

Host Sex Modulates the Energetics of Pathogen Proliferation and Its Dependence on Environmental Resources

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ABSTRACT: Sex differences in immunity are predicted to underlie much of the frequently observed sex differences in the prevalence or severity of infection. We propose the additional hypothesis that differences in the ability of males and females to acquire and use resources will also affect how readily a pathogen can convert host energy into transmission stages, thereby contributing to sex differences in infection dynamics. To test this we manipulated the resource environment of male and female *Daphnia magna* by altering the availability of food and then exposed hosts to a bacterial pathogen. We measured the production of transmission spores and virulence via the reduction in life span, together with feeding rates and changes in mass-independent metabolic rate, as a measure of the intake and expenditure of energy during infection. When raised in the presence of high resource levels, females more readily allowed for resources in the environment to be translated to pathogen exploitation, as represented by increased spore production, greater virulence, and higher energy use. In contrast, the traits of infected males were robust to changes in resource availability. High food availability thus exaggerated the degree of sexual dimorphism observed between the sexes. It also modified the relationship between host energy use, virulence, and pathogen spore production for each sex. These results suggest that a host's resource environment can affect how a male or female is exploited by a pathogen and may thus be an additional factor driving sex-specific patterns of disease susceptibility or severity.

Keywords: *Daphnia*, host-pathogen interactions, metabolic rate, *Pasteuria*, sexual dimorphism, virulence.

Introduction

Sex differences in the prevalence or severity of infection are a common feature of many species. Across the animal kingdom one sex is often described as the “sicker sex,” al-

though whether males or females suffer more from infection varies by taxon and species (Poulin 1996; Schalk and Forbes 1997; McCurdy et al. 1998; Sheridan et al. 2000; Zuk 2009; Cousineau and Alizon 2014). In explaining these differences, focus is frequently placed on the ways each sex differentially invests in immunity versus other components of fitness, such as reproduction (Rolff 2002; Zuk and Stoehr 2002; Stoehr and Kokko 2006; Zuk 2009), or on the role that different transmission pathways play (e.g., horizontal vs. vertical; Úbeda and Jansen 2016). These perspectives, however, capture only part of the complexity of how sex differences might influence disease outcomes (Duneau and Ebert 2012; Gipson and Hall 2016; Hall and Mideo 2018). Males and females also vary in the way they acquire, store, or expend energy (Stoehr and Kokko 2006; Boggs 2009; Zajitschek and Connallon 2017). For a pathogen, each sex thus represents a different quantity and quality of resource to be exploited, an effect that potentially contributes to the sexual dimorphism observed in both pathogen proliferation and the resulting damage caused to a host.

In exploring how resource availability affects the interplay between host harm and pathogen proliferation, Hall et al. (2009b) modeled the effects of a host's nutritional environment on multiple aspects of the infection process. They found that with increasing host condition (*sensu* Rowe and Houle 1996) and thus the net availability of resources for the pathogen to exploit, disease progressed more rapidly and resulted in greater pathogen reproduction and virulence. In contrast, hosts of lower condition were constrained in both growth and food intake, limiting the resources available for pathogen exploitation and thus pathogen reproduction. Here, we apply the insight of this model to the study of sex differences and pathogen exploitation. We aimed to assess whether the sex that provides a greater pool of resources to the pathogen, or the one that is more able to convert nutrients in the environment into

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accessible resources, is also the one that suffers an accelerated progression of infectious disease.

One way to explore how host sex and resources interact to influence infection dynamics is to manipulate food availability and characterize how changes in the acquisition and use of energy then translate into variation in disease symptoms. Many components of host and pathogen fitness are known to vary when food availability or quality is modulated (Pike et al. 2019), presumably owing to changes in immune investment (Brown et al. 2000; Seppälä et al. 2008) or the pool of resources that a pathogen can exploit (Bedhomme et al. 2004; De Roode et al. 2008). Yet resource availability is only one part of the flow of energy through hosts to pathogens (Nørgaard et al. 2021), as how each sex acquires and expends energy will also matter. Males and females, for example, often vary in both their food preferences and their feeding rates (Maklakov et al. 2008; Hite et al. 2017). Previous work has also found that the sexes differ in the way metabolic rates, a measure of energy transformation and use (Kearney and White 2012), respond to infection or immune activation (Evans et al. 2009; Arnold et al. 2013) and that these differences can be mediated through sex-specific physiology or energy use (Bashir-Tanoli and Tinsley 2014). Obtaining measures of how males and females acquire, transform, and expend energy is thus key to linking resource availability to the pathogen exploitation strategies observed in each sex.

In this study we utilize the *Daphnia magna*–*Pasteuria ramosa* model system (Ebert et al. 2016) to explore whether differences in the way each sex acquires and expends energy can contribute to the diversity of disease outcomes seen in sexually dimorphic species (Sheridan et al. 2000; Cousineau and Alizon 2014). In this system, female *Daphnia* are more susceptible to infection, allow for greater pathogen proliferation, and suffer a greater loss of life due to infection (i.e., virulence) than males, suggesting that females are the more readily exploitable sex (Gipson and Hall 2018; Hall and Mideo 2018; Gipson et al. 2019; Nørgaard et al. 2019). Previous research has hinted that energy availability and use is likely to play an important role in these differences. Females, for example, are known to be larger than males (Duneau et al. 2012) and thus likely to ingest resources at a greater rate. They also experience a substantial increase in mass-independent metabolic rates upon infection (Nørgaard et al. 2021). Comparable estimates, however, for the energetics of infection in males, and its link to food availability in the environment, are currently lacking.

