

Functional Ecology

Energetic scaling across different host densities and its consequences for pathogen proliferation

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Energetic scaling across different host densities and its consequences for pathogen proliferation

ABSTRACT

1. The spread of infectious disease is determined by the ability of a pathogen to proliferate within and spread between susceptible hosts. Processes that limit the performance of a pathogen thus occur at two scales: the availability of energy within a host, and the number of susceptible hosts in a patch. When the rate at which a host intakes and expends energy is density-dependent, these two processes are intimately linked.
2. By modifying how hosts compete for and expend resources, a shift in population density may contribute to differences in the flow of energy in a host-pathogen system, both in terms of the energy available for a host to grow, reproduce and fight infection, as well as the energy available for a pathogen to exploit. Energy flux, therefore, connects the two contrasting scales of within- and between-host dynamics by directly linking the proliferation of a pathogen to the number of hosts circulating within a patch.
3. We use the host *Daphnia magna* to explore the relationship between energy intake and expenditure at various population densities, as estimated by feeding and metabolic rates respectively. By infecting hosts with the bacterial pathogen *Pasteuria ramosa*, we then explore how infection changes the relative balance of energy intake and expenditure, and how this energy scope translates into production of transmission spores.

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4. Our work demonstrates that energy intake declines at a faster rate with density than does metabolic rate, leaving more excess energy (i.e., discretionary energy) available for both hosts and their dependent pathogens at low population densities. This energetic advantage translates positively into host and pathogen growth, with the production of mature transmission spores benefiting most from correlated changes in host body size, as well as a direct connection between energy scope and spore loads.
 5. Our findings reinforce how patch quality for a pathogen operates at two contrasting scales, with the within-host proliferation of a pathogen being optimised in energy rich, low density host populations, and opportunities for between-host transmission likely maximised in dense populations.

Keywords: Discretionary energy, energy scope, population density, energy intake and expenditure, feeding rate and metabolic rate, host-pathogen interactions, *Daphnia magna*, *Pasteuria ramosa*.

INTRODUCTION

As a pathogen spreads through a host population it inevitably experiences differences in the density of its target host. This variation in host density can arise via changes in habitat quality across a landscape, or as a result of range expansions (Phillips, 2009) and extinction-recolonisation dynamics (Hanski and Gilpin, 1991; Drake and Griffen, 2009). Any change in host density fundamentally alters the supply of susceptible hosts available for secondary infection, on which the further spread of an epidemic depends (Anderson and May, 1981). Yet, by modifying how intensely hosts compete for and utilise resources (DeLong and Vasseur, 2011), a shift in host density will also alter the flow of energy in a host-pathogen system, both in terms of the energy available for a host to grow, reproduce and fight infection, as well as the energy available for within-host pathogen exploitation (Cressler *et al.*, 2014; Pike, Lythgoe and King, 2019). Energy flux, therefore, links the number of susceptible hosts in a habitat patch to the within-host performance of a pathogen, bridging the two seemingly disparate scales that define pathogen transmission (Mideo, Alizon and Day, 2008; Hall and Mideo, 2018).

An understanding of the energy available to a pathogen during an invasion requires exploring the difference between rates of host energy intake and expenditure – the magnitude of which describes the scope for energy production or overall “discretionary energy” (Kearney and Porter, 2009; Buckley, Nufio and Kingsolver, 2014; Ghedini, White and Marshall, 2017). Intuitively, energy acquisition (as estimated by food consumption, Jenkins *et al.*, 1999) is expected to decline as the number of competitors in a habitat increase (Ban *et al.*, 2008; DeLong and Vasseur, 2011). However, it is now recognised that energy expenditure (metabolic rate, as estimated by oxygen consumption; White, 2011) also scales negatively with population density in many cases (see Table 1 in DeLong, Hanley and Vasseur, 2014), either as a direct response to reduced ingestion rates (Schmoker and Hernández-León, 2003; DeLong and Hanson, 2009), or as a consequence of lowered activity levels (Waters *et al.*, 2010), conspecific chemical cues (Lovass, Marshall and Ghedini, 2020), or metabolic depression due to increased stress (Guppy and Withers, 1999). Depending on the relative strengths at which per capita energy intake and expenditure decline with the number of hosts in a population, either more or less energy is accessible for a host or its dependent pathogen at any given host population density (see Fig. 1, modified from Ghedini, White and Marshall, 2017).

How any changes in discretionary energy along a density gradient might translate into the transmission of a pathogen depends on whether the host, or pathogen, benefits from an improved access to energy (Cressler *et al.*, 2014; Pike, Lythgoe and King, 2019). An increase in discretionary energy, for example, might dampen the spread of a pathogen if it also allows a host's resource intensive immune system to clear infection or limit pathogen proliferation (Sheldon and Verhulst, 1996; Cornet *et al.*, 2014). Conversely, if the flow of energy affords the pathogen either greater infection success or within-host proliferation, then the spread of disease will be accelerated under conditions that increase energy availability (Krist *et al.*, 2004; Frost, Ebert and Smith, 2008; Hall, Knight, *et al.*, 2009). Estimates of energy flow in a host-pathogen system, therefore, offer a mechanistic view of how population level processes can influence within-host dynamics, complementing research that have shown how host-parasite interactions are separately shaped by host feeding behaviours (Hite, Pfenning and Cressler, 2019), resource supplementation (Becker, Streicker and Altizer, 2015), or population dynamics (Nørgaard, Phillips and Hall, 2019).

