Parasitology

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Host food quality and quantity differentially affect *Ascogregarina barretti* parasite burden, development and within-host competition in the mosquito *Aedes triseriatus*

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Research Article

Cite this article: Westby KM, Sweetman BM, Adalsteinsson SA, Biro EG, Medley KA (2019). Host food quality and quantity differentially affect *Ascogregarina barretti* parasite burden, development and within-host competition in the mosquito *Aedes triseriatus*. *Parasitology* **146**, 1665–1672. https://doi.org/10.1017/S0031182019000994

Received: 12 February 2019
Revised: 8 July 2019
Accepted: 10 July 2019
First published online: 29 August 2019

Key words:
Aedes triseriatus; *Ascogregarina barretti*; detritus; gregarines; host condition; mosquito; tannins; with-in host competition

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Abstract

Host condition depends in large part on the quality and quantity of available food and heavily influences the outcome of parasite infection. Although parasite fitness traits such as growth rate and size may depend on host condition, whether host food quality or quantity is more important to parasite fitness and within-host interactions is poorly understood. We provided individual mosquito hosts with a standard dose of a gregarine parasite and reared mosquitoes on two food types of different quality and two quantities. We measured host size, total parasite count and area, and average size of parasites within each treatment. Food quality significantly influenced the number of parasites in a host; hosts fed a low-quality diet were infected with more parasites than those provided a high-quality diet. In addition, we found evidence of within-host competition; there was a negative relationship between parasite size and count though this relationship was dependent on host food quality. Host food quantity significantly affected total parasite area and parasite size; lower food quantity resulted in smaller parasites and reduced overall parasite area inside the host. Thus both food quality and quantity have the potential to influence parasite fitness and population dynamics.

Introduction

Food quality and quantity can influence a host's ability to resist and respond to infection through effects on host condition. Hosts in poor condition generally suffer greater effects of infection in terms of retarded development, reduced fitness and lower survival rates compared to hosts in relatively better condition (Brown *et al.*, 2000). Many studies have been able to quantify increases in immune function in hosts in relatively better condition and this may drive much of the improved outcomes observed in these hosts. Hosts that have access to sufficient and high-quality nutrition should have more resources to invest in defense, as mounting an immune response is metabolically costly (Zuk and Stoehr, 2002). For example, nutritional deprivation has been shown to reduce the melanization response (Suwanchaichinda and Paskewitz, 1998; Lee *et al.*, 2008) and antimicrobial gene upregulation in insects (Brunner *et al.*, 2014). Thus, well-fed hosts are generally able to invest more in immunity, fecundity and somatic maintenance in response to infection compared to their nutritionally deprived counterparts (Kopp and Medzhitov, 2009).

Many studies that investigate how host food affects the cost or outcome of parasitism focus on the amount of food available to a host (e.g. Brown *et al.*, 2000). While food availability is both crucial to host condition and highly variable in nature, food quality (e.g. micro and macro nutrients, plant secondary compounds) may be just as variable and important for generalist feeders. Generally, hosts feeding on a higher quality diet have greater measures of fitness proxies and infection outcomes. Protein, in particular, seems to increase host resistance to parasites and lead to more favorable outcomes for the host; for example protein supplementation increases body size and resistance to *Batrachochytrium dendrobatidis* infection in amphibians, and increases survival and resistance to nucleopolyhedrovirus in lepidopterans (Lee *et al.*, 2006; Venesky *et al.*, 2012). Additionally, the chemical composition and amount of secondary compounds produced by plants are known to alter insect herbivore-parasite interactions (reviewed by Cory and Hoover, 2006) and may also impact aquatic detritus-based communities (Davidson *et al.*, 2012; Stoler *et al.*, 2016). Despite evidence that both the amount of available food and the nutritive quality of food being important for hosts, less is known about their relative importance to each other or potential interactive effects.

