1 2 3	Alternate patterns of temperature variation bring about very different disease outcomes at different mean temperatures
4	Charlotte Kunze ^{1,2*} , Pepijn Luijckx ^{2*#} , Andrew L. Jackson ² , Ian Donohue ²
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6	¹ Institute for Chemistry and Biology of the Marine Environment [ICBM], Carl-von-Ossietzky
7	University Oldenburg
8	² Department of Zoology, School of Natural Sciences, Trinity College Dublin, Dublin, Ireland
9	*equal contribution
10	# communicating author
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16 Abstract

17 The dynamics of host-parasite interactions are highly temperature-dependent and may be modified by 18 increasing frequency and intensity of climate-driven heat events. Here, we show that altered patterns 19 of temperature variance lead to an almost order-of-magnitude shift in thermal performance of host and 20 pathogen life history traits over and above the effects of mean temperature and, moreover, that 21 different temperature regimes affect these traits differently. We found that diurnal fluctuations of $\pm 3^{\circ}$ C 22 lowered infection rates and reduced spore burden compared to constant temperatures in our focal host 23 Daphnia magna exposed to the microsporidium parasite Ordospora colligata. In contrast, a three-day 24 heatwave $(+6^{\circ}C)$ did not affect infection rates, but increased spore burden (relative to constant 25 temperatures with the same mean) at 16° C, while reducing burden at higher temperatures. We 26 conclude that changing patterns of climate variation, superimposed on shifts in mean temperatures due 27 to global warming, may have profound and unanticipated effects on disease dynamics.

28 Introduction

29 One of the major challenges of the 21st century is understanding how infectious diseases, 30 which have profound ecological and epidemiological impacts on human (Hotez et al., 2014), 31 agricultural (Chakraborty et al., 2011) and wildlife (Harvell et al., 2019) populations, will be affected 32 by climate change. It is now well-established that the interaction between hosts and their pathogens is 33 sensitive to temperature (Kirk et al., 2020; Rohr et al., 2013). For example, disease transmission (Ben-34 Horin et al., 2013), host immunity (Dittmar et al., 2014; Rohr et al., 2010) and pathogen growth 35 (Gehman et al., 2018; Kirk et al., 2018) can increase with temperature, while other host-pathogen life 36 history traits such as lifespan and fecundity can decrease (Altizer et al., 2013). The interaction 37 between temperature and multiple host and pathogen life history traits highlights the inherent 38 complexity of temperature effects on infectious diseases. Indeed, each host or pathogen trait may have 39 a unique dependency on temperature and it is their combined effect (that is, R_0 , disease outbreak, 40 virulence) that is often of interest. However, while a growing body of theoretical (Kirk et al., 2020; 41 Rohr et al., 2013) and empirical (Ben-Horin et al., 2013; Dallas et al., 2016; Gehman et al., 2018; Kirk 42 et al., 2020; Zhang et al., 2019) studies have quantified the effect of rising mean temperatures on host 43 and pathogen traits (such as, for example, within-host growth (Kirk et al., 2018), pathogen 44 transmission (Kirk et al., 2019) and epidemiology (Gehman et al., 2018; Shocket et al., 2018)), the 45 influence of variable temperature regimes such as heat waves and temperature fluctuations remains 46 unresolved (Claar et al., 2020; Rohr et al., 2013).

47 Climate change is predicted to increase not only mean temperatures, but also temperature 48 fluctuations and the frequency and intensity of extreme weather events (Schär et al., 2004; Vasseur et 49 al., 2014). Such changes in temperature variance have the potential to modify host-pathogen dynamics 50 (Franke et al., 2019; Rohr et al., 2013). For instance, diurnal temperature fluctuations have been 51 shown to increase malaria transmission at the lower end of the thermal range (Paaijmans et al., 2010), 52 while short-term temperature fluctuations led to reduced transmission success due to lower filtration 53 rates in a Daphnia-pathogen system (Dallas et al., 2016). The effect of extreme heat events on host 54 and pathogen traits is also highly variable and may depend on the magnitude, duration and intensity of 55 the applied heatwave (Landis et al., 2012; Schreven et al., 2017; Zhang et al., 2019). In a parasitoid-

insect interaction, a heatwave of 5 °C resulted in greater parasitoid development while a 10 °C increase reduced parasitoid growth (Schreven et al., 2017). These apparent contrasting results in response to variation in temperature (here used to refer both to fluctuating temperature regimes and extreme heat events), imply that alternate temperature regimes or exposure to temperature shifts of different magnitudes will have distinct impacts on host-pathogen interactions. Indeed, whether all temperature variation acts in the same way or leads to different disease outcomes has been identified as a key open question in the field (Rohr et al., 2013).

