Environmental variability can promote parasite diversity within hosts and transmission among hosts

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1.0.0. Abstract

While the mechanisms that govern disease emergence and spread among hosts are increasingly well-described, the mechanisms that promote parasite diversity within-hosts, affecting host outcomes and spillover potential, have been comparatively understudied. Furthermore, while attention has been paid to the effects of increasing temperatures on disease systems, the effects of environmental variability have been left underexplored, despite rising climatic variability and internal temperature variability in a prolific reservoir for disease. To investigate the impacts of environmental variability on parasite diversity within-hosts, we analyzed a model of within-host population dynamics wherein two parasites indirectly compete through the host's immune response. We simulated the model under constant, demographically stochastic, environmentally stochastic, and demographically and environmentally stochastic conditions, and analysed the viability and longevity of non-equilibrium parasite co-occurrence. We found that environmental stochasticity increased the viability and longevity of parasite co-

occurrence, suggesting that thermal variability arising from climatic change and as a physiological trait may promote parasite diversity within ectotherms and help explain bats' propensity to support diverse communities of parasites. Further, we found that under certain conditions, the transmissibility of co-occurring parasites can surpass the transmissibility of single parasites, suggesting that thermal variability may increase the transmissibility of co-occurring parasites.

2.0.0. Introduction

In recent years, it has become clear that climate and land-use change can facilitate novel species interactions and increase contact rates between wild populations and humans, resulting in devastating epidemics and enabling spillover events and disease emergence among wild and human hosts alike (Daszak et al., 2000, 2001; Patz et al., 2004; R. Plowright et al., 2020; R. K. Plowright et al., 2017). However, despite the consequential interplay between within- and among-host processes, the mechanisms that promote parasite diversity within hosts and create kindling for events observed among hosts have been understudied. In particular, while stabilizing mechanisms like niche differentiation have been employed to explain the maintenance of parasite diversity within hosts, the contributions of equalizing mechanisms, which decrease average fitness differences between competitors, have yet to be examined (Bashey, 2015; Chesson, 2000; Chesson & Huntly, 1997; Karvonen et al., 2006; Rynkiewicz et al., 2015).

An intermediate level of disturbance may promote species diversity by forestalling the competitive advantages allowed by low levels of disturbance, and the extinction risks brought on by high levels of disturbance (Chesson, 1994; Connell, 1978).

Expanding on the work of Connell (1978) and Chesson (1994), Roxburgh et al. (2004) posited that because different species respond to disturbances in different ways, there are myriad ways in which diversity may be generated, thereby interpreting the intermediate disturbance hypothesis not as a mechanism unto itself, but as a collection of mechanisms through which intermediately sized or timed disturbances in resource availability or environmental conditions may promote diversity (Roxburgh et al., 2004). Though the applicability of the intermediate disturbance hypothesis has been debated, positive and negative effects of environmental variability on diversity have been well-documented across systems (Laprise & Dodson, 1994; Righetti et al., 2019; Shurin et al., 2010; Vega-Rodriguez et al., 2015).

When considering the effects of environmental variability on parasite diversity within hosts, there arise a number of ways to conceptualize environmental variability. First, one may consider rising environmental variability as a feature of climate change (Stocker et al., 2013). While much attention has been paid to the effects of rising temperatures on disease systems, far less has been invested in exploring the effects of rising temperature variability, despite evidence of its outsized impacts on species outcomes (Dobson & Carper, 1992; Lafferty, 2009; Lawson et al., 2015; Vasseur et al., 2014). In ectothermic hosts, increasingly erratic external conditions will affect the internal conditions of the host, thereby also affecting any parasites the host may harbour (Cizauskas et al., 2017). A relationship between thermal variability and within-host parasite diversity may be of particular interest when considered alongside the long-debated relationship between climate change and widespread disease-mediated

amphibian declines (Cohen et al., 2019; Daszak et al., 1999, 2003; Lips et al., 2008; Pounds et al., 1999; Rohr et al., 2008; Rohr & Raffel, 2010).

Second, one may consider environmental variability as a physiological trait of hosts. In particular, bats (order *Chiroptera*) have long been acknowledged as prolific reservoirs for disease and have oft been implicated in zoonotic spillover events (Brook & Dobson, 2015; Calisher et al., 2006; Irving et al., 2021). Bats are also unique among mammals in their ability to vary their internal temperature according to their activity level (e.g. torpor, flight) (Fumagalli et al., 2021). It has been theorized that these variations in internal temperature may aid bats' immune responses, allowing them to carry microparasites without suffering outsized morbidity (Fumagalli et al., 2021; Luo et al., 2021). Extending on that idea, it seems pertinent to question how internal temperature variation may contribute to bats' propensity to support large communities of parasites.

We used a model to investigate the impacts of environmental variability on the viability and maintenance of parasite diversity within hosts. We began by modifying Antia et al.'s (1994) model of within-host population dynamics to include two parasite strains or species that indirectly interact via the host's immune system. We analyzed deterministic, demographically stochastic, environmentally stochastic, and demographically-and-environmentally stochastic implementations of the model. Within the environmentally stochastic implementations of the model, we varied the magnitude and frequency of thermal disturbance within the system. Between and among the deterministic and stochastic simulations of the model, we compared the ability of the secondary parasite to invade a system containing the primary parasite, and the duration of time for which the primary and secondary parasites co-occurred. Further, we

compared the transmission potentials of co-occurring parasites with the transmission potential of a single parasite. We found that compared to a constant environment, a variable environment tended to increase the ability of the secondary parasite to invade the system, facilitated longer periods of co-occurrence between the primary and secondary parasites, and increased the transmissibility of co-occurring parasites. These results (1) suggest that climatic changes may have significant effects on parasite diversity within ectothermic hosts, (2) provide insight on a host's ability to support large communities of parasites, and propose that variability within-hosts may influence transmission dynamics among-hosts.

3.0.0. Methods

3.1.0. Model Description

3.1.1. Within-Host Population Dynamics Model

We began with a model of within host population dynamics based on Antia et al., (1994) that represents the interaction between an infecting parasite and its host's immune system as analogous to the interaction between a prey item and its predator. The model assumes that the replication rate of the host's immune system is proportional to parasite density at low parasite densities, and saturates at high parasite densities, allowing the host's immune system to either extirpate or succumb to the infection (Antia et al., 1994). We adapted the model to include a second parasite that interacts indirectly with the primary parasite through the host's immune response (Eqns. 1-3, Table 1). Parameters values were not chosen to describe a specific system, but to replicate a dynamical behaviour, as described above and shown in Figure 3. Given that an infinite number of parameter value combinations could have produced this behaviour, we opted

to maintain the initial conditions and select parameter values proposed by Antia et al. (1994). However, we increased the rate at which parasites are destroyed by the host's immune system and decreased the maximum replication rates of the primary and secondary parasites to favour extirpation, and eliminate host death as an infection outcome (Table 1). We also conducted a sensitivity analysis to determine how variations in (1) the value of k and (2) the shape of the relationship between parasite replication rate and the temperature of the system would affect our results, and found the model to be sufficiently robust (see pages 2, 4-8 of supplement for complete results).

$$(1) \quad P_i(t+1) = (1 + r_i - \left(1 - e^{-kI(t)}\right))P_i(t)$$

$$(2) \quad P_j(t+1) = (1 + r_j - \left(1 - e^{-kI(t)}\right))P_j(t)$$

$$(3) \quad I(t+1) = (1 + \rho\left(\frac{P_i(t)}{P_i(t) + \phi} + \frac{P_j(t)}{P_j(t) + \phi}\right))I(t)$$

Table 1: Parameter definitions and values.

