Infection route alters the effect of host diet quality on host-parasite interactions

Charlotte Narr[1], Scott Binger[2], and Edward Hall[3]

[1]Southern Illinois University Carbondale College of Science
[2]Southern Illinois University Carbondale
[3]Colorado State University System

July 16, 2024

Abstract

Linking characteristics of parasites to host diet-induced shifts in host-parasite interactions is a critical step in predicting the abundance of specific parasites within an ecosystem. A parasite's infection route reflects a suite of parasite characteristics that could mediate the effects of host diet quality on host-parasite interactions, but the effect of infection route on the response of host-parasite interactions to host diet quality has not been quantified. We quantified these effects for the first time by experimentally manipulating a parasite capable of transmitting itself via multiple pathways utilized by a wide variety of parasites. We altered the diet phosphorus (P) content of Daphnia infected by the mixed mode transmitter Hamiltosporidian tvaerminnensis via one of two infection routes: horizontal, via the ingestion of spores from dead hosts, or vertical, via the infection of host offspring. Then, we quantified the effect of infection route on host diet-induced shifts in parasite load and virulence (i.e. host fecundity and survival). Overall, we found that horizontal infections were more sensitive to host diet than vertical infections. Low P diets decreased the parasite load and fecundity of all hosts, but had stronger negative effects on the loads of horizontally infected individuals than vertically infected ones. Likewise, horizontal infection reduced the survival of hosts fed low P (but not high P) diets while vertical infection did not affect survival. This increased sensitivity of horizontal infections to diet quality empirically supports the intuitive hypothesis that host diet quality exerts stronger effects on parasites that transmit via ingestion into a host's gut than on parasites that transmit via routes less directly related to host nutrient uptake. Through these effects, host diet quality may shape the ecology and evolution of parasite communities.

Introduction

The diet of an infected individual can influence the outcome of infection via a variety of mechanisms (Aalto et al., 2015; Bedhomme et al., 2004; Bize et al., 2008; Smith, 2007). Many of these mechanisms are likely mediated by characteristics of the host and parasite, but empirical work linking characteristics of either the host or the parasite to diet-induced shifts in their interaction are rare (Sanders & Taylor, 2018). Drawing these links could provide valuable insight regarding when and where specific parasites will be most successful. Linking the effect of host diet on disease outcomes to characteristics of the host (e.g. immune response or social structure) is important for determining which host populations are most at risk to disease (Becker et al., 2018). Drawing this link to characteristics of the parasite is a critical step in evaluating how host diet influences the ecology and evolution of parasites (Smith et al., 2015).

For hosts with relatively simple immune systems (e.g. most invertebrates), high quality diets are often expected to improve parasite outcomes (e.g. parasite load) by increasing the availability of resources to parasites (Frost et al., 2008; Pike et al., 2019), but parasite characteristics may exacerbate, ameliorate, or reverse this response. For example, a parasite's access to nutrients derived from a host's diet may be mediated by its infection site within a host (Bernot and Poulin 2018). The effects of host diet quality on the host-parasite interaction are likely also shaped by the parasite's nutritional requirements (Aalto et al., 2015; Narr & Krist, 2015) and the magnitude of the mismatch between parasite and host nutritional requirements (Frenken et al., 2021). While there is some evidence that parasite size and life stage influence these nutritional requirements (Paseka and Grunberg 2019, Stanley et al., 2024), empirical data on parasite characteristics relevant to the effects of host diet on disease is sparse. Moreover, there is a paucity of experimental work that directly tests how parasite characteristics mediate host-parasite responses to host diet quality (Narr et al. 2024). As a result, there is a need to link well-studied characteristics of parasites to the effects of host diet on outcomes of the host-parasite interaction.

One particularly well-studied parasite characteristic that is expected to mediate diet effects on the host-parasite interaction is infection route (Narr et al., 2019). Infection route describes the specific pathway taken by a parasite during infection and may include a variety of environmental (e.g. faecal-oral), vector, and additional intermediate host components (Antonovics et al., 2017). The mechanisms by which infection route might mediate host diet effects on infection are somewhat intuitive. For example, environmental nutrient availability can alter host-parasite outcomes by altering the abundance of intermediate hosts (Johnson et al., 2007). Likewise, many parasites transmit horizontally through host ingestion of parasite propagules that are then taken into the host via the gut. This infection route has the potential to increase the sensitivity of the parasite to shifts in host diet because these shifts can alter the ingestion rates of hosts, which, in turn, can influence the rate at which the host encounters parasites and alter the dose of infection (Hall et al., 2010). However, the effect of infection route on diet-mediated shifts in infection outcomes have not been explicitly tested. One reason for this may be because it is logistically challenging to experimentally isolate the effects of infection route from the effects of parasite identity.

Mixed mode transmitters provide a solution to this logistical issue because they transmit from one host to another via multiple transmission pathways. This makes them excellent study subjects to explore interactions between a host's environment and infection route because they enable the experimental manipulation of infection route in different host environments while controlling for variation in the identity of both the parasite and host. Moreover, understanding how diet influences the interaction between mixed mode transmitters and their hosts is valuable because mixed mode transmitters comprise a large fraction of the earth's symbionts, many of which are relevant to human disease (Ebert, 2013). A host's environment can influence the ecology and evolution of mixed mode transmitters by shifting the relative success of one transmission mode over another (Lipsitch, et al. 1996, Agnew and Koella 1999, Kaltz and Koella 2003). The investment of mixed mode transmitters in either horizontal (from infected host to an unrelated individual) or vertical (from parent to offspring) transmission can be assessed by comparing the horizontal fitness components (i.e. parasite load) and vertical fitness components (i.e. number of infected offspring produced) of a single host (Vizoso & Ebert, 2005b). There is some evidence that high resource conditions select for increased investment in vertical transmission relative to horizontal transmission, but the results are mixed, and these studies have focused on the effects of diet quantity (sometimes mediated by host density) rather than quality (Dusi et al., 2015; Magalon et al., 2010; Zilio et al., 2023). Based on these effects of diet quantity, we anticipate that low quality diets also strengthen the tradeoff between horizontal and vertical fitness components of infection and enhance investment in horizontal fitness components.