63 Here, we examine the effect of different types of temperature variation on host-pathogen 64 interactions across a broad range of mean temperatures. Specifically, we used the *Daphnia magna*— 65 Odospora colligata host-pathogen system to test experimentally how temperature variation alters the 66 thermal performance of both the host and the pathogen across their natural temperature range. 67 Daphnia are a well-established ecological model system (Miner et al., 2012) used frequently in 68 climate change studies (Dallas et al., 2016; Hector et al., 2019; Kirk et al., 2020), while Ordospora 69 transmission is representative of a classical environmentally-transmitted pathogen (that is, it mimics 70 diseases such as SARS-CoV-2 and Vibrio cholerae) and meets the assumptions of conventional 71 epidemiological models (e.g. infection following mass action (Kirk et al., 2019), continuous shedding 72 of infectious particles (Ebert, 2005) and little or no spatial structure within host populations). Our 73 microcosm experiment comprised three distinct temperature regimes: constant temperatures and two 74 variable temperature regimes with diurnal fluctuations of ± 3 °C and three-day heatwayes of six 75 degrees above ambient, all replicated over the natural temperature range of the model system (that is, 76 10-28 °C, Fig. 1). These variable temperature regimes were selected to mimic naturally-occurring 77 temperature events in habitats our study organisms encounter naturally (that is, small ponds and rock 78 pools) (Jacobs et al., 2008; Kuha et al., 2016).

During the experiment we measured host longevity, fecundity, infection status and the number of *O. colligate* spores within the host gut (see *Methods* for details). All measurements were conducted on individually-kept *Daphnia* with up to 18 replicates per measurement. To compare the three different temperature regimes (that is, constant, diurnal fluctuations, heatwave; Fig. 1) we fitted a Beta function (a function that can be used to capture thermal performance curves (Dowd et al., 2015)) using

a Bayesian framework. The advantage of using the Beta function is that each of its parameters has a clear a biological meaning, where F_m is the fitness at optimal performance for the fitted host or parasite trait, T_{opt} is temperature at optimal performance, and T_{min} and T_{max} are, respectively, the critical minimum and maximum temperatures over which fitness of the trait becomes unviable.





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90 Fig. 1. The three temperature regimes used in the experiment. Our experimental design comprised 91 seven constant temperature regimes with temperatures ranging from 10 °C to 28 °C, five variable 92 temperature regimes mimicking diurnal temperature fluctuations of ± 3 °C around the mean, and four 93 heatwave regimes where temperatures were identical to the equivalent constant treatment except 94 during a three-day period between days 20 and 23 when temperatures were raised by 6 °C. Constant 95 temperature regimes were replicated 12 times ($7 \times 12 = 84$ individuals), while in the variable 96 temperature regimes the number of replicates was increased to 18 as we expected increased mortality 97 in these treatments (5 \times 18 =90 and 4 \times 18 = 72, respectively for the fluctuating and heatwave 98 regime). Non-exposed controls, which received a placebo infection, were included for all treatments. 99 All animals were terminated after day 27 and fitness estimates were collected within three days. 100 101 **Results** 102 Diurnal temperature fluctuations narrowed the thermal performance curve for infectivity 103 compared with constant temperatures (Fig. 2A). The maximum temperature at which spores were able 104 to cause infections was 5 °C lower under fluctuating temperatures than under constant temperatures

105 (Fig. 2A; $T_{max} = 25$ °C for fluctuating vs. 30 °C for constant, confidence intervals for T_{max} do not

106 overlap). The thermal performance curve for infectivity under the heatwave, where temperatures were 107 raised by 6 °C for three days and then returned to constant temperature (Fig. 1), was almost identical 108 to that under constant temperature (all confidence intervals overlap, Fig 2A & Table S2). However, 109 unlike the constant temperature regime, the heatwave did not differ from the fluctuating regime, as 110 estimates for the maximum temperature had broad confidence intervals, likely caused by the lack of 111 data at the higher temperatures. Remaining parameter estimates of the Beta Equation were similar for 112 the three temperature regimes, with the highest rate of infection at 19 °C, a maximum infection rate of 113 ~95% infection and no infections under 10 °C (Fig. 2A & Table S2; confidence intervals overlap for 114 T_{opt} , F_m and T_{min}). Thus, while diurnal fluctuations led to less infection at higher temperatures, a 115 heatwave did not alter infection rates. 116



125 Fig. 2. Thermal performance curves of host and parasite life history traits across our three 126 temperature regimes. (a) Infection rates of Ordospora in its Daphnia host. (b) Mean number of spore 127 clusters in infected Daphnia at the end of the experiment. (c) Reproductive output of the host when 128 exposed to Ordospora (for a comparison of unexposed and exposed individuals see Fig. 3). For all 129 panels the constant temperature regime is in blue, the diurnally fluctuating regime in yellow and the 130 heatwave in red. Points present the observed mean values for the measured traits and dashed lines 131 provide the fit for the Beta Equation. 95% confidence intervals of minimum, optimal and maximum 132 temperature estimates (respectively, $T_{min}/T_{opt}/T_{max}$) are shown above the x-axis. The estimate for the 133 optimal value of the life history trait (F_m) and its 95% confidence interval is displayed to the right of

134 *each panel. Significant differences (non-overlapping 95% confidence intervals) in parameter*

135 estimates are highlighted with an asterisk. Error bars on data points indicate standard error. Beta

