

ARTICLE

The sicker sex is plastic: Thermal plasticity determines sex biases in pathogen transmission

Nathan J. Butterworth^{1,2,3}  | Jared Lush¹  | Kerri J. Moore¹ | Matthew D. Hall¹

¹School of Biological Sciences, Monash University, Clayton, Victoria, Australia

²School of Life and Environmental Sciences, Deakin University, Burwood, Victoria, Australia

³Department of Ecological, Plant and Animal Sciences, La Trobe University, Bundoora, Victoria, Australia

Correspondence

Nathan J. Butterworth

Email: nathan.b@deakin.edu.au

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Abstract

Sex differences are predicted to play an important role in the spread and evolution of pathogens. However, attempts to generalize the “sicker” sex have been challenged by intraspecific variability of sex biases across the infection process. Sex-specific plasticity provides a framework to resolve this by elucidating how infection is shaped at the sex-pathogen-environment interface. Using the *Daphnia magna* and *Pasteuria ramosa* system, we measure infection outcomes for males and females across three temperatures and seven pathogen densities to quantify how sex-specific plasticity shapes susceptibility, pathogen loads, and ultimately transmission. We find unique forms of plasticity at each stage of infection – including equivalent, sex-specific, and divergent plasticity. Integrating these into a single estimate of transmission reveals a clear pattern—male-biased transmission at cold temperatures, and female-biased transmission at warm temperatures. Sex-specific thermal plasticity can thus determine the “sicker” sex, with implications for pathogen spread and evolution in a warming world.

KEYWORDS

disease, host–pathogen interactions, pathogen transmission, phenotypic plasticity, sex-specific plasticity, sexual dimorphism, sexual selection, thermal ecology, virulence

INTRODUCTION

Sex differences in the outcome of infection have long had an important place in our understanding of mate choice (Adamo et al., 2001; Córdoba-Aguilar & Munguía-Steyer, 2013; Folstad & Karter, 1992; Hamilton & Zuk, 1982; Nunn et al., 2008; Rolff, 2002; Schwenke et al., 2016; Stoehr & Kokko, 2006; Zuk, 1990, 2009; Zuk & McKean, 1996) and are also fundamental in shaping the ecology and evolution of pathogens (Cousineau & Alizon, 2014; Duneau & Ebert, 2012; Gipson et al., 2019; Gipson & Hall, 2016; Hall & Mideo, 2018; Klein & Flanagan, 2016; Úbeda & Jansen, 2016). Yet attempts to generalize the identity of the “sicker” sex across the animal kingdom have revealed

substantial variation among species (Kelly et al., 2018; McCurdy et al., 1998; Poulin, 1996; Schalk & Forbes, 1997; Sheridan et al., 2000; Zuk & McKean, 1996); and it remains difficult to predict when and why a “sicker” sex should emerge. Indeed, opportunities for sex differences to arise occur multiple times during the contact between a host and pathogen (Hall et al., 2017; vom Steeg & Klein, 2016), from the initial attempt by the pathogen to establish in the host (susceptibility), through to its proliferation within the host (pathogen load), and any damage caused (virulence).

In some cases the sicker sex has been quantified as the sex that is most susceptible to infection (Butterworth et al., 2024; Duneau et al., 2012; Gray, 1998; Moloo, 1993; Morton & García-del-Pino, 2013; Sanchez et al., 2011; Zuk & McKean, 1996), in others as the sex with the

highest pathogen burden (Duneau et al., 2012; Gipson & Hall, 2018; Hillegass et al., 2008; Kailing et al., 2023; Rosso et al., 2020; Thompson et al., 2017; Zuk & McKean, 1996), or even still the sex which exhibits the greatest damage from pathogen virulence (Duneau et al., 2012, 2017; Gipson & Hall, 2018; Gipson et al., 2022; Stephenson et al., 2016; Taylor & Kimbrell 2007; Thompson et al., 2017; Úbeda & Jansen, 2016; Zuk, 1990, 2009). The sicker sex could also be framed as the one with the lowest resistance (i.e., the sex in which a pathogen has the highest fitness; sensu Roy & Kirchner, 2000), the lowest tolerance (i.e., the sex in which per-parasite virulence is maximized; sensu Råberg et al., 2008; Little et al., 2010), or the one most prone to autoimmune disease (Dunn et al., 2023; van Lunzen & Altfeld, 2014; Whitacre, 2001). This multifaceted nature of host–pathogen interactions means that a universally “sicker” sex is challenging, if not impossible, to define—and not all mechanisms will be directly related to the evolution and spread of disease.

Compounding the challenge of defining the sicker sex is that each potential pathway underlying male–female differences may be uniquely sensitive to different forms of environmental change. Host–pathogen interactions, in general, are highly sensitive to the environment (Aulsebrook et al., 2023; Civitello et al., 2015; Duffy et al., 2012; Gehman et al., 2018; Gervasi et al., 2015; Gipson et al., 2022; Hall et al., 2006; Kunze et al., 2022; Mordecai et al., 2019; Teffer et al., 2022; Vicente-Santos et al., 2023), with environmentally induced variation arising first at the initial contact between a host and pathogen, where the dose encountered can define the probability of infection and subsequent damage that occurs (Ben-Ami et al. 2010; Clay et al., 2021; Ebert et al., 2000; Lunn et al., 2019; McCallum et al., 2017; Regoes et al., 2002, 2003), and extending to any external condition that shapes the capacity of a host to fuel its immune system or influence within-host pathogen growth (Hall et al., 2006, 2007, 2009; Nørgaard et al., 2021; Pike et al., 2019). At each stage, however, the characteristics of infected males and females could respond in several distinct ways. Most simply, males and females could plastically respond to the changing environment in the same direction (i.e., equivalent plasticity, Figure 1A), maintaining the relative consistency in identity and fitness costs of the sicker sex. Alternatively, one sex could more plastically respond to the environment (i.e., sex-specific plasticity, Figure 1B) or both sexes could respond, but in opposing directions (i.e., divergent plasticity, Figure 1C), giving rise to either a change in the degree of male–female differences, and thus the magnitude of the sicker sex (Figure 1B), or the identity of the sicker sex in any given environment (Figure 1C) respectively.

Given substantial evidence that males and females differ in their extent of phenotypic plasticity (Brand et al., 2023; Bonduriansky, 2007; Fairbairn, 2005; Fernández-Montraveta & Moya-laraño, 2007; Hangartner et al., 2022; Polverino et al., 2023; Pottier et al., 2021; Rohner et al. 2018; Stillwell et al., 2010; Stillwell & Davidowitz, 2010), combining sex-specific phenotypic plasticity with our understanding of disease will help evaluate both the consistency of sex differences within species and the utility of comparing patterns across the animal kingdom. However, with growing interest in the consequences of male–female differences for pathogen fitness (Cousineau & Alizon, 2014; Duneau & Ebert, 2012; Gipson et al., 2019; Gipson & Hall, 2016; Hall & Mideo, 2018; Klein & Flanagan, 2016; Úbeda & Jansen, 2016), not just in patterns of sexual dimorphism, a more integrated approach is required to understand sexual dimorphism and sex-specific plasticity in the light of pathogen fitness. Models of the transmission process of host and pathogen combinations provide a way to achieve this by summarizing sex-specific outcomes across the stages of infection (which can vary among host–pathogen systems) into a single metric of pathogen performance which is comparable among systems—pathogen transmission (Anderson & May, 1979; De Jong et al., 1995; Grassly & Fraser, 2008; McCallum et al., 2001). Summarizing the varying aspects of the “sicker” sex in terms of the one most likely to transmit the pathogen to other individuals (i.e., “per-capita transmission”, Figure 1D) provides a useful way to interpret the overall consequences of sex-specific plasticity for the ecology and evolution of pathogens.

