Differences in Gregarine Parasite Load Between Male and Female *Calopteryx maculata*

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Abstract

Damselflies and other insects of the order Odonata are frequently parasitized by gregarine protists. In the trophozoite stage of the gregarine life cycle, the parasite feeds on the contents of the host's gut and negatively affect its reproductive success. Possibly as a result of its impacts on the host's reproductive system, levels of gregarine parasitism has been observed to differ between male and female damselflies. We aimed to measure relative levels of gregarine parasitism of male and female damselflies of the species *Calopteryx maculata*. In order to do this, we collected damselflies at multiple sites in Michigan's Maple River and dissected individuals to observe the presence of gregarine parasites. Our results indicate that females experience significantly higher levels of gregarine parasitism than their male counterparts. We propose that this difference is the result of increased levels of migratory behavior in female damselflies due to increased parental investment.

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Introduction

Ebony jewelwing damselflies (*Calopteryx maculata*) are a species of broad-winged damselfly commonly infected by parasitic protists of the subclass Gregarinasina (Hupalo et al. 2014). Gregarine oocysts can often be found attached to the legs of damselfly prey species, i.e. dipterans, and are ingested alongside the prey (Hecker et al. 2002). These oocysts contain sporozoites which develop into feeding trophozoites in the gut of the damselfly host (Hupalo et al. 2014). The trophozoites develop into gamonts which can mate and produce further oocysts, which are then passed out with the feces of the host, thus completing the life cycle of the gregarine (Hupalo et al. 2014).

The impacts that gregarines have on their hosts is well-studied. Gregarines have been observed to hinder ability to produce spermatophores in male field crickets and thus represent an important factor in sexual selection in the host species (Zuk 1987).The parasites have also been shown to impact the reproductive abilities of species in the order Odonata; in Lebellula damselflies, the presence of gregarine parasites decreases the ability of males to match muscle contractility to their energy storage. As a result, unparasatized males in populations with high levels of parasitism become territory holders while parasitized males act as satellite mates (Marden & Cobb 2004).

Parasitism is also known to impact males and females differentially. The conventional “sicker sex” theory posits that, because males invest more energy into mating efforts, they have less energy to invest into their immune systems, leaving them more vulnerable than females to parasitism (Córdoba-Aguilar and Munguía-Steyer 2013). While this male-biased infection hypothesis has been observed in several species of damselflies, including *Argia anceps, A. extranea, A. harknessi, A. pulla, A. tezpi, A. sp., Enallagma novahispaniae, Hetaerina americana, Protoneura cara* and *Tellebasis salva* (Córdoba-Aguilar and Munguía-Steyer 2013), the opposite, i.e. a population in which females are significantly more infected than males, has also been observed (Hecker et al. 2002).

In addition to differing effects of parasitism, important behavioral differences exist between the sexes in damselflies. Females are generally more mobile than males for a number of reasons. Because female damselflies are choosier than males (Ebony jewelwing, black-winged damselfly - Calopteryx maculata n.d.), they will travel across great distances through a number of male territories in search of a mate (Battin 1993). Furthermore, females are driven to travel further while foraging than males due to their increased caloric intake requirements while developing eggs (Introduction to the Odonata n.d.). This combination of factors forces females to travel to a greater variety of habitats, increasing their likelihood of coming into contact with parasites. In this study, we wanted to examine the differences in parasite load between male and female *C. maculata*. We hypothesized that females would have a higher parasite load than males due to their migratory behavior.

Methods

Study Site

This study took place at three sites along the Maple River in Emmet County, MI. The Maple River is a coldwater stream of high water quality that runs through Northern lower Michigan. It is characterized by two branches (West and East); a man-made dam is located just below the confluence of the two branches and is scheduled to be removed between Spring 2018 and Fall 2018 (Tip of the Mitt Watershed Council 2018). This study took place in the midst of dam removal. One site was located above the dam on the West Branch (45°33'01.5"N, 84°47'44.5"W). The second site was located just below the dam (45°31'40.7"N, 84°46'26.1"W). The third site was located 1.7 kilometers south/downstream of the dam near the dead-end of Pine Trail Road off of Maple River Road in Brutus, MI. Three sites were chosen as replicates to control for confounding variables (*Figure 1*).