136 Equation parameter estimates displayed in this figure can be found in tables S2-S4.

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138 Spore burden of the two variable temperature regimes deviated from both the constant 139 temperature regime and from each other (Fig. 2B). Consistent with infection rates, daily temperature 140 fluctuation led to a lower maximum temperature (by ~3 °C) for parasite growth within the host, 141 resulting in a narrowed thermal performance curve for burden compared with the other temperature 142 regimes (Fig 2B & Table S3, non-overlapping confidence intervals for T_{max}). This is supported further 143 by the consistently lower spore burden for the fluctuating regime when compared with the constant 144 temperature regime except near the optimum temperature of 19 $^{\circ}$ C, where spore burdens of both 145 temperature regimes were similar (confidence intervals for T_{opt} and F_m overlap). While infection rates 146 and burden showed a similar thermal performance for diurnal fluctuations (both narrowing), the 147 response to the heatwave differed between infection and burden (Fig. 2). Compared to the constant 148 temperature regime spore burden in the heatwave showed a shift in the optimum temperature (from 149 19.4°C to 15.7°C), and an increase in the number of spore clusters (Fig. 2B, confidence intervals for 150 T_{opt} , and F_m do not overlap). However, while spore burden was different at ~16 °C, at ~19 °C spore 151 burden was nearly identical for all three temperature regimes. Moreover, due to the opposite effects at 152 16 °C for both variable temperature regimes (that is, a narrowing of performance under fluctuating 153 temperatures, exacerbation under heatwave) spore burden at this temperature differed by almost an 154 order of magnitude (that is, 86 vs. 737 spore clusters).

155 Host fitness was generally reduced when exposed to *Ordospora* spores or when experiencing variable

156 temperature regimes. *Daphnia* exposed to the parasite had lower reproductive success near the

157 optimum temperature (~20 °C) compared to unexposed controls (non-overlapping confidence intervals

- 158 for F_m) and lost between 8% (constant) and 24% (diurnal fluctuation) of reproductive output (Fig. 3).
- 159 Comparing host performance among the different temperature regimes shows that exposed animals in
- variable temperatures had lower reproductive success (Fig. 2C & Table S4, non-overlapping 95%
- 161 intervals for F_m), with a small shift (1.1 °C) in their thermal optimum under the heatwave regime (Fig.

- 162 2C, non-overlapping 95% intervals for T_{opt}). Unexposed animals also had lower fitness when 163 experiencing the heatwave (Fig. 3, non-overlapping 95% intervals for F_m) and, while the reproduction 164 at the optimal temperature of the unexposed animals experiencing diurnal fluctuations was lower, 165 confidence intervals overlapped with the constant temperature regime (Fig 3). The host response to the 166 variable temperature regimes differed from that of the pathogen (compare thermal performance curves 167 for the heatwave and diurnal fluctuating regimes between figures 2A, B & C). While host performance 168 was reduced (lower F_m) under both variable temperature regimes, parasite traits showed either a 169 narrowing of the performance curve (for diurnal fluctuations) or no effect and greatly increased 170 performance (for infection and burden under the heatwave).
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172 173 Fig. 3. Reproductive success in exposed and unexposed Daphnia. Exposed Daphnia (dotted lines) 174 produce less offspring than unexposed individuals (solid lines). Lines are the fitted Beta Functions for 175 the different temperature regimes (constant temperature regime in blue, the diurnally fluctuating 176 regime in yellow and the heatwave in red). 95% confidence intervals of reproductive output (F_m) are 177 shown to the right, and the temperature where it is at its optimum (T_{out}) , are shown above the x-axis. 178 Significant differences in parameter estimates of the Beta Function are highlighted with an asterisk. 179 Estimates for minimum and maximum temperatures are not displayed as we used restrictive priors. 180 Error bars on data points indicate standard error. Beta Equation parameter estimates displayed in

181 *this figure can be found in table S4.*

182 183 Discussion

184 We show that not only does temperature variation alter the thermal performance of host and 185 pathogen life history traits in a unique way, driving a shift in performance up to order-of-magnitude 186 over and above the effect of mean temperature, but that the type of variation and the mean temperature 187 at which it occurs are also critical. Indeed, each of the life history traits we measured was affected 188 differently by thermal variation. While variable temperature regimes affected host and pathogen 189 performance, the direction and strength of this depended both on the type of variation and the mean 190 temperature to which it was applied. With global warming altering the mean and variance of 191 temperature around the world, how this affects diseases and their dynamics is a critical outstanding 192 question (Claar et al., 2020; Rohr et al., 2013). Our results demonstrate that the combined effect of 193 changing temperature mean and variance can be highly complex, and may alter the vulnerability of 194 host populations (Harvell et al., 2019), affect the evolution of host and parasites (Buckley et al., 2016) 195 and, therefore, impede our ability to accurately predict future disease outbreaks.