Temperature provides an ideal opportunity to assess the contribution of sex-specific plasticity to sex biases in disease characteristics and ultimately disease transmission. For one, understanding the plastic responses of both sexes and their pathogens to temperature is increasingly important, as climate change is expected to fundamentally alter the spread of disease (Gehman et al., 2018; Mordecai et al., 2019). Thermal plasticity has been shown to occur across every step of the infection process – from a host’s susceptibility, to pathogen growth rates, and virulence (Gehman et al., 2018; Hector et al., 2024; Kirk et al., 2019; Kunze et al., 2022; Mordecai et al., 2019; Shocket et al., 2019) with contrasting forms of plasticity arising at each step of infection (Hector et al., 2024; Kirk et al., 2018, 2019). Sex-specific responses to temperature have also been observed across the animal kingdom for body size (Stillwell et al. 2010; Stillwell & Davidowitz, 2010), movement activity (Brand et al., 2023) and thermal tolerance (Hangartner et al., 2022; Pottier et al., 2021). Surprisingly then, although it is well established that males and females exhibit differential

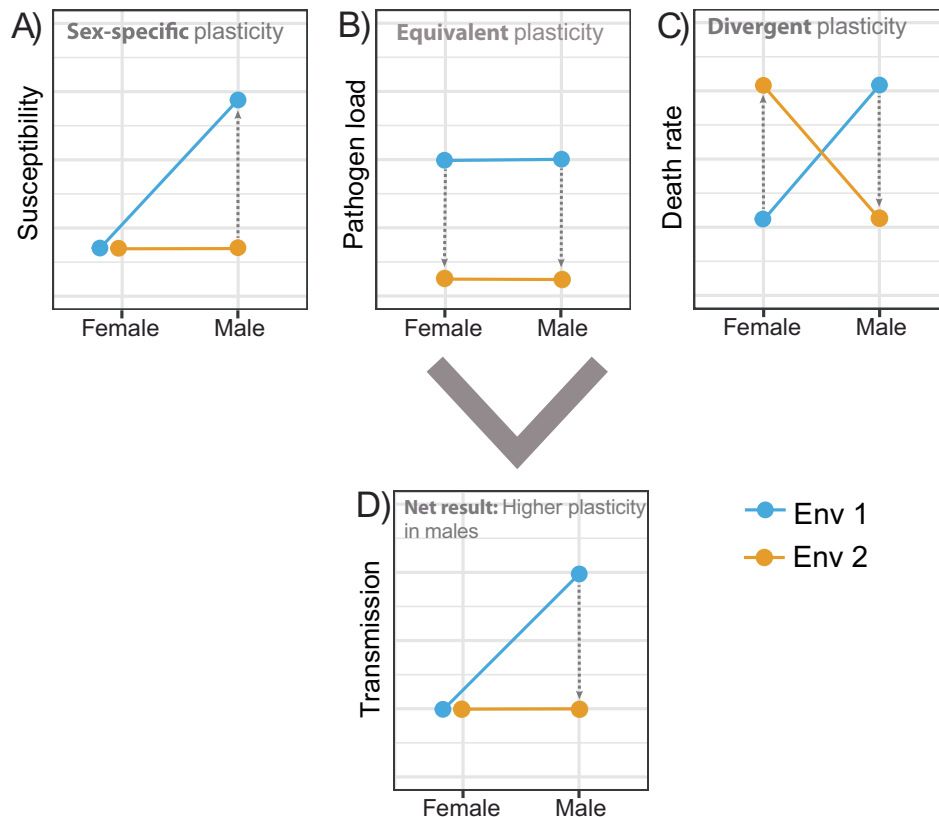


FIGURE 1 Some possible forms of sex-specific plasticity in each component of infection and how they may lead to non-intuitive predictions for sex biases in pathogen transmission (D). Using a horizontally transmitted pathogen as an example (e.g., a bacterial infection of *Daphnia magna* that is transmitted only at host death) the capacity for pathogen transmission through each sex will depend on the sex-specific differences in (A) host susceptibility, (B) pathogen loads, and (C) host death rates (e.g., Aulsebrook et al., 2023; Hall & Mideo, 2018). Hypothetically, each component of infection could have a unique form of sex-specific plasticity – susceptibility might be plastic only for males (i.e., sex-specific plasticity), pathogen loads equally plastic in both sexes in the same direction (i.e., equivalent plasticity), and death rates equally plastic in both sexes but in opposing directions (i.e., divergent plasticity). Yet non-intuitively, integrating each component into a single measure of disease spread would suggest a strong male bias in transmission in environment 1, but no sex bias at all in environment 2, and that this shift in the extent of the “sicker sex” (in regard to the sex where pathogen transmission is highest) would be underpinned entirely by plasticity in male transmission only (D).

plastic responses to temperature—it remains entirely unknown how such sex-specific plasticity combines with the thermal dependency of infection to shape the spread and evolution of pathogens.

To understand how sex biases in transmission emerge, and the role that the thermal environment might play, an essential consideration is the density of pathogen propagules encountered. Pathogen density (i.e., dose) fundamentally determines the likelihood and consequences of contact between a host and pathogen (Ben-Ami et al. 2010; Butterworth et al., 2024; Clay et al., 2021; Ebert et al., 2000; Lunn et al., 2019; McCallum et al., 2017; Regoes et al., 2002, 2003). Put simply, at low doses, encounters with the pathogen are rare, and immune systems remain unchallenged, providing little opportunity for sex differences in immunity and their thermal sensitivity to be expressed. Conversely, at high

doses, pathogen encounter rates are maximized and immune systems are overwhelmed, even for the most resistant sex. Both scenarios have been shown to obscure sex biases in susceptibility (Butterworth et al., 2024) and to alter the sensitivity of different components of infection to environmental change (e.g., Kirk et al., 2019). The dose dependency of infection must be considered because it directly determines the scope within which sex-specific thermal plasticity will emerge or be constrained.

Here, we investigated how sex-specific thermal plasticity emerges across the infection process and shapes sex biases in pathogen transmission, using the *Daphnia magna* and *Pasteuria ramosa* system (Ebert et al., 2016) to measure infection outcomes across three temperatures, seven pathogen densities, and 2100 individual clonal males and females. Previous studies have shown that thermal plasticity is sensitive to initial pathogen dose

(Kirk et al., 2019) and fundamentally determines infection rates in *Daphnia* (Kirk et al., 2019; Shocket et al., 2019). Males and females also exhibit strong sexual dimorphism across almost every measurable trait—males being significantly smaller (Duneau et al., 2012), having lower thermal tolerances when uninfected (Laidlaw et al., 2020), differing in susceptibility to infection (Butterworth et al., 2024; Duneau et al., 2012), and with lower pathogen burdens (Gipson et al., 2022). Considering the thermal sensitivity of the infection process, strongly sexually dimorphic infection outcomes, and differing thermal sensitivity between the sexes—the *Daphnia-Pasteuria* system provides the ideal opportunity to explore how sex-specific plasticity can shape the extent and identity of the “sicker” sex.