Data Collection

All data was collected on August 3rd, 2018 between 10:00 am and 3:00 pm. Weather conditions were sunny and clear, the average temperature during collection was 21.11° F. Damselflies were collected using a standard insect net with a polyester net bag 40 cm in diameter, 76 cm in depth with reinforced muslin at the top, and 1 m wooden handle. Damselflies were identified immediately upon capture by species and sex. Only *C. maculata* were collected. Males were identified by their emerald abdomen and fully dark-pigmented wings. Females were identified by their dark brown abdomen and dark, translucent wings featuring a white wing-spot (*Figure 2*). After each specimen was collected, each was placed in an individual wax envelope. All specimens were transported alive to the laboratory, and euthanized via freezer on the day of collection. After thawing, specimens were examined individually under a Leica EZ4 dissecting scope. The thorax and head were removed from the abdomen. Only the abdomen was dissected. The intestines within the abdomen were mixed with a drop of water and smeared on a slide. Gregarines were counted but not identified by species. Non-gregarine parasites were also counted, but not identified.

Data Analysis

Two-tailed t-tests were used to examine the total number of gregarine parasites found between males and females. In order to account for variation between sites, a One-way ANOVA was conducted to test the differences in number of gregarine parasites found between sexes across the different sites. The ANOVA was used to address assumed but unmeasured differences between collection sites. Without ANOVA results demonstrating a lack of differences in parasitism level between sites, the results of a lone t-test would be invalid. This is due to the possibility of confounding variables in the form of differences between sites, thus precluding us from treating different sites as functionally identical replicates. The exact measurements of differences are not directly relevant to the focus of this study, but it is necessary that differences observed between sites are not statistically significant. Post-hoc Tukey tests were conducted to isolate specific differences between populations, i.e. between males and females.

Results

We collected a total of 17 damselflies from three different sites; seven females and ten males. The females had an average of 32.86 gregarine parasites and 0.29 non-gregarine parasites, and the males had an average of 0.2 gregarine parasites and 1 non-gregarine parasite. There was a significant difference between the mean number of gregarine parasites in males versus females, with females having significantly more than males ($t = 2.98$, d.f. = 15, $p = 0.009$). This information is reproduced in *Figure 3*. There was not a significant difference between the mean number of non-gregarine parasites in males versus females ($t = 1.03$, $p = 0.32$, d.f. = 15). This information is reproduced in *Figure 4*.

From our one-way ANOVA, we found a significant difference in parasite load between the populations of females from Pine Trail, males from Pine Trail, males from Maple River Dam, and males from Maple River West (F-value = 109.5, *P*-value > 0.001) (Figure 5). We then ran a Post-hoc Tukey Test to isolate specific differences between populations. The females from our Maple River Dam site were not included due to their insufficient sample size of one specimen. We found that the abundance of gregarine parasites was greater in females from Pine Trail than males from the same site (F = 19.83, *P* > 0.001). The abundance of gregarine parasites in females from Pine Trail was greater than males from Maple River Dam (F = 19.17, *P*= 0.001). The abundance of gregarine parasites in females from Pine Trail was also greater than males from Maple River West (F = 19.83, *P*= 0.002). There was not a significant difference in the abundance of gregarine parasites in males from Pine Trail than in males from Maple River Dam. There was not a significant difference in the abundance of gregarine parasites in males from Pine Trail than in males from Maple River West. There was not a significant difference in the abundance of gregarine parasites in males from Maple River West than in males from Maple River Dam.

Discussion

Our results support our hypothesis that female *C. maculata* would have a higher load of gregarine parasites than male *C. maculata (Figure 3)*. This finding is consistent with the results of other similar studies. One likely explanation for these results is a suspected parasite resistance in male *Calopteryx*. Male *Calopteryx splendens* damselflies produce melanized "eye spots" on their wings as an ornament of sexual attraction to the opposite sex (Suhonen 2017). The melanin produced for these spots also functions as an immune defense against parasites, and males from populations of *C. splendens* with high levels of gregarine parasitism were found to have smaller eye spots than those from parasite-free populations (Suhonen 2017). These results suggest that female mate choice may be tied to a "good genes" type hypothesis in which females have a preference for males with larger, darker pigmented wing spots which signify good genes for gregarine parasite resistance.

Variation in the parasite load of female *C. maculata* ranged from a count of 9 to 111. While these numbers are quite variable, we were unable to statistically account for this due to small sample size. This variation in parasite load within collected females, and between collected males and females may be due to differences in age among specimens. Other similar studies have found that younger damselflies tend to have lower rates of parasitism than older individuals, due to less exposure and time to accumulate parasites (Córdoba-Aguilar & Munguía-Steyer. 2013).