In this study, we explored how rates of energy intake and expenditure both varied across a gradient of population densities and linked the resulting changes in discretionary energy to a key component of pathogen fitness; the production of transmission spores. To do so, we used the water flea *Daphnia magna* and its bacterial pathogen *Pasteuria ramosa*. *Daphnia* is a suitable candidate for density manipulations since natural populations undergo frequent extinction and recolonisation events and experience large fluctuations in population density and resource availability (Carvalho and Crisp, 1987; Ebert, 2005; Altermatt and Ebert, 2010). Many host traits, such as filter-feeding, growth, fecundity, and metabolic rate, often decline with population density, both as a response to reduced food availability, as well as due to conspecific chemical cues or physical crowding (Burns, 2000; Ban *et al.*, 2008; Delong, Hanley and Vasseur, 2014; Michel, Ebert and Hall, 2016). These processes in turn reduce the capacity of *P. ramosa* to proliferate by limiting the resource acquisition and growth of its infected carrier (Pulkkinen and Ebert, 2004; Clerc, Ebert and Hall, 2015; Nørgaard, Phillips and Hall, 2019). Both host and pathogen performance, therefore, are predicted to be influenced by the scaling relationship of energy intake and expenditure with population density.

To explicitly study the within-host dynamics of pathogen proliferation, we infected all hosts prior to establishment of density manipulations. We thus controlled for the ability of *Pasteuria* to infect its host, as infection success depends primarily on the pathogen's ability

to attach to the host oesophagus (but see Hall, Routtu and Ebert, 2019) and the dose of spores that reach this tissue (Ben-ami, Ebert and Regoes, 2010); a process shaped by feeding rates (Hall, Sivers-Becker, et al., 2007), but not energy availability per se (Duneau *et al.*, 2011). By quantifying the relative rates at which energy intake and metabolic rate declined with increasing host density, we then correlated the resulting changes in discretionary energy with both variation in host growth (i.e., mass) and the production of pathogen transmission spores. We discuss our findings in terms of how the patch conditions that maximise the within-host proliferation of a pathogen are unlikely to be the same as those that further the spread of infectious disease.

MATERIALS AND METHODS

The study system

Daphnia magna Straus is a filter-feeding cladoceran that reproduces via cyclical parthenogenesis. It is naturally infected by the endospore forming, gram-positive bacteria, *Pasteuria ramosa* Metchnikoff 1888. *P. ramosa* is horizontally transmitted and infection causes severe loss of fecundity, an increase in body size (gigantism), and reduced survival (Clerc, Ebert and Hall, 2015; Hall, Routtu and Ebert, 2019). Both host life-history and pathogen performance are influenced by host density and resource intake, with host fecundity and pathogen spore production reduced when food is limited or densities are high (Pulkkinen and Ebert, 2004; Cressler *et al.*, 2014; Nørgaard, Phillips and Hall, 2019; although responses can be genotype specific, see Michel, Ebert and Hall, 2016). In this study, we used the host genotype HU-HO-2 originating from Hungary, and two novel *P. ramosa* genotypes from Russia (C1) and Germany (C19).

Experimental design and infection procedure

Prior to the experiment, female *Daphnia* were collected from stock cultures and maintained under standard conditions (20°C, 16L:8D light cycle) for three asexual generations (reared in 60-mL jars with 50 mL artificial *Daphnia* media (ADAM, Ebert, Zschokke-Rohringer and Carius, 1998). From the standardised mothers we raised experimental animals with three individuals per jar in 60-mL jars filled with 20 mL media. At three days old, each animal was then randomly allocated to either the pathogen genotype C1, C19 or the unexposed control treatment groups, and on day three and four days old, each individual received either 20,000 spores (40,000 in total) or an equivalent volume of placebo-solution (crushed uninfected

hosts). All animals were therefore exposed to either a pathogen or a placebo dose before entering the density manipulations.

At seven days, animals were randomly (within exposure treatments) pooled and reared at six ecologically relevant population densities (e.g., Carvalho and Crisp, 1987) ranging from 20–300 individuals per litre (*i.e.* 1, 3, 6, 9, 12 or 16 individuals jar⁻¹), in 60-mL jars filled with 50 mL media. All populations were fed 10 million *Scenedesmus* algae cells daily, with juveniles removed and water changed three times weekly. The focal experiment was divided into three blocks containing four replicates of all density and exposure combinations (3 infection treatments x 6 densities x 3 blocks x 4 replicates = 216 experimental populations).

Concurrently, back-up jars with the same density and infection treatment combinations were established, and these individuals were used to replace focal individuals that either died during the density manipulation or remained uninfected after pathogen exposure. Thus, all animals used for metabolic and feeding assays spent the same duration in their respective density treatments.

Estimating energy expenditure via measurements of metabolic rates

Oxygen consumption rates (VO₂) were obtained by using a fluorescence-based respirometry system, and this measure was used as a proxy for metabolic rates (see White 2011). Oxygen consumption was measured at 21 days post-infection, since, at this point, infections are well established in the hosts and pathogens produce mature spores (Clerc, Ebert and Hall, 2015). All individuals from a given density replicate were first rinsed in sterilised media, and then loaded into single 20-mL glass vial with oxygen sensor spots at the bottom.

To measure oxygen consumption rates, we used four 24-channel PreSens oxygen readers (SDR SensorDish, Precision Sensing GmbH, Germany), each loaded with nine experimental populations (including all density and exposure combinations) and two blank vials containing media only (sensors were calibrated prior to measurements using sterile air-saturated media (100% air saturation) and media containing 2% sodium sulphite (0% air-saturation)).