Symbol	Parameter	Value used in Antia et al. (1994)	Value Used
P_i and P_j	Parasite abundance	1*	1*
I	Host immune cell abundance	1*	1*
r_i and r_j	Replication rates of parasites	0.1 – 10.0	0.1 – 2.0†
k	Rate at which parasites are destroyed by host's immune system	10^{-3}	10^{-2}
ρ	Maximum replication rate of host's immune system	1.0	1.0
ϕ	Parasite density at which replication rate of host's immune system is half its maximum	10^3	10^3
D	Lethal within-host parasite density	10^9	10^9

* Initial conditions

† Dependent on the temperature of system in environmentally stochastic simulations

To compare the impacts of different types of stochasticity on parasite diversity, we wrote deterministic (D), demographically stochastic (S_D), environmentally stochastic (S_E), and demographically-and-environmentally stochastic (S_{DE}) implementations of the model. Within the demographically stochastic implementation of the model, we wrote the model's growth terms as Poisson processes, and mortality term as a binomial process (Eqns. 4-6).

(4) $P_i(t+1) = P_i(t) + B_{P_i}(t) - D_{P_i}(t)$

(5) $P_j(t+1) = P_j(t) + B_{P_j}(t) - D_{P_j}(t)$

(6) $I(t+1) = I(t) + B_I(t)$

In Eqns. (4) and (5), $B_{P_i}(t)$ and $B_{P_j}(t)$ are Poisson random variables such that $B_{P_i}(t) \sim Poisson(1, \lambda_i)$, where $\lambda_i = r_i \cdot P_i(t)$, and $B_{P_j}(t) \sim Poisson(1, \lambda_j)$, where $\lambda_j = r_j \cdot P_j(t)$. In Eqn. (6), $B_I(t)$ is a Poisson random variable such that $I_B(t) \sim Poisson(1, \lambda)$, where $\lambda = \rho\left(\frac{P_i(t)}{P_i(t)+\phi} + \frac{P_j(t)}{P_j(t)+\phi}\right) I(t)$. In Eqns. (4) and (5), $P_{i,D}(t)$ and $P_{j,D}(t)$ are binomial random variables such that $D_{P_i}(t) \sim Binomial(1, P_i(t), p)$, where $p = 1 - e^{-kI(t)}$, and $D_{P_j}(t) \sim Binomial(1, P_j(t), p)$. To write the environmentally stochastic implementation of the model, we generated a stochastic temperature series and allowed the replication rates of each parasite to vary in response to the temperature of the system (Eqns. 7-9).

(7) $P_i(t+1) = (1 + r_i(T(t+1)) - (1 - e^{-kI(t)}))P_i(t)$

(8) $P_j(t+1) = (1 + r_j(T(t+1)) - (1 - e^{-kI(t)}))P_j(t)$

(9) $I(t+1) = (1 + \rho\left(\frac{P_i(t)}{P_i(t)+\phi} + \frac{P_j(t)}{P_j(t)+\phi}\right))I(t)$

We used first-order autocorrelated Gaussian noise to generate stochastic temperature series. We began with a value of 0, and generated the next value, $T(t+1)$, by multiplying the previous value by the coefficient of autocorrelation (AC = 0.25), and summing the resulting value with a value drawn from a normal random variable with a mean of zero. We varied the standard deviation of the normal random variable, as described below. We then added the average temperature of the system to each value in the series. Resulting values outside the thermal range of the system were re-drawn. We then converted the stochastic temperature series into series of replication rates using various parameterizations of the Sharpe-Schoolfield sub-model, as detailed below (Eqns. 10.1, 10.2; Table 2, Table S2).

$$(10.1) \quad r_i(T(t+1)) = r_{i,0} e^{\frac{-E_{r_i}}{k}\left(\frac{1}{T(t+1)} - \frac{1}{T_0}\right)} \cdot \left(1 + e^{\frac{E_{r_i}^L}{k}\left(\frac{1}{T_{r_i}^L} - \frac{1}{T(t+1)}\right)} + e^{\frac{E_{r_i}^H}{k}\left(\frac{1}{T_{r_i}^H} - \frac{1}{T(t+1)}\right)}\right)^{-1}$$

$$(10.2) \quad r_j(T(t+1)) = r_{j,0} e^{\frac{-E_{r_j}}{k}\left(\frac{1}{T(t+1)} - \frac{1}{T_0}\right)} \cdot \left(1 + e^{\frac{E_{r_j}^L}{k}\left(\frac{1}{T_{r_j}^L} - \frac{1}{T(t+1)}\right)} + e^{\frac{E_{r_j}^H}{k}\left(\frac{1}{T_{r_j}^H} - \frac{1}{T(t+1)}\right)}\right)^{-1}$$

Table 2: Parameter definitions. See Table A1 for parameter values.

Symbol	Unit	Parameter
T	K	Temperature of the system at time t
r_0		Baseline values of r_i or r_j at a reference temperature T_0
T_0	K	Reference temperature (set arbitrarily such that T^L << T_0 << T^L)
E_r	eV	Activation energy of r_i or r_j
E_r^L, E_r^H	eV	Low- and high-temperature inactivation energies of r_i or r_j
T_r^L, T_r^H	K	Low- and high-temperature inactivation thresholds for r_i or r_j
k		Boltzmann's constant

3.1.2. Sharpe-Schoolfield Sub-Model

The Metabolic Theory of Ecology (MTE) predicts how metabolic rates control ecological processes across levels of organization, and explains how metabolic rates

vary with body size and temperature (Brown et al., 2004). Building off the MTE, the Boltzmann-Arrhenius (BA) relation describes metabolism as a function of temperature. The Sharpe-Schoolfield model characterizes the performance of a trait as a function of temperature through a thermal performance curve (TPC) (Dell et al., 2011; Molnár et al., 2017; Schoolfield et al., 1981). When applied to disease systems, the Sharpe-Schoolfield model has been shown to describe relationships between temperature and species traits, and has proven useful in investigating the relationship between rising temperatures and disease transmission (Kirk et al., 2018; Molnár et al., 2013).

We used various parameterizations of the Sharpe-Schoolfield model to generate paired thermal performance curves relating the temperature of the system to the replication rates of two parasites (r_i and r_j) (Figure 1). We generated four paired thermal performance curves, representing four thermal scenarios: (1) the identical responses scenario, (2) the offset responses scenario, (3) the P_i-advantage scenario, and (4) the P_j-advantage scenario (Table S1). Each thermal scenario describes a different relationship between the temperature of the system and parasite replication rates. The identical responses scenario describes r_i and r_j as responding identically to the temperature of the system (Fig. 1). Under the offset responses scenario, r_i and r_j peak on opposite sides of the average temperature of the system (Fig. 1). To develop the P_i-advantage scenario, we decreased the average temperature of the system to give the primary parasite, P_i, a thermal advantage (Fig. 1). We considered two versions, (I) and (II), of the P_j-advantage scenario, each of which gave the secondary parasite, P_j, a thermal advantage. To develop (I), we increased the average temperature of the system to give the secondary parasite a quantitatively comparable thermal advantage (Fig. 1).

To develop (II), we replicated the P_i-advantage scenario, and assigned the TPC with the lower optimum to P_j, to give the secondary parasite the same thermal advantage given to the primary parasite and isolate the effects of asymmetry (Fig. S9). Given that (I) and (II) produced qualitatively comparable results, we will only discuss (I) in the main text (see supplement for complete results). Within each thermal scenario, the average temperature of the system was chosen to maintain quantitatively comparable (1) parasite replication rates between thermal scenarios and (2) differences between replication rates at the average temperature of the system.

