Infection affects guppy reproductive fitness and offspring parasite resistance

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Abstract: Infection imposes energetic costs on hosts. Hosts typically respond by shifting resources, potentially affecting the quantity and quality of offspring they produce. As the sexes differ in their optimal reproductive strategies, infection of mothers versus fathers may affect offspring quantity and quality in different ways. Here, we test how experimental infection of guppies *Poecilia reticulata* with the ectoparasite *Gyrodactylus turnbulli* affects parental reproductive fitness and the parasite resistance of offspring. We compared breeding pairs in which one or neither parent had previously been infected. Parental infection did not affect the size, body condition, or number of offspring produced, but previously infected mothers had 35-39% longer latency to birth. However, offspring of infected mothers experienced 105 fewer worm days than those of infected fathers: fathers, but not mothers, that experienced heavy infections themselves produced offspring that also experienced heavy infections. The parent-offspring regression for infected fathers is therefore consistent with previous evidence that parasite resistance is heritable in this system, whereas that among mothers provides novel insight that mothers may engage in transgenerational immune priming. The sexes may thus strike a different balance between offspring quantity and quality when faced with infection, with potentially broad implications for disease and population dynamics.

Keywords: Trinidadian guppy, *Gyrodactylus*, life history, transgenerational immune priming, heritable parasite resistance, parent-offspring regression, sex-specific response to infection

Background: Infected parents may change the quantity and quality of the offspring they produce, both because of the energetic constraints [1] or pathology [2–4] associated with infection, and because the optimal investment in defence [5–9] and reproductive strategy may differ under the threat of infection [10,11]. Intuitively, parental infection may induce the production of fewer, lower quality offspring due to decreased parental resources [12,13]. However, infection may also induce the production of more offspring as infected parents 'terminally invest': in the face of probable death, hosts increase reproductive output at the expense of their own defence against the infection [14]. Infection may also induce the production of higher quality offspring, particularly if they are likely to be exposed to the same parasite, through parental investment in the immunological defences of their offspring, known as "transgenerational immune priming", or TGIP [15]. TGIP can last from shortly after birth, (in the case of e.g. maternal antibodies [16,17]) to subsequent generations (in the case of epigenetic changes [18]), depending on the host species and the disease to which they are responding [15]. TGIP is costly to both parents and offspring, however: manufacturing parentally donated factors such as proteins or antibodies requires resources and energy, and any form of priming may require offspring to mount an immune response, even in the absence of the disease [15].

We might expect mothers and fathers to take different strategies in adjusting their investment. In the absence of infection, males should optimally invest in offspring quantity, and females, quality [19,20]: they may also differ in how they shift their investment post-infection. Indeed, there is some preliminary evidence for sex-specific effects of infection on parental care. Female blue tits that are infected with avian malaria *Plasmodium spp.* provision their young significantly less

than uninfected conspecifics [21], indicating an infection-induced trade-off between parental investment and parental disease survival. However, male great tits infected with *Plasmodium spp.* provisioned their chicks significantly more than uninfected males, resulting in a positive relationship between disease prevalence in males and offspring provisioning [22].

As well as ultimate drivers, the sexes may differ in the proximate mechanisms through which they can affect offspring quantity or quality. For example, in many systems, mothers have more opportunity to affect the phenotypes of their offspring [23,24], and as a result offspring phenotypes are often strongly correlated with maternal phenotypes, for example in live-birthing fish [25] and humans [26]. This may hold true for TGIP: although the underlying mechanisms have not been fully elucidated, promising candidates include parentally donated proteins [27] that are likely too large to be transferred by sperm [23,28]. Indeed, no relationships among antibody titres were detected in vertebrates when fathers but not mothers were primed [29,30]. However, other mechanisms, such as epigenetic modifications [31] or nonnuclear effects [32], could be mediated by either parent. Indeed, a recent meta-analysis of TGIP in invertebrates found that the sex of the parent primed did not affect the strength of TGIP [33], indicating the prevalence of such mechanisms in this group. Such mechanisms likely function among vertebrates, too: among pipefish, both offspring and grand-offspring of primed parents show altered gene expression [34]. However, more broadly, work in vertebrates has historically been heavily biased towards investigating maternal effects [15,35]. Indeed, evidence for paternally-mediated TGIP in vertebrates is limited to sex-role reversed species [35], or those with some paternal care [36].

Here, we test how infection with *Gyrodactylus turnbulli* affects the reproductive fitness of male and female guppies, and the quality of the offspring produced. This highly tractable and

manipulable system has been used extensively [37–40], and has characteristics that make it particularly appropriate for understanding transgenerational effects. Exposure to predators has transgenerational effects mediated by both parents [41]. For infection, resistance to *G. turnbulli* is heritable in the Trinidadian guppy [42,43], enabling us to partially control for host genotype when investigating other potential sources of resistance. The parasite is associated with lower survival [44,45] and body condition [46] of wild guppies, indicating it depletes host resources. Further, the key conditions for TGIP are present in this system [15]: *G. turnbulli*-can be directly transmitted from mother to offspring during birth, so offspring of infected mothers are likely to be exposed to the same infection; and *G. turnbulli* primarily reproduce asexually [47], indicating infections in natural populations have some genotypic stability.

The biology of our system suggests that the sexes may differ in how infection with *G. turnbulli* affects parental investment. First, the parasite interacts with the host sexes differently: males are less likely to be infected in nature [48], and experience lower infection loads both in the wild [37,48] and during experimental infections [43] than females. Despite this, females may be more likely than males to survive their infections in nature [45,46,49], and appear better able to maintain the same behavioural phenotypes as uninfected fish [50,51]. Teleost fish also provide some of the most robust evidence for maternally [15] and paternally-derived (among sex-role reversed pipefish [34,35]) TGIP. Female guppies may be well-positioned to prime their offspring: they give birth to live offspring whose eggs are provisioned only at the time of fertilization, and female phenotype affects offspring phenotype [52]. However, in other fish, maternally-provisioned antibodies often disappear after offspring pass certain developmental stages such as absorbing the yolk sac [16,17] which, in livebearers such as the guppy, usually happens before they are born [53]. Male guppies may also be able to directly affect offspring phenotype through sperm quality: in an artificial insemination experiment, sperm from males fed a high quality diet was of a higher quality itself, and produced offspring that were heavier at birth [54].

We also know that infection with *G. turnbulli* affects DNA methylation in guppies [55], raising the possibility of epigenetically-mediated (and thus potentially bi-parental) TGIP. Such epigenetic changes can last generations after an initial immune challenge [18].

Based on parental investment theory, we predicted that males should prioritize offspring number, whereas females should prioritise offspring quality. We quantified offspring number as the total number of offspring a pair produced during our observation period, and latency to birth: together these predict how many offspring a female (and her mate in monogamous pairs, such as in our experiment) may produce in her lifetime, assuming all else is constant. We quantified offspring quality as how heavily infected they became during experimental infection.

Methods:

(a) Fish Origin and Maintenance

We used laboratory-bred descendants of guppies from the Caura River in Trinidad. Fish were maintained and bred in 20 L tanks of mixed sexes, under standard conditions, as follows. Tanks were on a recirculating system (Aquaneering) with approximately 20% daily water changes, a lighting schedule of 12L:12D, and an average temperature of 25±1°C. In these systems, wastewater from tanks passed through foam, sand, and ultraviolet filters before re-entering other tanks, preventing unintentional parasite transmission. Fish were fed flake and Artemia daily.

