Oviposition Preference in the Dark-Winged Damselfly (*Colopteryx maculata*)

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Abstract

Our study investigated preference of oviposition in *Calopteryx maculata*, the dark-winged damselfly. Artificial damselfly territories were created using several leaves of *Sparganium americanum* fixed in place in a river, at known flow rates for 48 hours. On each leaf total eggs were counted and algal coverage was measured. We found that *C. maculata* oviposits more frequently in areas of intermediate flow rate, approximately 0.2-0.4 m/s. We also found that algal coverage was more common in this flow rate range, suggesting that damselflies oviposition choice is not affected by the presence of algae and that there is a relatively narrow window of optimum flow rate for oviposition.

Introduction

Mating behavior in *Calopteryx maculata* has been studied extensively. This is likely because *C. maculata* is an ideal organism for studying female choice and mating behavior. Damselflies spend a significant amount of time away from their stream or river displaying non-mating behavior. When attempting to mate, males display territorial behavior by defending patches of vegetation. Conflicts between males determine which individual remains in a given territory (Waage, 1973). Females fly into a male’s territory and either accept the males attempt to mate, or prevent copulation by unfolding her wings (Johnson, 1962).

After copulation, the female lays her fertilized eggs within the floating plant vegetation. Females oviposit along the length of a leaf in a two parallel and staggered rows of eggs. Eggs are placed endophytically, or within the plants tissue (Corbet, 1980). After larvae hatch, they enter the water and begin their aquatic life stage (Siva-Jothy et al., 1995). Oviposition in damselflies likely depends on many factors. Past studies have shown ovipositioning in *Calopteryx splendens xanthostoma* to be more frequent in areas of faster flow rates (Siva-Jothy et al., 1995). The areas of the stream with faster flow rates have been suggested to be beneficial because of higher oxygen concentration and decreased algal coverage (Gibbons and Pain, 1992).

This study’s purpose was to investigate preference in ovipositioning in *C. maculata*. There was a specific interest in how flow rate and algal coverage affected the number of eggs females oviposited. If there was a preference for a fasterr flow rate then females would oviposit more frequently and in greater numbers on territories in faster flowing water. If preference was due to lower algal coverage then we would expect to see lower algal coverage correlated with greater number of eggs.

Materials and Methods

The study site was in the Maple River, near Pellston, Michigan. The study site was on University of Michigan Biological Station property, directly south of Douglas Lake Rd.. The river is rather slow moving, with peak flow rates near .7 m/s. Our study was conducted between July 27th and July 31st 2008.

Many field observations of female dark-winged damselflies ovipositing were made in order to design an appropriate experiment. Females were typically observed to oviposit along the length of one leaf, beginning on the floating portion of a given leaf and sometimes continuing below the surface of the water. Females were only observed ovipositing on *Sparganium americanum*, the most prevalent floating plant within our study site. Leaf scars are readily visible to the naked eye after oviposition occurs, however they typically turn brown after a period of 24-48 hours, making counting even easier. Chlorophyll was eluted from leaves known to have been oviposited on by female dark-winged damselflies. Upon inspection using a compound light microscope, it was determined that each leaf scar in the bleached leaves corresponded to one endophytic egg.

To measure oviposition preference, artificial territories were created using samples of *S. americanum*, fishing line, wooden dowels. Leaves were collected on site, with all algal coverage being removed prior to use. Each leaf was visually inspected for leaf scarring to ensure no previous ovipositions had occurred. Fishing line was used to pierce the bottom of each leaf and then tied to create a length of fishing line of approximately 20 cm with five to seven leaves at the one end. The opposite end of the fishing line was then tied to a short dowel rod. To install artificial oviposition sites, the dowel rods were inserted into the riverbed, and the fishing lines were tightened so that the bottoms of each leaf were held underwater while at least half of each leaf remained floating at the surface (Figure 1).

Each artificial damselfly territory was left in the stream for 48 hours. There were a total of two 48 hour trials, each of which had 10 territories. Several leaves were dislodged from the apparatus, for a total of 95 leaves that were collected for data. Distance from shore was measured upon placement. Flow rates were measured using a “Flo-mate” before and after collection, and an average of the two values was used to represent an assumed constant flow rate. Flow rates were measured approximately 10 cm upstream of the artificial territories 1 cm below the surface of the water.