196 Infection rates were reduced at higher temperatures when animals experienced diurnal 197 fluctuations but not after experiencing a heatwave. In Daphnia, filtration rates determine the contact 198 rate between host and pathogen, and a reduction in filtration can thus lead to reduced levels of 199 infection (Hall et al., 2010). As filtration rates of *Daphnia magna* decline at higher temperatures (Kirk 200 et al., 2019), average infection rates under diurnal temperature fluctuations would thus be expected to 201 be lower due to the non-linear nature of the thermal performance curve (that is, Jensen's inequality 202 (Dowd et al., 2015)). In addition, infection probability in our study system decreases sharply when 203 temperatures surpass 22 °C (Kirk et al., 2019), reducing infection rates under fluctuating temperatures 204 that exceed this temperature (again, due to Jensen's inequality). In systems where immune function 205 depends on temperature (e.g. insects, mosquitos and ectotherms in general (Paaijmans et al., 2013), 206 heatwaves may interact with the immune system in complex ways (Murdock et al., 2012), particularly 207 when the heatwave occurs early in the infection process. However, given that our heatwave occurred 208 twenty days post-infection and that *Daphnia* are not known to recover from infection (Ebert, 2005), 209 the effect of the heatwave on established infections may have been limited. Absence of an effect of a 210 heatwave on infection rates has also been found for a pipefish-trematode host-parasite system (Landis

et al., 2012). However, though the heatwave did not affect infection rates in our experiment, it didaffect parasite burden.

213 Our results show that different types of temperature variation can alter parasite burden and 214 thus affect pathogen growth within the host. While diurnal temperature fluctuations and heatwaves 215 brought about an almost order of magnitude difference in spore burden at a mean temperature of 16 216 °C, no differences were observed at ~19 °C. Generally, similar to infection rates, the thermal 217 performance curve for spore burden narrowed under fluctuating temperatures, as predicted by 218 averaging over the non-linear thermal performance curve (Denny, 2017; Dowd et al., 2015). The 219 impact of diurnal temperature fluctuations on parasite fitness has been studied previously, with 220 multiple studies suggesting a shift in the thermal performance of parasite fitness under fluctuating 221 temperatures (Dallas et al., 2016; Duncan et al., 2011; S. E. Greenspan et al., 2017; Paaijmans et al., 222 2010). Indeed, our findings that Ordospora has a narrower thermal performance for spore burden and 223 infectivity under fluctuating temperatures adds to a growing body of evidence (Dallas et al., 2016; S. 224 E. Greenspan et al., 2017; Hector et al., 2019; Roth et al., 2010) suggesting that estimates and 225 predictions that ignore temperature variation may over- or underestimate disease burden and 226 prevalence (Sasha E. Greenspan et al., 2017; Raffel et al., 2013; Rohr et al., 2013). Moreover, with 227 almost an order-of-magnitude difference between both our two variable temperature regimes at some 228 — though not all — temperatures, our results highlight that both the context and type of temperature 229 variance needs to be considered when trying to understand how pathogen performance may be 230 affected by climate change.

231 Spore burden increased following heatwaves, but the effect depended on the mean 232 temperature to which the heatwave was applied. Indeed, the heatwave had either higher, similar or 233 lower spore burden compared to the equivalent constant temperature regime. It was shown recently in 234 a fish-tapeworm host-parasite system that parasite growth, egg production and the number of first-235 stage larvae increased after a one-week exposure to higher temperatures (increase up to 7.5 °C) 236 (Franke et al., 2019). Our findings corroborate that heatwaves associated with climate change may, 237 under some conditions, increase disease burden. Indeed, we found a considerable increase in spore 238 burden and a shift in the optimum temperature following a three-day increase in temperature of 6 °C at 239 16 °C. Although some studies have reported increased disease susceptibility following heatwaves 240 (Dittmar et al., 2014; Roth et al., 2010), others found no effect on immune function (Stahlschmidt et 241 al., 2017) or reduced disease performance after exposure to high temperatures (Fayer et al., 1998). Our 242 results may explain these conflicting findings — we found that the effects of a heatwave on spore 243 burden are contingent on the mean temperature to which the heatwave is applied. That is, our results 244 show that the heatwave has either lower or higher burden than equivalent constant temperatures. This 245 context-dependency of heatwaves is supported further by studies in both plant-endoparasite (Schreven 246 et al., 2017) and herbivore-parasitoid (Zhang et al., 2019) systems, which showed that the effect of a 247 heatwave on parasite traits depended on the amplitude of the extreme event. As highlighted by a recent 248 review (Claar et al., 2020), effects of warming events on disease traits remain difficult to generalise, 249 and more studies and insight into underlying principles and mechanisms is needed to forecast the 250 effect of extreme heat events on disease dynamics. Indeed, while it is clear from our experiment that a 251 short, three-day increase in temperature can drastically alter the thermal performance curve for 252 parasite burden, the exact mechanism(s) underlying this change remains unidentified.

253 Differences in acclimatisation speeds between hosts and pathogens may explain the observed 254 increase in burden of Ordospora at 16 °C following a heatwave. According to the temperature 255 variability hypothesis (Raffel et al., 2013; Rohr et al., 2013), parasites, which have faster metabolic 256 rates due to their smaller size, should acclimatise more rapidly to changing temperatures than their 257 larger hosts. In unpredictable variable environments, such as our heatwave regime, parasites thus 258 should have an advantage over their hosts. Moreover, host resistance may also decrease as a result of a 259 trade-off between the energy demand for acclimatisation and immunity (Nelson et al., 1996). That 260 varying temperature can lead to higher infection prevalence has been established in Cuban tree frogs, 261 red-spotted newts and abalone (Ben-Horin et al., 2013; Raffel et al., 2013). While this hypothesis may 262 explain our observation of high burden for the heatwave near 16 °C, it does not, however, explain why 263 the response depends on the mean (that is, lower performance at higher temperatures). Though 264 Ordospora should have an overall advantage under the temperature variability hypothesis, the realised 265 advantage may be smaller as its thermal range is more restricted than its host (Kirk et al., 2018). The 266 heatwave may thus cause proportionally more stress in the parasite than the host at high temperatures,

