Effects of Water Mite Parasitism on the Fitness of *Enallagma hageni*

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Abstract:

Damselflies are entwined in a parasitic relationship with water mites. This parasitic relationship between organisms benefits one while harming the other – the water mite adversely affects the damselfly. As the frequency of water mites on its host increases, the subsequent the fitness of a damselfly decreases. Damselfly mass and date of collection served as independent variable to investigate the parasitic relationship between the water mite of genus *Arrenurus* and the damselfly, *Enallagma hageni*. This study was accomplished by capturing *E. hageni* on different days at the same test site and quantifying mite frequency, mating status, and body weight in each sample. After tabulation of collected specimens, data was analyzed in Excel and SPSS, to determine relationships among tested variables.

The study of parasitism disclosed an inverse correlation between damselfly weight and water mite frequency. As concluded by the Poisson test, the study was biased regarding the formation of a parasitic relation between water mites and damselflies. Studies also revealed a negative temporal relationship between the date water mites parasitize a host and mite load. Based on this, damselflies were concluded to lose fitness with an increasing number of mites due to reduced damselfly mass and mass-related reproductive success. The study also deduced damselfly immunity increases with date of mite parasitizing damselflies, correlating an increase in fitness.

Introduction:

Parasitism is the relationship between two different species in which one benefits at the expense of others. This relation may or may not result in host death. In order to avoid the latter outcome, parasites establish themselves to live among a stable set of hosts. Parasites limit their interaction to specific organisms based on habitat selection (Rolff 2001). The water mite is a parasite found in freshwater environments. All insects are potential hosts for a cycle of water mites (Rolff 2001). Literature supports a strong taxon bias of the arrenurid mites towards parasitizing Diptera and Odonata (Rolff 2001); the genus *Arrenurus* is specialized to parasitize Odonata (Smith 1988). Furthermore, mites such as these only parasitize adult damselflies (Forbes 1991). The dispersal of the *Arrenurus* mite is restricted to habitats with emergence substrate, such as water plants (Corbet 1999).

Water mites subsist by feeding off their environment in addition to their hosts. During their larval form, before they parasitize a host, water mites feed off their own eggs (Rolff 2001). Their transition from a self sufficient environment to a living host serves as their second source of nourishment. Water mites attack damselflies when the Odonata transition from an aquatic to a terrestrial habitat – the time damselflies are most susceptible to parasitic attacks (Rolff *et al.* 2001; Wohltmann 1999). When they live off their hosts, they can growth up to 500 times their original form (Davids 1997). Mites eventually end their coexistence with a host in order to complete their life cycle (Smith 1998). These mites detach themselves either when they are fully fed or when hosts return to a body of water for reproduction (Robb and Forbes 2006; Smith 1988).

Water mites alter many aspects of an adult damselfly's lifestyle. Since 1975, water mites have been investigated as a factor affecting host fitness due to their parasitic nature parasites (Lanciani). The mites play an important role in damselfly reproduction, reducing host reproductive output when present. The degree of parasitism can be affected by host behavior as well. Melatonic encapsulation due to environmental temperature is one external defense against mites (Robb and Forbes 2005). Internal defenses are mainly comprised of haemoctye and humoral defense systems (Rolff and Siva-Jothy 2004). The various aspects of host fitness water mites influence and their readily identifiable presence make them good candidate to analyze (Rolff 1999; Rolff 2000).

The objective for this study is to determine the effects of parasitism from water mites on the fitness level of male *Enallagma hageni*. We will examine the extent this parasitic relationship affects damselflies by studying distributions of damselfly mass and percent parasitism between mating and non-mating male damselflies. A point of investigation within this fact is to see whether this trend varies based on mating status. Furthermore, we will see if this parasite-host relationship is affected temporally within a given location of study. We hypothesize water mites have a negative effect on the damselflies fitness and that damselfly mass should decrease with increasing mite load. In addition, we predict that this lowered fitness should be more prevalent earlier in the damselfly mating season and diminish over the season. If levels of parasitism deviate from a negative correlation with mite loads, then stated hypothesis may be rejected.