Leaves were stored in water filled Ziploc bags to ensure that they remained green. After waiting several days for leaf scars to brown, algal coverage was measured on the leaves. Algal coverage occurred in patches that covered the whole width of each leaf and only on one side. To calculate algal coverage the length of leaf covered by algae was divided by the total leaf length. Upon removal of algae, leaf scars were counted using dissecting microscopes. Each leaf scar was counted as an endophytic egg, according to previous findings using elution and light microscopy.

Data were entered into a Microsoft Excel spreadsheet. Flow rates were sorted into categories of 0.0-0.2, 0.2-0.4, and 0.4-0.6 m/s or slow, intermediate and fast respectively. Percent algal coverage was sorted into categories of 0-20%, 20-40%, and 40-60% coverage. Two distance categories were defined as less than 250 cm from shore or greater than 250 cm from shore.

One-way ANOVA’s were performed comparing distance, flow rate, and algae percent groupings with egg number. One way ANOVA’s were also performed using the various grouping categories and egg presence or absence. These sets of ANOVA's were performed to test if variation in the frequency of egg laying was statistically significant. Independent sample t-tests and post-hoc tests were performed between individual groups if it was of interest what groups gave rise to statistically significant variation.

Results

Statistical analysis of our data yielded many interesting results. Of all leaves in our experiment 22.3% were oviposited on by dark-winged damselflies and 28.7% had algae coverage present. The number of eggs oviposited ranged from 0 to 289, with the average number of eggs per leaf being 18.7. The percent algae coverage ranged from 0% to 55%, with the average being 6.7%. Flow rates ranged from .015 to .53 m/s, with an average value of .261 m/s.

A one-way ANOVA using number of eggs oviposited and flow rate was performed to determine if number of oviposits was affected by flow rate. This ANOVA gave a significance value of .008 and an F-value of 5.04. This showed that there is a significant difference in the number of eggs laid between the flow rate groups (Figure 2). The three independent-samples t-tests that were done between number of eggs in each of the three flow rate groups showed that significant variation was due a relatively high average number of eggs per leaf in the intermediate flow rates. An independent-samples t-test comparing slow flow rate and intermediate flow rate showed that significant differences existed between the groups. A similar result was found for a comparison between intermediate and fast flow rates. When an independent-samples t-test was performed between slow and fast flow rate groups, there was no significant difference found. There was a statistical difference found between intermediate flow rates and any other group of flow rates.

A one-way ANOVA was performed using number of eggs and the two distance groupings. A significance value of .464 and a F-value of .540 were obtained. This demonstrated that there was no significant difference in number of eggs oviposited between the two distances.

A one-way ANOVA using number eggs oviposited and algal coverage was performed to determine if the number of eggs oviposited depended on flow rate. This ANOVA gave a significance value of .209 and an F-value of 1.595. This demonstrated that there is not a significant difference in the number of eggs laid between the percent algal coverage groups (Figure 3). Independent-sample t-tests were done between all percent algae groups and yielded no significant results.

An additional one-way ANOVA was performed using presence of algae or absence of algae and flow rate groupings to determine if flow rate had an effect on algal presence. This ANOVA yielded a significance value of .042 and an F-value of 3.273This demonstrated that there was significant variation of algal presence between flow rate categories. Scheffe post-hoc tests were used to determine what groupings were performed to determine where most of this variation existed. The frequency of algae presence was higher in the intermediate flow rate category than both the slow and fast categories. An independent-samples t-test comparing frequency of algae presence between slow and intermediate flow rate groups yielded a significance value of .011. The frequency of algal presence for slow, intermediate, and fast flow rate categories were found to be .125, .4, and .294, respectively.