Measurements took place in a dark, temperature-controlled room at 20⁰ C for 2.5 hours with oxygen concentration measured every two minutes. Animals were left to acclimate for 20 minutes before the recording process began. To allow for accurate testing of both energy intake and expenditure on the same day, animals were starved for 14 hours prior to oxygen consumption measurements.

The rate of oxygen consumption ($\dot{V}O_2$) was calculated following Alton et al. (2007) from the change in oxygen saturation over time ($\% h^{-1}$) as $\dot{V}O_2 = -1 \times [(m_a - m_c)/100] \times V \times \beta O_2$, where m_a is the rate of change in oxygen saturation for an experimental treatment, m_c is the per run average rate of change for the blank controls, βO_2 is the oxygen capacitance of air-saturated water at 20° C (6.40, Cameron 1986), and V is the water volume of the vials (0.020 L). The parameters m_a and m_c were estimated using the *LoLinR* package in R (Olito et al., 2017). $\dot{V}O_2$ estimates ($mL O_2 h^{-1}$) were then converted to metabolic rate ($Joules h^{-1}$) using the calorific conversion factor of 20.08 J $mL^{-1} O_2$ (Lighton, 2008).

Estimating energy intake via feeding rates

After oxygen consumption measurements, all experimental populations were transferred into 60-mL glass jars filled with 20 mL fresh autoclaved media. We then measured feeding rates by adding 40 million algal cells to each population and gently homogenised. *Daphnia* were then allowed to feed for 1.5 hrs ($T_{1.5}$), after which all jars were again gently homogenised and sampled for subsequent algae counts. We also sampled control jars (no animals) at the start and end of the energy intake assay to use as a representative of the starting algae concentration (T_0) and potential decay (difference between control T_0 and $T_{1.5}$). Algae concentrations were measured using Accuri BD flow cytometer (BD Biosciences, San Jose, USA) following protocols as outlined below for the characterisation of spore load.

Energy intake was calculated from estimates of algae consumption (difference between algae concentration at start and end of feeding trials; *i.e.* $T_0 - T_{1.5}$) by converting the known carbon content of algae (2.85×10^{-8} mg C $cell^{-1}$) to calories, where 1 mg C approximates 11.4 calories (Platt and Irwin, 1973). To avoid overestimation of energy intake (energy available for utilisation) we assumed a 70% assimilation efficiency (Nielsen and Olsen 1989).

Following feeding rate measurements, we measured body size of each individual using a dissecting microscope and converted to dry weight ($mg = 0.00535 \times \text{body size}^{2.72}$) following Yashchenko et al. (2016). Infected animals were then frozen individually for later spore counts.

Estimating pathogen spore production

To estimate pathogen spore production, frozen infected animals were thawed, homogenised, and then their spore loads measured using an Accuri C6 flow cytometer. 10 μL homogenised sample were diluted into 190 μL of 5 mM EDTA, mixed and loaded into one well of a round-bottomed PPE 96-well plate for analysis using flow cytometry. To identify mature spores, we

used custom gates based on fluorescence to distinguish algae cells from pathogen spores, and side scatter to separate mature spores from pre-spores and animal debris. Finally, spore load was measured twice per infected individual and averaged.

Statistical analyses and energy calculations

All statistical analyses were performed in R (v. 3.3.3; R Development Core Team). From our experimental approach, we obtained direct estimates of energy intake and expenditure, host dry weight (from body size) and pathogen spore loads. To subsequently estimate discretionary energy, we subtracted the metabolic rate from energy intake per jar. We likewise estimated mass-specific energy intake, expenditure and discretionary energy by dividing by the average dry weight per jar. Prior to analysis, we excluded 29 samples for which we could not estimate energy intake (lost due handling errors or problems with the assay) and outlier estimates, giving a total of 164 measurements of energy intake. Similarly, for metabolic rate, we lost five measures due to handling errors, and removed outliers (near zero or negative estimates) leading to a total of 209 measures of metabolic rate.

For each trait, we modelled the change in the mean trait values (per jar) with increasing population density as a power-law relationship ($trait = a \times density^b$), where b is the scaling exponent (i.e., slope when log-log transformed), and a is the normalisation constant (or proportionality constant) that accounts for differences in the absolute size of the traits with respect to density for a given a value (i.e., log-log intercept, Niklas and Hammond, 2019). We first used the *drc* package (Ritz *et al.*, 2015) to compare the fit of a power curve model versus linear or quadratic polynomial regression models, and in all cases the power curve model proved to be a better fit (see Table S1 in Supporting Information). We then used a non-linear mixed effect model (*nlme*, using restricted maximum likelihood (Pinheiro and Bates, 2000)), to incorporate block as a random effect in the power curve model (via the normalisation constant, akin to random-intercept models). To test for differences between infection treatments in the scaling relationship between population density and our traits of interest, we included infection treatment (uninfected, pathogen C1 and C19, except for spore loads where only pathogen C1 and C19 were included) as a fixed effect, allowed parameters a and b to vary by treatment, and jointly tested the significance of the interaction terms as per Pinheiro and Bates (2000).