Here, we quantify the effects of diet quality and infection route on outcomes of the interaction between *Daphnia magna* and the mixed mode transmitter *Hamiltosporidium tvaerminnensis* using a fully crossed experimental design. *H. tvaerminnensis* is a model system for the study of microsporidian ecology and evolution (Haag et al., 2011), in part, because it is transmitted both horizontally (through the ingestion of spores from dead hosts) and vertically (likely transovarial) creating two host phenotypes: vertically infected*Daphnia* that have large spore loads, but experience low levels of virulence (i.e., are rarely castrated), and horizontally infected*Daphnia* that have smaller spore loads, but are frequently sterilized (Vizoso & Ebert, 2005b). A tradeoff between spore load and host offspring production indicates that ecological factors may alter the evolution of virulence in this system by shifting the role of horizontal vs vertical transmission in maintaining parasite abundances (Vizoso & Ebert, 2005b). The effects of phosphorus (P) on this system are particularly relevant because host diet phosphorus content exhibits substantial variation in the rock pools where this parasite ranges in prevalence from 0-100%, and *H. tvaerminnensis* prevalence increased with particulate nitrogen (N):P ratios (Narr et al., 2019). To evaluate how infection routes mediates host diet effects on this host-parasite interaction and, overall, how diet influences each fitness component within an individual host, we assessed the interactive effects of diet and infection route on fecundity, spore load, and survival rates of *Daphnia* .

Methods and Materials

Experimental design

We infected a single *Daphnia magna* clone (OER 3-3) with the microsporidian parasite *H. tvaerminnensis* which primarily resides in fat cells in the host's gut and ovaries (Vizoso and Ebert 2005). To quantify the effects of diet and infection route on the spore load, fecundity, and survival of individual hosts, we raised horizontally infected, vertically infected, and uninfected *Daphnia* on high and low P diets of the algae *Scenedesmus obliquus* . To create these diets, we, first, spiked the algal media with 16.5 mg/L (high P) and 1.65 mg/L (low P) of NaH_2PO_4, then mixed algae from these cultures to create diets with C:P ratios 1).

One generation prior to the experiment, we collected uninfected*Daphnia* neonates (<24 h old) from stock, uninfected brood moms, horizontally infected them via exposure to the crushed bodies of*H. tvaerminnensis* -infected *D. magna* , and then maintained them on high P diets fed *ad libitum* . We obtained 51 female*D. magna* that were vertically infected with *H. tvaerminnensis* by collecting the 4-5th brood of these mothers. Vertical transmission to parthenogenetic offspring is nearly 100% effective (Vizoso and Ebert, 2005). We assigned 21 of these neonates to the high P diet treatment and 30 to the low P diet treatment. To create our horizontally infected and uninfected treatments, we collected 97 female *Daphnia* neonates from uninfected brood mothers and infected 58 of them with *H. tvaerminnensis* by exposing them (from days 0-6) to the crushed bodies of infected *Daphnia* to achieve a high dose of uninfected neonates were exposed to homogenized uninfected*Daphnia* . Eighteen of these horizontally infected individuals were assigned to the high P treatment and 40 were assigned to the low P treatment. Thirty eight females were left uninfected. Of these 38 females, 9 were assigned to the high P diet and 29 were assigned to the low P diet. Uneven numbers of replicates in each treatment reflect the removal of male *Daphnia* from our experiment after the treatments were assigned (when males could be visibly differentiated with the naked eye). We also assigned more neonates to low P diets, especially in the more virulent horizontal infection treatments, because previous work indicates that mortality can be higher in this system when hosts are fed low P diets (Narr & Frost, 2016).

Neonates were raised individually in 20 mL (days 0-6) and then 40 mL (days 6-28) of COMBO (Kilham et al., 1998) and fed 2 mg C/L (days 0 and 2), 4 mg C/L (day 4), and 8 mg C/L (days 6-28) of their algal diet every other day. Starting on day six, we transferred *Daphnia* to new media every 4 days and collected their neonates and recorded mortality every other day. Neonates were preserved in ethanol (70%) so that total fecundity of each individual could be counted at the end of the experiment. On day 28 of the experiment, *Daphnia* were measured, rinsed, and then crushed in ultrapure water. Spore loads were determined for a subset of infected *Daphnia* in each treatment by dividing the average total number of spores in each sample, estimated from three 16 µL aliquots on a hemacytometer, by the *Daphnia* 's weight (calculated using length-weight regression from Ku et al. 2022).

Statistical Analysis

To determine if diet affected the spore load of horizontally infected individuals differently than vertically infected individuals, we regressed spore load against diet, infection route, and their interaction using a general linear model fit with a gamma distribution and log link. Then, we conducted posthoc comparisons within each infection route using tukey's HSD to determine if the effects of diet within each infection route were significant. Individuals for whom we were unable to detect spores were not included in this analysis.

To determine if diet influenced how virulent horizontal and vertical infections were, we examined the effects of diet on differences in the fecundity and mortality rates of uninfected *Daphnia* compared to*Daphnia* infected by each infection route. We used a general linear model with a Poisson distribution (with a scale parameter, generally referred to as quasipoisson) to predict individual*Daphnia* fecundity as a function of diet, infection type, and their interaction. To determine if differences between each infection type were significant within each diet, we conducted posthoc comparisons within each diet using Tukey's HSD. We compared survival rates of*Daphnia* infected via each infection route to those of uninfected*Daphnia* fed the same diet using separate Kaplan-Meier models. We used a right-censored design with time-at-risk based on the time (days) since the beginning of the experiment and a log-log ratio to test for significance (Murray & Bastille-Rousseau, 2020). We used the "survival" package in R for this part of this analysis.