METHODS

Study organisms

The freshwater crustacean *Daphnia magna* Straus (Cladocera: Anomopoda) reproduces via facultative parthenogenesis and can produce genetically identical male and female clones. In its natural environment it is frequently parasitized by a range of organisms (Ebert, 2005), including the gram-positive bacteria *Pasteuria ramosa* Metchnikoff (Green, 1974), which attaches to the host esophagus and hindgut and reproduces within the hemolymph to produce transmission spores (Bento et al., 2020; Ebert et al., 2016). Infected females suffer severely reduced fecundity and lifespan, and an increase in body size (Clerc et al., 2015; Hall et al., 2019). Males suffer less with fewer spores and a smaller reduction in longevity (Duneau et al., 2012; Gipson et al., 2022). Transmission is exclusively horizontal, only occurring after the release of spores from dead hosts (Aulsebrook et al., 2023; Hall & Mideo, 2018).

Production of experimental animals

This experiment used stocks of *D. magna* of a single genotype BE-OHZ-M10 which was originally hatched from a resting egg (ephippia) collected from Oud Heverlee, Belgium (coordinates: 50° 50' 14", 4° 39' 48"). The *P. ramosa* genotype C1 was used to infect all animals. This pathogen was originally isolated from a single female infected with pond sediment from Moscow, Russia (Luijckx et al., 2011). This host-pathogen combination was chosen because it is compatible and has been shown to result in sex biases in pathogen loads (Laidlaw et al., 2020). To minimize

variation in maternal effects, female *D. magna* were raised individually for three generations in 70 mL jars filled with 50 mL of artificial *Daphnia* medium (ADaM—Klüttgen et al., 1994; modified by Ebert et al., 1998) and were maintained under standard conditions (20°C, 16L:8D).

Genetically identical male and female *D. magna* were produced by exposing the third generation of standardized females to the crustacean juvenile hormone methyl farnesoate (300 µg L; product ID: S-0153, Echelon Biosciences, Salt Lake City, UT) which promotes the production of male offspring. Following established protocols, adult females were transferred to 20 mL of hormone after the release of their first clutch (Gipson et al., 2022; Thompson et al., 2017). The second clutch produced after exposure to the hormone (their third lifetime clutch), composed of a mixture of male and female offspring, was then collected and immediately returned to normal artificial media and placed into the relevant temperature treatment (15, 20, or 25°C). This method of producing both male and female *Daphnia* has no detectable impact on either the lifespan or fecundity of control animals (Thompson et al., 2017). Male and female *Daphnia* offspring were identified by the presence/absence of the sexually dimorphic appendage used for clasping onto females (Ebert, 2005).

Experimental design

To assess how the sensitivity of thermal plasticity varies across environmental doses, we performed a dose-response experiment across three temperatures. Males and females to be infected were housed individually at their respective temperature (15, 20, or 25°C), in 70 mL jars, filled with 50 mL of artificial media. Animals were fed daily throughout the entire experiment with increasing numbers of algal cells (*Scenedesmus* sp.)—1 million cells (at 1 day old), 2.5 million cells (at 2–5 days old), 3 million cells (at days 5–6 days old), and 5 million cells (from day 7 onwards). Animals were changed into 50 mL of fresh ADaM every 3 days throughout the entire experiment.

To infect the animals, *P. ramosa* spores were set up as follows: the highest spore dose of 1,250,000 spores was subsequently diluted seven times by a factor of five to produce the other doses, resulting in seven dose levels of (A) 80, (B) 400, (C) 2000, (D) 10,000, (E) 50,000, (F) 250,000, and (G) 1,250,000 spores. At 4 days of age, the animals were transferred individually into jars containing 20 mL of ADaM and exposed to one of the seven *P. ramosa* spore doses and immediately returned to their respective temperature cabinet.

After 4 days of exposure to the spores, animals were moved to fresh jars filled with 50 mL of ADaM and then water was changed as usual afterward. Each treatment consisted of 50 individual replicates for a total of 2100 experimental animals (2 sexes $\times$ 3 temperatures $\times$ 7 doses $\times$ 50 replicates).

Quantifying infection rates, pathogen loads, death rates, and virulence

Experimental animals were monitored daily throughout the experiment until death at which point their lifespan was recorded, and they 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 load by hemocytometer counts of pathogen spores under a phase-contrast microscope. Death rate was calculated as the inverse of lifespan ($1/\text{lifespan}$). Virulence was calculated for each combination of dose and temperature as the difference between the mean lifespan of uninfected animals taken from the lowest dose (80 spores) and a given infected individual. The experiment started with 2100 animals, and 2004 were successfully assayed; 96 animals were removed due to being lost or mislabeled during the experiment. All following statistical analyses were performed in R (ver. 4.0.2; R Development Core Team 2025).

Assessing the sex-specific plasticity of susceptibility

To test how sex biases in susceptibility to infection emerge across temperatures, we first modeled the change in the proportion of hosts infected with increasing spore dose as a three-parameter logistic non-linear model via the package *drc* (where $Y = d / (1 + e^{-b(X-e)})$; Ritz et al., 2016), allowing the partitioning of susceptibility into three components: e , the relative ED50 (the dose at which 50% of susceptible hosts are infected), b , the slope (related to the variance in susceptibility), and d , the max asymptote (the maximum proportion of infected hosts). For each temperature, we compared the fit of a model where the parameters were constrained to be equal between the sexes with one where we allowed the parameters to vary by sex (sensu Butterworth et al., 2024). At the four doses with appreciable infection rates (10,000, 50,000, 250,000, and 1,250,000 spores), we analyzed the probability of infection using a binomial generalized linear model with a logit link function from “glm” function

using the “stats” package (v 4.2.2) with sex, temperature, and dose as fixed effects.

Analyzing the plasticity of sex-biases across the entire infection process

Next, we subset the data to only the doses with appreciable infection rates (10,000, 50,000, 250,000, and 1,250,000 spores) and assessed how sex biases in host lifespan, virulence, and total spore loads varied among exposure dose, sex, and temperature treatments. For death rates we used square root transformation and a linear model with sex, temperature, and dose as fixed effects. For virulence, we used a linear model with the “lm” function from the “stats” package with sex, temperature and dose specified as fixed effects. For spore loads, we used a square root transformation and a generalized least squares model with sex, temperature and dose as fixed effects using the “gls” function from the “nlme” package (v. 3.1, Pinheiro et al., 2023), and allowed for residual variance to vary independently for each temperature treatment to account for heteroscedasticity using “VarIdent”. We then used “Anova” type III from the “car” package (v. 3.1-1, Fox & Weisberg, 2019). Predicted mean infection rates, spore loads, death rates, and virulence and standard errors were extracted from each model with the “emmean” function from the “emmeans” package (v. 1.8.3, Lenth et al., 2024).

Quantifying sex biases in pathogen transmission

To understand how sex biases in pathogen transmission were mediated by sex-specific plasticity, we use the composite transmission rate ($\sigma = \beta\phi\omega D$) adapted from Hall and Mideo (2018) and following Aulsebrook et al. (2023), which describes the per capita rate at which new infections are created based only on the characteristics of infected individuals. In the *Daphnia*–*Pasteuria* system, for pathogens to be transmitted they must first be ingested from the environment by a host, successfully bind and infect the host, proliferate within the host, then kill the host to be released back into the environment for subsequent horizontal transmission (Ebert et al., 2016). These features are all captured by the simple composite transmission function (σ) (Hall & Mideo, 2018) which will increase with an increase in environmental transmission rate (per-spore infectivity) (β), spore loads at host death (ω), or the rate at which infections end owing to both pathogen-induced virulence and background host

mortality (D). It also depends on the effective rate of spore release from dead hosts (ϕ).