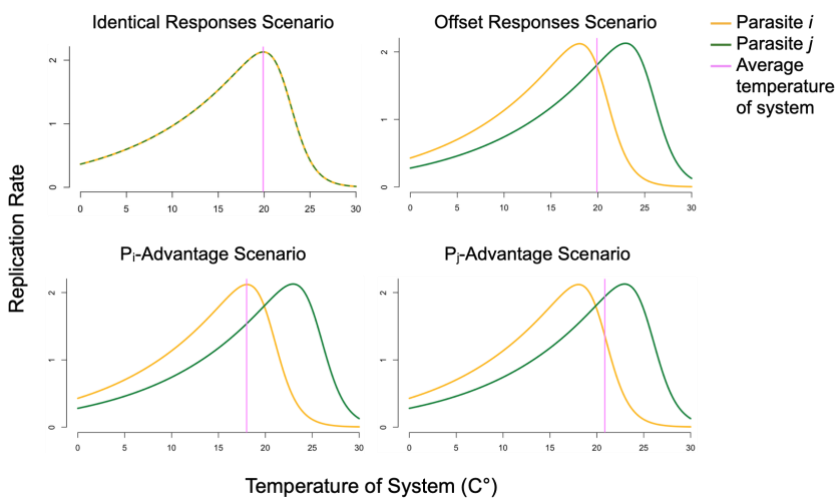

Figure 1: Sharpe-Schoolfield thermal performance curves relating the temperature of the system to the replication rates (r_i and r_j) of two parasites (P_i and P_j). Yellow lines show the relationship between the temperature of the system and the replication rate (r_i) of P_i. Green lines show the relationship between the temperature of the system and the replication rate (r_j) of P_j. Pink lines denote the average temperature of the system. Each panel (A-E) shows a different thermal scenarios.

3.2.0. Model Simulations

Within each thermal scenario, we simulated the deterministic model (D), and each of the three stochastic models (S_D, S_E, S_{DE}) for fifty days with a discretization time step of 0.1, which was sufficient to capture the full course of parasite infection and clearance across all simulations. We began each set of simulations with the primary parasite and the host's immune system in the system, at initial abundances of 1 as the

initial condition. We then considered all cases for the introduction of the secondary parasite from its introduction at the onset of infection, to its introduction on the fourty-ninth day of infection.

For each environmentally stochastic model (S_E, S_{DE}) simulation, we generated a new temperature series and two corresponding series of replication rates. When determining the ways in which new temperatures would be selected, we considered two axes of thermal disturbance: magnitude and frequency. To vary magnitude of disturbance, we varied the standard deviation of the normal random variable in the temperature autocorrelation function, starting from zero (no thermal disturbance) and increasing by two until the effect of magnitude on co-occurrence was fully characterized (SD = 30), resulting in fifteen discrete magnitudes of disturbance. To vary frequency of disturbance, we re-assigned the temperature of the system every 10, 9, 8, 7, 6, 5, 4, 3, and 2 days, once per day, and 2, 5, and 10 times per day, resulting in thirteen discrete frequencies of disturbance. We conducted 1000 simulations per stochastic model and treatment (Fig. 2). Two hundred such simulations are shown in Figure 3.

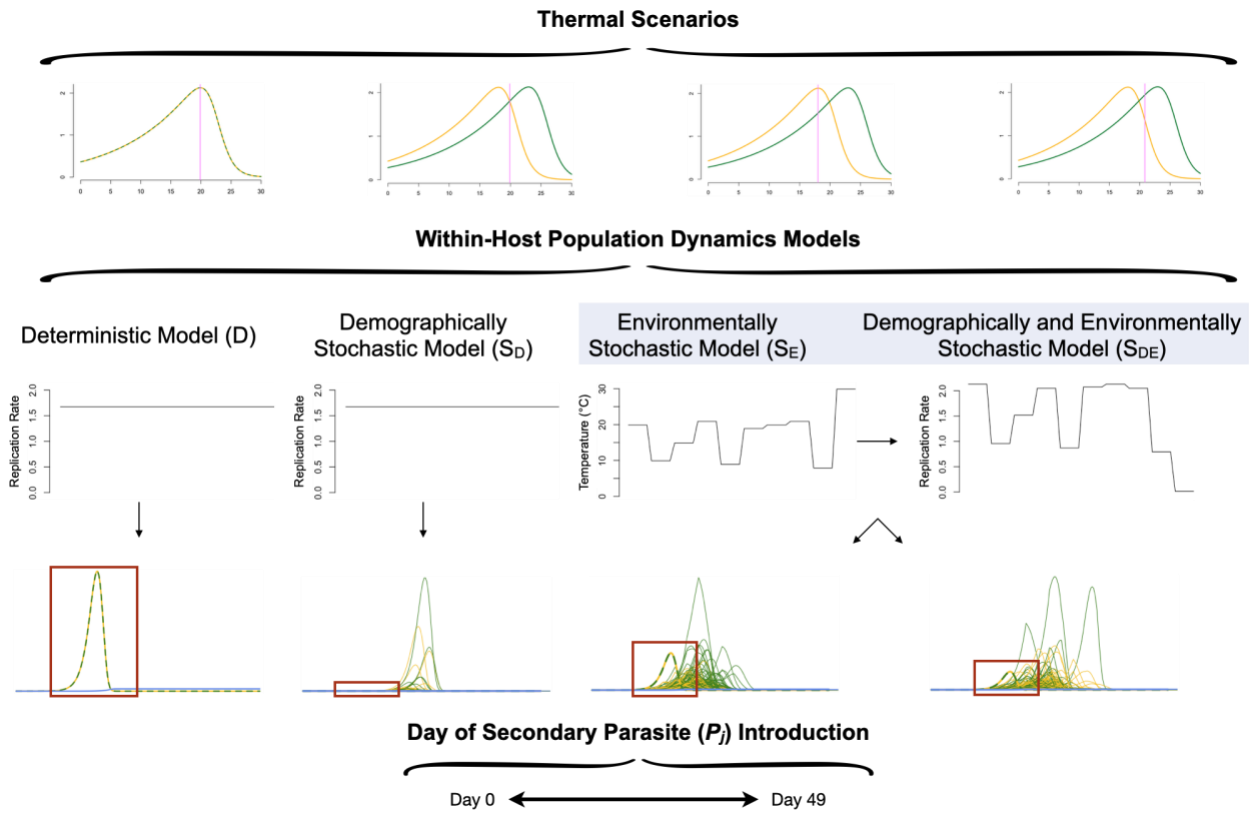

Figure 2: Schematic detailing simulation experiment. We considered four thermal scenarios, and four models (deterministic, demographically stochastic, environmentally stochastic, and demographically and environmentally stochastic). When simulating the environmentally stochastic models, we considered 195 disturbance regimes. When simulating each of the four models, we considered 50 days on which the secondary parasite could be introduced. We conducted 1000 simulations per stochastic model and treatment.

