EXPERIMENTALLY ASSESSING THE INFLUENCE OF RESOURCE AVAILABILITY AND SOCIAL AGGREGATION ON THE PARASITES OF RACCOONS

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

EXPERIMENTALLY ASSESSING THE INFLUENCE OF
RESOURCE AVAILABILITY AND SOCIAL AGGREGATION
ON THE PARASITES OF RACCOONS

presented by Ryan Joseph Monello,

a candidate for the degree of doctor of philosophy,

and hereby certify that, in their opinion, it is worthy of acceptance.

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DEDICATION

My family not only made it a lot more entertaining and fun to talk about parasites and raccoons over the last five years, but my wife literally deserves credit for my and our family's very existence. If it were not for a fortuitous series of events between 2000 and 2003 – which included meeting her and going along with her insistence that we apply for the Peace Corps – I would have never walked into the doctor's office seven years ago and found out my heart was in urgent need of some serious work. I have been grateful every day since, and cannot thank her enough for her endless and enthusiastic support throughout our relationship and this dissertation. As one can imagine, there were some interesting moments amidst the pursuit of nocturnal raccoons, deadly parasites, and raising two young boys. I also want to thank my entire family, especially my parents, who taught me how to think independently and have always stood by and supported any direction I have chosen.

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DISSERTATION ABSTRACT

I had three overall objectives in this research. **(1)** I measured the relative importance of host characteristics (age, sex, weight) and abiotic variables (site, season, year) on ectoparasite prevalence (% hosts infested) and abundance and endoparasite species richness across 12 unmanipulated sites in mid-Missouri. In each case, I developed and tested *a priori* hypotheses using an information-theoretic framework. Ectoparasites had distinct patterns that were related to their host specificity, mobility, and ability to survive off the host. Tick (adult *Dermacentor variabilis*) abundance was dependent on the month of collection, as they are only active in summer and peak in July. However, the abundance of engorged ticks, which need to be present on hosts for 7-10 days prior to obtaining a full blood meal, was primarily related to host age and sex, with older raccoons and males infested by more ticks (Chapter 1). Lice (*Trichodectes octomaculatus*) are highly dependent on raccoons and their prevalence and abundance were best predicted by host age and sex. In particular, male raccoons were infested by 2-3x the number of lice compared to females. Fleas (*Orchopeas howardi*), which can use multiple hosts and survive off hosts for weeks at a time, displayed only a weak seasonal pattern of infestation (Chapter 2). Among endoparasites, infection patterns diverged according to their life history; directly transmitted parasites declined over the life of the host while indirectly transmitted parasites increased (Chapter 4). Collectively, these results highlight the need to consider parasite characteristics and simultaneously assess the relative importance of multiple ecological variables between parasite species when describing general trends and constraints of host-parasite associations.

(2) I investigated how experimental increases in social aggregation and resource availability affected ectoparasite prevalence and intensity (number of parasites on infested hosts only) and endoparasite species richness of raccoons. Twelve independent raccoon populations were randomly subjected to differential resource provisions for two years; a clumped food distribution to aggregate hosts (n = 5 populations), a dispersed food distribution to control for the effects of food without aggregating hosts (n = 3), and a no food treatment (n = 4). The intensity of ticks was greater in aggregated populations, particularly among male raccoons. Conversely, the intensity of lice on male raccoons declined in aggregated populations due to greater overdispersion of lice and a larger number of male hosts harboring fewer parasites. The intensity of fleas did not differ among treatments and displayed no correlation with host characteristics (Chapter 3). Among endoparasites, there was strong evidence that food additions decreased the number of indirectly transmitted parasites, particularly among the oldest age classes at sites with clumped food. Conversely, food and social aggregation had little to no impact on the species richness of directly transmitted parasites (Chapter 4). These results suggest that the effects of increased resources and social aggregation of hosts are parasite-specific and can be dependent on parasite mobility and route of transmission, as well as sex-related differences in host behavior or physiology.

(3) I determined sampling constraints of measuring stress hormones (fecal glucocorticoid metabolites, FGM) of raccoons and conducted a parasite-reduction experiment to determine if nematodes and ectoparasites affect baseline levels of FGM in adult free-ranging raccoons. Parasite reduction treatments reduced the prevalence and

abundance of the most widespread ectoparasites, the prevalence of most nematodes, and the number of parasite species per individual. No differences in FGM values were observed within individuals or between treatment and control groups following parasite reduction treatments, indicating that the reductions in nematodes and ectoparasites had no effect on stress hormone levels of raccoons during summer (Chapter 5). Because this study coincided with the most common and energetically expensive ectoparasite in the region (*Dermacentor variabilis*), I conclude that ectoparasites do not affect glucocorticoid levels of raccoons. However, given that helminth parasites are one of the most likely groups to influence the endocrine system, further experimental work should focus on methods that can more effectively reduce all endoparasite species and measure their influence on stress hormone levels across seasons.

PREFACE

This research was conducted from 2005 to 2008. Portions of it were published during the research process and different data sets were used in each chapter. Chapters 1 and 2 analyze parasite data from unmanipulated sites. The sample sizes of these chapters differ because Chapter 1 analyzes data on ticks collected from May to August in 2005-06, while Chapter 2 examines data on lice and fleas from all available months between 2005-07. Both of these chapters have been published (see below) as they appear here.

Chapters 3 and 4 analyze the results of a two year field experiment that was conducted during 2006-07. Sample sizes differ between these chapters because they cover different parasite taxa (ecto- and endoparasites) and the ability to acquire these types of samples and their relevant constraints differed. Sample sizes also differ within these chapters due to sampling constraints; e.g., Chapter 3 limits data on ticks to late spring and summer months (the only time this ectoparasite occurs), but does not limit data on lice and fleas because they occur on animals year-round. Finally , Chapter 5 examines stress hormones and is a completely separate experiment from the above work and was conducted in 2007-08.

Publications to date include (Chapters 1 and 2):

Monello, R.J., and M.E. Gompper. 2007. Biotic and abiotic predictors of tick (*Dermacentor variabilis*) abundance and engorgement on free-ranging raccoons (*Procyon lotor*). Parasitology 134:2053-2062.

Monello, R.J., and M.E. Gompper. 2009. Relative importance of demographics, locale, and seasonality underlying louse and flea parasitism of raccoons (*Procyon lotor*). Journal of Parasitology 95:56-62.

CHAPTER 1: BIOTIC AND ABIOTIC PREDICTORS OF TICK (*DERMACENTOR VARIABILIS*) ABUNDANCE AND ENGORGEMENT ON FREE-RANGING RACCOONS (*PROCYON LOTOR*)[1]

ABSTRACT

I examined the relative importance of abiotic and biotic factors on the ability of adult *Dermacentor variabilis* ticks to attach and engorge with blood across 10 populations of free-ranging raccoons (*Procyon lotor*). I developed *a priori* models that represented explicit hypotheses based on the literature and tested the ability of these models to explain non-replete and replete (fully engorged with blood) tick infestation using generalized linear models and Akaike's Information Criterion. Abiotic models that included month and site of collection clearly provided a better fit for non-replete tick abundance data, while biotic models with host age and sex covariates best fit the replete tick data. Abiotic models of non-replete abundance were superior to biotic models because of large seasonal and site fluctuations in non-replete abundance that masked differences due to host characteristics. Conversely, best-fitting models of replete tick abundance included only age and sex and suggest that once a tick has reached a host, host-parasite interactions are the primary determinant of engorgement by female ticks. Host population structure may have a large influence on potential cohort size of ticks by reducing or increasing the total number and proportion that can become engorged and molt or lay eggs.

INTRODUCTION

The survival, population dynamics, and disease vector competency of ticks are a function of the ability of individuals to (1) survive while off the host until a host is found, and (2) once a host is found, to persist on the host for a sufficient period to gain a blood meal (Strickland et al. 1976, Allan 2001). Factors that influence the ability of ticks to survive off-host and successfully infest and survive on a host are likely to be different. The former has been found to be a function of local site characteristics such as habitat and microclimate (Daniel 1978, Lindström and Jaenson 2003) or host diversity (LoGiudice et al. 2003). Thus, factors affecting the number of ticks that ultimately find a host are likely to be intrinsic to the local environment, with the host itself playing a secondary role. Once a host has been found, however, environmental effects may be of secondary importance relative to the density of ticks on a host (competition among ticks resulting in density dependency), and the host behavioral and physiological response to the attachment (factors intrinsic to the host). Together these factors result in characteristics of hosts being correlated with parasite prevalence and abundance.

Differences due to sex and weight are well documented in a diversity of mammalian host-parasite associations, including helminths (Poulin 1996, Morales-Montor et al. 2004), arthropods (Schalk and Forbes 1997), and unicellular parasites (Moore and Wilson 2002). Male-biased rates of parasitism are primarily attributed to the immunosuppressive effects of male sex hormones (Zuk and McKean 1996, Cox and John-Alder 2007), larger body sizes that support more parasites (Folstad and Karter 1992), or differences in grooming or movement patterns (Mooring et al. 1996, Zuk and

McKean 1996). While sex biases in host parasitism have been detected for ticks (Gallivan et al. 1995, Mooring et al. 1996, Gompper 2004), few studies have examined patterns in the persistence of ticks once on a host, especially in free-ranging host populations. Yet such information is critical to understanding the likelihood of an individual tick obtaining a blood meal, and thereby surviving to reproduce (Wilson et al. 1990), as well as remaining on a host for sufficient time to act as a competent disease vector (Piesman et al. 1987).

In addition to general correlations with host sex and size, three contrasting relationships between host age and parasite abundance have been described: a continual increase over the life of the host; an initial increase until the number of parasites reaches an asymptote and levels off; and an initial increase and peak, followed by a decline in older animals due to host immune response, death of heavily parasitized animals, or other age-related factors (Hudson and Dobson 1995). A continual increase or leveling off of parasite burden with age has been observed for a variety of helminth species in mammals (Halvorsen 1986, Quinnell 1992) and birds (Hudson 1992), but few studies have documented an initial increase in young animals and subsequent decline in older animals (but see Gregory et al. 1992). Logistic difficulties associated with obtaining such data in the field may have contributed to the lack of such observations (Wilson et al. 2003).

Correlation of parasitism to local environmental factors and to host-intrinsic factors such as age or sex and weight suggest the need to study these factors in concert to gain a more refined understanding of the likelihood of ticks persisting on hosts long enough to obtain a blood meal and fully act as a disease vector. To gain insights into

biotic and abiotic factors that may underlie the likelihood of ticks persisting on hosts to obtain blood meals, I examined data on parasitism by the tick *Dermacentor variabilis* from ten host populations of raccoons (*Procyon lotor*). *Dermacentor variabilis* is the largest and most abundant ectoparasite of raccoons in the Midwest U.S. (Whitaker 1982, Kollars et al. 2000). It is a three host tick that is only found on animals while feeding or mating. Once a blood meal is obtained, *D. variabilis* drop off the host, molt to the next stage or lay eggs, and if not an adult, seek another host. This life history results in two distinct 'habitats' or 'environments' during each stage: free-living ticks that are seeking a host (questing) and ticks that have obtained a host and are attempting to mate or become fully engorged with blood (i.e., replete).

My objective is to measure the relative influence of abiotic variables and host characteristics on two components of the tick population, male and non-replete female ticks (hereafter referred to as non-replete) and replete female ticks. Adult female *D. variabilis* must feed continuously for 7-10 days to become fully engorged and markedly distended (Atwood and Sonenshine 1967). I hypothesized that different factors affect non-replete and replete female tick abundance on raccoons. Because free-living adult *D. variabilis* ticks are primarily influenced by local site conditions (Campbell and MacKay 1979, Sonenshine 1991) and time of collection (Kollars et al. 2000), I predicted non-replete infestation will primarily be related to abiotic factors such as site or month of data collection that influence questing ticks. Conversely, I predicted the ability of a female tick to remain on their host and become fully engorged is more likely to be influenced by host characteristics such as age or sex. Such effects are expected to be particularly

important if testosterone is suppressing the immune system, if acquired immunity is occurring, or if other age-related factors predict the extent of parasitism.

MATERIALS AND METHODS

Host Species and Tick Quantification

Raccoons were sampled during summer 2005 and 2006 at 10 locations in central Missouri. All sites were located on state, federal, or university conservation or research areas within 60 km of Columbia, MO. Sites consisted of second growth oak (*Quercus* spp.) and hickory (*Carya* spp.) forest with a maple (*Acer* spp.) and cedar (*Juniperus virginiana*) understory. All sites were >10 km apart, with the exception of two areas that had two sites each that were 4 km apart. To reduce confounding effects of seasonal fluctuations in tick abundance, only animals captured between 20 May and 5 August were included in analyses. Trapping extended beyond these dates, but tick infestations were not consistently observed across all study sites before mid-May or after early August in 2005 or 2006. This is consistent with other studies of raccoons and *D. variabilis* in Missouri (Kollars et al. 2000), and I assumed all animals included in analyses were susceptible to infestation during this time period. Data from recaptured animals were not included in analyses. Research was carried out under Missouri Department of Conservation permit #12869 and University of Missouri Animal Care and Use Protocol #3927.

Trapping occurred at all ten areas in 2005 but I only included data from four of the areas in 2006 because food supplementation treatments were initiated in the other six

areas (see chapter 3). These areas were not included because raccoon behavior and resource availability in these areas was altered, which may influence parasite prevalence and abundance (Wright and Gompper 2005). Traps were baited with mackerel and checked daily. Raccoons were immobilized with an injection of ketamine hydrochloride and xylazine (Evans 2002), marked with metal ear tags, weighed, sexed, and aged by body size, genital morphology, and tooth eruption and wear (Grau et al. 1970, Larson and Taber 1980). Grau et al. (1970) provides tooth wear patterns for five age classes; I = 0-14 months, II = 15-38 months, III = 39-57 months, IV = 58-86 months, and V = over 86 months. I combined ages IV and V due to difficulty distinguishing between these groups (hereafter referred to as IV+), and included a cub category (0-5 months) based on date of capture, weight, and dental characteristics.

Adult *D. variabilis* ticks are distinct, relatively large (3-5 mm in length), and readily found and identified on animals in the field without magnification. I quantified adult *D. variabilis* by a thorough search of the entire body (Kollars et al. 2000, Kollars and Kengluecha 2001), and classified each tick as non-replete (i.e., not engorged with blood), replete (engorged), or semi-replete. I considered a tick to be non-replete if they were not discolored (brown) and similar in width (2-3 mm) to questing adult *D. variabilis* ticks found off host. I considered a tick to be replete when it was obviously engorged with blood, $\geq$ 5 mm width, and discolored (pale, white). Ticks that were not clearly in either category were noted as semi-replete and not included in analyses.

Prevalence and abundance were estimated, respectively, as the number of animals infested by ticks divided by the total number of animals examined, and as the total

number of ticks observed on each individual animal (Bush et al. 1997). I also calculated the ratio of replete:non-replete ticks for each individual host; this measure approximates a proportion of ticks that encounter a host that persist to the point of fully obtaining a blood meal. I used Mann-Whitney U tests to examine sex-related differences in tick abundance in each age category. Separate comparisons were conducted within age classes to ensure statistical differences due to sex would not be masked or biased by divergent patterns in older or younger individuals and reveal the nature of sex*age interactions. Because there were five age-class analyses for each of non-replete tick abundance, replete tick abundance, and the ratio of replete to non-replete abundance, I used a Bonferroni correction ($\alpha = 0.05/5 = 0.01$) to qualify statistically supported relationships. Thus, values of $P \leq 0.01$ were deemed significant, and values of $0.05 > P > 0.01$ were deemed weakly significant.

Model Selection

I used information-theoretic model selection to test *a priori* models predicting non-replete tick abundance and replete tick abundance. Model selection refers to the process of using the observational data to evaluate a suite of models that represent hypotheses. Models are ranked and weighted to evaluate the probability that the model in question is the best fitting model. Although rarely applied in ecological parasitology, model selection has been increasingly used in the broader ecological literature over the last 20 years (Johnson and Omland 2004), and can provide a powerful tool to identify the

most relevant ecological factors influencing dependent variables such as parasite abundance.

I erected a series of explicit hypothesis-based models that relate abiotic and host characteristics to the abundance of parasitizing ticks (Table 1), and ranked the fit of these models using an information-theoretic approach. Such an approach allowed me to identify not only the support for an array of hypotheses, but also to rank the hypotheses and thereby discern the model with the greatest support in explaining parasitism by ticks. Model covariates included three abiotic (site, month, year of collection) and three biotic variables (sex, age, weight of host). The same set of models were used to predict non-replete and replete abundance to evaluate differences in predictive abilities across tick populations as a whole. The number of non-replete ticks was not included as an independent parameter in models of replete tick abundance as I was specifically interested in whether and how biotic and abiotic factors influence replete tick abundance, and including non-replete ticks as a parameter for replete tick models would likely confound the test of the hypothesis that temporal and spatial abiotic factors are important drivers of tick abundance (Burg 2001, see Results).

I tested if non-replete and replete ticks differed from a negative binomial distribution using the maximum-likelihood method of Bliss and Fisher (1953) with the program Quantitative Parasitology 3.0 (Rozsa et al. 2000). I used generalized linear models with a negative binomial distribution for model selection. Tick abundance of individual raccoons was the sample unit and all covariates were fixed effects. I calculated Akaike's Information Criterion (corrected for small sample size; AIC_c) to rank

the models and calculated the difference between the best approximating model (i.e., the model with the lowest AIC_c) and all other models (ΔAIC_c) in the candidate set. Only models with an AIC_c value within two points of the best-fitting model were considered to have substantial empirical support as a best-fitting model (Burnham and Anderson 2002).

RESULTS

Quantitative Patterns

I captured 177 individual raccoons, with an average of 17.7 ± 2.63 (S.E.) animals caught per study area. Prevalence of non-replete ticks did not vary between males and females (92% for both) and, with the exception of cubs (56%), was $\geq$85% for all age classes. Prevalence of replete ticks was also similar among males (63%) and females (65%). Prevalence of replete ticks increased in older animals, ranging from 11-38% for cubs and age class I animals, and 75-78% for age class II and above (Table 2).

Tick distributions did not differ from a negative binomial distribution (non-replete $\chi^2 = 21.67$, df = 22, P = 0.520; replete $\chi^2 = 10.09$, df = 13, P = 0.310). The exponent of the negative binomial (k) indicated that both non-replete (k = 0.890) and replete (k = 0.600) ticks were highly aggregated on raccoons. Replete ticks displayed a greater degree of aggregation, with 10% (n = 18) of hosts harboring 51% of the replete *D. variabilis* (Figure 1). Non-replete tick abundance across all sites combined ranged from 0-95 and averaged 16.27 ± 1.35 (S.E.). Replete tick abundance ranged from 0-21 and averaged 2.54 ± 0.28. For individual across sites, the range of mean ticks per animal was 3.63 to 30.23 for non-replete and 0.42 to 3.69 for replete ticks. There were temporal

differences in the mean abundance for non-replete and replete ticks; both peaked in July, but replete abundance displayed less variation than non-replete abundance (Figure 2). The ratio of replete:non-replete did not differ by month (Kruskal-Wallis $H' = 5.990$, df = 3, $P = 0.112$).

Non-replete tick abundance increased with age, with the exception of females in age class IV+ (Figure 3a). In age class II, males supported a greater number of non-replete ticks than females (Mann-Whitney U test, $P = 0.001$), and there was a weakly significant pattern ($P = 0.037$) for age class IV+. No other sex-related differences were detected among non-replete ticks ($P \geq 0.160$ for all other comparisons), although males consistently had higher tick burdens. Replete tick abundance displayed an increasing trend with age of host; however, both females and males in age class IV+ exhibited declines in replete tick abundance when compared to age class III (Figure 3b). No sex-related differences were detected among replete tick abundance in any age class ($P \geq 0.181$ for all comparisons), although like for non-replete ticks, males consistently had higher tick burdens (excepting cubs).

There was a significant difference among age classes in the ratio of replete to non-replete ticks (Kruskal Wallis $H' = 18.54$, df = 4, $P = 0.001$) (Figure 3c). Post-hoc comparisons indicated significant differences between age classes I and II (Mann-Whitney U test, $P = 0.001$) and I and III ($P = 0.001$), and weakly significant differences between age classes III and IV+ ($P = 0.025$). Within each age class, no sex-related differences were detected for the ratio of replete to non-replete ticks (Mann-Whitney U test, $P \geq 0.054$ for age class II and $P \geq 0.432$ for all other age classes).

Model Selection

Non-replete tick abundance was best predicted by abiotic models (Table 3). The non-replete$_{month+site}$ model was the best fitting model of the data, with no other models falling within 2 AIC_c units; thus this model represents the only model considered to have substantial empirical support as the best fitting model. The weight of evidence (i.e., probability) in favor of non-replete$_{month+site}$ being the best model was 0.68. The non-replete$_{month+site+year}$ model ranked second (ΔAIC_c.= 2.21) and had a weight of 0.22. Global and host characteristic (biotic) models had little support as a best-fitting model (AIC_c weight $\leq$ 0.04 in all cases; Table 3).

Conversely, biotic models provided a better fit for replete tick data. The top five models were based on factors intrinsic to the host, with replete$_{age}$ and replete$_{age+sex}$ both having substantial empirical support as the best fitting model (Table 4). The AIC_c values for these models differed little and both had similar weight of evidence as the best fitting model (replete$_{age}$ AIC_c weight = 0.45, replete$_{age+sex}$ AIC_c weight = 0.36). The only other model with substantial support based on weight of evidence was replete$_{age+sex+weight}$ (AIC_c weight = 0.11). None of the abiotic models had an AIC_c weight > 0.01 (Table 4).

DISCUSSION

I observed clear differences in factors predicting the abundance of non-replete and replete *D. variabilis* on raccoons. Non-replete abundance was primarily a function of month and site of collection, and while a relationship between abundance and host age and sex was observed, these relationships were of secondary importance in predicting

tick abundance on hosts. In contrast, replete tick abundance was best predicted by biotic covariates, in particular host age and sex. Such differences are surprising because age profiles of non-replete and replete abundance were similar, both displaying a positive relationship with age. However, seasonal fluctuations of non-replete abundance were >400% greater than replete abundance. This suggests that for non-replete ticks seasonal and site specific effects overshadow age and sex-specific differences in susceptibility to tick attachment, but factors intrinsic to the host are more important for predicting the abundance of ticks that are able to both reach a host by questing and subsequently persist on the host long enough to become fully engorged.

Previous research has found host-seeking adult *D. variabilis* display large seasonal fluctuations in abundance and are aggregated in habitats and locations where the likelihood of survival and host attachment are best. Kollars et al. (2000) sampled free living adult *D. variabilis* in Missouri and observed a peak in July with similar magnitudes of difference between months to that observed in this study. These patterns are also consistent with those observed in the northeastern North America (Campbell 1979, McEnroe 1979, McEnroe and Specht 1987). In contrast, adult *D. variabilis* in the Southeast U.S. display bimodal or multimodal activity patterns due to the large numbers of adult ticks that successfully overwinter and attach to hosts in spring (Sonenshine and Stout 1971, McEnroe 1974, Sonenshine 1979, Newhouse 1983, Carroll and Nichols 1986, McEnroe and Specht 1987, Burg 2001). Based on seasonal fluctuations in this study and Kollars et al. (2000), Missouri populations appear to primarily consist of overwintering larvae that result in summer cohorts of adults. Burg (2001) suggests

summer cohorts of *D. variabilis* may have greater energy reserves than overwintering adults. Future research should determine if this results in higher rates of host attachment, feeding success, or transovarial disease transmission via greater egg mass size and zoonotic amplification of tick-borne pathogens such as Rocky Mountain spotted fever (*Rickettsia* spp).

Site and month of collection were the primary influence on non-replete tick abundance, but there were consistent increases in tick burden through age class III (Figure 3) (declines in age class IV+ are discussed below). This suggests there may be age-related behavioral or physiological factors that influence tick infestation (as there are with sex-related differences). The most likely explanation is that the youngest animals (age classes C and I) may receive the benefits of grooming from adult females that they den and travel with, while for older animals (age classes II – IV+) greater movements may increase susceptibility to infestation. Therefore, on a smaller scale (within a site vs. between sites), age is of greater relative importance than sex when considering tick infestation in general. Hudson (1992) found tick intensity declined in red-grouse chicks after two weeks when Louping ill virus was present, but continually increased in the absence of this disease. A variety of patterns have described for helminths and mammals (e.g., Halvorsen 1986, Gregory et al. 1992, Quinnell 1992); however, I know of no studies that have examined the influence of age on tick acquisition in free-living mammals. Further investigation is needed to determine whether such relationships are common.

The finding that the best-fitting models of replete tick abundance included age and sex and no abiotic covariates suggest that once a tick has reached a host, host-parasite interactions are the primary determinant of full engorgement by female ticks, independent of where the host is found. This is also supported by the observations that replete tick abundance exhibited little difference between months and a greater degree of aggregation than non-replete abundance. Several mechanisms may underlie the prominent role of host characteristics, including acquired immunity, tick-associated mortality of hosts, or the effect of density dependence on numbers of ticks on a host. Previous research has found a variety of mammals to acquire resistance to tick infestation (Wikel 1996, Hughes and Randolph 2001, Castagnolli et al. 2003), including *D. variabilis* (Trager 1939, denHollander and Allen 1985). Raccoons have been found to develop immunity to *Ixodes scapularis* (Craig et al. 1996), but this has not been examined for *Dermacentor* spp. These results do not, however, support acquired immunity as a reason for including age in the best-fit model of replete ticks, for although I observed a decline in the absolute number and proportion of replete tick abundance in older age classes (Figures 3,4), acquired immunity is predicted to occur at a much earlier age. Craig et al. (1996) found a significant decline in the proportion of engorged *Ixodes scapularis* larvae within two months of initial tick infestation, and antibody production displayed a two- to ten-fold increase within weeks of infestation. Other studies also suggest immunity to ticks develops in mammals and birds within weeks or months (Hudson 1992, Hughes and Randolph 2001, Castagnolli et al. 2003). Acquired immunity might be perceived as underlying the decline in the abundance of replete compared to non-replete ticks

observed between months (excepting August for which sample sizes were small; Figure 2). However, this data disassociated the abundance of these two classes of ticks on individual hosts. When the ratio of replete:non-replete ticks on individual hosts is examined across months, there is no temporal decline in the likelihood of a tick persisting to become engorged.

The decline of replete ticks in the oldest age class suggests the most heavily infested older animals may die earlier, perhaps due to persistently high tick burdens or due to causes that correlate with, or result in, higher tick burdens. *Dermacentor* spp. are known to cause tissue damage, anemia, and paralysis in domestic animals (Strickland et al. 1976, Allan 2001). Hawlena et al. (2006) observed age-related differences in flea induced mortality of rodents; thus while tick-driven effects have not been evaluated in natural hosts, older animals are likely to be more susceptible to such impacts. Alternatively, ticks may be more abundant on less healthy animals which have higher mortality rates. Ticks may also have indirect effects by transmitting diseases or creating sites suitable for bacterial infection. Problematically for the explanation that declines in replete tick abundances in older age classes may be a function of host mortality, however, is the finding that non-replete tick burdens also decline among the oldest females (but not males). Such a pattern seems unlikely to derive from host mortality.

Lack of variation in replete compared to non-replete abundance may be due to ticks reaching their carrying capacity and exhibiting density dependence due to limited host resources or intraspecific interactions. Competition between ectoparasites is not expected to have a significant influence on rates of infestation because many species

spend long periods of time off the host, and thus it has received little research attention (Krasnov et al. 2005). Adult *Dermacentor* ticks exhibit high prevalence (Kollars et al. 2000; this study), however, and are located almost exclusively on the head of raccoons, primarily on the back of the neck and in and around the ears (R. Monello, unpublished observation). Raccoons can presumably self-groom all other locations and thus space may be limited for tick attachment sites. In addition, several studies have documented the effect of density dependence on numbers of ticks on hosts that can develop acquired immunity (Randolph 1994) and competition for food between and within hematophagous flea species (Tripet and Richner 1999; Krasnov et al. 2005).

Although differences in parasitism between males and female raccoons were not statistically significant within most age classes, the consistent trend of greater tick burdens in males was important in model formulation. The lack of large sex-related differences within age classes is not surprising as male raccoons in Missouri are only ~12% larger than females (Lotze and Anderson 1979) and the degree of male-biased parasitism is often associated with sexual dimorphism (Moore and Wilson 2002). In addition, *D. variabilis* parasitize raccoons pre- and post-parturition, and the immunosuppressive effects of hormones or energy required during pregnancy or lactation can increase rates of parasitism in females (Festa-Bianchet 1989, Dobson and Meagher 1996).