Host food can not only influence host condition and infection outcomes, but can potentially influence parasite fitness. There is considerable debate in the literature as to whether well-fed, good-condition hosts or ill-fed, poor-condition hosts will be better habitats for the parasites that exploit them. In some systems, poor quality hosts have parasites that produce more infectious stages or have faster growth rates (Ezenwa, 2004; Vale *et al.*, 2013), whereas the reverse is true in other systems (Pulkkinen and Ebert, 2004; Logan *et al.*, 2005; Dube *et al.*, 2018). It is not hard to conceive why these discrepancies exist in the literature; parasites of well-fed hosts will have a

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host with plenty of resources to exploit but also a host capable of mounting a strong immune response (Bize *et al.*, 2008; Seppälä *et al.*, 2008). Thus, the specific mode of transmission, host immune strategy, host lineage, degree of environmental heterogeneity and evolutionary history of host and parasite likely will influence whether well-fed hosts produce more or less fit parasites (Lafferty and Holt, 2003; Bedhomme *et al.*, 2004). Direct measures of parasite fitness, such as the basic reproductive number R_0, are difficult to determine experimentally as they require measuring many aspects of a parasite's lifetime fitness (Hartemink *et al.*, 2008). Thus, more easily determined proxy measures of fitness, such as parasite size, are often used and are correlated with fitness (Tseng and Myers, 2014) and many parasite taxa have strong size–fecundity relationships (Sasall and Morand, 1998; Holfeld, 2000; Rowe *et al.*, 2008; Costanzo *et al.*, 2018).

In recent decades, a growing body of work has demonstrated that both inter- and intra-specific competition for resources within hosts may have important consequences for parasite fitness correlates and transmission dynamics (Tompkins and Hudson, 1999; Wedekind *et al.*, 2000; de Roode *et al.*, 2003). Resource competition in free-living organisms is well known to be context-dependent (Taniguchi and Nakano, 2000; Murrell and Juliano, 2008). Little research, however, has been conducted to understand under which conditions with-in host competition occurs. There may be strong seasonal patterns where density-dependent competition is only detected during certain times of the year (Irvine *et al.*, 2001), may depend on host age and activation of the immune system (Patterson and Viney, 2002; Chylinski *et al.*, 2009), or may be altered by the supplementation of a nutrient required for parasite development (Wale *et al.*, 2017). As host food quantity and quality impact host and parasite fitness, it is likely they also impact within-host dynamics such as competition.

Gregarine parasites (phylum Apicomplexa: *Ascogregarina*) are common obligate parasites of invertebrates. For gregarine species that infect mosquitoes, the infection begins when free-living oocysts are ingested by larvae in the aquatic habitat. The oocysts then migrate to the larval midgut where they release sporozoites that enter the epithelial cells of the gut and develop into trophozoites. New oocysts are formed in the malpighian tubules of adults and the infection cycle continues as new oocysts are returned to the larval habitat when infected adults or pupae die in or on the water, when adults defecate upon emergence, or when they return to lay eggs (more life-cyle detail available in Beier and Craig, 1985; Lantova and Volf, 2014). *Aedes triseriatus*, the Eastern tree-hole mosquito, is parasitized by *Ascogregarina barretti*, and though there is little evidence that *As. barretti* infection causes direct mortality in *Ae. triseriatus*, infection can alter larval behavior (Soghigian *et al.*, 2017), prolong female development time (Walker *et al.*, 1987) and decrease adult size (Walker *et al.*, 1987; Siegel *et al.*, 1992), reducing fecundity and population growth (Soghigian and Livdahl, 2017).

In this paper, we tested the effects of host food quality and quantity on fitness proxies for both host and parasite in addition to within-host competition among parasites using the larval stage of the mosquito *Ae. triseriatus* and its protozoan parasite *As. barretti*. We predicted that if competition were occurring we would see a reduction in parasite size with increasing parasite counts. Using a model-selection approach, we were able to determine which host and parasite metrics influenced each other in terms of host size, parasite number, parasite size and extent of infection.

Materials and methods

Experimental design

Each experimental unit consisted of one second instar *Ae. triseriatus* larva placed in 12 mL of infusion in a 15 mL glass vial with 21.5 μL oocyst suspension containing approximately 7000 gregarine oocysts. There were 30 larvae per treatment at the start of the experiment (see Supplementary Table S1). Larvae were reared on infusions of one of four food treatments; (1) quality (animal or leaf detritus) and (2) quantity (high or low) at the following concentrations: animal-low (0.005 g per larva, $n = 12$), animal-high (0.01 g per larva, $n = 21$), leaf-low (0.084 g per larva, $n = 19$) and leaf-high (0.144 g per larva, $n = 22$). These two detritus types and quantities were chosen because they have been shown to have differential effects on mosquito growth and host quality (Yee and Juliano, 2006) and preliminary trials in our laboratory showed that they were sufficient to allow for development to the fourth instar with minimal death. Leaves and insect carcasses are common inputs in these aquatic container habitats where mosquito larvae live and become infected (Fish and Carpenter, 1982; Beier and Craig, 1985; Daugherty *et al.*, 2000). The animal infusion consisted of camel crickets (*Ceuthophilus* spp.) collected at Tyson Research Center (Eureka, MO) dried at 50 °C for 48 h, and homogenized in a kitchen blender. The leaf infusion consisted of mixed species of senescent oak leaves (*Quercus* spp.) collected from the forest floor, dried for 1 week at 50 °C and stored at room temperature. Leaves were cut into one inch squares with galls and petioles removed. The four infusions were steeped for 8 days at 25 °C to allow the growth of microorganisms. Experimental mosquito larvae were reared in a growth chamber at 25 °C with a 14:10 day:light photoperiod.