Materials and Methods:

The study was divided into two collections. Male *E. hageni* were captured from waterfronts near the Biological Station Cabin located on Sugar Island, Michigan. The first three collections of damselflies were taken on three different days in 2004: July 9, July 16 and July 23. On July 9, 104 males were caught. On July 16, 299 damselflies were captured and on Jul 23, 124 males were caught. The fourth collection of damselflies was taken on July 24, 2007 in which 157 males were obtained. Each sampling day, the damselflies were collected using butterfly nets and then stored in glycine envelopes. Damselflies were frozen in order to easily identify and count their attached parasites. Data taken from each damselfly included water mite frequency, sex, and weight. Water mite counts were taken using dissecting microscopes and magnifying lenses. They were taken from two locations: the thorax of the damselfly or within the glosine envelopes if they detached. Mite counts were recorded and damselflies were cleaned of water mites and weighed. The results were tabulated using Excel and SPSS.

Using SPSS, mite counts and their intensities per collection date were tabulated. The prevalence (percent of individuals parasitized) and intensity (mean number of parasites per individual for all test subjects) were computed by collection date (Bush *et al*. 1997). Applying this data in a Poisson distribution, the expected distribution of water mites per damselfly was found. In order to determine whether the difference between observed and expected water mites was significant, the Poisson calculations were used to conduct a chi-square test. The combination of these tests was used to determine if parasitism is a randomly occurring event among their male hosts. The effect of time on mite counts was also investigated using an independent sample t-test. A Wilcoxon test was also used to see if mating status (mating or non-mating) presented a relation between damselfly mass versus mite count and the data sets between mating status was graphed and linear regressions calculated. Using Excel, data was further sorted by mating status and investigated the specific relationships of time on mite counts and mass separately. These were analyzed using the Mann-Whitney U test using the Wilcoxon Signed Ranks Test in SPSS.

Results:

The data showed consistent trends throughout each day of collection. A negative relationship between the number of mites attached to an individual and the reflected frequency of damselflies (Figure 1 a, b). The greatest percentage of damselflies with at least one mite was 13.7% and occurred on July 23, 2004. The largest percentage of damselflies without mites occurred on July 24, 2007 and measured 83.4%.

Comparing the four collections was comprised of a greater percentage of non-mating than mating pairs. Although the data was comprised of many non-parasitized individuals, those that were parasitized allowed for the computation of prevalence and intensity (Table 1).

A Poisson distribution using a combined data of the individuals collected in 2004 showed great variance between the observed versus the expected distributions of the frequency of individuals with a number of water mites (Table 2). These values were used in a chi-square test and applied to a degree of freedom of 7. The resultant p-value was much lower than 0.001 and deemed the data significant.

A non-parametric, independent samples test was conducted in order to compare the mite counts between different collection days. All tests except the one conducted between July 16 and July 23 were statistically significant. (July 9 and July 16) ($Z = -3.776$, $p < 0.001$) ; (July 9 and July 23) ($Z = -3.465$, $p < 0.001$) ; (July 9 and July 24) ($Z = -3.465$, $p < 0.001$) ; (July 16 and July 23) ($Z = -2.59$, $p = 0.759$) ; (July 16 and July 24) ($Z = -3.695$, $p < 0.001$) ; (July 23 and July 2) ($Z = -3.05$, $p = 0.002$). Among all the tests, earlier collection dates showed greater mite counts than later collections.

The graphs correlating damselfly weight versus mite count both had indirect, linear relationships; as the frequency of parasitized mite increased, the damselfly weight decreased. The effect was more prevalent among the mating mites compared to non-mating mite. This was evident in the slope correlating the relationship between these two variables; the weight for mating males varies 3 magnitudes more than the weight for non-mating males with respect to mite counts (Figure 1).

Mite counts between mating and non-mating status for damselflies showed significance in data sets of both years (2004) ($Z = -10.43$, $p < 0.001$) ; (2007) ($Z = -2.328$, $p = 0.02$). The difference in data between the two years of collection showed levels of parasites can vary temporally. A negative ranks test shows significance for the 2004 data (Wilcoxon $Z = -4.401$, $p < 0.001$) but not for the 2007 data (Wilcoxon $Z = -1$ $p = 0.317$) (Table 3). It shows the distribution of parasites differs between mating status in the 2004 data set but not for the 2007 data.