Lastly, we explored how any change in pathogen spore load is related to dry weight and discretionary energy of an infected carrier. Data for this analysis comes from the per block

expected averages per capita for each trait and density combination, as spore loads, dry mass, and discretionary energy were not always available for every jar. We regressed $\log_{10}$ -transformed spores against the two $\log_{10}$ transformed and standardised predictor variables (mean 0, standard deviation 1, to obtain comparable parameter estimates). This model estimates the partial effects of increasing discretionary energy on changes in spore load, after controlling for the effect of a host's dry weight (and vice-versa). To test whether the relationship between our covariates and spore loads was the same for each genotype, we included pathogen genotype as a fixed effect and compared the fit of this model to one that also included the interactions between pathogen genotype and both dry weight and discretionary energy (using partial-F tests). The results were visualised for each trait (dry weight and discretionary energy) using the partial residuals obtained via the *visreg* package (Breheny and Burchett, 2017).

RESULTS

Scaling of energy intake and expenditure with population density

Using a non-linear modelling approach, we found negative density-dependence of energy intake and expenditure for both infected and uninfected *Daphnia* (Fig. 2A, B). The relationship between per capita energy intake and population density did not differ significantly between any of the exposure treatments (i.e. uninfected and infected with pathogen C1 and C19; $F_{4, 156} = 1.820$, $p = 0.128$), although hosts infected with pathogen C1 appear to have lower energy intake at all densities (Fig. 2A, left panel). In contrast, the scaling relationship between per capita energy expenditure and population density varied by exposure treatment ($F_{4, 201} = 4.790$, $p = 0.001$). Overall, uninfected animals had lower energy expenditure at all densities (Fig. 2A, right panel), with the trends for the two pathogen genotypes not significantly different from each other ($F_{2, 134} = 0.716$, $p = 0.490$).

For all treatments, energy intake declined at a faster rate than expenditure, as demonstrated by higher scaling exponents (-0.440 to -0.526 versus -0.337 to -0.371) and normalization constants (0.837 to 1.588 versus 0.080 to 0.123) for all trait and exposure combinations (see Table S2). The rapid decline in energy intake and gradual decline in expenditure across density meant that discretionary energy declined with host density (Fig. 2C). However, the lower energy expenditure of uninfected animals did not translate into a higher availability of discretionary energy in the absence of a pathogen. Instead, we found that the change in discretionary energy with density did not differ between the exposure treatments ($F_{4, 151} =$

1.379, $p = 0.244$), with the availability of discretionary energy, on average, predicted to be 4.5 times higher for individuals at the lowest densities (0.27 ± 0.02 at 20 individuals L^{-1} versus 0.06 ± 0.01 at 300 individuals L^{-1}).

Density effects on host and pathogen traits

We found that the growth of both host (change in dry weight) and pathogen (production of transmission spores) also scaled non-linearly with population density (see Table S2 for parameter estimates). Hosts were larger (estimated by dry weight) at low density, and size then declined with increasing density (Fig. 3A). However, the relationship between size and population density differed between the infection treatments ($F_{4, 208} = 8.451$, $p < 0.001$), with the body mass of uninfected animals declining faster than those infected (scaling exponent -0.200 versus -0.177, Table S2). The trends for both pathogens were similar ($F_{2, 138} = 0.301$, $p = 0.741$), with infected hosts, on average, larger than uninfected animals at all densities, consistent with the increase in body size (i.e. gigantism) that is associated with infection in this system (Clerc, Ebert and Hall, 2015).

Both pathogen genotypes produced more spores when replicating within hosts at low densities (~2.5-fold). However, pathogen C19 produced more spores than pathogen C1 at all densities (Fig. 3B), leading to a significant difference between the genotypes in the way spore loads vary with increasing population density ($F_{2, 138} = 12.249$, $p < 0.001$), largely accounted for by differences in the normalisation constant (5.570 versus 4.457, Table S2). Importantly, even when correcting for the weight of infected hosts, we still observed a negative density-dependence in mass-specific spore loads (Fig. 3C, Table S.2). Both pathogens were able to more efficiently convert resources from hosts at low density leading to a higher number of spores per gram of host mass, although pathogen C19 maintained higher mass-specific spore production overall ($F_{2, 138} = 13.238$, $p < 0.001$). Similar results were found when correcting energy intake, expenditure and discretionary energy for average body size, indicating that both hosts and pathogens are typically performing at a level greater than expected based on host mass alone when maintained at low density (see Fig. S1 and Table S2).

Discretionary energy and consequences for pathogen fitness

Finally, we explored the joint effect of host growth and discretionary energy on pathogen spore loads. In this system, pathogen spore production and host growth are tightly correlated. As part of the infection process, *P. ramosa* castrates its host and by limiting reproductive opportunities, at least temporarily (Hall, Routtu and Ebert, 2019), the excess resources are

predicted to flow into host growth (i.e., gigantism) and pathogen proliferation (Clerc, Ebert and Hall, 2015). In this study, we found that spore loads were highest in low density hosts and positively correlated with the availability of discretionary energy ($r = 0.786$, 95% CI [0.506, 0.845], $p < 0.001$). However, as discussed, it is unclear whether this correlation was a consequence of the pathogen having access to more discretionary energy within the host, or the exploitation of larger hosts overall.

Using an analysis of covariance, we found that the influence of host density on pathogen spore production was mediated by correlated changes in both discretionary energy and host body size, albeit in a genotype specific manner (pathogen genotype by trait interactions ($F_{2, 30} = 3.676$, $p = 0.037$). For pathogen genotype C19, the availability of discretionary energy predicted an increase in pathogen spore loads even after controlling for host size (and vice versa, Fig. 4), but the overall effect of host size on spore production being much stronger than the effect of discretionary energy alone (standardised weight: $\beta = 0.236 \pm \text{SE } 0.034$, $p < 0.001$; standardised discretionary energy: $\beta = -0.130 \pm \text{SE } 0.036$, $p < 0.003$). In contrast for pathogen C1, the effect of discretionary energy was eliminated once host size was controlled for (standardised weight: $\beta = 0.315 \pm \text{SE } 0.065$, $p < 0.001$; standardised discretionary energy: $\beta = -0.038 \pm 0.063$, $p = 0.557$), indicating that the connection between host growth and spore loads is more general than that of discretionary energy.