To calculate transmission (σ) for each temperature, dose, and sex combination we quantified the relevant components using data from the experimental animals that were observed from birth until death. For transmission, spore loads at host death (ω) were available for each infected individual and we estimated the rate at which infections end owing to host death (D) as the inverse of measured host lifespan ($1/\text{days}$). The environmental transmission rate (β) was estimated using the numbers of infected and uninfected individuals from each treatment, where the probability of remaining uninfected (P) depends on the density of pathogen spores (Z , Dose per 20 mL) and the length of the infection period (t , 4 days), such that $P = e^{-\beta Z t}$ (following Shocket et al., 2018).

For all traits we used the Stan modeling language (Stan Development Team 2023) as implemented via the CmdStanR package v. 0.5.3 (Gabry et al., 2023) in R, to calculate Bayesian posterior distribution estimates for each metric (see also Shocket et al., 2018). We used the default sampling settings of Stan and assumed uniform priors based on a plausible range of values (following Aulsebrook et al., 2023). In all following analyses, we arbitrarily set $\phi = 0.01$ (following Hall & Mideo, 2018) as it was not measured in this experiment. To calculate the composite transmission function (σ) we fed the posterior samples of each of the metrics described above into the composite transmission rate equation, and then characterized the resulting distribution via its mean and 90% credible intervals (capturing that 90% of the mass in this interval is above or below the outer values).

RESULTS

The more susceptible sex is determined by sex-specific thermal plasticity

To characterize how sex biases in susceptibility are mitigated by the relationship between environmental temperature and pathogen dose we first estimated each dose–response curve for the probability of becoming infected using a three-parameter log-logistic model. At 15°C, we found that males were broadly more susceptible to infection (Figure 2A), as allowing parameters to vary by sex significantly improved the fit of the dose–response model ($F_{3,8} = 8.468$, $p = 0.007$). At 20°C we found no statistical support for differences between males and females in their dose–response curves ($F_{3,8} = 0.711$, $p = 0.572$). Thus, sex differences in susceptibility did not appear to arise at 20°C (Figure 2A). However, at 25°C, we again

found that allowing parameters to vary by sex significantly improved the fit of the dose–response model ($F_{3,8} = 50.091$, $p < 0.001$). Yet the opposite trend was observed relative to the 15°C treatment, with females now more susceptible to infection across most doses (Figure 2A).

To further interrogate how thermal plasticity in sex biases arises at each dose, we next analyzed only the doses where rates of infection were appreciable ($>10,000$ spores, Figure 2B). After doing so, we found infection rates depended on a three-way interaction between dose, temperature, and sex (Table 1). Thus, while temperature directly mediates the magnitude and direction of sex biases in infection rates, the pattern was not consistent across doses. At the lowest and highest doses there were no sex biases in susceptibility and only an effect of temperature on overall infection likelihood (Figure 2B, Table 1). Instead, sex biases emerged at the intermediate doses (50,000 and 250,000) and this is where the effect of temperature was felt most strongly on plasticity in sex biases. As expected by the dose–response curves (Figure 2A), the 15°C treatment favored higher infection rates in males, while the 25°C favored higher infection rates in females, leading to a two-way interaction between sex and temperature at each dose (Table 1).

Patterns of plasticity were not reinforced in downstream components of infection

Downstream of infection, the effects of temperature on sex biases were not felt equally for subsequent infection outcomes—with each component showing a unique functional form of plasticity. For the death rates of infected animals, there was a significant dose-by-temperature interaction (Figure 3A; Table 1) but no overall sex bias with death rates simply increasing with temperature (Figure 3B). Broadly then, the death rate of infected hosts is sensitive to both dose and temperature but shows equivalent thermal plasticity between the sexes (i.e., no overall sex-by-temperature interaction, Table 1). In contrast, spore loads at host death showed clear differences in the thermal plasticity of each sex, and the degree of sex biases occurring at each temperature and dose as a result (Figure 4, and sex-by-temperature interactions in Table 1). As temperature rises, female spore loads increase by fifteen-fold, especially at low doses. Males, in contrast, are much less sensitive increasing only four-fold. As a result, sex biases in pathogen load are driven by greater thermal plasticity in females (Figure 4B). The significant three-way interaction between dose, temperature, and sex further reflects the subtle effect that pathogen dose has on the interaction between temperature and sex (Figure 4A; Table 1).