A one-way ANOVA comparing percent algal coverage between flow rate groups was also performed. This ANOVA yielded a significance value of .077 with an F-value of 2.639. This demonstrated that there is no statistically significant variation of percent algal coverage between flow rate groups. Due to the fact that the ANOVA showed a nearly significant amount of variation, independent-samples t-tests were used to compare means of algal coverage between specific flow rate groups. The independent sample t-test between slow and intermediate flow rates yielded a significance value of .031. This showed that there is a significant difference in algal coverage between slow and intermediate flow rate groups. No other t-tests analyzing algal coverage between flow rate groups reported significant values.

Discussion

Our study found that *C. maculata* showed higher oviposition rates in areas of intermediate flow rate. This preference for intermediate flow rates existed despite the higher frequency of algal presence in intermediate flow rates. This suggests that previous researcher's suggestions that oviposition preference was due to lower algal coverage may have been incorrect.

There was significant variation of number of eggs oviposited between flow rate groups (Figure 2). Comparison of means between flow rate groups showed that the average number of oviposition sites per leaf was higher in the intermediate flow rate group. Assuming that number of eggs laid is an indicator of preference, this demonstrates that dark-winged damselflies prefer to oviposit in territories with intermediate flow rate more than in territories with slow or fast flowing water. Preference in *C. splendens xanthostoma* was shown to favor areas of faster flow rate (Siva-Jothy et al., 1995). This suggests that oviposition preference in *C. maculata* may be different than oviposition in *C. splendens xanthostoma.* To determine if there is a difference in preference a comparitive study of the two species should be performed using the same experimental setup.

Mating behavior in damselflies has been extensively studied due to the complex interactions between male competition and female choice (Waage, 1973). Oviposition preference had not been previously studied in *C. maculata* and finding that there is a significant preference for intermediate flow rates is a significant achievement.

Future studies of oviposition preference in *C. maculata* could expand on our own by including both behavioral observations and a larger sample size. Observation of ovipositing females would allow data to be collected on how many eggs each female is ovipositing, and possibly provide a better estimation of actual preference. A larger sample size could shed light on the near significances found in our study.

Our study demonstrated that in *C. maculata* there is a preference to oviposit in areas of intermediate flow rate despite higher average algal coverage. This is in contradiction to the suggestions from previous researchers that oviposition preference may be due to algae avoidance (Siva-Jothy et al., 1995). Our results showed oviposition rates were significantly higher in the same flow rate grouping that had the highest average algal coverage. Future study is needed to properly determine how other environmental factors can affect oviposition preference.

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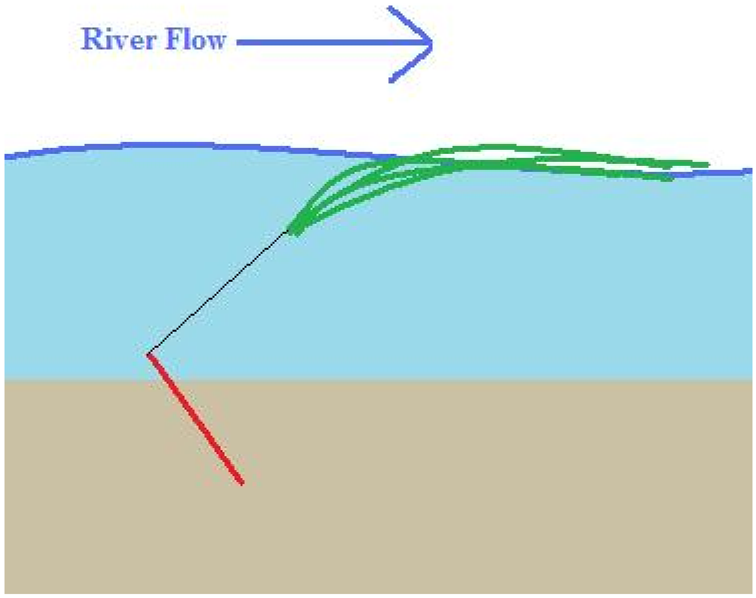