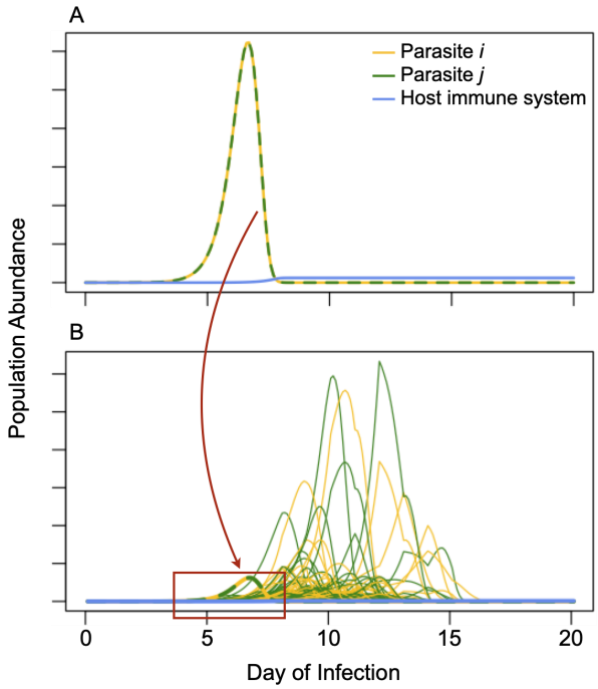

Figure 3: Example of deterministic and stochastic population trajectories. Panel A shows a simulation of the deterministic model. Parasite replication rates were given by the identical responses scenario, and the temperature of the system was held at the average temperature of the system (19.9°C). Panel B shows two hundred S_{DE} model simulations. Parasite replication rates were given by the identical responses scenario, and thermal disturbances were intermediately sized and timed. In both cases, P_i and P_j were introduced simultaneously.

3.3.0. Co-Occurrence Analyses

We quantified longevities of co-occurrence by counting the number of time steps for which primary and secondary parasite population abundances were equal to or greater than one within each simulated timeseries. We summarised these results as distributions for each stochastic model and treatment. Using the S_D model as a baseline, we investigated the effects of environmental variability on the viability and maintenance of parasite diversity by comparing the ability of the secondary parasite to invade a single-parasite system, and the duration of time for which the primary and secondary parasites co-occurred across stochastic treatments. Additionally, we analysed (1) the proportion of stochastic simulations per model and treatment that resulted in longer periods of co-occurrence relative to the deterministic model to quantify rarity, and (2) the competitive outcomes of each treatment to gain insight on underlying mechanisms.

3.4.0. Transmissibility Analyses

To explore the impacts of co-infection on transmission potential, we compared the lifetime replicative outputs of co-occurring parasites with those of a single parasite in constant and variable environments. As per Antia et al., (1994), we determined the total transmission (q_i, q_j, q, where q is the total transmission of the single parasite) of a parasite (P_i, P_j, P, where P is the single parasite) from an infected host by taking the integral of the rate of transmission (u) of each parasite from the host over the duration of infection (Eqns. 11.1-11.3). For simplicity, we set the rate of transmission to one.

$$(11.1) \quad q_i = \int_0^\infty uP_i(t)dt$$

$$(11.2) \quad q_j = \int_0^\infty uP_j(t)dt$$

$$(11.3) \quad q = \int_0^\infty uP(t)dt$$

To compare constant and variable environments, we analysed multi-parasite and single-parasite versions of the S_D and S_{DE} models.

4.0.0. Results

Analytically, the secondary parasite could invade ($P_{j(t+1)} > P_{j(t)}$) when $I(t) < \frac{-\ln(1-r_j)}{k}$. Our parameterization of the S_D model allowed the secondary parasite to invade when introduced before the tenth to twelfth day of infection, resulting in 8.4 to 9.2 days of co-occurrence, depending on thermal scenario (Fig. 4, Supplementary Datafile 1-1:10).

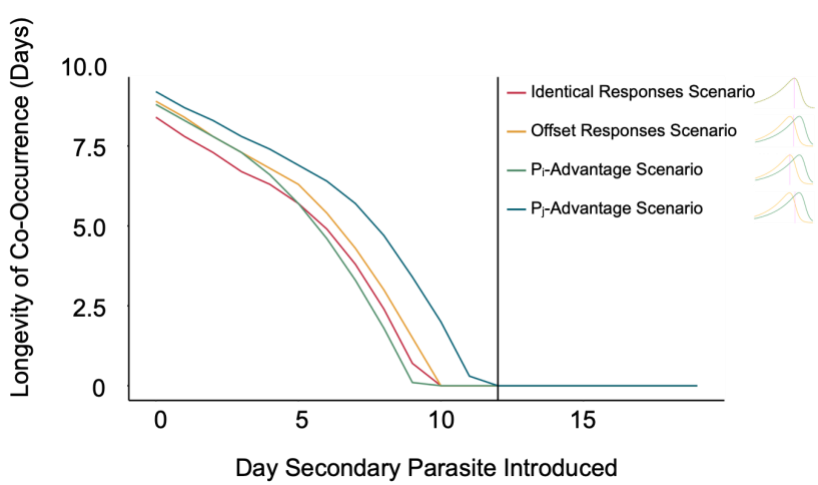

Figure 4: Longevity of co-occurrence as per the deterministic model. The plot shows the number of time steps for which P_i and P_j have population abundances greater than one, by introduction day, as per the deterministic model. The black line denotes the first day on which the introduction of the secondary parasite, P_j, does not result in any co-occurrence between the two parasites.

4.1.0. Co-occurrence Analysis

Simulation outputs show that compared to a deterministic baseline, the addition of demographic stochasticity has little effect on co-occurrence (Supplementary Datafile 2-1:10), while the addition of thermal variability increases the ability of the secondary parasite to invade the system and facilitates longer periods of co-occurrence between the primary and secondary parasites. While longevities of co-occurrence are maximised under the P_j-advantage scenario, the difference between deterministic and environmentally stochastic implementations is not consistently maximised by any one thermal scenario. Additionally, while larger and more frequent disturbances tend to promote longer periods of co-occurrence, increasingly large disturbances produce a less steep effect than do increasingly frequent disturbances. These findings are common among thermal scenarios.

When both parasites were introduced simultaneously, S_D model simulations resulted in periods of co-occurrence that were, on average, 4.5% to 3.1% shorter (0.4 to 0.3 days) than deterministically simulated periods of co-occurrence (Figs. 5, S10). Under the same conditions, simulations of the environmentally stochastic models (S_E, S_{DE}) resulted in periods of co-occurrence that were up to 46.2% (S_E) and 36.0% (S_{DE}) longer (3.9 (S_E) and 3.0 (S_{DE}) days) than deterministically simulated periods of co-occurrence (Figs. 5, S10). Across all 195 disturbance regimes, simulations of the S_E model resulted in mean periods of co-occurrence that were between 2.7% shorter (0.2 days) and 46.2% longer (3.9 days) than their deterministic counterparts (Figs. 5, S10). The identical responses scenario promoted the shortest and longest mean periods of co-occurrence (Figs. 5-6, S10-S11). Simulations of the S_{DE} model resulted in mean periods of co-occurrence that were between 6.8% shorter (0.6 days) and 36.0% longer

(3.0 days) than deterministically simulated periods of co-occurrence (Figs. 5, S10). Again, the identical responses scenario promoted the shortest and longest mean periods of co-occurrence (Figs. 5-6, S10-S11). Across thermal scenarios, mean periods of co-occurrence were shortest when disturbances were large (standard deviation of the normal random variable in the temperature autocorrelation function = 22 to 30) and frequent (once every 7 to 9 days) (Figs. 6, S11). Mean periods of co-occurrence were longest when disturbances were large (SD = 24 to 30) and infrequent (five to ten times per day) (Figs. 6, S11).

When isolating the effect of size of disturbance (frequency of disturbance fixed at an intermediate value (once per day)), simulations of the S_E model resulted in mean periods of co-occurrence that were between 2.2% and 37.8% longer (0.2 and 3.2 days) than deterministically simulated periods of co-occurrence (Figs. 5-7, S10-S12, S14). The P_i-advantage and identical responses scenarios promoted the shortest and longest mean periods of co-occurrence, respectively (Figs. 5-7, S10-S12, S14). Simulations of the S_{DE} model resulted in mean periods of co-occurrence that were between 2.9% shorter (0.3 days) and 32.3% longer (2.7 days) than deterministically simulated periods of co-occurrence (Figs. 5-7, S10-S12, S14). The P_j-advantage and identical responses scenarios promoted the shortest and longest mean periods of co-occurrence, respectively (Figs. 5-7, S10-S12, S14). Periods of co-occurrence were shortest when disturbances were small (SD = 2), and longest when disturbances were large (SD = 24 to 30) (Figs. 5-7, S10-S12, S14).