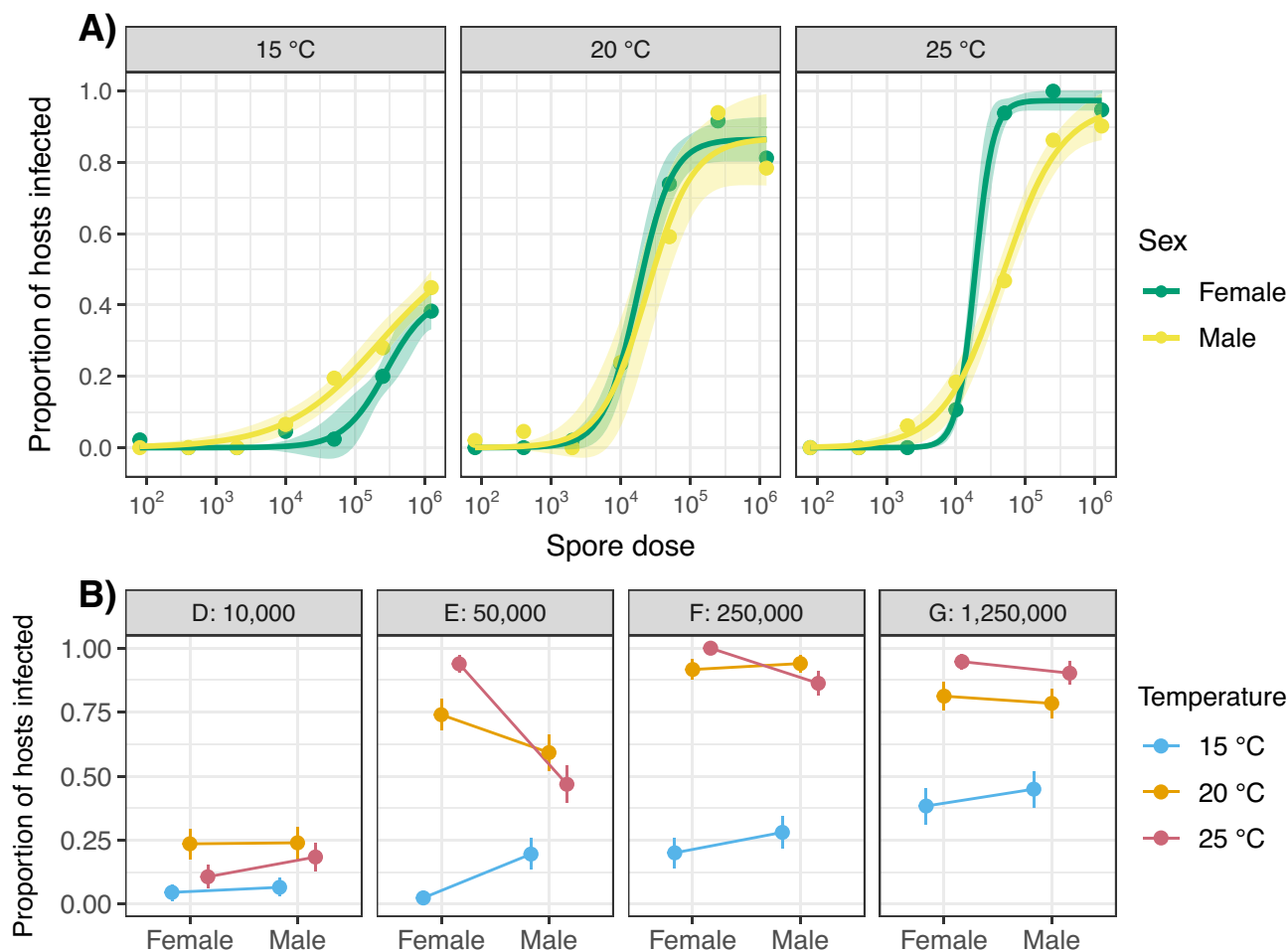


FIGURE 2 (A) The non-linear relationship between pathogen dose and infection success. Shown is the change in proportion of infected *Daphnia magna* (genotype: BE-OHZ-M10) hosts in relation to the dose of the pathogen *Pasteuria ramosa* (genotype: C1), as modeled by a three-parameter logistic model with $\ln$ spore dose as the explanatory variable. Hosts were exposed to the pathogen when 4 days old and at one of three temperatures (15, 20, 25 °C). Yellow shading represents 90% CIs for males. Green shading represents 90% CIs for females. (B) Predicted means and standard errors of infection rates from the binomial generalized linear model at each of the four doses where infection rates were appreciable. Doses D, E, F, G correspond to the four highest spore doses—10,000, 50,000, 250,000 and 1,250,000 spores, respectively.

Dose-increased female spore loads only at 15 °C, but showed the reverse trend in females at 20 and 25 °C (Figure 4A), which explains why the emergence of sex biases in spore loads at 15 °C only occurs at the highest doses (Figure 4B).

Mirroring the observed variation in spore loads was the reduction in host lifespan caused by the pathogen (i.e., virulence, Figure 5, relative to the lifespan of the lowest “A” dose treatment; Appendix S1: Figure S1). Accounting for the significant three-way interaction between dose, temperature and sex biases, there was a rank order change across doses in the temperature with the greatest reduction in female lifespan—which was 15 °C at low doses, but 20 °C at the highest dose (Figure 5A; Table 1). This reflects the greater sensitivity of virulence in females to both dose and temperature, as there was a clear

dose-induced reduction in virulence at 15 °C, but an increase at 20 °C (Figure 5A). Although the death rates of infected animals did not differ between the sexes (see above and Figure 3A), the lifespan of control males and females did at each temperature (see Appendix S1: Figure S1). This allowed for sex biases in virulence to emerge, which again were due to greater female sensitivity to temperature (Figure 5B).

Warmer temperatures increase sex-biases in transmission through greater female plasticity

Integrating each characteristic of infection into a single measure of pathogen performance, reveals that sex biases in

TABLE 1 Result of the analysis of variance (type III) for the effects of dose (4 levels: 10,000, 50,000, 250,000, and 1,250,000 spores), sex (2 levels: Male or female), and temperature (3 levels: 15, 20, and 25°C) on infection prevalence (binomial generalized linear model on the probability of being infected), death rate (linear model on square root death rate), virulence (linear model on the reduction in lifespan relative to the control average), and spore load (generalized least squares on the square root total number of spores at host death).

	Infection rate (Figure 2B)			Death rate (Figure 3B)			Spore load (Figure 4B)			Virulence (Figure 5B)		
	χ^2	df	p	F	df	p	F	df	p	F	df	p
Full model												
Dose	296.72	3	<0.001	0.45	3576	0.718	5.49	3570	0.001	5.12	3576	<0.001
Temp	248.78	2	<0.001	46.56	2576	<0.001	81.72	2570	<0.001	105.98	2576	0.002
Sex	2.4	1	0.122	0.01	1576	0.956	0.49	1570	0.482	776.77	1576	<0.001
Dose × Temp	33.77	6	<0.001	2.23	6576	0.039	3.62	6570	0.002	6.96	6576	<0.001
Dose × Sex	4.55	3	0.208	0.58	3576	0.626	2.72	3570	0.044	3.12	3576	<0.001
Temp × Sex	19.25	2	<0.001	0.28	2576	0.759	19.56	2570	<0.001	64.37	2576	0.025
Dose × Temp × Sex	20.67	6	0.002	0.79	6576	0.576	3.59	6570	0.002	3.03	6576	0.006
D: 10,000												
Temp	13.24	2	0.001	10.55	2,36	<0.001	220.19	2,36	<0.001	18.94	2,36	<0.001
Sex	0.67	1	0.413	0.04	1,36	0.851	1.92	1,36	0.175	151.34	1,36	<0.001
Temp × Sex	0.67	2	0.717	0.71	2,36	0.497	48.81	2,36	<0.001	7.67	2,36	0.002
E: 50,000												
Temp	89.22	2	<0.001	10.72	2135	<0.001	237.14	2135	<0.001	27.94	2135	<0.001
Sex	10.58	1	0.001	0.02	1135	0.883	0.01	1135	0.934	112.49	1135	<0.001
Temp × Sex	27.29	2	<0.001	0.25	2135	0.781	52.21	2135	<0.001	24.61	2135	<0.001
F: 250,000												
Temp	145.73	2	<0.001	34.84	2201	<0.001	296.39	2198	<0.001	102.07	2201	<0.001
Sex	0.23	1	0.635	1.69	1201	0.196	47.56	1198	<0.001	713.91	1201	<0.001
Temp × Sex	10.27	2	0.006	0.31	2201	0.733	34.74	2198	<0.001	45.57	2201	<0.001
G: 1,250,000												
Temp	68.52	2	<0.001	83.79	2204	<0.001	248.99	2201	<0.001	40.78	2204	<0.001
Sex	0.01	1	0.956	0.22	1204	0.642	44.53	1201	<0.001	548.17	1204	<0.001
Temp × Sex	1.26	2	0.532	3.44	2204	0.034	25.35	2201	<0.001	40.01	2204	<0.001

Note: Bold numbers indicate significant values.

pathogen transmission depend on the interaction between dose and temperature (Figure 6). Warmer temperatures and higher doses led to higher transmission in general (Figure 6A), with females most plastic in their response to temperature at every dose, leading up to a 48-fold increase towards female-biased transmission at intermediate doses. This result reflects the higher thermal plasticity females showed in key components of pathogen transmission— infection rates (Figure 2) and spore loads at host death (Figure 4). However, transmission was not exclusively female biased. At low temperatures (15°C), there was up to a 13-fold shift towards male-biased transmission at lower doses (particularly at the 50,000 spores dose) and a lack of dimorphism across other doses (Figure 6B). Overall, the most pronounced manifestation of sex differences occurs at dose E (50,000 spores) across all temperatures.