Finally, we examined the potential for diet to influence the relationship between fecundity and spore load. To do this, we used model comparison based on Akaike's information criteria (Burnham et al., 2011) to examine how well individual fecundity was explained by linear models including 1) spore load alone, 2) spore load plus diet and/or infection route, and 3) spore load times diet and or infection route. Models with a delta AICc <2 were considered top models. All posthoc comparisons were computed using the package emmeans, and all statistics were conducted in R version 4.4.0.

Results

Spore load of infected *Daphnia* was higher in vertical infections than horizontal infections (coef = 5.56, t = 13.14, $p < 0.001$), and lower for hosts fed low P diets (coef = -1.91, t = -3.80, $p < 0.001$, Figure 1). However, an interaction between infection route and host diet P content indicated that low P diets reduced the spore loads of horizontally infected *Daphnia* more than those of vertically infected *Daphnia* (coef = 1.27, t = 2.15, p = 0.041). Posthoc comparisons within each infection route show that low P diets significantly reduce the spore loads of horizontally infected*Daphnia* (t ratio =3.79, p = 0.0006), but only marginally reduced the spore load of vertically infected *Daphnia* (t ratio =2.0, p = 0.054).

Daphni a fecundity was reduced by vertical infection (coef = -0.33, t = -3.15, p =0.0022), horizontal infection (coef= -0.36, t = -2.94, p =0.0041), and low P diets (coef= -1.10, t = -9.10, $p << 0.001$, Figure 2). Within the low P diet, horizontal infection further reduced host fecundity (coef = -0.76, t = -3.21, p = 0.0018) and vertical infection marginally reduced it (coef = -0.35, t = -1.95, p = 0.054). Post hoc comparison indicates that, within the high P diet, both horizontal (z = 2.94, p = 0.0092) and vertical (z = 3.147, p = 0.0047) infection reduced fecundity relative to uninfected individuals, but there was no difference between the fecundities of*Daphnia* infected via each route (z=-0.26, p = 0.96). Likewise, within the low P diet, both vertical (z = 4.72, $p < 0.001$) and horizontal (z = 5.85, $p < 0.001$) infections reduced fecundity, but there was no difference between the fecundities of *Daphnia* infected via each route (z = -2.024, p = 0.11). Relative to uninfected*Daphnia* fed high P diets, the survival of *Daphnia* fed high P diets was not affected by either horizontal infection (chisq = 2.62, p = 0.11) or vertical infection (chisq = 0.017, p = 0.90, Figure 3). Relative to uninfected *Daphnia* fed low P diets, the survival of*Daphnia* fed low P diets was reduced by horizontal infection (chisq = 8.98, p = 0.0027), but not by vertical infection (chisq =0.18, p = 0.67).

The top model predicting fecundity as a function of spore load included an effect of diet and an interaction between spore load and infection route (Supplementary Material, Table 2, Akaike model weight = 0.83). The output of this model indicated that fecundity was reduced by low P diets (coef = -69.80, t = -14.40, $p << 0.001$), higher for vertically infected hosts than horizontally infected ones (coef = 82.24, t = 3.47, p = 0.0014), and, when hosts were vertically infected, fecundity declined with spore load (coef = -11.09, t =-2.62, p = 0.013, Figure 4).

Discussion

Our study provides empirical evidence that infection route can shape the response of both the host and parasite to shifts in host diet quality. In our system, host diet quality exerted stronger effects on horizontally transmitted infections and their hosts than on vertically transmitted infections and their hosts (Figures 1 and 2). We suspect that the mechanisms for these differential effects are common to other parasites using similar infection routes in invertebrate hosts. As a result, we anticipate that the results presented here will help identify which host-parasite systems are most likely to respond to shifts in host diet. Moreover, for mixed mode transmitters like *H. tvaerminnensis* , the reduced spore load and survival of horizontally infected hosts fed low P diets suggests that low quality host diets might limit the role of horizontal infection in maintaining the parasite's prevalence.

Our experiment also tested the possibility that low quality diets cause mixed mode transmitters to invest in horizontal fitness components at the cost of vertical fitness components. Resource availability has been shown to alter the tradeoff between horizontal and vertical fitness in some systems (Dusi et al., 2015; Magalon et al., 2010; Zilio et al., 2023), but we did not find evidence that low quality host diets produce the same shift over the duration of our experiment. While we detected a tradeoff between horizontal and vertical fitness components in vertically infected individuals (spore load was inversely related to fecundity), diet quality did not influence the strength or shape of this tradeoff. Rather, our results are support the hypothesis that high quality host diets benefit parasites infecting hosts with simple immune systems (Johnson et al., 2007, Frost et al., 2008, Pike et al. 2019, Figure 1). Low P diets reduced both the horizontal and vertical fitness components of *H. tvaerminnensis* in *Daphnia* , regardless of infection route. Below, we discuss the potential mechanisms for the observed effects of diet quality on each infection route and fitness component and consider the potential consequences of these effects for infected populations over longer timescales.