To investigate how host sex and resource availability interact to influence the energetics of pathogen proliferation, we began by utilizing a feature of *Daphnia* biology, whereby offspring sex is determined by environmental cues (Ebert 2005), to produce genetically identical males

and females. We raised each sex on two different levels of resource availability, as manipulated by the number of green algal cells provided to an animal each day. We then infected males and females from each resource treatment with one of two pathogen genotypes and measured the resulting production of transmission spores and the changes in host life span, mass-independent metabolic rates, and feeding rates. For each sex and resource level combination, we explored how changes in energy expenditure upon infection (the increase in mass-independent metabolic rate) interacts with virulence (the reduction in life span) to predict pathogen spore loads. With this design we aimed to understand how sexual dimorphism in infection is shaped by the way a pathogen might differentially exploit each sex when resources vary in an environment.

Material and Methods

Daphnia magna Straus is a freshwater crustacean that produces genetically identical male and female offspring via cyclic parthenogenesis (Ebert 2005). *Daphnia* filter feed on planktonic algae, which brings them in contact with the spore-forming bacterial pathogen, *Pasteuria ramosa* Metchnikoff (Ebert et al. 2016). Once taken up by *Daphnia* hosts, *P. ramosa* proliferates and fills the body cavity, causing a severe reduction in fecundity and life span, before being released upon host death (Ebert et al. 2016; Hall et al. 2019). This experiment utilized male and female *Daphnia* from genotype HU-HO-2, originating from a pond in Hungary, and novel *P. ramosa* genotypes C20 and C24 (from ponds in Belgium and England, respectively; see Luijckx et al. 2010), which vary in pathogen-induced reduction of host life span and the production of transmission spores (Clerc et al. 2015; Hall and Mideo 2018). Before the experiment, all animals were maintained under standardized conditions for three generations to minimize any potential maternal effects (see the supplemental PDF, available online).

Production of Experimental Animals

Genetically identical male and female *Daphnia* were produced by exposing the standardized females to a short pulse of the hormone methyl farnesoate ($300 \mu\text{g L}^{-1}$; product ID: S-0153, Echelon Biosciences, Salt Lake City, UT). Following previously established protocols (Thompson et al. 2017), animals were transferred to the hormone treatment upon the release of their first clutch. The third clutch produced after exposure to the hormone was collected and returned to normal artificial *Daphnia* medium (ADaM; Klüttgen et al. 1994; modified by Ebert et al. 1998). Male *Daphnia* were identified by the presence of a modified appendage used for clasping onto females (Ebert 2005). This method

of producing male *Daphnia* has no detectable impact on either the life span and fecundity of control animals or the virulence and spore production exhibited by infected individuals (Thompson et al. 2017).

Infection Experiment Design and Measures of Pathogen Fitness

Male and female *Daphnia* were randomly exposed either to pathogen C20 or C24 or to a control suspension and then assigned either to a high-resource-availability treatment or to a low-resource-availability treatment of 4 million or 1 million algal cells daily (*Scenedesmus* sp.). Similar manipulations have been used in this system previously, whereby females raised on a diet of 1 million algae cells exhibit reduced size and delayed production of their first clutch, as well as lower overall host fecundity and pathogen spore production (Ben-Ami et al. 2010; Hall and Ebert 2012). Each treatment combination consisted of 80 individuals, with an additional 50 males and 50 females assigned as uninfected controls for each resource treatment (2 sex $\times$ 2 pathogen genotypes $\times$ 2 resource levels $\times$ 80 replicates + (2 sex $\times$ 2 resource levels $\times$ 50 control replicates) = 840 individuals).

Daphnia were infected over the course of 2 days at 4 and 5 days old. Infections took place in 60-mL vials filled with 20 mL of ADaM, and 20,000 pathogen spores were added daily (40,000 spores total). On day 6, animals were transferred to fresh jars filled with 50 mL of ADaM and maintained under standardized conditions (see the supplemental PDF). Survival was monitored daily, and upon death individuals were frozen in 500 μ L of purified water for later determination of infection status and measurement of transmission spores. All *Daphnia* were thawed, crushed with a pestle, and sampled individually for infection status and spore count using an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA), following previous protocols (Gipson et al. 2019).