DISCUSSION

Sex-biases in transmission are predicted to fundamentally shape the spread and evolution of pathogens in species with pronounced sexual dimorphism (Cousineau & Alizon, 2014; Hall & Mideo, 2018; Ubeda & Jansen, 2016). A hallmark of male–female differences for many species, however, is their differential plasticity in response to environmental change (Bonduriansky, 2007; Hangartner et al., 2022; Pottier et al., 2021; Rohner et al., 2018; Stillwell et al., 2010; Stillwell & Davidowitz, 2010). Here, we integrate sex-specific plasticity with an understanding of sexual dimorphism in pathogen transmission. By manipulating the thermal environment and density of pathogens, we show that temperature fundamentally determines the extent of sex-biases in many metrics of infection, and ultimately transmission. The identity of

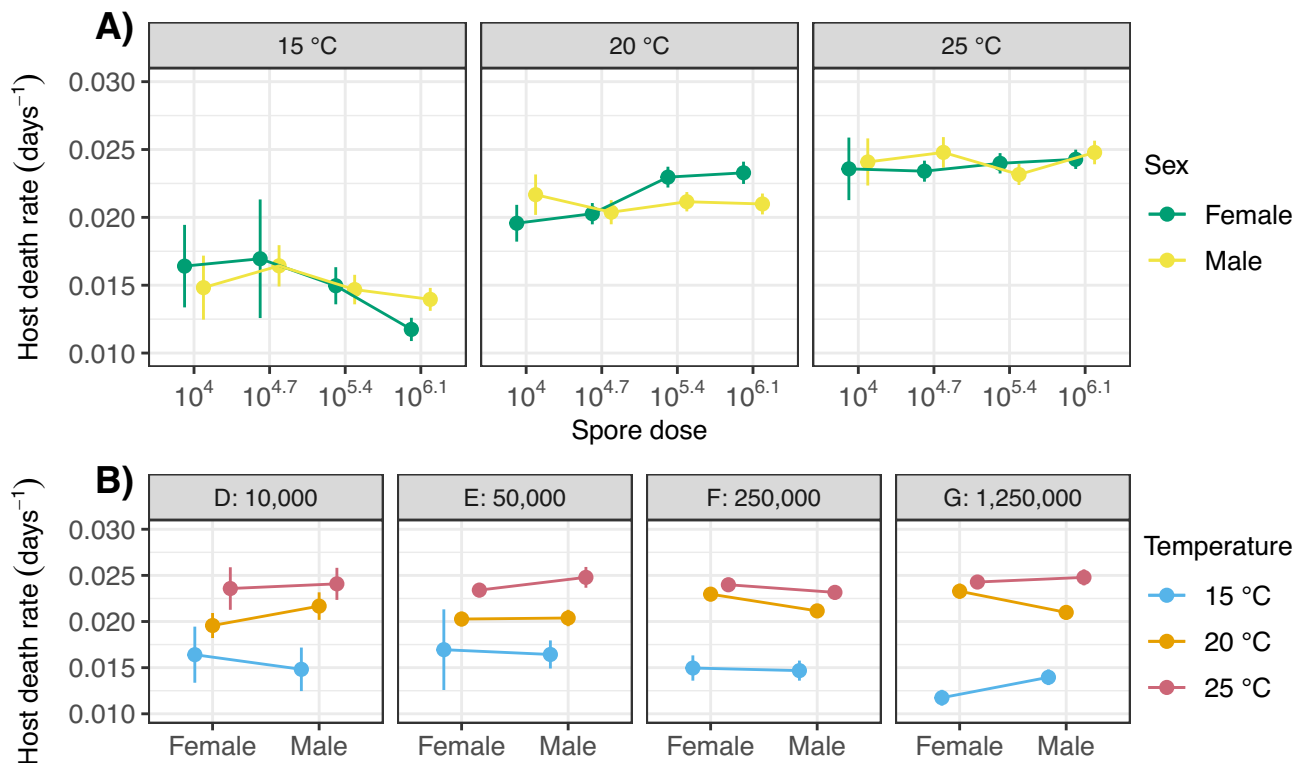


FIGURE 3 Thermal plasticity of host death rate as measured for *Daphnia magna* (genotype: BE-OHZ-M10) exposed to the pathogen *Pasteuria ramosa* (genotype: C1). Shown are (A) dose responses reflected by predicted treatment means ($\pm$ SE) where each facet represents one of three temperature treatments and (B) the same data instead presented as male–female reaction norms of the predicted treatment means ($\pm$ SE). Each facet represents one of the four exposure doses where infection rates were appreciable as per Figure 2A. Doses D, E, F, G correspond to the four highest spore doses—10,000, 50,000, 250,000, and 1,250,000 spores, respectively.

the “sicker” sex thus depends not only on the species in question (McCurdy et al., 1998; Poulin, 1996; Schalk & Forbes, 1997; Sheridan et al., 2000; Zuk & McKean, 1996) but will be determined by the environmental context, and is likely highly variable even within species.

Rather than reinforce the notion of a static and generalizable “sicker” sex within species, our results instead highlight the remarkable plasticity of many metrics used to define the sicker sex. In this single host–pathogen system we saw every possible form of sex-specific plasticity that could arise (e.g., Figure 1). For some components of infection, such as death rate, there was no sex bias and each sex responded equally to thermal change (e.g., Figure 3B) leading to a general rise in death rates with warmer temperatures (see Figure 3A). For other components, plasticity was sex-specific (e.g., Figure 1B), with females far more sensitive to temperature changes than males. In the case of pathogen load, rising temperature drove ever-increasing differences between the sexes (Figure 4B), while the reverse pattern was observed for the reduction in lifespan (Figure 5B). Finally, for infection rates, we found evidence for divergent sex-specific plasticity (e.g., Figure 1C), as males had generally higher

infection rates at 15°C and females higher infection rates at 25°C (Figure 2A), although these patterns were conditional on initial pathogen dose (Figure 2). Sex biases in infection outcomes thus appear to be both entirely trait-dependent and highly modifiable by environmental change (see also Butterworth et al., 2024; Gipson et al., 2022). An absence of a sex bias under one environmental condition thus does not preclude the emergence of a male or female bias in another.

Integrating the different components of infection into a single measure of transmission, derived from formal epidemiological models (Aulsebrook et al., 2023; Hall & Mideo, 2018), made it evident that warmer temperatures favored increasingly female-biased pathogen transmission, driven by the greater sensitivity of females to warming (Figure 6). Only at the coldest temperature, and the lowest initial doses, did we observe a shift towards male-biased transmission (Figure 6). In terms of the spread of infectious disease, therefore, the brunt of rising temperatures could most heavily be felt though females in this system (see also Gipson et al., 2022 for a similar argument regarding resource fluctuations). Although neither males nor females are universally