DISCUSSION

An understanding of energy flux in a host-pathogen system offers the opportunity to connect the two contrasting scales on which pathogen fitness depends (within- and between-host, Mideo, Alizon and Day, 2008), by directly linking the proliferation of a pathogen to the number of hosts within a patch (see also Hite and Cressler, 2018). Yet, rarely are direct estimates of energy intake and expenditure simultaneously available for host-pathogen combinations. Feeding rates (Hall *et al.*, 2010; Hite, Pfenning and Cressler, 2019), resource supplementation and fertilizer use (Becker, Streicker and Altizer, 2015; Civitello *et al.*, 2018), and nutrition (Pike, Lythgoe and King, 2019) have all been linked to the growth of a host population and the transmission and spread of a pathogen; but capture only one side of the energy flux in a system. In contrast, studies of host-pathogen interactions using dynamic energy budgets (Hall, Becker and Cáceres, 2007; Hall, Simonis, *et al.*, 2009) explicitly consider energy intake, storage, and expenditure within a host, but not necessarily in a manner that extends readily to empirical estimates of host growth, pathogen proliferation, and their dependency on population density. We provide a complementary approach for

quantifying energy availability across a density gradient, and test whether an increase in energy translates into increased pathogen proliferation, or a greater capacity for the host to fight infection.

By estimating rates of energy intake and expenditure at various population densities, we found that both infected and uninfected hosts at low density maintained higher amounts of discretionary energy (Fig. 2), achieved by a greater intake of energy at low density, relative to the energy expended on metabolic processes. This supports previous findings by Ghedini, White and Marshall, 2017, where decreasing discretionary energy across a density gradient was identified in colonies of the sessile colonial bryozoan, *Bugula neritina*. In their study, the decline in individual body size with increasing population density could be predicted by estimates of discretionary energy, with colonies reducing their energy expenditure per gram of mass in the presence of an increasing number of competitors. We find similar patterns: per gram of body mass, energy intake, energy expenditure, and discretionary energy all decrease with increasing density (Fig. S1).

Our results reiterate the importance of feeding rates in this system, not necessarily in terms of absolute energy intake per se, but rather how the variation in feeding rates far exceeds that found in metabolic rates. It is this disparity in variation across host densities that leads to greater discretionary energy at low densities (scenario A, Fig. 1). Given that Ghedini, White and Marshall, 2017 found similar patterns, this may be a common feature of aquatic filter-feeding species (Fig. 1A and C). For other species, however, alternate scenarios whereby metabolic rates play a far greater role (as outlined in Fig. 1) are just as possible. Indeed, in a review of the literature, DeLong, Hanley and Vasseur (2014) found that the scaling relationship between metabolic rate and density (i.e., b , the scaling exponent) can vary by up to two orders of magnitude, in many cases easily exceeding the scaling exponent we observed between feeding rates (and thus energy intake) and population density. They also found that, although rare, metabolic rates do not always scale negatively with population density (Stoltz, Andrade and Kasumovic, 2012). As a consequence, viewing either feeding rates or metabolic rates in isolation may lead to a distorted picture of how energy flows change with host density.

Infection by either of the two pathogens – pathogens that otherwise vary in their proliferation and host exploitation strategies (Clerc, Ebert and Hall, 2015; Hall and Mideo, 2018) – did not fundamentally alter the scaling relationship between density and discretionary energy, nor the dominant role of feeding rates. For both pathogen genotypes, infection induced an increase in

metabolic rates at all densities (Fig. 2A) but did not result in any statistically significant changes in energy intake (despite pathogen C1 appearing to result in lower feeding rates). This led to a slight reduction in overall discretionary energy for infected individuals, but no changes in the rate at which discretionary energy declines with population density (Fig. 2C). The higher discretionary energy in low density hosts appears to be reflected in pathogen fitness; both pathogens produced more spores in resource rich low-density hosts (Fig. 3B). Even when we corrected for host size, we observed that both pathogens produced more transmission spores per gram of host mass at lower population densities (Fig. 3C), indicating that pathogens were able to exploit this additional discretionary energy.

Our findings suggest that increased discretionary energy in a host does not always facilitate a stronger immune-response to curtail the proliferation of the pathogen (c.f. Sheldon and Verhulst, 1996; Cressler *et al.*, 2014). Perhaps the capacity of the host to clear infection (see Hall, Routtu and Ebert, 2019) may have been improved by greater access to discretionary energy, although by standardising the infection procedure for all animals we excluded this possibility in our study (but see Cressler *et al.*, 2014). We also found no overt evidence of pathogen induced anorexia in this system, whereby hosts modulate their energy intake as a mechanism of defence (Hite, Pfenning and Cressler, 2019), as feeding remained similar between uninfected and infected animals at all densities. However, the trend towards lower feeding rates for one pathogen genotype suggests that further work may be needed to determine if pathogen induced anorexia occurs in this system more generally. Instead, we show that the higher amount of discretionary energy in hosts at low density is associated with an increase in the production of transmission spores. This flow of energy appears to predominately affect pathogen performance through correlated changes in a host's body size (Fig. 4). Only for pathogen C19 did we see that discretionary energy directly predicted spore production, a phenomenon that may help explain why this pathogen maintained a higher number of spores per mg of host than pathogen C1, at each host density (Fig. 3C).

