Parasitism of damselflies (*Enallagma boreale*) by gregarines: sex biases and relations to adult survivorship

K.R. Hecker, M.R. Forbes, and N.J. Léonard

Abstract: We studied host damselflies *Enallagma boreale* (Odonata: Coenagrionidae) and their gregarine parasites (Apicomplexa: Eugregarinidae) to elucidate the causes and consequences of any sex biases in parasitism of adult hosts. Larvae of both sexes were highly infected, but there was no difference between male and female larvae in either prevalence or intensity of gregarine infections. Newly emerged adults had few or no parasites, thereby setting the stage for investigating accumulation of parasites by adults. Adult females had a higher prevalence and intensity of infection by gregarines than did males, but only on 1 (of 2) days when the potential confounding factor of host age was controlled for. Both adult males and females showed a positive correlation between longevity under conditions of food stress and the number of gregarines they initially carried. This finding may be explained if the food ingested with the infective cysts is more beneficial than the parasites are harmful, and it also has implications for investigating sex biases in numbers of trophically transmitted parasites of such insects.

Résumé : Nous avons étudié des demoiselles *Enallagma boreale* (Odonata : Coenagrionidae) en tant qu'hôtes de grégarines (Apicomplexa : Eugregarinidae) dans le but d'élucider les causes et les conséquences d'une sélection des hôtes adultes en fonction de leur sexe. Les larves des deux sexes étaient très infectées, mais il n'y avait pas de différence entre les larves mâles et les larves femelles quant à la fréquence et à la gravité des infections. Les adultes récemment émergés avaient peu ou n'avaient pas de parasites, une condition idéale pour étudier l'accumulation des parasites chez les adultes. Chez les femelles adultes, la fréquence et la gravité des infections de grégarines étaient plus élevées que chez les mâles, mais seulement au cours de l'une des 2 journées où l'âge de l'hôte, un facteur potentiel de confusion, a été contrôlé. Chez les adultes, mâles et femelles, nous avons trouvé une corrélation positive entre la longévité dans des conditions de stress alimentaire et le nombre initial de grégarines parasites. Ce résultat peut s'expliquer si l'ingestion de nourriture contenant des kystes infectés comporte plus de bénéfices que les parasites ne causent d'effets nuisibles et il faudra en tenir compte lors de l'étude de la sélection d'un hôte en fonction de son sexe chez des parasites d'insectes transmis par les aliments.

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Introduction

Parasites can infect and affect conspecifics differentially. For example, males and females can differ in encounter rates with parasites (Bundy 1988) or in their immune responses to parasites (Schalk and Forbes 1997). In extreme instances, only one sex acts as host to particular species of parasites, as with blood-feeding mosquitoes and malarial protozoans. The degree to which parasites can harm different hosts is important for assessing their impact on populations (Anderson and May 1981). In most models of parasite–host interactions, only female hosts are considered. The impact of macroparasites on fecundity and survivorship, relative to aggregation of parasites on individual hosts, should determine the impact of parasites on host populations (Jaenike 1996).

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For these reasons, researchers have become interested in differential susceptibility and (or) immune responses of individuals (often males and females) to parasitic infections. Sex differences in immune function and exposure to parasites are well documented for vertebrates and may explain sex differences in prevalence or intensity of parasitism (Poulin 1996; Schalk and Forbes 1997). For invertebrates, sex differences in parasitism are generally not observed (Sheridan et al. 2000); when they are observed, their causes are not well understood (Zuk and McKean 1996; but see Wedekind and Jakobsen 1998). In this study, we examined whether sex differences in either prevalence or intensity of infection by gut protozoans exist for the damselfly host *Enallagma boreale* (Selys). Prevalence is the proportion of hosts infected by a particular parasite species or higher taxon, whereas intensity is the mean number of parasites per infected host in the sample (Bush et al. 1997). We also examined whether infections by these gregarines were more costly to either sex.

We studied this parasite–host association for several reasons related to both parasite and host biology. First, although the taxonomy of insect gregarines is not well understood, keys to gregarine parasites of damselflies are under construction (Perkins et al. 2001). Second, the basic ecology and life cycle of gregarines parasitic on odonate insects is fairly well understood. Gregarines appear to be ingested in

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their infective stage, along with insect prey, by feeding damselflies and dragonflies. Gregarine oocysts (the infective stage) have been found attached to the legs of chironomid midges (Åbro 1974); such insects can be completely macerated by damselflies and ingested as a bolus (within which the oocysts are embedded).