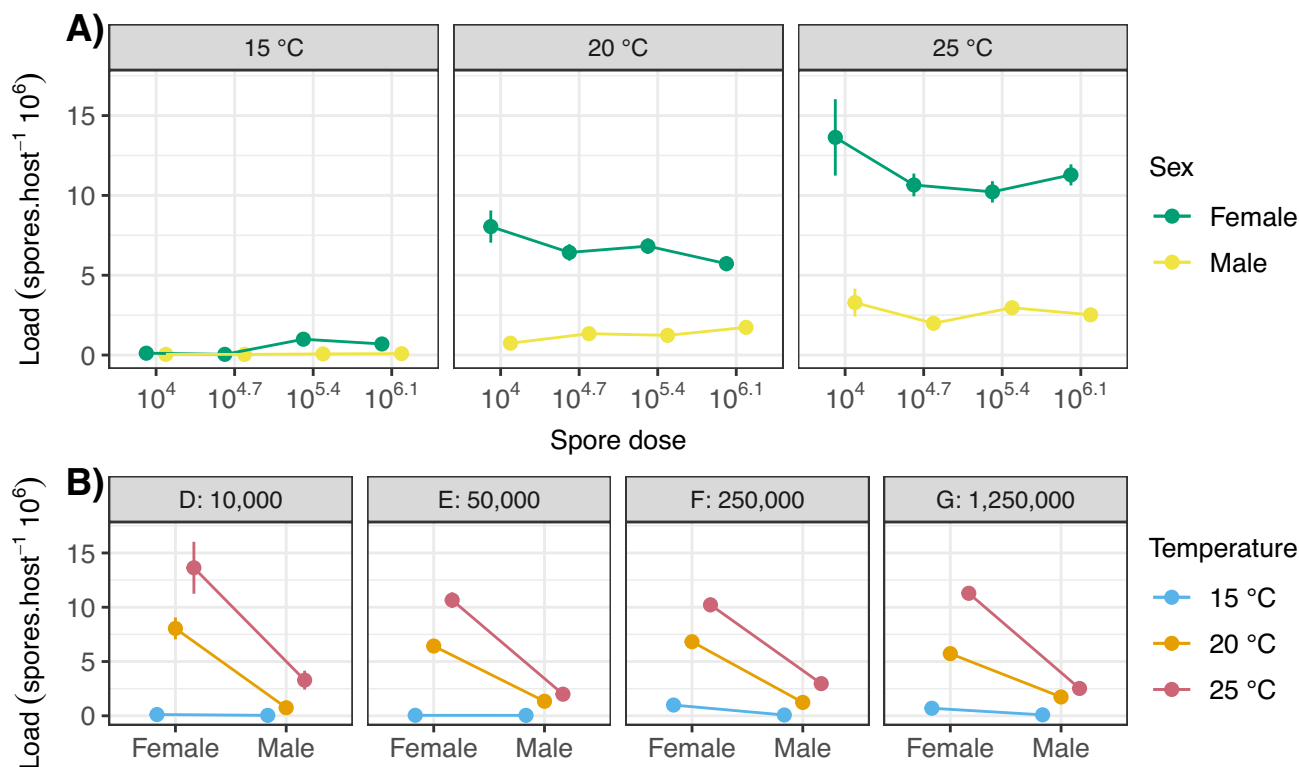


FIGURE 4 Thermal plasticity of the number of mature spores present within the host upon death as measured for *Daphnia magna* (genotype: BE-OHZ-M10) exposed to the pathogen *Pasteuria ramosa* (genotype: C1). Shown are (A) dose responses reflected by predicted treatment means ($\pm$ SE) where each facet represents one of three temperature treatments and (B) the same data instead presented as male–female reaction norms of the predicted treatment means ($\pm$ SE). Each facet represents one of the four exposure doses where infection rates were appreciable as per Figure 2A. Doses D, E, F, G correspond to the four highest spore doses—10,000, 50,000, 250,000, and 1,250,000 spores, respectively.

more responsive to warming on average (i.e., Hangartner et al., 2022; Pottier et al., 2021), for a subset of species, females have been shown to be the more thermally plastic sex for ecologically relevant functions such as thermal tolerances (e.g., for five of 11 *Drosophila* species, Mitchell & Hoffmann, 2010) and growth rates (Stillwell et al., 2010). Indeed, females suffering the greatest consequences of warming on traits underlying pathogen transmission may be a widespread trend in species with female-biased sexual size dimorphism. However, there are also many examples where males exhibit greater plasticity to temperature in their thermal tolerance (e.g., two of 11 *Drosophila* species, Mitchell & Hoffmann, 2010) or other fitness components (e.g., body size, Stillwell & Fox, 2007; locomotor activity, Brand et al., 2023). Thus, in other systems where males are the more thermally plastic sex, we may see a shift towards male-biased pathogen transmission as temperatures rise.

Why might female *D. magna* show greater thermal plasticity in infection outcomes? Thermal plasticity has been shown to scale with body size across species

(see Rohr et al., 2018), with a greater capacity for phenotypic plasticity occurring for the larger sex in several other invertebrate species with strong sexual dimorphism (Bonduriansky, 2007; Rohner et al., 2018; Stillwell et al., 2010; Teder & Tammaru, 2005). In *D. magna*, females are significantly larger than males (Duneau et al., 2012), suggesting a greater capacity for plasticity already (sensu Rohr et al., 2018), but they also display higher and more environmentally sensitive feeding rates (e.g., Gipson et al., 2022). Because feeding rate is linked directly to pathogen contact rate in this system (Hall et al., 2007) and warming directly increases feeding rates (Shocket et al., 2018; Shocket et al., 2019), it is likely that females intake disproportionately more resources and pathogens compared with males as temperatures rise. Thus, thermal sensitivity is higher for both female infection rates (likely due to greater increases in pathogen contact rates with warming for females, Hall et al., 2007; Shocket et al., 2019) and spore loads (likely due to greater increases in available energy for pathogens with warming in females; Nørgaard et al., 2021).

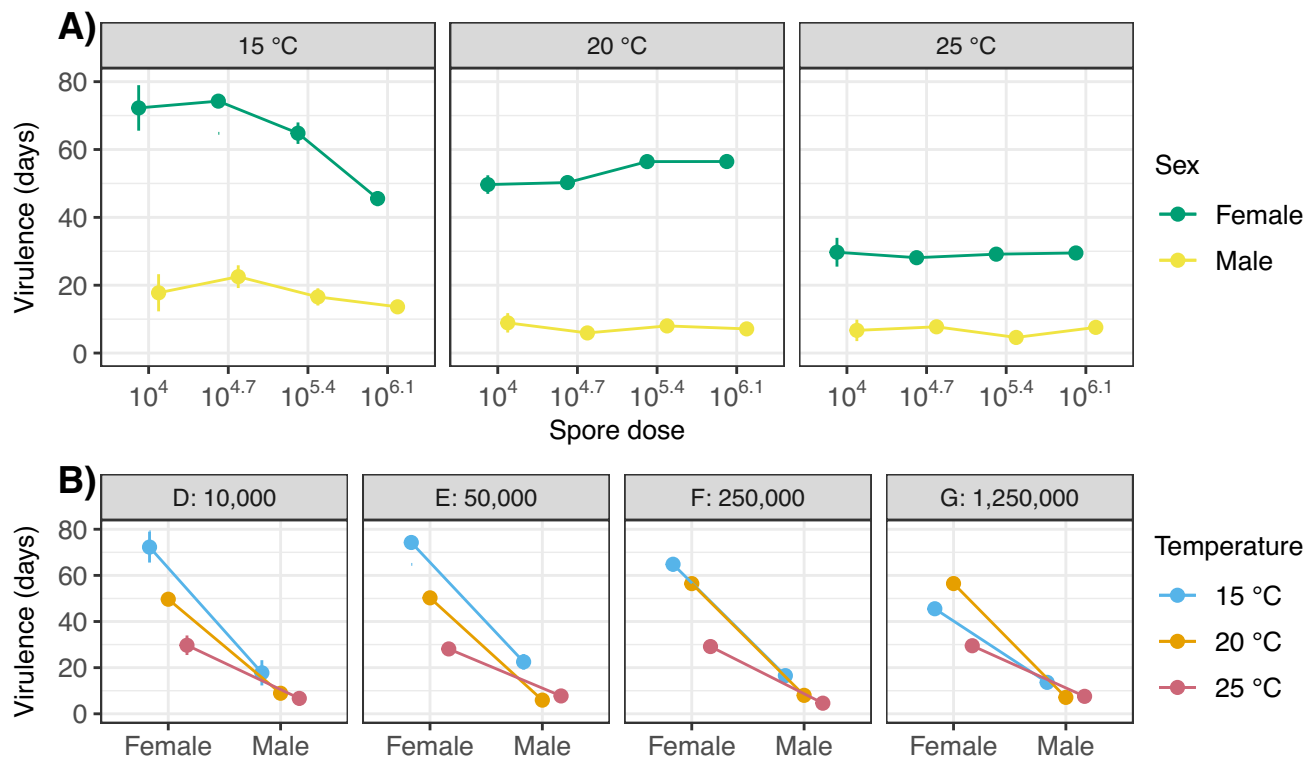


FIGURE 5 Thermal plasticity of virulence (reduction in lifespan relative to uninfected hosts) as measured for *Daphnia magna* (genotype: BE-OHZ-M10) exposed to the pathogen *Pasteuria ramosa* (genotype: C1). Shown are (A) dose responses reflected by predicted treatment means ($\pm$ SE) where each facet represents one of three temperature treatments, and (B) the same data instead presented as male–female reaction norms of the predicted treatment means ($\pm$ SE). Each facet represents one of the four exposure doses where infection rates were appreciable as per Figure 2A. Doses D, E, F, G correspond to the four highest spore doses—10,000, 50,000, 250,000, and 1,250,000 spores, respectively.