(b) Parasite origin and maintenance

Our parasite line was established by transferring one worm from a commercially obtained guppy to an uninfected host from a mixed laboratory-bred stock, a ‘culture fish’. This parasite line was maintained on groups of three to six culture fish per 1.8 L tank under standard conditions. Twice per week, all culture fish were anesthetized under anaesthetic (tricaine methanesulfonate

‘MS222’; 4g per L) and parasites were counted using a dissecting microscope. Uninfected fish were added to replace recovered or dead fish.

(c) Parental infection and breeding design

Parental fish were anaesthetized (using MS222 as above) and placed in close proximity to a dead, highly infected culture fish until at least two worms transmitted (mean±SEM=2.8±0.27), as observed under a dissecting microscope. Once infected, parental fish were isolated in 1.8 L tanks maintained under standard conditions for the duration of their infection. We counted worms every three days for 15 days to record individual infection trajectory over time. After 15 days, we treated fish with levamisole (0.002g per L) to clear infection. We recorded the length and weight of the parents before and after infection.

We set up breeding pairs of one male and one female using the recovered fish and parasite naïve fish in three different treatments: recovered mother, naïve father; recovered father, naïve mother; and both parents naïve. These breeding pairs were housed in 1.8 L tanks under standard conditions and checked daily for the presence of offspring. Each brood produced from a pair was moved to a separate 1.8 L tank.

(d) Offspring infection

Once they reached adulthood, offspring were separated into individual 1.8 L tanks. We used two batches of fish, one in August 2021 (with an average age at infection of approximately 4.4 months) and one in May 2022 (with an average age at infection of approximately 7.8 months). According to the needs of other concurrent experiments, some of these fish were infected manually, as described under parental infection, and others were infected via “co-housing”: they were housed in 1.8 L tanks with an infected conspecific and screened for infection every 24 hours. Once infected, these fish were isolated in a 1.8 L tank and treated the same as the

manually infected offspring and parents from that point forwards. As noted below, we included infection type as a fixed effect in the statistical models and found that it did not explain significant variation in any of our response variables. On all infected offspring, we counted the worms every three days for 12 days and then sacrificed the host.

(e) Data Analysis

We used R statistical software [56] to analyse the data, and provide the code and output in the supplement. We used the data from this experiment to address two main questions: 1) Does infection affect reproductive fitness? 2) Does parent infection affect offspring response to infection? We addressed each question using a) data from all pairs, whether or not one parent had been infected; and b) only data from pairs in which one parent had been infected. This enabled us to evaluate the extent to which parental exposure to infection affected reproductive fitness and offspring infection (a); and the extent to which the intensity of the parent's infection explained variation in these two outcomes (b). As our measure of the parent's infection intensity, and offspring response to infection, we used the area under the curve of infection load over time ('infection integral').

As our metrics of reproductive fitness, we used: the latency between the parent's infection and the birth of the offspring; the total number of offspring a pair produced during the observation period; and the body length and body condition of those offspring at adulthood. We used each of these four metrics as the response variable in separate linear (mixed) models (Gaussian error family, identity link function) in glmmTMB [56] using first the data from all pairs, and then data from only pairs in which one parent had been infected (i.e., a total of eight models). Each of these eight models included as fixed effects the parental infection treatment (mother, father, or neither parent infected), mother's body condition, mother's body length, and father's body condition. For response variables relevant to the level of the individual offspring (birth latency,

body length, body condition), the models included mother identity as a random factor as many pairs produced multiple offspring. Because male and female guppies typically differ in body condition metrics and length, we included sex of the offspring as a fixed effect in those models. For the total number of offspring models, we used only one observation per pair. The four models using only data from pairs in which one parent had been infected additionally included the (log transformed) infected parent's infection integral and the interaction with parental infection treatment as fixed effects.

We used two separate linear mixed models to test how a) parental exposure to infection, and b) the severity of that infection, affects offspring response to infection (Gaussian error family, identity link function in glmmTMB [56]). Both models included as fixed effects the sex, body condition, body length (residuals on sex), age at infection, the method of experimental infection (manual vs. cohoused), and the number of parasites establishing an infection on the offspring. Both models also included the parental infection treatment (mother, father, neither), and the interaction between parental infection treatment and offspring age at infection. As random effects, both models included the mother's identity to control for the non-independence of data from multiple offspring from the same pair. In addition, the model including parental infection severity included parental infection integral (log transformed), and its interaction with parental infection treatment. We checked the fit of all models using DHARMa [57], and the figures were plotted using visreg [58] and ggplot2 [59].

Heritability was estimated via the slope of the parent-offspring regression [60]. While the animal model of heritability can control for more variables, it would assume the first generation of fish to be completely unrelated to one another [61]. Our fish do not meet this assumption: they have been maintained in the lab since 2012 and are more likely to share an ancestor than their wild

counterparts (Kramp et al, in prep.).

Results:

Does infection affect reproductive fitness?

We found that previously infected and parasite naïve guppies produced the same number of offspring, and these did not differ in either size or body condition at adulthood (all P>0.2, see supplement). We did find that infection, at least of mothers, substantially increased the number of days between (sham) infection and birth. Mothers who had previously been infected took 34.7% and 38.7% longer to give birth (147.9 days) than pairs in which the father (96.6 days), or neither parent (89.9 days), had been infected, respectively (Fig. 1, Chisq = 14.42, df = 2, P=0.0007). We tested post-hoc whether this effect was due to infection negatively impacting maternal body condition and found no evidence to support this idea: maternal body condition was not affected by infection (P>0.4), whereas that of fathers was (Chisq= 10.59, df=2, P=0.005).

Similarly, we found that the intensity of parental infection did not significantly affect the number of offspring a pair produced, or the size or body condition of those offspring at adulthood (P= 0.06-0.97, see supplement). Some of these results were marginally non-significant: infected mothers produced fewer offspring (mean±SEM= 2.6±0.7) than infected fathers (mean±SEM= 5.1±1; Chisq = 2.99, df=1, P=0.084), and parents that experienced heavier infection loads produced offspring with higher body condition at adulthood (Chisq = 3.51, df=1, P=0.061). Again, we found that the number of days between (sham) infection and offspring birth was affected by parental infection treatment, and this effect depended on the intensity of that infection (Fig. 2; interaction between parental infection treatment and integral: Chisq = 5.13, df=1, P=0.024).

Does parent infection affect offspring response to infection?

We found that the effect of parental infection treatment on offspring response to infection depended on the age of the offspring at infection, and the parental infection treatment: among offspring with a previously infected father, younger offspring experienced lower infection intensities than older offspring (Fig. 3; interaction between parental infection treatment and offspring age at infection: Chisq = 9.13, df = 2, P = 0.010). We confirmed that the correlation in Fig. 3 is only significantly different from 0 for offspring of infected fathers using *post-hoc* tests (see supplement). We also found that the more parasites initially established on the fish, the higher their infection integral (Chisq = 9.62, df = 1, P = 0.002).