Regarding hosts, male and female *E. boreale* differ in their foraging ecology. Males of many coenagrionid damselflies, including *E. boreale*, appear to spend enough time foraging away from ponds to ensure that they have sufficient energy for daily mate searching (Anholt 1992). In comparison, females are energy maximizers and spend longer away feeding in order to mature clutches of eggs and avoid harassment from mate-searching males (Anholt 1992). Determination of this difference in foraging ecology was based on measures of gut fullness (Anholt 1992). This difference is further believed to be responsible for biases in operational sex ratios at ponds, with mate-searching males outnumbering receptive females (Anholt 1992; cf. Fincke 1994). Based on this sex difference in foraging ecology, we predicted that females would be more heavily parasitized by gregarines than males of the same age, simply because females ingest more food.

Primarily we wanted to assess whether males and females of the same age differ in their gregarine numbers as a result of differences in their feeding ecology. Related to this larger question, we had four specific objectives. First, we examined whether larval males and females differ in their degree of parasitism by gregarines. Sex differences in habitat use and foraging ecology are not usually studied in larval damselflies, although at least one study on another coenagrionid damselfly has indicated that males and females may differ in their development times (Baker et al. 1992). As indicated, we expected differences in parasitism of adult sexes due to differences in their foraging ecology. However, we first had to rule out pre-existing biases in gregarine parasitism of the larval stages of *E. boreale*. Secondly, we were interested in whether larval damselflies might lose any resident gregarines during transition to the adult stage. Thus, we were interested in whether pre-reproductive newly emerged damselflies (hereinafter teneral adults) start their adult life with few or no gregarines and acquire them through time, since this is the basis for expecting the adult sexes to differ in prevalence or intensity measures.

Our third and main objective was to compare variation in prevalence and intensity of gregarines infecting adult *E. boreale* across two sites and two sampling periods. We chose two study sites because we expected that spatial variation in parasitism might exist and because sex biases might only be detectable when prevalence of parasitism was high. In another study of gut protozoans in insects, Arnqvist (1992) showed that host population densities were related positively to prevalence. More specifically, Åbro (1974) showed both spatial and temporal variation in gregarine numbers within damselfly hosts, as did Taylor and Merriam (1996). If the prevalence of gut protozoans is low, sex biases may not be observed unless very large samples are collected. Thus, we expected to find sex biases in parasitism only when age of hosts could be reasonably controlled for, and when enough infective stages of gregarines were present so that infection and accumulation of parasites for individual hosts were likely.

Like other parasites, gregarines are expected to change in numbers over the lifetime of individual hosts. In fact, age-related profiles of parasitism are well documented for many parasite–host associations in general (Scott 1988) and for insect–gregarine associations in particular (Zuk 1988). Thus, researchers have to be sure that hosts included in samples for comparison are the same or similar in age. Otherwise, any sex biases may be obscured by age variation within samples of hosts.

Finally, we examined whether gregarines affected adult males differently than adult females by bringing damselflies (of unknown infection status) into the laboratory and subjecting them to food shortages. Food shortages often occur indirectly at our study location during a series of cool rainy days, when damselflies are forced to roost and forgo feeding (Forbes and Baker 1991; Leung and Forbes 1997). Previous experiments with a related species (*Enallagma ebrium* (Hagen)) showed that males survived less well than females under seminatural conditions (Forbes and Leung 1995). Male damselflies might survive less well with a given number of gregarines simply because they have fed less than females. As indicated below, any sex differences in survivorship can have implications for detecting sex biases in parasitism in natural populations. Our study, however, does not assess sex differences in survivorship associated with costs of more feeding (cf. Anholt 1992).

Methods

Study species

Enallagma boreale is a common and widespread coenagrionid damselfly that typically inhabits beaver ponds and freshwater marshes in Eastern Ontario, Canada (Forbes 1991). As in many other *Enallagma* species, the mating system is defined as scramble mate competition (Fincke 1994). Although females show a suite of male-rejection behaviours, these typically follow oviposition (which occurs under water in the absence of the male) (Fincke 1994). After the female has laid her eggs, she resurfaces and soon flies away from the breeding pond, often chased by single males at the pond margin (Forbes 1994).