Larval developmental stage (instar) was monitored daily by looking for the exuviae shed between each molt. Midguts were dissected from fourth instar larvae 9–10 days after the initial hatch and were examined for the presence of gregarine trophozoites (Munstermann and Wesson, 1990). The midguts were photographed at 100× power for further analysis. Larval heads were also removed and stored in 70% ethanol until they could be photographed at 100× magnification and were used as a measure of mosquito size (Alto *et al.*, 2009; Murrell and Juliano, 2013). The images of both midguts and heads were given obscure names so that the researcher measuring them was blind to the treatment identity of the photos.

We measured four response variables for each mosquito larva: total parasite count, total parasite area, mean size of individual parasites and larval head width. Larval mosquito head width (mm) was measured from photos using imageJ (Schneider *et al.*, 2012) and was measured at the outer edge of the eyes which is the furthest two points on the larval head from the dorsal perspective in *Aedes* mosquitoes. The larval head capsule size is fixed soon after eclosion and is a reliable correlate of larval size at the fourth instar (Daly, 1985).

The body size (area, mm^2) of individual parasites was measured from photos using imageJ (Schneider *et al.*, 2012). Parasites were first classified into one of three size categories, (1) large enough to measure with imageJ's automated tracing tool ($n = 1778$, $\geqslant 1.86 \times 10^{-4}$ mm^2), (2) too small to measure with the tracing tool ($n = 2475$, mean $= 1.85 \times 10^{-4}$, standard error $= 9.41 \times 10^{-6}$ mm^2) and (3) discernably smaller than category two ($n = 3381$, mean $= 5.89 \times 10^{-5}$, standard error $= 3.51 \times 10^{-6}$ mm^2). It was possible to measure parasites in the two former categories individually, but due to the time required to complete this, it was not considered feasible. In order to include the two smaller size classes in the analyses, we calculated the average parasite size for the two smaller size classes by individually measuring all of the smaller size-class parasites present in three randomly chosen mosquito larvae; the class two average was calculated among 39 individually measured parasites and the class three average was calculated among 42 individually measured parasites. Once an individual parasite was deemed too small to use the imageJ tracing tool, the parasite was recorded as falling into either size class two or three, determined by visual

https://doi.org/10.1017/S0031182019000994 Published online by Cambridge University Press

observation by one researcher for all parasites. Thus, the response variable 'total parasite area' in a mosquito larva is the sum of all of the individually measured category one parasites, plus the number of category two and three parasites multiplied by their average size. The response variable 'mean parasite size' within a larva is the average size of all the parasites from within individual a mosquito larva. This was calculated by averaging across all size classes by including the sizes of each individually measured category one parasites, in addition to sizes for each of class two and class three parasites where their individual sizes were assigned the average value calculated for each size category (two and three). In addition to calculating mean parasite size, we also tallied the number of parasites within each of the three categories to determine whether the quantity of parasite within each size class was more or less than expected.

Statistical analyses

We restricted our analysis to individual mosquito larvae for which we had collected data for all 4 response variables (total parasite count, total parasite area, mean size of individual parasites and larval head width; $n = 74$). Data were not captured for individual larvae if they died before reaching the forth instar, the midgut dissection was unsuccessful, the head was destroyed during dissection, or if they failed to reach the fourth instar (see Supplementary Table S1 for the distribution between treatment groups).