Measurements of Host Metabolic Rate

Oxygen consumption rates ($\dot{V}_{O_2}$) were used as a proxy for metabolic rate. Measurements were conducted via a 24-channel fluorescence-based respirometry system (SDR SensorDish, Precision Sensing, Regensburg, Germany) using *Daphnia* at 3 weeks after exposure to *P. ramosa*. This age was selected because it captures an intense phase of within-host competition between host and pathogen for resources (Clerc et al. 2015; Hall and Mideo 2018; Gipson et al. 2019). *Daphnia* were first rinsed in ADaM and loaded individually into 4-mL vials with nonconsumptive oxygen sensor spots adhered to the bottom. Before measurement, oxygen sensor spots were calibrated using air-saturated ster-

ile ADaM (100% air saturation) and sterile ADaM containing 2% sodium sulfite (0% air saturation). Each run (8 $\times$ 24-channel readers at a time) occurred in a dark, temperature-controlled room at 20°C for 1.5 h, with oxygen consumption measurements taken once every minute. Animals were left to acclimate for 40 min before the recording process began.

For each 24-channel reader, we randomly allocated 20 animals and four blank controls containing only sterile ADaM. *Daphnia* from each treatment were represented in every oxygen reader. $\dot{V}_{O_2}$ was calculated from the change in oxygen saturation over time (% h⁻¹) according to White et al. (2011) as $\dot{V}_{O_2} = -[(m_a - m_c)/100] \times V \times \beta_{O_2}$, where m_a is the rate of change of oxygen saturation for an animal, m_c is the per-run average rate of change for the controls, β_{O_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 (0.004 L). The parameters m_a and m_c were estimated from the oxygen consumption data using the LoLinR package in R (Olito et al. 2017). $\dot{V}_{O_2}$ estimates (mL O₂ h⁻¹) were then converted to metabolic rate (mJ h⁻¹) using the calorific conversion factor of 20.08 J mL O₂⁻¹ (Lighton 2008). Following any measurement, the body size of each individual was measured using a dissecting microscope from the eye to the base of the caudal spine and converted to dry weight (mg) per Yashchenko et al. (2016).

Feeding Trials

In a follow-up experiment, we quantified how differences in energy intake, as estimated via feeding trials (see Nørgaard et al. 2021), might help explain the observed patterns of pathogen spore production, virulence, and energy expenditure. Infected animals were generated following the process outlined above (2 sex $\times$ 2 pathogen genotypes $\times$ 2 resource levels $\times$ 30 replicates + (2 sex $\times$ 2 resource levels $\times$ 30 control replicates) = 360 individuals). Feeding trials began using infected and control animals at 21 days after exposure. For each trial, we placed three individuals from the same treatment within a 50-mL centrifuge tube filled with 20 mL of ADaM. We added 1.5 mL of algae (~20 million cells mL⁻¹) and allowed the animals to feed for 4 h, with the tubes gently inverted every 30 min to help keep the algae in suspension. Ten animal-free control vials were also established to use as a measure of the starting algae concentration that also accounts for any potential decay over the trial.

Immediately following the feeding trials, the animals were removed, and algae concentrations were measured using Accuri BD flow cytometer following protocols outlined above. We estimated feeding rates (mL individual⁻¹ h⁻¹) as $FR = [\log(\bar{C}_t) - \log(C_{if})] \times V \times N^{-1} \times t^{-1}$, where $\bar{C}_t$

is the average concentration at time t in the animal-free controls (cells mL⁻¹), C_{if} is the algae concentration at time t in the experimental treatment jars (cells mL⁻¹), V is the testing water volume (mL), N is the total number of the experimental animals per vial (individual), and t is the trial duration (h). We removed any negative feeding rate estimates, as these represent technical errors. In total we obtained 61 independent estimates of feeding rates across the sex, resource level, and infection treatment combinations.

Statistical Analyses

All statistical analyses were performed in R (ver. 4.0.2; R Development Core Team) using data deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.tdz08kpxz>; Gipson et al. 2021). Approximately 600 animals (85 male and 81 female controls, 120 males and 137 females infected by C20, and finally, 69 males and 111 females infected by C24) split evenly across the low- and high-resource treatments (308 and 295 individuals, respectively) were successfully assayed for their metabolic rate at 3 weeks after exposure. These differences in sample sizes arose as a result of variation in infection rates, handling errors, and the death of animals before they could have their metabolic rate assayed. Traits were analyzed using a full factorial analysis of variance (type III, white adjusted to correct for unequal variances between the sexes) with host sex (male or female), pathogen genotype (C20 or C24), resource availability (low or high), and their interactions as fixed effects, as implemented using the car package (Fox and Weisberg 2011). Residuals from all fitted models were approximately normally distributed.

As females typically live longer and are larger than males, we converted the relevant raw traits into estimates that are directly comparable between males and females. First, mass-independent metabolic rates (i.e., residual metabolic rate) were derived from the residuals of a nonlinear regression of raw metabolic rates on mass, modeled separately for each sex as a sex-specific power-law relationship via the drc package (Ritz et al. 2015). Next, we estimated the relative changes in life span and mass-independent metabolic rates that result from infection by calculating the difference between an individual's trait value and the mean of the corresponding control animals (sex and resource availability matched). For the change in mass-independent metabolic rates, positive values indicate that this trait increased following infection, relative to control animals of the same sex and resource availability. For the changes in life span, positive values instead correspond to a greater reduction in life span (i.e., virulence; Gipson et al. 2019). We also calculated spore density (spores mg⁻¹) using the dry weight of hosts as described above.