Factors that influence the ability of female ticks to feed to repletion may impact tick population and disease dynamics because female egg mass is related to the amount of blood ingested (Strickland et al. 1976, Allan 2001), and transovarial pathogen

maintenance of *Rickettsia* spp. (including *R. rickettsii*, i.e., Rocky Mountain spotted fever) and *Francisella tularensis* has been documented in *Dermacentor variabilis* (Macaluso et al. 2002, Goethert and Telford 2005, Parola et al. 2005). Thus, while environmental factors are of importance during free-living stages, host population structure may have a large influence on potential cohort size of ticks by reducing or increasing the total number and proportion that can become engorged and molt or lay eggs. Such effects may be particularly important when the host is the primary feeding source for the tick and when individuals exhibit large differences in susceptibility or response to parasitism as a function of sex and age. Further research on host-tick interactions should focus on mechanisms underlying these patterns and the ability of ticks to become engorged.

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Table 1. *A priori* models used to estimate non-replete and replete *Dermacentor variabilis* tick abundance of raccoons in Missouri (β_0 = intercept, $\beta_i(X)$ are the parameters of independent variables).

Hypothesis	Model	Model structure
Host characteristics		
Differences are due to host characteristics	Age + Sex + Weight	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Sex}) + \beta_3(\text{Weight})$
Differences are due to host age	Age	$\beta_0 + \beta_1(\text{Age})$
Differences are due to host sex	Sex	$\beta_0 + \beta_1(\text{Sex})$
Differences are due to host weight	Weight	$\beta_0 + \beta_1(\text{Weight})$
Differences are due to host age and sex	Age + Sex	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Sex})$
Age-related differences are related to host sex	Age*Sex	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Sex}) + \beta_3(\text{Age*Sex})$
Differences are due to host weight and sex	Weight + Sex	$\beta_0 + \beta_1(\text{Weight}) + \beta_2(\text{Sex})$
Weight-related differences are related to host sex	Weight*Sex	$\beta_0 + \beta_1(\text{Weight}) + \beta_2(\text{Sex}) + \beta_3(\text{Weight*Sex})$
Abiotic factors		
Differences are due to abiotic factors	Month + Site + Year	$\beta_0 + \beta_1(\text{Month}) + \beta_2(\text{Site}) + \beta_3(\text{Year})$
Differences are due to month and study site	Month + Site	$\beta_0 + \beta_1(\text{Month}) + \beta_2(\text{Site})$
Differences are due to month	Month	$\beta_0 + \beta_1(\text{Month})$
Global		
Differences are due to host characteristics and abiotic factors	Age + Sex + Weight + Month + Site + Year	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Sex}) + \beta_3(\text{Weight}) + \beta_4(\text{Month}) + \beta_5(\text{Site}) + \beta_6(\text{Year})$

Table 2. Prevalence (%) of *Dermacentor variabilis* on raccoons. Raccoon age class designations are cub = 0-5 months, I = 5-14 months, II = 15-38 months, III = 39-57 months, IV+ = $\geq$ 58 months Sample sizes (*n*) represent number of individual raccoons.

Ticks	All	Males	Females	Cubs	I	II	III	IV+
Non-replete	92%	92%	92%	56%	95%	95%	97%	85%
Replete	64%	63%	65%	11%	38%	75%	78%	75%
n	177	75	102	9	37	79	32	20

Table 3. Ranking of *a priori* models estimating abundance of non-replete *Dermacentor variabilis* adult ticks on raccoons. Rankings are based on Generalized Linear Models with a negative binomial distribution (n = 177). *A priori* models not included in the table had an AIC_c weight = 0.

Model	log (l)[a]	k[b]	ΔAIC_c[c]	AIC_c weight
Month + Site	-657.09	13	0.00	0.68
Month + Site + Year	-657.01	14	2.21	0.22
Weight + Sex	-670.92	3	5.56	0.04
Month	-670.26	4	6.35	0.03
Weight	-672.52	2	6.69	0.02
Age + Sex + Weight	-668.95	7	10.15	0.00

[a]Maximized log-likelihood value

[b]The number of model parameters

[c]The lowest AIC_c score was 1342.41

Table 4. Ranking of *a priori* models estimating abundance of replete female *Dermacentor variabilis* adult ticks on raccoons. Rankings are based on Generalized Linear Models with a negative binomial distribution (n = 177). *A priori* models not included in the table had an AIC_c weight = 0.

Model	log (l)[a]	k[b]	ΔAIC_c[c]	AIC_c weight
Age	-413.86	5	0.00	0.45
Age + Sex	-413.18	6	0.48	0.36
Age + Sex + Weight	-413.16	7	2.91	0.11
Weight	-419.54	2	5.06	0.04
Weight + Sex	-419.35	3	6.77	0.02
Month	-418.47	4	7.08	0.01
Age*Sex	-412.16	10	7.56	0.01
Weight*Sex	-419.23	4	8.61	<0.01

[a]Maximized log-likelihood value

[b]The number of model parameters

[c]The lowest AIC_c score was 838.08

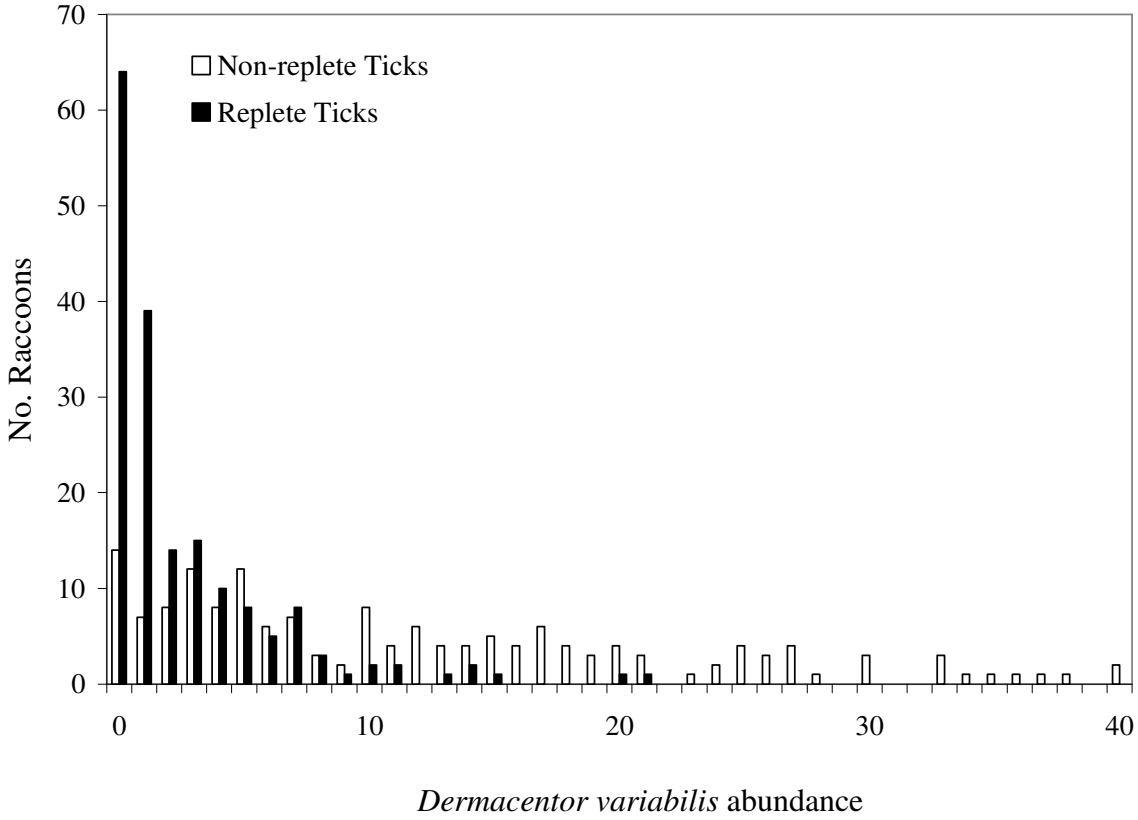

Figure 1. Number of non-replete and replete *Dermacentor variabilis* adult ticks infesting raccoons in mid-Missouri. Note: 15 raccoons had > 40 non-replete ticks (range = 41 to 95). These data were removed for figure clarity.

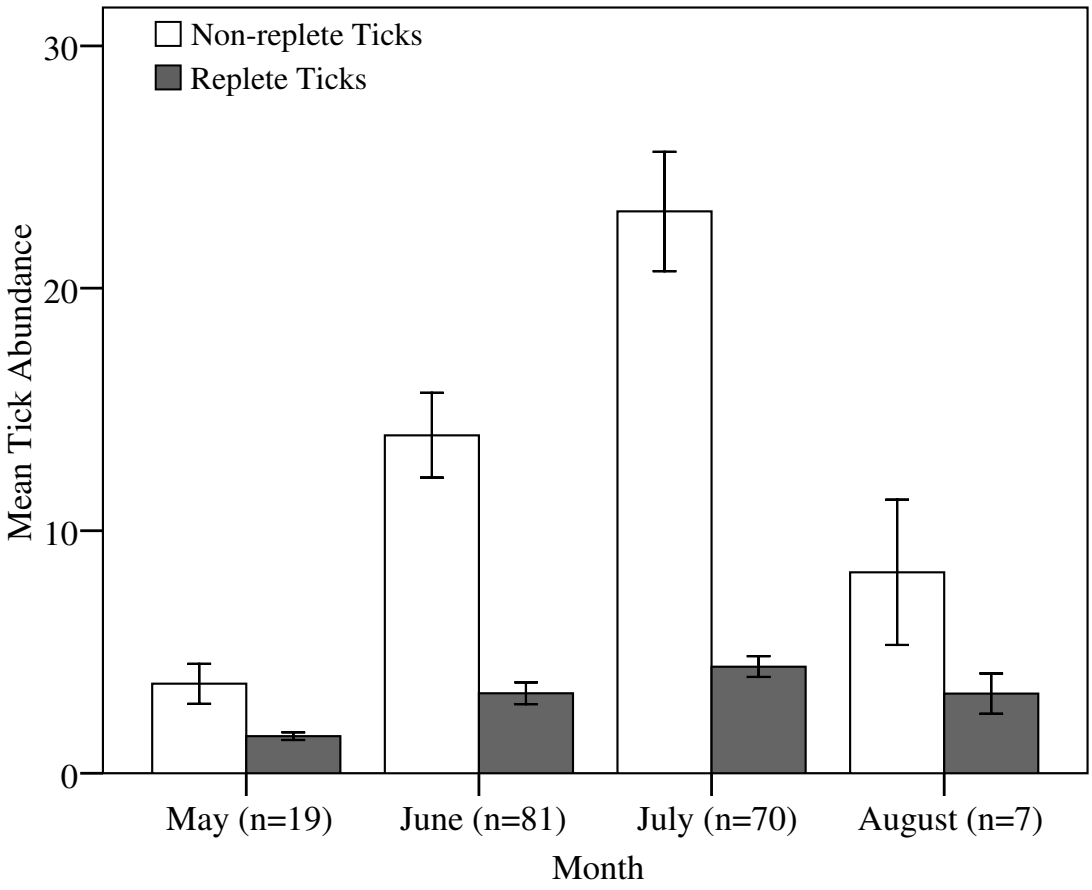

Figure 2. Mean (± S.E.) seasonal abundance of *Dermacentor variabilis* adult ticks on raccoons in Missouri during 2005 and 2006. Sample sizes represent number of animals captured in each month.

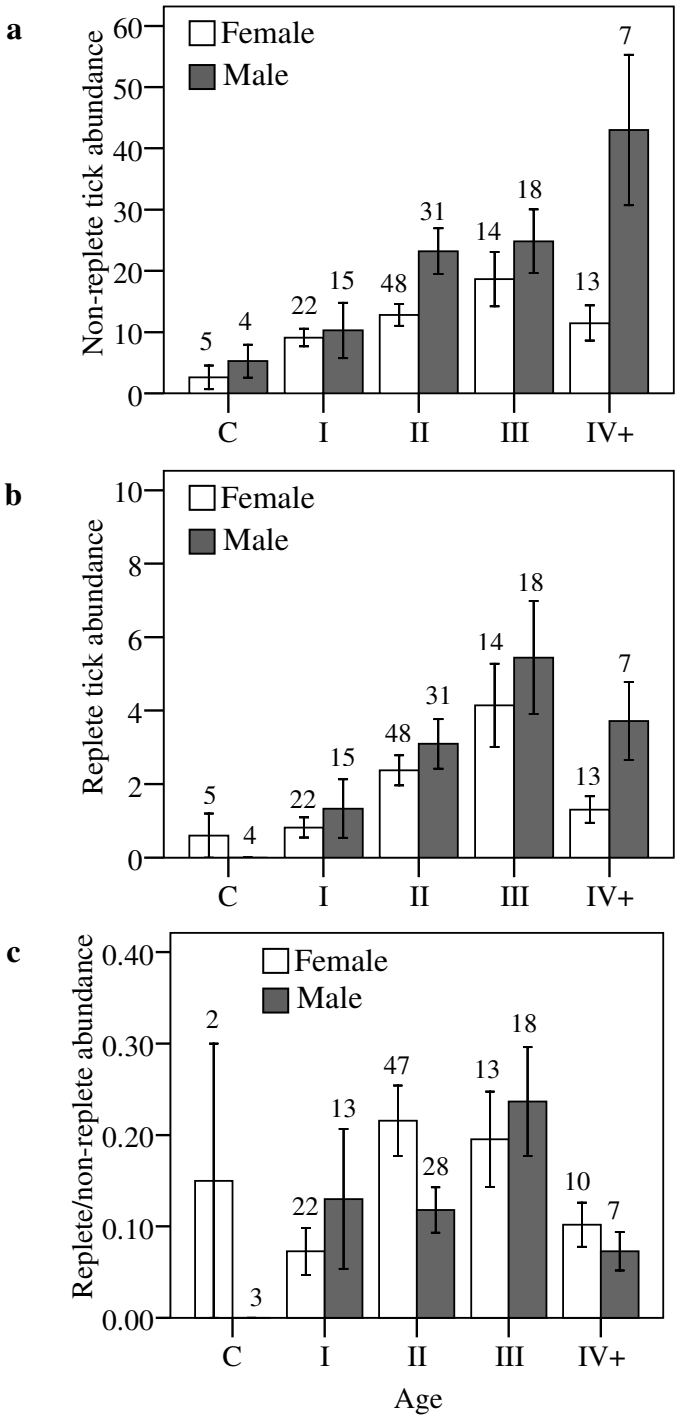

Fig. 3. Mean (± S.E.) abundance of (a) non-replete and (b) replete *Dermacentor variabilis* ticks; and (c) the ratio (± S.E.) of replete to non-replete *D. variabilis* ticks on raccoons. Sample sizes are shown above S.E.

CHAPTER 2: RELATIVE IMPORTANCE OF DEMOGRAPHICS, LOCALE, AND SEASONALITY UNDERLYING LOUSE AND FLEA PARASITISM OF RACCOONS (*PROCYON LOTOR*)

ABSTRACT

A variety of demographic, seasonal, and site-specific variables may influence parasitism, but the relative importance of these variables is generally unclear. I measured the relative ability of host characteristics, season, and site to explain louse (*Trichodectes octomaculatus*) and flea (*Orchopeas howardi*) infestation across 10 populations of raccoons (*Procyon lotor*). Lice are highly dependent on specific hosts and predicted to display a relatively strong relationship with factors intrinsic to the host when compared to fleas, which can infest multiple species and survive off host for weeks without feeding. I developed *a priori* models that represented explicit hypotheses and contrasted their ability to predict infestation patterns. While the abundance of lice was seasonal, models that included solely host age and sex best predicted prevalence and abundance, in part because males were infested with 3 times the number of lice than females. Conversely, flea prevalence and abundance, which peaks sharply in the spring, was best predicted by season; factors intrinsic to the host were relatively unimportant for predicting abundance. These and other recent findings emphasize the need to simultaneously assess the relative importance of multiple ecological variables between parasite species when attempting to describe general trends and constraints of host-parasite associations.

INTRODUCTION

A variety of host characteristics and environmental factors are correlated with macroparasite prevalence and abundance. Identifying such correlations is fundamentally important because predicting the likelihood of an individual being parasitized is a tenet of ecological parasitology and central to understanding if and how these parasites influence host populations. However, correlation identification is frequently done in isolation, with little attention given to the relative influence of important covariates. For example, in mammals, males tend to exhibit greater parasite burdens than females (Wilson et al. 2002), but these differences may be ecologically insignificant when compared to the influence of other abiotic and biotic factors. Recent work indicates, for instance, that models of tick abundance that are based on spatial or temporal factors can be superior to models based on host characteristics even when obvious correlations between the extent of parasitism and age and sex exist. Furthermore, for subsets of the same parasite population, such as those ticks that remain on a host long enough to gain a blood meal, the reverse is true; factors such as age and sex of the host become of primary importance (Monello and Gompper 2007).

Here, I expand on this theme by addressing the relative importance of multiple biotic and abiotic factors for predicting ectoparasite abundance. Ectoparasites such as lice (Phthiraptera) and fleas (Siphonaptera) must cope with both host defenses and environmental influences and are, therefore, well suited for evaluation of the relative import of such factors. Comparisons between species that co-exist on the same host are particularly useful for determining if differences exist between orders that have direct life

cycles, but vary in their ecological constraints. Lice are permanent, host-specific parasites that cannot survive off the host for more than a few hours. Their entire life cycle typically takes place on a single host and consists of eggs, larvae, and adults. Transfer of lice between mammals only occurs when two hosts are in close contact (Durden 2001). Conversely, fleas can infest multiple species of hosts and are relatively mobile compared to lice (Marshall 1981). Their life cycle includes eggs, larvae, pupae, and adults, with only the adults found on hosts. Adult fleas are obligate blood feeders but may frequently leave their host and have been described as permanent satellites (Krasnov et al. 2002), which can persist off host for weeks or months without feeding (Marshall 1981).

The abundance of lice and fleas has been correlated with an array of independent factors, including season, site, and host characteristics. Fleas often display strong seasonal trends that are correlated with extreme temperature or humidity (Osácar et al. 2001, Soliman et al. 2001a, Krasnov et al. 2002), but may also differ among habitats or as a function of related factors such as site productivity (Krasnov et al. 1997, Krasnov et al. 1998). Conversely, apparent seasonal differences in the prevalence and abundance of lice have been ultimately attributed to factors such as fluctuations in host density or population size (Soliman et al. 2001a), although few studies have addressed this topic.

Studies on sex-biased patterns of parasitism by fleas and lice have primarily been conducted on small mammals and results have been inconsistent. There is support for both male (Schalk and Forbes 1997, Soliman et al. 2001b, Anderson and Kok 2003, Perez-Orella and Schulte-Hostedde 2005) and, to a lesser degree, female-biased patterns

of parasitism (Dick et al. 2003, Krasnov et al. 2005). Sex-related differences may also be seasonal (Krasnov et al. 2005) or non-existent (Clemons et al. 2000, Hawlena et al. 2006). Age-biases in the likelihood and extent of parasitism also occur, although this topic has received less attention. Parasites have generally been suggested to follow one of three patterns of age-related parasitism: a continual increase over the life of the host; an initial increase until the number of parasites reaches an asymptote and levels off; or an initial increase and peak, followed by a decline in older animals due to host immune response, death of heavily parasitized animals, or other age-related factors (Hudson and Dobson 1995). However, these general patterns are primarily based on helminth or microparasite infections. Few studies have documented age-related patterns of ectoparasitism. Krasnov et al. (2006) found that flea abundance increased with host age among small mammals, but the number of fleas per unit body size decreased, suggesting that parasitism exhibited a relative decline in older age classes. Conversely, Hawlena et al. (2006) observed juvenile gerbils (*Gerbillus andersoni*) had greater infestations of fleas than adults. Other ectoparasites (ticks, mites) exhibit greater infestation levels among adults, sometimes with decreases in the oldest age classes (Hawlena et al. 2006, Monello and Gompper 2007).

My objective was to identify correlations as well as measure the relative influence of host characteristics (age, sex, size), site, and season on louse and flea infestations of free-ranging raccoons (*Procyon lotor*). Raccoons are considered to be the sole host of the louse *Trichodectes octomaculatus* (Whitaker 1982). The most common flea (*Orchopeas howardi*) of raccoons is typically found on squirrels (*Sciurus* spp*., Tamiasciurus*

hudsonicus, Glaucomys volans), but has also been observed on at least 5 other mammals (Mohr and Morlan 1959, Whitaker 1982, Kollars et al. 1997). Ecological relationships between these ectoparasites and raccoons, or indeed any carnivores, have not been well described. I hypothesized that although both fleas and lice display direct life cycles, different factors will affect their prevalence and abundance due to differing abilities to use alternate hosts and persist off host. Because lice are host-specific and dependent on their host for survival at all life stages and times, I predicted that infestation patterns would be closely related to host characteristics. Conversely, I predicted flea abundance and prevalence would be relatively independent of host characteristics compared to lice.

MATERIALS AND METHODS

Louse and Flea Quantification

Raccoons were sampled from March to November 2005-2007 at 10 locations in central Missouri. All sites were located on forested state, federal, or university conservation or research areas within 60 km of Columbia, Missouri. Sites consisted of second growth oak (*Quercus* spp.) and hickory (*Carya* spp.) forest with a maple (*Acer* spp.) and cedar (*Juniperus virginiana*) understory. All sites were >10 km apart, with the exception of two areas that had two sites each, that were 4 km apart. Over the course of the study, no animals were observed to move between sites.

Trapping occurred at all 10 study sites in 2005, but I only included data from four of the sites in 2006 and 2007 because food supplementation treatments were initiated in the other six sites (see chapter 3). These sites were not included because raccoon

behavior and resource availability in these sites were altered, which may influence parasite prevalence and abundance (Wright and Gompper 2005). Traps were baited with mackerel and checked daily. Raccoons were immobilized with an injection of ketamine hydrochloride and xylazine (Evans 2002), marked with metal ear tags, weighed, sexed, and aged by body size, genital morphology, and tooth eruption and wear (Grau et al. 1970, Larson and Taber 1980). Grau et al. (1970) provided tooth wear patterns for five age classes; I=0–14 mo, II=15–38 mo, III=39–57 mo, IV=58–86 mo, and V=over 86 mo. I combined ages IV and V due to difficulty distinguishing between these groups (hereafter referred to as IV+), and included a cub category (0–5 mo) based on date of capture, weight, and dental characteristics. Data from recaptured animals were not included in analyses.

I quantified the relative abundance of lice and fleas via 10 strokes with a flea comb from the base of the neck to the base of the tail on the dorsal region of raccoons (similar to Clayton and Drown 2001). Animals that were wet or muddy were not included in the study. Ectoparasite samples were immediately separated from the flea comb in a plastic bag, which was sealed and frozen within eight hours. In the laboratory, lice and fleas were examined using a dissecting scope and identified to species (Whitaker 1982).

The measures of parasitism used here follow the definitions of Bush et al. (1997): prevalence was estimated as the number of animals infested by lice or fleas divided by the total number of animals examined; relative abundance represented the total number of lice or fleas observed on each individual animal; and relative intensity was equal to the

total number of lice or fleas observed on each infested animal. The relationship of site, season, host sex, age, and weight to the presence of lice or fleas was evaluated by entering all variables into a single logistic regression model for each ectoparasite. Three seasons were used in the analyses; spring (March-May), summer (June-August), and fall (September-November).

I tested for sex-related differences in the relative abundance of lice and fleas. To ensure I was measuring the effects of sex and not size-related differences, I tested for differences in the relative abundance of lice or fleas per 1 cm^2 of body surface by dividing the number of lice or fleas by body mass (in g) to the power of 0.667 (Krasnov et al. 2006). I used Mann-Whitney *U*-tests and made separate comparisons within age classes I-IV+ to ensure statistical differences due to sex would not be masked or biased by divergent patterns in older or younger individuals and reveal the nature of sex*age interactions. Because there were 4 age class analyses for louse and flea relative abundance per 1 cm^2 of body surface (cubs were not examined), I used a Bonferroni correction ($\alpha = 0.05/4 = 0.0125$) to qualify statistically supported relationships. Thus, values of $P \leq 0.0125$ were deemed significant.

Model Selection

I used information-theoretical model selection to test the ability of *a priori* models to explain the variation of louse and flea abundance. Model selection uses observational or experimental data to evaluate a series of models that each represent distinct hypotheses. Models are ranked and weighted to assess the probability that the model in

question is the best fitting model. Model selection approaches are rarely applied in ecological parasitology, but are increasingly used in the broader fields of theoretical and applied ecology (Johnson and Omland 2004), and provide a powerful mechanism for identifying which ecological factors best explain the variability in a dependent variable such as ectoparasite abundance.

I constructed a series of explicit hypothesis-based *a priori* models that related host characteristics (age, sex, weight), site, and season to the abundance of lice or fleas (Table 1), and ranked the fit of these models using an information-theoretic approach. The same set of models were used to examine louse and flea abundance to contrast the relative importance of these independent parameters for the two taxa. Such an approach allowed me to identify not only the support for an array of hypotheses, but also to rank the hypotheses and thereby discern the model with the greatest support in explaining parasitism by lice or fleas. Models were initially divided into host characteristics and site or season to examine the relative importance of these model types. Once the best host and site or season models were determined, I also examined a post-hoc model that combined these to determine if their fit could be further improved. For lice, the post-hoc model was age+sex+season and for fleas it was sex+season (see also Table 1).

I tested whether lice and fleas differed from a negative binomial distribution using the maximum-likelihood method of Bliss and Fisher (1953) with the program Quantitative Parasitology 3.0 (Rozsa et al. 2000). I used generalized linear models with a negative binomial distribution for model selection. The use of a negative binomial distribution ensures that model selection is conducted with an error structure that is

consistent with the overdispersed nature of parasite populations (Wilson and Grenfell 1997). Louse or flea abundance (+1) of individual raccoons was the sample unit and all covariates were fixed effects. I increased the relative abundance of each sample by +1 to facilitate model convergence, which was initially problematic due to the large number of zeros in the dataset. I calculated Akaike's Information Criterion (corrected for small sample size; AIC_c) to rank the models and calculated the difference between the best approximating model (i.e. the model with the lowest AIC_c) and all other models (ΔAIC_c) as well as Akaike's weights for each model in the candidate set (Burnham and Anderson 2002). Models with an AIC_c value within two points of the best-fitting model were considered to have substantial empirical support as a best-fitting model. Akaike's weights for each model represent the probability of a model being the best approximating model of those evaluated (Burnham and Anderson 2002). I used SPSS 15.0 (Chicago, Illinois) for all model selection procedures.

RESULTS

Infestation Patterns

I captured 252 individual raccoons, with a mean of 45.8 ± 21.04 (S.E.) raccoons per study site. Capture effort was not equal among seasons, with 77% of captures (n = 196) occurring in summer, and 11% in both the spring (n = 29) and fall (n = 27). One chewing louse (*Trichodectes octomaculatus*, prevalence = 50%) and 2 flea species (*Orchopeas howardi,* prevalence = 11%*; Chaetopsylla lotoris*, prevalence = 5%) were found on raccoons (Table 2). Subsequent results only focus on *T. octomaculatus* and *O.*

howardi due to the low prevalence of *C. lotoris*. *Trichodectes octomaculatus* were present at all study sites and *O. howardi* was identified at 9 of the 10 study sites. Prevalence of *T. octomaculatus* was 72% for males and 33% for females, and was lowest among cubs (6%), highest in age class I (67%), and generally stable after age class II (age classes II-IV+ = 46-53%). *Orchopeas howardi* infested 13% of males and 9% of females and exhibited similar age patterns as lice, with no cubs infested (n = 17)**,** and age class I exhibiting the highest prevalence (16%) (Table 2). Prevalence of *T. octomaculatus* displayed relatively little variation from spring to fall (41-51%), while prevalence of *O. howardi* was greatest in spring (48%) and ranged from 4-6% in the summer and fall seasons. Logistic regression models indicated that age ($P < 0.001$) and sex ($P < 0.001$) explained a significant amount of the variation in *T. octomaculatus* presence ($\hat{g}[\chi] = 1.028+24.540[age]+26.786[sex]$), and season ($P < 0.001$) explained a significant amount of the variation in *O. howardi* presence ($\hat{g}[\chi] = -18.846+17.203[season]$).

The distribution of louse and flea relative abundance did not differ from a negative binomial distribution (*T. octomaculatus* variance/mean ratio = 15.48, $\chi^2 = 16.55$, $P = 0.554$; *O. howardi* variance/mean ratio = 2.04, $\chi^2 = 0.78$, $P = 0.677$). Relative abundance of *T. octomaculatus* ranged from 0 to 68 and averaged 3.25 ± 0.45 (S.E.) per animal (mode of relative abundance = 0; mode of relative intensity = 2). Relative abundance of *O. howardi* ranged from 0 to 4 per animal and averaged 0.18 ± 0.04 per animal (mode of relative abundance = 0; mode of relative intensity = 1). Both species exhibited temporal fluctuations in abundance, in particular *O. howardi,* for which

abundance was more than an order of magnitude greater in the spring than summer and fall (Figure 1). Patterns of relative intensity were nearly identical to those of relative abundance described here.