Diet effects modulated by infection route

We suspect that horizontal infections were more strongly influenced by host diet than vertical ones because of differences in infection site, within host replication rate, and secondary infection effects. If these mechanisms were responsible for the results we observed, our results may inform predictions regarding the effects of host diet quality in a range of host-parasite systems. We suggest that spore loads in horizontally infected individuals may have been more sensitive to host diet quality for two principle reasons 1) nutrient availability at the infection site of horizontal infection was more variable than that of vertical infections, and 2) horizontal infections grew faster than vertical ones. Like those of many parasites transmitted horizontally via ingestion, horizontal infections of *H. tvaerminnensis* develop from the host's gut while vertical infections of *H. tvaerminnensis* , like many other vertically transmitting parasites, are likely transovarial (Dunn et al., 2001; Vizoso & Ebert, 2004). Within crustaceans, the nutrient content of specific tissues differ from one another, and, in*Daphnia* , egg nutrient content remains homeostatic despite fluctuations in the nutrient content of a Daphnia's diet (Faerovig & Hessen, 2003), suggesting that nutrient availability with the ovaries is relatively constant. In comparison, nutrient availability within host infection sites more tightly involved in digestion and absorption of nutrients (e.g., the gut) is likely more tightly coupled to the quality of the host's diet (Stanley et al., 2024). If so, parasites infecting digestive sites would be more susceptible to declines in host diet quality. We suspect that horizontal infections also grew faster than vertical ones. Because horizontal infections are acquired later in the host's life cycle than vertical infections, and because horizontal infections are less impacted by their effects on host fitness, horizontal infections can develop faster than vertical infections (Vizoso & Ebert, 2004). Therefore, the potential for nutrient limitation of horizontal infections may be further exacerbated by elevated growth rates compared to vertical infections. Greater nutrient uptake during periods of intense growth are expected for a variety of single-celled organisms (Droop, 1973). Unfortunately, very few studies have quantified variation in nutrient availability within hosts, and the nutritional requirements of microparasites are largely unexplored (but see Narr et al. 2024)).

The reduced survival of horizontally (but not vertically) infected*Daphnia* fed low quality diets is consistent with observations that, for this host-parasite system, host survival rates are dictated by secondary effects of infection rather than direct effects of parasite production (i.e. damage to cells upon penetration of the host, Vizoso and Ebert 2004). Previous work on the *Daphnia* clone used in our experiment shows that *Daphnia* compensate for low P diets by increasing ingestion rates (Narr & Frost, 2016). Increased ingestion rate would result in an increased ingestion of *H. tvaerminnensis* spores and increased damage to cells as more spores penetrated the host. Indeed, higher infection doses have been shown to increase mortality in other horizontally transmitted parasites of *Daphnia* that damage the gut during penetration (Ebert et al., 2000). Because many animals compensate for declines in diet quality by increasing their consumption rates (Darchambeau & Thys, 2005), and many horizontally transmitted parasites infect their hosts through ingestion, potentially damage their host during penetration (Antonovics et al., 2017), we find it likely that low quality diets increase the virulence of a wide variety of horizontally transmitted parasites by increasing their ingestion rate and thus encounter frequency between parasite and host.

Diet effects on fitness components that were not influenced by infection route

For some mixed mode transmitters, increased investment in one transmission mode leads to a decreased investment in the other, so that environments that limit the transmission of one mode enhance the other (Lipsitch, et al. 1996, Agnew and Koella 1999, Dusi et al. 2015, Zilio et al. 2023). We observed evidence for this tradeoff, in the form of a significant relationship between spore load (the horizontal fitness component of *H. tvaerminnensis*) and fecundity (the vertical fitness component), when *Daphnia* were vertically infected, but not when they were horizontally infected (Figure 4). Given that previous work has detected this tradeoff in individuals infected with *H. tvaerminnensis* via both routes (Vizoso & Ebert, 2005b), we suspect that we did not observe this tradeoff because horizontal infection resulted in very small spore loads, and did not achieve a high enough level to burden the host in a way that influenced fecundity beyond the strain caused by the initial infection. We expected that low quality diets would strengthen this tradeoff by limiting the parasite's vertical fitness component, but we found no evidence that diet quality influenced the slope of the relationship between individual spore load and fecundity. While low P host diets reduced the vertical fitness component of this parasite, they also reduce the horizontal one.

Negative effects of poor quality host diets on spore production are consistent with other work on horizontally transmitted parasites (e.g. Frost et al., 2008) and generally thought to be caused by a reduced ability of the parasite to access resources. The precise mechanism by which host diet influences parasite load is unclear, especially when, as is the case in our study, parasite load is calculated as a function of host biomass. Recent evidence from the same host-parasite system suggests that the nutritional quality of the host's diet directly influences the nutrient content of *H. tvaerminensis* spores with the C:P ratio of *H. tvaerminnensis* spores increasing with the C:P ratio of the host's diet (Narr et al. 2024). Thus, it's possible that the parasite's spore load is a function of its nutritional state which is directly affected by the nutritional status of its host. However, more work is needed to fully understand how stoichiometric shifts in parasite nutrient content are related to parasite growth and replication.

Consistent with a large body of work, our results show that low P diets reduced the fecundity of both infected and uninfected *Daphnia (Lürling & Van Donk, 1997; Sterner et al., 1993)* , and that*H. tvaerminnensis* reduces the fecundity of the *Daphnia* it infects relative to uninfected *Daphnia* fed the same diet (Bieger & Ebert, 2009). Our results provide new evidence that parasite-induced reductions in host fecundity exist when *Daphnia* are fed both high and low quality diets (Figure 2). Previous work on the same host-parasite system suggests that higher quality diets increase the differences between the fecundities of uninfected and *H. tvaeriminnensis* -infected *Daphnia* (Narr & Frost, 2016). We suspect that different outcomes between our experiment and the previous experiment (Narr and Frost, 2016) reflect the age of the *Daphnia* when each experiment was ended: our current experiment was ended 10 days later than the earlier experiment. The extended duration of the current study likely provides the more realistic estimate of these effects, but the discrepancy between these two experiments highlights the value of collecting more finely resolved life history data over the entire "lifetime" of the host. While our results should be interpreted with some caution, the implications of host diet effects on the relative competitive ability of infected hosts their uninfected counterparts are key to predicting the effects of diet quality on vertically transmitted parasites. The magnitude of the difference in fecundity between infected and uninfected hosts is a critical outcome for vertically transmitted parasites because it dictates the outcome of competition between infected and uninfected hosts. If competitive differences within populations are present, the magnitude and direction of those differences could shape epidemics, much in the way that competitive differences between species can (Orlansky & Ben-Ami, 2023). For*H. tvaerminnensis* , competition between infected and uninfected*Daphnia* drives the parasite to extinction when horizontal transmission is prevented (Lass & Ebert, 2006). The diet-induced differences in fecundity that we observed indicate that low P diets enhance the competitive disadvantage of *H. tvareminnensis* infected hosts which should drive the host to extinction faster within populations if horizontal transmission is prevented.