Figure 1: Experimental Setup

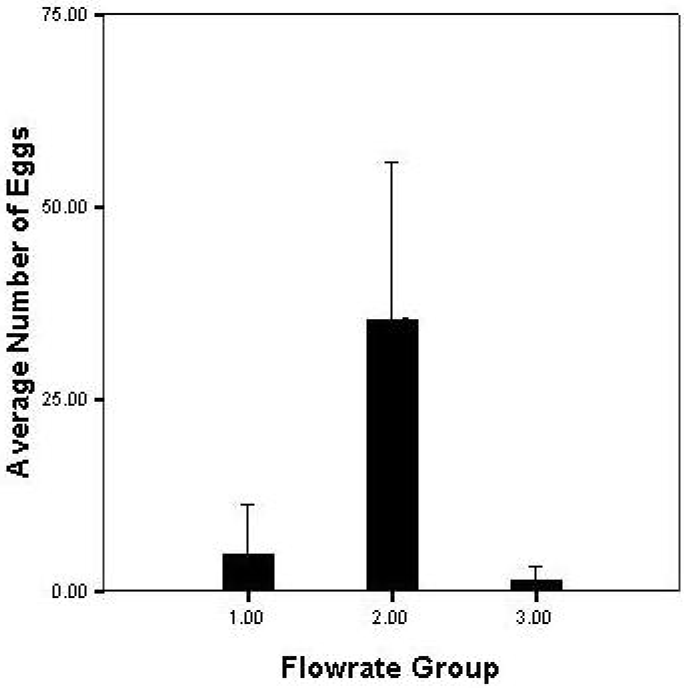

Figure 2 : Average Egg Number in Flow Rate Groups-Flowrate, Groups 1=0.0-0.2 m/s, 2=0.2-0.4 m/s, 3=0.4-0.6 m/s

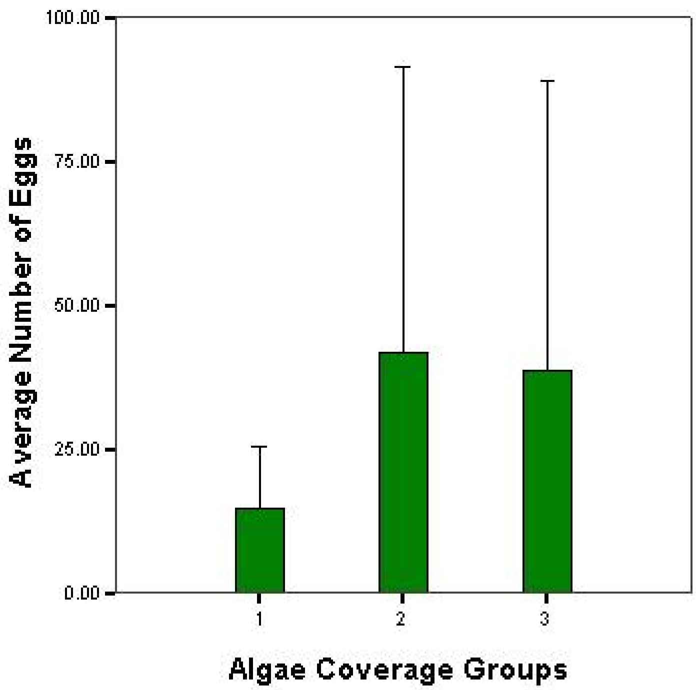

Figure 3: Average Egg Number in Algae Coverage Groups, Groups 1=0-20% coverage, 2=20-40%, 3=40-60%

Literature Cited

Corbet, P. 1980. Biology of odonata. Annual Review of Enotmology. 25: 189-217.

Gibbons, D.W., and D. Pain. 1992. The influence of river flow rate on the breeding behaviour of Calopteryx damselflies. The Journal of Animal Ecology 61: 283-289.

Johnson, C. 1962. Breeding behavior and oviposition in *Calopteryx maculatum* (Beauvais) (Odonata: Calopterygidae).American Midland Naturalist. 68: 242-247.

Siva-Jothy, M., D.W. Gibbons, D. Pain. 1995. Female oviposition-site preference and egg hatching success in the damselfly *Calopteryx splendens xanthostom*. 1995. Behavioral Ecology and Sociobiology. 37: 39-44.

Waage, J. 1973. Reproductive behavior and it's relation to territoriality in Calopteryx maculata (Beauvois) (Odonata:Calopterygidae). Behavior. 47. 240-256.