Our results reinforce that patch quality for a pathogen can be mediated by processes occurring at two scales. Within-host proliferation will be accelerated when energy availability is greatest, such as when resources are locally abundant or competition between hosts for resources is low (Hall, Knight, *et al.*, 2009; Nørgaard, Phillips and Hall, 2019). Conversely, between-host dynamics are likely to be maximised in high density populations (Anderson and May, 1981). However, several processes in natural populations may modify this balance (as per Cressler *et al.*, 2014; Hite and Cressler, 2018). In dense populations,

interspecific competition and physical interactions will reduce individual filtering-rates, as observed here, and thus parasite encounter rates via ingestion (Penczykowski *et al.*, 2014; Nørgaard, Phillips and Hall, 2019). By reducing transmission rates, this potentially offsets the between-host advantage that high density populations provide (and conversely increases the advantage at low densities where feeding rates are higher). Alternatively, the between-host advantage of a high-density population may be enhanced if the reduction in energy leads to higher infection rates, counteracting the reduction in within-host proliferation. Both nutrient limitation and crowding stress (e.g. Lindsey *et al.*, 2009; Cornet *et al.*, 2014; Michel, Ebert and Hall, 2016), for example, can make hosts more susceptible to infection, which, in the context of our study, would further increase transmission rates in high density populations, where competition for resources is most intense.

In summary, rates of energy intake and expenditure provide a common currency to link processes that shape population dynamics, such as competition for resources, to the growth and performance of a host and its pathogen (see also Cressler *et al.*, 2014; Hite and Cressler, 2018). We have shown that both the host and pathogen benefit from increased discretionary energy at low population densities; the hosts received more energy for growth, but with larger body size, pathogen spore production also increased. Our findings reinforce how patch quality operates at two contrasting scales, where conditions that optimise within-host proliferation correspond to energy rich, low density populations, whereas between-host transmission is likely maximised in high density populations that facilitate higher encounter rates with susceptible hosts. Whether other host-pathogen systems display similar patterns of energy flux remains to be explored, but understanding how pathogen transmission relates to host energy intake and expenditure provides a powerful tool to link community- and population-level processes to the evolution, spread, and severity of infectious disease in a host-pathogen system.

Figures

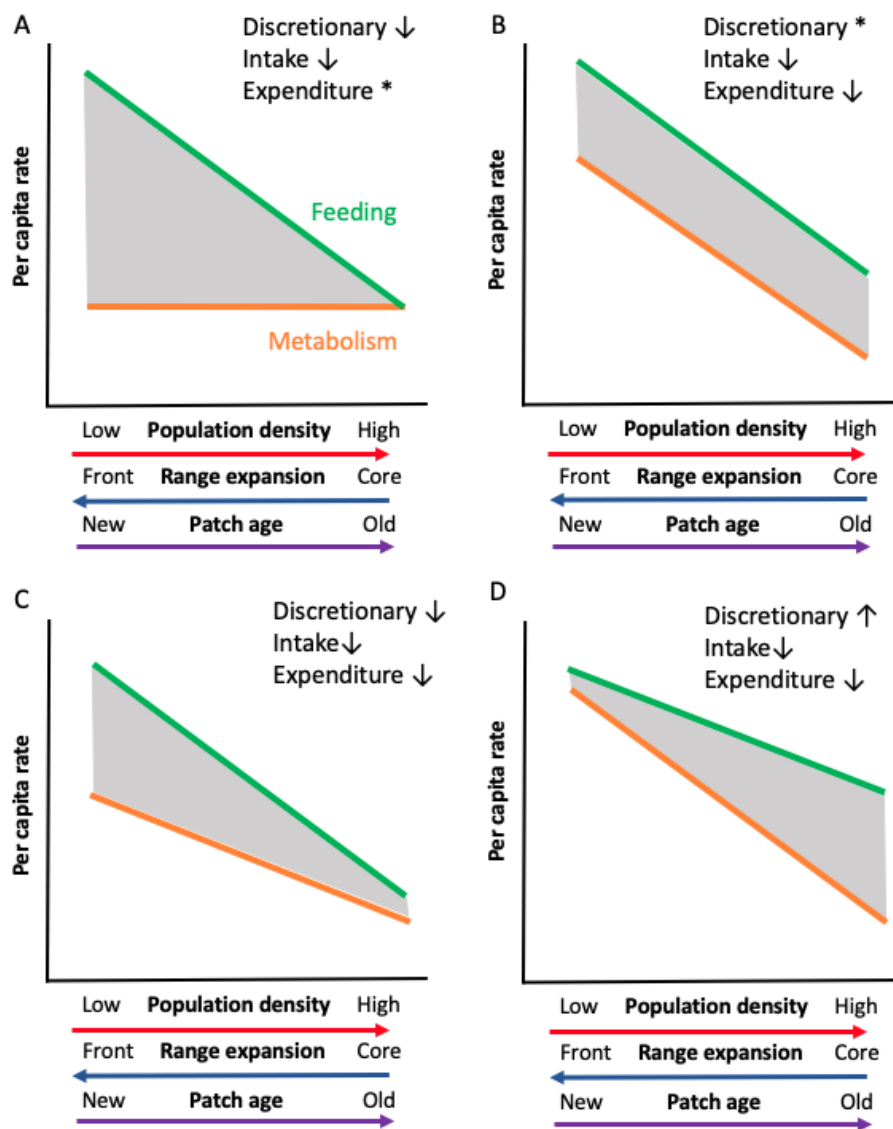


Figure 1. Predictions for the scaling relationship between energy intake (feeding rate – green line) and energy expenditure (metabolic rate – orange line) at different population densities (red arrow), or analogously, along a gradient of range expansion (blue arrow), or as a function of patch age since initial colonisation (purple arrow), and how this influences discretionary energy (grey). A) with increasing population density, feeding rate declines, but metabolic rate is stable, causing a net reduction in discretionary energy. B) both feeding and metabolic rate declines with density, leaving a constant amount of discretionary energy. C) feeding rate declines faster than metabolic rate, leaving less discretionary energy at high density. D) metabolic rate declines faster than feeding, leaving more discretionary energy at high density. Figure is modified from Ghedini, White and Marshall, 2017.