When isolating the effect of frequency of disturbance (magnitude of disturbance fixed at an intermediate value (SD = 10)), simulations of the S_E model resulted in mean

periods of co-occurrence that were between 2.3% shorter (0.2 days) and 35.2% longer (3.0 days) than deterministically simulated periods of co-occurrence (Figs. 5-7, S10-S12, S15). The identical responses scenario promoted the shortest and longest mean periods of co-occurrence (Figs. 5-7, S10-S12, S15). Simulations of the S_{DE} model resulted in mean periods of co-occurrence that were between -6.0% shorter (0.5 days) and 25.3% longer (2.1 days) than deterministically simulated periods of co-occurrence (Figs. 5-7, S10-S12, S15). Again, the identical responses scenario promoted the shortest and longest mean periods of co-occurrence (Figs. 5-7, S10-S12, S15). Periods of co-occurrence were shortest when disturbances were infrequent (once every 7 to 8 days), and longest when disturbances were frequent (five to ten times per day) (Fig. 5-7, S10-S12, S15).

More generally, as disturbances increased in size, periods of co-occurrence increased in length towards a saturation point (standard deviation of the normal random variable in the temperature autocorrelation function ≈ 24-30, depending on thermal scenario) (Figs. 7A, S12, S16, Supplementary Datafiles 3-1:5, 4-1:5). Additionally, when disturbances were infrequent (once every seven to ten days), periods of co-occurrence were similar in length to deterministically simulated periods of co-occurrence. Per the deterministic model, P_i and P_j can only co-occur for a maximum of 9.2 days. Thus, systems subjected to infrequent disturbances were functionally similar to systems subjected to a constant environment, making the outcome of a simulation reliant on the initial conditions of the system. Interestingly, when disturbances were intermediately frequent (once per day to once every few days), mean periods of co-occurrence were shorter than deterministically simulated periods of co-occurrence (Fig. 7B, S12, S19).

When disturbances were frequent (twice to ten times per day), mean periods of co-occurrence were longer than deterministically simulated periods of co-occurrence. Finally, within a given disturbance regime, distributions of longevities of co-occurrence were normally distributed (Figs. 7, S12-S13, S20).

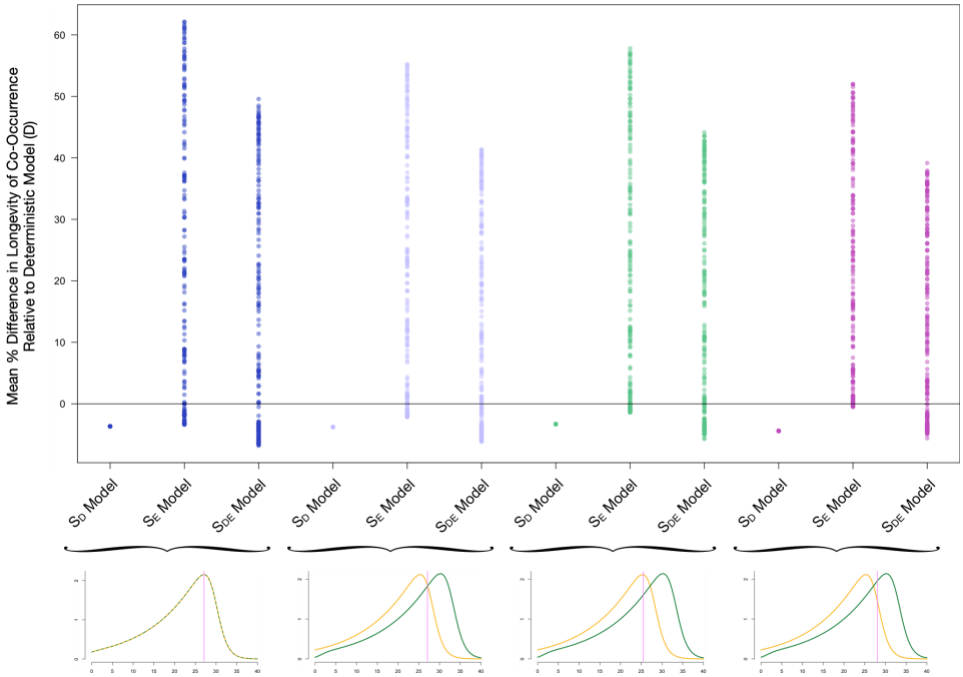

Figure 5: The mean percent difference in longevity of co-occurrence relative to deterministically simulated periods of co-occurrence, when both parasites are introduced at the same time. Each point represents the mean percent difference in longevity of co-occurrence relative to deterministically simulated periods of co-occurrence across 1000 stochastic simulations, as per a given environmental disturbance regime. As such, there are 195 points above each "S_E Model"/"S_{DE} Model" label.

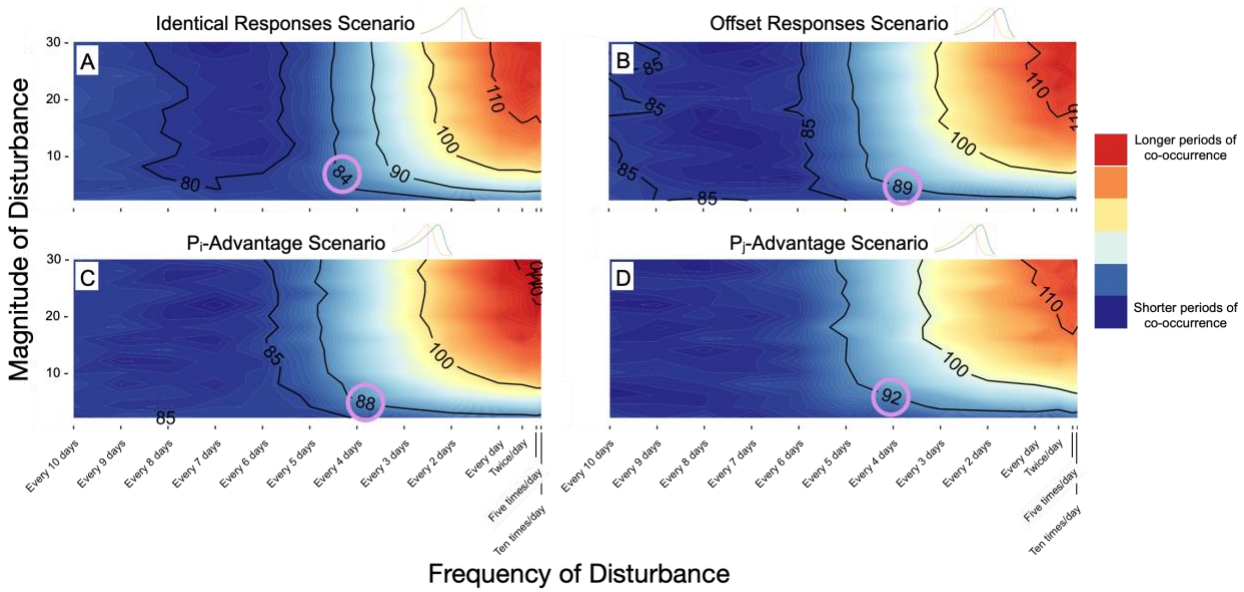

Figure 6: Fully factorial representations of the impacts of varyingly sized and timed disturbances on the longevity of co-occurrence. The plot shows the mean number of time steps (10 time steps/day) for which P_i and P_j have population abundances greater than one as per the S_{DE} model. The circled numbers denote the number of time steps for which P_i and P_j can co-occur, per the deterministic model.