Relative abundance of *T. octomaculatus* was lowest in the cub age class**,** but increased sharply thereafter, with similar values of abundance occurring in age classes I and greater (Figure 2a). Age-related patterns of relative abundance for *O. howardi* were similar, although the S.E.:mean ratios were greater than for *T. octomaculatus* for most age and sex classes (Figure 2b). Males supported a greater number of *T. octomaculatus* per 1 cm^2 than females in age classes II and III (Mann-Whitney *U*-test, $P < 0.001$ for both comparisons), and there was a weakly significant pattern for age class I and IV+ ($P \leq 0.017$ for both comparisons) (Figure 2a). Overall, male raccoons harbored more than 3 times the abundance of lice than female raccoons (males = 5.32 ± 0.73; females = 1.16 ± 0.52). No sex-related differences were detected for *O. howardi* abundance per 1 cm^2 in any of the within age comparisons ($P \geq 0.269$ for all 4 comparisons; Figure 2b). Patterns for louse and flea relative intensity for each age class was nearly identical to those graphs of relative abundance unadjusted for body size (see Figure 2).

Model Selection

Relative abundance of *T. octomaculatus* was best predicted by host characteristics; all best fitting models included host age and sex, and one included weight (Table 3). The weight of evidence, i.e., the probability that the best fitting model was either age+sex or age*sex was 0.76. The only other model with substantial support was

age+sex+weight (weight of evidence = 0.22). However, raccoon weight was not well correlated with *T. octomaculatus* abundance ($r^2 \leq 0.051$ for linear, quadratic, and cubic curve fits). The performance of the age+sex+weight model was likely because cubs were not infested with lice (thus animals with the lowest weights all had a relative abundance of 0-1) and because this model only increased the number of parameters in the model by a value of one (increasing parameters are penalized in AIC rankings), resulting in a fit similar to the age+sex model. The intercept-only model, post-hoc model, and models that included site and season were all irrelevant; none were within 10 AIC_c units of the best-fitting model and none had any weight of evidence as a best fitting model (Table 3).

Conversely, *O. howardi* relative abundance was best predicted by the season-only model (Table 4). The probability that season alone was the best model was 0.44. Several other models also had support as the best fitting model, including the intercept-only model (weight of evidence = 0.25), sex-only model (weight of evidence = 0.11), and weight only model (weight of evidence = 0.11) (Table 4). The post-hoc model (sex+season) did not improve the fit of the best-fitting model (season), but did have some support as a best fitting model when evaluated against other candidate models (ΔAIC_c = 1.89, weight of evidence = 0.15).

DISCUSSION

Ecological correlates of louse and flea infestations exhibited a clear divergence and were in agreement with initial predictions based on the extent to which these taxa can survive off host and on alternate hosts. Louse prevalence and abundance was best

predicted by the age and sex of their host. Although lice displayed seasonal differences, this variation was relatively unimportant in a predictive framework. The close relationship with host characteristics and lack of seasonal variability in lice is consistent with the observation that lice are host-specific and cannot survive off host. Conversely, flea prevalence and abundance were best predicted by season, with the largest infestations occurring in spring. Although sample sizes during the spring were small compared to summer, it is highly unlikely that this was a sampling artifact; abundance was an order of magnitude higher in spring than summer, and smaller sample sizes consistently underestimate, rather than overestimate, parasite burden (Gregory and Woolhouse 1993). The seasonality of parasitism by fleas observed here is likely a conservative estimate and larger sample sizes would be predicted to increase the weight of evidence for the season only model. These results are consistent with the observation that *O. howardi* abundance on gray squirrels (*Sciurus carolinensis*) is greatest during the spring (Durden 1980). The finding that a variety of other models had support as the best-fitting model indicates flea infestations are likely influenced by a variety of factors and a large amount of the variation in this dataset cannot be adequately explained.

Large differences in louse relative abundance were observed between male and female raccoons. Numerous host-parasite associations have displayed a similar relationship among terrestrial mammals, but the majority of these studies have documented relatively small differences between males and females ($\leq$ 20% difference in parasite prevalence and intensity; Poulin 1996, Schalk and Forbes 1997, Krasnov et al. 2005, Monello and Gompper 2007). Sex-biased parasitism is complex and can be due to

multiple factors (Krasnov et al. 2005), but is commonly attributed to differences in size, physiology, or behavior. Size is not a likely explanation for the differences observed in this study because I found no correlation between louse abundance and size of raccoons**;** indeed, males are only ~12% larger than females (Lotze and Anderson 1979). Males are also suggested to be more susceptible to parasitism due to the immunosuppressive effects of testosterone (Schalk and Forbes 1997). However, *Trichodectes* spp. feed primarily on skin debris and dried blood and belong to the suborder Ischnocera. This taxon is not known to be affected by the host immune system in birds (Marshall 1981, Whiteman et al. 2006). Moreover, it is unknown whether mammalian hosts elicit an effective physiological immune response to *Trichodectes* spp. or other biting lice.

Movement patterns (e.g., Guégan et al. 2005), contacts with conspecifics (Wright and Gompper 2005), or grooming activities (Gompper 2004, Mooring et al. 2004) can also influence parasite populations and may result in sex-biased parasitism (Krasnov et al. 2005). Although raccoons are generally considered to be solitary, adult males (but not females) sometimes form semi-permanent dyads that travel and den together for at least several months (Gehrt and Fritzell 1998, Chamberlain and Leopold 2002, Gehrt 2004). I captured adult males in the same trap five times during this study. In addition, I never trapped adult females together and found that adult males, but not females, den together in a concurrent radio-telemetry study (R. Monello and M. Wehtje, unpublished observation). Males may be more likely to be infested by lice if they have increased contact with other infested animals. The presence of multiple young animals in the same

den also makes it possible that female louse infestations are 'diluted' by the presence of cubs (e.g., Freeland 1977, Rubenstein and Hohmann 1989, Mooring and Hart 1992).

Consistency in within-sex ectoparasite abundance in age classes I and above suggests that acquired immunity does not shape the louse or flea infestation patterns in these populations and that parasite acquisition and parasite mortality are constant between host age classes (Hudson and Dobson 1995, Wilson et al. 2002). This pattern is expected for chewing lice; however, other mammals can develop a strong physiological immune response to fleas (Khokhlova et al. 2004). Moreover, *O. howardi* are primarily fleas of *Sciurus* spp. (Whitaker 1982) and their periodic low prevalence and abundance may not be great enough to warrant or detect an energetically expensive immune response in raccoons. The lack of a convex parasite burden, where the youngest and oldest animals have the lowest abundance of parasites, suggests that neither of these ectoparasites has a strong detrimental influence on host health (Grenfell et al. 1995). This is not surprising given the low relative abundance of fleas and the observation that lice rarely affect host health (Durden 2001).

In conclusion, results of the present study are consistent with louse and flea life history strategies. A primary goal of ecological parasitology is to describe general patterns that exist within parasite populations and communities (Poulin 2007). Recent comparative work within and between parasite species indicates that parasite prevalence, abundance, or diversity often depends on both host and environmental characteristics**.** Examining the relative importance of these independent parameters should generate insights regarding the fundamental ecological factors underpinning parasitism (Monello

and Gompper 2007, Krasnov et al. 2008) and parasite microevolution (Clayton et al. 2004, Whiteman et al. 2007). Further efforts to elucidate such patterns will facilitate determining whether the recent patterns observed within and between taxa can be generalized. It is important to emphasize that such patterns may be host-specific and comparisons between hosts should, therefore, also be made. For example, the abundance of *O. howardi* may be more closely related to host characteristics when their primary hosts (*Sciurus* spp.) are examined, due to the potential for an immune response (e.g., Khokhlova et al. 2004). Nevertheless, given the similarity in life history and transmission strategies among closely related parasites, wide host-parasite patterns may exist and be identified when the relative importance of multiple abiotic and biotic covariates are simultaneously evaluated among parasite taxa.

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Table 1. *A priori* models used to estimate louse (*Trichodectes octomaculatus*) and flea (*Orchopeas howardi*) abundance of raccoons in Missouri. Three seasons were included; spring (March-May), summer (June-August), and fall (September-November).

Hypothesis	Model[a]
Host characteristics	
Differences are due to host characteristics	Age + Sex + Weight
Differences are due to host age	Age
Differences are due to host sex	Sex
Differences are due to host weight	Weight
Differences are due to host age and sex	Age + Sex
Age-related differences are related to host sex	Age*Sex
Differences are due to host weight and sex	Weight + Sex
Site and season	
Differences are due to study site and season	Site + Season
Differences are due to study site	Site
Differences are due to season	Season
Global model	Age + Sex + Weight + Site + Season

[a]Structure was similar for all models; e.g., Age + Sex + Weight was $\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Sex}) + \beta_3(\text{Weight})$ and Age *Sex was $\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Sex}) + \beta_3(\text{Age*Sex})$. β_0 = intercept and $\beta_i(X)$ = the parameters of independent variables.

Table 2. Prevalence (%) of the chewing louse *Trichodectes octomaculatus* and the fleas *Orchopeas howardi* and *Chaetopsylla lotoris* on raccoons from 10 sites in Missouri. Raccoon age class designations are cub = 0-5 mo, I = 5-14 mo, II = 15-38 mo, III = 39-57 mo, IV+ = $\geq$ 58 mo. Sample sizes (*n*) represent number of individual raccoons.

Ticks	All	Males	Females	Cubs	I	II	III	IV+
T. octomaculatus	50%	72%	33%	6%	67%	53%	47%	46%
O. howardi	11%	13%	9%	0%	16%	9%	13%	9%
C. lotoris	5%	4%	6%	9%	2%	2%	11%	6%
n	252	111	141	17	51	96	53	35

Table 3. Ranking of models estimating relative abundance of lice (*Trichodectes octomaculatus*) on raccoons from 10 sites in Missouri. Rankings are based on generalized linear models with a negative binomial distribution (n = 252). *A priori* models not included in the table had an AIC_c weight = 0.

Model	log (l)[a]	k[b]	ΔAIC_c[c]	AIC_c weight
Age + Sex	-612.66	6	0.00	0.45
Age*Sex	-608.75	10	0.75	0.31
Age + Sex + Weight	-612.30	7	1.40	0.22
Weight*Sex	-618.90	4	8.28	0.01
Weight + Sex	-620.30	3	9.30	<0.01

[a]Maximized log-likelihood value

[b]The number of model parameters

[c]The lowest AIC_c score was 1237.67

Table 4. Ranking of models estimating relative abundance of fleas (*Orchopeas howardi*) on raccoons from 10 sites in Missouri. Rankings are based on Generalized Linear Models with a negative binomial distribution (n = 252). *A priori* models not included in the table had an AIC_c weight = 0.

Model	log (l)[a]	k[b]	ΔAIC_c[c]	AIC_c weight
Season	-376.69	3	0.00	0.44
Intercept-only	-379.31	1	1.15	0.25
Sex	-379.07	2	2.70	0.12
Weight	-379.14	2	2.85	0.11
Weight + Sex	-379.03	3	4.67	0.04
Weight*Sex	-379.01	4	6.53	0.02
Age	-379.00	5	8.76	0.01
Age + Sex	-378.79	6	10.44	<0.01

[a]Maximized log-likelihood value

[b]The number of model parameters

[c]The lowest AIC_c score was 759.48

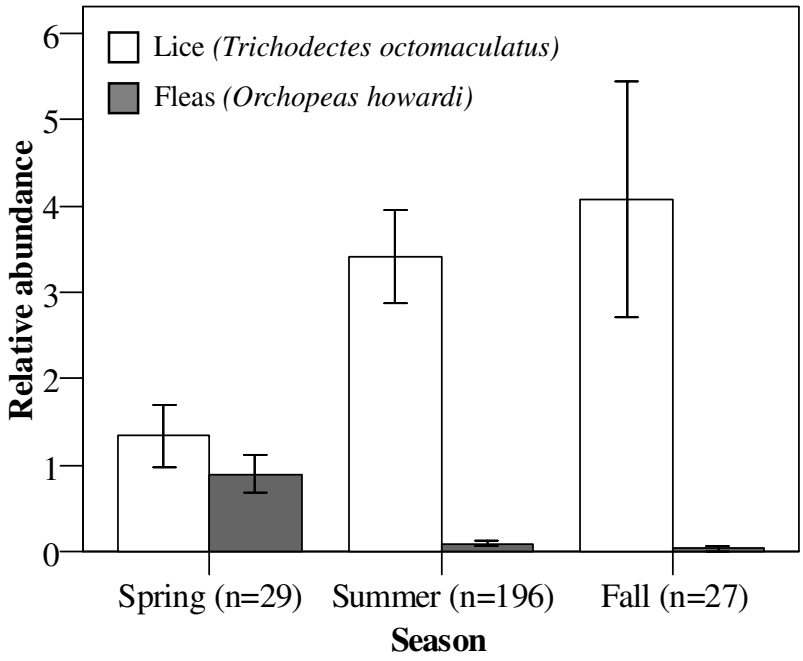

Figure 1. Mean (± S.E.) seasonal relative abundance of lice (*Trichodectes octomaculatus*) and fleas (*Orchopeas howardi*) on raccoons in Missouri. Sample sizes represent number of individual animals captured in each month.

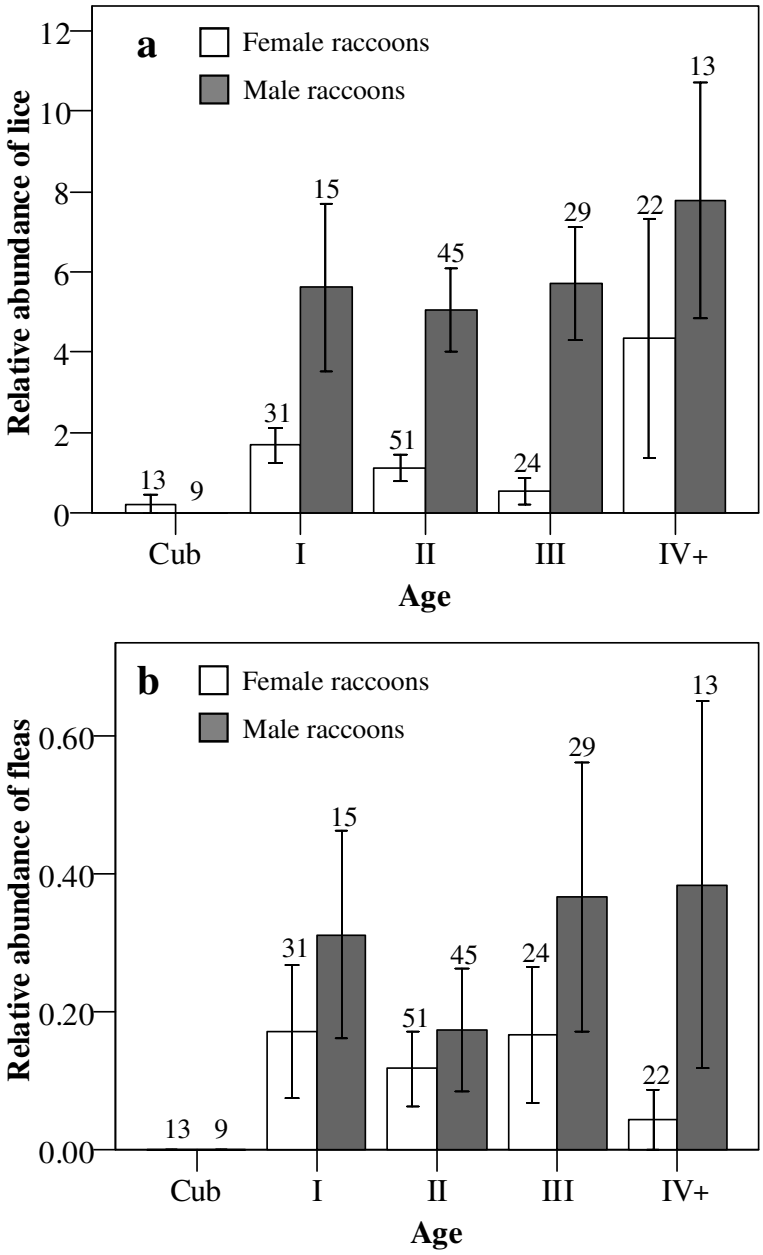

Figure 2. Mean (± S.E.) relative abundance of (a) lice (*Trichodectes octomaculatus*) and (b) fleas (*Orchopeas howardi*) on raccoons in Missouri. Raccoon age class designations are cub = 0-5 mo, I = 5-14 mo, II = 15-38 mo, III = 39-57 mo, IV+ = $\geq$ 58 mo. Sample sizes are shown above S.E. bars.

CHAPTER 3: THE EFFECTS OF EXPERIMENTALLY INDUCED SOCIAL AGGREGATION ON ECTOPARASITES OF RACCOONS

ABSTRACT

I investigated how experimental increases in social aggregation affected ectoparasite prevalence and intensity on free-ranging raccoons (*Procyon lotor*). Twelve independent raccoon populations were subjected to differential resource provisions for two years: a clumped food distribution to aggregate hosts (n = 5 populations), a dispersed food distribution to control for the effects of food without aggregating hosts (n = 3), and a no food treatment (n = 4). The intensity of non-replete and replete (engorged with blood) adult American dog ticks (*Dermacentor variabilis*) were greater in aggregated populations, primarily due to greater infestations of male raccoons. Conversely, the intensity of lice (*Trichodectes octomaculatus*) on male raccoons declined in aggregated populations due to greater overdispersion of lice and a larger number of male hosts harboring fewer parasites. The intensity of lice on female raccoons was threefold lower than on males and did not differ due to treatment. The intensity of fleas (*Orchopeas howardi*) did not differ among treatments and displayed no correlation with host characteristics. Differential responses among treatment categories can be attributed to parasite mobility and host specificity, and sex-related differences in host behavior or physiology. These results provide evidence that the effects of increased social aggregation among hosts are parasite-specific and can be largely dependent on host sex.

INTRODUCTION

A central tenet of ecological parasitology is that the transmission of macroparasite populations are positively correlated with the rate of contact among hosts. The relationship between host contact and parasite transmission is largely based on theoretical models that unequivocally predict that higher rates of contact result in a greater prevalence and abundance of parasites (Anderson and May 1978, Dobson 1990). Although there is empirical support for this outcome (Krasnov et al. 2002), several other studies have found no change or a decline in parasite burden under greater host densities or rates of contact (Fauchald et al. 2007, Vicente et al. 2007). The relationship between the behavioral ecology of hosts and their parasite burden remains uncertain in part because causal relationships have not experimentally examined in natural settings. As such, the direction and magnitude of shifts among parasite populations due to alterations in host behavior remains poorly understood.

There are several reasons why increases in the density or contact rate of hosts may fail to increase parasite transmission. The response of parasite prevalence and abundance may differ as a function of the route of transmission (Arneberg 2001, Fenton et al. 2002, Whiteman and Parker 2004) or dilution effects (Mooring and Hart 1992). Long-term increases in parasite burden due to increased host density or contact are even more difficult to predict, as host species may display density-dependent disease resistance (Wilson et al. 2002a), acquired immunity (Craig et al. 1996), or alterations in behaviors that reduce risk and extent of infection (Loehle 1995).

To my knowledge, only one study has directly altered the social behavior of a free-ranging vertebrate to measure the response of their parasites. Wright and Gompper (2005) examined the effects of altered contact on raccoons (*Procyon lotor*) by contrasting two populations, one of which was manipulated with food to induce hosts to aggregate. The prevalence of directly transmitted endoparasites increased due to aggregation, but little to no differences were observed among ectoparasites and indirectly transmitted endoparasites. However, this study was not replicated and did not fully explore treatment effects of food supplementation or the age and sex of hosts. Here I expand on Wright and Gompper (2005) by incorporating multiple study sites and an expanded experimental design.

My objective is to determine how host aggregation and food supplementation influence ectoparasite infestation patterns of raccoons, and to do so in light of other relevant ecological factors that have been identified from previous work. For example, many parasites display strong age or sex-related infestation patterns among hosts (Wilson et al. 2002b, Monello and Gompper 2007, 2009). I examined the response of the American dog tick *Dermacentor variabilis*, the louse *Trichodectes octomaculatus*, and the flea *Orchopeas howardi*, which collectively represent the primary ectoparasites that infest raccoons in Missouri, USA (Kollars et al. 1997, 2000, Monello and Gompper 2007, 2009). The life history and ecological constraints of these parasites allowed for general predictions of how greater host contact will affect their parasite burden. *Dermacentor variabilis* attach and drop off hosts after feeding. They obtain a single blood meal from a variety of hosts in each of their three life stages (larvae, nymph, adult), but raccoons are

the primary host species for adult *D. variabilis* in Missouri (Kollars et al. 2000). Because ticks are not transmitted between animals, I predicted that increases in host aggregation would have little impact on adult tick infestation levels of raccoons. Conversely, lice are host species-specific and cannot survive off-host for more than a few hours (Durden 2001). The ability of lice to infest new individuals is dependent on host contact, and so I predicted that increased host aggregation should result in greater prevalence and louse population size. Fleas have been shown to be sensitive to the density of their primary hosts (Krasnov et al. 2002), but they also have the ability to live on multiple hosts and survive off-host for extended periods of time. Thus, I predicted that flea populations will not be affected by host contact to the same extent as lice, but should display a stronger relationship with host aggregation than ticks.

MATERIALS AND METHODS

Study System

Study sites (n = 12) were located on state or federal lands within 60 km of Columbia, Missouri, USA (Figure 1). Sites consisted of second growth oak (*Quercus* spp.) and hickory (*Carya* spp.) forest with a maple (*Acer* spp.) and cedar (*Juniperus virginiana*) understory. All sites were more than 6 km apart, with the exceptions of three areas that had two sites each within 4 km of each other. I considered all sites to be independent of one another, as I captured >700 individuals with >500 recapture events from 2005-2007, and no animals were observed to move between sites.

Each site was assigned to one of three treatments in January 2006; a permanent feeding station receiving 35 kg/wk of dog food at a single location to aggregate raccoons (n = 5 populations), a highly dispersed distribution of the same quantity of dog food to control for the effects of food addition but not aggregate hosts (control with food, n = 3 populations), and a no food treatment (control without food, n = 4 populations). Treatments were assigned randomly within geographically defined subsets that were used to assure that treatments were interspersed and to control for unidentified regional effects (Figure 1). The control sites with food were provisioned by placing food in 0.25 kg piles (~140/wk) that were randomly moved each week throughout a 4 km^2 portion of each study site. All provisions were maintained from January through September in 2006 and 2007.

Host Sampling

Traplines of 15 pairs of Tomohawk box traps (30 total) were established at each site. A single trap was place ~50 m on each side of a 1 km transect, with adjacent traps spaced 75 to 100 m apart. Raccoons were trapped for $\geq$10 days at each site two to three times per year between March and November. Traps were baited with mackerel and checked daily. Raccoons were immobilized with ketamine hydrochloride and xylazine (Evans 2002), tagged, weighed, sexed, and aged by body size, genital morphology, and tooth eruption and wear (Grau et al. 1970). This resulted in five age classes; cub = 0-5 mo, I = 0–14 mo, II = 15–38 mo, III = 39–57 mo, and IV+ = over 58 mo. Data from

cubs, which were generally free of ectoparasites (Monello and Gompper 2007, 2009), and recaptured animals were not included in analyses.

Remote cameras (DeerCam DC-300) were used to monitor feeding station use and group aggregation sizes in experimental (hereafter referred to as aggregated sites) and control sites. One camera was maintained from January to August at each permanent feeding station in aggregated sites. Five cameras were maintained in control sites with and without food for 10-15 days during both the spring and summer. Cameras were placed in front of a small pile of food (control with food) or bait (control without food) and relocated every 5 days. Aggregation size was recorded as the maximum number of raccoons, excluding cubs, observed per night and site to enhance independence between photos.

Because of the potential for the treatments to influence population density and body condition, which in turn might influence ectoparasites, I quantified population density and relative body condition. I calculated population density of each site by estimating adult ($\geq$ age class I) population size in 2007 using closed population models in Popan-5 (Arnason and Schwarz 1999), and divided these values by the effective trapping area. I estimated the effective trapping area by multiplying the linear length of the trapline by a buffer of ¾ of the median summer home range of raccoons (Kenward 1985) from rural sites in Illinois (Prange et al. 2004), which were comparable to preliminary home range estimates from sites in this study (M. Wehtje, unpublished data). Population density was estimated for 2007 only because this is when the maximum treatment effect on density should occur and capture-recapture data was more robust in 2007 than 2006. I

used the residuals from a linear regression of body mass on body size to assess individual body condition (Schulte-Hostedde et al. 2005).

Parasite Quantification

Adult *D. variabilis* are relatively large (3–5 mm in length) and readily found on animals in the field without magnification. I quantified adult *D. variabilis* by a thorough search of the entire body, and classified each tick as non-replete, semi-replete, or replete. I considered ticks to be non-replete if they were similar in width (2–3 mm) and color (brown) to questing adult *D. variabilis* ticks found off host. I considered a tick to be replete when it was engorged with blood, $\geq$5 mm width, and clearly discolored (pale or white). Ticks that were not clearly in either category (< 9% of all ticks) were noted as semi-replete and not included in analyses. Analyses only included tick counts that were conducted between April and August, which corresponds to the time period when replete ticks were observed on animals. However, graphs of tick intensity include all data to show the complete range of seasonal variation.

I quantified the relative abundance of lice and fleas via 10 strokes with a flea comb from the base of the neck to the base of the tail on the dorsal region of raccoons (Monello and Gompper 2009). Animals that were wet or muddy could not be combed and were excluded. Hair and ectoparasites were immediately placed in a plastic bag, sealed, and frozen within eight hours. In the laboratory, lice and fleas were identified to species and quantified under a dissecting scope.

Measures of parasitism follow Bush et al. (1997); prevalence was the percentage of hosts infested and intensity represents the population size of a parasite species among infested animals (i.e., uninfeseted animals are not included). I used generalized linear models with a binomial distribution and logit transformation to determine if the presence or absence (i.e., prevalence) of each ectoparasite differed between treatment categories. I also examined the following interactions because prior research (Monello and Gompper 2007, 2009) indicated their importance and potential to interact with my study design: month*treatment for non-replete ticks; age*treatment and sex*treatment for replete ticks; and sex*treatment for lice. I included treatment as a single factor with three levels (control without food, control with food, aggregated). Pairwise comparisons were used for all-level comparisons if a significant difference was found ($\alpha = 0.05$).

Model Selection – Parasite Intensity

I used information-theoretic model selection to evaluate the ability of treatments, host characteristics, and month to explain the intensity of each ectoparasite species. Model factors included aggregation, food supplementation, host age and sex, and month of capture. *A priori* model formulation was based on previous research (Monello and Gompper 2007, 2009) which identified predictors of natural patterns of ectoparasite infestation in the absence of experimental manipulations. I conducted separate analyses for each ectoparasite species, as well as for non-replete and replete ticks. The best-fit models from previous research (Monello and Gompper 2007, 2009) were included in all analyses. In addition, I included aggregation and food supplementation as separate

factors with previously identified variables of importance. This resulted in a total of 23 models (Table 1), although I restricted this to 13 models for fleas by excluding models with age because this variable was unimportant to *O. howardi* in previous work and fewer raccoons harbored fleas, resulting in a smaller sample size than tick and lice data sets. Intercept-only and global models were also included in model selection procedures for each ectoparasite. Site was not included in models because the goal was to assess treatment effects, and including site as a variable in the model would assign individual model estimates to each site and likely mask treatment effects. Further, the treatments were randomly applied to sites, thereby precluding sites with higher or lower parasite populations from being assigned to a particular treatment category. Year was not included as a factor because no significant model effects of year were found for any of the ectoparasite species ($P \geq 0.086$ in all cases).

I used generalized linear models with a negative binomial distribution and log identity for model selection (Wilson and Grenfell 1997, Hardin and Hilbe 2007). Ectoparasite intensity of individual raccoons was the sample unit. I conducted a goodness-of-fit test to assess the ability of model factors to explain ectoparasite intensity by comparing the global model against the intercept-only model for each ectoparasite species (Franklin et al. 2000). I calculated Akaike's Information Criterion (corrected for small sample size; AIC_c) and ranked the models based on differences between the best approximating model and all other models (ΔAIC_c) in the candidate set. Models with a ΔAIC_c value ≤ 2 of the best-fitting model were considered to have substantial empirical support as a best-fitting model (Burnham and Anderson 2002). I calculated the

overdispersion parameter of the best-fit model for each ectoparasite species to assess model structure (Burnham and Anderson 2002). I also calculated model weight (i.e., the probability that each model is the best-fit model) and model averaged estimates and odds ratios for each variable in the 90% confidence set of models (Σmodel weights $\geq$ 0.90).