Discussion:

The results of the study support and show a negatively relation for the effects of parasitism on damselflies. The negative trends illustrate mite load has adverse effects on damselfly fitness regardless of mating status (Figure 1 a, b, c, d). Damselfly mortality is directly related to mite load (Rolff and Schroder 1999). Thus, with increasing abundance of water mites, fewer adult damselflies with that mite frequency are present. This can be explained due to the parasitic nature of water mites. The primary resources for these mites are the bodily fluids of their hosts. A greater number of mites feeding for a longer period of time have higher detrimental effects to the host – reducing host mass proportionally. With a decreased mass, males would have lower mating success since they have less total energy to expend (Rolff and Siva-Jothy 2004). Mating success has been shown to be inversely related to the degree of parasitism (Forbes 1991). The subsequent increase in parasitism and reduced host mating success correlates with a reduced survivorship and fitness in damselflies (Forbes and Baker 1991).

The distribution of water mites in the environment is an issue with less definitive results. The Poisson distribution tested for this spread of water mites among damselflies in the environment. A significant p-value obtained from the Chi-square test following the Poisson distribution concluded water mite attacks were random. This means all mites are not equally apt to parasitize one host compared to another host (Smith 1988). This is one possible explanation reflecting why mite prevalence in the 2007 damselfly collection differed from previous years. The mites may have been parasitizing hosts based on fluctuating features compared to hosts in the past. The prevalence for the 2007 set was 16.56% while the prevalence for the combined 2004 data set was 35.29%. Comparing the different years, the significant difference in levels of parasitism shows a differential selection for hosts by water mites. If the observed prevalences were a majority of the total percent, then the expected mite prevalence could have been better emulated by a Poisson distribution and there would not be an observable host preference by water mites (Anderson 1982).

Individual accounts of prevalence were more informative after further studies through comparative analyses. The different samples of damselflies were taken over a period of one month. As the summer progressed, the prevalence of parasites on *E. hageni* decreased (Table 1). This change in damselfly parasitism within a generation could have result from external and internal defenses of the damselfly. External factors such as temperature can lead to seasonal increases in mite resistance. Though this reason may be applicable in certain environments, mites parasitizing damselflies have also been thought spent longer periods of time searching for larval hosts as the summer progresses (Robb and Forbes 2005). The analysis of mating and non-mating pairs of damselflies comparing the early seasonal sampling in 2004 relative to the later sampling in 2007 shows damselfly mite counts varied initially (Table 3). As the season progress, no significant difference between the damselflies was found because of an increased mite resistance (Yourth *et al.* 2002). The increases immunity can lead to a lower mortality and can cause of greater damselflies fitness; however, the results of this study are not fully conclusive and may be flawed due to the 2007 data set. It is important to note that data set had very few parasitized individuals and in low mite loads compared to other collection dates. This inconsistency may lead to a result bias for percentage based results and skewing comparative conclusions.

Reasonable conclusions regarding damselfly fitness were possible from the study, but not without certain flaws. The span of three years in between data collections may have allowed for a change in the environment. A possible cause for the irregular data set collected in 2007 may be a result damselflies parasitism by different water mites. Future studies could further this topic by investigating populations of damselflies with different species of water mites per population. One topic of investigation could pertain to damselfly responses and subsequent responses due to different water mites. There could be more than 20 species of *Arrenurus* in a body of water, so it is harder to attribute changes in host reproduction or survival as the cause due to a single mite species (Forbes *et al.* 2004). In regards to the 2004 data set, the one with greater data set provided logical conclusions. Among them was the verification of the hypothesis: damselfly mass appears to decrease with greater levels of parasitism. Based on combined data of the four collections, the effects of parasitism are increased earlier in the season and correlate inversely to damselfly immunity and thus fitness.

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Figure legends:

Table 1 – General statistics of all data collections

	N	N Parasitized	Prevalence	Intensity
July 9, 2004	104	53	50.96%	4.5962
July 16, 2004	299	84	28.09%	2.4849
July 23, 2004	124	39	31.45%	1.8952
Combined 2004	527	186	35.29%	2.7628
July 24, 2007	157	26	16.56%	.6497

Table 2 – Poisson distribution for the combined 2004 collections

Parasite Frequency	Expected	Observed	Chi-Square
0	33.26488735	341	2846.872696
1	91.90090429	64	8.470650712
2	126.9473141	23	85.11439719
3	116.9057816	15	88.83040839
4	80.7439007	10	61.98238434
5	44.6142349	6	33.42115224
6	20.54262446	8	7.658097855
≥ 7	12.08133189	60	190.0617229
Total	527	527	3322.41151

Table 3 – Temporal effect on damselfly body mass.

a.