Conclusion

More work is needed to determine how the interactive effects of infection route and diet that we observed scale up to populations of the host and parasite, infected ecosystems, and longer timescales. Direct scaling of these relationships may be obfuscated by a variety of mechanisms that occur in natural systems including the potential for double infections via multiple infection modes, feedbacks between resources and hosts or host densities, and the nuanced effects of horizontal infection on the persistence of mixed mode transmitters. For example, the prevalence of *H. tvaerminnensis* in populations of*D. magna* was positively related to particulate N:P ratios in natural rock pools, and *H. tvaerminnensis* spore load was highest in completely infected mesocosms without P additions (Narr et al., 2019). We suspect that our observations in individual hosts may differ from those in more complex systems because, in natural systems, vertically infected individuals could also become horizontally infected, which can increase spore load (Vizoso & Ebert, 2005a). It is also possible that, over long timescales, tradeoffs between horizontal and vertical fitness components become more pronounced. Indeed, while individual hosts in low P mesocosms had higher spore loads, low P mesocosms also achieved lower host densities than those in high P mesocosms (Narr et al., 2019). Our experiment indicates that these lower population sizes could have been caused by reduced fecundity, enhanced mortality from secondary horizontal infections or both. We highlight the utility of work conducted at the level of the individual host, as much of this work has informed our understanding of the mechanisms that produced the effects we observed, without which generalizing our predictions to other host-parasite systems would not be possible. However, we also suggest the need for additional research exploring the link between treatments effects on host-parasite outcomes at the individual scale and those at the population level.

Overall, our results indicate that low quality diets negatively affect*H. tverminnensis* at the scale of the individual host, but this effect is influenced by infection route. Our results are relevant to a wide variety of parasites capable of transmitting via one or both of these infection routes, and we suspect that a systematic investigation of diet-mediated shifts in host-parasite systems using additional routes could yield additional insight into mechanisms that govern these shifts. Moreover, quantifying the effects of other parasite characteristics (e.g. life history characteristics, mode of resource acquisition, etc.) on diet-induced shifts in host-parasite systems should facilitate our ability to predict the effects of host diet on disease. At present, diet quality has only been manipulated for a few different host-parasite systems, so this task remains daunting.