Finally, on the basis of the predictions of Hall et al. (2009b), we explored how changes in energy expenditure upon infection (the increase in mass-independent metabolic rate) interact with virulence (pathogen-induced reduction in life span) to predict the fitness of a pathogen. To do so we used response surface analysis, as estimated using second-order polynomial regression (as in Hall et al. 2008) and subsequently visualized via nonparametric general additive models (mgcv; Wood 2017). This approach focuses on how sex and resource availability change the relationship among traits, not their mean value. To compare between the sexes, spore production was first converted to a relative fitness measure by dividing by the mean for each sex. Within each sex, we then standardized all of the other traits to a mean of zero and standard deviation of one. A series of models were fitted that increased in complexity, from a model that estimated no sex- or resource-specific response surfaces to one that estimated separate response surfaces for each treatment combination. Models were ranked based on Akaike information criterion scores, and differences between specific response surfaces were assessed using partial F -tests.

Results

Host Sex and Resource Availability Interact to Shape Disease Outcomes

In all measured traits, we found that interactions between resource levels and host sex defined variation in disease outcomes (table 1), with the results characterized by the greater response of females to the high-resource treatment. High resource levels in females resulted in a near doubling of spore loads and spore density, a reduction in host life span (i.e., virulence), and an increase in mass-independent metabolic rates following infection (i.e., energy use). In contrast, males displayed greater differentiation among the pathogen genotypes, with either no effect of resource availability on spore loads and spore density or, conversely, a small reduction in virulence (reduction in life span) and energy use (change in mass-independent metabolic rates) when more resources were available.

Relationship between Pathogen Exploitation and Resource Availability

We next explored whether the way a pathogen exploits host resources might vary by sex and resource level. To do so we related the production of spores by a pathogen to both the reduction in life span of a host (i.e., virulence) and the changes in mass-independent metabolic rates (energy expenditure) via a response surface analysis. We found that the best-fitting response surface was one that

Table 1: Summary of the three-factor analyses of variances (white corrected, type III) describing the effects of host sex, parasite genotype, and resource availability on the production of transmission spores, the density of spores per milligram dry weight of a host, and the relative changes in host life span and mass-independent metabolic rate compared with controls

	<i>F</i>	df	<i>P</i>
Production of transmission spores (millions):			
Sex	316.689	1,429	<.001*
Pathogen genotype	1.710	1,429	.192
Resource availability	59.802	1,429	<.001*
Sex × pathogen	1.330	1,429	.249
Sex × resource	46.660	1,429	<.001*
Pathogen × resource	.327	1,429	.568
Sex × pathogen × resource	1.908	1,429	.168
Spore density (millions mg ⁻¹):			
Sex	4.129	1,429	.043*
Pathogen genotype	14.721	1,429	<.001*
Resource availability	20.515	1,429	<.001*
Sex × pathogen	3.047	1,429	.082
Sex × resource	12.653	1,429	<.001*
Pathogen × resource	.498	1,429	.481
Sex × pathogen × resource	.614	1,429	.434
Reduction in host life span (days):			
Sex	339.664	1,421	<.001*
Pathogen genotype	40.228	1,421	<.001*
Resource availability	34.328	1,421	<.001*
Sex × pathogen	6.952	1,421	.009*
Sex × resource	127.237	1,421	<.001*
Pathogen × resource	.156	1,421	.693
Sex × pathogen × resource	2.954	1,421	.086
Increase in mass-independent metabolic rate (mJ h ⁻¹):			
Sex	36.118	1,429	<.001*
Pathogen genotype	.648	1,429	.421
Resource availability	19.290	1,429	<.001*
Sex × pathogen	.225	1,429	.636
Sex × resource	41.200	1,429	<.001*
Pathogen × resource	.086	1,429	.770
Sex × pathogen × resource	1.636	1,429	.202

* Significant effect ($\alpha = .05$).

allowed for an interaction between host sex and resource treatment (table 2). We then tested whether the response surfaces varied (1) between resource levels within a given sex and (2) between sexes within a given resource level.

Within a given sex, the surfaces were not significantly different between the high-resource treatment and the low-resource treatment for males (df = 5,171, $F = 0.928$, $P = .464$), but they were significantly different for females

Table 2: Candidate models describing the effects of host sex and resource availability treatments on the response surface of relative pathogen spore production, as predicted by the standardized increase in mass-independent metabolic rate and the standardized reduction in life span of the host

Candidate response surfaces	No. new model terms	AIC	Δ AIC	AIC weight
1. No response surface	0	597.678	144.346	<.001
2. Single response surface for all treatments	5	506.155	74.302	<.001
3. Response surface varies by sex only	6	503.430	50.100	<.001
4. Response surface varies by resources only	6	514.122	60.790	.001
5. Response surface varies by sex and resource	12	453.331	...	1.000

Note: The least complex model estimates no sex- or resource-specific response surface, and the most complex model estimates separate response surfaces for each treatment combination. Models were ranked by Akaike information criterion (AIC) and the best-fitting model was selected by AIC weight.