Among offspring with one infected parent, we found their infection integral was significantly predicted by whether the mother or the father had been infected, but this effect depended both on offspring age at infection, and the intensity of the parent's infection. As in the model including all offspring, younger offspring experienced lower infection intensities than older offspring, but only among those whose father had been infected (parental infection treatment interacts with offspring age at infection: Chisq = 3.85, df = 1, P = 0.0498). We also found a significant correlation between the father's infection integral and that of the offspring, but no significant correlation between mother and offspring infection integral (Fig 4.; interaction between parental infection integral and parental infection treatment: Chisq = 11.54, df = 1, P = 0.0007). From this parent-offspring regression, we estimate heritability to be 0.21.

Discussion:

We found that parental infection does affect the quantity and quality of offspring a pair produces, depending on which parent was infected. Pairs in which the father was infected showed no increase in birth latency, whereas those in which the female was infected took almost 40%

longer to produce offspring. Under natural conditions this latency would likely translate to infected females producing far fewer offspring over their lifetimes, even if this latency to birth is transient. Females appear to balance this reduction in offspring quantity with an increase in quality: offspring of fathers who experienced heavy infections also experienced heavy infections, whereas offspring of mothers showed no such pattern. On average, offspring of infected mothers experienced 105 fewer worm days than offspring of infected fathers. Paternal infection load therefore appeared to be heritable, meaning that heavily infected fathers quickly produced offspring that also became heavily infected. Heavily infected mothers, on the other hand, took longer to produce offspring that did not become heavily infected. This finding, similar to previous work in teleosts [36], suggests that maternally mediated TGIP may be occurring in the system. Overall, our results give a broad overview of how parasites may change reproductive investment strategies, and how this affects the next generation of hosts.

The observation that infected mothers took longer to give birth is contrary to other studies, including in humans, in which infection and disease cause premature birth [62]. There are several possible explanations for this result. First, previously infected mothers may have resisted breeding and simply not become pregnant for longer than uninfected conspecifics, as female guppies have been shown to be less receptive to mating when exposed to our parasite [63]. Second, previously infected mothers may mate at the same frequency but store sperm for longer than uninfected mothers. Female sperm storage creates more opportunities for competition between males' sperm and therefore could be an effective mechanism for ensuring the production of the healthiest offspring [64]. In times of physiological stress, such as infection, this may be a beneficial strategy to improve offspring fitness when exposed to the same stressor. Third, infected mothers may be more likely to cannibalize their offspring, a behaviour that is common in healthy guppies but may be exacerbated as a result of the energetic costs of

infection [65]. However, infection did not affect maternal body condition, which would be a clear indication of significant energetic costs and thus more reason to cannibalize young.

In contrast, previously infected fathers produced broods at the same rate as uninfected pairs and produced more offspring than previously infected mothers (mean±SEM= 5.1±1 vs. 2.6±0.7, though this result was marginally non-significant), though these offspring appear to be less parasite resistant than those of previously infected mothers. Our data therefore indicate that in contrast to females, males pay no fecundity cost of infection with these parasites, at least when paired with a female with no other option (if females were given a choice, previously infected males may not have fathered any children due to pre- and post-copulatory sexual selection). That males therefore appear to produce more offspring despite apparently experiencing more severe costs of infection than females [45,46,48] suggests that males may terminally invest in maintaining reproductive output at the expense of maintaining other physiological systems [14]. This terminal investment appears to have been in offspring quantity rather than quality, as offspring of infected fathers experienced more worm days than offspring of infected mothers, and our data suggest that mothers, but not fathers, primed their offspring to resist *G. turnbulli* infection.

While they broadly support classic parental investment theory, there is some circularity in our results that raises some non-mutually exclusive explanations. We found that: 1) among infected fathers, those with heavier infections produced offspring quicker (Fig. 2), so these would be among the oldest to have been infected according to our experimental design; 2) we found that older offspring of infected fathers tended to get heavier infections (Fig. 3); and 3) we found a significant positive correlation between father infection integral and offspring infection integral, but no such correlation among mother-offspring infection integral (Fig. 4). Our results may therefore be driven by older fish overall becoming more heavily infected. This seems unlikely,

given that age only predicts infection intensity among those with a previously infected father. Alternatively, fathers who become heavily infected may mate more quickly than those with lighter infections (Fig. 2) and thus their broods are produced earlier; perhaps either the lower quality of their sperm [54] or reduced investment in their broods by the female [66,67] result in their offspring's weak response to infection. Finally, fathers who become heavily infected may generally have a faster pace of life, investing heavily in reproduction and little else, and their offspring inherit this phenotype: the positive parent-offspring regression we observe is consistent with previous findings that resistance to *G. turnbulli* is heritable in guppies [42,43,68].

Despite the evidence for the heritability of resistance to this parasite, we did not observe a significant mother-offspring regression (Fig. 4), indicating that TGIP may be occurring from mothers to offspring: offspring of mothers that experienced severe infections had light infections comparable to those of offspring of mothers who experienced less severe infections. Thus, any parasite load may be sufficient to induce TGIP to the same protective level, or perhaps TGIP is compensatory to load and thus allows all offspring of infected mothers to benefit from a similar level of resistance. This priming appears to last indefinitely, as age did not impact the mother-offspring regression in infection severity, which suggests the underlying mechanism is likely not maternally donated proteins: maternal antibodies, mRNAs, and proteins are typically absent from guppy offspring within a few days post-birth [16,17,53]. As it did not appear to wane, the maternally mediated TGIP we observed may have an epigenetic basis. Indeed, epigenetic changes in response to infection have been observed in female guppies [69] and these changes may be conserved from generation to generation [70].

Our results indicate yet another factor in the complex interactions between disease dynamics and host and parasite evolution in natural communities. In our system, populations differ in their prevalence of *Gyrodactylus* spp. and the mean intensity of infection, and these are likely not

stable through time [44]. In populations and at times when prevalence is high, our results indicate that more mothers may prime their offspring, who would develop overall lower infection loads, potentially reducing transmission [71], and thus affecting population-level prevalence and mean intensity. If female TGIP has a measurable impact on disease dynamics in nature, it may therefore slow the evolution of host resistance by reducing the strength of parasite-mediated selection. However, host defences do appear to evolve rapidly in response to these parasites [68], so TGIP may not be important. Potentially, those that develop the heaviest infections comparable to those we observed here do not successfully breed. Indeed, observed natural infection loads tend to be low [48], and patterns of aggregation strongly suggest that heavily infected hosts are missing from our surveys, presumed dead [49]. The long latency to breed among previously infected females, i.e., the ones who produce the primed offspring, would exacerbate this problem. Similarly, the impact of TGIP may be overwhelmed by the fact that males are able to sire offspring (that inherit their parasite resistance) even posthumously [72], and engage in coercive mating so will likely mate [73], even though females discriminate against males who are infected [74], or those who may lack resistance [75]. Our results therefore demonstrate that the sexes pay different costs of infection with this parasite, with potentially important implications for the evolution of host defences.