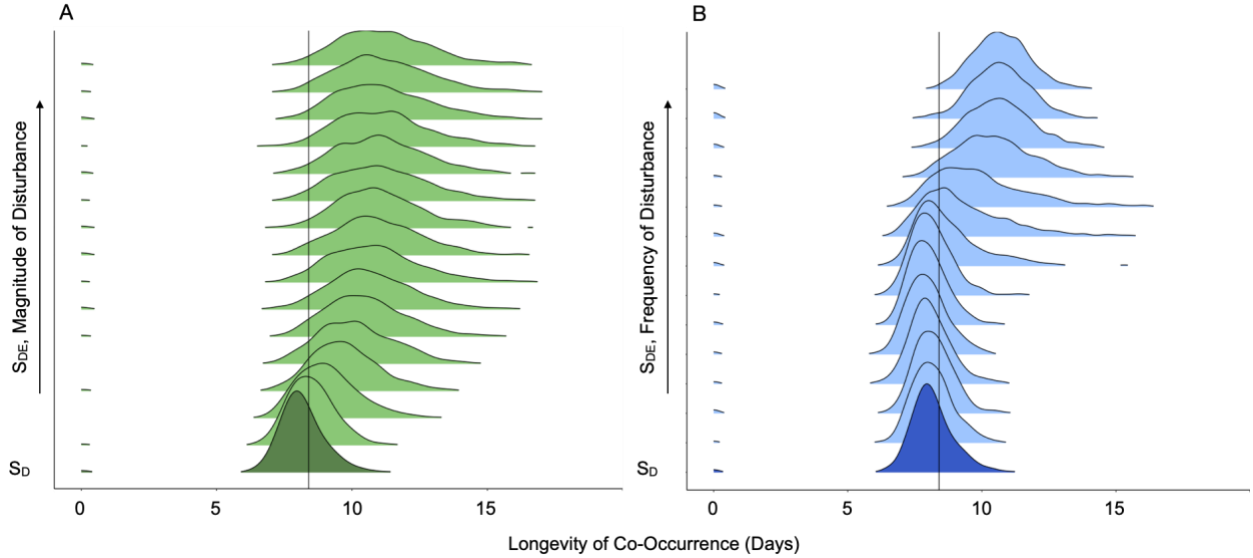

Figure 7: Distributions of outcomes across select disturbance regimes when both parasites are introduced at the same time. "S_D" refers to longevities of co-occurrence as per the S_D model. "S_{DE}" refers to longevities of co-occurrence as per the S_{DE} model. Panel A shows the effects of increasingly large disturbances (frequency of disturbance fixed (once per day)). Panel B shows the effects of increasingly frequent disturbances (magnitude of disturbance fixed (SD = 10)). Black vertical lines represent deterministic reference points. This figure shows longevities of co-occurrence under the identical responses scenario. See Fig. S12 for full results.

4.2.0. Rarity and Competitive Outcomes

Analyzing the proportion of environmentally stochastic simulations in which longevities of co-occurrence exceeded deterministically simulated periods of co-

occurrence revealed that the effect of environmental variability on the viability and maintenance of parasite co-occurrence was not rare. When the secondary parasite was introduced before or on the invasion threshold (10th or 12th day of infection, depending on thermal scenario), 29%-71% (S_E) and 46%-58% (S_{DE}) of environmentally stochastic simulations resulted in longer periods of co-occurrence relative to deterministic simulations. When the secondary parasite was introduced after the invasion threshold, the likelihood that an environmentally stochastic simulation resulted in some period of co-occurrence sharply declined. When the secondary parasite was introduced one to twelve days after the invasion threshold, 1%-31% (S_E) and 1%-46% (S_{DE}) of environmentally stochastic simulations resulted in some period of co-occurrence. Thus, environmental variability extended the invasion threshold by 50%-75% (S_E) and 100%-120% (S_{DE}), depending on thermal scenario (Fig. 8).

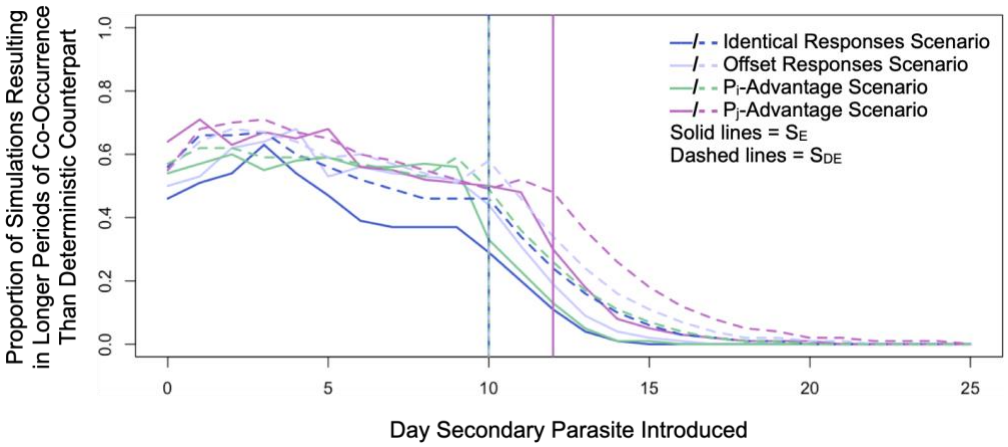

Figure 8: Proportion of environmentally stochastic simulations (SD = 10, frequency of disturbance = once per day) resulting in longer periods of co-occurrence relative to deterministic simulations by the day on which the secondary parasite was introduced. Vertical lines show the first day on which the introduction of the secondary parasite does not result in any co-occurrence between the two parasites, as per the deterministic model (D).

Analyzing competitive outcomes at intermediate levels of disturbance (SD = 10, frequency of disturbance = once per day) revealed that under the offset responses scenario, when P_i and P_j were introduced at the same time, P_j outlasted P_i 65.5% (S_E)

and 63.4% (S_{DE}) of the time (Fig. S20-S21). Additionally, under the P_i-advantage scenario, when P_i and P_j were introduced at the same time, P_i and P_j were equally likely to outlast one another (P_j outlasts P_i 49.9% (S_E) and 51.0% (S_{DE}) of the time), but under the P_j-advantage, P_j was much more likely to outlast P_i (P_j outlasts P_i 76.6% (S_E) and 70.4% (S_{DE}) of the time) (Fig. S19-S20). Across thermal scenarios, when the secondary parasite, P_j, was introduced in the middle of the infection period, P_i was more likely to outlast P_j. When P_j was introduced late in the infection period, P_j was more likely to outlast P_i (Fig. S20-S21). Regardless of when P_j was introduced, simulations resulting in especially long periods of co-occurrence are equally likely to show either parasite outlasting the other (Fig. S20-S21).

