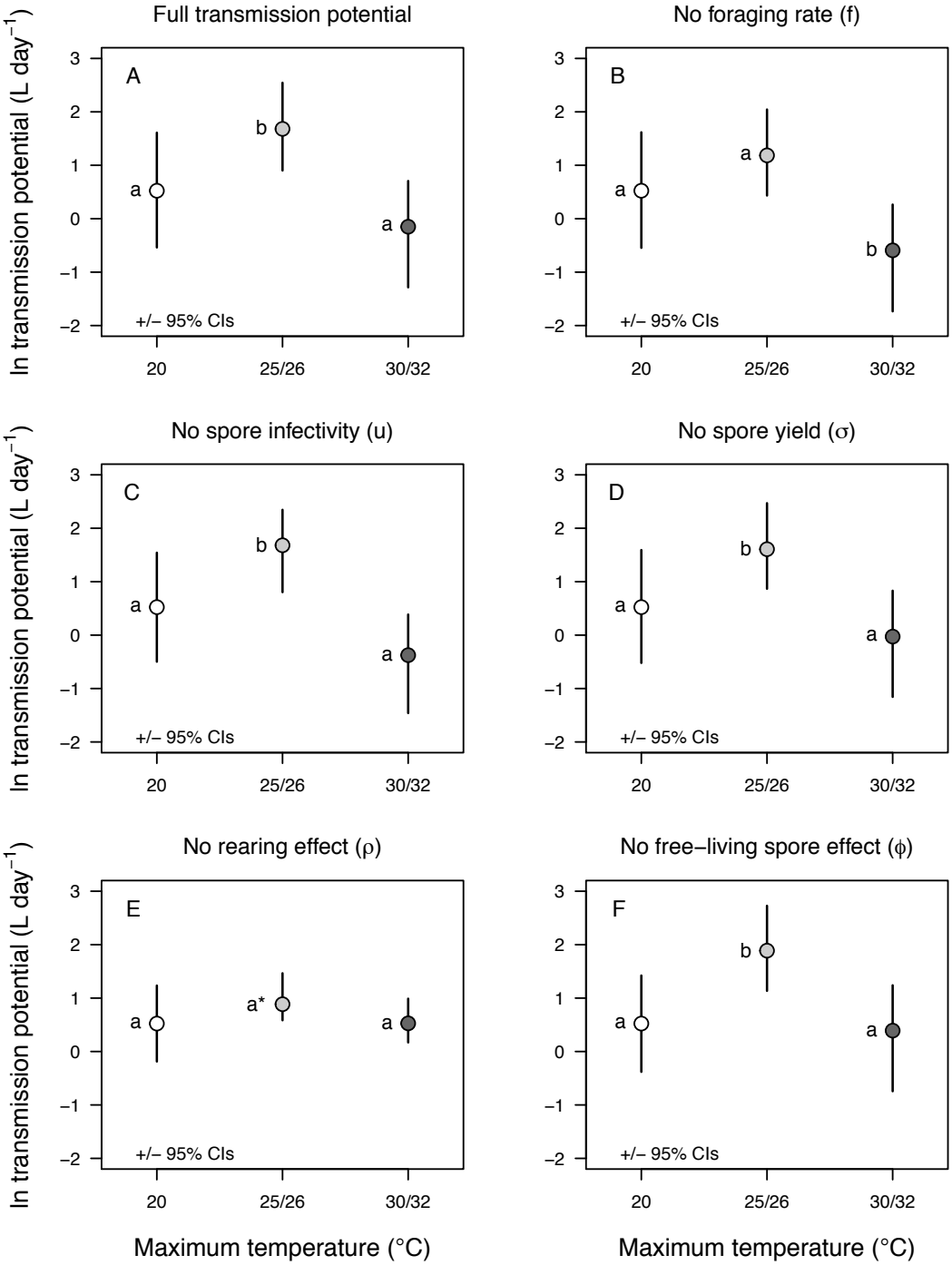

Figure 5: High temperature impacts on transmission potential. (A) Transmission potential ($fu\sigma\rho\varphi$) responds unimodally, increasing from 20°C (white) to 25/26°C (light grey) and decreasing from 25/26°C to 30/32°C (dark grey). (B-F) Transmission potential with each mechanism held constant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