Gregarines used in this study were *Hoplorhynchus* spp. (R. Clopton, personal communication). These are septate gregarines (Eugregarinidae) with one host in their life cycle. The infective oocytes are ingested by the host, and once in the midgut, they split to produce eight sporozoites per oocyte (Åbro 1976). The sporozoites then attach to the gut wall as trophozoites and absorb digesta. After growing for some time, the trophozoites detach from the gut epithelium and develop into gamonts that move about freely in the gut. Gamonts find a mate and undergo syzygy, after which the two attached individuals are enclosed in a protective covering and become a gametocyst. Depending on temperature, the gametocyst is excreted approximately 2 weeks after ingestion of the oocysts (Åbro 1974). Each gametocyst undergoes development and eventually releases many oocysts; in some species, development of oocysts follows an obligatory period of low temperatures (Åbro 1976).

Table 1. Comparison of prevalence of gregarine infection in *Enallagma boreale* larvae and teneral adults.

	Site	Sex	*n*	Prevalence (%)	χ^2	*p*
Larvae	JM	Male	30	93.3 ± 9.1	0.15	0.69
	JM	Female	32	90.6 ± 10.2		
Teneral adults	JM	Male	59	23.7 ± 10.7	0.01	0.94
	JM	Female	82	23.2 ± 9.1		
	UD	Male	24	4.2 ± 7.8	1.89	0.17
	UD	Female	39	15.4 ± 11.2		

Note: JM and UD refer to the study sites Jack's marsh and Upper Dowsley, respectively. Larvae were collected on 16–20 May 1997. Teneral adults were collected from 30 May to 2 June 1997; *n* is the sample size. Prevalence estimates are given with the confidence interval (CI).

Study sites

Our study sites were Jack's marsh and Upper Dowsley pond (hereinafter JM and UD, respectively). Both sites are flooded beaver ponds located less than 10 km from the Queen's University Biological Station near Chaffeys Lock, Ontario, Canada (44°34′N, 79°15′W). Both support dense populations of *E. boreale* (at UD, M.R. Forbes estimated close to 10 000 damselflies emerging in one day; unpublished data). JM is roughly oval in shape and ca. 1 ha in size, whereas UD is <1 ha and approximately round in shape. Ponds at both sites have grassy margins with sedges and are bordered by mixed deciduous forests. Emergent vegetation at both sites is largely cattails (*Typha* spp.) and sedges (*Carex* spp.), although there are many dead trees in JM and these serve as emergence sites for *E. boreale*. A fuller description of these sites is presented in Forbes (1991).

Age of damselflies

Determining accurately the ages of some damselflies is difficult. Recapture methods yield a low recapture rate when applied to damselflies (e.g., *E. boreale* in Eastern Ontario, Canada; M.R. Forbes, unpublished data). Counting daily growth rings based on cuticular sections has been used to age some insects, including odonates (Neville 1967; Zuk 1988); however, this method is prone to error after the pre-reproductive period for *E. boreale*. One way to ensure a reduction in age variation is to survey ponds and collect samples at the start of the flight season, when the first mature damselflies return to ponds and are approximately the same age (Corbet 1999). Another indirect method is to sample only those individuals that have nearly to fully engorged mites (*Arrenurus drepaniphorus* Cook), since these adults are approximately the same age (10–14 days old; Forbes 1991). We employed both of the latter methods in order to include only young adults in samples collected during early June 1997 at JM and UD and in the sampling done in 1998. Damselflies collected later in June 1997 included both old and young adults.

Collections and dissections

Larvae and adults were collected from 16 May to 12 June 1997 and from 6 May to 23 May 1998 (Tables 1–4). Final-instar larvae (with wingpads extending to the fourth abdominal segment) were collected using an aquatic D-frame net. Both teneral and mature adults were caught in aerial insect nets. Adults were classified as teneral if the wings had a rainbow sheen and the body was soft and grey to brown in colour (Walker 1953). In comparison, reproductively mature adults had clear, hardened wings and brightly coloured, firm bodies. Over all samples, 62 larvae, 204 teneral adults, and 520 reproductively mature adults were collected (Tables 1–4).