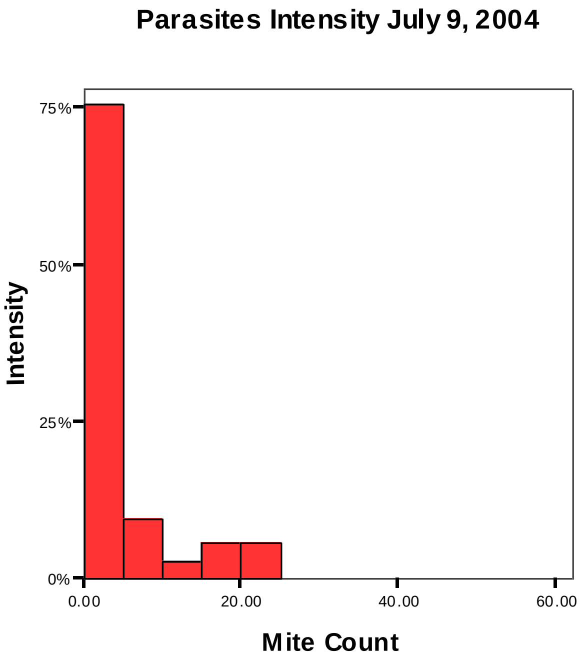
2004	Mite Count	Prevalence
Non-Mating	160	35.60%
Mating	25	33.30%
Z = -4.041	p < .001	

b.

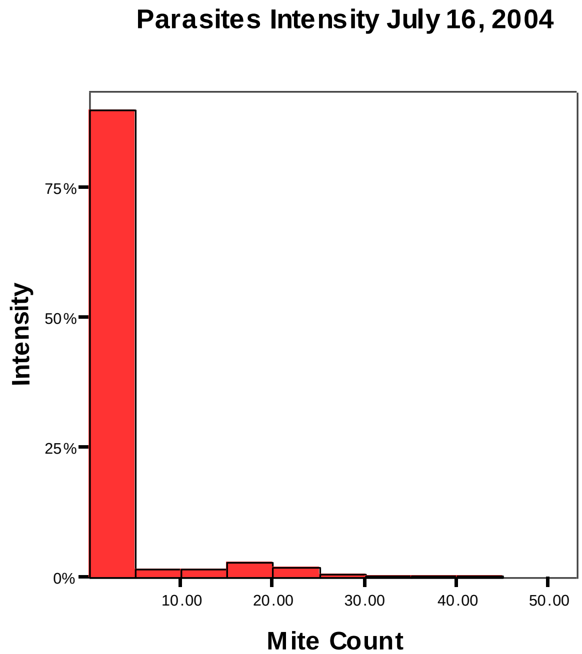
2007	Mite Count	Prevalence
Non-Mating	25	11.10%
Mating	1	5.30%
Z = -1	p = .317	

Parasite Intensity Graphs

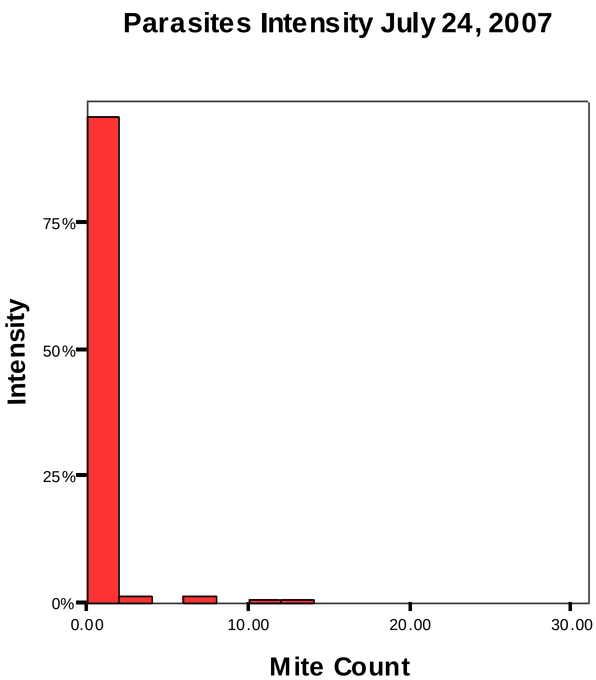
a.



b.



c.



d.