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Literature cited:

1. Tizard I. 2008 Sickness behavior, its mechanisms and significance. *Anim. Health Res. Rev.* **9**, 87–99.
2. Khan I, Agashe D, Rolff J. 2017 Early-life inflammation, immune response and ageing. *Proc. Biol. Sci.* **284**. (doi:10.1098/rspb.2017.0125)
3. Armour EM, Bruner TL, Hines JK, Butler MW. 2020 Low-dose immune challenges result in detectable levels of oxidative damage. *J. Exp. Biol.* **223**. (doi:10.1242/jeb.220095)
4. Billi AC, Kahlenberg JM, Gudjonsson JE. 2019 Sex bias in autoimmunity. *Curr. Opin. Rheumatol.* **31**, 53–61.
5. Boots M, Bowers RG. 2004 The evolution of resistance through costly acquired immunity. *Proc. Biol. Sci.* **271**, 715–723.
6. Viney ME, Riley EM, Buchanan KL. 2005 Optimal immune responses: immunocompetence revisited. *Trends Ecol. Evol.* **20**, 665–669.
7. Tschirren B, Richner H. 2006 Parasites shape the optimal investment in immunity. *Proc. Biol. Sci.* **273**, 1773–1777.
8. Houston AI, McNamara JM, Barta Z, Klasing KC. 2007 The effect of energy reserves and food availability on optimal immune defence. *Proc. Biol. Sci.* **274**, 2835–2842.

9. Graham AL. 2013 Optimal immunity meets natural variation: the evolutionary biology of host defence. *Parasite Immunol.* **35**, 315–317.
10. Sheldon BC, Verhulst S. 1996 Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* **11**, 317–321.
11. Schmid-Hempel P. 2003 Variation in immune defence as a question of evolutionary ecology. *Proc. Biol. Sci.* **270**, 357–366.
12. Vantaux A, Dabiré KR, Cohuet A, Lefèvre T. 2014 A heavy legacy: offspring of malaria-infected mosquitoes show reduced disease resistance. *Malar. J.* **13**, 442.
13. Hagmayer A, Furness AI, Pollux BJA. 2020 Parasite infestation influences life history but not boldness behavior in placental live-bearing fish. *Oecologia* **194**, 635–648.
14. Duffield KR, Bowers EK, Sakaluk SK, Sadd BM. 2017 A dynmaic threshold model for terminal investment. *Behav. Ecol. Sociobiol.* **71**, 185.
15. Roth O, Beemelmanns A, Barribeau SM, Sadd BM. 2018 Recent advances in vertebrate and invertebrate transgenerational immunity in the light of ecology and evolution. *Heredity* **121**, 225–238.
16. Brown LL, Evelyn TPT, Iwama GK. 1997 Specific protective activity demonstrated in eggs of broodstock salmon injected with rabbit antibodies raised against a fish pathogen. *Dis. Aquat. Organ.* **31**, 95–101.
17. Breuil G, Vassiloglou B, Pepin JF, Romestand B. 1997 Ontogeny of IgM-bearing cells and changes in the immunoglobulin M-like protein level (IgM) during larval stages in sea bass (Dicentrarchus labrax). *Fish Shellfish Immunol.* **7**, 29–43.
18. Beemelmanns A. 2019 *Biparental Immune Priming in the Broad-nosed Pipefish (Syngnathus Typhle)*. Universitätsbibliothek Kiel.
19. Rolff J. 2002 Bateman's principle and immunity. *Proc. Biol. Sci.* **269**, 867–872.
20. Stoehr AM, Kokko H. 2006 Sexual dimorphism in immunocompetence: what does life-history theory predict? *Behav. Ecol.* **17**, 751–756.

21. Knowles SCL, Palinauskas V, Sheldon BC. 2010 Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *J. Evol. Biol.* **23**, 557–569.
22. Richner H, Christe P, Oppliger A. 1995 Paternal investment affects prevalence of malaria. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 1192–1194.
23. Wassarman PM, Jovine L, Litscher ES. 2001 A profile of fertilization in mammals. *Nat. Cell Biol.* **3**, E59-64.
24. Wolf JB, Wade MJ. 2009 What are maternal effects (and what are they not)? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **364**, 1107–1115.
25. Hagmayer A, Furness AI, Reznick DN, Pollux BJA. 2018 Maternal size and body condition predict the amount of post-fertilization maternal provisioning in matrotrophic fish. *Ecol. Evol.* **8**, 12386–12396.
26. Zhang C *et al.* 2018 Maternal obesity and longitudinal ultrasound measures of fetal growth: Findings from the NICHD Fetal Growth Studies-Singletons. *JAMA Pediatr.* **172**, 24.
27. Adrian-Kalchhauser I, Sultan SE, Shama LNS, Spence-Jones H, Tiso S, Valsecchi CIK, Weissing FJ. 2020 Understanding'non-genetic'inheritance: insights from molecular-evolutionary crosstalk. *Trends Ecol. Evol.* **35**, 1078–1089.
28. Arnqvist G, Rowe L. 2005 *Sexual conflict*. Princeton, NJ: Princeton University Press.
29. Gasparini J, McCoy KD, Tveraa T, Boulinier T. 2002 Related concentrations of specific immunoglobulins against the Lyme disease agent *Borrelia burgdorferi sensu lato* in eggs, young and adults of the kittiwake (*Rissa tridactyla*). *Ecol. Lett.* **5**, 519–524.
30. Reid JM, Arcese P, Keller LF, Hasselquist D. 2006 Long-term maternal effect on offspring immune response in song sparrows Melospiza melodia. *Biol. Lett.* **2**, 573–576.
31. Vilcinskas A. 2021 Mechanisms of transgenerational immune priming in insects. *Dev. Comp. Immunol.* **124**, 104205.

32. Simms EL, Triplett JK. 1996 PATERNAL EFFECTS IN INHERITANCE OF A PATHOGEN RESISTANCE TRAIT IN IPOMOEA PURPUREA. *Evolution* **50**, 2178–2186.
33. Rutkowski N-AJ, McNamara KB, Jones TM, Foo YZ. 2023 Trans-generational immune priming is not mediated by the sex of the parent primed: a meta-analysis of invertebrate data. *Biol. Rev. Camb. Philos. Soc.* **98**, 1100–1117.
34. Beemelmanns A, Roth O. 2017 Grandparental immune priming in the pipefish Syngnathus typhle. *BMC Evol. Biol.* **17**, 44.
35. Roth O, Klein V, Beemelmanns A, Scharsack JP, Reusch TBH. 2012 Male pregnancy and biparental immune priming. *Am. Nat.* **180**, 802–814.
36. Pizzolon M, Giacomello E, Marri L, Marchini D, Pascoli F, Mazzoldi C, Rasotto MB. 2010 When fathers make the difference: efficacy of male sexually selected antimicrobial glands in enhancing fish hatching success. *Funct. Ecol.* **24**, 141–148.
37. Walsman JC *et al.* 2022 Shoaling guppies evade predation but have deadlier parasites. *Nat Ecol Evol* **6**, 945–954.
38. Stephenson JF, Perkins SE, Cable J. 2018 Transmission risk predicts avoidance of infected conspecifics in Trinidadian guppies. *J. Anim. Ecol.* **87**, 1525–1533.
39. Madhavi R, Anderson RM. 1985 Variability in the susceptibility of the fish host, Poecilia reticulata, to infection with Gyrodactylus bullatarudis (Monogenea). *Parasitology* **91**, 531–544.
40. Harris PD, Lyles AM. 1992 Infections of Gyrodactylus bullatarudis and Gyrodactylus turnbulli on guppies (Poecilia reticulata) in Trinidad. *J. Parasitol.* **78**, 912–914.
41. Stein LR, Hoke K. 2022 Parental and individual experience with predation risk interact in shaping phenotypes in a sex-specific manner. *Anim. Behav.* **191**, 75–89.
42. Madhavi R, Anderson RM. 1985 Variability in the susceptibility of the fish host, *Poecilia reticulata*, to infection with *Gyrodactylus bullatarudis* (Monogenea). *Parasitology* **91**, 531–544.