267 consistent with the thermal stress hypothesis, which suggests that a shift in temperature may reduce 268 performance of either host or parasite (Paull et al., 2015). Indeed, that thermal stress can affect host 269 and pathogen performance has been well supported (Gehman et al., 2018; Kirk et al., 2019; Schreven 270 et al., 2017; Zhang et al., 2019). Alternatively, the observed increase in parasite burden due to 271 heatwaves may be system-specific and not explained by differences in acclimatisation speed. 272 Estimates show that growth rates of Ordospora increase by a factor of five between 20 °C and 24 °C 273 before declining again (Kirk et al., 2018). While the optimal performance of Ordospora occurs around 274 19 °C, due to the balance of thermal performance curves of other host and pathogen traits (e.g. 275 mortality, infectivity, etc), a temporary increase to 22 °C, as occurred under our heatwave at 16 °C, 276 may thus have exacerbated pathogen growth, particularly if different traits react differently to a 277 temperature disturbance, which may have disrupted the balance between host and parasite. 278 Changes in host fecundity in response to temperature variation differed to the response of both 279 parasite traits (that is, infectivity and spore burden) we measured. While infectivity and burden had 280 either a narrower thermal performance curve or showed a heightened and shifted peak, temperature 281 variation lowered reproductive output of the host near the thermal optimum. A reduction in 282 reproductive output of the host under variable temperatures is consistent with previous work both on 283 Daphnia (Schwartz et al., 2016) and in other systems (Craig et al., 1983; Uvarov et al., 2011). 284 Similarly, a reduction in host fecundity due to parasitism is well established (Ebert, 2005). Infection 285 may also reduce the thermal tolerance of the host (Hector et al., 2019), which would explain the small 286 shift of the thermal optimum for host reproduction under the heatwave regime. While host responses 287 are thus consistent with expectations, the distinct responses to the different temperature regimes of the 288 different life history traits we measured (that is, host fecundity, parasite infectively and parasite 289 burden) highlight that the effects of temperature variation on host-pathogen systems are complex. 290 When trying to model disease dynamics and outbreaks, we often include a multitude of host and 291 pathogen traits, each with their own thermal dependencies. Recent studies have made advances in 292 predicting disease growth and spread under rising mean temperatures, integrating approaches and 293 identifying mechanisms that can capture and predict the thermal performance of host and pathogen 294 traits within epidemiological models (e.g., metabolic theory) (Kirk et al., 2020). It remains to be seen,

however, whether such modelling frameworks can be extended to incorporate temperature variation,
especially considering the distinct responses for the life history traits we measured to each of our
variable temperature regimes.

298 Our study shows that temperature variation alters the outcome of host-pathogen interactions in 299 complex ways. Not only does temperature variation affect different host and pathogen life history 300 traits in a distinct way, but the type of variation and the mean temperature to which it is applied also 301 matters, with up to an order of magnitude change between diurnal fluctuations in temperature and 302 extreme heat events. With global warming altering both the mean and variance of temperature around 303 the world, we can thus expect to see unanticipated changes in disease dynamics of host-pathogen 304 systems. Indeed, extreme temperature events like *El Niño* have been linked to disease-driven collapses 305 of keystone predators (Harvell et al., 2019), increases in diseases such as dengue and cholera 306 (Anyamba et al., 2019), and shifts in the geographic distribution of pathogens (Claar et al., 2020). 307 While temperature variation can thus affect disease dynamics in human, wildlife and livestock 308 populations — with potentially devastating economic and health consequences (Altizer et al., 2013) — 309 the complexity of the effects of temperature and its variation currently limits our ability to move 310 beyond system-specific predictions, in particular for extreme temperature events (Claar et al., 2020). 311 We conclude that improving our mechanistic understanding of the role of temperature variation on 312 disease dynamics, and exploring the generality of its effects and how it affects thermal performance 313 curves of both hosts and parasites (Claar et al., 2020), are critical to predicting disease dynamics in a 314 warming world.