WORKS CITED

Allen, D. E., & Little, T. J. (2011). Dissecting the effect of a heterogeneous environment on the interaction between host and parasite fitness traits. *Evolutionary Ecology*, *25*(2), 499–508. doi:10.1007/s10682-010-9413-2

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., & Rohani, P. (2006). Seasonality and the dynamics of infectious diseases. *Ecology Letters*, *9*(4), 467–84. doi:10.1111/j.1461-0248.2005.00879.x

Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate change and infectious diseases: from evidence to a predictive framework. *Science*, *341*(6145), 514–9. doi:10.1126/science.1239401

Auld, S. K., Hall, S. R., Housley Ochs, J., Sebastian, M., & Duffy, M. A. (2014). Predators and patterns of within-host growth can mediate both among-host competition and evolution of transmission potential of parasites. *American Naturalist*, *184*(S1), S77-90. doi:10.1086/676927

Berger, L., Speare, R., Hines, H. B., Marantelli, G., Hyatt, A. D., McDonald, K. R., … Tyler, M. J. (2004). Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinary Journal*, *82*(7), 434–439. doi:10.1111/j.1751-0813.2004.tb11137.x

Bolker, B. M., & R Development Core Team. (2017). bbmle: Tools for General Maximum Likelihood Estimation.

Carruthers, R. I., Larkin, T. S., & Firstencel, H. (1992). Influence of thermal ecology on the mycosis of a rangeland grasshopper. *Ecology*, *73*(1), 190–204. doi:10.2307/1938731

Civitello, D. J., Forys, P., Johnson, A. P., & Hall, S. R. (2012). Chronic contamination decreases disease spread: a Daphnia-fungus-copper case study. *Proceedings of the Royal Society B: Biological Sciences*, *279*(May), 3146–3153. doi:10.1098/rspb.2012.0684

Civitello, D. J., Pearsall, S., Duffy, M. A., & Hall, S. R. (2013). Parasite consumption and host interference can inhibit disease spread in dense populations. *Ecology Letters*, *16*(5), 626–34. doi:10.1111/ele.12089

Civitello, D. J., Penczykowski, R. M., Smith, A. N., Shocket, M. S., Duffy, M. A., & Hall, S. R. (2015). Resources, key traits and the size of fungal epidemics in Daphnia populations. *Journal of Animal Ecology*, *84*, 1010–1017. doi:10.1111/1365-2656.12363

Cohen, J. M., Venesky, M. D., Sauer, E. L., Civitello, D. J., McMahon, T. A., Roznik, E. A., & Rohr, J. R. (2017). The thermal mismatch hypothesis explains host susceptibility to an emerging infectious disease. *Ecology Letters*, *20*(2), 184–193. doi:10.1111/ele.12720

Cornet, S., Bichet, C., Larcombe, S., Faivre, B., & Sorci, G. (2014). Impact of host nutritional status on infection dynamics and parasite virulence in a bird-malaria system. *Journal of Animal Ecology*, *83*(1), 256–265. doi:10.1111/1365-2656.12113

Dell, A. I., Pawar, S., & Savage, V. M. (2011). Systematic variation in the temperature dependence of physiological and ecological traits. *Proceedings of the National Academy of Sciences*, *108*(26), 10591–10596.

Dowd, W. W., King, F. A., & Denny, M. W. (2015). Thermal variation, thermal extremes and the physiological performance of individuals. *Journal of Experimental Biology*, *218*(12), 1956–1967. doi:10.1242/jeb.114926

Duffy, M. A., & Hunsberger, K. K. (2019). Infectivity is influenced by parasite spore age and exposure to freezing: do shallow waters provide Daphnia a refuge from some parasites? *Journal of Plankton Research*, *41*(1), 12–16. doi:10.1093/plankt/fby046

Ebert, D. (2005). *Ecology, epidemiology, and evolution of parasitism in* Daphnia. Bethesda: National Library of Medicine (USA), Center for Biotechnology Information.