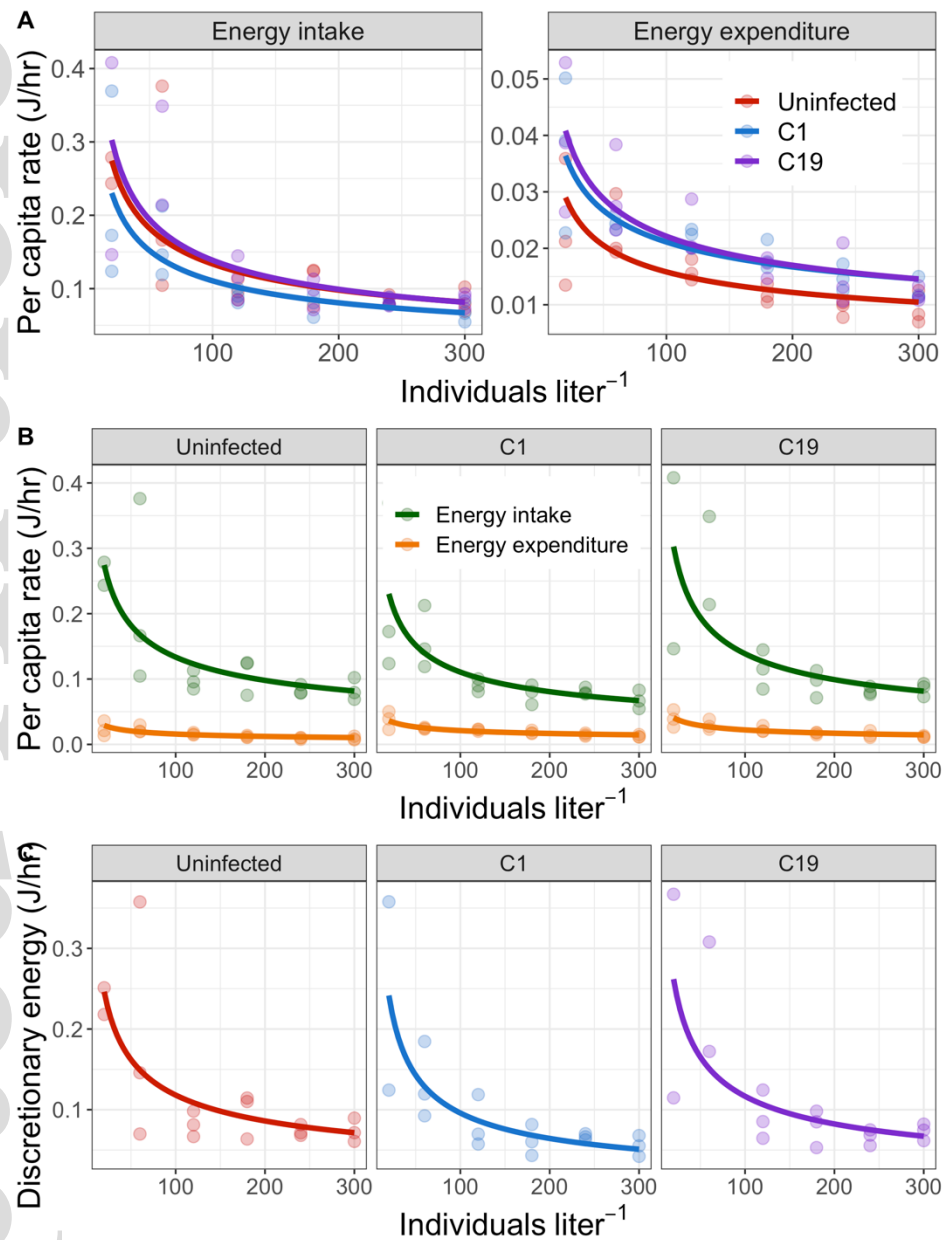


Figure 2. The relationship between population density and A) energy intake (left panel) and energy expenditure (right panel) (J/hr) for all exposure treatments, B) density dependence of energy intake and expenditure in uninfected and infected (C1 and C19) individuals, and C) discretionary energy (J/hr) across population densities, calculated as the difference between energy intake and expenditure. Lines represent the power-law relationship fit using individual jars as replicates, while points show the raw mean values for each experimental block (Note: the statistical analysis were performed on the jar level data with experimental block modelled as a random effect).