For the response variables total parasite count, total parasite infection area, mean parasite size and larval head width, we built generalized linear models to determine the effect of detritus quality and quantity on each variable. Additionally, we added our other response variables into the models as covariates (total parasite count, total parasite area and larval head size) where we had *a priori* hypotheses about how larval growth would affect parasite development and how parasitism would affect larval size (Table 1). For example, we predicted that evidence for within-host competition would manifest in a negative correlation between parasite count and individual parasite size. For each model, we chose the distribution that best fit the data and had minimal overdispersion as evaluated by the deviance for each model. For instance, for models fit using a Poisson or a negative binomial error distribution, the distribution that fit the data with lower deviance was selected for the entire model set for that response variable. We used the negative binomial distribution with a log link function for the total parasite count and linear models with normally distributed error for the remainder of the response variables. We created post-hoc qq plots and compared the distribution of model residuals to a randomly generated distribution (either binomial or normal) using chi-square diagnostics in R. We used information theoretic model selection to rank models for each response variable using Akaike's Information Criterion adjusted for a small sample size (AICc) (Burnham *et al.*, 2010). Where appropriate, we conducted post-hoc Tukey's analyses to determine differences in pairwise comparisons for top models.

As a complement to the generalized linear models assessing mean parasite area, we also evaluated frequencies of parasites within each of the three size classes by food type and quantity in a multi-way contingency table using hierarchical log-linear models in R with the base package (Gotelli and Ellison, 2004). We assembled a suite of nine hierarchical models including a fully saturated model and models with each of the variables removed, calculated G^2 statistic for each and ranked individual models using AIC (Table 3, Burnham and Anderson, 2002). To determine whether more or fewer parasites than expected within a given size class were present in larvae exposed to each food type

Table 1. Complete AIC table for the effects of detritus quality and quantity on parasite count, total area of parasites inside a host, mean parasite size, developmental time and larval head width

Effects included	AICc	Delta AICc	Weight of evidence
Total parasite count			
Null	802.1	18.7	0.00
T, A	**783.4**	**0**	**0.56**
T, A, T × A	785.5	2.1	0.19
T, A, HW	785.5	2.2	0.18
T, A, T × A, HW	787.9	4.5	0.06
T, A, T × A, HW, HW × A	789.25	5.85	0.03
T, A, T × A, HW, HW × T	790.21	6.81	0.02
Total parasite infection area			
Null	−354.1	27.4	<0.001
T, A	−380.1	1.4	0.19
T, A, T × A	**−381.4**	**0.0**	**0.37**
T, A, HW	−377.8	3.6	0.06
T, A, T × A, HW	−380.3	1.1	0.21
T, A, T × A, HW, HW × A	−377.9	3.5	0.06
T, A, T × A, HW, HW × T	−378.7	2.7	0.10
Mean parasite size			
Null	−1032.2	45.3	0.00
T, A	−1046.9	30.6	0.00
T, A, T × A	−1052.6	24.9	0.00
T, A, PC, HW	−1059.2	18.3	0.00
T, A, T × A, HW	−1053.0	24.5	0.00
T, A, T × A, HW, HW × A	−1050.6	27	0.00
T, A, T × A, HW, HW × T	−1050.6	27	0.00
T, A, T × A, PC	−1057.2	20.3	0.00
T, A, T × A, PC, PC × A	−1055.1	22.5	0.00
T, A, T × A, PC, PC × T	−1076.6	0.9	0.39
T, A, T × A, HW, PC	−1057.8	19.7	0.00
T, A, T × A, HW, PC, PC × A	−1055.5	22.1	0.00
T, A, T × A, HW, PC, PC × T	**−1077.6**	**0.0**	**0.61**
Larval mosquito head width			
Null	−188.9	44.4	0.00
T, A	−201.3	32.1	0.00
T, A, T × A	**−233.3**	**0.0**	**0.25**
T, A, PC, PA	−197.7	35.7	0.00
T, A, T × A, PA	−232.3	1.1	0.15
T, A, T × A, PA, PA × A	−230.7	2.7	0.07
T, A, T × A, PA, PA × T	−230.0	3.3	0.05
T, A, T × A, PC	−231.0	2.4	0.08
T, A, T × A, PC × A	−229.2	4.1	0.03
T, A, T × A, PC × T	−228.6	4.7	0.02
T, A, T × A, PA	−232.3	1.1	0.15
T, A, T × A, PC	−231.0	2.4	0.08
T, A, T × A, PA, PC	−232.2	1.1	0.14

Models in bold are the best model determined by the lowest AIC score. T, detritus type (quality); A, detritus amount (quantity); HW, head width; PC, parasite count; PA, parasite area. Some models included covariates that were also response variables where we had *a priori* hypotheses about how they might affect the data we collected.

https://doi.org/10.1017/S0031182019000994 Published online by Cambridge University Press

Table 2. Model output and parameter estimates for the best model, determined by the lowest AICc value, for each of the response variables