Elderd, B. D., & Reilly, J. R. (2014). Warmer temperatures increase disease transmission and outbreak intensity in a host-pathogen system. *Journal of Animal Ecology*, *83*(4), 838–849. doi:10.1111/1365-2656.12180

Gehman, A.-L. M., Hall, R. J., & Byers, J. E. (2018). Host and parasite thermal ecology jointly determine the effect of climate warming on epidemic dynamics. *Proceedings of the National Academy of Sciences*, *115*(4), 744–749. doi:10.1073/pnas.1705067115

Hall, S. R., Duffy, M. A., Tessier, A. J., & Cáceres, C. E. (2005). Spatial heterogeneity of daphniid parasitism within lakes. *Oecologia*, *143*, 635–644. doi:10.1007/s00442-005-0005-8

Hall, S. R., Knight, C. J., Becker, C. R., Duffy, M. A., Tessier, A. J., & Cáceres, C. E. (2009). Quality matters: resource quality for hosts and the timing of epidemics. *Ecology Letters*, *12*(2), 118–128. doi:10.1111/j.1461-0248.2008.01264.x

Hall, S. R., Simonis, J. L., Nisbet, R. M., Tessier, A. J., & Cáceres, C. E. (2009). Resource Ecology of Virulence in a Planktonic Host-Parasite System: An Explanation Using Dynamic Energy Budgets. *The American Naturalist*, *174*(2), 149–162. doi:10.1086/600086

Hall, S. R., Sivars-Becker, L., Becker, C., Duffy, M. A., Tessier, A. J., & Cáceres, C. E. (2007). Eating yourself sick : transmission of disease as a function of foraging ecology. *Ecology Letters*, *10*, 207–218. doi:10.1111/j.1461-0248.2006.01011.x

Hall, S. R., Tessier, A. J., Duffy, M. A., Huebner, M., & Cáceres, C. E. (2006). Warmer does not have to mean sicker: temperature and predators can jointly drive timing of epidemics. *Ecology*, *87*(7), 1684–95.

Hite, J. L., Penczykowski, R. M., Shocket, M. S., Strauss, A. T., Orlando, P. A., Duffy, M. A., … Hall, S. R. (2016). Parasites destabilize host populations by shifting stage-structured interactions. *Ecology*, *97*(2), 439–449. doi:10.1890/15-1065.1

Kirk, D., Jones, N., Peacock, S., Phillips, J., Molnár, P. K., Krkošek, M., & Luijckx, P. (2018). Empirical evidence that metabolic theory describes the temperature dependency of within-host parasite dynamics. *PLOS Biology*, *16*(2), e2004608. doi:10.1371/journal.pbio.2004608

Kooijman, S. A. L. M. (2009). *Dynamic Energy Budget Theory for Metabolic Organisation* (3rd ed.). New York, New York: Cambridge University Press.

Lafferty, K. D. (2009). The ecology of climate change and infectious diseases. *Ecology*, *90*(4), 888–900. doi:10.1890/09-1656.1

Lafferty, K. D., & Mordecai, E. A. (2016). The rise and fall of infectious disease in a warmer world. *F1000Research*, *5*.

Laine, A. L. (2007). Pathogen fitness components and genotypes differ in their sensitivity to nutrient and temperature variation in a wild plant-pathogen association. *Journal of Evolutionary Biology*, *20*(6), 2371–2378. doi:10.1111/j.1420-9101.2007.01406.x

Lampert, W. (1989). The adaptive significance of diel vertical migration of zooplankton. *Functional Ecology*, *3*(1), 21–27.

Lampert, W., & Trubetskova, I. (1996). Juvenile growth rate as a measure of fitness in Daphnia. *Functional Ecology*, *10*(5), 631–635.