After collection, each damselfly was sexed. Following decapitation, their complete gut was removed by grasping the posterior abdominal segment with forceps and gently pulling. The gut was then preserved in sugared ethanol (1 L water : 1 L 95% ethanol : 40 g sucrose) and stored individually and temporarily in an Eppendorf microcentrifuge tube. Using a dissecting microscope, the gut was later located, excised from the body wall, and then dissected with 00 insect pins. The pins were used to break open the gut wall and separate gregarines from the gut and digesta. A drop of 2.5% pharmaceutical iodine placed on the dissected gut dyed the gregarines a unique shade of dark red, increasing their contrast with the gut and allowing accurate enumeration. Gregarine trophozoites, gamonts, and oocytes were counted.

To compare the effects of parasites on adults of both sexes, 31 males and 28 females were collected on 2 June 1997. Individuals were placed singly in plastic cups containing 2 mL of water beneath window-screen mesh and a wooden dowel for a perch. Each cup was covered with perforated aluminum foil. The cups were placed in a dark, cool room (between 14.0 and 19.5°C) until the individuals became moribund or died. As mentioned, these conditions simulated a natural period of food deprivation resulting from prolonged cool, cloudy weather (Forbes and Baker 1991; Leung and Forbes 1997). Individuals were classified as moribund if they were unable to move when lightly touched. We checked the cups every 12 h for moribund or dead individuals. Most individuals were moribund and were quickly dispatched as above. Both moribund and dead individuals were dissected for enumeration of their gregarines. We measured the forewings of damselflies from the nodus to the tip (Forbes 1991). Wing length relates to survivorship of *Enallagma* spp. under such conditions in other studies (Leung and Forbes 1997).

Statistics

Gregarine prevalence was compared between male and female larvae and adults using 2×2 χ^2 tests. To allow direct comparisons between samples, we also calculated 95% confidence limits for prevalence of infections, following Snedecor and Cochran (1980). We applied ln transformation (number of gregarines + 1) to approximate normal distributions for intensity data and to allow the use of parametric tests because untransformed data for intensity did not conform to normal distributions ($p < 0.05$ using Shapiro–Wilk tests). We then used unpaired *t* tests or two-way ANOVAs to compare mean intensities between the sexes of larvae or sexes of adults, controlling for site where applicable.

For the survivorship experiment we performed linear or nonlinear regressions between the number of days males or females survived and ln(number of gregarines + 1). We did this analysis to examine the effect of gregarines on survivorship while controlling for wing length as a covariate (Zar 1996).

Table 2. Comparison of intensity (ln-transformed) of gregarine infection in *E. boreale* larvae and teneral adults.

	Site	Sex	*n*	ln(intensity)	BT mean*	*t*	*p*
Larvae	JM	Male	28	2.25	9.5	–0.30	0.76
	JM	Female	29	2.32	10.3		
Teneral adults	JM	Male	14	1.39	4.0	0.089	0.93
	JM	Female	19	1.37	13.9		
	UD	Male	1	1.09	93.0	na	na
	UD	Female	6	1.36	83.9		

Note: JM and UD refer to the study sites Jack's marsh and Upper Dowsley, respectively. Larvae were collected on 16–20 May 1997. Teneral adults were collected from 30 May to 2 June 1997; *n* is the sample size; "na" signifies that a *t* test was not possible because of a low sample size for one category (males). However, intensity in the one parasitized male teneral adult was within the 95% CI of intensity for female teneral adults.

*Back-transformed mean numbers of gregarines.

Table 3. Comparison of prevalence of gregarine infection between *E. boreale* adult males and females.

Site	Date(s)	Sex	*n*	Prevalence (%)	χ^2	*p*
JM	2 June 1997	Male	42	14.3 ± 10.5	0.01	0.93
		Female	36	13.9 ± 11.3		
	11 June 1997	Male	37	78.4 ± 13.3	3.32	0.069
		Female	50	92.0 ± 7.52		
UD	2 June 1997	Male	52	48.1 ± 13.6	17.9	0.000
		Female	61	85.2 ± 8.9		
	12 June 1997	Male	50	80.0 ± 11.1	0.38	0.54
		Female	40	85.0 ± 11.1		
JM	19–23 May 1998	Male	88	58.6 ± 10.0	2.8	0.096
		Female	64	71.4 ± 11.0		

Note: JM and UD refer to the study sites Jack's marsh and Upper Dowsley, respectively. For other details see Table 1.