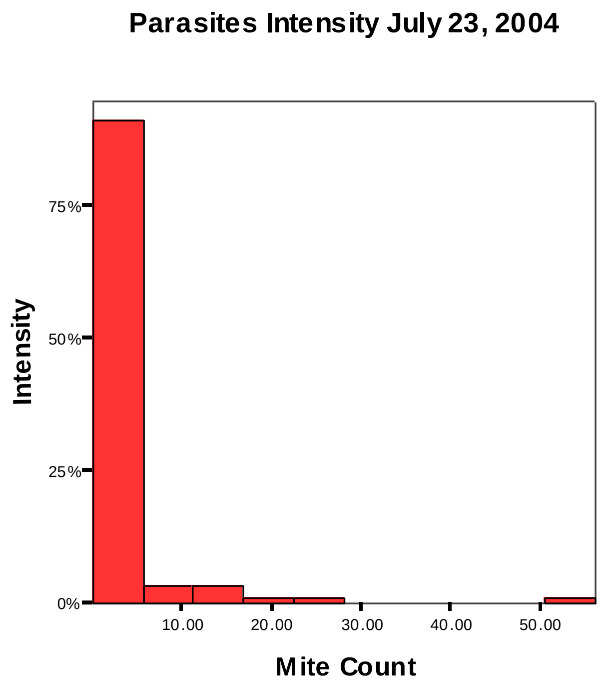
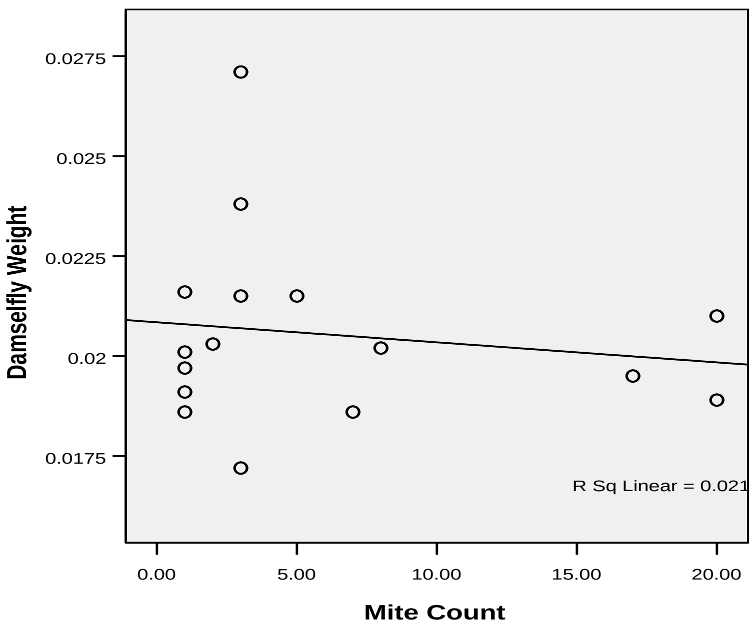


Figure 1.

Weight vs. Mite Count for mating and non-mating damselflies

a. Mating



b. Non-Mating

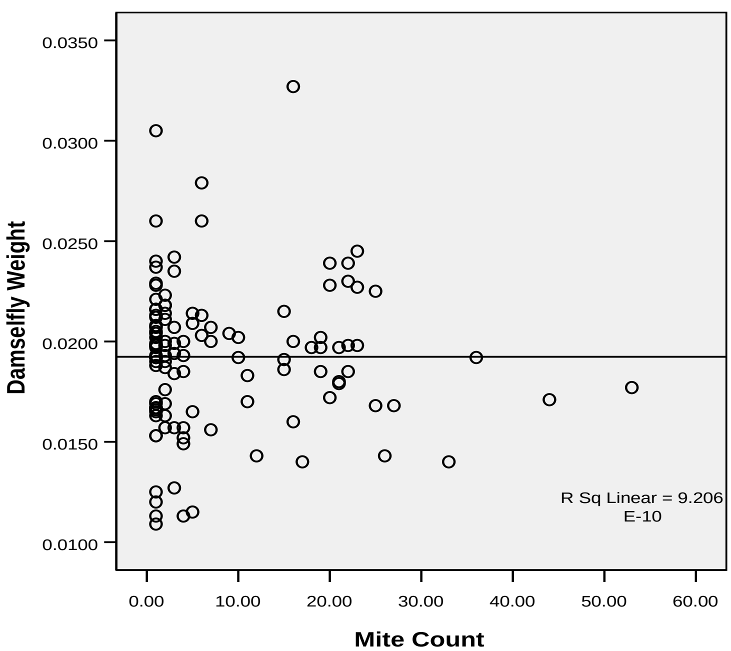


Figure 2.