4.3.0. Transmissibility

Comparing the transmission potentials of co-occurring and single parasites revealed that under certain conditions, the transmissibility of co-occurring parasites can exceed the transmissibility of single parasites. Across thermal scenarios, simulations of the multi-parasite S_D model produced lifetime replicative outputs (LROs) (median LRO of P_i ranged between 1.49E+04 and 9.12E+05; median LRO of P_j ranged between 2.59E+04 and 6.09E+05) that were one to two orders of magnitude lower than those produced by simulations of the single-parasite S_D model (median LRO of P ranged between 3.62E+06 and 5.13E+07) (Supplementary Datafiles 5-1:16). Similarly, under the first three thermal scenarios, simulations of the multi-parasite S_{DE} model produced LROs (median LRO of P_i ranged between 1.02E+04 and 1.05E+06; median LRO of P_j ranged between 8.36E+03 and 3.00E+05) that were one to two orders of magnitude lower than those produced by simulations of the single-parasite S_{DE} model (median

LRO of P ranged between 6.45E+04 and 3.41E+07). However, under the P_j-advantage scenario and among 62 of 195 (32%) disturbance regimes, the average transmission potential of the co-occurring secondary parasite, P_j, exceeded that of a single parasite (Fig. 9B). Among these disturbance regimes, the transmission potential of P_j exceeded that of a single parasite by an average of 2% to 135%. The transmission potential of P_j was maximised when disturbances were large (SD = 20) and infrequent (once every eight days) (Fig. 9A, Supplementary Datafiles 5-8). The transmission potentials of co-occurring parasites peaked when disturbances were infrequent (once every ten days) to intermediately frequent (once every six days) (Fig. S22). While large and frequent disturbances maximally increased longevities of co-occurrence, such infections did not result in particularly high transmission potentials, indicating that especially long periods of co-occurrence may be facilitated by lower lifetime replicative outputs that do not trigger a large immune response, and thus allow for more persistent infections.

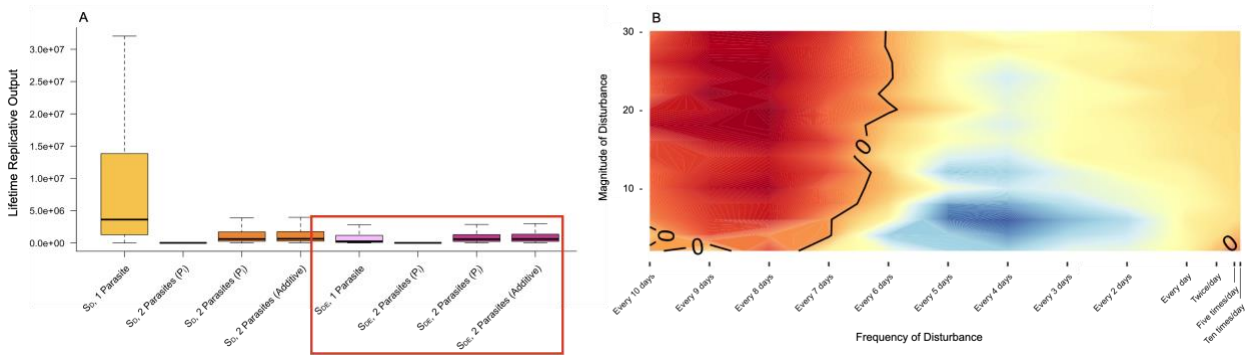

Figure 9: The impacts of environmental variability on transmission potential under the P_j-advantage scenario. Panel A shows the total transmission of parasites by model (S_D, S_{DE}) and structure (multi-parasite, single-parasite), when disturbances are large (SD = 20) and infrequent (once every eight days). Panel B shows a fully factorial representation of the impacts of thermal disturbance on the transmission potential of P_j, relative to the equivalent single-parasite treatment. The black line indicates disturbance regimes under which the average transmission potential of P_j was equal to that of a single parasite. Colours warmer than denoted by the black line indicate disturbance regimes under which the average transmission potential of P_j exceeded that of a single parasite; colours cooler than denoted by the black line indicate the opposite.

5.0.0. Discussion

Despite increasing rates of disease emergence, the mechanisms that promote parasite diversity within hosts and create kindling for emergences and epidemics among hosts have been understudied (Baker et al., 2022; Jones et al., 2008; Taylor et al., 2001). Here, we investigated the potential for environmental variability, both as a feature of climate change and as a physiological trait unique to a prolific reservoir for disease to affect the viability and maintenance of within-host parasite diversity. Our work provides theoretical evidence that relative to a constant environment, a variable environment can increase the ability of a secondary parasite to invade a single-parasite system, promote longer periods of co-occurrence between parasites, and increase the transmissibility of co-occurring parasites. In more detail, thermal disturbances of various sizes and frequencies allowed a secondary parasite to invade a single-parasite system up to twelve days after the invasion threshold, extending the invasion threshold by 100% to 120% (S_{DE} model, Fig. 8). Large and frequent thermal disturbances enabled parasites to co-occur for up to 37.8% longer, relative to a constant environment (S_{DE} model, Figs. 5, S10).

Across thermal scenarios and disturbance regimes, the relationship between disturbance size and longevity of co-occurrence was initially positive and eventually saturating (Fig. S16). We determined that increasing magnitudes of disturbance produced a saturating response rather than the expected parabolic response due to the structure of the Sharpe-Schoolfield sub-model, which cannot generate negative replication rates. When we introduced negative replication rates, larger disturbances began to have negative effects on parasite populations, resulting in the expected

parabolic response, and implying that intermediate magnitudes of disturbance maximised the viability and longevity of parasite co-occurrence (Figs. S17-S18).

The relationship between climate change and disease-mediated amphibian declines has long been debated. While some argue that climatic changes may impede immune function or result in performance gaps between parasites and their hosts, and thus make amphibians more susceptible to infection, others argue that insufficient evidence should preclude a causal link (Cohen et al., 2019, 2019; Daszak et al., 1999, 2003; Lips et al., 2008; Pounds et al., 1999; Rohr et al., 2008). While much of the literature proposes mean-climate hypotheses, some evidence suggests that intensifying El Niño events are increasing thermal variability, which may in turn affect amphibians' susceptibility to or ability to defend against infection (Rohr & Raffel, 2010). Similarly, emerging evidence suggests that in addition to rising mean temperatures, daily temperature fluctuations may affect the incubation period of malaria parasites and related vital rates, altering malaria transmission patterns (Paaijmans et al., 2010, 2013). Congruently, our findings provide theoretical evidence that thermal variability promotes the viability and maintenance of within-host parasite diversity, and thus suggest that as the climate continues to change, attention should be paid to shifts in microparasite communities and their impacts on ectothermic hosts. In particular, well-documented climate-driven increases in disease incidence among a wide variety of marine organisms may constitute an important starting point for future work (Bates et al., 2009; Bruno et al., 2007; Carilli et al., 2012; Drinkwater et al., 2003; Harvell et al., 2001; Tracy et al., 2019).

Across thermal scenarios and disturbance regimes, intermediate and high frequencies of disturbance impeded and promoted the viability and longevity of parasite co-occurrence, respectively (Figs. 6, S11, S19). Given the transient nature of infection specified by the underlying model, intermediate frequencies of disturbance (once every seven days, for example) resulted in the temperature of the system being disturbed towards the end of the infection period, when parasite populations were small and especially susceptible to unfavourable conditions. Conversely, high frequencies of disturbance resulted in the temperature of the system being disturbed throughout the duration of the infection period, and as such, neither favourable nor unfavorable conditions lasted long enough for one parasite to gain a decisive advantage over and exclude the other. While it has been theorized that variations in internal temperature may aid bats' immune responses, allowing them to carry microparasites without suffering outsized morbidity (Fumagalli et al., 2021; Luo et al., 2021), these findings suggest that variations in internal temperature may also facilitate the co-occurrence of parasites within bats, and thus may help explain bats' propensity to support diverse communities of parasites. Further, these results, combined with the regularity with which bats are implicated in spillover events and epidemics, suggest that the observed effects of environmental variability on parasite diversity within hosts will scale up to have effects on between-host processes like transmission, as discussed below.