Langwig, K. E., Frick, W. F., Reynolds, R., Parise, K. L., Drees, K. P., Hoyt, J. R., … Kilpatrick, A. M. (2015). Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1799), 20142335. doi:10.1098/rspb.2014.2335

Lazzaro, B. P., Flores, H. A., Lorigan, J. G., & Yourth, C. P. (2008). Genotype-by-environment interactions and adaptation to local temperature affect immunity and fecundity in *Drosophila melanogaster*. *PLoS Pathogens*, *4*(3), 1–9. doi:10.1371/journal.ppat.1000025

Little, T., Birch, J., Vale, P., & Tseng, M. (2007). Parasite transgenerational effects on infection. *Evolutionary Ecology Research*, *9*(3), 459–469.

Mangal, T. D., Paterson, S., & Fenton, A. (2008). Predicting the impact of long-term temperature changes on the epidemiology and control of schistosomiasis: a mechanistic model. *PloS One*, *3*(1), e1438. doi:10.1371/journal.pone.0001438

McCallum, H., Fenton, A., Hudson, P. J., Lee, B., Levick, B., Norman, R., … Lello, J. (2017). Breaking beta: deconstructing the parasite transmission function. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*, 20160084. doi:10.1098/rstb.2016.0084

Molnár, P. K., Kutz, S. J., Hoar, B. M., & Dobson, A. P. (2013). Metabolic approaches to understanding climate change impacts on seasonal host-macroparasite dynamics. *Ecology Letters*, *16*(1), 9–21. doi:10.1111/ele.12022

Mordecai, E. A., Caldwell, J. M., Grossman, M. K., Lippi, C. A., Johnson, L. R., Neira, M., … Villena, O. (n.d.). Thermal biology of mosquito-borne disease. *Ecology Letters*.

Niehaus, A. C., Angilletta, M. J., Sears, M. W., Franklin, C. E., & Wilson, R. S. (2012). Predicting the physiological performance of ectotherms in fluctuating thermal environments. *The Journal of Experimental Biology*, *215*(Pt 4), 694–701.

doi:10.1242/jeb.058032

Overholt, E. P., Hall, S. R., Williamson, C. E., Meikle, C. K., Duffy, M. A., & Cáceres, C. E. (2012). Solar radiation decreases parasitism in Daphnia. *Ecology Letters*, *15*(1), 47–54. doi:10.1111/j.1461-0248.2011.01707.x

Pascual, M., & Dobson, A. (2005). Seasonal patterns of infectious diseases. *PLoS Medicine*, *2*(1), 0018–0020. doi:10.1371/journal.pmed.0020005

Paull, S. H., Lafonte, B. E., & Johnson, P. T. J. (2012). Temperature-driven shifts in a host-parasite interaction drive nonlinear changes in disease risk. *Global Change Biology*, *18*(12), 3558–3567. doi:10.1111/gcb.12018

Penczykowski, R. M., Hall, S. R., Civitello, D. J., & Duffy, M. A. (2014). Habitat structure and ecological drivers of disease. *Limnology and Oceanography*, *59*(2), 340–348. doi:10.4319/lo.2014.59.2.0340

Penczykowski, R. M., Lemanski, B. C. P., Sieg, R. D., Hall, S. R., Housley Ochs, J., Kubanek, J., & Duffy, M. A. (2014). Poor resource quality lowers transmission potential by changing foraging behaviour. *Functional Ecology*, *28*(5), 1245–1255. doi:10.1111/1365-2435.12238

R Core Team. (2017). *R: A language and for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Raffel, T. R., Michel, P. J., Sites, E. W., & Rohr, J. R. (2010). What Drives Chytrid Infections in Newt Populations? Associations with Substrate, Temperature, and Shade. *EcoHealth*, *7*(4), 526–536. doi:10.1007/s10393-010-0358-2

Rakus, K., Ronsmans, M., & Vanderplasschen, A. (2017). Behavioral fever in ectothermic vertebrates. *Developmental & Comparative Immunology*, *66*, 84–91. doi:https://doi.org/10.1016/j.dci.2016.06.027

Robert, V. A., & Casadevall, A. (2009). Vertebrate Endothermy Restricts Most Fungi as Potential Pathogens. *Journal of Infectious Diseases*, *200*(10), 1623–1626. doi:10.1086/644642