Table 4. Comparison of intensity of gregarine infection between *E. boreale* adult males and females.

Site	Date(s)	Sex	*n*	ln(intensity)	BT mean	*t*	*p*
JM	2 June 1997	Male	6	1.65	5.2	–0.043	0.97
		Female	5	1.67	5.3		
	11 June 1997	Male	29	2.21	9.1	0.42	0.67
		Female	46	2.11	8.3		
UD	2 June 1997	Male	25	2.18	8.8	–0.24	0.81
		Female	52	2.24	9.4		
	12 June 1997	Male	40	1.89	6.6	0.79	0.43
		Female	34	1.72	5.6		
JM	19–23 May 1998	Male	52	2.31	10	2.8	0.80
		Female	46	2.23	9.3		

Note: JM and UD refer to the study sites Jack's marsh and Upper Dowsley, respectively. For other details see Table 2.

Results

General observations

Gregarines could often be seen through the wall of the excised gut. The parasites were most often in the midgut; however, a few individuals (of thousands counted) were in the hindgut, and in one host, one gregarine was found in the foregut. The occasional presence of gregarines in the hindgut and foregut may be an artifact of processing of individuals. Within hosts, one or all of the various stages of their life cycle were evident (see above). Trophozoite sizes were

normally distributed and ranged from 0.3 to 2.5 mm (n = 91). Damselflies containing gregarines did not appear to be unhealthy (i.e., they had no midgut discoloration, lesions, or ruptures).

Prevalence and intensity of parasitism of larvae and teneral adults

Larvae at JM had a higher prevalence of gregarine infection than damselflies in any life stage at either site (91.9 ± 6.7% infected (mean ± SE)). We found no significant difference between larval males and females from JM in either prevalence (Table 1) or intensity of infection (Table 2). On average, male and female larvae from JM had approximately 12 and 15 gregarines, respectively. The results from teneral damselflies were illuminating. They showed a low overall prevalence of infection by gregarines (20.0 ± 5.5%) compared with larvae. Male and female teneral adults did not differ in either prevalence or intensity of infection at either JM or UD (Table 2).

Prevalence and intensity of parasitism of mature adults

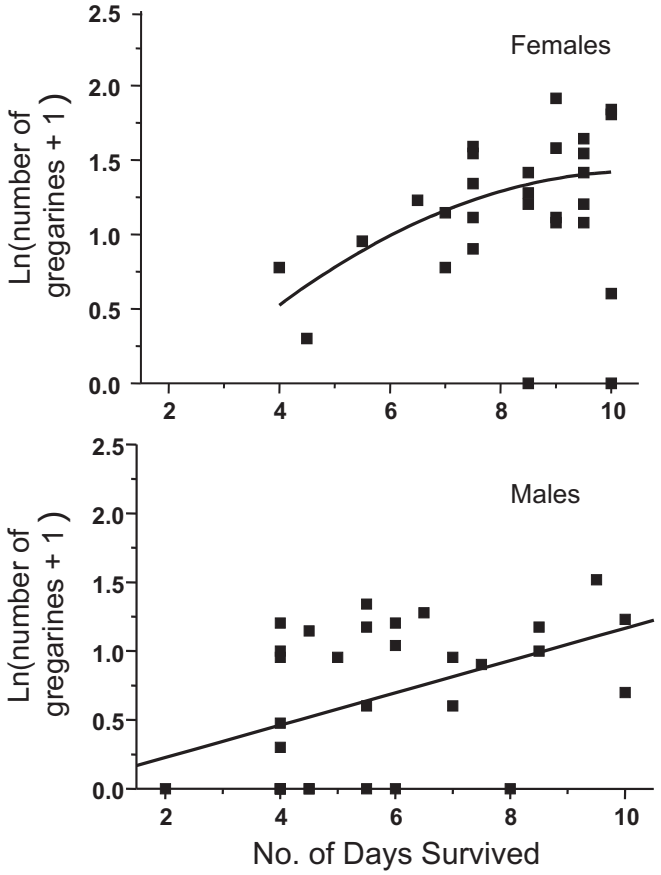
Over all sampling dates and sites, mature damselflies had a high prevalence of infection (64.4 ± 4.9%); females were more likely to be parasitized than males once all samples were combined (73.3 ± 6.3 and 55.2 ± 7.2%, respectively; $\chi^2 = 13.0$, $p < 0.001$). Although adult females generally had a higher prevalence than adult males, the difference in only one of four site-by-date samples in 1997 was significant (specifically on 2 June at UD; Table 3). There were also no sex differences in intensity of parasitism of mature adults by gregarines in any site-by-date comparison (Table 4). The same result was obtained when a two-way ANOVA was performed for each site, with sex and date as main effects and ln-transformed intensity (numbers of gregarines from infected individuals) as the dependent variable. Sex did not account for significant variation in transformed numbers of gregarines at JM ($F_{[1,82]} = 0.00012$, $p > 0.1$), nor did date ($F_{[1,82]} = 2.56$, $p > 0.1$). The interaction term also was not significant ($F_{[1,82]} = 0.0064$, $p > 0.1$). At UD, however, date accounted for significant variation in intensity ($F_{[1,147]} = 7.20$, $p < 0.01$), although neither sex ($F_{[1,147]} = 0.12$, $p > 0.1$) nor the interaction between sex and date was significant ($F_{[1,147]} = 0.51$, $p > 0.1$).