($df = 5, 231$, $F = 2.394$, $P = .032$). Within a given resource treatment, the exploitation surfaces were not significantly different between the sexes at high resources levels ($df = 5, 190$, $F = 0.901$, $P = .459$), but they were significantly different at low resource levels ($df = 5, 211$, $F = 3.328$, $P = .007$).

Our results suggest that pathogens infecting either males at any resource level or females with access to high resources will exploit their host in a similar manner. Low-diet females, however, represented a shift in how energy expenditure and virulence are translated into pathogen spore loads. The parameter estimates underlying the best-fitting response surfaces are shown in table 3 and visualized via cubic splines in figure 2. For both males overall and females with high resources, we found that pathogen spore loads were positively associated with the increases in mass-independent metabolic rates caused by infection and negatively associated with the reduction in life span that might follow (fig. 2A, 2B). In each context, therefore, pathogen proliferation was highest within hosts who simultaneously maximized energy expenditure (greatest increase in mass-

independent metabolic rates) and minimized virulence (lowest reduction in life span). In contrast, for females in the low-resource treatment the production of pathogen spores peaked at middle to low values for the reduction in life span (as indicated by a significant quadratic term; table 3) and was not related to variation in mass-independent metabolic rates (fig. 2C).

Evidence for Sex Differences in Feeding Rates and Resource Acquisition

The greater robustness of infected males to varying resource levels suggests that differences in the ability of each sex to acquire resources from the environment might be playing a role. In a follow-up experiment, we assayed the feeding rates of males and females from each of the treatment combinations. Feeding rates were found to be influenced by the sex of the host ($F_{1,49} = 8.985$, $P = .004$) and to a lesser extent an interaction between resource availability and infection treatments ($F_{2,49} = 3.196$, $P = .050$; for the full model, see table S1, available online). Most notably, the feeding rate of females, irrespective of their infection treatment, was significantly higher than that of males (0.286 vs. 0.196 mL individual⁻¹ h⁻¹). There was no clear trend (outside of pathogen genotype C20) for feeding rates to be higher for individuals raised in high-resource environments.

Discussion

A host's ability to acquire and expend energy can have far-reaching effects on the outcome of disease, as well as the relationship between host and pathogen fitness characteristics (Brown et al. 2000; Bedhomme et al. 2004; De Roode et al. 2008; Seppälä et al. 2008; Pike et al. 2019). In this study we explored how the sex of a host can modify the relationship between resource availability, the flow of energy from environment to pathogen, and the capacity of pathogens to exploit their host. We found that females more readily allowed for resources in the environment to be translated to pathogen exploitation, as represented by increased pathogen spore production, spore density, virulence, and energetic demand when raised in the presence of high resource levels (table 1; fig. 1). These patterns were qualitatively consistent with the model predictions of Hall et al. (2009b), where increasing resources of the host should relate to greater pathogen reproduction and virulence. In contrast, infected males expressed near-constant spore loads and densities across the same resource levels and even a slight decline in virulence and energy use at higher resource levels (fig. 1).

Our results suggest that resource variation in natural environments may result in evolutionary hot or cold spots

Table 3: Summary of second-order polynomial response surfaces describing how the standardized reduction in host life span caused by the pathogen (virulence [VR]) and the standardized increase in mass-independent host metabolic rate following infection (MR change) predict relative pathogen spore production for both males and females ($\alpha = .05$)

	Estimate	SE	P
Males, both resource availability levels:			
VR	-.129	.039	.001**
MR change	.076	.028	.007**
VR × MR change	.043	.031	.056
VR ²	-.051	.027	.340
MR change ²	-.020	.020	.171
Females, high resource availability:			
VR	-.137	.037	<.001***
MR change	.142	.036	<.001***
VR × MR change	.051	.038	.182
VR ²	-.073	.032	.024*
MR change ²	-.036	.024	.138
Females, low resource availability:			
VR	-.095	.066	.148
MR change	.084	.050	.093
VR × MR change	.028	.051	.584
VR ²	-.141	.043	.001**
MR change ²	-.047	.039	.244

* $P < .05$.