References

Aalto, S. L., Decaestecker, E., & Pulkkinen, K. (2015). A three-way perspective of stoichiometric changes on host–parasite interactions.*Trends in Parasitology* , *31* (7), 333–340. https://doi.org/10.1016/j.pt.2015.04.005Agnew, P., & Koella, J. C. (1999). Life history interactions with environmental conditions in a host-parasite relationship and the parasite's mode of transmission.*Evolutionary Ecology* , *13* (1), 67–91. https://doi.org/10.1023/A:1006586131235Antonovics, J., Wilson, A. J., Forbes, M. R., Hauffe, H. C., Kallio, E. R., Leggett, H. C., Longdon, B., Okamura, B., Sait, S. M., & Webster, J. P. (2017). The evolution of transmission mode. *Philosophical Transactions of the Royal Society B: Biological Sciences* , *372* (1719), 7–11. https://doi.org/10.1098/rstb.2016.0083Becker, D. J., Streicker, D. G., & Altizer, S. (2018). *Using host species traits to understand the consequences of resource provisioning for host – parasite interactions* . *June 2016* , 511–525. https://doi.org/10.1111/1365-2656.12765Bedhomme, S., Agnew, P., Sidobre, C., & Michalakis, Y. (2004). Virulence reaction norms across a food gradient. *Proceedings of the Royal Society B: Biological Sciences* , *271* (1540), 739–744. https://doi.org/10.1098/rspb.2003.2657Bernot, R. J., & Poulin, R. (2018). Ecological Stoichiometry for Parasitologists. *Trends in Parasitology* , *34* (11), 928–933. https://doi.org/10.1016/j.pt.2018.07.008Bieger, A., & Ebert, D. (2009). Expression of parasite virulence at different host population densities under natural conditions. *Oecologia* , *160* (2), 247–255. https://doi.org/10.1007/s00442-009-1297-xBize, P., Jeanneret, C., Klopfenstein, A., & Roulin, A. (2008). What makes a host profitable? Parasites balance host nutritive resources against immunity.*American Naturalist* , *171* (1), 107–118. https://doi.org/10.1086/523943Burnham, K. P., Anderson, D. R., & Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons.*Behavioral Ecology and Sociobiology* , *65* (1), 23–35. https://doi.org/10.1007/s00265-010-1029-6Darchambeau, F., & Thys, I. (2005). In situ filtration responses of Daphnia galeata to changes in food quality. *Journal of Plankton Research* , *27* (3), 227–236. https://doi.org/10.1093/plankt/fbh171Droop, M. R. (1973). Some thoughts on nutrient limitation in algae. *Journal of Phycology* ,*9* (3), 264–272.Dunn, A. M., Terry, R. S., & Smith, J. E. (2001). Transovarial transmission in the microsporidia. *Advances in Parasitology* , *48* , 57–100. https://doi.org/https://doi.org/10.1016/S0065-308X(01)48005-5Dusi, E., Gougat-Barbera, C., Berendonk, T. U., & Kaltz, O. (2015). Long-term selection experiment produces breakdown of horizontal transmissibility in parasite with mixed transmission mode. *Evolution* ,*69* (4), 1069–1076. https://doi.org/10.1111/evo.12638Ebert, D. (2013). The Epidemiology and Evolution of Symbionts with Mixed-Mode Transmission. *Annual Review of Ecology, Evolution, and Systematics* , *44* (1), 623–643. https://doi.org/10.1146/annurev-ecolsys-032513-100555Ebert, D., Zschokke-rohringer, C. D., Carius, H. J., & Carius, H. J. (2000). Dose Effects and Density-Dependent Regulation of Two Microparasites of Daphnia magna. *Oecologia* , *122* (2), 200–209.Faerovig, P. J., & Hessen, D. O. (2003). Allocation strategies in crustacean stoichiometry: the potential role of phosphorus in the limitation of reproduction. *Freshwater Biology* , *48* (10), 1782–1792. https://doi.org/10.1046/j.1365-2427.2003.01128.xFrenken, T., Paseka, R., González, A. L., Asik, L., Seabloom, E. W., White, L. A., Borer, E. T., Strauss, A. T., Peace, A., & Van de Waal, D. B. (2021). Changing elemental cycles, stoichiometric mismatches, and consequences for pathogens of primary producers. *Oikos* , *130* (7), 1046–1055. https://doi.org/10.1111/oik.08253Frost, P. C., Ebert, D., & Smith, V. H. (2008). Responses of a bacterial pathogen to phosphorus limitation of its aquatic invertebrate host. *Ecology* , *89* (2), 313–318. https://doi.org/10.1890/07-0389.1Haag, K. L., Larsson, J. I. R., Refardt, D., & Ebert, D. (2011). Cytological and molecular description of Hamiltosporidium tvaerminnensis gen. et sp. nov., a microsporidian parasite of Daphnia magna, and establishment of Hamiltosporidium magnivora comb. nov. *Parasitology* , *138* (4), 447–462. https://doi.org/10.1017/S0031182010001393Hall, S. R., Becker, C. R., Duffy, M. a, & Cáceres, C. E. (2010). Variation in resource acquisition and use among host clones creates key epidemiological trade-offs.*The American Naturalist* , *176* (5), 557–565. https://doi.org/10.1086/656523Johnson, P. T. J., Chase, J. M., Dosch, K. L., Hartson, R. B., Gross, J. A., Larson, D. J., Sutherland, D. R., & Carpenter, S. R. (2007). Aquatic eutrophication promotes pathogenic infection in amphibians. *Proceedings of the National Academy of Sciences of the United States of America* , *104* (40), 15781–15786. https://doi.org/10.1073/pnas.0707763104Kaltz, O., & Koella, J. C. (2003). Host growth conditions regulate the plasticity of horizontal and vertical transmission in Holospora undulata, a bacterial parasite of the protozoan Paramecium caudatum. *Evolution* , *57* (7), 1535–1542. https://doi.org/10.1111/j.0014-3820.2003.tb00361.xKilham, S. S., Kreeger, D. A., Lynn, S. G., Goulden, C. E., & Herrera, L. (1998). COMBO: A defined freshwater culture medium for algae and zooplankton.*Hydrobiologia* , *377* (1–3), 147–159. https://doi.org/10.1023/a:1003231628456Ku, D., Chae, Y. J., Choi, Y., Ji, C. W., Park, Y. S., Kwak, I. S., Kim, Y. J., Chang, K. H., & Oh, H. J. (2022). Optimal Method for Biomass Estimation in a Cladoceran Species, Daphnia Magna (Straus, 1820): Evaluating Length–Weight Regression Equations and Deriving Estimation Equations Using Body Length, Width and Lateral Area. *Sustainability (Switzerland)* ,*14* (15). https://doi.org/10.3390/su14159216Lass, S., & Ebert, D. (2006). Apparent seasonality of parasite dynamics: analysis of cyclic prevalence patterns. *Proceedings. Biological Sciences / The Royal Society* , *273* (1583), 199–206. https://doi.org/10.1098/rspb.2005.3310Lipsitch, Marc, Siller, S., & Nowak, M. (1996). The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution* , *50* (5), 1729–1740. https://doi.org/10.1525/california/9780520280540.003.0006Lurling, M., & Van Donk, E. (1997). Life history consequences for Daphnia pulex feeding on nutrient-limited phytoplankton. *Freshwater Biology* ,*38* (3), 693–709. https://doi.org/10.1046/j.1365-2427.1997.00242.xMagalon, H., Nidelet, T., Martin, G., & Kaltz, O. (2010). Host growth conditions influence experimental evolution of life history and virulence of a parasite with vertical and horizontal transmission. *Evolution* , *64* (7), 2126–2138. https://doi.org/10.1111/j.1558-5646.2010.00974.xMurray, D. L., & Bastille-Rousseau, G. (2020). Estimating survival and cause-specific mortality from continuous time observations. In D. L. Murray & B. Sandercock (Eds.), *Population Ecology in Practice: Underused, Misused, and Abused Methods* .Narr, C.F. Binger, S., Sedlacek, E., Anderson, B. Shoemaker, G., Stanley, A., Stokoski, M., Hall, E. (2024). Measuring the Stoichiometry of Microbial Parasite Infrapopulations One Cell at a Time using Energy Dispersive Spectroscopy