RESULTS

Raccoon Aggregation, Body Condition, and Density

Aggregation size of adult raccoons was greatest in aggregated sites (Kruskal-Wallis H′ = 230.948, df = 2, P < 0.001; aggregated > control sites with food > control sites without food, all comparisons P $\leq$ 0.006 based on Mann Whitney *U* test), with up to 10 individuals simultaneously visiting the food plots in aggregated sites (Figure 2; mean number of individuals per photo ± 95% C.I.; control without food = 1.00 ± 0.00; control with food = 1.16 ± 0.06; aggregated = 3.22 ± 0.19). Raccoon density did not differ among treatments (Kruskal-Wallis H′ = 0.50., df = 2, P = 0.778), averaging 29.4 ± 7.3 (S.E.) in control without food sites (range 22.4 to 33.6, *n* = 4), 34.8 ± 7.6 in control with food sites (range 29.3 to 35.2, *n* = 3), and 32.32 ± 8.50 in aggregated sites (range 12.9 to 46.4, *n* = 5). Supplemental food increased the weight and improved the relative body condition of female raccoons (mean body condition ± 95% C.I.; control without food = -0.54 ± 0.21; control with food = -0.17 ± 0.16; aggregated = 0.03 ± 0.15), but no differences were observed among males (control without food = 0.26 ± 0.25; control with food = 0.40 ± 0.27; aggregated = 0.35 ± 0.17; Figure 3).

Ectoparasite Prevalence

I captured 406 raccoons during 2006 and 2007. The male:female capture ratio was similar in each treatment category during both years of the experiment (mean of both years ± S.E.; control without food = 1.05 ± 0.28, control with food = 1.13 ± 0.226, aggregated = 1.16 ± 0.09; Kruskal-Wallis test, $P \geq 0.585$ during both years). Prevalence of non-replete *D. variabilis* averaged 92 ± 3% (mean ± 95% C.I.; all estimates of data dispersion hereafter represent 95% C.I.) across all sites and did not differ between treatments (treatment, $\chi^2 = 3.429$, df = 2, P = 0.163; month*treatment, $\chi^2 = 8.212$, df = 12, P = 0.767) (Table 2). Replete ticks infested 55 ± 5% overall and also did not differ by treatments or display an age*treatment or sex*treatment interaction ($P \geq 0.113$ for all comparisons). The prevalence of lice did not differ due to treatment ($\chi^2 = 2.401$, df = 2, P = 0.301), but exhibited a sex*treatment interaction ($\chi^2 = 71.886$, df = 3, $P > 0.001$). Pairwise comparisons indicated that male raccoons had a greater prevalence of lice than females regardless of treatment category ($P > 0.001$ for all comparisons). Overall, almost 3x more male raccoons (71 ± 6%, *n* = 217) harbored lice than females (28 ± 7%; *n* = 197). However, no within sex treatment differences were detected for males or females ($P > 0.185$ for all within sex treatment comparisons). Prevalence of the flea *O. howardi* was 14 ± 3% overall and did not differ due to treatment (P = 0.823) (Table 2).

Ectoparasite Intensity

Infested animals in aggregated populations harbored more non-replete ticks (mean intensity = 30.7 ± 4.9, range 1-140, *n* = 127) than those in control without food (18.2 ±

3.6, range 1-89, n = 81) and control with food populations (19.4 ± 3.8, range 1-107 n = 87). High intensities of non-replete ticks were maintained from May to July in aggregated populations, whereas intensity in control with and without food populations increased through the summer, peaked in July, and consistently displayed a lower intensity than aggregated populations (Figure 4a). Non-replete tick intensity was greatest among males in aggregated populations, surpassing the non-replete tick intensity of males and females in control with and without food populations. Among females, animals in aggregated sites had higher non-replete tick intensity than those in control without food sites, but not control with food sites (Figure 5a). Overall, the mean intensity of non-replete ticks was 26.0 ± 4.2 on males (n = 147) and 21.9 ± 3.3 on females (n = 148), with relatively small but consistent increases from age I (22.8 ± 6.2, n = 60) to age IV+ (25.0 ± 5.4, n = 75).

Infested animals in aggregated populations also harbored more replete ticks (mean intensity = 4.5 ± 0.9, range 1-24, n = 82) than those in control without food (3.0 ± 0.8, range 1-14, n = 49) and control with food populations (3.3 ± 0.9, range 1-12, n = 46). Aggregated and non-aggregated populations both reached maximum intensity in May and there were no temporal differences as with non-replete ticks (Figure 4b). Similar differences among treatments were observed among replete and non-replete ticks (Figure 5a,b), with the notable exception that males displayed a lower replete tick intensity than females. Overall, replete tick intensity was 3.2 ± 0.6 on males (n = 82) and 4.3 ± 0.9 on females (n = 95). Replete ticks varied with age but did not consistently increase; age

class I had the highest (4.4 ± 1.4, n = 35) intensity and age class II had the lowest (3.0 ± 0.5, n = 53).

Intensity of lice was lower in aggregated populations (control without food = 6.7 ± 2.1, n = 55; control with food = 7.0 ± 2.3, n = 56; aggregated = 5.0 ± 0.8, n = 94). However, there were sex-specific treatment differences; female raccoons exhibited little difference in louse intensity among treatments, while males in aggregated populations had a lower intensity of lice than those in control with and without food populations (Figure 5c). The intensity of lice on male raccoons in aggregated treatments exhibited greater overdispersion (variance:mean = 12.99) than the intensity in control with food (variance:mean = 9.61) and control without food (variance:mean = 9.30) categories, with a greater number of hosts in aggregated treatments harboring fewer lice (Figure 6). The distribution of lice on females was similar among treatments (Figure 6).

Intensity of fleas did not exhibit any patterns among treatments (control without food = 1.8 ± 0.5, n = 16; control with food = 1.7 ± 1.2, n = 17; aggregated = 2.0 ± 0.6, n = 25) or host sex (males = 2.0 ± 0.7, n = 37; females = 1.7 ± 0.6, n = 21). Seasonal variation of flea intensity was not estimated due to low prevalence.

Model Selection

Goodness-of-fit tests that compared the global and intercept-only model found a significant difference for non-replete ticks (Likelihood ratio chi-square = 55.182, df = 10, $P < 0.001$) and lice (Likelihood ratio chi-square = 32.188, df = 14, P = 0.004). There were no differences between the global and intercept-only model for replete ticks

(Likelihood ratio chi-square = 12.989, df = 10, P = 0.224) or fleas (Likelihood ratio chi-square = 6.635, df = 13, P = 0.920).

The best-fit models of non-replete tick intensity were month+aggregation+sex and month+aggregation (Table 3; overdispersion parameter of best-fit model = 0.715). The combined weight of evidence for these two models was 0.74. No other model was within 2 AIC_c units of these best-fit models, and all models with a weight of evidence > 0.01 included month. The best-fit model with food supplementation was the global model, which had a weight of evidence of 0.12. Model averaged estimates indicated that aggregation increased non-replete tick intensity, as non-replete tick intensity in non-aggregated populations was 0.47-0.77 that of aggregated populations (Table 4). The months May through July all increased non-replete tick intensity when included in the model, with the greatest increases occurring in July (Table 4).

The best-fit model for replete tick intensity was aggregation+sex (overdispersion parameter = 0.602). No other model was within 2 AIC_c units. The aggregation+sex model had a weight of evidence of 0.38, and the only other model with a weight of evidence > 0.10 was the aggregation-only model (Table 3). A wide variety of models had support as the best-fitting model; e.g., the food+sex model had a weight of evidence of 0.09, but all models with food instead of aggregation had a lower weight and explained less variation. Similar to non-replete ticks, model averaged estimates indicated aggregation increased replete tick intensity, as replete tick intensity in non-aggregated populations was 0.47-0.93 that of aggregated populations (Table 5). Females were also

positively related to replete tick burden, with replete tick intensity of females 1.02 to 2.01 times greater than males when the factor sex was included in models (Table 5).

Louse intensity was best explained by aggregation+sex and the sex-only model (Table 6; overdispersion parameter of best-fit model = 1.348). All models with a weight of evidence $\geq$ 0.01 included sex as a factor. The weight of evidence that the age+sex was the best model was 0.54 and the sex-only model had a weight of 0.24. Although food was included in some models, these consistently underperformed compared to models with aggregation (Table 7). Model averaged estimates indicated sex was the most important variable, with the intensity of lice on females being 0.32 to 0.64 that of males when included in the model. Aggregation had the opposite relationship as found with ticks, with non- aggregated populations having louse populations that were 0.99 to 1.81 times greater than aggregated populations (Table 7).

None of the model factors adequately explained flea intensity. The best-fit model was the intercept-only model with a weight of evidence of 0.40 (Table 6). Models that included only sex, aggregation, or food performed equally well, but their poor performance relative to the intercept-only model indicated that none of them adequately explained flea infestation patterns and thus model averaged estimates were not calculated.

DISCUSSION

I observed large shifts in parasite intensity due to host aggregation. The direction and magnitude of these effects was parasite-specific and inconsistent with my original predictions. Tick intensity increased to maximum levels earlier in the season and was

higher on animals in aggregated populations, whereas louse intensity was lower in aggregated populations and fleas exhibited no differences among treatments. Such findings suggest the outcome of increases in host contact or aggregation are highly variable and dependent on the host-parasite association in question. Other studies on ecto- and endoparasites have also attributed increases (Arneberg et al. 1998, Morand and Poulin 1998, Krasnov et al. 2002) and declines (Mooring and Hart 1992, Fauchald et al. 2007, Vicente et al. 2007) in macroparasite abundance or intensity to population density and group size, but to my knowledge this is the first study to examine such questions in an experimental manner across ectoparasite taxa within the same system and host. These findings indicate that even in the absence of density alterations, host social behavior and spacing can alter the infestation levels of directly transmitted ectoparasites. The interaction between host social behavior, spacing, and ectoparasites appears to be considerably more complex than the current understanding, which is primarily based on models of endoparasite abundance (Anderson and May 1978, Dobson 1990; but see Krasnov et al. 2002).

Tick intensity on raccoons in aggregated sites increased earlier in the season and was greater overall than non-aggregated populations. This trend was particularly apparent for non-replete ticks, but replete ticks also displayed greater intensity on animals in aggregated sites. There are several reasons for higher tick intensity in aggregated sites: ticks may seek out the established feeding areas; stationary raccoons may be more likely to be found and infested by questing ticks; and raccoons may be transporting ticks to the site, where larvae and nymphs have a greater probability of dropping off, molting, and

later reinfesting raccoons at the food plots. These hypotheses are non-exclusive and supported by the literature. Adult *D. variabilis* concentrate and focus questing behavior in areas that exhibit greater wildlife and human activity (Burg 2001), and can move up to 100 m to reach such areas (Carroll and Nichols 1986). *Dermacentor* spp. are also able detect and seek out sources of CO_2 (e.g., Garcia 1965) and are likely attracted to CO_2 plumes from the large, consistent host aggregation at the food plots.

Aggregation interacted with different factors for non-replete and replete ticks. Month of sampling and aggregation were the most important variables for explaining non-replete tick intensity, while sex and aggregation were most important to replete tick intensity. This is consistent with previous research that found that parasitism by non-replete ticks was primarily influenced by month of collection, while parasitism by replete ticks was primarily influenced by host characteristics (Monello and Gompper 2007). The importance of aggregation relative to other variables in this experiment suggests that changes in host social behavior or space use can overshadow other factors that typically influence tick intensity. Higher tick intensities that are maintained at their maximum level for a longer period of time could also alter pathogen transmission between ticks and hosts, with the direction and magnitude of effect dependent on whether the host is an efficient reservoir for the pathogen (Brunner and Ostfeld 2008).

Results from this study suggest the potential for opposing trends in sex bias between two segments of the same parasite population. Male raccoons had a greater overall intensity of non-replete ticks. This was primarily due to the disproportionate increase of non-replete ticks among males in aggregated populations (Figure 5a). Sex

was included as a factor in the best-fit model of non-replete tick intensity, but it was relatively unimportant compared to monthly fluctuations and the effect of aggregation (Tables 3,4; Figure 5a). Conversely, females had a greater overall intensity of replete ticks. Sex was included in the best-fit model and had a positive relationship with replete tick intensity (Tables 3,6; Figure 5b). There is support for both male and female-biased patterns of parasitism, which are generally attributed to sex-related differences in size, physiology, diet, or behavior (Poulin 1996, Schalk and Forbes 1997, Wilson et al. 2002b). To my knowledge, however, this is the first example of opposing trends in sex bias between two segments of the same parasite population. The most likely mechanisms to reduce tick burdens are direct removal through auto- or allogrooming (Levin and Fish 1998) or an increased immune response elicited by higher tick burdens (Craig et al. 1996). Adult male raccoons are frequently observed to form semi-permanent dyads (R. Monello and M. Wehtje unpublished data; Gehrt and Fritzell 1998), which could increase grooming opportunities. Further, females infested by adult *D. variabilis* just prior to and after parturition may not be able to reduce their replete tick burden due to energetic constraints or immunosuppressive effects associated with reproduction (Festa-Bianchet 1989).

The intensity of lice was lower on males in aggregated sites, but no treatment differences were observed among females. Fewer lice on males in aggregated sites was due to greater overdispersion, which resulted in a larger proportion of hosts with smaller louse populations in comparison to non-aggregated sites (Figure 6). Smaller per capita parasitism in larger social groups (i.e., parasite dilution) has been observed in other

studies, but these observations are primarily from ungulates and mobile ectoparasites that have a negligible population response to greater host numbers (Côté and Poulin 1995, Fauchald et al. 2007). Dilution has not been observed and is not predicted to occur among host specific parasites that require close host contact for transmission and cannot survive off host (Mooring and Hart 1992). Indeed, aggregation is predicted to increase the prevalence and intensity of lice, which complete their life cycle within 30-40 days (Durden 2001) and should benefit from increased transmission opportunities. However, dilution could be responsible for the patterns observed in this study if it results in a greater number of smaller louse populations (due to increased transmission) that are less likely to persist or be detected with the methods used here. Additionally, physical changes in the substrate of the skin due to increased nutrition (Watson 1998) may cause parasite death and lower the intensity of chewing lice (Nelson et al. 1975) or persistence of small populations.

It is surprising that treatment effects were sex-specific. Females may not have exhibited a decline in lice because of initial lower intensity. Thus, the ability to cause a measurable decline via dilution or other route is reduced. It is also possible that treatment-induced alterations in louse intensity among females were too small to detect with the methods used here. Overall, the tick and louse burden of males were affected by aggregation in a disproportionate manner compared to females. Sex-biased use of the food plots in aggregated sites might explain this, but several lines of evidence suggest this was not the case. The proportion of males and females captured was similar among

treatment categories, and a separate telemetry-based study found no sex-related differences in use of the food plots (M. Wehtje, unpublished data).

No relationships were observed between fleas and treatment categories. The intercept-only model was superior to all other models because inclusion of aggregation, food, age, sex, or month did not increase the ability of the model to account for variability in flea abundance and AIC_c rankings penalize models for additional parameters. I suggest one reason that differences among treatments did not occur is because *Sciurus* spp. are the primary hosts of *O. howardi* (Durden 1980) and alterations to raccoon ecology have little or no impact on the relatively low levels of prevalence and intensity observed in this study. This is consistent with previous work from these sites in the absence of experimental manipulations that found flea abundance peaked during the spring, but no other abiotic or biotic correlates of flea infestation (Monello and Gompper 2009; see also Durden 1980).

Results from this study suggest parasite life history characteristics - including route of transmission, ability to survive off host, and host specificity - are critical in predicting how ectoparasite populations will respond to alterations in host demographics or behavioral ecology. Models of directly transmitted endoparasites that predict a positive relationship between parasite abundance and host contact (Anderson and May 1978, Dobson 1990) have been found to fit some host-ectoparasite interactions (Krasnov et al. 2002). However, my results suggest additional host-parasite relationships need to be investigated prior to assuming such relationships for other ectoparasites. Ectoparasites are subjected to both environmental and host influences and exhibit vastly different

constraints between taxa in terms of movement abilities, survival off host, and routes of reproduction. Yet even when such characteristics are taken into account, it is difficult to accurately predict the general response to host aggregation or contact rates. Ticks are not reliant on host contact or aggregation for successful infestation, yet in this study they displayed the greatest treatment-related differences, perhaps due to their ability to seek out hosts and a life cycle that includes bouts of host detachment and attachment that likely moved them closer to aggregation sites. The response to social aggregation may differ within as well as between parasite species. To elucidate such patterns among diverse host and parasite types, I emphasize the need for more empirical research on this topic in a manner that directly compares the responses of multiple parasite species.

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Table 1. *A priori* models of ectoparasite intensity of raccoons subjected to aggregation and resource treatments (β_0 = intercept, $\beta_i(X)$ are the parameters of independent variables).

Hypothesis	Model structure
The effects of aggregation	
Differences are due to aggregation	$\beta_0 + \beta_1(\text{aggregation})$
Differences are due to aggregation and age	$\beta_0 + \beta_1(\text{aggregation}) + \beta_2(\text{age})$
Differences are due to aggregation and month	$\beta_0 + \beta_1(\text{aggregation}) + \beta_2(\text{month})$
Differences are due to aggregation, month, and age	$\beta_0 + \beta_1(\text{aggregation}) + \beta_2(\text{month}) + \beta_3(\text{age})$
Differences are due to aggregation and sex	$\beta_0 + \beta_1(\text{aggregation}) + \beta_2(\text{sex})$
Differences are due to aggregation, month, and sex	$\beta_0 + \beta_1(\text{aggregation}) + \beta_2(\text{month}) + \beta_3(\text{sex})$
Differences are due to aggregation, age, and sex	$\beta_0 + \beta_1(\text{aggregation}) + \beta_2(\text{age}) + \beta_3(\text{sex})$
The effects of food supplementation	
Differences are due to food supplementation	$\beta_0 + \beta_1(\text{food})$
Differences are due to food and age	$\beta_0 + \beta_1(\text{food}) + \beta_2(\text{age})$
Differences are due to food and month	$\beta_0 + \beta_1(\text{food}) + \beta_2(\text{month})$

Differences are due to food, month, and age	$\beta_0 + \beta_1(\text{food}) + \beta_2(\text{month}) + \beta_3(\text{age})$
Differences are due to food and sex	$\beta_0 + \beta_1(\text{food}) + \beta_2(\text{sex})$
Differences are due to food, month, and sex	$\beta_0 + \beta_1(\text{food}) + \beta_2(\text{month}) + \beta_3(\text{sex})$
Differences are due to food, age, and sex	$\beta_0 + \beta_1(\text{food}) + \beta_2(\text{age}) + \beta_3(\text{sex})$
Additive effects of aggregation and food	
Differences are due to aggregation and food	$\beta_0 + \beta_1(\text{aggregation}) + \beta_2(\text{food})$
Differences are due to aggregation, food, age, and sex	$\beta_0 + \beta_1(\text{aggregation}) + \beta_2(\text{food}) + \beta_3(\text{age}) + \beta_4(\text{sex})$
Differences are due to age	$\beta_0 + \beta_1(\text{age})$
Differences are due to sex	$\beta_0 + \beta_1(\text{sex})$
Differences are due to month	$\beta_0 + \beta_1(\text{month})$
Differences are due to age and sex	$\beta_0 + \beta_1(\text{age}) + \beta_2(\text{sex})$
Differences are due to sex and month	$\beta_0 + \beta_1(\text{sex}) + \beta_2(\text{month})$
Differences are due to age, sex, and month	$\beta_0 + \beta_1(\text{age}) + \beta_2(\text{sex}) + \beta_3(\text{month})$
Differences are due to sex and month	$\beta_0 + \beta_1(\text{sex}) + \beta_2(\text{month})$

Table 2. Prevalence (% hosts infested) of non-replete (NR) and replete (R) adult ticks (*Dermacentor variabilis*), lice (*Trichodectes octomaculatus*), and fleas (*Orchopeas howardi*) among raccoon populations subjected to aggregation and resource treatments.

Species	*n*	Control without food	Control with food	Aggregated	P
NR Ticks	322	91%	88%	95%	0.163
R Ticks	322	55%	46%	61%	0.126
Lice	406	55%	47%	50%	0.301
Fleas	406	16%	14%	13%	0.823

Table 3. Ranking of *a priori* models estimating intensity of non-replete and replete ticks (adult *Dermacentor variabilis*) on raccoons from sites subjected to aggregation and resource treatments. Rankings are based on generalized linear models (negative binomial distribution, log identity) and only models in the 90% confidence interval are shown.

Model	Log-likelihood	No. Parameters	ΔAIC_c	AIC_c weight
Non-replete ticks (n = 295)				
$I_{aggregation+month+sex}$	-1213.25	7	0.00	0.42
$I_{aggregation+month}$	-1214.57	6	0.55	0.32
$I_{aggregation+month+sex+food+ age}$	-1210.22	11	2.49	0.12
$I_{month+sex+food}$	-1214.96	7	3.41	0.08
Replete ticks (n = 177)				
$I_{aggregation+sex}$	-429.80	3	0.00	0.38
$I_{aggregation}$	-431.89	2	2.11	0.13
$I_{food+sex}$	-431.20	3	2.80	0.09
I_{sex}	-432.64	2	3.61	0.06
$I_{aggregation+sex+age}$	-428.53	6	3.81	0.06
I_{food}	-432.82	2	3.97	0.05
$I_{aggregation+food}$	-431.82	3	4.04	0.05
$I_{intercept}$	-433.93	1	4.15	0.05
$I_{aggregation+age}$	-430.40	5	5.40	0.03

Table 4. Model averaged estimates and odds ratios of parameters included in the 90% confidence set of models used to estimate the intensity of non-replete adult *Dermacentor variabilis* ticks.

Parameter[a]	Model averaged estimate	Unconditional standard error	Odds ratio	Lower 95% C.I.	Upper 95% C.I.
No aggregation	-0.43	0.13	0.60	0.47	0.77
April	0.24	0.25	1.29	0.77	2.16
May	0.77	0.21	2.26	1.47	3.46
June	0.83	0.21	2.39	1.59	3.61
July	0.99	0.20	2.87	1.95	4.21
Sex (female)	-0.13	0.09	0.82	0.65	1.04
No food	-0.08	0.07	0.73	0.53	1.02
Age I (5-14 mos.)	-0.03	0.04	0.75	0.52	1.08
Age II (15-38 mos.)	-0.02	0.03	0.83	0.60	1.14
Age III (39-57 mos.)	-0.01	0.02	0.90	0.64	1.27

[a]The parameters aggregation, month (August), sex (male), food, and age (IV) were redundant and set to 0 in model averaged estimates and 1 in odds ratios.

Table 5. Model averaged estimates and odds ratios of parameters included in the 90% confidence set of models used to estimate the intensity of replete adult *Dermacentor variabilis* ticks.

Parameter[a]	Model averaged estimate	Unconditional standard error	Odds ratio	Lower 95% C.I.	Upper 95% C.I.
No aggregation	-0.25	0.15	0.66	0.47	0.93
Sex (female)	0.20	0.13	1.43	1.02	2.01
No food	-0.05	0.06	0.72	0.49	1.05
Age I (5-14 mos.)	0.01	0.02	1.11	0.69	1.80
Age II (15-38 mos.)	-0.02	0.03	0.82	0.53	1.28
Age III (39-57 mos.)	0.01	0.02	1.17	0.72	1.90

[a]The parameters aggregation, sex (male), food, and age (IV) were redundant and set to 0 in model averaged estimates and 1 in odds ratios.

Table 6. Ranking of *a priori* models estimating intensity of lice (*Trichodectes octomaculatus*), and fleas (*Orchopeas howardi*) on raccoons from sites subjected to aggregation and resource treatments. Rankings are based on generalized linear models (negative binomial distribution, log identity) and only models in the 90% confidence interval are shown.

Model	Log-likelihood	No. Parameters	ΔAIC_c	AIC_c weight
Lice (n = 205)				
$I_{aggregation+sex}$	-577.50	3	0.00	0.54
I_{sex}	-579.33	2	1.61	0.24
$I_{food+sex}$	-578.87	3	2.75	0.14
Fleas (n = 58)				
$I_{intercept-only}$	-107.41	1	0	0.40
I_{sex}	-107.29	2	1.90	0.16
$I_{aggregation}$	-107.34	2	1.99	0.15
I_{food}	-107.41	2	2.14	0.14
$I_{aggregation+sex}$	-107.19	3	3.92	0.06
$I_{food+sex}$	-107.29	3	4.12	0.05

Table 7. Model averaged estimates and odds ratios of parameters included in the 90% confidence set of models used to estimate the intensity of the louse *Trichodectes octomaculatus*.

Parameter[a]	Model averaged estimate	Unconditional standard error	Odds ratio	Lower 95% C.I.	Upper 95% C.I.
No aggregation	0.16	0.11	1.34	0.99	1.81
Sex (female)	-0.74	0.18	0.45	0.32	0.64
No food	0.02	0.03	1.18	0.84	1.64

[a]The parameters aggregation, sex (male), and food were redundant and set to 0 in model averaged estimates and 1 in odds ratios.

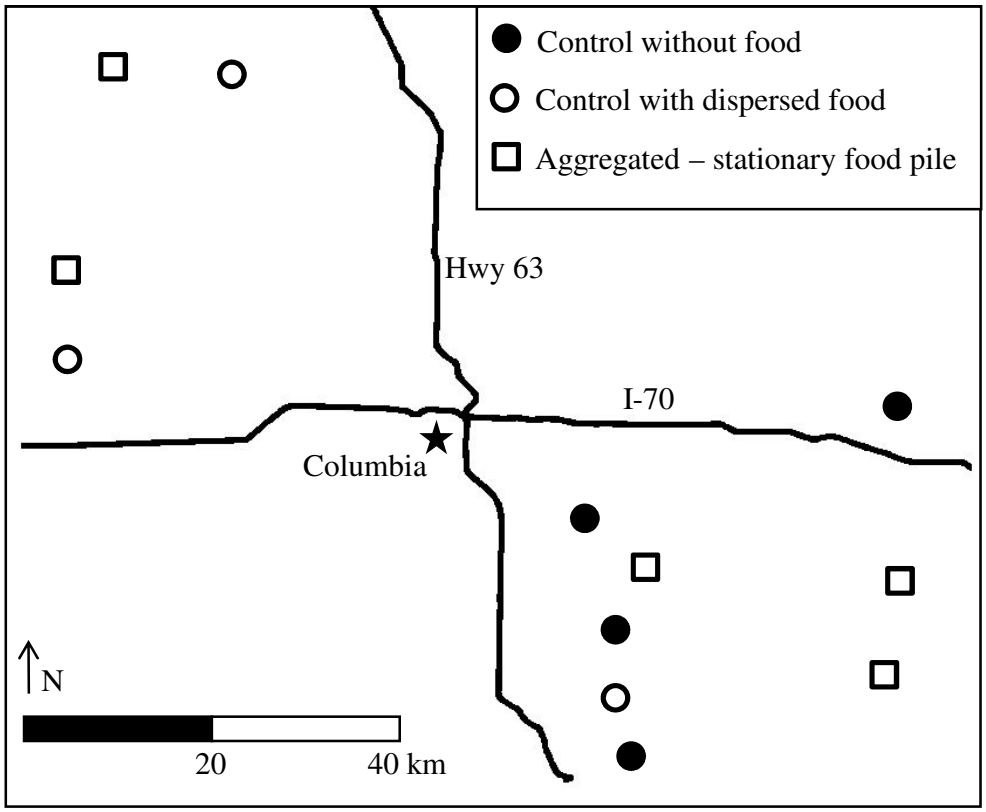

Figure 1. Location and treatment category of study sites in mid-Missouri.