Sarnelle, O., & Wilson, A. E. (2008). Type III functional response in *Daphnia*. *Ecology*, *89*(6), 1723–1732. doi:10.1890/07-0935.1

Searle, C. L., Ochs, J. H., Cáceres, C. E., Chiang, S. L., Gerardo, N. M., Hall, S. R., & Duffy, M. A. (2015). Plasticity, not genetic variation, drives infection success of a fungal parasite. *Parasitology*, *142*(06), 839–848. doi:10.1017/S0031182015000013

Shocket, M. S., Strauss, A. T., Hite, J. L., Šljivar, M., Civitello, D. J., Duffy, M. A., … Hall, S. R. (2018). Temperature Drives Epidemics in a Zooplankton-Fungus Disease System: A Trait-Driven Approach Points to Transmission via Host Foraging. *The American Naturalist*, *191*(4), 435–451. doi:10.1086/696096

Shocket, M. S., Vergara, D., Sickbert, A. J., Walsman, J. M., Strauss, A. T., Hite, J. L., … Hall, S. R. (2018). Parasite rearing and infection temperatures jointly influence disease

transmission and shape seasonality of epidemics. *Ecology*. doi:10.1002/ecy.2430

Shocket, M. S., Verwillow, A. B., Numazu, M. G., Slamani, H., Cohen, J. M., El Moustaid, F., … Mordecai, E. A. (n.d.). Transmission of West Nile virus and other temperate mosquito-borne viruses occurs at lower environmental temperatures than tropical mosquito-borne diseases.

Springate, S., & Thomas, M. B. (2005). Thermal biology of the meadow grasshopper, Chorthippus parallelus, and the implications for resistance to disease. *Ecological Entomology*, *30*(6), 724–732. doi:10.1111/j.0307-6946.2005.00743.x

Stahlschmidt, Z. R., & Adamo, S. A. (2013). Context dependency and generality of fever in insects. *Naturwissenschaften*, *100*(7), 691–696. doi:10.1007/s00114-013-1057-y

Stewart Merrill, T. E., & Cáceres, C. E. (2018). Within-host complexity of a plankton-parasite interaction. *Ecology*.

Strauss, A. T., Civitello, D. J., Cáceres, C. E., & Hall, S. R. (2015). Success, failure and ambiguity of the dilution effect among competitors. *Ecology Letters*, *18*(9), 916–926. doi:10.1111/ele.12468

Thomas, M. B., & Blanford, S. (2003). Thermal biology in insect-parasite interactions. *Trends in Ecology & Evolution*, *18*(7), 344–350. doi:10.1016/s0169-5347(03)00069-7

Tseng, M. (2006). Interactions between the parasite's previous and current environment mediate the outcome of parasite infection. *The American Naturalist*, *168*(4), 565–571. doi:10.1086/507997

Vale, P. F., Stjernman, M., & Little, T. J. (2008). Temperature-dependent costs of parasitism and maintenance of polymorphism under genotype-by-environment interactions. *Journal of Evolutionary Biology*, *21*(5), 1418–1427. doi:10.1111/j.1420-9101.2008.01555.x

Wilson, M. J., Digweed, A. J., Brown, J., Ivanonva, E. S., & Hapca, S. H. (2015). Invasive slug pests and their parasites—temperature responses and potential implications of climate change. *Biology and Fertility of Soils*, *51*(6), 739–748. doi:10.1007/s00374-015-1022-3

Zbikowska, E., Wrotek, S., Cichy, A., & Kozak, W. (2013). Thermal preferences of wintering snails Planorbarius corneus (L.) exposed to lipopolysaccharide and zymosan. *Journal of Invertebrate Pathology*, *112*(1), 57–61. doi:10.1016/j.jip.2012.08.011

SUPPORTING INFORMATION

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

Appendix S1: Methods, Figures, and Tables

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

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

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

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

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

Table S4: p-values from randomization tests.

Table S5: p-values and ΔAIC from model selection.

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

Table S7: PD (probability density) values for transmission potential 'sensitivity analysis' calculations.