Source of variation	Estimate	Std. error	*Z* value	*P* value
Total parasite count				
Intercept	3.78	0.18	21.05	<0.001
Detritus type	1.11	0.22	5.09	<0.001
Detritus quantity	−0.39	0.22	−1.77	0.08
Total parasite area			*T* value	
Intercept	0.009	0.004	2.35	0.02
Detritus type	0.03	0.005	5.60	<0.001
Detritus quantity	−0.005	0.006	−0.80	0.43
Detritus type × detritus quantity	−0.02	0.008	−1.89	0.06
Mean parasite size			*T* value	
Intercept	-1.04×10^{-4}	4.05×10^{-4}	−0.026	0.80
Detritus type	-2.52×10^{-4}	8.13×10^{-5}	−3.10	0.003
Detritus quantity	-2.74×10^{-4}	7.18×10^{-5}	−3.82	<0.001
Larval mosquito size (HW)	6.98×10^{-4}	3.91×10^{-4}	1.79	0.08
Total parasite number	-3.15×10^{-6}	5.50×10^{-7}	−5.72	<0.001
Detritus type × detritus quantity	1.79×10^{-4}	9.61×10^{-5}	1.86	0.07
Detritus type × total parasite number	3.06×10^{-6}	6.32×10^{-7}	4.85	<0.001
Larval head width			*Z* value	
Intercept	0.97	0.11	92.05	<0.001
Detritus type	0.12	0.02	7.48	<0.001
Detritus quantity	0.12	0.02	6.36	<0.001
Detritus type × detritus quantity	−0.16	0.03	−6.41	<0.001

and quantity, we assessed complete independence by comparing a saturated model (all possible interactions) and to one containing only additive effects of the three variables. To assess conditional independence among the variables, we compared pairs of models to one another within the hierarchical set; for instance, to determine conditional independence between food type and size class, we compared a model containing the all three possible interactions (food type × size class + food quantity × size class + food type × food quantity) to a similar model with the interaction between the variable of interest removed (e.g. food type × size class + food quantity × size class; a result of non-independence here would indicate the variables food type and food amount are not independent).

Collection of mosquitoes and parasites

Mosquito eggs were collected on seed germination paper taped above the water line in previously established water barrels in the forest at Tyson Research Center. Papers with eggs were dried and stored at 25° C with a 14:10 light:dark photoperiod until use (no longer than 2 months). Eggs were hatched by submersing egg papers in a solution of 0.35 g Difco™ Nutrient Broth (Becton, Dickinson and Company, Sparks, MD) per 1 L deionized water for 24 h. The hatched first instar larvae were rinsed and placed communally in a 7 g L^{-1} mixed-species oak leaf infusion with a small addition of a 1:1 mix of lactalbumen: liver-powder (MP Biomedicals, Solon, OH). After 72 h the larvae had molted to second instar, the first size at which they can be reliably identified to species (personal observation), and *Ae. triseriatus* larvae were identified and added individually to each of 120 experimental microcosms.

A gregarine oocyst suspension was prepared using modified methods of Beier and Craig (1985). To do this, we collected *Ae. triseriatus* larvae from extant water barrels at Tyson Research Center that had previous evidence of gregarine parasite occurrence (unpublished data), in addition to hatching mosquito larvae from field-collected eggs and rearing them in the lab with a previously prepared gregarine oocyst suspension. Pupae were allowed to eclose in a flask covered with netting with the adults left to die on the surface of the water. Contents of the Erlenmeyer flask were homogenized using a kitchen blender and the homogenate was sieved through progressively smaller filters (160 μm, 80 μm, 66 μm and 20 μm). The sieved suspension was transferred to 50 mL conical tubes and concentrated in an Eppendorf 5810R benchtop centrifuge for 7 min at 2500 rpm (978 ×***g***). The precipitate was collected, combined and the number of oocysts per 1 μL were quantified using a haemocytometer.