Abstract. *BioRxiv* , 1–17. https://biorxiv.org/cgi/content/short/2024.01.11.575226v1Narr, C. F., Ebert, D., Bastille-Rousseau, G., & Frost, P. C. (2019). Nutrient availability affects the prevalence of a microsporidian parasite.*Journal of Animal Ecology* , *88* (4). https://doi.org/10.1111/1365-2656.12945Narr, C. F., & Frost, P. C. (2016). Exploited and excreting: Parasite type affects host nutrient recycling. *Ecology* , *97* (8). https://doi.org/10.1002/ecy.1437Narr, C. F., & Krist, A. C. (2015). Host diet alters trematode replication and elemental composition.*Freshwater Science* , *34* (1). https://doi.org/10.1086/679411Orlansky, S., & Ben-Ami, F. (2023). The parasites of my rival are my friends. *Frontiers in Microbiology* ,*14* (May), 1–7. https://doi.org/10.3389/fmicb.2023.1135252Paseka, R. E., & Grunberg, R. L. (2019). Allometric and trait-based patterns in parasite stoichiometry. *Oikos* , *128* (1), 102–112. https://doi.org/10.1111/oik.05339Pike, V. L., Lythgoe, K. A., & King, K. C. (2019). *On the diverse and opposing effects of nutrition on pathogen virulence* .Sanders, A. J., & Taylor, B. W. (2018). Using ecological stoichiometry to understand and predict infectious diseases.*Oikos* , *127* (Smith 2007), 1399–1409. https://doi.org/10.1111/oik.05418Smith, V. H. (2007). Host resource supplies influence the dynamics and outcome of infectious disease.*Integrative and Comparative Biology* , *47* (2), 310–316. https://doi.org/10.1093/icb/icm006Smith, V. H., Holt, R. D., Smith, M. S., Niu, Y., & Barfield, M. (2015). Resources, mortality, and disease ecology: Importance of positive feedbacks between host growth rate and pathogen dynamics. *Israel Journal of Ecology and Evolution* ,*61* (1), 37–49. https://doi.org/10.1080/15659801.2015.1035508Sterner, R. W., Hagemeier, D. D., Smith, W. L., & Smith, R. F. (1993). Phytoplankton nutrient limitation and food quality for Daphnia. *Limnology and Oceanography* , *38* (4), 857–871. https://doi.org/10.4319/lo.1993.38.4.0857Vizoso, D. B., & Ebert, D. (2004). Within-host dynamics of a microsporidium with horizontal and vertical transmission: Octosporea bayeri in Daphnia magna.*Parasitology* , *128* , 31–38. https://doi.org/10.1017/S0031182003004293Vizoso, D. B., & Ebert, D. (2005a). Mixed inoculations of a microsporidian parasite with horizontal and vertical infections. *Oecologia* , *143* (1), 157–166. https://doi.org/10.1007/s00442-004-1771-4Vizoso, D. B., & Ebert, D. (2005b). Phenotypic plasticity of host-parasite interactions in response to the route of infection. *Journal of Evolutionary Biology* ,*18* (4), 911–921. https://doi.org/10.1111/j.1420-9101.2005.00920.xZilio, G., Kaltz, O., & Koella, J. C. (2023). Resource availability for the mosquito Aedes aegypti affects the transmission mode evolution of a microsporidian parasite. *Evolutionary Ecology* , *37* (1), 31–51. https://doi.org/10.1007/s10682-022-10184-7

Figure Legends

Figure 1: Spore load (log spores per µg of *Daphnia* dry weight) of*Daphnia magna* fed high (green) and low (orange) phosphorus diets that were infected by *Hamiltosporidium tvaerminnensis* either horizontally or vertically. Letters denote significant ($p < 0.05$) differences based on Tukey HSD post hoc comparisons within in each infection route.

Figure 2: Total fecundity of uninfected *Daphnia magna* (blue) and *D. magna* infected either horizontally (red) or vertically (purple) by *Hamiltosporidium tvaerminnensis* after 28 days. Letters denote significant ($p < 0.05$) differences based on Tukey HSD post hoc comparisons within in each diet treatment.

Figure 3: Survival of uninfected *Daphnia magna* (blue) and*D. magna* infected either horizontally (red) or vertically (purple) by *Hamiltosporidium tvaerminnensis* over 28 days. Shaded areas represent 95% confidence intervals.

Figure 4: Total fecundity as a function of spore load (spores per µg of host dry weight) for *Daphnia magna* infected either horizontally (red) or vertically (purple) by *Hamiltosporidium tvaerminnensis* and fed high (circles) or low (triangles) phosphorus diets for 28 days.