43. Dargent F, Rolshausen G, Hendry AP, Scott ME, Fussmann GF. 2016 Parting ways: parasite release in nature leads to sex-specific evolution of defence. *J. Evol. Biol.* **29**, 23–34.
44. Stephenson JF, Kinsella C, Cable J. 2016 A further cost for the sicker sex? Evidence for male-biased parasite-induced vulnerability to predation. *Ecology*
45. van Oosterhout C, Mohammed RS, Hansen H, Archard GA, McMullan M, Weese DJ, Cable J. 2007 Selection by parasites in spate conditions in wild Trinidadian guppies (*Poecilia reticulata*). *Int. J. Parasitol.* **37**, 805–812.
46. Stephenson JF, van Oosterhout C, Cable J. 2015 Pace of life, predators and parasites: predator-induced life-history evolution in Trinidadian guppies predicts decrease in parasite tolerance. *Biol. Lett.* **11**. (doi:10.1098/rsbl.2015.0806)
47. Schelkle B, Faria PJ, Johnson MB, van Oosterhout C, Cable J. 2012 Mixed Infections and Hybridisation in Monogenean Parasites. *PLoS One* **7**, e39506.
48. Stephenson JF, Van Oosterhout C, Mohammed RS. 2015 Parasites of Trinidadian guppies: evidence for sex-and age-specific trait-mediated indirect effects of predators. *Ecology*
49. Clark DR, Young KA, J. K, Moore PJ, Evans A, Stephenson JF. 2023 Aggregation of symbionts on hosts depends on interaction type and host traits. *Ecography*
50. Jog MG, Sackett ME, Kisty SD, Hansen JA, Stephenson JF. 2022 The behaviour of infected guppies depends on social context, parasite tolerance and host sex. *Anim. Behav.* **187**, 97–104.
51. Stephenson JF. 2019 Parasite-induced plasticity in host social behaviour depends on sex and susceptibility. *Biol. Lett.* **15**, 20190557.
52. Shikano T, Taniguchi N. 2005 Relationships between brood size and offspring body size in an ovoviviparous fish: maternal effects and genetic trade-off. *J. Exp. Zool. A Comp. Exp. Biol.* **303**, 635–642.

53. Norazmi-Lokman NH, Purser GJ, Patil JG. 2016 Gravid Spot Predicts Developmental Progress and Reproductive Output in a Livebearing Fish, Gambusia holbrooki. *PLoS One* **11**, e0147711.
54. Evans JP, Lymbery RA, Wiid KS, Rahman MM, Gasparini C. 2017 Sperm as moderators of environmentally induced paternal effects in a livebearing fish. *Biol. Lett.* **13**. (doi:10.1098/rsbl.2017.0087)
55. Hu J, Pérez-Jvostov F, Blondel L, Barrett RDH. 2018 Genome-wide DNA methylation signatures of infection status in Trinidadian guppies (Poecilia reticulata). *Mol. Ecol.* **27**, 3087–3102.
56. Foundation for Statistical Computing RR. In press. R: A language and environment for statistical computing. *RA Lang Environ Stat Comput*
57. Hartig F. In press. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. *R package version 0.3*
58. Breheny P, Burchett W. 2017 Visualization of Regression Models Using visreg. *R J.* **9**, 56.
59. Ginestet C. 2011 ggplot2: Elegant Graphics for Data Analysis. *J. R. Stat. Soc. Ser. A Stat. Soc.* **174**, 245–246.
60. Falconer DS, Mckay TFC. In press. Quantitative Genetics (Vol. 4). *Essex, England: Longman Group Ltd*
61. Wilson AJ, Réale D, Clements MN, Morrissey MM, Postma E, Walling CA, Kruuk LEB, Nussey DH. 2010 An ecologist's guide to the animal model. *J. Anim. Ecol.* **79**, 13–26.
62. Romero R, Espinoza J, Gonçalves LF, Kusanovic JP, Friel L, Hassan S. 2007 The role of inflammation and infection in preterm birth. *Semin. Reprod. Med.* **25**, 21–39.
63. López S. 1999 Parasitized female guppies do not prefer showy males. *Anim. Behav.* **57**, 1129–1134.

64. Cardozo G, Devigili A, Antonelli P, Pilastro A. 2020 Female sperm storage mediates post-copulatory costs and benefits of ejaculate anticipatory plasticity in the guppy. *J. Evol. Biol.* **33**, 1294–1305.
65. Bose APH. 2022 Parent-offspring cannibalism throughout the animal kingdom: a review of adaptive hypotheses. *Biol. Rev. Camb. Philos. Soc.* **97**, 1868–1885.
66. Cunningham EJ, Russell AF. 2000 Egg investment is influenced by male attractiveness in the mallard. *Nature* **404**, 74–77.
67. Reynolds JD, Gross MR. 1992 Female mate preference enhances offspring growth and reproduction in a fish,Poecilia reticulata. *Proc. Biol. Sci.* **250**, 57–62.
68. Phillips KP, Cable J, Mohammed RS, Herdegen-Radwan M, Raubic J, Przesmycka KJ, van Oosterhout C, Radwan J. 2018 Immunogenetic novelty confers a selective advantage in host-pathogen coevolution. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 1552–1557.
69. Hu J, Pérez-Jvostov F, Blondel L, Barrett RDH. 2018 Genome-wide DNA methylation signatures of infection status in Trinidadian guppies (Poecilia reticulata). *Mol. Ecol.* **27**, 3087–3102.
70. Heard E, Martienssen RA. 2014 Transgenerational epigenetic inheritance: myths and mechanisms. *Cell* **157**, 95–109.
71. Stephenson JF, Young KA, Fox J, Jokela J, Cable J, Perkins SE. 2017 Host heterogeneity affects both parasite transmission to and fitness on subsequent hosts. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **372**. (doi:10.1098/rstb.2016.0093)
72. Lopez-Sepulcre A, Gordon SP, Paterson IG, Bentzen P, Reznick DN. 2013 Beyond lifetime reproductive success: the posthumous reproductive dynamics of male Trinidadian guppies. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **280**, 20131116.
73. Magurran AE. 2005 *Evolutionary ecology: the Trinidadian guppy*. New York: Oxford Univeristy Press.

74. Kennedy CEJ, Endler JA, Poynton SL, McMinn H. 1987 Parasite load predicts mate choice in guppies. *Behav. Ecol. Sociobiol.* **21**, 291–295.