We controlled for age within another sample when we examined prevalence of gregarines for males and females at JM in 1998. As mentioned, we compared only males and females that retained their mites (*A. drepaniphorus*), as a way of approximating the age of hosts. In that sample, prevalence of gregarine infection in females (71.4 ± 11%) was higher than, but not significantly different from, that of similarly aged males (58.6 ± 10%) ($\chi^2 = 2.8$, $p = 0.096$). Furthermore, there was no significant difference in mean intensity between males and females (number of gregarines = 10.1 ± 2.7 (mean ± SE) for males and 9.3 ± 1.4 for females; $F = 0.56$, $p > 0.1$).

Survivorship

Intensity data for individuals used in the survivorship experiment were normally distributed when ln-transformed (Shapiro–Wilk test, $W = 0.9$, $p > 0.1$), so parametric regression analyses were used. Males showed a significant and positive linear relationship between number of gregarines and number of days survived ($r^2 = 0.20$, $F_{[1,29]} = 7.3$, $p < 0.01$) (Fig. 1). Females did not show a significant linear relationship between these variables ($r^2 = 0.089$, $F_{[1,26]} = 2.6$, $p > 0.1$); however, the second-order polynomial relationship between number of days survived and number of gregarines was significant ($r^2 = 0.22$, $F_{[1,26]} = 3.4$, $p < 0.05$) (Fig. 1). Females survived for a longer period, on average, than males (8.2 ± 0.3 days for females and 5.8 ± 0.4 for males; $t = -5.02$, df = 57, $p < 0.001$).

Fig. 1. Relationship between time survived and intensity of gregarine infection in *Enallagma boreale*. Moribund animals were treated as dead and dispatched quickly to enumerate gregarines.



Discussion

Our results confirm that larvae of each sex show equal parasitism by gregarines; this may be due to similarities in their foraging ecology. In fact, the sex of odonate larvae is often not considered to be important in studies of the effects of predators or population density on foraging rates and individual condition (Johnson 1991; but for an exception see Baker et al. 1992). However, the high prevalence and intensities of infection that we observed in larvae were somewhat unexpected. Åbro (1974, 1987, 1990) and others (Taylor and Merriam 1996) have not reported gregarines from larval damselflies, although gregarines have been reported and described

from other odonate larvae (Corbet 1999). We also found that newly emerged adult damselflies have low levels of infection, indicating that the majority of parasites are lost sometime around eclosion. This may be a result of the gregarines completing their life cycle and being excreted as infective cysts (the life cycle is approximately 2 weeks; Åbro 1976). This loss may also be due to shedding of the gut wall during eclosion. To our knowledge, metamorphosis has not been proposed as a mechanism for reducing endoparasite numbers. Other explanations cannot be ruled out, including direct parasite-induced mortality of larvae or parasite-associated increases in susceptibility of larvae to predators.