** $P < .01$.

*** $P < .001$.

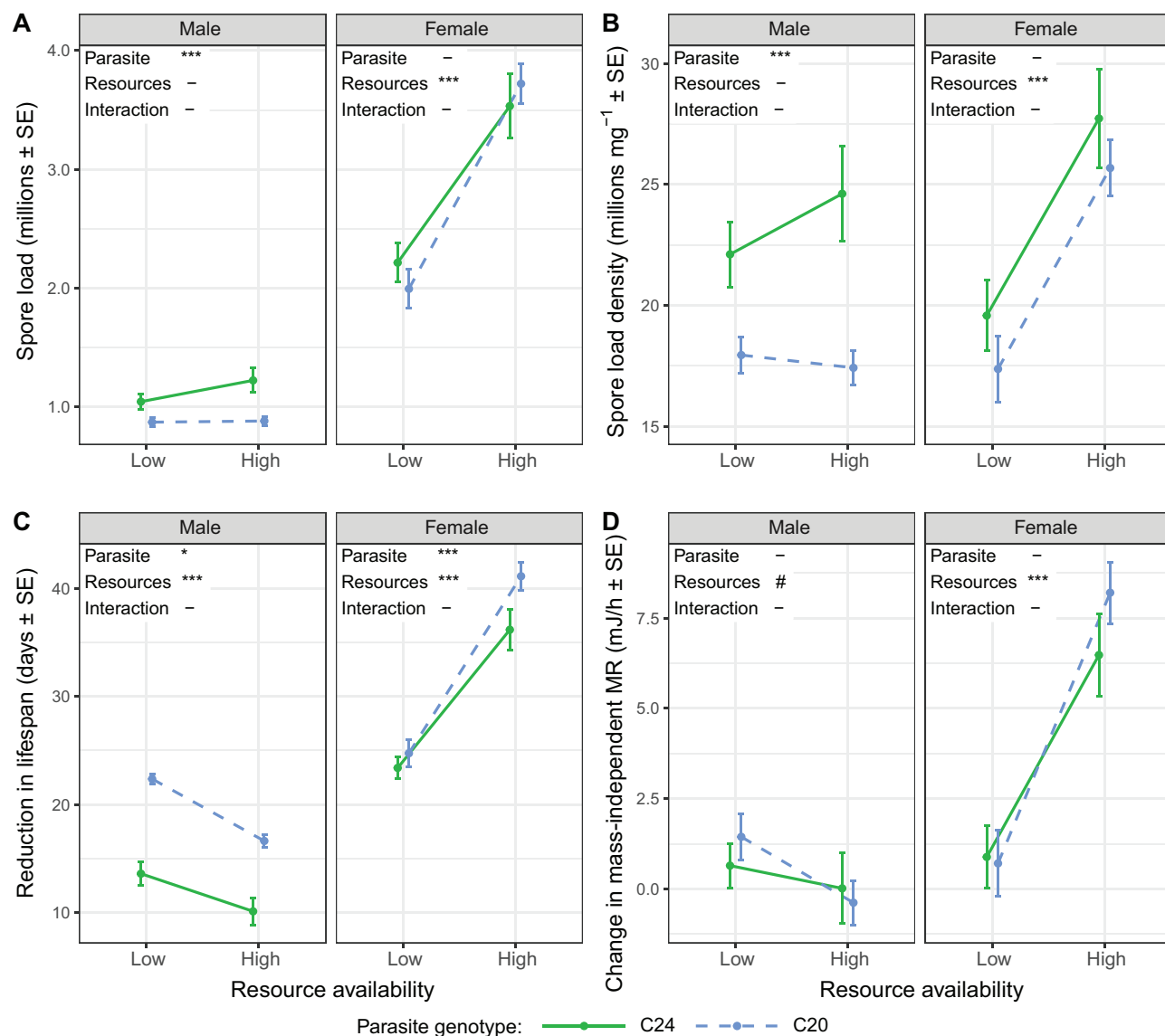


Figure 1: Influence of host sex and resource availability on the production of transmission spores (A) and the density of spores per milligram dry weight of a host (B) and, relative to controls, the reduction in host life span caused by infection (C) and the increase in mass-independent metabolic rate of the host following infection (D). Shown are treatment means and standard errors for each treatment. Asterisks indicate significant effects of the two-factor analysis of variances conducted separately for each panel ($\alpha = .05$). * $P < .10$; ** $P < .05$; *** $P < .001$.

(Thompson 1994; Gomulkiewicz et al. 2000), where the speed or intensity of host or pathogen evolution will depend on an interaction between resource acquisition and host sex. As the shifts from low to high resource levels were associated with subtle changes within males versus strong differences within females for all traits (fig. 1), we observed far greater divergence between the sexes at higher resource levels. High food availability thus exaggerated the degree of sexual dimorphism between the sexes in key pathogen fitness components, such as virulence and spore production,

a consequence that may increase the likelihood of sex-specific pathogen adaptation (Duneau and Ebert 2012; Gipson and Hall 2016). For example, assuming that spore loads are a strong predictor of transmission success (but see Hall and Mideo 2018), high-resource environments should increase the rate of pathogen adaptation to female hosts owing to the greater reproductive value (i.e., expected genetic contribution to future generations; Frank 1996) that this host type can afford a pathogen (sensu Gandon 2004).