75. Stephenson JF, Stevens M, Troscianko J, Jokela J. 2020 The size, symmetry, and color saturation of a male guppy's ornaments forecast his resistance to parasites. *Am. Nat.* **196**, 597–608.

Figure legends:

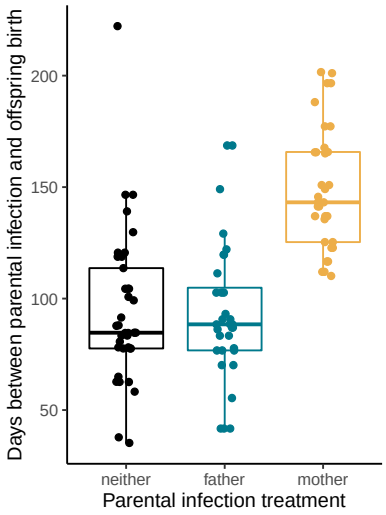


Fig. 1: Pairs in which the mother had been infected had longer latency to offspring birth than pairs in which the father, or neither parent had been infected. Points represent the partial residuals from the model described in the main text. Bold bars give the median, boxes the interquartile range, and whiskers the data falling within 1.5 x the median value.

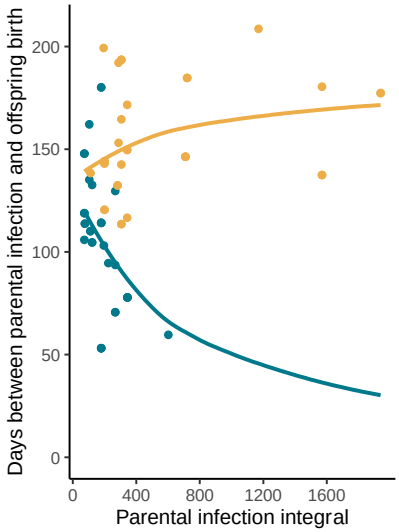


Fig. 2: Pairs in which fathers (darker blue) had more intense infections produced broods quicker, whereas the intensity of the mother's (lighter yellow) infection did not affect birth latency. Points represent the partial residuals from the model described in the main text,

lines give the model fits, and shading the 95% confidence intervals. We quantified infection intensity using ‘infection integral’, or the area under the curve of infection load over time.

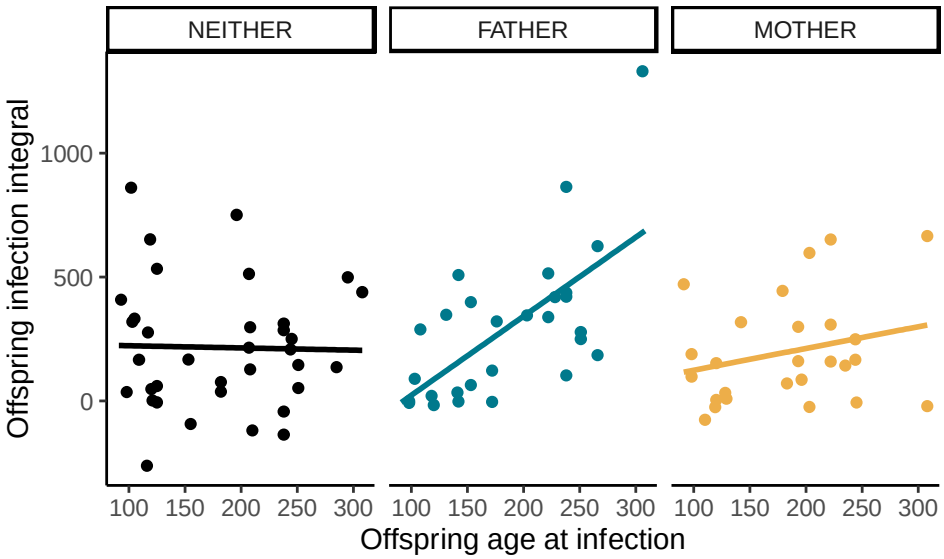


Fig. 3: Among offspring with an infected father, older fish tended to experience more intense infections, but no such relationship exists among offspring of infected mothers, or those of two parasite-naïve parents. Points represent the partial residuals from the model described in the main text, lines give the model fits, and shading the 95% confidence intervals. We quantified infection intensity using ‘infection integral’, or the area under the curve of infection load over time.