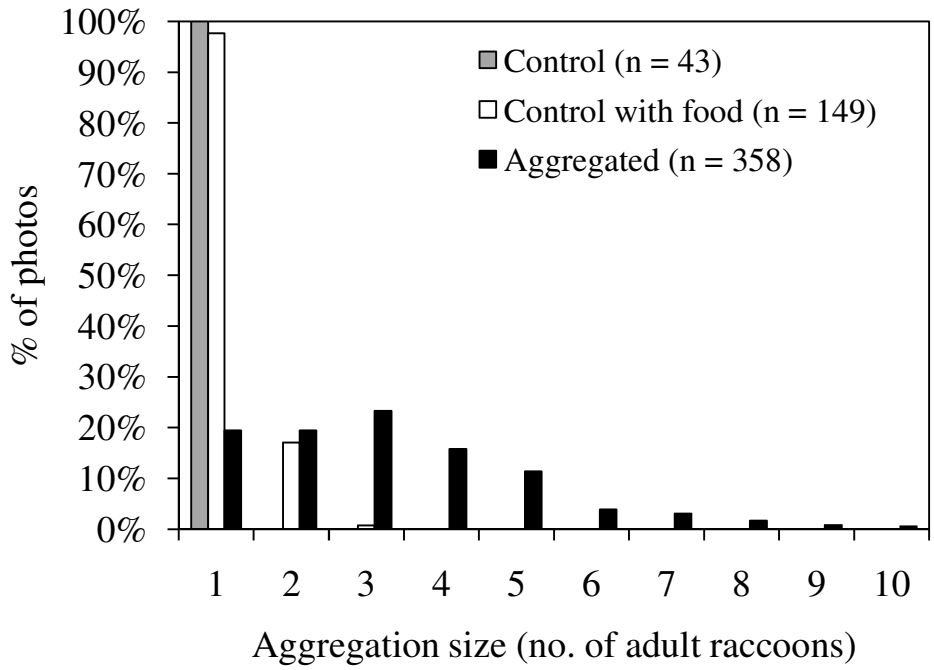

Figure 2. Maximum nightly aggregation size of raccoons observed at control with and without food and aggregated sites (*n* = no. of photos). Multiple raccoons were observed in 18% of the photos from the control with food sites (*n* =149 photos), but these appeared to be mothers accompanied by sub-adults that had yet to disperse. Photos from aggregated sites ranged from 1-10 adult raccoons, but this underestimates the number of individual raccoons visiting the site because I only used a single photo per night as a data point. Photos of ear-tagged animals indicated that even on nights when 8-10 individuals were observed in a single photo, a minimum of 1-3 additional raccoons used the food pile during that night. *Note:* Photo capture effort in the control with and without food sites (215-330 photo nights/year/site) exceeded the aggregated sites (70-105 photo nights/year/site) because cameras in the aggregated sites always ran out of film (but not food) within three days.

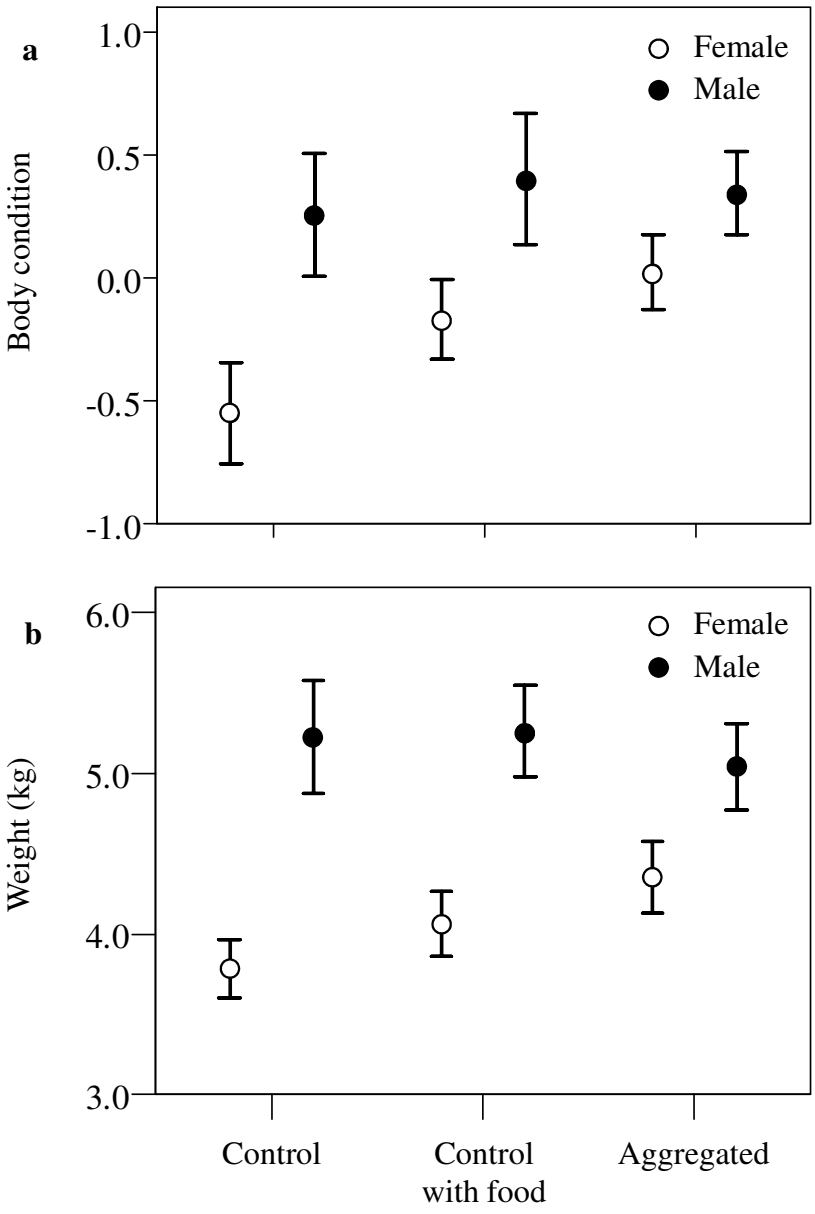

Figure 3. Mean (±95% C.I.) (a) body condition index and (b) weight of male (n = 207) and female (n = 183) raccoons in control with and without food and aggregated sites. Body condition was assessed using the residuals from a linear regression of body mass on body size; higher mass-size residuals represent better body condition.

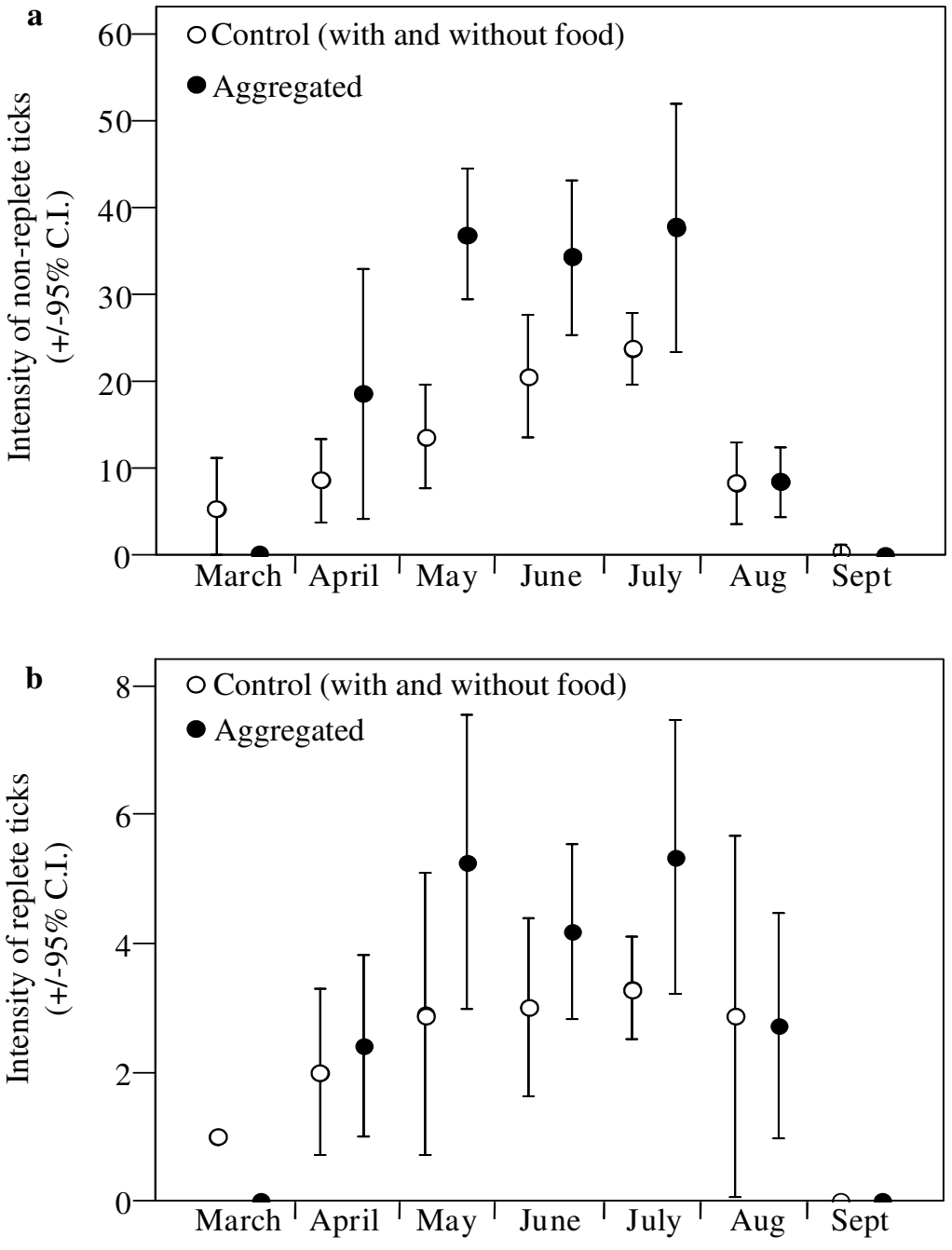

Figure 4. Monthly intensity of (a) non-replete (not engorged with blood) adult ticks (*Dermacentor variabilis*) and (b) replete (engorged with blood) adult ticks observed on raccoons in control with and without food and aggregated sites.

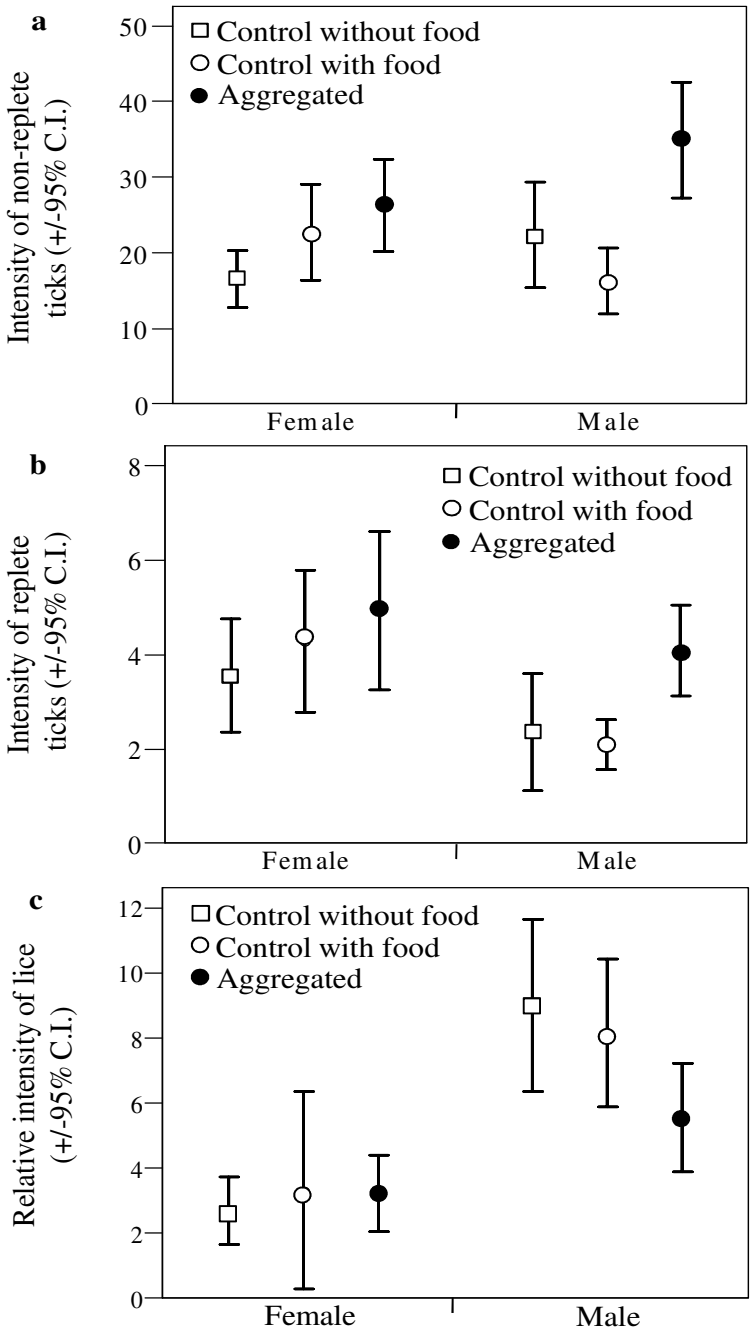

Figure 5. The mean intensity of (a) non-replete (not engorged with blood) adult ticks (*Dermacentor variabilis*), (b) replete (engorged with blood) adult ticks, and (c) lice (*Trichodectes octomaculatus*) observed on male and female raccoons in control with and without food and aggregated sites.

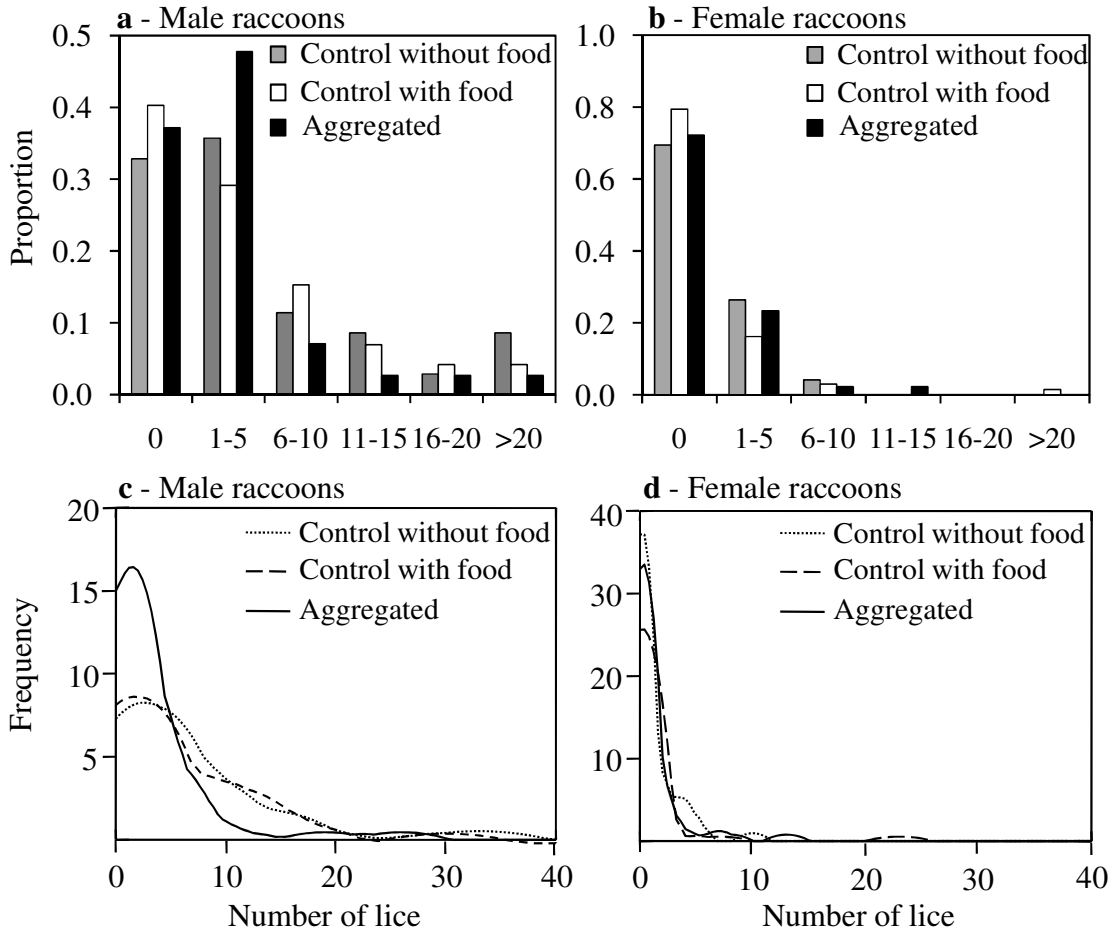

Figure 6. The observed distribution of lice (*Trichodectes octomaculatus*) among (a) male and (b) female raccoons and smoothed kernel density curve of lice among (c) male and (d) female raccoons. Equal sample sizes were used to estimate kernel density curves for each treatment category by randomly selecting the same number of animals within each sex and category (n = 42 males, n = 42 females).

CHAPTER 4: EFFECTS OF RESOURCE AVAILABILTY AND SOCIAL AGGREGATION ON ENDOPARASITE SPECIES RICHNESS OF RACCOONS

ABSTRACT

Few experimental studies have examined the role of host ecology on the parasite species richness of mammals in a natural setting. I measured the influence of host characteristics and study site on endoparasite species richness, and then experimentally assessed the effects of adding food supplements and inducing social aggregation. Twelve independent raccoon populations were subjected to differential resource provisioning for two years: a clumped food distribution to aggregate hosts (n = 5 populations), a dispersed food distribution to add food without aggregating hosts (n = 3), and a no food treatment (n = 4). I considered endoparasites with direct and indirect life cycles separately and evaluated *a priori* models using an information-theoretic framework. In unmanipulated sites, cubs were devoid of parasites but rapidly acquired similar species richness values to adults. There was strong evidence that food decreased the number of indirectly transmitted parasites, particularly among the oldest age classes who inhabited sites with clumped food. Conversely, food and social aggregation had little to no impact on the richness of directly transmitted parasites. Directly transmitted parasites may not have responded to increases in aggregation due to the relatively high densities of raccoons, and possibly rates of contact, that naturally exist in these study sites even in the absence of aggregation. These results suggest food availability and diversity can influence

transmission of indirectly transmitted parasites, while the effects of increased rates of host contact or social aggregation on directly transmitted parasites may depend on inherent patterns such as host spacing and population density.

INTRODUCTION

A primary objective of ecology is to better understand factors that drive patterns of biodiversity. Among parasites, there have been a large number of comparative studies that have assessed the influence of geography, habitat, host type and social system, and host and parasite phylogeny on the parasite species richness of different host species (the parasite component community). In contrast, few field studies have examined the role of host or ecosystem attributes on parasite species richness within host populations (the parasite infracommunity). There is a need to better understand this relationship, as regional or generalized cross-species trends do not adequately describe and can contradict the described ecological relationships that may occur within a host population (Poulin 1997, Bordes and Morand 2008).

Comparative studies have found host traits such as longevity, size, social organization, density, and life history can all correlate with parasite component communities (Gregory 1997, Morand et al. 2000, Nunn et al. 2003, Ezenwa et al. 2006). For example, among vertebrates, host weight or body size is frequently identified as an important factor that is positively correlated with parasite species richness (Gregory et al. 1996, Ezenwa et al. 2006). This makes intuitive sense as a larger body size results in more habitat for parasites and greater opportunities for exposure to parasites due to

increased food intake or home range (Poulin 1995, Ezenwa 2006). However, this conclusion is based on the comparison of multiple host species whose size can differ by several orders of magnitude. While such findings are important, they are primarily relevant to understanding the evolutionary ecology of hosts and parasites and are not necessarily relevant to individual species that occur in a particular ecosystem. When examining a single host species within a site or region, one is asking a fundamentally different question – do the subtle differences in size or weight observed within this particular species alter the parasite infracommunity of an animal? Furthermore, how does any such relationship compare to other characteristics such as sex or age? Such assessments are necessary to differentiate behavioral or exposure related influences (represented by age) from size-based influences.

In this study, I examined factors that influence endoparasite species richness of raccoons (*Procyon lotor*). I considered two measures of species richness by considering parasites with direct life cycles and parasites with indirect life cycles separately. Those with direct life cycles are transmitted directly between raccoons via close contact or contact with feces or fomites. Some directly transmitted parasites can also be transmitted through a different host, although this is not required. Parasites with indirect life cycles must infect at least one additional host (often a prey species such as an insect or gastropod) before they can infect raccoons and complete their development and reproduction. Previous studies have observed only endoparasites with direct life cycles to correlate with, or respond to, changes in the behavioral ecology or density of hosts (Altizer et al. 2003, Wright and Gompper 2005). Indirect parasites are generally not

thought to be sensitive to alterations or behavioral differences in host ecology because they are constrained by an additional intermediate host.

I had two primary objectives in this study. The first was to determine how host characteristics (age, sex, weight) or host use of a particular study site correlates with parasite species richness. This was done in the absence of experimental manipulations to identify naturally occurring relationships between the extent of parasitism and the life history and demographic states of the host. Many of the measures included in this study, such as host age, sex, and weight, have also been used in comparative studies (Poulin 1995, Altizer et al. 2003, Bordes and Morand 2008) or the examination of single host-parasite interactions (Wilson et al. 2002) which allow for general predictions to be made. I predicted species richness to increase with age and weight, and for males to exhibit higher parasite burdens than females. I did not expect site to influence parasite species richness, as regional differences should be minor or non-existent within the same host species (Bordes and Morand 2008).

The second objective was to experimentally assess the effects of food resources and social aggregation on parasite species richness. The experimental design consisted of a clumped food distribution to aggregate hosts, a dispersed food distribution that added the same amount of food but did not aggregate hosts, and a no food treatment. Food additions and aggregation were predicted to affect parasites with direct and indirect life cycles in a different manner. Many comparative studies on parasite component communities have suggested that the diversity of food intake, food resources, and differing levels of social contact can impact on the number of parasites (Kuris et al. 1980,

Pacala and Dobson 1988, Poulin 1995, Gregory 1997, Morand et al. 2000, Altizer et al. 2003, Nunn et al. 2003, Wright and Gompper 2005, Ezenwa et al. 2006). For this study, I predicted the number of directly transmitted species would increase in aggregation treatments due to higher contact between hosts. However, I expected the number of indirectly transmitted species to decline in aggregated treatments, and in food-augmented populations, because it would decrease the need for other food sources, including alternative hosts that are necessary to obtain indirectly transmitted species.

MATERIALS AND METHODS

Study Design

Raccoons were sampled from March to November 2005-2007 at 12 locations across ~2400 km^2 in central Missouri, U.S.A. (Table 1, Figure 1). All sites were located on forested state, federal, or university conservation or research areas within 60 km of Columbia, Missouri. Sites consisted of second growth oak (*Quercus* spp.) and hickory (*Carya* spp.) forest with a maple (*Acer* spp.) and cedar (*Juniperus virginiana*) understory. All sites were more than 6 km apart, except three areas that had two sites each within 4 km of each other. Sites were considered independent of each other; over the course of the study I captured >700 individuals and >500 recapture events, none of which were observed to move between sites.

Each site was assigned to one of three treatments in January 2006; receipt of a permanent feeding station receiving 35 kg/wk of dried commercial dog food to aggregate raccoons (aggregated, $n = 5$ populations), receipt of a dispersed distribution of the same

quantity of dog food so as not to aggregate hosts (control with food, n = 3 populations), and a no food treatment (control, n = 4 populations). Treatments were assigned randomly to sites within geographically defined site subsets that were used to assure that treatments were interspersed and to control for unidentified regional effects (Figure 1). The control with food sites were provisioned by placing food in 0.25 kg piles (~140/wk) that were randomly placed every week throughout each 4 km^2 study site. All provisions were maintained from January through September in 2006 and 2007.

Traplines of 15 pairs of Tomohawk box traps (30 total) were established at each site. A single trap was place ~50 m on each side of a 1 km transect, with adjacent traps spaced 75 to 100 m apart. Raccoons were trapped for $\geq$10 days at each site two to three times per year between March and November. Traps were baited with mackerel and checked daily. Raccoons were immobilized with ketamine hydrochloride and xylazine (Evans 2002), tagged with metal Hasco ear tags, weighed, sexed, and aged by body size, genital morphology, and tooth eruption and wear (Grau et al. 1970). Animals were categorized into one of five age classes; cub $\leq$5 mo, I = 5–14 mo, II = 15–38 mo, III = 39–57 mo, or IV+ = over 58 mo.

Fresh feces was collected from within or below traps, homogenized, and stored in 10% formalin. Only defecations that were less than 16 hours old were used; no feces collected with fecal loops were included in these analyses, as these samples are relatively small and underrepresented the number of parasites found in a host (R. Monello, unpublished data). Endoparasite ova and oocysts were identified by standard fecal floatation procedures at the University of Missouri and the Cornell University Diagnostic

Center using sugar and zinc sulfate centrifugation techniques (Bowman 1999). Only one sample per animal was included in this study. When multiple samples of an individual were obtained due to recapture events, one sample was randomly selected for analysis. Although using additional samples may give a more accurate parasite species richness index for an animal, I did not include them because it would have resulted in a disproportionately larger sampling effort for animals that were recaptured. Fecal samples cannot be assumed to provide a complete endoparasite inventory of each host, as some species are not represented because adult parasites present in the host may not have recently released eggs or reproduced. Thus, I assumed that by using one sample per individual and sampling each area with equal effort across seasons, the likelihood of finding parasites in fecal samples would be equal across all individuals, sites, and treatments.

Remote cameras (DeerCam DC-300) were used to monitor feeding station use and aggregation sizes in experimental (hereafter referred to as aggregated sites) and control sites. One camera was maintained from January to August at each permanent feeding station in aggregated sites. Five cameras were maintained in control sites with and without food for 10-15 days during both the spring and summer. Cameras were placed in front of a small pile of food (control with food) or bait (control without food) and relocated every 5 days. Aggregation size was recorded as the maximum number of raccoons, excluding cubs, observed per night and site to enhance independence between photos.

Because of the potential for the treatments to influence population density and body condition, which in turn might influence endoparasite species richness, I quantified population density and body condition. I calculated the population density of each site by estimating adult (≥ age class I) population size in 2007 using closed population models in Popan-5 (Arnason and Schwarz 1999) and divided these values by the effective trapping area. I estimated effective trapping area by multiplying the linear length of the trapline by a buffer of ¾ of the median summer home range of raccoons (Kenward 1985) from rural sites in Illinois (Prange et al. 2004), which were comparable to preliminary home range estimates from the sites in this study (M. Wehtje, unpublished data). Population density was estimated for 2007 only because this is when the maximum treatment effect on density should occur and capture-recapture data was more robust than 2006. I used the residuals from a linear regression of body mass on body size to assess the relative body condition of individuals (Schulte-Hostedde et al. 2005).

Model Selection

I used information-theoretic model selection to determine ecological correlates of endoparasite species richness in unmanipulated (i.e., baseline or 'natural' conditions) and experimental sites. I considered parasites with a direct and indirect life cycle separately. This resulted in four individual model selection analyses for parasite species richness; directly transmitted species in unmanipulated sites, indirectly transmitted species in unmanipulated sites, directly transmitted species in experimental sites, and indirectly transmitted parasites in experimental sites (Table 1). In each case, I first conducted a

goodness-of-fit test to assess the ability of model factors to explain endoparasite species richness by comparing the global model against the intercept-only model (Franklin et al. 2000).

Unmanipulated sites included all of the sites in 2005 and the control sites (no food) in 2006 and 2007. Generalized linear models with a normal distribution were used to test for model effects and selection in all analyses (Burnham and Anderson 2002). *A priori* model formulation for unmanipulated sites was based on previous findings from studies of single parasite species and comparative measures of parasite species richness. Briefly, studies of individual parasites as well as comparative studies indicated that age, sex, weight, and study site location can all be important drivers of parasite presence and abundance (Wilson et al. 2002). Thus, models for unmanipulated sites included the factors age, sex, and weight of the animal, and study site location. The factor year was included in all models of directly or indirectly transmitted species when significant model effects were found ($P < 0.05$). I constructed models to test distinct hypotheses (Table 2); for example, age and weight were not included in the same model (except the global model) because they are correlated (weight increases with age through class III) and inclusion of both could lead to a model that includes an irrelevant factor. Moreover, their separation allows a clear interpretation of which parameter is more important. This resulted in 11 *a priori* models and an intercept-only and global model (Table 2).

Experimental comparisons included control, control with food, and aggregated sites that were treated and sampled in 2006-07 (Table 1). *A priori* model formulation built on the results of the independent assessments of factors underpinning species

richness in unmanipulated sites (see Results). Thus, models for experimental sites included the factors age, sex, food, and aggregation. Cubs were not included during analyses of experimental sites because results from the unmanipulated sites indicated that cubs had fewer parasites than older age classes and this trend could dominate experimental analyses (as in the analyses of unmanipulated sites, see Results). Study site was also not included because differences between sites were not apparent in unmanipulated sites (see Results) and I assumed the randomly applied treatments precluded sites with higher or lower parasite burdens or species richness from being assigned to a particular treatment category. Finally, weight was not included because it displayed little relationship with endoparasite species richness in unmanipulated sites (see Results). The factors food and aggregation were considered separately (versus a treatment category) to compare their relative importance. Year was included in all models of directly or indirectly transmitted species when significant model effects were found ($P < 0.05$). *A priori* model formulation for experimental sites was based on previous findings from single parasite species and comparative studies of parasite species richness (Wilson et al. 2002), as well as data that indicated treatment differences were dependent on sex of the host (Chapter 3). This resulted in 12 *a priori* models and an intercept-only and global model (Table 3).