This study does not go without limitations. The small sample size was our most limiting factor. In total, we were only able to collect 17 *C. maculata*. Because of this, our data was limited in the amount and types of statistical analyses we were able to conduct. For example, females from the Maple River Dam site were excluded from the ANOVA test because there was a sample size of 1, which happened to be an outlier. Also, no females were collected from the West Branch site. Data collection was limited to weather conditions. The week of planned collection was rainy and cloudy, so collection was limited to a single day although multiple days had been spent attempting to collect. Differences in conditions between sites may have also influenced the prevalence of *C. maculata*. For example, the West Branch is much colder than the East branch as well as areas below the dam.

Future studies could examine the difference in parasite load between *C. maculata* and another species of damselfly that is parasitized by gregarines, like the river jewelwing damselfly (*Calopteryx aequabilis*) which have some rare sympatric occurrences with *C. maculata* population along the Maple River. We could also examine how parasite load affects the survival and reproductive success of different individuals.

Acknowledgments

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Figures

Figure 1. *ArcGIS map showing the three data collection sites along the Maple River in Emmet County, MI. Yellow dots represent collection sites, labels describe site name.*

Figure 2. *Side by side comparison of male and female C. maculata body minus abdomen.*

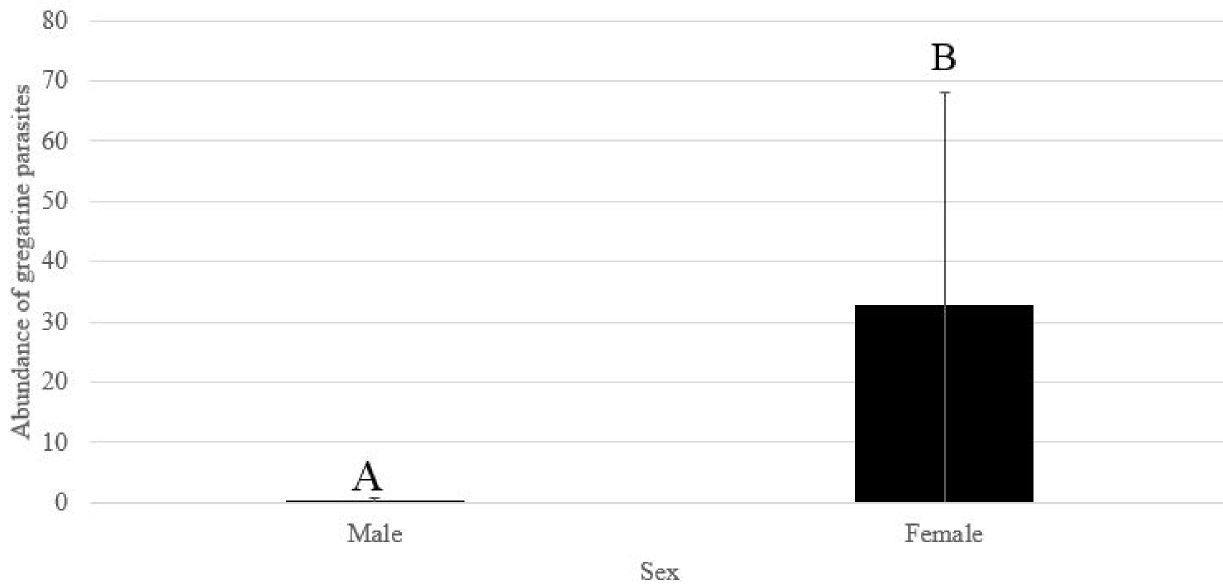

Figure 3. *Mean number of gregarine parasites between sexes. Separate letter designations represent statistically significant differences between populations. Error bars represent one standard deviation above and below the mean.*