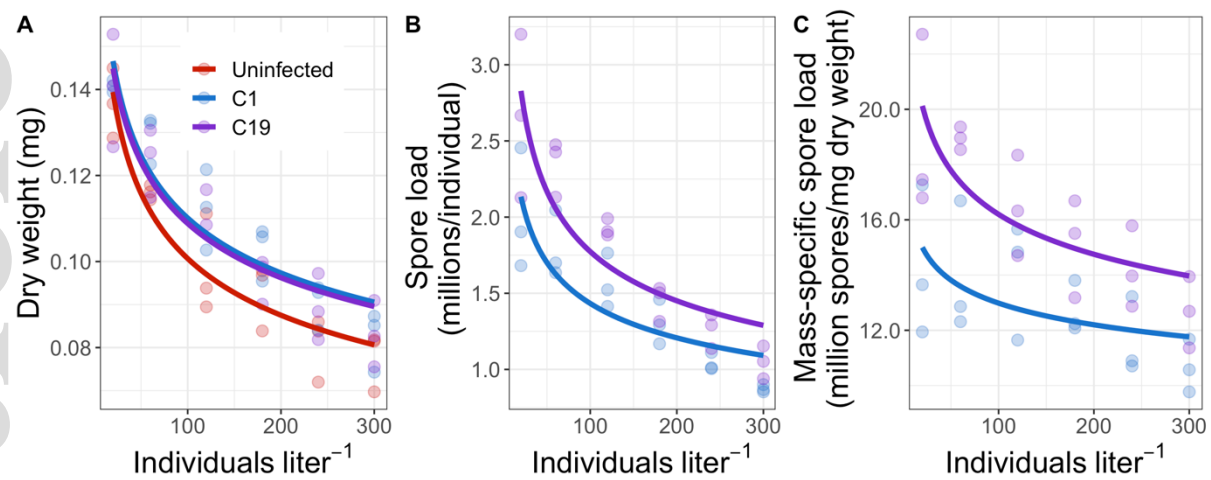


Figure 3. The relationship between population density and A) dry weight of hosts (all exposure treatments), B) spore load in infected carriers (million spores/individual), and C) mass-specific spore load (million spores/mg dry weight). Lines represent the power-law relationships fit using individual jars as replicates, and points are the raw mean values for each experimental block.

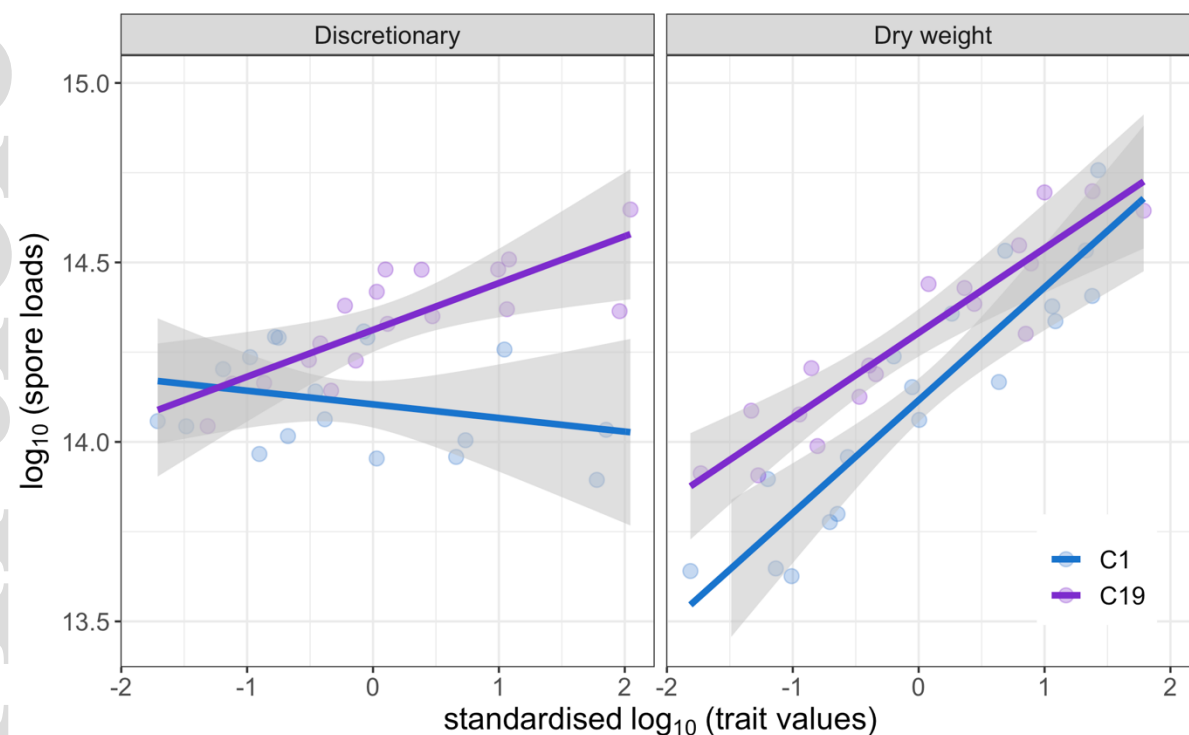


Figure 4. The partial effects of discretionary energy and dry weight on spore loads in infected hosts. Estimates of discretionary energy are obtained by subtracting energy expenditure from energy intake. Shown are the partial residuals and predicted model fit (and 95% confidence intervals) for the analysis of covariance for each trait-pathogen combination based on per block expected averages.

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Data accessibility:

All data used in this manuscript are available on Figshare repository.

<http://doi.org/10.6084/m9.figshare.13151567>.

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SUPPORTING INFORMATION

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

Figure S1: The relationship between population density and mass-specific energy intake, expenditure and discretionary energy

Table S1: Model comparison between a power curve, polynomial and linear regression on all measured traits.

Table S2: Parameter estimates for the effect of host density on all measured traits.