Results

The best model for total parasite count was the simplest model which included only the main effects of detritus quality and quantity (Table 1). Detritus quality significantly affected the number of parasites but the quantity was not significant (Table 2). Larvae reared with leaf detritus had more than twice the number of parasites than those reared with animal detritus (Fig. 1a). The best model for predicting total parasite area included the effects of detritus quality, quantity and the quality × quantity interaction, although the interaction was not significant (Tables 1 and 2). However, larvae reared with the higher quantity of leaf detritus had higher total parasite area in their guts compared to those reared with the lower amount (Fig. 1b). The best model for

https://doi.org/10.1017/S0031182019000994 Published online by Cambridge University Press

Table 3. Hierarchical models for multi-way contingency analyses of frequencies of each parasite size class within each treatment combination

Model	Model	G^2	Df	*P* value	AIC
1	T + A + SC	5903.8	235	<0.001	5433.8
2	T + A + SC + T × A	5891.8	234	<0.001	5423.8
3	T + A + SC + T × SC	5448.1	233	<0.001	5312.1
4	T + A + SC + A × SC	5814.5	233	<0.001	5348.5
5	T × A + T × SC	5766.1	232	<0.001	5302.1
6	T × A + L × SC	5802.4	232	<0.001	5338.4
7	T × SC + A × SC	5670.1	231	<0.001	5208.1
8	T × A + T × SC + L × SC	5665.7	230	<0.001	5205.7
9	T × A × SC	5663.7	228	<0.001	0

T, detritus type (quality); A, detritus amount (quantity); HW, head width; PC, parasite count; PA, parasite area.
The G^2 statistic results from a likelihood ratio test comparing observed counts of parasites within each size class with the distribution of expected counts. The lowest AIC value indicates the 'best' model fitting the data; the saturated model (model 9) by definition has the best fit/lowest AIC because it accounts for all possible variation.

mean parasite size was the model with the effects of detritus quality, quantity, the quality × quantity interaction, larval head size, total parasite count and the parasite count × detritus quality interaction (Tables 1 and 2). Parasites in larvae reared on high detritus levels were larger than in larvae reared on low detritus levels (Table 2). Hierarchical log-linear models evaluating counts of parasites within size classes revealed significant associations between food type and quantity. A test of complete independence revealed food quality, quantity and size class were not independent (comparison of models 1 *vs* 8, Table 3; $P < 0.001$). Tests of conditional independence revealed food type and food quantity were not independent (comparison of models 7 *vs* 8, Table 3; $P = 0.04$), and food quantity and size class were not independent (comparison of models 6 *vs* 8, Table 3; $P < 0.001$). These significant associations are driven by a higher than expected frequency of size class one and a lower than expected frequency of size class three in the high-quantity leaf food source, in addition to a lower than expected frequency of size class one and higher than expected frequency of size class three in the low-quantity leaf food source (Table 4).

The best model for larval head width included the effects of detritus quality, quantity and the quality × quantity interaction (Table 1). Larvae were largest when reared on the higher quality animal detritus, and larvae reared on leaf detritus were larger in the low quantity of food (Table 2 and Fig. 1c).

Discussion

In this study, we have demonstrated that both the quality and quantity of detritus in aquatic larval habitats affects the number of parasites in a host, the total parasite area within a host, the average size of individual parasites and the relationship between the number of parasites and the size of individual parasites. While detritus quality and quantity were both important, our results showed that each impacted host–parasite interactions in different ways.

We found that gregarine-infected mosquito larvae reared on a high quantity of animal detritus grew larger than those reared on plant detritus or a lower amount of animal detritus. Animal carcasses, primarily dead invertebrates, are a common and important detritus source in larval habitats (Bara *et al*., 2014), and strong positive effects of animal detritus on larval mass and survivorship have been detected in other studies (Daugherty *et al*., 2000; Bara *et al*., 2014; Yee and Juliano, 2006). In addition to animal detritus, plant detritus is a ubiquitous and important component of the food web in both natural and anthropogenic habitats of tree-hole mosquitos like *Ae. triseriatus* (Walker *et al*., 1987; Walker and Merritt, 1988). Larvae feed on the microorganisms (e.g. bacteria, fungi and protists) that decompose detritus in the larval habitat (Fish and Carpenter, 1982). Leaf detritus has been shown to be an inferior resource compared to animal detritus; larvae reared on leaf detritus have decreased mass, survivorship and fitness compared to larvae reared on animal detritus of the same concentration (Yee and Juliano, 2006). The leaching of secondary compounds such as tannins from high concentrations of leaf detritus can also be toxic to aquatic organisms (Mercer, 1993; Mercer and Anderson, 1994; Earl *et al*., 2015). Tannins from leaf detritus have been shown to increase mortality, the duration of the larval period and decrease the adult size of *Aedes albopictus* mosquitoes (Sota, 1993). Thus, larvae reared on higher amounts of animal detritus are generally well-fed hosts likely in better condition than their counterparts reared in either leaf detritus or on lower amounts of animal detritus. In this study, we did not include uninfected control larvae, but we did observe that larvae reared on the highest amount of animal detritus were the largest of the four treatment groups, and for insects, large size is indicative of higher fitness (Kingsolver and Huey, 2008; Costanzo *et al*., 2018).