Models were ranked using Akaike's Information Criterion (AIC_c) and differences between each model and the best-fit model (ΔAIC_c) and model weights (probability of a model being the correct model) were calculated for all models. For all model selection procedures I calculated model averaged estimates, unconditional standard errors, and

odds ratios (±95% C.I.) to determine the magnitude and direction of effects among model factors on parasite species richness (Burnham and Anderson 2002). All factors in the 90% confidence set of models (Σmodel weights $\geq$ 0.90) were included in model averaging procedures, which corresponded to all models with a $\Delta AIC_c \leq 5$. I also approximated the overdispersion parameter of each best-fit model to assess model structure (Burnham and Anderson 2002).

RESULTS

I detected 16 endoparasite species among 460 individual raccoon samples from 2005-2007 (Table 4). The most common parasites were a capillarid roundworm (*Capillaria procyonis*, prevalence = 86-90%) and an intracellular parasite (*Eimeria nutalli*, prevalence = 81-91%). Five parasites detected in this study are known to reproduce in raccoons and have direct life cycles (*Eimeria nutalli, Eimeria procyonis, Placoconus lotoris, Molineus barbatus,* and *Baylisascaris procyonis*) (Table 4). I considered these five species to be directly transmitted and all other species to be indirectly transmitted. Of the remaining 11 endoparasites, nine are known to only have an indirect life cycle, and two have unknown life cycles in raccoons (*C. procyonis* and *Cruzia* spp.) (Table 4). I considered both of these species to have indirect life cycles. When *C. procyonis* and *Cruzia* spp. were classified as directly transmitted, support for the model results reported below only increased. Thus, the classification of parasites and corresponding results of this study should be considered conservative.

The distribution of endoparasite species richness among unmanipulated study sites exhibited a normal distribution (skewness = 0.207 ± 0.179 (SE); kurtosis = 0.049 ± 0.179 (SE); Figure 2). A normal distribution was also exhibited among species richness measures of parasites with direct (skewness = 0.155 ± 0.179; kurtosis = -0.493 ± 0.356) and indirect (skewness = 0.345 ± 0.179; kurtosis = -0.029 ± 0.356) life cycles when they were considered separately (Figure 2).

Maximum aggregation size of adult raccoons was greatest in aggregated sites (Kruskal-Wallis H′ = 230.948, df = 2, $P < 0.001$; aggregated > control sites with food > control sites without food, all comparisons $P \leq 0.006$ based on Mann Whitney *U* test), with up to 10 individuals simultaneously visiting the food plots in aggregated sites (Figure 3; mean number of individuals per photo ± 95% C.I.; control without food = 1.00 ± 0.00; control with food = 1.16 ± 0.06; aggregated = 3.22 ± 0.19). Raccoon density did not differ among treatments (Kruskal-Wallis H′ = 0.50., df = 2, P = 0.778), averaging 29.4 ± 7.3 (S.E.) in control without food sites (range 22.4 to 33.6, $n = 4$), 34.8 ± 7.6 in control with food sites (range 29.3 to 35.2, $n = 3$), and 32.32 ± 8.50 in aggregated sites (range 12.9 to 46.4, $n = 5$). Supplemental food increased the weight and improved the relative body condition of female raccoons (mean body condition ± 95% C.I.; control without food = -0.54 ± 0.21; control with food = -0.17 ± 0.16; aggregated = 0.03 ± 0.15), but no differences were observed among males (control without food = 0.26 ± 0.25; control with food = 0.40 ± 0.27; aggregated = 0.35 ± 0.17).

Model Selection – Unmanipulated Sites

Goodness-of-fit tests that compared the global and intercept-only model found a significant difference for parasites with a direct (Likelihood ratio chi-square = 42.957, df = 19, P = 0.001) and indirect life cycle (Likelihood ratio chi-square = 44.024, df = 19, P = 0.001). Year of sample collection had significant model effects on parasites with direct (χ^2= 21.683, df = 2, p<0.001) and indirect life cycles (χ^2 = 13.193, df = 2, P = 0.001), and was included in all model selection procedures (Table 5). Based on 95% confidence intervals, the number of directly transmitted parasites was greater in 2007 (mean ± 95% C.I.; 2.36 ± 0.37, *n* = 42) compared to 2005 (1.52 ± 0.18, *n* = 96) and 2006 (1.78 ± 0.28, *n* = 46). The number of indirectly transmitted parasites was lowest in 2005 (1.84 ± 0.24, *n* = 96), with greater values in 2006 (2.59 ± 0.37, *n* = 46) and 2007 (2.40 ± 0.45, *n* = 42).

Age was the most important host characteristic for both directly and indirectly transmitted parasites, and was included in all models within 0-2 ΔAIC_c units (Table 5; overdispersion parameter = 1.041 for best-fit models of both parasite types). Model averaged estimates and odds ratios indicated that cubs had a negative relationship with species richness of parasites with direct and indirect life cycles (Table 6, Figure 4). Weight was also included in the 90% confidence set of models (Table 5) and exhibited a positive relationship with species richness, regardless of parasite life history. However, model averaged estimates and odds ratios indicated the overall effect of weight was small relative to the cub age class (Table 6), and models with weight were always ranked lower than those with age (Tables 5). Age classes I-IV and sex had odds ratio with a 95% C.I. that overlapped the value one, indicating these factors explained little of the variation in

parasite species richness data (Table 6). Site of collection was not included in any models with a model weight $\geq$ 0.001, indicating that parasite species richness did not differ between study sites.

Although ages I-IV did not have a strong effect on predicting parasite species richness, a divergent trend among directly and indirectly transmitted parasites occurred in older age classes. Cubs harbored few parasite species of either transmission types, averaging less than one per animal with a 95% C.I. that did not overlap with age class I and above (Figure 4). However, species richness of parasites with a direct life cycle doubled between age classes cub and I, reaching maximum species richness during age classes I and II and trending downward in age classes III and IV. This differs from indirectly transmitted parasites, which exhibited a similar increase between the age classes cub and I, but trended upward in older animals and diverged from directly transmitted species in age classes III and IV (Figure 4).

Model Selection – Experimental Sites

Goodness-of-fit tests that compared the global and intercept-only model found a significant difference for parasites with a direct (Likelihood ratio chi-square = 39.217, df = 7, $P < 0.001$) and indirect life cycle (Likelihood ratio chi-square = 16.550, df = 6, P = 0.011). Year of sample collection had significant model effects on parasites with direct (χ^2= 26.576, df = 1, $P < 0.001$) but not indirect life cycles (χ^2= 0.126, df = 1, P = 0.723), and was therefore only included in model selection procedures of directly transmitted parasites (Tables 7,8). The number of directly transmitted parasites was greater in 2007

(mean ± 95% C.I.; 2.52 ± 0.17, n = 140) than 2006 (1.91 ± 0.16, n = 149), whereas the number of indirectly transmitted parasites was similar in 2006 (2.40 ± 0.22, n = 149) and 2007 (2.46 ± 0.20, n = 140).

The best fit models of directly transmitted parasites (ΔAIC_c of 0-2) all included age, even though cubs were excluded from model analyses of the experimental study sites (Table 7; overdispersion of best-fit model = 1.021). Model averaged estimates and odds ratios indicated that age class III had a positive effect on species richness of directly transmitted parasites in the first year of the experiment (Table 9, Figure 5a), and that this effect only occurred in sites with food additions (Figure 5b). When age class III is examined by treatment in 2006 only, results indicate the increase in species richness of directly transmitted parasites primarily occurred in aggregated sites (mean ± 95% C.I.: aggregated = 3.10 ± 0.53, n = 10; control with food = 2.30 ± 0.83, n = 10; control = 2.07 ± 0.67, n = 13). These differences were not apparent in the second year of the experiment (2007), as species richness of endoparasites with direct life cycles increased across all age classes compared to 2006 (Figure 5a), as well as the values observed in unmanipulated sites (Figure 4).

The best fit models of indirectly transmitted parasites (ΔAIC_c of 0-2) included all four factors (age, sex, food, aggregation) from the *a priori* model set (Table 8). The top model was age + food, with a model weight of 0.438 and overdispersion parameter of 1.018. Model averaged estimates and odds ratios indicated age class I and food had the largest negative effects on the species richness of indirectly transmitted parasites (Table 9).

The role of age and food additions on indirectly transmitted species richness is best understood when considered together. In control sites with no food additions , age classes I and II had the lowest number of parasites and their 95% C.I. overlapped little with the larger number of parasites supported by age classes III and IV (Figure 5c). This trend is similar to what was observed in the unmanipulated sites; species richness of indirectly transmitted parasites increased over the lifetime of the animal (Figure 4). Conversely, much of the 95% C.I. of all age classes overlapped in sites with food, and age classes III and IV exhibited divergent patterns in sites with versus without food (Figure 5c). The lower species richness among older age classes in sites with food was primarily due to aggregated sites (mean ± 95% C.I. of ages III and IV combined: aggregated = 2.18 ± 0.34, $n = 44$; control with food = 2.57 ± 0.36, $n = 47$; control = 3.17 ± 0.39, $n = 48$).

DISCUSSION

Parasite species richness of raccoons in mid-Missouri was relatively high and consistent across the 12 sites in this study. In unmanipulated sites, cubs were devoid of parasites but rapidly acquired similar species richness values to adults. In experimental sites, the direction and effect of food and aggregation treatments was dependent on parasite life cycles. There was little evidence that these treatments increased the number of directly transmitted parasites, but strong support that food decreased the number of indirectly transmitted parasites, particularly among the oldest age classes that harbored the most parasites.

Alterations in resource availability led to a decline in the number of indirectly transmitted parasites per raccoon. Mean parasite species richness indicated that animals from the aggregated category were the primary driver of such effects; however, odds ratios from model selection procedures indicated that the addition of food was more important. This apparent discrepancy is due to the intermediate declines observed among animals in control with food sites and can be resolved by considering the implications of food dispersal; aggregated food piles were large and predictable in location, whereas dispersed food piles were small and unpredictable. Raccoons in aggregated treatments were able to travel directly to the same spot to obtain food, while those in control with food sites still had to search for food, increasing the likelihood of coming into contact with and consuming intermediate hosts that harbor infectious parasites. Two alternative explanations are also plausible. First, increased nutrition can decrease susceptibility to parasitism (Ezenwa 2004, Hines et al. 2007). I consider this unlikely in this study because similar declines in species richness were not observed among directly transmitted parasites. Further, only female body condition improved in sites with food additions, yet there were no differences in the number of indirectly transmitted parasites detected between male and female raccoons. Second, food or social aggregations could alter the distribution of intermediate hosts. This is also unlikely because such effects would be localized in aggregated sites (where the primary decline of indirectly transmitted parasites occurred); food plots were less than 5 m in diameter and visibly altered areas were less than 20 m in diameter (R. Monello, personal observation). Thus, a

decline in the diversity of diet is the most plausible reason for the observed decline in species richness of parasites with indirect life cycles.

Species richness of parasites with a direct life cycle was generally not altered by treatments. The only notable difference was that animals in age class III displayed greater species richness of directly transmitted parasites in aggregated sites during the first year of the experiment (based on 95% C.I.; Figure 5). It is unclear why this would occur only in age class III animals. Overall, these findings do not concur with other research that has found aggregation increases parasite burden (Wright and Gompper 2005, Hines et al. 2007). However, the interaction between aggregation and population density has not been studied. The density of raccoons across all of my study sites was on the upper end of rural raccoon populations from the Midwestern U.S. (Blackwell et al. 2004, Gehrt and Fox 2004, Prange et al. 2004). Animals in this study may be exposed to infectious parasites at a relatively high rate and aggregation cannot further increase it. This is consistent with Prange et al. (2004), who found that raccoons in high density populations do not temporally segregate themselves from each other and suggested that, even in the absence of social aggregation, such populations are at risk for greater parasite transmission.

Data from unmanipulated sites indicated that cubs have lower infection rates than older animals. This is not surprising as cubs (0-5 mo) remain in their den for the first two months and do not travel as far as adults (Schwartz and Schwartz 2002), and are thus less likely to be exposed to infectious parasites. Species richness of parasites with both direct and indirect life cycles exhibited the largest increase between the cub and age class I

category. This suggests that young animals are quickly exposed to a variety of parasites, and supports the premise that relatively high exposure rates between hosts and parasites are inherent in these sites. The smaller parasite burdens observed in the youngest age class is consistent with Negovetich et al. (2006), who found impala (*Aepyceros melampus*) lambs to harbor the fewest parasites. However, subsequent increases in the parasite species richness of juveniles and adults were relatively modest (<20%) compared to the values observed in this study (80-100%).

Separate model selection runs of parasites with direct and indirect life cycles indicated that differentiating animals into age classes I through IV played little to no role in predicting species richness values observed in unmanipulated sites. However, when viewed in concert, there is a divergent trend between these two types of parasites. Those with direct life cycles remained at the same level or declined over the course of the host's life when compared to those with indirect life cycles, which tended to increase (Figure 4). The decline in directly transmitted parasites (relative to indirectly transmitted species) suggests older animals are either more resistant or develop behavioral patterns that make them less likely to come into contact with infectious parasites (e.g., Snyder and Fitzgerald 1987, Kazacos 2001, Gompper and Wright 2005). Slight increases among indirectly transmitted parasites in older age classes are likely due to increased exposure; older animals are more likely to have consumed a larger amount and variety of food sources than younger animals.

These results further emphasize the need to consider parasite life cycles when infra- and component community processes are examined. Distinct differences were

apparent in this study when parasites with direct and indirect life cycles were considered separately. Further research is needed to determine the effects of experimentally increased rates of contact or social aggregation among a variety of species and populations. The results in this study suggest the outcome among infracommunities may largely depend on ecosystem properties that determine animal contact, such as spacing and population density. If contact rates are sufficiently high under natural conditions, further increases due to anthropogenic or other activities may not alter the parasite community. However, measures such as parasite abundance may differ from the infracommunity response and should also be investigated.

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Table 1. Study site names, treatment category (in 2006-07), and UTM coordinates. Unmanipulated sites consisted of all sites in 2005 and control without food sites in 2006-2007.

Study Site[a]	2005	2006-2007	UTM
USFS Road 354	Baseline	Control without food	15 S 574888 4297472
USFS Road 398	Baseline	Control without food	15 S 570740 4283954
Whetstone CA	Baseline	Control without food	15 S 613593 4312405
Baskett WRA-Central	Baseline	Control without food	15 S 568506 4289694
Rudolf Bennit CA-East	Baseline	Control with dispersed food	15 S 549314 4346208
Davisdale CA-South	Baseline	Control with dispersed food	15 S 532957 4316522
Baskett WRA-South	Baseline	Control with dispersed food	15 S 569425 4287553
Reform CA	Baseline	Aggregated – stationary food pile	15 S 605900 4287249
Prairie Forks CA	Baseline	Aggregated – stationary food pile	15 S 610114 4305483
Rudolf Bennit CA-West	Baseline	Aggregated – stationary food pile	15 S 545427 4345848
Davisdale CA-North	Baseline	Aggregated – stationary food pile	15 S 531605 4319320
Baskett WRA-North[b]	Baseline	Aggregated – stationary food pile	15 S 570794 4291836

[a]Abbreviations: USFS = U. S. Forest Service; CA = Conservation Area, Missouri Department of Conservation; Baskett WRA = Baskett Wildlife Research Area, University of Missouri

[b]This area also included U.S. Forest Service land that was adjacent to the north side of Baskett WRA (note: stationary food pile was in Baskett WRA)

Table 2. *A priori* models used to estimate the number of endoparasites with direct and indirect life cycles among raccoons in unmanipulated (2005) and control without food sites (2006-07) (β_0 = intercept, $\beta_i(X)$ are the parameters of independent variables). Year of sample collection (Yr) was included in all models of direct and indirect parasites (model effects of year were $P \leq 0.001$ for both).

Hypothesis	Model	Model structure
Differences are due to host age	Age + Yr	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Yr})$
Differences are due to host sex	Sex + Yr	$\beta_0 + \beta_1(\text{Sex}) + \beta_2(\text{Yr})$
Differences are due to host weight	Weight + Yr	$\beta_0 + \beta_1(\text{Weight}) + \beta_2(\text{Yr})$
Differences are due to age and sex	Age + Sex + Yr	$\beta_0 + \beta_1(\text{Age}) + \beta_3(\text{Sex}) + \beta_2(\text{Yr})$
Differences are due to weight and sex	Weight + Sex + Yr	$\beta_0 + \beta_1(\text{Weight}) + \beta_2(\text{Sex}) + \beta_3(\text{Yr})$
Differences are due to study site	Site + Yr	$\beta_0 + \beta_1(\text{Site}) + \beta_2(\text{Yr})$
Differences are due to site and age	Site + Age + Yr	$\beta_0 + \beta_1(\text{Site}) + \beta_2(\text{Age}) + \beta_3(\text{Yr})$
Differences are due to site and sex	Site + Sex + Yr	$\beta_0 + \beta_1(\text{Site}) + \beta_2(\text{Sex}) + \beta_3(\text{Yr})$
Differences are due to site and weight	Site + Weight + Yr	$\beta_0 + \beta_1(\text{Site}) + \beta_2(\text{Weight}) + \beta_3(\text{Yr})$
Differences are due to age, sex, and site	Age + Sex + Site + Yr	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Sex}) + \beta_3(\text{Site}) + \beta_4(\text{Yr})$
Differences are due to weight, sex, and site	Weight + Sex + Site + Yr	$\beta_0 + \beta_1(\text{Weight}) + \beta_2(\text{Sex}) + \beta_3(\text{Site}) + \beta_4(\text{Yr})$

Table 3. *A priori* models used to estimate the number of endoparasites with direct and indirect life cycles among raccoons in control, control with food, and aggregated sites (2006-07) (β_0 = intercept, $\beta_i(X)$ = parameters of independent variables). Year of sample collection (Yr) was included in all models of directly transmitted parasites (model effects of year were $P < 0.001$), but not indirectly transmitted parasites ($P = 0.723$).

Hypothesis	Model	Model structure
Differences are due to host age	Age	$\beta_0 + \beta_1(\text{Age})$
Differences are due to host sex	Sex	$\beta_0 + \beta_1(\text{Sex})$
Differences are due to aggregation	Aggregation	$\beta_0 + \beta_1(\text{Aggreg})$
Differences are due to food additions	Food	$\beta_0 + \beta_1(\text{Food})$
Differences are due to age and sex	Age + Sex	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Sex})$
Differences are due to age and aggregation	Age + Aggregation	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Aggreg})$
Differences are due to age and food	Age + Food	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Food})$
Differences are due to sex and aggregation	Sex + Aggregation	$\beta_0 + \beta_1(\text{Sex}) + \beta_2(\text{Aggreg})$
Differences are due to sex and food	Sex + Food	$\beta_0 + \beta_1(\text{Sex}) + \beta_2(\text{Food})$
Differences are due to age, sex, and aggregation	Age + Sex + Aggregation	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Sex}) + \beta_3(\text{Aggreg})$
Differences are due to age, sex, and food	Age + Sex + Food	$\beta_0 + \beta_1(\text{Age}) + \beta_2(\text{Sex}) + \beta_3(\text{Food})$

Table 4. Prevalence (% infected) of endoparasites detected among raccoons in unmanipulated sites (pre-experiment, 2005 only) and control, control with food, and aggregated sites during 2006-07 (*n* = number of individual raccoons). Parasites with * are known to reproduce in raccoons and have a direct life cycle (references[a]).

Parasite Species	Unmanipulated (*n* = 171)	Control (*n* = 93)	Control w/food (*n* = 89)	Aggregated (*n* = 107)
Capillaria procyonis	86%	90%	87%	86%
*Eimeria nutalli**	81%	81%	91%	88%
Capillaria puttori	44%	51%	51%	42%
*Placoconus lotoris**	41%	39%	36%	29%
Eurytrema procyonis	37%	52%	21%	25%
*Molineus barbatus**	28%	43%	42%	31%
*Eimeria procyonis**	27%	35%	55%	50%
Capillaria plica	19%	25%	30%	23%
Crenosoma spp.	18%	23%	22%	14%
Physaloptera spp.	15%	15%	9%	19%
Atriotaenia procyonis	8%	1%	1%	1%
*Baylisascaris procyonis**	6%	9%	16%	20%
Macracanthorhynchus ingens	3%	2%	2%	3%
Cruzia spp.	3%	1%	1%	1%
Sarcocystis spp.	2%	0%	2%	0%
Alaria spp.	1%	1%	0%	0%

[a]*Eimeria* spp. (Yakimoff and Matikaschwili 1933, Inabnit et al. 1972); *P. lotoris* (Balasingam 1958); *M. barbatus* (Chandler 1942, Gupta 1939); *B. procyonis* (Kazacos 2001); *Capillaria* spp. (Butterworth and Beverley-Burton 1980); *E. procyonis* (Denton 1942); *Crenosoma* spp. (Dougherty 1946); *Physaloptera* spp. (Morgan 1941); *A. procyonis* (Gallati 1959); M. *ingens* (Moore 1946); *Cruzia* spp. (Bartholomew and Crites 1964); *Sarcocystis* spp. (Stanek et al. 2002); *Alaria* spp. (Shoop and Corkum 1981)

Table 5. Ranking of *a priori* models estimating the number of directly and indirectly transmitted endoparasite species among raccoons in unmanipulated and control without food sites (n = 184 individuals for both analyses). Models above the dashed line represent the 90% confidence set (Σweight $\geq$ 0.90) used in model averaging procedures. *A priori* models not included in the table had an AIC_c weight = 0.

Model	log (l)	K	ΔAIC_c	Weight
Directly transmitted species				
Age + Year	-238.719	8	0.00	0.532
Age + Sex + Year	-238.130	9	1.041	0.316
Weight + Year	-243.662	5	3.382	0.098
Sex + Weight + Year	-243.661	6	5.523	0.034
Sex + Year	-245.257	5	6.574	0.020
Indirectly transmitted species				
Age + Year	-282.798	8	0.000	0.367
Age + Sex + Year	-281.877	9	0.377	0.304
Sex + Weight + Year	-285.171	6	0.386	0.302
Weight + Year	-288.703	5	5.307	0.026
Sex + Year	-292.115	5	12.130	0.001

Table 6. Model averaged estimates and odds ratios of parameters included in the 90% confidence set of models used to estimate the number of directly and indirectly transmitted endoparasite species among raccoons in unmanipulated and control sites.

Parameter[a]	Model averaged estimate	Unconditional standard error	Odds ratio	Lower 95% C.I.	Upper 95% C.I.
Directly transmitted parasites					
Year (2005)	-0.813	0.174	0.420	0.297	0.594
Year (2006)	-0.639	0.197	0.504	0.339	0.750
Age cub	-0.709	0.282	0.437	0.239	0.797
Age I	0.101	0.200	1.120	0.706	1.775
Age II	0.196	0.174	1.261	0.848	1.875
Age III	0.040	0.192	1.061	0.682	1.651
Sex (F)	0.050	0.057	0.854	0.643	1.135
Weight	0.089	0.081	1.115	1.001	1.241
Indirectly transmitted parasites					
Year (2005)	-0.577	0.296	0.558	0.358	0.870
Year (2006)	0.099	0.259	1.086	0.653	1.807
Age cub	-1.066	0.439	0.201	0.093	0.435
Age I (5-14 mos.)	-0.261	0.219	0.685	0.379	1.236
Age II (15-38 mos.)	-0.067	0.175	0.903	0.543	1.501
Age III (39-57 mos.)	0.025	0.195	1.014	0.576	1.786
Sex (female)	0.241	0.186	1.287	0.895	1.850
Weight	0.087	0.065	1.333	1.150	1.547

[a]The parameters year (2007), age (IV), and sex (male) were redundant and set to 0 in model averaged estimates and 1 in odds ratios.

Table 7. Ranking of *a priori* models estimating the number of directly transmitted endoparasite species among raccoons in control, control with food, and aggregated sites. Rankings are based on ΔAIC_c values from generalized linear models with a normal distribution (n = 289). Models above the dashed line represent the 90% confidence set (Σweight $\geq$ 0.90) used in model averaging procedures.

Model (directly transmitted parasites)	log (l)	K	ΔAIC_c	Weight
Age + Food + Year	-404.405	7	0.00	0.333
Age + Year	-405.871	6	0.832	0.219
Age + Sex + Food + Year	-404.332	8	1.970	0.124
Age + Sex + Year	-405.833	7	2.857	0.080
Age + Aggregation + Year	-405.835	7	2.862	0.080
Age + Sex + Aggregation + Food + Year	-404.075	9	3.586	0.055
Age + Sex + Aggregation + Year	-405.789	6	4.884	0.029
Food + Year	-410.039	4	5.010	0.027
Aggregation + Food + Year	-409.461	5	5.926	0.017
Sex + Year	-410.940	4	6.814	0.011
Aggregation + Year	-410.955	4	6.842	0.011
Sex + Food + Year	-409.985	5	6.974	0.010
Sex + Aggregation + Year	-410.928	5	8.860	0.004
Intercept-only	-423.683	2	28.201	0.000

Table 8. Ranking of *a priori* models estimating the number of indirectly transmitted endoparasite species among raccoons in control, control with food, and aggregated sites. Rankings are based on ΔAIC_c values from generalized linear models with a normal distribution (n = 289). Models above the dashed line represent the 90% confidence set (Σweight $\geq$ 0.90) used in model averaging procedures.

Model (indirectly transmitted parasites)	log (l)	K	ΔAIC_c	Weight
Age + Food	-478.339	6	0.000	0.438
Age + Sex + Food	-478.339	7	2.101	0.153
Age	-481.051	5	3.337	0.083
Age + Aggregation	-480.139	6	3.599	0.072
Food	-483.386	3	3.880	0.063
Age + Sex + Aggregation + Food	-478.326	8	4.190	0.054
Sex + Food	-483.385	4	5.334	0.030
Age + Sex	-481.043	6	5.409	0.029
Aggregation + Food	-483.251	4	5.667	0.026
Age + Sex + Aggregation	-480.138	7	5.699	0.025
Aggregation	-485.030	3	7.168	0.012
Intercept-only	-486.601	2	8.267	0.007
Sex + Aggregation	-485.030	4	9.224	0.004
Sex	-486.583	3	10.274	0.003

Table 9. Model averaged estimates and odds ratios of parameters included in the 90% confidence set of models used to estimate the number of directly and indirectly transmitted endoparasite species among raccoons in control, control with food, and aggregated sites (2006-07).

Parameter[a]	Model averaged estimate	Unconditional standard error	Odds ratio	Lower 95% C.I.	Upper 95% C.I.
Directly transmitted parasites					
Year (2006)	-0.521	0.115	0.570	0.454	0.716
Age I (5-14 mos.)	-0.228	0.165	0.781	0.551	1.108
Age II (15-38 mos.)	-0.051	0.144	0.946	0.698	1.282
Age III (39-57 mos.)	0.293	0.156	1.392	1.003	1.932
No food	-0.113	0.085	0.807	0.633	1.031
Sex (female)	0.011	0.035	1.046	0.831	1.317
No aggregation	0.002	0.023	0.968	0.763	1.228
Indirectly transmitted parasites					
Age I (5-14 mos.)	-0.3627	0.236	0.639	0.408	1.002
Age II (15-38 mos.)	-0.1676	0.186	0.811	0.548	1.204
Age III (39-57 mos.)	0.1990	0.204	1.272	0.833	1.942
No food	0.2803	0.193	1.456	1.063	1.994
Sex (female)	0.0004	0.041	0.999	0.742	1.344
No aggregation	0.0170	0.041	1.236	0.909	1.681

[a]The parameters year (2007), age (IV), sex (male), food, and aggregation were redundant and set to 0 in model averaged estimates and 1 in odds ratios. Cubs and the parameter weight were not included in these analyses.