315

316 Materials and Methods

317 Study system

The crustacean *Daphnia magna* plays a key role in ecosystem functioning. *Daphnia* are filter feeders that consume planktonic algae and other microorganisms, thus promoting water transparency and helping to prevent algal blooms (Miner et al., 2012). They are a key food source for planktivorous fish and thus constitute a major part in the food web (Ebert, 2005) and play a key role in nutrient cycling (Elser et al., 2000). Over its entire range, *Daphnia* is affected by a broad variety of pathogens. 323 Here, we use *Odospora colligata*, a widely distributed microsporidium parasite that is only known to 324 infect Daphnia magna. This gut parasite has been recently used as a model to understand how changes 325 in mean temperatures under global warming may affect host-parasite systems (Kirk et al., 2020). 326 However, effects of temperature variance remain unstudied. Daphnia become infected when they 327 accidently ingest water borne spores of Ordospora while filter feeding. After successful establishment, 328 spores divide intracellularly in the gut epithelium of *D. magna* (Larsson et al., 1997) until they form a 329 cluster of 32 to 64 spores. Spores are then released either to the environment or go on to infecting 330 neighbouring cells after O. colligata lyses the cell. 331 332 Experimental set-up 333 In the laboratory, we established water baths with temperatures ranging from 10-28 °C. Each 334 bath was regulated with a temperature controller (Inkbird ITC-308) that interfaced with cooling 335 (Hailea HC300A) and heating (EHEIM JÄGER 300W) units. Pumps (Micro-Jet Oxy) were used to 336 create constant flow, which ensured equal temperature distribution within the water baths. Each bath 337 held up to 99 microcosms and was kept under natural lighting conditions (16:8 light:dark). 338 Temperature and light intensity were recorded using HOBO loggers which were housed in the spare 339 microcosms. Each microcosm was filled with up to 80 ml of Artificial Daphnia Medium (ADaM, 340 modified to use only 5% of the recommended seleniumdioxide concentration (Klüttgen et al., 1994)). 341 To test for the effect of changing both mean temperature and patterns of temperature variation 342 in our host-parasite system, we created three different temperature regimes: one constant and two 343 variable temperature regimes, the latter comprising diurnal temperature fluctuations and a heatwave 344 (Fig. 1). In the constant temperature regime, individual Daphnia were kept at one of seven 345 temperatures for the whole experimental period (that is, 10, 13, 16, 19, 22, 25 and 28 °C). The diurnal 346 fluctuation regime comprised five temperature levels, which experienced the same mean temperature 347 as the constant regimes but with a fluctuation of +3 °C every 12 hours (that is, 10 - 16 °C, 13 - 19 °C, 348 16 – 22 °C, 19 – 25 °C, 22 – 28 °C), mimicking diurnal fluctuations in small rock pools (Jacobs et al., 349 2008). The heatwave was performed at four different temperature levels (13, 16, 19, 22 °C), with 350 conditions identical to the constant regime except for an increase of 6°C for 72 hours, 20 days after 351 animals were exposed to the parasite, mimicking a short heatwave (Kuha et al., 2016). We chose these

352 temperature levels because of their relevance for our host and pathogen system, as no infection occurs 353 below 12 °C and hosts have high mortality above 30 °C (Kirk et al., 2018, 2019). Animals were kept 354 individually in microcosms, organised into trays and repositioned daily to avoid positioning effects. In 355 each temperature regime half of the microcosms were exposed to the parasite while the other half 356 served as controls. For each of the constant temperature levels, we used 12 replicates for both exposed 357 and control animals. However, as we expected increased mortality in the variable temperature regimes 358 (Régnière et al., 2012), we increased the number of replicates of these regimes to 18. We based this 359 number of replicates on experience with previous temperature experiments with the Daphnia-360 Ordospora system (Kirk et al., 2019).

361 The *Daphnia* genotype (clone FI-OER-3-3) we used was previously isolated from a rock pool 362 at Tvärminne archipelago, Finland and propagated clonally in the laboratory. To generate sufficient 363 animals for the experiment, we grew Daphnia asexually under standardized conditions for three 364 weeks. Animals were raised in small populations (twenty 400 ml microcosms, 12 animals per 365 microcosm) under continuous light at 20 °C. The medium (ADaM) was replaced at least twice a week 366 and Daphnia were fed ad libitum with Scenedesmus algae (Scenedesmus sp.), which was grown in 367 batch cultures at 20 °C in WC Medium (Kilham et al., 1998) under nutrient- and light-saturated 368 conditions. The experiment was initiated by collecting a cohort of female juveniles (~600 females up 369 to 72 hours old) from the small population microcosms. Individual juveniles were then randomly 370 transferred into 100 ml glass microcosms filled with 40 ml ADaM. These glass microcosms were 371 placed into their assigned water baths and, after an acclimation period of 24 hours, the animals were 372 exposed to the parasite by adding 1 ml medium containing ~10000 spores of O. colligata. This spore 373 solution was prepared by crushing 3560 infected D. magna individuals with known average burden 374 (determined by using phase contrast microscopy on a sub-sample), using mortar and pestle and 375 diluting down the resulting spore slurry. The unexposed controls received a placebo exposure 376 consisting of crushed uninfected animals diluted in medium. Animals were exposed either to the 377 parasite or placebo for six days and were transferred subsequently to clean microcosms with fresh 378 medium (80 ml of ADaM) twice a week until the end of the experiment. Animals were fed four times 379 a week with increasing amount of algae to accommodate the increased food demand of the growing

animals (from 4 million algae ml⁻¹ at the start of the experiment to 10 million algae ml⁻¹ by day ten
which was maintained until the end of the experiment). Between transfers, evaporation of the medium
was offset by refilling microcosms daily with 50-50 ADaM-distilled water.