The quality of larval food source had a greater effect on the total parasite count than did the quantity of food. Larvae reared on animal detritus contained significantly fewer parasites than those reared on the lower quality leaf detritus. As all larvae in this experiment received the same infectious dose from the same population of parasites, the number of parasites present in each host is essentially the number of parasites that were able to infect and establish in the host's midgut. This is a measure of host susceptibility, and is not necessarily a measure of future relative parasite fitness. The high-quality, high-protein animal food diet led to hosts that were less susceptible to infection than hosts fed the lower quality leaf diet.

The total area the parasites occupied in the host was also substantially influenced by the quality of food available, but not by the quantity. Parasites in hosts reared on higher amounts of food had a larger total area than those reared on the lower amount of food. This implies that host susceptibility, in our study system, may be driven by the quality of food, but once a parasite is established in a host, the quantity of available food may be more important for parasite growth.

Both food quality and quantity influenced the average size of individual parasites. Parasites were smaller when in hosts reared on the greater amount of food for both types and were the largest when reared on the high amount of the low-quality food. Parasites were larger in larger hosts overall. Significant positive

https://doi.org/10.1017/S0031182019000994 Published online by Cambridge University Press

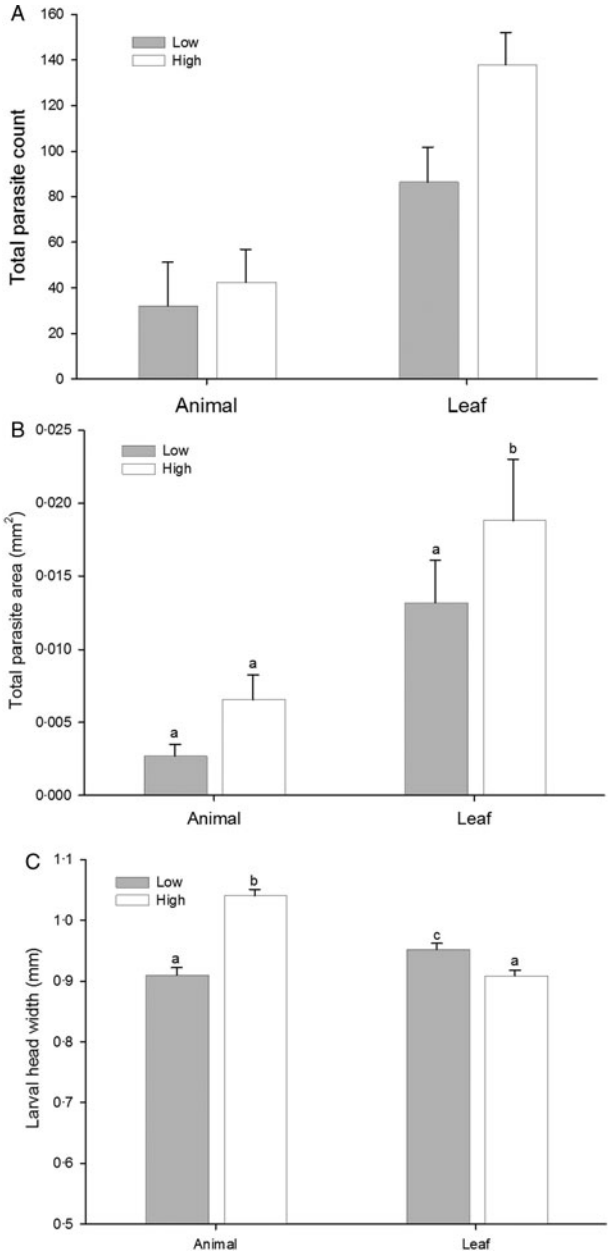

Fig. 1. (A) Means and standard errors for the effect of the two detritus types (animal and leaf) and quantities (low and high) on the total number of parasites in the host, (B) on the total area the parasites occupied in the host, and (C) host size (larval head width). Lower case letters indicate significant differences between bars (Tukey's HSD) and are not indicated for the total parasite count as there was not an appropriate post-hoc test with the negative binomial.

relationships between host and parasite size have been documented in many taxa (Taylor, 1988; Sasall and Morand, 1998; Holfeld, 2000; Tsai *et al.*, 2001; Rowe *et al.*, 2008) and many parasites exhibit strong size–fecundity relationships (Sasall and Morand, 1998; Holfeld, 2000; Rowe *et al.*, 2008). While we do not know if such a size–fecundity relationship exists for *As. barretti*, the gregarine parasite used in this study, it is reasonable to assume that larger individuals of this species have been able to extract more resources and enjoyed more rapid development than their smaller counter parts. We did not measure parasite fecundity in this experiment, but it is probable that larger individuals may also contribute more propagules to the subsequent generation.