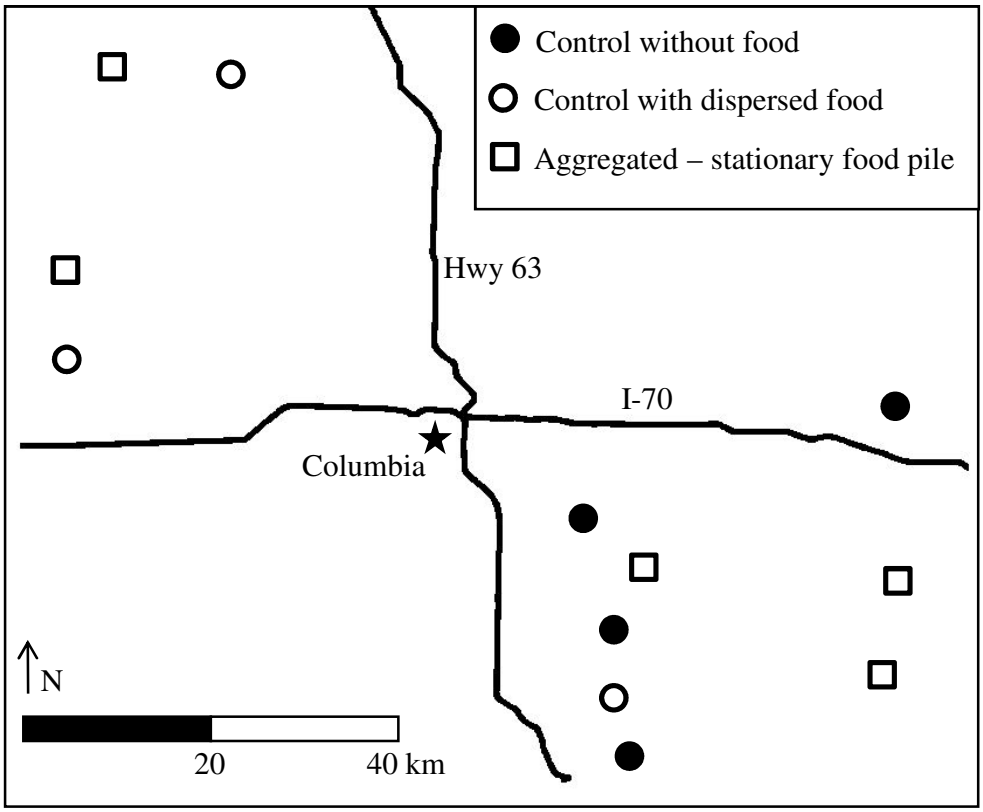

Figure 1. Location and treatment category of study sites in mid-Missouri.

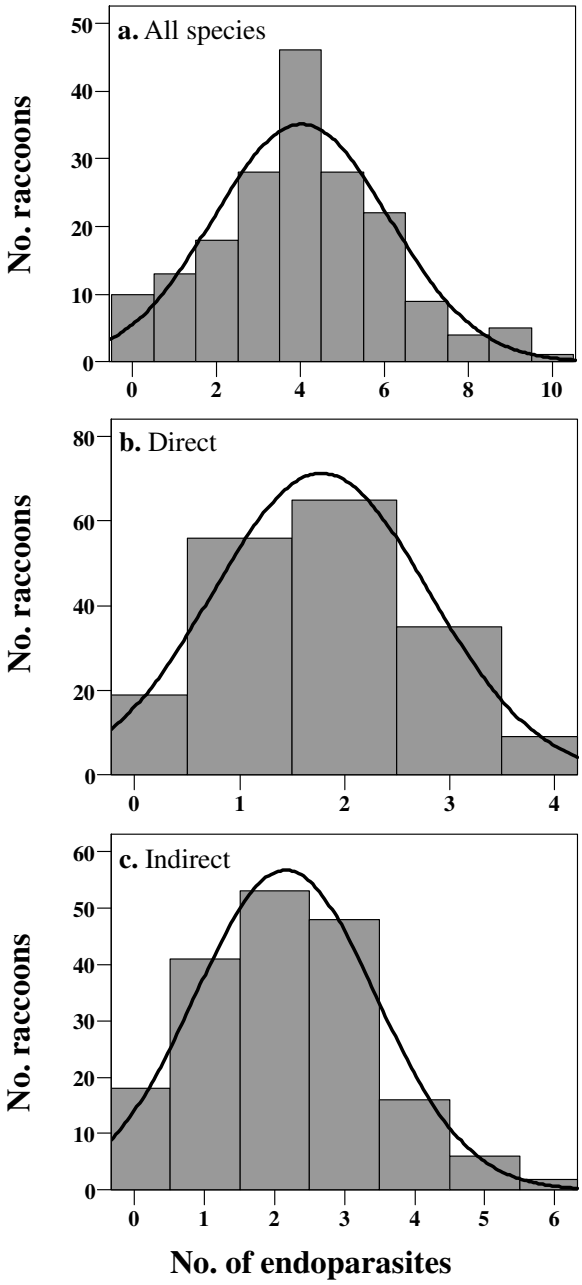

Figure 2. Number of endoparasites detected among individual raccoons (n = 184 individuals). The black line represents the normal curve for (a) all endoparasite species detected, (b) endoparasites with a direct life cycle, and (c) endoparasites with an indirect life cycle.

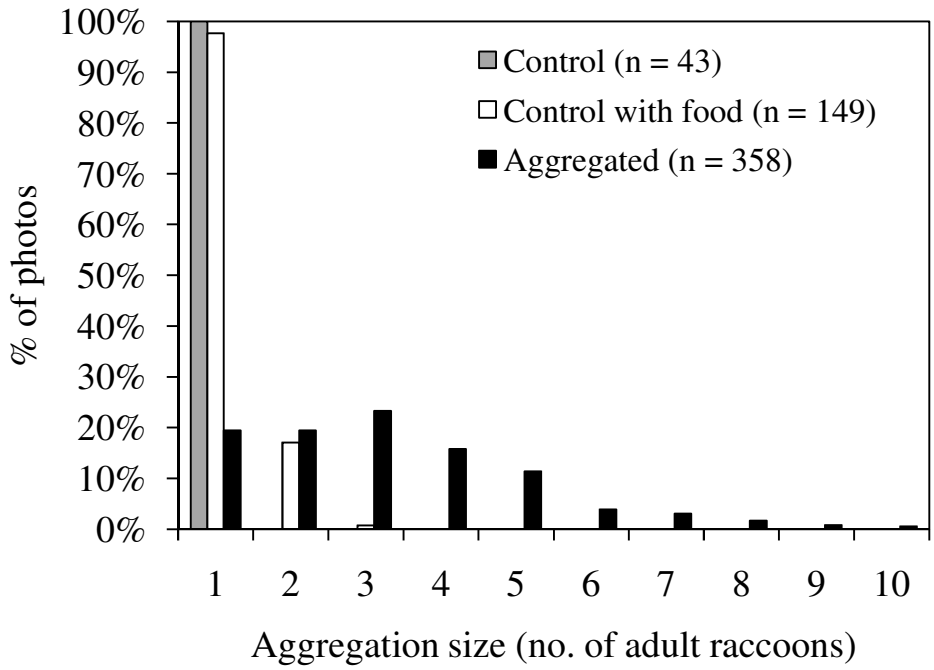

Figure 3. Maximum nightly aggregation size of raccoons observed at control with and without food and aggregated sites (*n* = no. of photos). Multiple raccoons were observed in 18% of the photos from the control with food sites (*n* =149 photos), but these appeared to be mothers accompanied by sub-adults that had yet to disperse. Photos from aggregated sites ranged from 1-10 adult raccoons, but this underestimates the number of individual raccoons visiting the site because I only used a single photo per night as a data point. Photos of ear-tagged animals indicated that even on nights when 8-10 individuals were observed in a single photo, a minimum of 1-3 additional raccoons used the food pile during that night. *Note:* Photo capture effort in the control with and without food sites (215-330 photo nights/year/site) exceeded the aggregated sites (70-105 photo nights/year/site) because cameras in the aggregated sites always ran out of film (but not food) within three days.

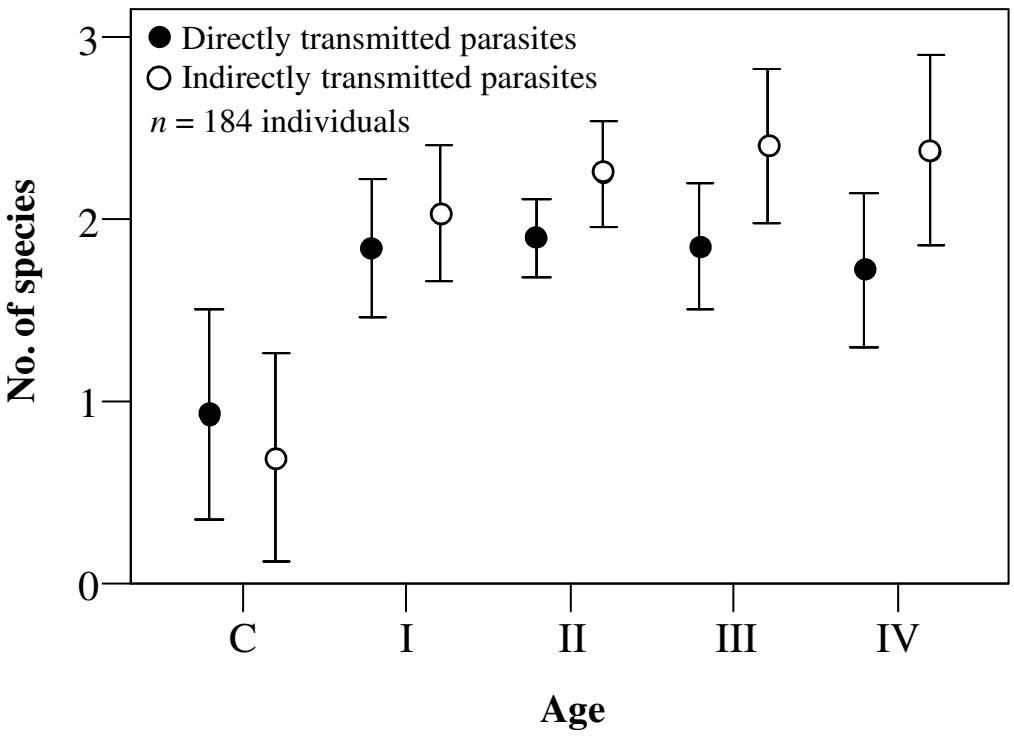

Figure 4. Average number of directly and indirectly transmitted parasite species (±95% C.I.) detected per raccoon in unmanipulated sites (2005) and control sites without food (2006-07). Raccoon age classes are C = cub, I = 5–14 months, II = 15–38 months, III = 39–57 months, IV+ = ≥58 months.

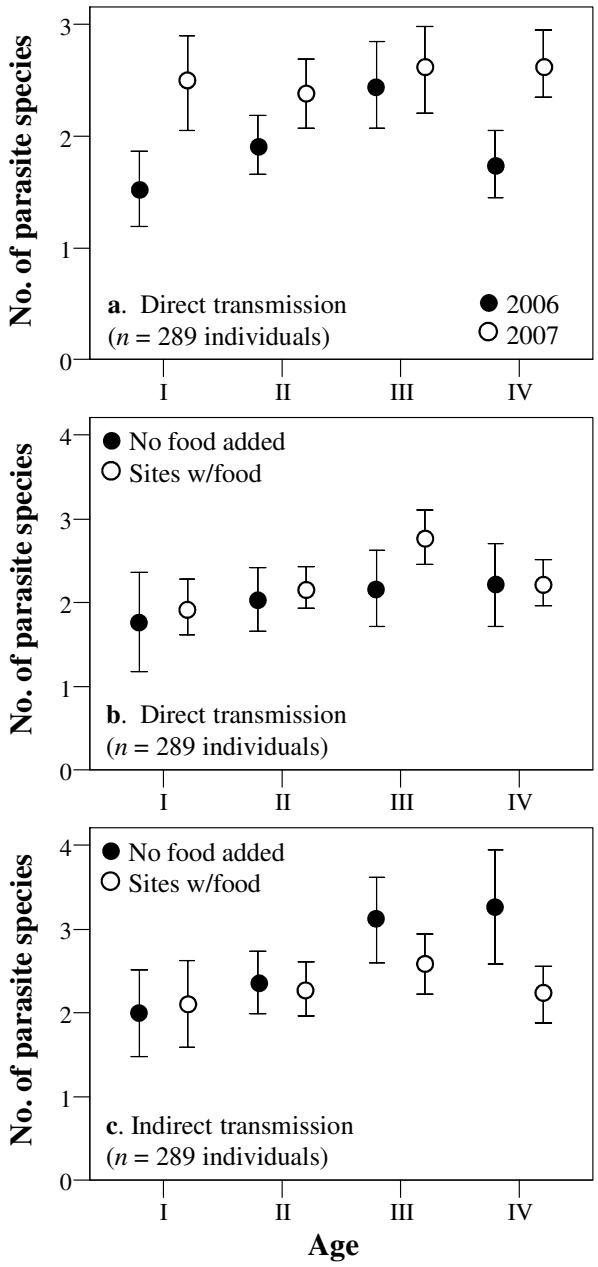

Figure 5. Average number of directly transmitted parasites detected per raccoon (± 95% C.I.) in (a) 2006 and 2007 and (b) at sites with food (control with food, aggregate) and without food (control); and (c) the number of indirectly transmitted parasite species per raccoon (± 95% C.I.) in sites with and without food.

CHAPTER 5: THE INFLUENCE OF PARASITES ON GLUCOCORTICOID METABOLITE LEVELS OF RACCOONS: AN EXPERIMENTAL ASSESSMENT IN A NATURAL SETTING

ABSTRACT

There are expected interactions between stress hormones and parasites because glucocorticoids can enhance or depress the immune system. Yet this subject has received relatively little attention among free-ranging animals and experimental research in natural settings is equivocal. I determined sampling constraints of measuring fecal glucocorticoid metabolites (FGM) in raccoons and conducted a parasite-reduction experiment to determine if nematodes and ectoparasites affect baseline levels of FGM in adult raccoons. Parasite reduction treatments occurred by treating raccoons with ivermectin to control nematodes, acanthocephalans, and ectoparasites, as well as applying Frontline Plus® to further control ectoparasites. Raccoons were recaptured from May to July and parasites and FGM were remeasured within 30 days of treatment. Parasite reduction treatments reduced the prevalence of most nematodes and the number of nematode species per individual, although there was no difference in the number of endoparasites in treated and control individuals when protozoans, trematodes, and cestodes were also included. Parasite reduction treatments were also highly effective at eliminating or reducing ectoparasites. The prevalence and abundance of the most widespread tick and louse species was lower in treated animals, and the number of

ectoparasite species per animal was lower in the treated versus control group. No differences in FGM values were observed within individuals or between treatment and control groups following parasite reduction treatments, indicating that the reductions in nematodes and ectoparasites had no effect on stress hormone levels of raccoons during summer. Because this study coincided with the most common and energetically expensive ectoparasite in the region (*Dermacentor variabilis*), I conclude that ectoparasites do not affect glucocorticoid levels of raccoons. However, given that helminths are one of the most likely groups to influence the endocrine system, further experimental work should focus on methods that can more effectively reduce all endoparasite species and measure their influence on stress hormone levels across seasons.

INTRODUCTION

Free-ranging animals must cope with a variety of environmental and social stressors that can influence survival and fitness. A number of behavioral or life history strategies may be employed to deal with such stress, but the common physiological response of vertebrates is activation of the hypothalamic-pituitary-adrenal axis, which increases the secretion of glucocorticoid hormones. Short-term increases of glucocorticoids are considered adaptive as it mobilizes energy and the immune system, while simultaneously inhibiting physiological processes that are not essential for immediate survival (Wingfield et al. 1995). However, chronic stressors, such as a drought or severe winter, lead to a long-term elevation of these hormones, which can suppress the immune (Fowles et al. 1993, Saino et al. 2003) and reproductive systems

(Sapolsky 1985, Orr and Mann 1992, Wingfield and Farner 1993, Dunlap and Schall 1995, Sapolsky et al. 2000, Kitaysky et al. 2003) and decrease survival (Brown et al. 2005).

In nature, baseline stress hormone measures are known to be correlated with age, sex, and social rank of some vertebrates (Millspaugh et al. 2001, Creel 2005, Sapolsky 2005). There are also expected interactions between stress hormones and parasites because glucocorticoids can enhance or depress the immune system. This subject has received relatively little attention, and experimental results from natural settings have not been consistent. Raouf et al. (2006) observed glucocorticoid metabolite levels of cliff swallows (*Petrochelidon pyrrhonota*) were lower in fumigated (ectoparasite free) colonies, but only when colony size was large. Pedersen and Greives (2008) found removal of nematodes with ivermectin reduced stress hormone levels of white-footed mice, but only when food was not limiting. Conversely, lungworm (*Protostongylus* spp.) removal in bighorn sheep (*Ovis canadensis canadensis*) did not alter glucocorticoid metabolite levels (Goldstein et al. 2005). Together, these results suggest alterations in stress hormones can be dependent on social factors and nutrition, and may not be present for some types of parasites or hosts. There may also be differences due to the parasite(s) removed; blood-feeding swallow bugs are known to have large, direct impacts on swallow survival and fitness, while lungworms are not known to have significant impacts on adult health or survival. For most species of wildlife it will be difficult to identify and target specific, energetically expensive parasite species. Thus, to better understand the interaction of stress hormones and parasites, reduction of parasite community size will be

the most beneficial route to determine if parasites have a consistent and general influence on the hypothalamic-pituitary-adrenal axis.

The nature of the relationship between stress hormone levels and parasite infection in natural settings also remains in doubt because glucocorticoid-parasite interactions are rarely studied in free-ranging animals. The general view is that prolonged elevations of stress hormone levels make an animal more susceptible to infection due to the immunosuppressive effects of glucocorticoids (Bly et al. 1997). There are, however, at least two alternative possibilities: increased stress hormone levels may occur after infection as a defensive response by the host, or elevated stress hormone levels do not increase susceptibility and may even enhance immunity to parasites (Hanley and Stamps 2002).

This study had two objectives. The first was to determine the sampling constraints of measuring fecal glucocorticoid metabolites (FGM) in free-ranging raccoons (*Procyon lotor*). This included validating the use of FGM for raccoons and conducting a challenge experiment to determine the temporal effects of trapping on FGM. The second objective was to determine if nematodes and ectoparasites (i.e., macroparasites) affect baseline levels of FGM in adult raccoons. This included a parasite reduction experiment to test for a causal relationship between macroparasites and stress hormone levels. I hypothesized that reduction of nematodes and ectoparasites would decrease baseline FGM levels of treated versus control animals.

MATERIALS AND METHODS

All field work took place at the University of Missouri Baskett Wildlife Research Area near Ashland, Missouri (38°45′ N, 92°12′ W). The area encompasses 917 ha and primarily consists of second growth oak (*Quercus* spp.) and hickory (*Carya* spp.) forest with a maple (*Acer* spp.) and cedar (*Juniperus virginiana*) understory. Research was carried out under Missouri Department of Conservation permit #13643 and University of Missouri Animal Care and Use Protocol #4231.

Challenge Experiment

I conducted an Adrenocorticotropin hormone (ACTH) challenge during May to July 2007. A trapline of 20 pairs of Tomohawk box traps was placed ~5 m from each side of road, with adjacent traps spaced 100 m apart. Traps were set at 20:00 and checked every 15 minutes using a spotlight from a vehicle. Once trapped, I immediately checked for feces in and below the trap and thereafter within 30 minute intervals until 6:00. Adult raccoons were randomly assigned to one of three categories: control (no injection), a sham injection of saline solution (Bloomer et al. 1995), or ACTH injection (Cortrosyn™; Amphastar Pharmaceuticals Inc., Rancho Cucamonga, California). Animals assigned to the ACTH injection category received two intramuscular injections in the hind quarters; the first dose consisted of 1 International Unit (IU)/kg of ACTH and was given immediately on capture, and the second dose consisted of 0.5 IU/kg of ACTH and was given 30 minutes after the first dose (1 IU = .01 mg of Cortrosyn™, see also Washburn et al. 2003). All ACTH injections were given in a saline vehicle solution.

Sham injections of saline were delivered at the same time (at capture and 30 minutes later) and consisted of the same volume as each ACTH dose.

Fecal samples were homogenized, placed in plastic vials on dry ice within 15 minutes of collection, and transferred to a -20°C freezer within four hours. I classified fecal samples obtained within 90 minutes as baseline samples and assumed they were not affected by trapping. The timing of greater FGM following a stressful event is dependent on the passage rate of the digestive system (Palme et al. 1996). I considered 90 minutes to be conservative given that FGM of similar-sized mammals, including Carnivora, do not peak for at least 8 hours and are not increased for ~180 minutes following ACTH injection (Wasser et al. 2000, Schatz and Palme 2001, Young et al. 2004). In addition, data from this study indicated no significant or consistent increase in FGM until 220 minutes post-capture (see Results).

I processed each raccoon starting at 6:00. Raccoons were immobilized with ketamine hydrochloride and xylazine (Evans 2002), tagged with metal ear tags, weighed, sexed, and aged by body size, genital morphology, and tooth eruption and wear (Grau et al. 1970). This resulted in five age classes; cub = 0-5 mo, I = 0–14 mo, II = 15–38 mo, III = 39–57 mo, and IV+ = over 58 mo. I only included adults in this study, defined as age classes II and above with one of the following characteristics: descended testes, evidence of lactation, or a weight of at least 4.5 kg.

Parasite Reduction Experiment

I conducted a parasite reduction experiment from May to July 2008 using an expanded but similar trap design and the same animal processing methods as described above. Animals were assigned to a treatment or control category on their initial capture. Ivermectin (1 mg/kg, Ivomec injectable; Merial; Hill et al. 1991, Bauer and Gey 1995) and Frontline Plus® (9.8% w/w fipronil and 8.8% w/w S-methoprene; Merial) or a sham injection of saline (i.e., the control category; Bloomer et al. 1995) was given randomly to adults and distributed evenly among males and females. Trapping continued through July to recapture animals and assess treatment effects on FGM levels and parasite burdens. Treatments were reapplied to recaptures that had not been treated within 20 days.

I collected parasite data from all animals to evaluate the efficacy of the parasite reduction treatment. Fecal samples were homogenized, with half placed in 10% formalin and the other half collected for stress analysis. Endoparasite ova and oocysts were identified by standard fecal floatation procedures using sugar and zinc sulfate centrifugation techniques (Bowman 1999). I quantified ectoparasites by direct count (adult ticks) and collecting hair samples via 10 strokes with a flea comb from the base of the neck to the base of the tail (lice, fleas) (Monello and Gompper 2007, 2009). Animals that were wet or muddy were excluded. Hair and ectoparasite samples were sealed in a plastic bag and placed in a -20°C freezer within eight hours. In the laboratory, I identified lice and fleas to species and quantified them under a dissecting scope.

I evaluated the efficacy of the parasite reduction treatment by comparing treated and control animals using all available parasite data. This included prevalence (% infected) of each endoparasite detected, prevalence and abundance of each ectoparasite detected, and three measures of species richness; number of endoparasites (all species detected), number of acanthocephalan and nematode species, and number of ectoparasites. The number of acanthocephalan, nematode, and ectoparasite species were evaluated separately because these are the primary species that should be eliminated or reduced with ivermectin and Frontline (Hill et al. 1991, Young et al. 2003, Bauer and Gey 1995). For treated animals, I limited data used in statistical tests to samples obtained within 30 days of an ivermectin and Frontline delivery because labels on these products indicated re-application is necessary after this time. This did not skew data towards earlier dates because animals were retreated after 20 days, allowing recaptures from late June and July to also be used in the analyses. I used all data from control animals. When multiple parasite samples were obtained for an individual, I included all endoparasites observed and averaged the abundance of each ectoparasite species to more accurately determine parasite burden. I tested for treatment differences ($\alpha = 0.05$) with Fisher's exact test (prevalence) and Mann Whitney *U* tests (abundance, number of species per individual).

Results from the 2007 challenge experiment indicated that FGM are artificially increased ~220 minutes after raccoons are trapped (see Results). I set traps at 20:00 and checked each one at least every 150 minutes to ensure baseline stress hormone levels were measured (versus an artificially high level due to trapping). I only used fecal

samples that were found the first time an animal was observed in a trap on a given night, and from animals that had not been captured in the previous five days. In addition, because FGM levels exhibit daily rhythms (Millspaugh & Washburn 2004), I only used defecations that occurred between 20:00 and 2:00. Fecal samples were homogenized and placed on dry ice within 15 minutes, where they remained for up to 10 hours until transferred to a -20°C freezer.

FGM Sample Preparation

I placed frozen fecal samples in a lyophilizer (Freeze-dry Specialties, Inc. Osseo, Minnesota, USA) for 24 hours. Freeze dried samples were sifted through a stainless steel mesh to remove large debris, with ~0.20 g placed in a test tube with 2.0 ml of 90% methanol and vortexed at high speed in a multi-tube vortexer for 30 minutes. Samples were then centrifuged at ~1900 *g* for 20 minutes, and the supernatant stored at -84°C until assayed.

Radioimmunoassay Procedures and Assay Validation

Corticosterone I^{125} double antibody radioimmunoassay (RIA) kits (Cat. #07120103, MP Biomedicals, Solon, Ohio) were used to quantify raccoon FGM concentrations. Fecal samples were analyzed in five assays. The ICN protocol for the corticosterone I^{125} RIA was followed, except all reagent volumes were halved (Wasser et al. 2000).

I conducted a standard assay validation including assessment of parallelism, recovery of exogenous analyte, intra- and interassay precision, and assay sensitivity (Jeffcoate 1981, Grotjan and Keel 1996, O'Fegan 2000) to confirm the assay accurately and precisely measured corticosterone metabolites in raccoon feces. Parallelism and recovery of exogenous corticosterone validation assays were conducted on two pooled fecal extract samples (each pool consisted of feces from five individuals). Parallelism ensures the assay maintains linearity under dilution, and recovery of exogenous corticosterone verifies accurate measurement throughout the working range of the assay (Jeffcoate 1981). I added exogenous corticosterone to the low and high pooled fecal extracts to obtain corticosterone values under higher dilution levels (each pool consisted of feces from five individuals). I used tests for equal slopes (parallelism) to determine if log-transformed curves of serially diluted pooled fecal extracts were parallel to log-transformed corticosterone standard curves. I used the low and high controls provided with the kit and analyzed them in the all assays. Interassay variation was calculated from these two controls by averaging the coefficient of variation of replicate tubes from 20 randomly chosen samples.

Statistical Analyses

I evaluated naturally occurring differences due to sex or season using control animals and the values observed among treatment animals during their first capture event (i.e., 'prior' to treatment). Multiple FGM measures from the same individual and month were averaged when they met sampling requirements (within 150 minutes of capture,

more than five days apart). When data spanned more than one month for an individual, I randomly selected one sample for analyses to assure independence between data points. I tested for treatment differences ($\alpha = 0.05$) between males and females with a Mann Whitney *U* test and between months with a Kruskal-Wallis test. I also used untreated animals to determine if total parasite species richness (including all endo- and ectoparasites) was correlated with FGM values.

I used FGM to determine the effects of the parasite reduction treatment on baseline stress hormone levels. I evaluated this in two ways. First, I measured the proportional change in FGM values of individuals in both the treatment and control groups before and after delivery of ivermectin and Frontline or the sham injection. Second, I evaluated differences by comparing FGM values of treatment and control groups. For treated animals, I only used fecal samples obtained within 30 days of ivermectin and Frontline delivery. I used all data from control animals. I averaged multiple FGM measures from the same individual (excluding the initial capture of treated animals) because no differences were observed between months (see Results), resulting in one value per individual. I tested for treatment differences ($\alpha = 0.05$) in FGM data with a Mann Whitney *U* test.

RESULTS

Validation and Challenge

Serial dilutions of raccoon fecal extracts ranging from 1:64 to 1:2048 resulted in displacement curves that were parallel to the corticosterone standard curve ($P > 0.05$, Figure 1). Mean recovery of added exogenous corticosterone (0.25-1.25 ng/ml) was 110% ± 1.92% (S.E., $n = 20$). Acceptable recovery ranges (90-110%) and parallelism indicate no sample matrix effects (Jeffcoate 1981, Grotjan and Keel 1996, O'Fegan 2000). Assay sensitivity was 1.25 ng/g. The manufacturer's reported cross-reactivity of the antisera was 100% with corticosterone and <1% for other steroids. Inter-assay variation for the assays was 7.4% and average intra-assay variation was 1.8%.

For the ACTH challenge, 23 animals with multiple fecal samples (mean per individual ± S.E.; 2.35 ± 0.12, $n = 54$ samples) met requirements for inclusion in the analyses (control $n = 14$ animals, saline $n = 4$, ACTH $n = 5$). Most samples were from females (20 of 23), as males had a lower capture rate. Across all treatment categories, raccoon FGM were 6.6% ± 16% (S.E., $n = 5$) higher through 220 minutes, but displayed marked increases 235-285 minutes after capture (Figure 2). The average rate of change from baseline FGM in the control category was -7% between 100-200 minutes post capture ($n = 3$ individuals), 32% between 200-300 minutes ($n = 4$), 242% between 300-400 minutes ($n = 4$), and 236% after 400 minutes ($n = 6$). However, the 32% increase observed during the 200-300 minute mark was relatively small (average change = 286.25 ng/g) given the variation observed in the baseline samples (mean ± 95% C.I.; 1032.73 ± 659.21 ng/g).

Baseline FGM measures varied widely, ranging from 169.43 to 4482.49 ng/g (mean ± 95% C.I.; 1032.08 ± 480.70 ng/g). The largest measures occurred at 15 minutes (3513.73 ng/g), 45 minutes (2142.45 ng/g), and 60 minutes (4482.49 ng/g) post capture. The six baseline samples collected between 75-90 minutes post capture ranged from 269.67-837.93 ng/g (see also Figure 2).