383

384 Measurements of host and parasite life history traits

385 To obtain fitness estimates for the host, we counted the offspring produced and checked 386 mortality of all animals daily. Infection status and spore burden (that is, the number of spores inside 387 the host) were assessed upon death by dissecting individuals and counting the number of spore clusters 388 (each cluster holds up to 64 parasite spores) in the gut with phase contrast microscopy (400x 389 magnification). Any animals that remained alive until the end of the experiment (day 27) were 390 terminated within three days, dissected and their infection status and burden were determined without 391 the observer being aware of the identity of the sample. Because infections cannot be diagnosed 392 accurately in early infection stages, animals that died before day 11 were not considered in analyses. 393 Any male *Daphnia* that were misidentified as female at the start of the experiment were also excluded. 394 In addition, to prevent potentially confounding effects of animals that died early (where the parasite 395 had less time to grow) as having lower spore burden, we included only animals from the last day of 396 the experiment in the analysis for spore burden. Note that, to facilitate good estimates for spore 397 burden, we terminated most hosts before natural death occurred, which limits our ability to assess the 398 effects of virulence (host mortality, reduced fecundity).

399 Data analysis

Analyses were performed using R version 3.6.1(R Core Team, 2018) interfacing with JAGS
(Lunn et al., 2009; Plummer et al., 2006), and used datafiles and code are available at
<u>https://github.com/charlyknz/HostParasite.git</u>. A Beta Function was fitted to each of our different
fitness estimates (that is, host fecundity, parasite infectivity and burden) for each of the three
temperature regimes, as:

$$f = F_m \left(\frac{T_{max} - T}{T_{max} - T_{opt}} \right) \left(\frac{T - T_{min}}{T_{opt} - T_{min}} \right)^{\left(\frac{T_{opt} - T_{min}}{T_{max} - T_{opt}} \right)}$$

405 where f is fitness at temperature T, F_m is estimated fitness at optimal performance for the fitted host or 406 parasite trait, T_{opt} is temperature at optimal performance, and T_{min} and T_{max} are, respectively, the critical 407 minimum and maximum temperatures over which fitness of the trait becomes unviable. This non-408 linear function has been shown to capture thermal performance accurately (Niehaus et al., 2012) and 409 has the advantage that all four parameters in the equation have clear biological meaning. 410 To determine the effect of both mean and variation in temperature on host and pathogen traits, 411 we used a Poisson distribution for reproductive output (number of offspring per individual) and spore 412 burden (number of spore clusters produced by the parasite). For pathogen infectivity we used a 413 binomial distribution. Models were fitted using the MCMC fitting algorithm called from R. All 414 models were fitted in a Bayesian framework with JAGS (Lunn et al., 2009; Plummer et al., 2006), 415 while allowing for separate parameter values for each of the different temperature regimes. Priors for 416 temperature effects were specified in order to satisfy the necessary condition $T_{min} \le T_{opt} \le T_{max}$ and 417 informed by previous work (See Table S1 for the priors) (Kirk et al., 2018, 2019, 2020). The posterior 418 distribution of all parameters was estimated using 3 chains, 10000 posterior draws which were then 419 thinned by five to yield 6000 samples (3*10000/5). Model convergence was checked using the 420 Gelman-Rubin diagnostic. 421 422 423 Acknowledgements 424 We thank Dieter Ebert and Jürgen Hottinger for provision of the biological materials, Alison Boyce 425 for technical assistance in creating the water baths and Maren Striebel for helpful comments on the 426 manuscript. ALJ was funded by an Irish Research Council grant IRCLA/2017/186. PL was funded by 427 a Science Foundation Ireland Frontiers for the Future grant 19/FFP/6839. The authors have no 428 conflicts of interest to declare. 429

430 Author contributions

- 431 CK, PL and ID designed the experiment. CK and PL conducted the experiment with assistance of ID.
- 432 ALJ, CK and PL conducted the analyses. CK and PL wrote the first draft of the manuscript and all
- 433 authors contributed to revisions.

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599 Table S1: Priors for each of the parameters in the Beta Function for each of the different (j) 600 temperature regimes (constant, fluctuating, heatwave) and life history traits (infection rate, spore 601 burden, reproductive output) which were all drawn from the uniform distribution with specified limits. 602 Priors for the minimum, optimal and maximum temperature satisfy the necessary condition 603 $T_{min} \leq T_{opt} \leq T_{max}$ and were informed by previous work (Kirk et al., 2018, 2019). Priors for the scaling 604 parameter F_m were restricted to be positive and less than ten on the log10 scale for both spore burden 605 and host reproductive output and between 0 and 1 for infection rates. A Poisson likelihood was used 606 for the observed reproductive output of the host and spore burden and the rate parameter λ was 607 modelled as a function of temperature with a log link function, with different parameter values for 608 each of the three temperature regimes. For infection rates we used a similar approach but using a 609 binomial likelihood where the probability p was estimated from the beta function constrained so that 0 610 $\leq p \leq 1$ and N was the number of *Daphnia* in each temperature regime.

parameter	Infection rate	Spore burden	Host reproductive output
	(min, max)	(min, max)	(min, max)
F_m [j]	0,1	0,10	0,10
<i>T_{min}</i> [j]	5, 15	5,14	0,14
T_{opt} [j]	$T_{min}[j] + 0, T_{min}[j] + 20$	$T_{min}[j] + 0, T_{min}[j] + 10$	$T_{min}[j] + 0, T_{min}[j] + 25$
<i>T_{max}</i> [j]	$T_{opt}[j] + 0,35$	$T_{opt}[j] + 0, 30$	$T_{opt}[j] + 0, 40$

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613 Table S2: Estimates of the parameters of the Beta Function for infection rate over the different 614 temperature regimes. Provided are the mean thermal minimum (T_{min}) , maximum (T_{max}) and thermal

615 optimum (T_{opt}), as well as the maximum infection rate (F_m) with 95% confidence interval (lower CI, 616 2.5% and upper 97.5%). The sample sizes for these estimates were respectively 82, 85 and 63 for 617 constant, fluctuating and heat wave regimes.