We found evidence of within-host competition; there was a negative relationship between the total number of parasites in a host and the average size of parasites. This is consistent with results from a similar mosquito-gregarine system where parasite

Table 4. Multi-way contingency table for counts of parasites within each size class by food quality and quantity

		Parasite size class		
Food treatment	Quantity	I	II	III
Animal	High	169	349	543
	Low	**48****	143	270
Leaf	High	**933***	994	**1107****
	Low	**331****	499	**814***

Associated hierarchical models testing independence among categories and levels are provided in Table 2. Counts in bold represent observed frequencies that are either significantly higher (*) or lower than expected (**) based upon tests of complete and conditional independence between category levels.

growth rates were reduced under high parasite doses (Soghigian and Livdahl, 2017). Previous studies have shown that the addition of competing parasites of the same or different strains can reduce the size of cogeners with-in a host (Wedekind *et al.*, 2000; Fredensborg and Poulin, 2005; Lagrue and Poulin, 2008). There was a significant interaction between food quality and total parasite count which implies that the relationship between parasite count and size is dependent on host food quality. Within-host competition is a widely accepted phenomenon (de Roode *et al.*, 2005; Mideo, 2009), but under which conditions it is expected to occur is much less studied. Additionally, it is not well understood if, or when, these density-dependent effects are driven by competition for limited resources or by top-down effects of the host immune system. Density-dependent effects in another study were more intense for malaria parasites in mosquito hosts that had supplemented nutrition (Wale *et al.*, 2017). However, nematodes infecting rats do not show signs of density dependent regulation in immunocompromised hosts even at different infectious doses (Patterson and Viney, 2002) implying a role for the host's immune system.

While we did not measure any parameters related to host immune function, we speculate that the reduced size of parasites in the animal detritus treatment could be due to an increase in host immune function constraining parasite growth. One important component of the insect immune response against parasites, including gregarines (Comiskey *et al.*, 1999), is encapsulation and melanization (Siva-Jothy *et al.*, 2005). This immune response was shown to decrease linearly as food levels declined in larvae of the mosquito *Anopheles gambiae* (Suwanchaichinda and Paskewitz, 1998). The total number of haemocytes, cells pivotal in many insect immunological pathways, in female *Aedes aegypti* decreased when larvae were reared under nutritional stress (Telang *et al.*, 2012). Additionally, high levels of tannins and other polyphenolic compounds leached from oak leaf detritus (used in this study) degrade the larval midgut epithelium, an important component of larval immune systems (David *et al.*, 2000). Tannins can also inhibit the growth of microorganisms that serve as the food source for larvae and sequester protein in the larval aquatic habitat (Hättenschwiler and Vitousek, 2000). Diets low in protein have been shown to reduce immunological activity in hymenopterans (Brunner *et al.*, 2014) and amphibians (Venesky *et al.*, 2012). Alteration to the immune system, due to high tannin concentrations or reductions in available protein, in hosts fed higher amounts of leaf detritus may also explain the differences we observed in parasite area between the two food quantities.

In this experiment, we provide an example where hosts fed a high-quality diet are inferior hosts for their parasites. We also show that both food quality and quantity impact measures of parasite performance. Quality affected parasite number more

https://doi.org/10.1017/S0031182019000994 Published online by Cambridge University Press

than quantity, while quantity had a greater effect on parasite size. Food quality and quantity are variable in nature, both spatially and temporally. This variability will influence disease dynamics in addition to, as our data suggest, within-host dynamics. Future studies should expand the work presented here by testing many more combinations of food types and amounts to better understand the likely complicated interplay between host nutrition and host–parasite interactions.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182019000994.

Acknowledgements. We thank Thomas Van Horn, Lexie Beckermann and Delilah Sayer for laboratory assistance and Susan Flowers for program support.

Financial support. This work was funded by Tyson Research Center.

Conflicts of interest. None.

Ethical standards. Not applicable.

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