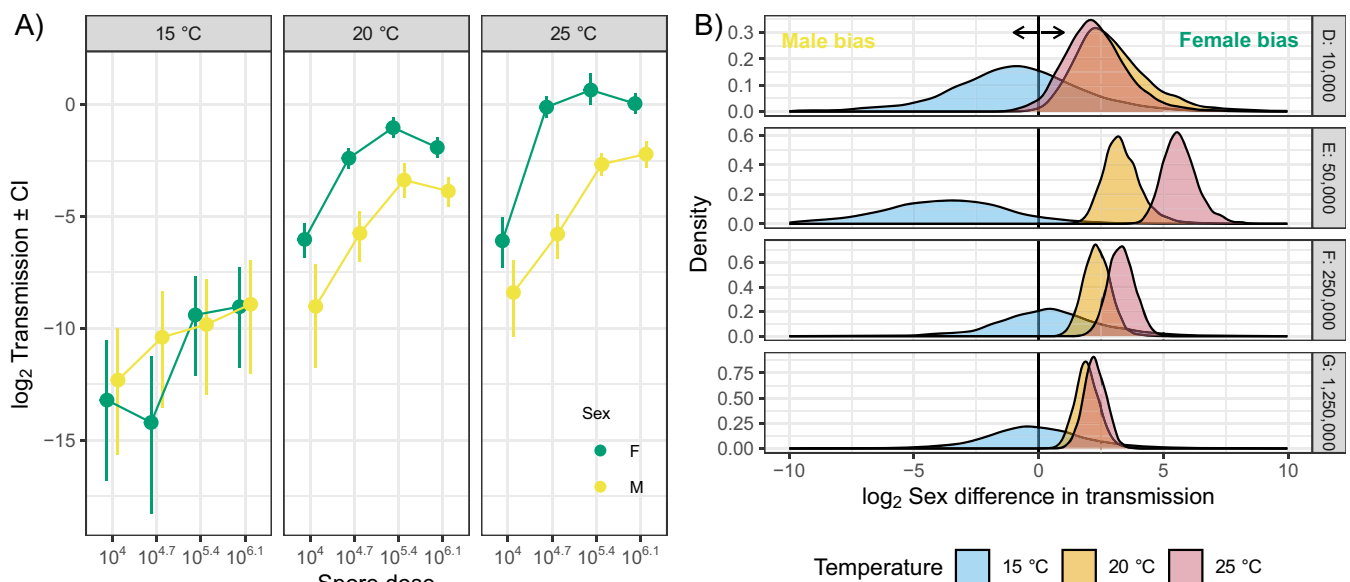


FIGURE 6 Thermal plasticity in transmission (calculated as the composite transmission rate from Hall & Mideo, 2018; Aulsebrook et al., 2023) as displayed on the $\log_2$ scale. Shown are (A) dose responses reflected by treatment means and 90% credible intervals for each of the four doses that had appreciable infection rates, where each facet represents one of three temperature treatments (B) the extent of sex difference in transmission calculated as female value minus the male value for each of the four doses that had appreciable infection rates (as per Figure 2A). Doses D, E, F, G correspond to the four highest spore doses—10,000, 50,000, 250,000, and 1,250,000 spores, respectively.

That the direction and magnitude of sex differences in pathogen transmission depend so strongly on the environmental context also has important consequences for the evolutionary trajectories of pathogens. When pathogens can encounter and infect multiple types of hosts (i.e., host heterogeneity), including males and females (Cousineau & Alizon, 2014; Úbeda & Jansen, 2016; see also Duneau & Ebert, 2012; Gipson & Hall, 2016), theory suggests pathogens will become strongly adapted to the host type which they most frequently encounter and through which they have the highest transmission (see Gandon, 2004; Osnas & Dobson, 2012). For example, when it is warm and transmission is maximized predominantly in females, such as observed here in *Daphnia*, females are predicted to have a greater impact on pathogen evolution, with any impact of host sex more evenly shared under cooler conditions (or even slightly male-biased). In *Daphnia*, at least, females are expected to favor more virulent pathogen genotypes (Gipson et al., 2019), which our results suggest would likely lead to a rise in virulence under warming. However, our conclusions are based on the combination of a single host and a single pathogen genotype and broader patterns will require testing across multiple genotypes and taxa. For other systems the evolution of overall virulence will depend on the magnitude of between-sex transmission and the relative timing of propagule production and mortality during infection (Cousineau & Alizon, 2014; Úbeda & Jansen, 2016). Importantly, temperature can also influence sex allocation and sex ratios, which can shift dramatically over seasons (Camp et al., 2019; Mrosovsky et al., 1984; Roy et al., 2003). Thus, warming can alter both the availability of males and females and the sex that drives pathogen adaptation, with significant implications for the evolution of pathogen virulence – an aspect often overlooked in the context of global change.

In conclusion, we have revealed that the identity of the “sicker” sex within a species is not fixed, but rather can be contingent upon the environment and the patterns of sex-specific plasticity that emerge at each stage of the infection process. As global temperatures continue to rise, our results show how sex-specific plasticity can dictate whether pathogen transmission rates skew towards males or females. However, whether these patterns hold across diverse taxa – from bees, to guppies, to humans—remains unknown. Nevertheless, recognizing the important role of sex-specific plasticity not only broadens the search for evolutionary generalizations of sex biases in infection outcomes (as per Folstad & Karter, 1992; Hamilton & Zuk, 1982; Rolff, 2002; Zuk, 1990; Zuk, 2009; Zuk & McKean, 1996) but also implies that similar sex-specific disease dynamics may

also extend to other environmental factors (i.e., resource limitation, Gipson et al., 2022; pollution, Aulsebrook et al., 2023, Polverino et al., 2023). Building this enhanced understanding of sex-specific plasticity and shifting sex biases into studies of pathogen transmission will be essential to improve our capacity to anticipate the trajectory of infectious diseases in a sexually dimorphic and changing world.

AUTHOR CONTRIBUTIONS

Nathan J. Butterworth conceived the idea, collected data, and performed statistical analyses. Jared Lush collected data. Kerri J. Moore collected data and maintained experimental animals. Matthew D. Hall conceived the idea and performed modeling work. Nathan J. Butterworth wrote the first draft of the manuscript and all authors contributed substantially to revisions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Butterworth et al., 2025) are available in Figshare at <https://doi.org/10.6084/m9.figshare.30311638.v1>.

ORCID

Nathan J. Butterworth  <https://orcid.org/0000-0002-5679-2700>

Jared Lush  <https://orcid.org/0009-0001-1289-5708>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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