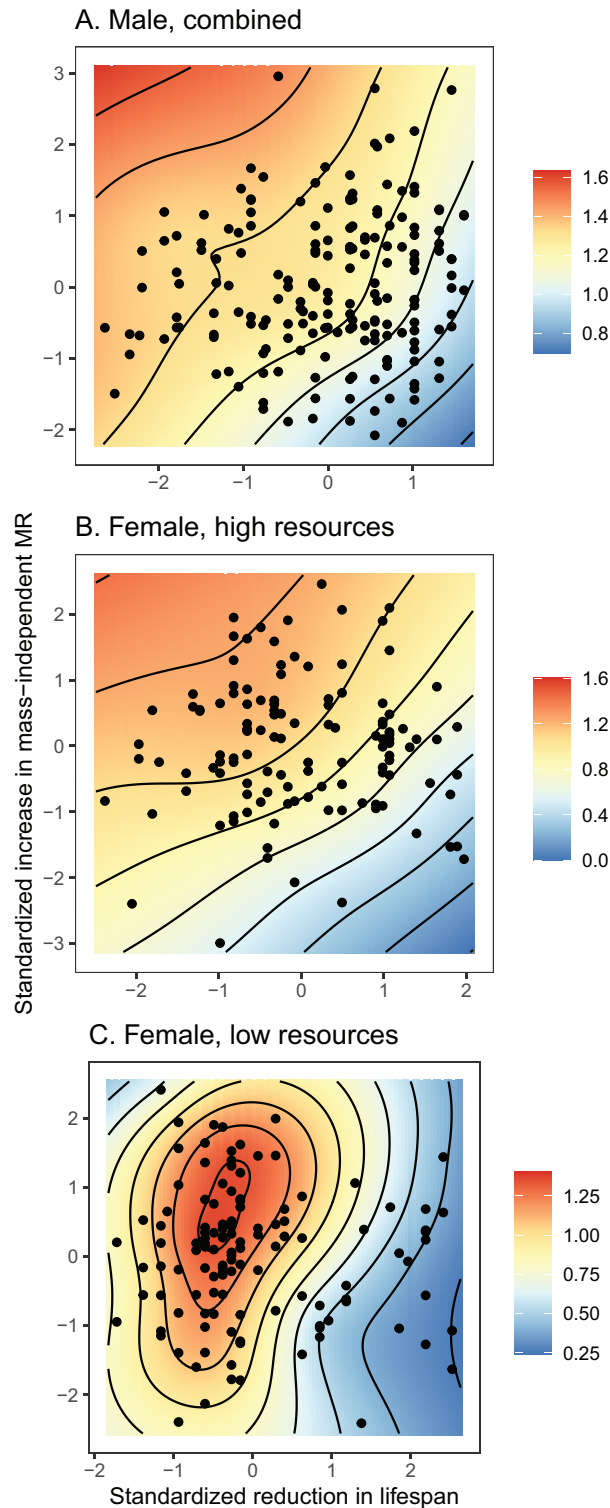


Figure 2: Nonparametric contour plots describing how virulence (standardized reduction in host life span) and energy expenditure (standardized increase in mass-independent metabolic rates [MRs]) predict relative pathogen spore production (mean spore loads per treatment combination). Separate contour plots are shown for in-

Underlying the different sensitivity of each sex to resource variability were also changes in the way pathogens exploited each sex. In males from either resource treatment or females raised specifically on a high-resource diet, we found that a greater reduction in host life span and a smaller increase in mass-independent metabolic rates after infection reduced pathogen proliferation (fig. 2A, 2B). Low virulence and high host energetic demand were thus best for the pathogen in these contexts. Yet this pattern changed within females when they were raised under limited resources. Now an increase in pathogen proliferation was favored at moderate reductions in host life span (fig. 2C) and thus intermediate virulence, irrespective of any variation in metabolic rates and energy use. Collectively, these results suggest that what best predicts pathogen proliferation will change depending on patterns of resource acquisition within females yet will remain robust to this variation within males. In this way the resource environment affects how a male or female is exploited by a pathogen and may thus be an additional factor driving sex-specific outcomes of infection.

Contributing to the sex differences observed in this study will be variation in the ability of male and female *Daphnia* to acquire resources from the environment, as well as their general energetic requirements. In a follow-up experiment, we found that females were more readily able to acquire resources than males owing to a much higher feeding rate (fig. 3; see also Hite et al. 2017). As a result, our food supply treatment may simply not have stressed male *Daphnia* to the extent that it stressed females. Females are also the larger sex (Duneau et al. 2012) and exhibit substantial resource investment in producing a clutch of offspring every 3 days after reproductive maturity (Ebert 2005). We have previously shown that host body size is a key predictor of pathogen spore loads (Clerc et al. 2015; Nørgaard et al. 2021), but even after correcting for size differences, the greater sensitivity of females to resource variation was still evident in the patterns of spore density (fig. 1B). Accounting for sex differences in reproductive investment is more challenging. Yet our estimates of the change in mass-independent metabolic rates upon infection are benchmarked against male and female control animals (see “Material and Methods”). Estimates for the energetics of host exploration are thus relatively equitable between the sexes, despite the different role each sex plays in reproduction.

Overall, our results highlight how the flow of resources from the environment to a pathogen population can occur disproportionately through female hosts, beginning

ected males at both resource levels (A), high-resource females (B), and low-resource females (C). Red or blue coloration represents high or low relative spore production, respectively.