With regards to competitive outcomes, under the offset responses scenario, P_j often outlasted P_i, while under the P_i-advantage scenario, P_i and P_j were equally likely to outlast each other (Fig. S20-S21). Under the P_j-advantage scenario, P_j was much more likely to outlast P_i (Fig. S20-S21). These outcomes are consequences of the

asymmetrical structure of the thermal performance curves (Sinclair et al., 2016). As an example, within the offset responses scenario, colder than average temperatures resulted in a slightly higher replication rate for P_i and a slightly lower replication rate for P_j, while warmer than average temperatures resulted in a much lower replication rate for P_i and a slightly higher replication rate for P_j. In short, P_i was more disadvantaged by unfavourable conditions than was P_j. Further, regardless of when P_j was introduced, simulations resulting in especially long periods of co-occurrence were equally likely to show either parasite outlasting the other. Taken together, these results suggest that to predict the competitive outcome of a multiple infection, one may need to consider the interplay between how well adapted a parasite is to its host, priority effects, and the responses of biological rates to environmental factors. While one might expect a specialist or well-adapted parasite (here, a thermally advantaged parasite) to exploit its host more efficiently than, and thus outcompete, a generalist or novel parasite, the results of this work suggest that environmental variability can even the scales, and allow a generalist or novel parasite to establish and persist, as predicted by the intermediate disturbance hypothesis (Chesson, 1994; Connell, 1978; Roxburgh et al., 2004). Similarly, while one might additionally expect a colonizing parasite (here, the primary parasite) to benefit from an initial lack of competition, the results of this work again suggest that environmental variability and disturbance may contribute to the structuring of communities by allowing latecomers to establish and persist more often and for longer than they otherwise would (Jiang & Patel, 2008; Munguia et al., 2010; Symons & Arnott, 2014).

While the metabolic theory of ecology posits that certain biological rates (e.g. metabolic rate, replication/growth rate, etc.) will exhibit an asymmetrical and unimodal response to temperature, other vital rates may exhibit symmetrical or bimodal responses to temperature (Dell et al., 2014; Molnár et al., 2017; Sinclair et al., 2016). This work suggests that the shapes of thermal performance curves can meaningfully affect the nature and outcome of an infection. As anthropogenic changes alter host-parasite interaction networks, understanding how evolutionary and environmental factors combine to affect interactions between parasites will be essential in predicting and managing spillover and emergence in wildlife and human populations (Paull et al., 2012; Pedersen & Fenton, 2007).

From an array of parasites circulating among ectotherms to Hendra virus, emerging coronaviruses, and other parasites carried by bats, developing an understanding of how the impacts of environmental variability on parasite diversity within hosts may scale to affect among-host processes and events, including transmission, spillover, and emergence will be essential to the maintenance of wildlife, human, and ecosystem health (Allocati et al., 2016; Daszak et al., 2003; Halpin et al., 2000; Johnson, 2006). While increased within-host parasite diversity provides more strains or species of a parasite or parasites an opportunity to transmit, within-host competition may result in decreased parasite loads, thus decreasing the transmission probability of any one strain or species, and altering expected transmission patterns (Johnson et al., 2013; Pedersen & Fenton, 2007). Similarly, our findings provide theoretical evidence that when environmental variations are very large and very frequent, within-host competition may decrease the transmission rates of coinfecting

parasites, thereby decreasing transmission probability. However, our findings also suggest that when thermal disturbances are variably sized and infrequent, the lifetime replicative outputs of coinfecting parasites can exceed those of single parasites, indicating that the effects of environmental variability may not be limited to the promotion of within-host parasite diversity, but may also promote transmission between hosts (Fig. 9B). Additionally, we found that while large and frequent disturbances may maximally increase the amount of time for which two parasites co-occur, such infections do not result in particularly high transmission potentials, indicating that especially long periods of co-occurrence may be facilitated by lower replicative outputs that do not trigger a large immune response, and thus allow for more persistent infections. Conversely, disturbances of intermediate size and low to intermediate frequency may constitute an anomalous region within which environmental variation can increase the amount of time for which two parasites co-occur, while allowing parasites to achieve high replicative outputs, resulting in longer and more transmissible infections (Fig. 9B).

While both Antia et al.'s (1994) model and our modified model should be broadly applicable to an array of within-host multi-parasite systems, including multi-strain or cross-species SARS-CoV-2 and influenza infections, both models are relatively simple as to remain generalizable, and thus cannot represent chronic infections, systems within which parasites exhibit more complex interactions with their host or their host's immune system (ex. malaria, HIV), nor systems within which parasites directly interact with one another (as is often observed in trematode coinfection and in hosts coinfected with multiple strains of the bacterium *Pseudomonas aeruginosal*) (Al Khatib et al., 2020; Bashey, 2015; Kim et al., 2021; Saenz et al., 2010). As such, it may be interesting to

validate the theoretical insights of this work in an empirical system. It may be of particular interest to explore the impacts of environmental variability on within-host parasite diversity in amphibians, mosquitos, ectothermic marine organisms, or, given the risk they pose to public health, bats (Bates et al., 2009; Bruno et al., 2007; Carilli et al., 2012; Drinkwater et al., 2003; Paaijmans et al., 2010; Rohr & Raffel, 2010; Tracy et al., 2019).

This work is also limited by the scope with which thermal dependency was considered. Given the thermal sensitivity of immune function in ectotherms and endotherms, it will be worthwhile to consider the thermal dependency of immune processes like response time and replication rate in tandem with the thermal dependency of parasite replication rates (Butler et al., 2013; Wright & Cooper, 1981). Similarly, using nonlinear averaging to assess the response of a nonlinear process to variability is contentious. While many theoretical works and some empirical studies support the use of nonlinear averaging and find evidence for Jensen's inequality, borne out as disproportionate effects of thermal variability on performance, many empirical studies have contested an implicit assumption of nonlinear averaging: that traits should respond instantaneously to change, without acclimation (Bernhardt et al., 2018; Estay et al., 2011; Krichel et al., 2023; Paaijmans et al., 2010; Sinclair et al., 2016). Thermal acclimation through phenotypic plasticity alters thermal performance curves, and as such, produces a more complex relationship between biological rates and temperature that may not be well-characterized by nonlinear averaging (Altman et al., 2016; Schulte et al., 2011; Sckrabulis et al., 2022). With regards to host-parasite interactions, the temperature variability hypothesis posits that owing to their small relative size, parasites

should acclimate more quickly than their hosts, though the speed at which various parasites may acclimate remains unclear (Kunze et al., 2022; Raffel et al., 2013; Rohr et al., 2013). Interestingly, some empirical studies of the impacts of environmental variability on host-parasite interactions have considered thermal acclimation, yielding a range of conclusions including decreased pathogen prevalence, reduced infection prevalence, and decreased resistance in hosts (Dallas & Drake, 2016; Duncan et al., 2011; Raffel et al., 2013).

This work provides theoretical evidence that through climate change and as a physiological trait, environmental variability may promote parasite diversity within hosts and transmission among hosts, and thus create kindling for the spillover events, disease emergences, and epidemics we observe among wild and human hosts alike. Anthropogenic changes have allowed the next pandemic to leave the hypothetical and become a looming spectre. Camus wrote that plagues, despite their commonality, find us unprepared (Camus, Albert, 1991). Improving our understanding of how environmental variability will impact parasite diversity within- and between-hosts will allow us to expand our understanding of the mechanisms that can facilitate disease spread and emergence, which will allow us to make robust predictions, and develop and implement mitigation strategies as we move into an increasingly uncertain future. In brief, we will be found *less* unprepared.

6.0.0. Acknowledgements

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7.0.0. References

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