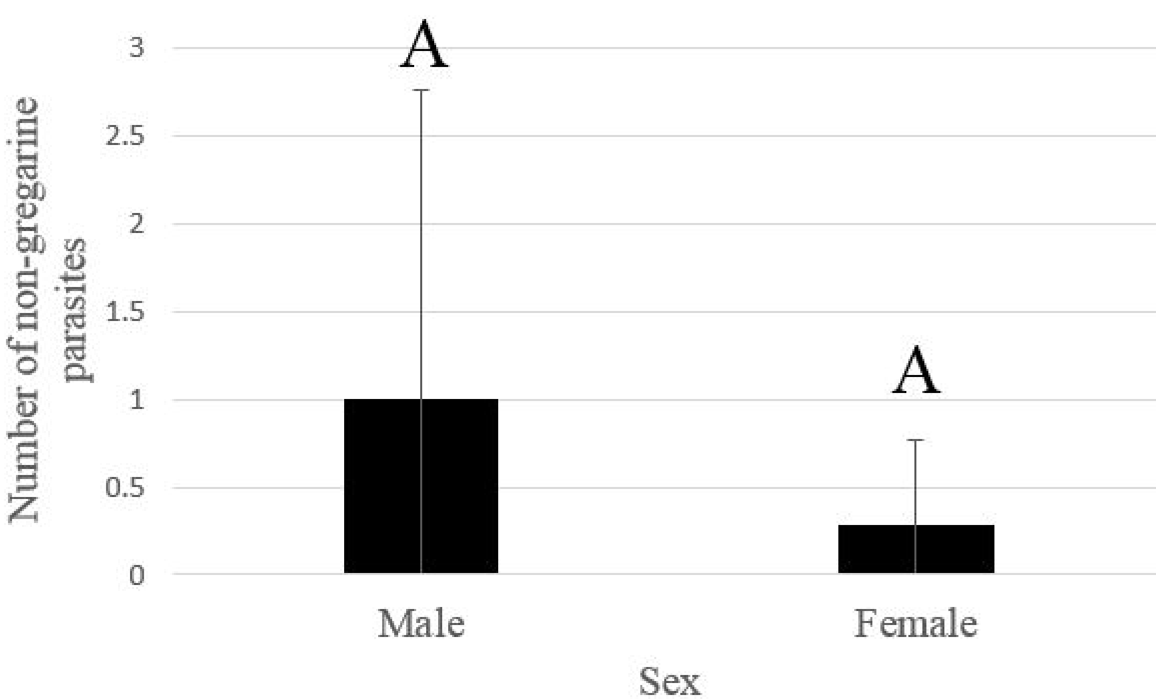

Figure 4. *Mean number of non-gregarine parasites between sexes. Separate letter designations represent statistically significant differences between populations. Error bars represent one standard deviation above and below the mean.*

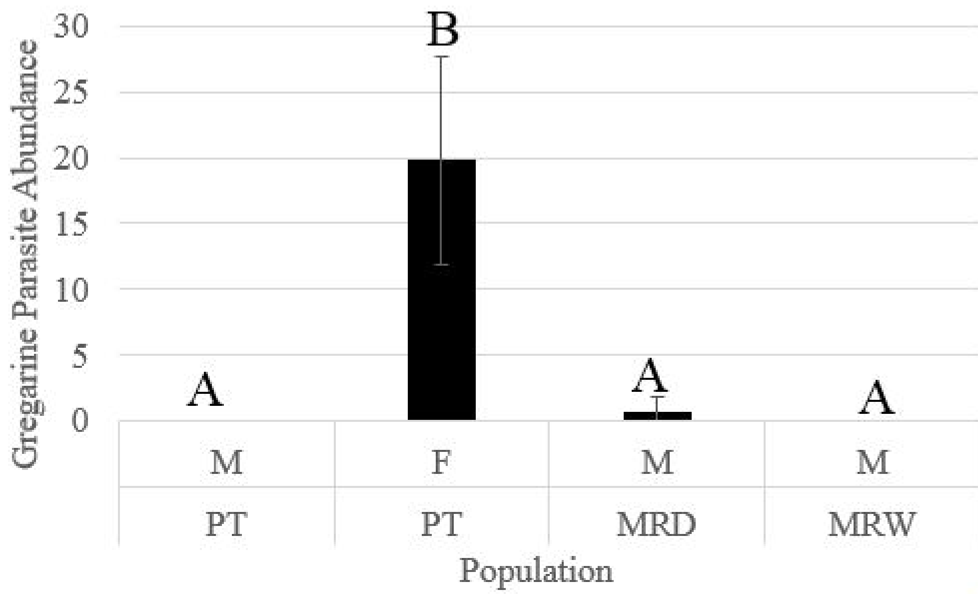

Figure 5. *Abundance of gregarine parasites across populations. The first row shows sex, with "M" representing males and "F" representing females. The second row shows sampling locations, with "PT" representing Pine Trails, "MRD" representing Maple River Dam, and "MRW" representing Maple River West Branch. Separate letter designations above bars represent statistically significant differences in mean gregarine parasite abundance. Error bars represent one standard deviation above and below the mean.*

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