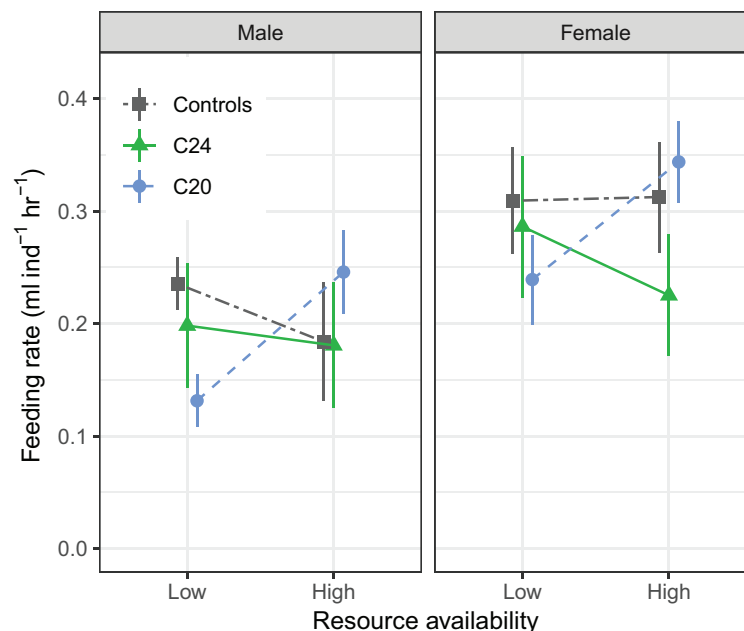


Figure 3: Influence of host sex and resource availability on the feeding rates of control animals and animals infected by pathogen genotype C24 or C20. Shown are treatment means and standard errors for each treatment.

with their elevated ingestion of food. Variation in food availability, coupled with naturally varying sex ratios (*Daphnia* populations can vary from 0% to 50% males throughout a season; Duncan et al. 2006; Galimov et al. 2011), will thus strongly influence pathogen reproduction and virulence in female-biased populations and lessen as the proportion of males increases. If the sexes typically vary in how resources are acquired and made available for pathogen exploitation, as they are observed here, then one sex may have a strong impact on the timing of epidemics, whereas the other may be less influential even as resources change (for a perspective on resource quality and epidemic timing, see Hall et al. 2009a). However, to truly understand the interplay between host sex and resource availability requires a more nuanced exploration of resource variation. Work by Civitello et al. (2020) has shown how variation in resources arising from periodic fluctuations can lead to very different infection dynamics than changes in a constant supply, something that has yet to be explored in the context of how well a pathogen can exploit the resources offered by each sex.

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Statement of Authorship

S.A.Y.G. and M.D.H. conceived of and designed the study. A.K.P. advised on the use of the respirometry equipment and analysis of the oxygen consumption data. S.A.Y.G. and L.H. collected the data. S.A.Y.G. and M.D.H. analyzed the data and led the writing of the manuscript. All authors contributed critically to the manuscript drafts and gave final approval for publication.

Data and Code Availability

Data used in this publication are available in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.tdz08kpxz>; Gipson et al. 2021).

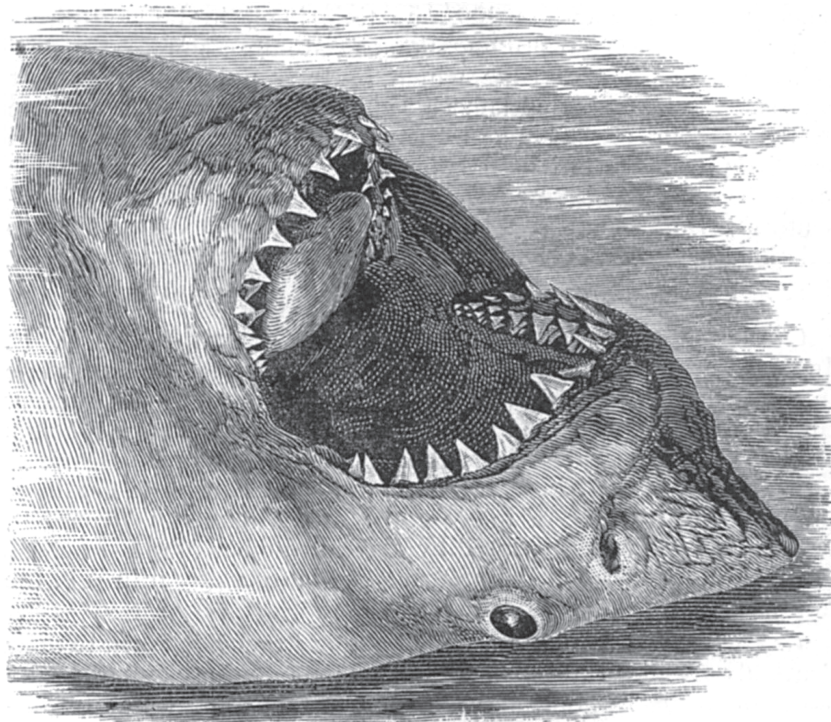
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“The experienced fishermen of Nantucket, with all their opportunities for investigating this interesting family of fishes, were ignorant of the correct name of this specimen, and they regarded it as a rare species in the waters of that vicinity.” From “The ‘Man-eater Shark,’ *Carcharodon carcharias*” by W. D. Stevenson (*The American Naturalist*, 1884, 18:940–941).