Parasite Reduction Experiment

Seven nematodes and one acanthocephalan (*Macracanthorhynchus ingens*) were detected during the experiment. The prevalence of six of seven nematode species was lower in the treatment group, and three of these were statistically significant ($P < 0.05$), including two that were eliminated from the host population (Table 1). Four endoparasite species that were not acanthocephalans or nematodes were also observed, consisting of two protozoans (*Eimeria* spp.), one trematode (*Eurytrema procyonis*), and one cestode (*Atriotaenia procyonis*). All of these species exhibited 6-16% higher prevalence in the treatment group, although none of these differences were statistically significant ($P > 0.12$ in all cases, Table 1). As a result, parasite reduction treatments halved the number of nematode and acanthocephalan species per individual ($P < 0.001$, Table 2), but there was no difference in the number of endoparasites in treated and control individuals when protozoans, trematodes, and cestodes were also included ($P > 0.23$, Table 2).

The prevalence of all six ectoparasites was lower in the treatment group, with the tick *Dermacentor variabilis* and louse *Trichodectes octomaculatus* exhibiting statistically significant declines ($P < 0.001$) and both flea species eliminated (Table 3). Abundance of

each ectoparasite was also lower in the treatment group, with the ticks *D. variabilis* and *Ixodes texanus* and louse *T. octomaculatus* exhibiting statistical declines ($P < 0.047$, Table 3). The number of ectoparasites detected per individual was >50% lower in the treated versus control group ($P < 0.001$, Table 2).

Baseline FGM levels of females were significantly higher than males (Mann-Whitney *U* test, $P = 0.006$, $n = 32$ females, 9 males) (Figure 3). Fecal glucocorticoid metabolites did not differ between month (Figure 3), regardless of whether males and females were considered together (Kruskal-Wallis $H' = 0.708$, $df = 2$, $P = 0.702$, $n = 41$ individuals) or separately ($P > 0.398$ for both). There was no correlation between FGM and total parasite species richness ($F_{1,26} = 0.037$, $P = 0.848$, $r^2 = 0.001$).

Measures taken before and after parasite reduction treatments indicated the proportional change in FGM within individuals did not differ between treatment and control groups (Mann-Whitney U test, $P = 0.624$). Overall individual measures of both groups displayed a similar change in FGM values; treated animals increased 26% ($n = 5$) and control animals increased 38% ($n = 4$).

Fecal glucocoriticoid metabolites did not differ between treatment and control groups, regardless of whether males and females were considered together (Mann-Whitney U test, $P = 0.236$, $n = 45$ individuals) or separately ($P \geq 0.259$) (Figure 4). Treated animals displayed more variation and had a higher mean FGM level than control animals (Figure 4a), but these effects were due almost entirely to three individuals with elevated stress hormone levels (Figure 4b).

DISCUSSION

Parasite reduction treatments lowered the prevalence and number of nematode and ectoparasite species in raccoons. Declines were observed in six of seven nematode species, and only one nematode (*Capillaria procyonis*) maintained a prevalence > 20% in the treatment group. Conversely, the prevalence of five nematodes was > 20% among animals in the control group. Treated animals also had a lower number of nematode species per individual. The parasite reduction treatment was also highly effective at removing or reducing ectoparasites. Only three species occurred on more than 45% of the control animals; the prevalence of two of these species was significantly reduced (*D. variabilis*, *T. octomaculatus*) and the third was reduced from 49% to 25% (*I. texanus*). In addition, the prevalence of several other species was at or near 0% and the three most abundant species all exhibited significantly lower abundance in the treated group. The number of ectoparasites per individual was also lower in the treatment group, with treated animals infested by one species each on average. In most cases, the species that maintained or reinfested treated animals was the tick *D. variabilis*, which still infested 75% of treated raccoons. However, the abundance of *D. variabilis* was markedly reduced from ~19 to 3.4 per raccoon in the treatment group. In addition, none of the ticks observed on treated animals (n = 24) were replete (i.e., engorged with blood), whereas 74% (42/57) of the raccoons in the control group had replete ticks (R. Monello, unpublished data). This suggests ticks on treated animals were recently acquired and unable to remain attached long enough to obtain a blood meal (7-10 days per Atwood and Sonenshine 1967).

No differences in FGM values were observed within individuals or between treatment and control groups following parasite reduction treatments. In addition, I detected no relationship between parasite species richness and FGM of raccoons. Because this work coincided with the peak abundance of *D. variabilis* ticks, the most common and energetically expensive ectoparasite in the region (Allan 2001, Monello and Gompper 2007), I conclude it is unlikely that any ectoparasite has a measurable effect on the stress hormone levels of raccoons.

Results also suggest that most nematodes in this study (with the potential exception of *C. procyonis* for which prevalence remained high) also had no influence on FGM levels of raccoons. This finding is consistent with Goldstein et al. (2005), who found lungworm removal did not affect the FGM levels of bighorn sheep. However, one limitation of my study is that it was conducted entirely in summer, and the prevalence and abundance of nematodes or their impact on raccoons may be greater in other seasons. Other studies have found that differences in stress hormone levels due to parasites are only manifested under certain social or resource conditions (Raouf et al. 2006, Pedersen and Greives 2008). Additional work that is able to assess the influence of endoparasites under a variety of conditions and seasons may be more likely to detect differences in stress levels if they exist.

Interestingly, the number of endoparasites per individual did not differ when protozoans, trematodes, and cestodes were included because all of these non-nematode parasites increased in the treatment group. Pedersen (2005) observed the same pattern

when mice (*Peromyscus* spp.) were treated with ivermectin. It is unknown why these parasites would increase when nematodes are reduced; one possibility that should be further explored is if a nematode induced innate immune response decreases non-nematode parasites. These results also indicate that the lack of differences in FGM levels between treatment and control groups in this study should be attributed to nematodes and not the entire endoparasite community. One additional limitation of this data is that I cannot accurately estimate the abundance of these endoparasites with fecal samples, as one adult female parasite can produce a large number of ova.

Because the treated animals had a higher mean FGM level (Figure 4a), one may be tempted to infer that a greater sample size would result in treated values that are statistically larger than control animals. There are two reasons why I consider this unlikely. First, the upper range of values observed in the treatment group were also observed in the baseline values of animals during the challenge experiment during 2007. This indicates values observed in the parasite reduction treatment were within the natural range of variation of untreated animals. Second, the inflated mean value of stress in the treated animals was primarily due to just three individuals. Together these findings suggest the lack of values >2500 ng/g in the control group was due to chance and not because parasites prevented such a response. In addition, although parasites can regulate host immune systems at the molecular level (Maizels et al. 2004), I am not aware of evidence that parasites can directly or indirectly down-regulate FGM production. Hanley and Stamps (2002) observed lower stress hormone levels among iguanas (*Ctenosaura similis*) parasitized with ticks or blood parasites, but subsequent experimental work did

not allow them to attribute this difference to parasite regulation of stress hormones. Conversely, studies across a variety of taxa have found a positive relationship between parasite infection and the stress hormone values of hosts (Maier and Watkins 1999, Belden and Kiesecker 2005, Muehlenbein 2006, Raouf et al. 2006, Arriero et al. 2008).

Sex and life history stages can influence baseline stress hormone levels (Reeder and Kramer 2005, Keay et al. 2006). In this study, female raccoons had higher FGM values than males, a finding that is frequently observed among mammals (Reeder and Kramer 2005). Differences between males and females are pronounced during and after pregnancy (Kenagy and Place 2000, Reeder et al. 2004) because higher glucocorticoids are positively correlated with mammary gland development and lactation (Voogt et al. 1969, Walker et al. 1992). The majority of females in this study were lactating (95%), and it was likely an important factor in the sex differences observed here. Higher baseline stress values did not prevent further increases in FGM, as females with the highest FGM values in the challenge experiment still exhibited increases of 285-520% after 300 minutes in a trap (see also Place and Kenagy 2000). It is feasible that the greater FGM production associated with lactation obscured a decline in baseline FGM during parasite removal, but I consider this unlikely because males also displayed no decline when parasites were removed.

It is difficult to examine ecological relationships of glucocorticoid metabolites in species that need to be captured for identification because trap-induced stress can prevent acquisition of baseline measures (Reeder and Kramer 2005). To overcome this, several studies have constantly monitored traps and sampled blood within three minutes of

capture to obtain baseline measures (Kenagy and Place 2000, Place and Kenagy 2000, Reeder et al. 2004). To my knowledge, this is the first study to compare the stress response of trapping to an ACTH challenge in a mammal. Results indicate that defecations within ~200 minutes of being trapped represent baseline measures. One limitation of this approach is that few individual raccoons defecated within 90 minutes of capture and then again within 100-200 minutes of capture (n = 3, all between 180-190 minutes). There was no increase in FGM among these individuals, but further work will refine the timing of usable samples. Once FGM did increase, the response among most of the control animals was markedly less than injected animals (saline or ACTH). This suggests trapping, in the absence of other stressful stimuli (e.g., an injection), may underestimate the magnitude of stress response. Thus, even relatively small increases in average FGM values among control animals, such as the 32% increase observed between 200-300 minutes, should be assumed to be due to stress that is trap-induced when no other stressful stimuli such as injections are present.

In conclusion, I found no evidence that glucocorticoid metabolites differ due to ectoparasite prevalence, abundance, or species richness. Most nematodes were eliminated or reduced in treated raccoons, and also had no effect on glucocorticoid values. However, further work is needed to determine if multiple anthelmintic drugs could be used in combination to reduce or eliminate endoparasites in free-ranging animals and measure the response of FGM across seasons. Given the myriad of ways that helminths can influence hosts (Maizels et al. 2004), this is one of the most likely

group of parasites that could regulate the endocrine system and consequently, glucocorticoid metabolites.

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Table 1. Prevalence (% infected) of endoparasite species detected among adult raccoons in control and treated categories. Parasites listed above the dashed line are nematodes or acanthocephalans, which are typically treatable by ivermectin. Treated animals were re-measured within 30 days of ivermectin and Frontline delivery. Different letters within the same row represent significant differences for Fisher's exact test (prevalence data, $P < 0.05$).

Parasite Species	Control (n = 27 individuals)	Treated (n = 16 individuals)
Capillaria procyonis	76%	63%
Molineus barbatus	55%	19%
Capillaria puttori	45%[a]	13%[b]
Crenosoma spp.	27%[a]	0%[b]
Capillaria plica	21%[a]	0%[b]
Placoconus lotoris	18%	6%
Physaloptera spp.	3%	13%
Macracanthorhynchus ingens	0%	6%
Eimeria nutalli	94%	100%
Eurytrema procyonis	64%	81%
Eimeria procyonis	58%	75%
Atriotaenia procyonis	3%	19%

Table 2. Mean number (±S.E.) of endo- and ectoparasite species detected among adult raccoons in control and treated categories. All sample sizes represent individual animals. Different letters within the same row represent significant differences (Mann Whitney *U* test, $P \leq 0.001$ in all cases).

Parasite Species	Control	Treated
Nematode, acanthocephalan spp. per individual ($n = 43$)	2.48 ± 0.25^{a}	1.19 ± 0.23^{b}
Endoparasite spp. (all) per individual ($n = 43$)	4.67 ± 0.31	3.94 ± 0.31
Ectoparasite spp. per individual ($n = 81$)	2.30 ± 0.13^{a}	1.08 ± 0.13^{b}

Table 3. Prevalence (% infected) of ectoparasites and number of ectoparasite species per individual (± S.E.) among adult raccoons in control and treated categories. Treated animals were remeasured within 30 days of ivermectin and Frontline delivery. Different letters within the same row and category represent significant differences for Fisher's exact test (prevalence data, $P < 0.001$).

Parasite Species	Control Prevalence ($n = 57$)	Treated Prevalence ($n = 24$)	Control Abundance ($n = 57$)	Treated Abundance ($n = 24$)
Dermacentor variabilis	100%[a]	75%[b]	19.25 ± 2.47[a]	3.44 ± 0.84[b]
Ixodes texanus	49%	25%	1.87 ± 0.49[a]	0.58 ± 0.25[b]
Amblyomma americanum	19%	4%	0.26 ± 0.07	0.04 ± 0.04
Orchopeas howardi	7%	0%	0.09 ± 0.05	0 ± 0.00
Chaetopsylla lotoris	5%	0%	0.07 ± 0.04	0 ± 0.00
Trichodectes octomaculatus	47%[a]	4%[b]	2.24 ± 0.68[a]	0.08 ± 0.08[b]

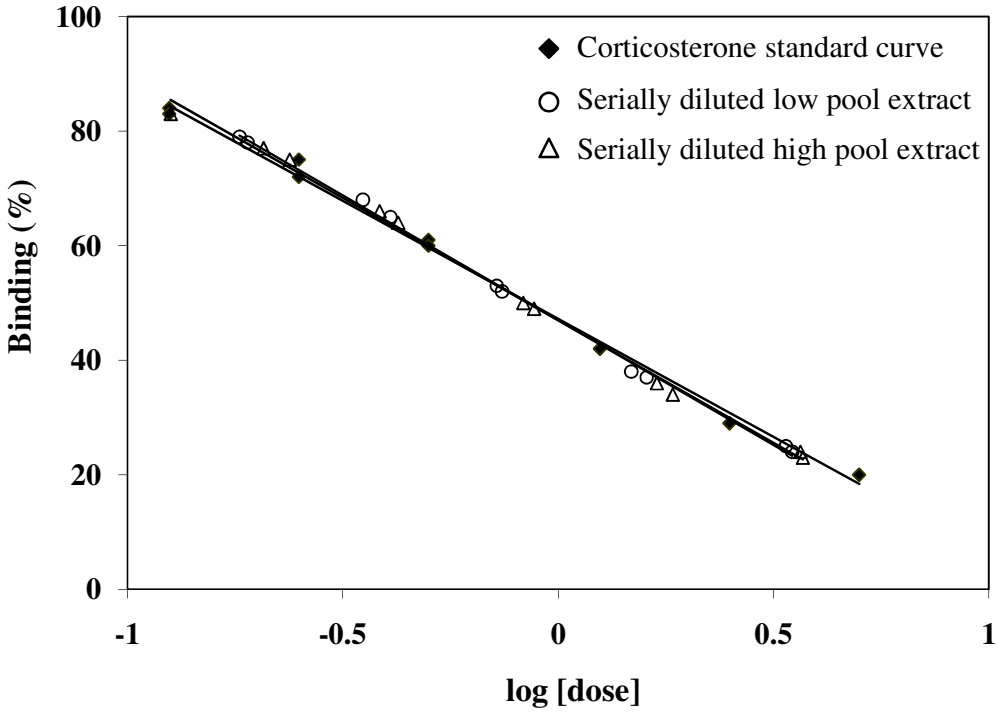

Figure 1. Parallelism of fecal glucocorticoid metabolite results for fecal extracts from raccoons (*Procyon lotor*). Curves of % binding of I^{125} tracer versus serially diluted (log-trans doses of 1:64 to 1:2048) low pool and high pool fecal extracts were parallel (n = 2 per pool; test of equal slopes, all $P > 0.05$) to corticosterone standard curves (log transformed doses of 0.125 to 5.0 ng ml^{-1}).

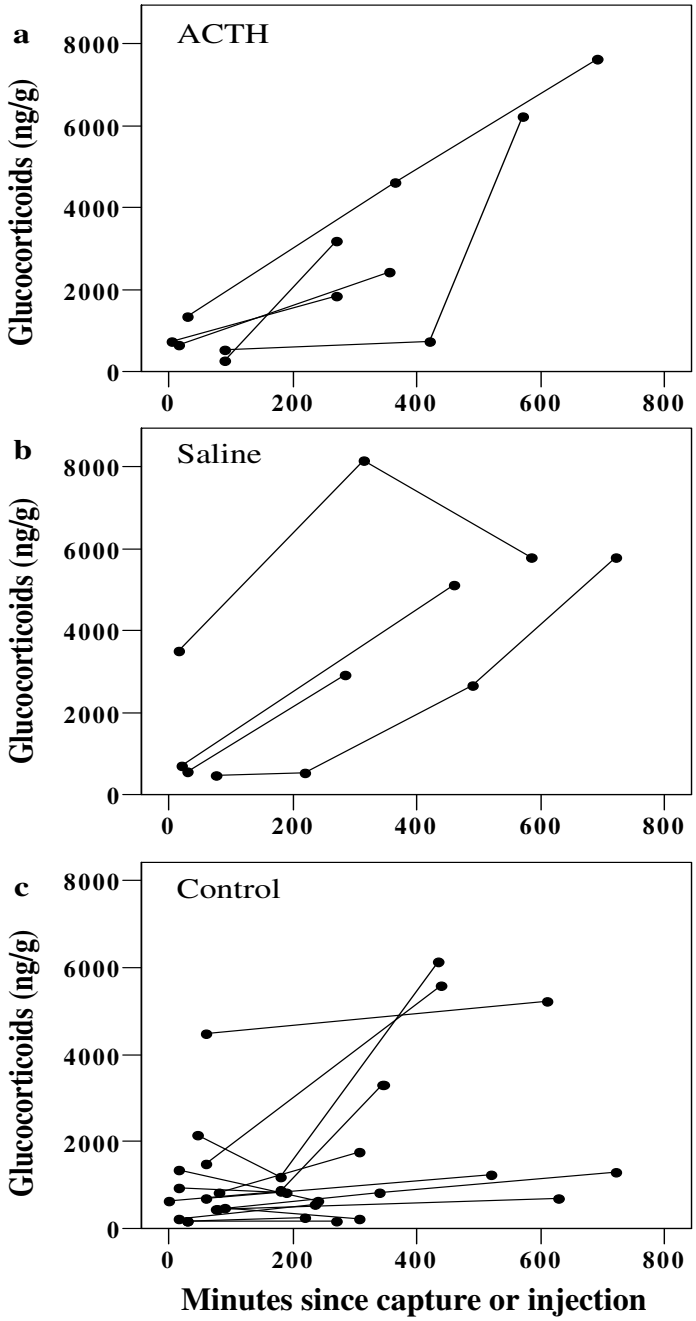

Figure 2. Glucocorticoid values of 23 adult raccoons assigned to a) Adrenocorticotropin hormone (ACTH) challenge, b) saline injection, or c) no injection (control). Lines show samples from the same animal and do not represent a rate of change.

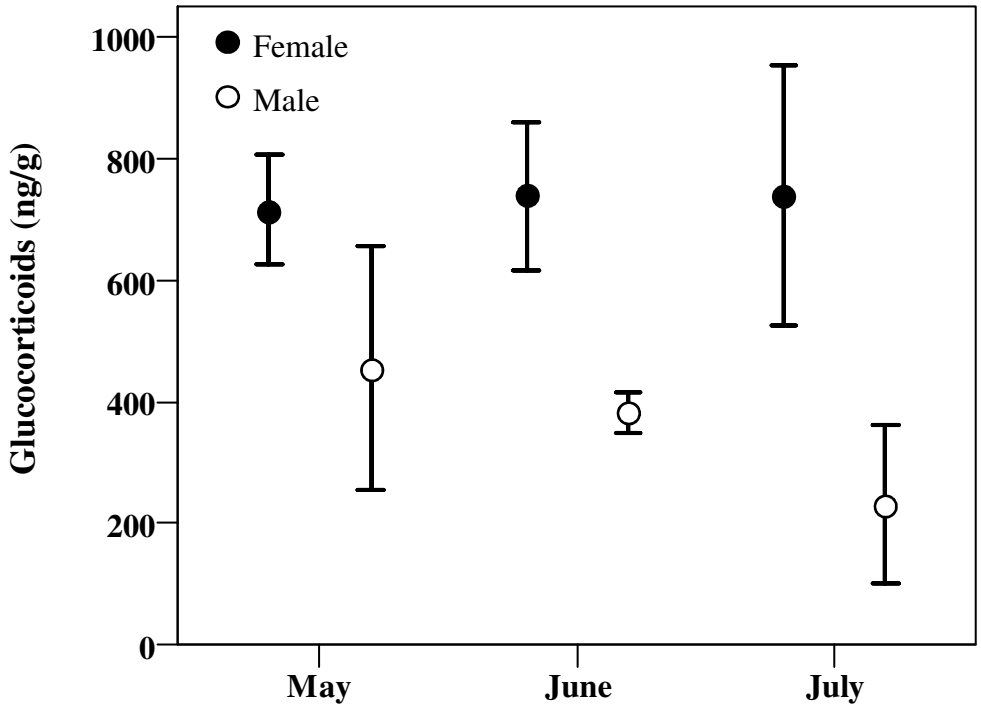

Figure 3. Fecal glucocorticoid metabolites (± S.E.) among male and female adult raccoons from May to July (n = 41 individuals).

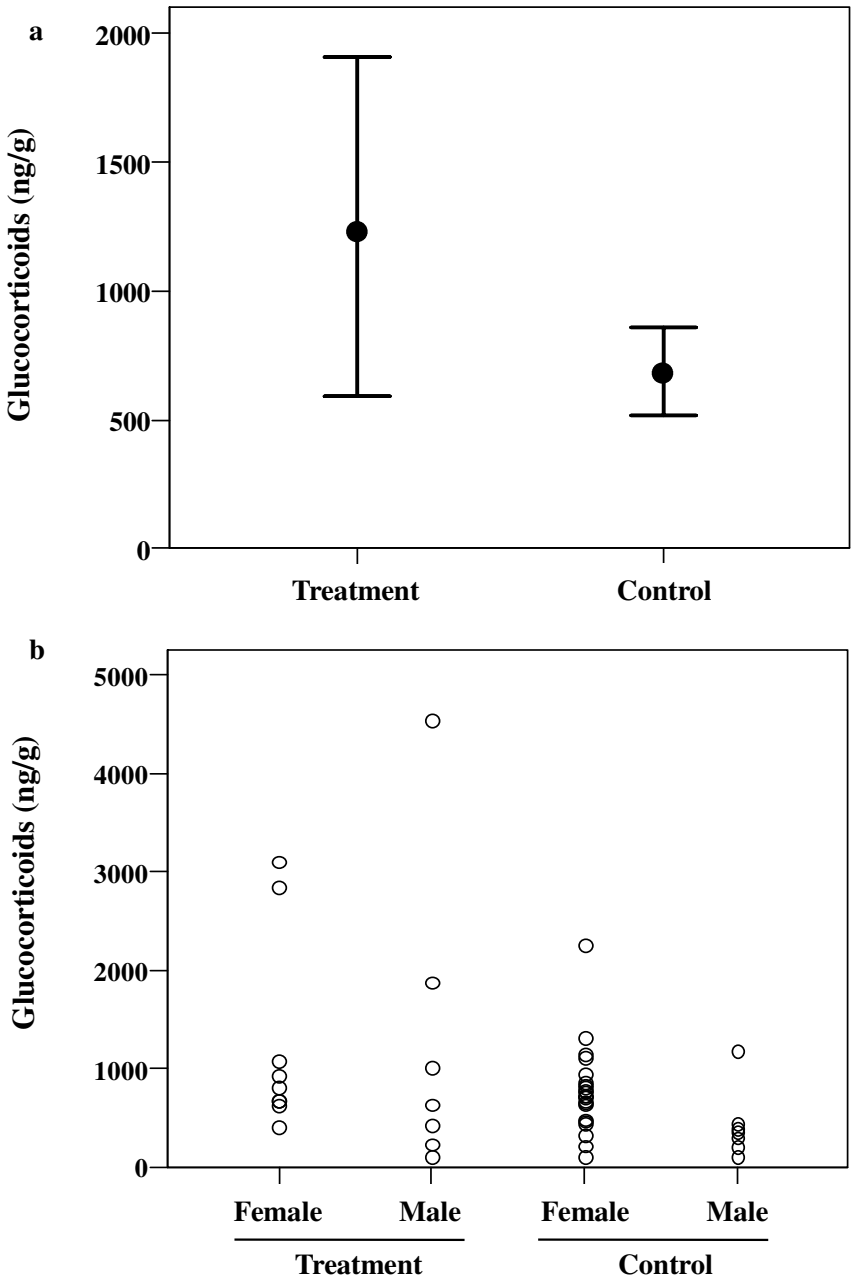

Figure 4. Fecal glucocorticoid metabolites of adult raccoons treated with ivermectin and Frontline or a saline injection (control) (n= 45 individuals): a) mean ± 95% C.I. of each group; and b) individual data points of females and males. Glucocorticoids of treated animals were measured within 30 days of a treatment application.

MANAGEMENT AND RESEARCH IMPLICATIONS

A central theme of this research was to determine if and how free-ranging animals can influence their parasite populations and community. It is clear from this work that multiple factors are important in natural settings and the relationship between host ecology and parasites is as diverse as the parasite assemblage itself. However, there were a number of implications beyond the scope of any particular chapter in this dissertation. Here I expand on these and discuss potential applications to the management and research of parasites and their wildlife hosts.

Ticks were the only macroparasite in this study that displayed clear and consistent increases due to host aggregation. Ticks are of interest because they can transmit bacteria (i.e., tick-borne illnesses) among animals and humans. An increase in the number of replete *Dermacentor variabilis* ticks on their primary host (as observed here) could result in a higher number of egg masses and larvae in the environment, particularly near areas where raccoons aggregate. This could increase or decrease the transmission of tick-borne illnesses, depending on whether or not raccoons or *D. variabilis* are efficient vectors for the bacteria. Sufficient information does not currently exist to make this determination; however, *Dermacentor* spp. and raccoons can harbor a diverse array of bacteria and it is prudent to assume that disease facilitation will occur in such a scenario. It is also likely that such effects would be most important to domestic animals and humans because anthropogenic activities are a primary cause of raccoon aggregations. Interestingly, such effects would only be expected to occur near natural or semi-natural environments that

support tick populations, as tick populations are generally smaller or not present in heavily urbanized areas.

Consistent with other research, I found the age and sex of a host can have large ramifications on their parasite burden. This suggests management strategies that shift the age or sex structure of the population may also influence parasite abundance and richness. For example, a shift towards a younger population with more females may reduce parasites over a short-term time frame (i.e., when trapping starts), but it could also increase the number of new migrants and susceptible animals (those with no previous exposure or resistance to some parasites) in the population and result in a higher parasite burden in the long-term. Further, aside from ticks, most of the short-term declines in parasites that would be associated with a younger population would occur among parasite species that have relatively little known importance to other host species, including humans (e.g., a variety of endoparasites such as *Capillaria* spp., lice). Generalist, virulent parasites - such as *Baylisascaris procyonis*, canine distemper, and rabies – are likely to increase with a younger age structure. Management strategies would create such a scenario if older animals are selectively removed from a population, or if removal of animals is likely to bring in new, young migrants.

It is perplexing that lice and directly transmitted endoparasites did not exhibit increases in sites where raccoons aggregated. The finding that maximum louse infestations and endoparasite species richness were attained in age class I (regardless of treatment) supports the hypothesis that these parasites are transmitted at a relatively high rate in these study sites. But what conditions create such high contact between raccoons

and these parasites? The populations in this study exist at a relatively high density (20-40 raccoons/km^2), and this likely facilitates contact with directly transmitted endoparasites, which are transmitted through feces. Because no alterations in density were observed, contact between raccoons and directly transmitted endoparasites may have been unaffected by a single large food pile or point of contact. This is supported by the finding that, despite extensive searches, feces were rarely found near the food plots in aggregated sites. Raccoons also frequently form semi-permanent dyads that travel and den together. Males were occasionally caught together in the same trap (meaning they were travelling directly next to one another) and denned together (based on radio-telemetry data) in this study. The propensity for males to den together could have contributed to higher louse burdens (relative to female raccoons); lice may require prolonged contact for transmission (as in a den) and this was unlikely to occur in aggregated sites.

It is interesting to consider the alterations in behavior observed at the aggregated food plots over the course of the study. Initially, raccoons were only observed to feed with conspecifics or in the presence of opossums. As the experiment progressed, raccoons were frequently found feeding with coyotes and foxes for prolonged periods of time (>30 mintues). This suggests raccoons can adapt behaviorally and determine that the perceived benefit outweighs the risk of feeding near other, potentially dangerous species. This could have important disease ramifications to other species where raccoons aggregate, as the most common source of disease-related die-offs in low density carnivores is due to transmission of microparasites such as rabies or canine distemper from an abundant to less common carnivore.

VITA

Ryan Monello graduated with a B.A. in Biology from Benedictine College (1995) and a M.S. in Wildlife Resources from the University of Idaho (1998). His interest in disease ecology began as a Research Associate at University of Idaho, where he conducted research on pneumonia epizootics in bighorn sheep. In 1999, he began to work as a Biologist for the National Park Service at Rocky Mountain National Park (1999-2003, 2005-current) and National Park of American Samoa (2003-2004). Ryan began his doctoral work in August 2004 and will continue to pursue topics from this work as a Postdoctoral Associate at the University of Missouri.