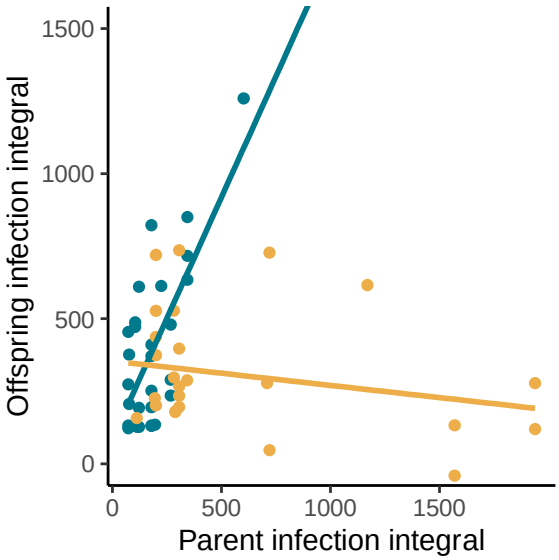
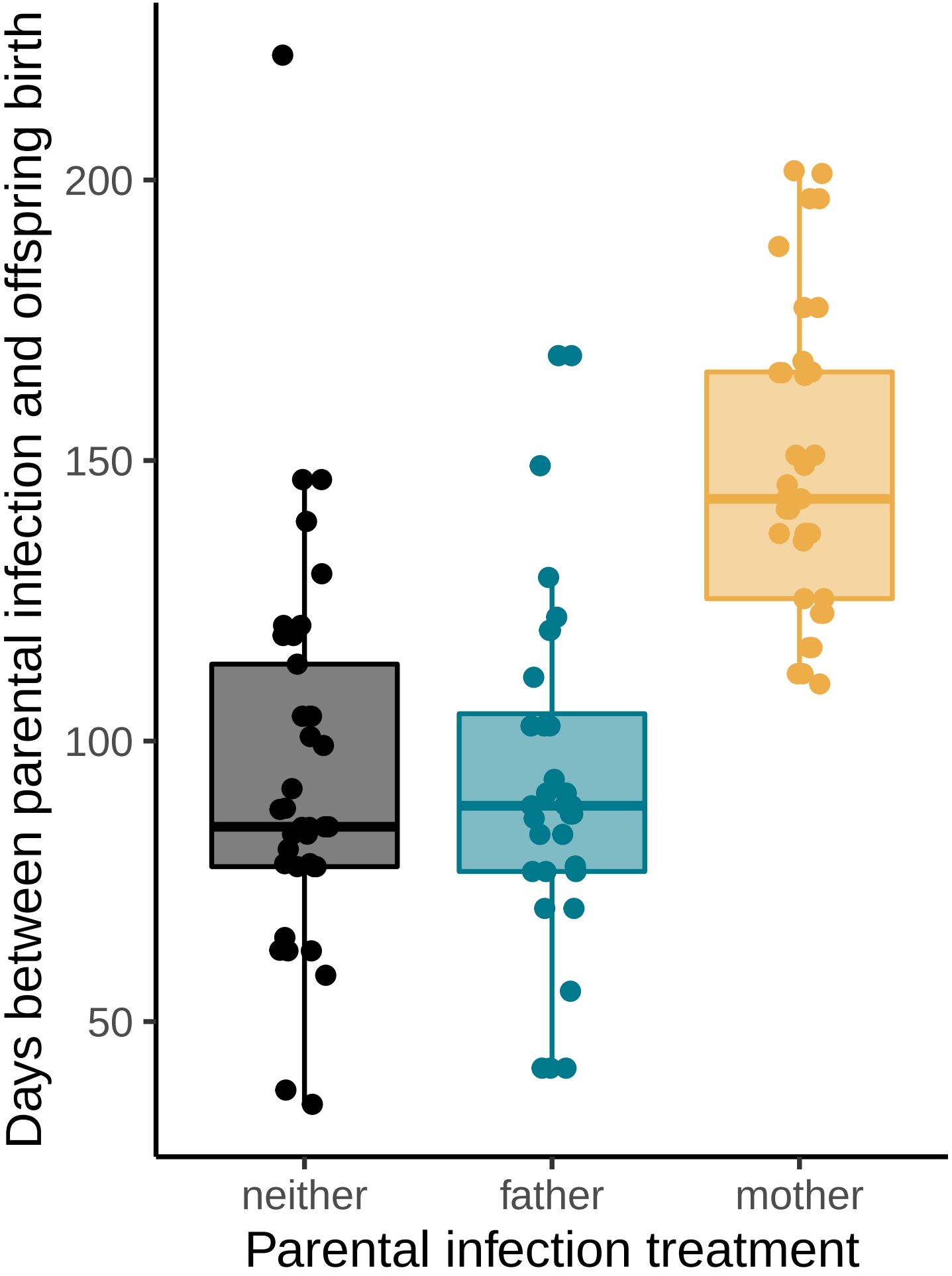
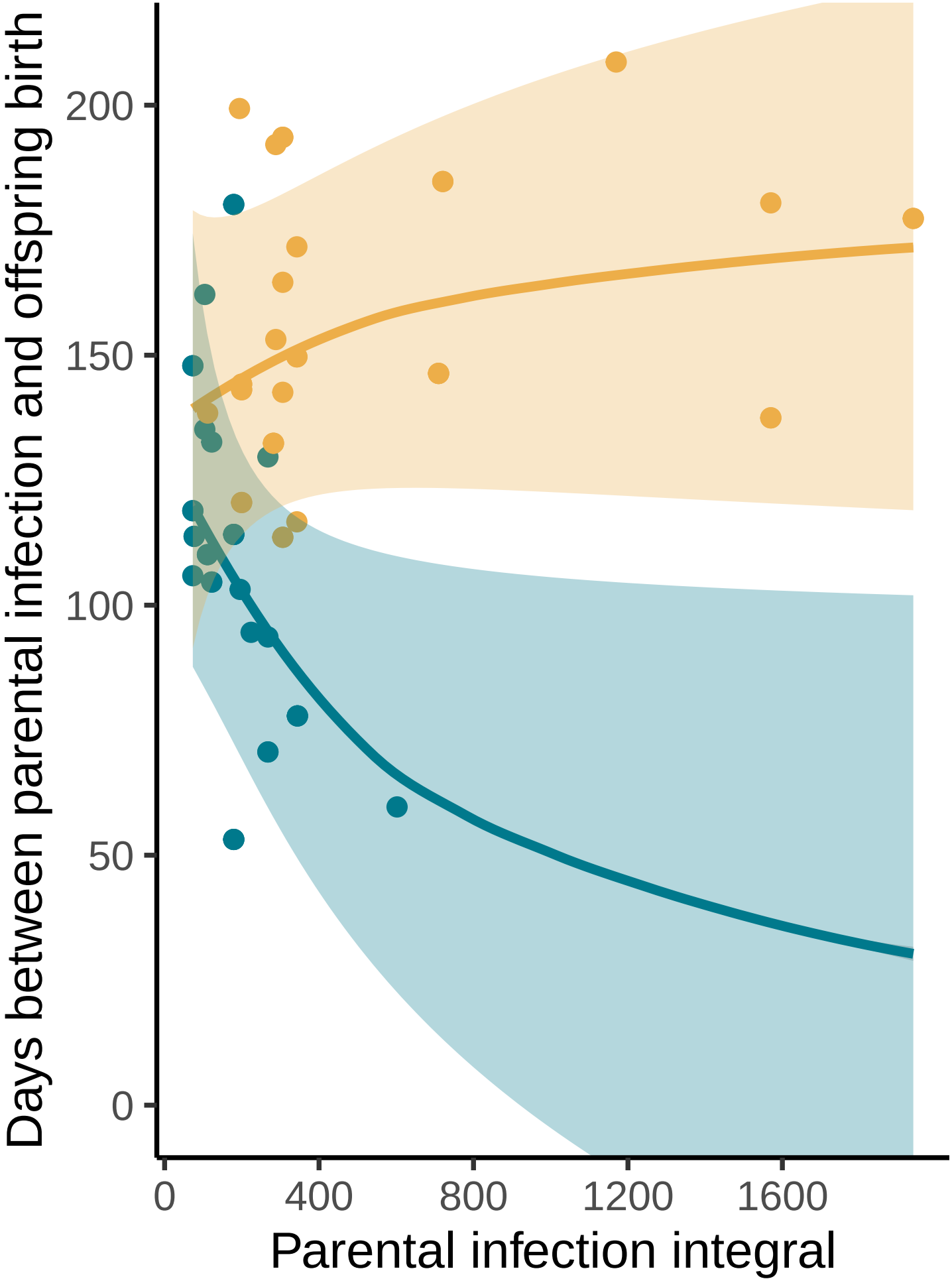
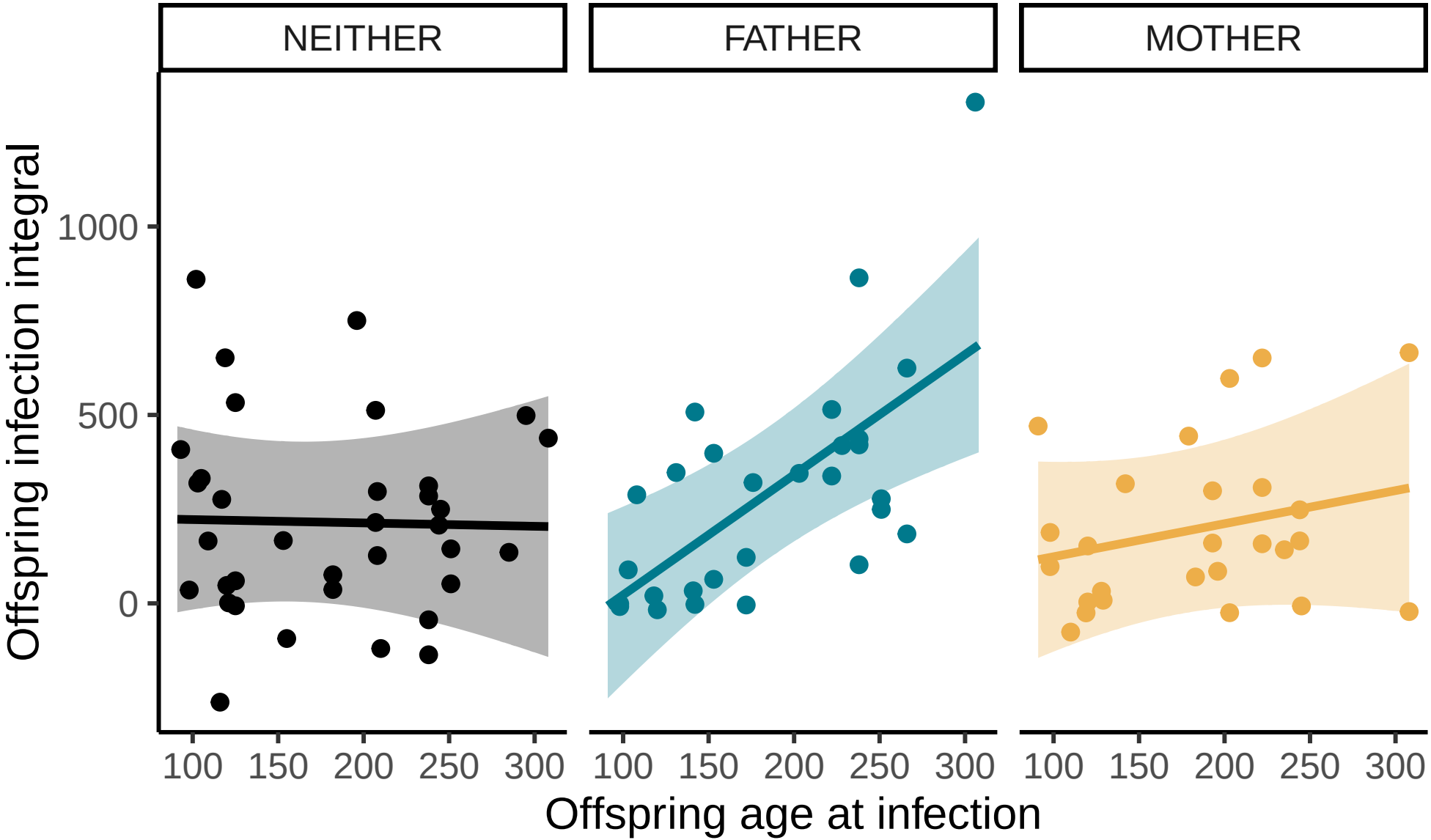
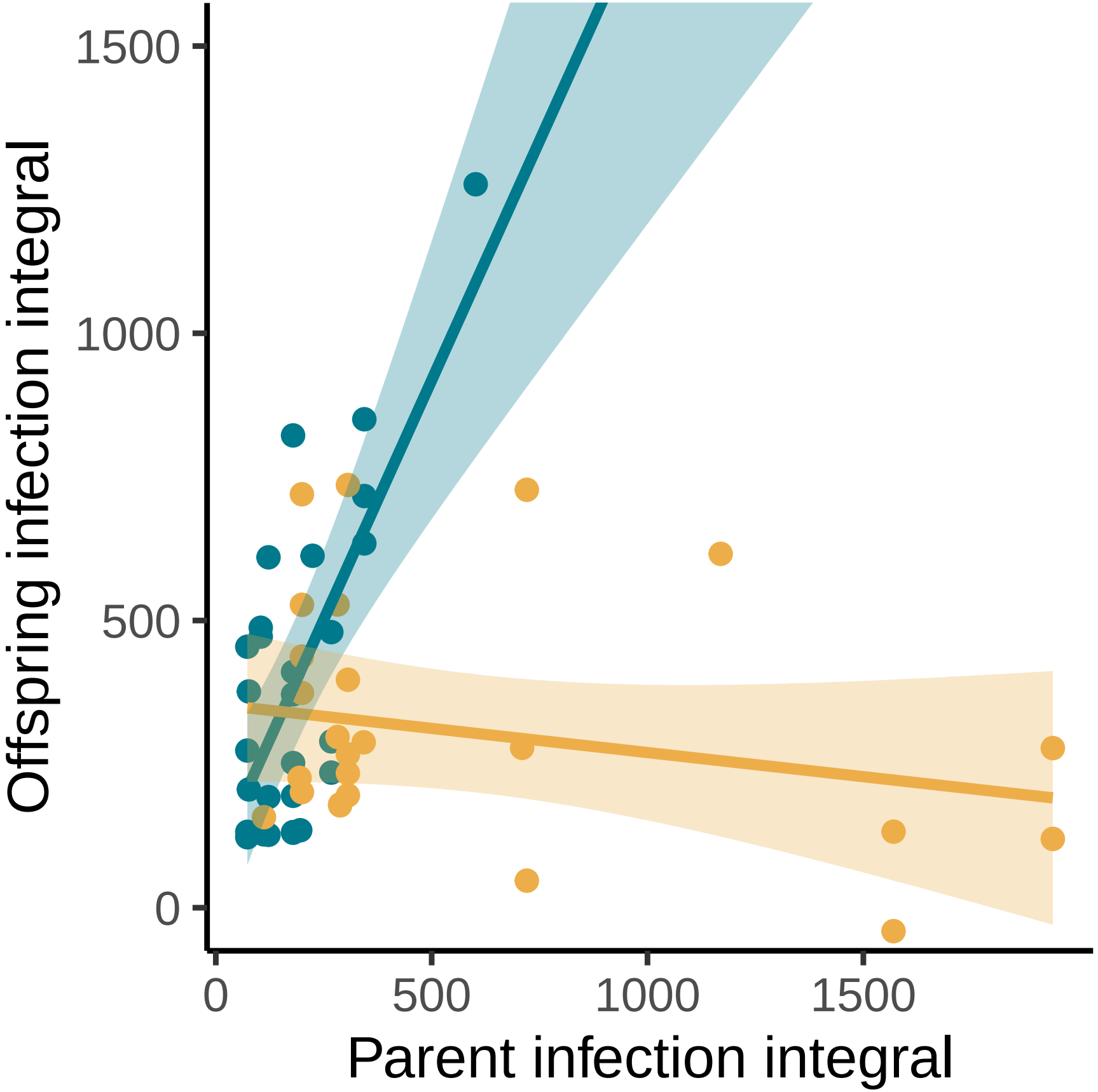


Fig. 4: A father's infection intensity significantly predicts the infection intensity that his offspring experience, but no such relationship exists between the infection intensity experienced by a mother and that of her offspring. Points represent the partial residuals from the model described in the main text, lines give the model fits, and shading the 95% confidence intervals. We quantified infection intensity using 'infection integral', or the area under the curve of infection load over time.


Days between parental infection and offspring birth
200
150
100
50
neither
father
mother
Parental infection treatment


Days between parental infection and offspring birth
200
150
100
50
0
0
400
800
1200
1600
Parental infection integral


NEITHER
FATHER
MOTHER
Offspring infection integral
1000
500
0
100
150
200
250
300
Offspring age at infection


Offspring infection integral
1500
1000
500
0
0
500
1000
1500
Parent infection integral