As mentioned, we attempted to control for age variation within samples using two approaches for approximating the ages of damselflies (collecting early in the season and collecting damselflies with nearly to fully engorged mites). We hypothesized that differences in the feeding ecology of adult males and females would cause the females to have more parasites than same- or similar-aged males. Our results partially supported this idea. Males and females at UD differed in prevalence of gregarine infection on the first sampling date but did not differ as expected on the later sampling date. However, males and females at JM did not differ on either date. The prevalence of infection at JM on the first sampling date (early season) was probably too low to conduct an adequate test using these sample sizes. We suspect that the lack of difference on the later sampling dates was due to the high variability of prevalence and intensity of infection, which would occur in a sample of individuals of differing ages.

In the second year, we compared males and females that retained their mites (as already indicated, this controls for approximate age). In that year we found that females had a higher prevalence of gregarines; this result was not significant, but noteworthy ($0.05 < p < 0.1$). Intensity of infection did not differ between males and females in that sample, as was found for the early sample collected at UD in the previous year.

Our results, while seemingly variable, can be explained in the following manner. We found evidence of sex differences in prevalence of gregarines, but only for two samples where prevalence of gregarines was high and for which relatively young males and females were compared (only one of these comparisons was significant, the other approached significance). We did not expect males to differ from females in samples collected later in the season during the first year. At that time, ages of hosts were not controlled for. The other early sample had a lower overall prevalence, but if the effect of foraging ecology on exposure to gregarines was strong, we should have detected a sex difference in prevalence (and we did not). Thus, a sex difference in prevalence of gregarines is difficult to detect using these methods. Perhaps most importantly, the difference is only in the likelihood of becoming infected, not in the numbers of gregarines once infection occurs (as our data on intensity of infection indicate).

Parasites that exert sublethal effects, or weaken their host only under certain conditions, may have a subtle and largely unrecognized influence on natural selection on a population (Levin and Pimental 1981). A common way of testing effects of parasites on hosts is to subject the hosts to stressful conditions and measure their longevity relative to their degree of infection (Zuk 1987). However, we found a significant positive relationship between number of gregarines and survivorship for both sexes. Condition of an individual damselfly is influenced by the quantity of food eaten. And gregarines, as parasites, should harm the host to some extent. However, if the amount of food eaten by a damselfly is roughly proportional to the number of gregarines ingested, then as the individual host eats more food it ingests more oocysts (or increases its probability of ingesting oocysts). However, the host's condition could improve more from feeding than it would decline from the effects of the gregarines (a similar point has been raised for other trophically transmitted parasites by Lafferty 1992).

That males survived less well than females is important for two reasons. It is entirely consistent with the idea that males feed less (for comparisons of the foraging ecology between the sexes in odonates see Anholt 1992, 1997), and sheds light on an important issue in testing for sex differences in parasitism: if males survive bouts of cool rainy weather less well than females, the populations sampled following these bouts will contain proportionately more older females than older males and this may give the impression of a sex bias in parasitism by gregarines. This may explain Åbro's (1996) finding of an apparent female bias in gregarine infection for another damselfly species. Of course, such risks from starvation have to be considered alongside risks from elevated foraging, which as Anholt (1992) notes, should increase mortality of females relative to males.

Finally, our results show that natural infections of gregarines in *E. boreale* were not lethal, or even obvious. This contrasts with the reports of many authors who found gregarines to be detrimental to their hosts. Gregarines appear to contribute to reduced average longevity in some damselflies (Åbro 1971) and reduced fat accumulation in males of a calopterygid (Siva-Jothy and Plaistow 1999). Åbro (1974) found that *Coenagrion hastulatum* (Charp.), *Enallagma cyathigerum* (Charp.), and *Pyrrhosoma nymphula* (Sulz.) were heavily infected, and feeble discoloured specimens exhibited extensive damage to the midgut, which likely progressed to bacterial septicemia (see also Åbro 1990). However, in the same study, *Sympetrum danae* was not affected by gregarines. Åbro (1987) suggests that there is gregarine-induced susceptibility to predation in some species of damselfly, as infected individuals are more feeble fliers. The effects of gregarines on odonates appear to vary widely (Åbro 1974; Taylor and Merriam 1996), perhaps like the effects of water mites, which are also odonate parasites (Andrés and Cordero 1998). If gregarines do harm *E. boreale*, the effects are subtle and likely masked by other factors, such as age of host and seasonal or environmental factors.

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