Variable	Temperature	Infection	Mean	CI 2.5%	CI 97.5%
	regime	status			
F_m	constant	exposed	0.96	0.87	1.00
F_m	fluctuating	exposed	0.94	0.84	1.00
F_m	heat wave	exposed	0.95	0.85	1.00
T _{max}	constant	exposed	30.23	27.98	34.04
T_{max}	fluctuating	exposed	24.98	24.32	26.41
T_{max}	heat wave	exposed	29.49	24.96	34.56
T_{min}	constant	exposed	9.86	6.39	10.70
T_{min}	fluctuating	exposed	10.75	6.10	12.33
T_{min}	heat wave	exposed	10.88	5.42	13.79
Topt	constant	exposed	19.72	18.13	21.27
T_{opt}	fluctuating	exposed	19.06	17.74	20.15
Topt	heat wave	exposed	19.23	17.49	20.95

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621	Table S3: Estimates of the parameters of the Beta Function for parasite burden over the different
622	temperature regimes. Provided are the mean thermal minimum (T_{min}) , maximum (T_{max}) and thermal
623	optimum (T_{opt}) , as well as the estimated number of spores at the thermal optimum (F_m) with their 95%
624	confidence interval (lower CI, 2.5% and upper 97.5%). The sample sizes for these estimates were
625	respectively 51, 46 and 46 for constant, fluctuating and heat wave regimes.

Variable	Temperature	Infection	Mean	CI 2.5%	CI 97.5%
	regime	status			
F_m	constant	exposed	489.78	478.63	501.19
F_m	fluctuating	exposed	467.74	446.68	478.63
F_m	heat wave	exposed	794.33	758.58	831.76
T _{max}	constant	exposed	27.70	27.45	27.95
T_{max}	fluctuating	exposed	24.80	24.52	25.07
T_{max}	heat wave	exposed	28.73	28.17	29.37
T _{min}	constant	exposed	11.49	10.75	12.11
T_{min}	fluctuating	exposed	11.48	10.18	12.32
T_{min}	heat wave	exposed	13.91	13.90	13.91
Topt	constant	exposed	19.44	19.34	19.54
T_{opt}	fluctuating	exposed	19.30	19.23	19.39
T_{opt}	heat wave	exposed	15.76	15.53	15.97

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629Table S4: Estimates of the parameters of the Beta Function for the reproductive output of the host over
the different temperature regimes. Provided are the mean thermal minimum (T_{min}) , maximum (T_{max})
and thermal optimum (T_{opt}) , as well as the estimate for the highest reproductive output (F_m) with their
and 95% confidence interval (lower CI, 2.5% and upper 97.5%). The sample size for these estimates
were 81 and 81 for constant, 85 and 87 for fluctuating, and 64 and 61 for heat wave regimes for
infected and uninfected individuals respectively.

Variable	Temperature	Infection	Mean	CI 2.5%	CI 97.5%
	regime	status			
F_m	constant	exposed	101.39	97.44	105.20
F_m	constant	unexposed	109.65	105.74	113.76
F_m	fluctuating	exposed	81.28	78.71	84.14
F_m	fluctuating	unexposed	106.41	102.85	110.15
F_m	heat wave	exposed	92.90	89.79	96.16
F_m	heat wave	unexposed	101.39	97.37	105.68
T _{max}	constant	exposed	36.22	35.37	37.16
T_{max}	constant	unexposed	37.58	36.73	38.51
T_{max}	fluctuating	exposed	38.90	36.54	39.97
T_{max}	fluctuating	unexposed	31.06	30.49	31.72
T_{max}	heat wave	exposed	39.46	38.11	39.99
T_{max}	heat wave	unexposed	34.68	30.79	39.38
T _{min}	constant	exposed	10.50	10.22	10.68
T_{min}	constant	unexposed	10.59	10.34	10.70
T_{min}	fluctuating	exposed	6.66	3.57	8.45
T_{min}	fluctuating	unexposed	0.63	0.02	2.18
T_{min}	heat wave	exposed	13.09	12.72	13.36
T_{min}	heat wave	unexposed	7.00	0.95	10.57
Topt	constant	exposed	19.61	19.43	19.80
Topt	constant	unexposed	20.22	20.05	20.39
Topt	fluctuating	exposed	20.50	20.12	20.89
Topt	fluctuating	unexposed	20.26	20.12	20.40
Topt	heat wave	exposed	18.52	18.23	18.83
T_{opt}	heat wave	unexposed	21.16	20.74	21.69