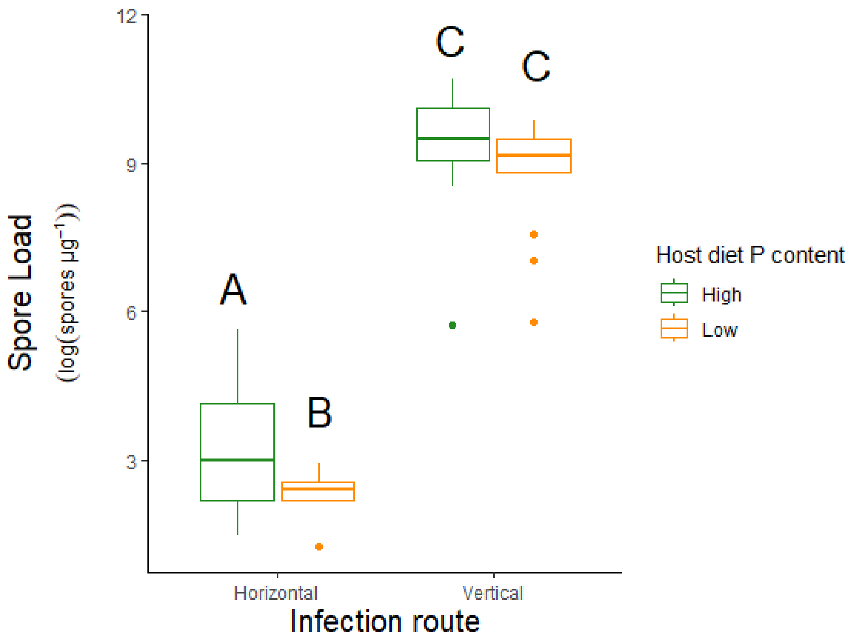

Figure 1

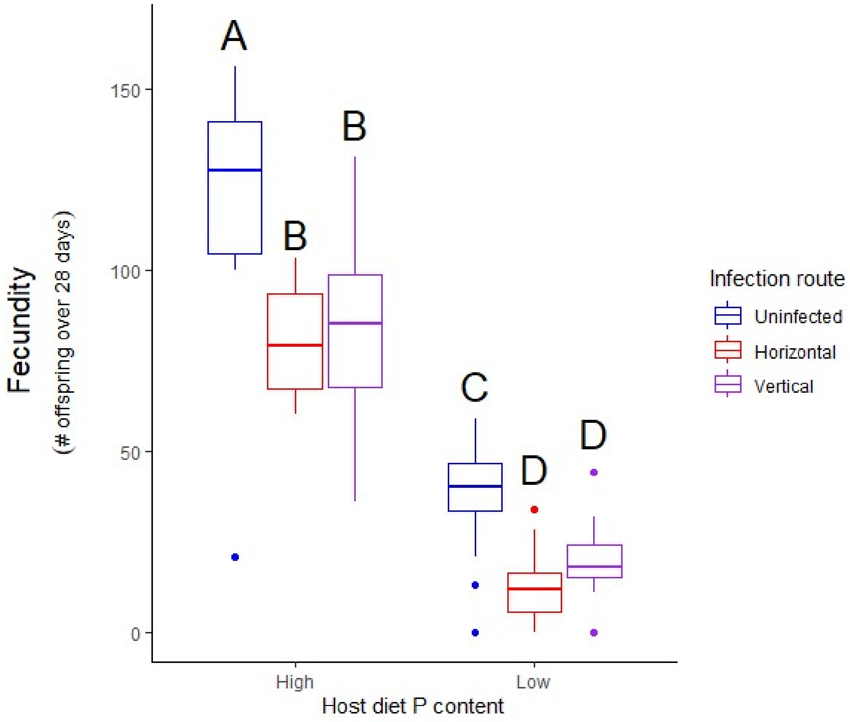

Figure 2

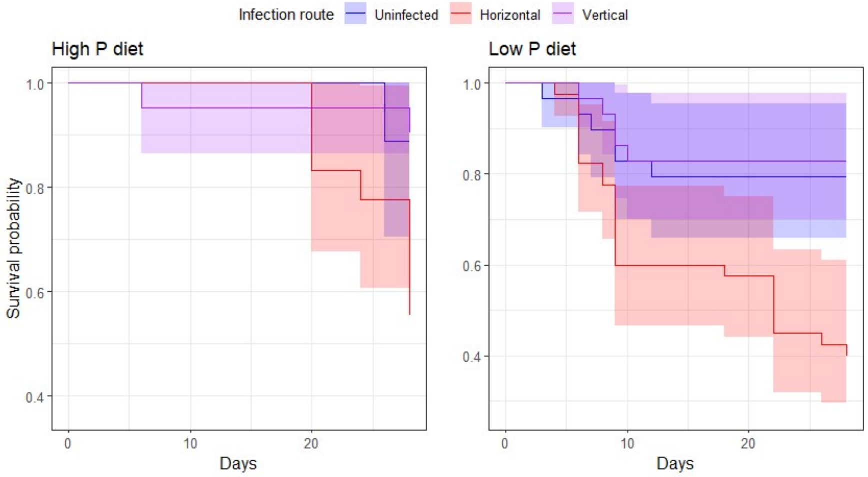

Figure 3

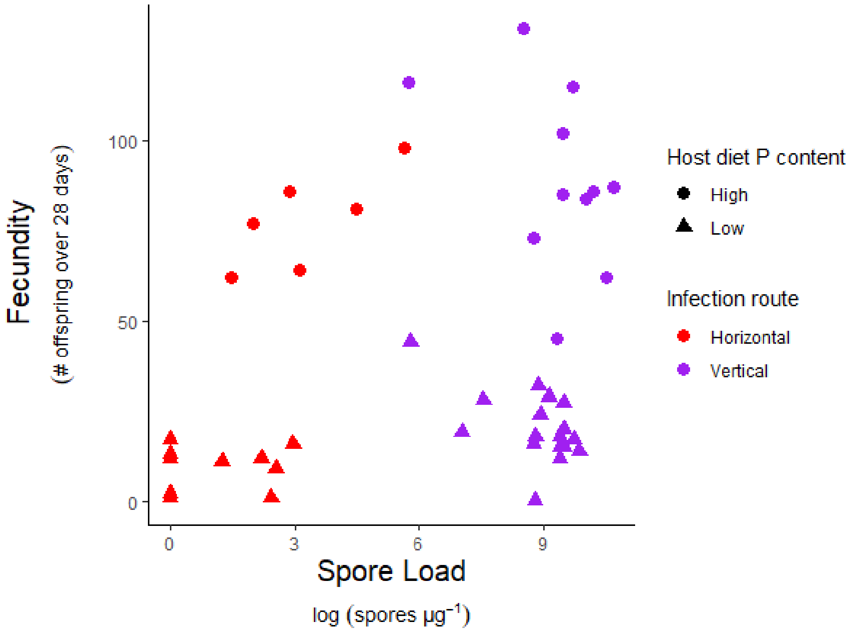

Figure 4