The Effects of Algae Leaf Coverage and Flow Rate on Oviposition Choice in *Calopteryx maculata*

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ABSTRACT

Female *Calopteryx maculate* are endophytic egg layers; they place their eggs within floating aquatic vegetation. In this study artificial oviposition leaf patches were created and placed in different sites along the Maple River near Pellston, MI. The varying flow rates were recorded and after 48 hours the leaf patches were collected. The egg number or oviposition sites were counted on each leave along with the percent algae leaf coverage. The effect of algae leaf coverage and flow rate on oviposition preference were statistically measured; algae leaf coverage did not affect oviposition frequency ($p>0.05$) and intermediate stream flow was preferred over slower and faster flow rates as oviposition sites ($p<0.05$). The reason for this correlation is unknown, however, it could relate to the optimal conditions for egg growth and development. It is impossible to know whether oviposition flow-rate preference is the result of the male or female's choice. More research of egg development and observational data would provide more insights into the reasoning for intermediate flow oviposition preference.

INTRODUCTION

The dark-winged damselfly (*Calopteryx maculata*) is a common specimen for studies involving sexual selection, territoriality, and mating systems. However, very little has been studied regarding the placement and preference of oviposition sites. Female *Calopteryx maculata*, like other Calopterids, are endophytic egg layers (Corbet, 1980). The female abdomen pierces the floating aquatic vegetation to lay her eggs inside the leaf mesophyll. The females make tracks of their eggs in a zigzag pattern down the length of the leaf (Siva-Jothy et al, 1995). There is a large amount of variation between oviposition sites because many physical and behavioral parameters affect oviposition placement, which make comprehensive studies very difficult (Gibbons and Pain 1992).

Previous studies have examined different oviposition site variables. Waage (1987) experimented with site size and the presence of other females. Female *Calopteryx maculata* oviposited eggs more frequently on larger oviposition sites compared to smaller sites. However, when other females were present on a smaller, adjacent site, females preferred the site with other females present. Siva-Jothy, Gibbons, and Pain (1995) examined flow rate effects in oviposition choice in *Calopteryx splendens xanthostoma*. By controlling the rate of water flow past oviposition patches, they were able to test which patches had higher oviposition frequencies. They also tested the effect of algae on oviposition choice and egg survivorship. They concluded that C. s. xanthostoma preferred to oviposit in fast waters to maximize female fitness with lower egg mortality at these sites (Siva-Jothy et al. 1995). Gibbons and Pain (1992) used observational and quantitative flow-rate data to document the breeding behavior at different flow-rates.

The purpose of this study is to examine the effects of flow rate and algal leaf coverage on oviposition preference in *Calopteryx maculata*. We hypothesize that oviposition will be more frequent in fast water on leaves with the least algae leaf cover to optimize egg viability.

MATERIALS AND METHODS

Data collection

Field work was conducted between July 25 and July 29, 2008. The field site was located at the Maple River in Pellston, Michigan 300 yards downstream of the Douglas Lake Road overpass. Artificial leaf patches were designed for the females to oviposit. Each artificial patch was constructed with a 12 inch dowel rode, fishing line, and 5-7 *Sparganium americanum* leaves (Figure 1). By using leaves picked at the bottom of the stream, we could ensure that the only eggs laid were a result of the experiment and not from previous oviposits. The stiff rods allowed easy placement into the gravel and sand of the river bed and could be buried deep enough so that the rod did not disrupt stream flow. The fishing line was used to pierce and hold the leaves in place floating on the surface. Flags were placed behind each artificial patch to mark the site.

A different artificial patch was placed every 10-15 yards where dark-winged damselflies were observed. At each placement the flow rate and distance from the nearest shore were measured (flow rate was measured using a Flo-Mate meter and wading pole).The placements varied across the width and length of the river to achieve varied flow rates. On the first day, 10 trials were placed in the river. After 48 hours, the trials were collected. Flow rate was measured at the time of collection to get an average flow rate over the 48 hour period. This procedure was repeated farther downstream in 10 different sites over the next 48 hours.

Once the leaves were collected, they sat in water for 24 hours. This allowed the oviposition sites to change color and become easily visible. Eggs were counted using dissecting microscopes. The length of algae cover was recorded and compared to the leaf length to get percent algae leaf coverage. This measurement was used because the algae usually covered the entire width of the leaf and only varied on the length of the leaf; it was also an easy way to calculate leaf size or area without complicated surface area calculations using non-geometric shapes.

Statistical analysis

Both parameter groups had to be split into categories to allow statistical analysis. Algae cover was divided into 3 groups: 0-20%, 20-40%, and 40-60% algae coverage. Flow rate was divided into three flow rate categories: 0.0-0.2 m/s, 0.2-0.4 m/s, and 0.4-0.6 m/s. ANOVA Post Hoc tests were used to calculate statistical significance between the parameter groups and the number of eggs (SPSS v. 15.0).

RESULTS

Algae cover and number of eggs were not significant ($p=.556$). Algae cover and flow rate showed some significance. Algae group 1 (0-20%) and 2 (20-40%) had significant differences between their flow rates ($p=.031$) but algae group 1(0-20%) and 3 (040-60%) and group 2 and 3 did not show significance ($p=0.311$ and $p=0.361$) (Figure 3). 9.72% of the leaves in flow rate group 2 had leaves with algae cover where flow group 1 and flow group 2 had 2.85% and 5.98% algae coverage respectively (Table 1).

Egg number and flow rate were significant. Flow rate group 1 and 2 and 2 and 3 were statistically significant ($p<0.05$), but flow rate group 1 and 3 were not statistically significant ($p=0.974$) (Figure 2). Oviposit sites in flow group 2 (0.2 m/s-0.4 m/s) occurred at a frequency of 0.33 while flow group 1 and 3 had oviposit frequencies of 0.13 and 0.12 respectively (Table 2).

DISCUSSION

While previous studies have found that damselflies prefer to oviposit in fast-flowing water (Siva-Jothy *et al*. 1995, Gibbons 1992), our results indicate intermediate flow rates had more oviposits than slower or faster flow rates (Figure 2). Why this flow-rate is preferred is unknown. Studies show that eggs placed in fast flowing water developed significantly faster and had lower mortality than those lain in slow-flowing water (Siva-Jothy *et al* 1995). This does not explain why the fastest flow group has fewer oviposition sites. The fast-flow might have increase egg fitness but it may also prevent females from being able to land on the vegetation. Although each setup was standardized, the faster flow rates caused the floating vegetation to submerge deeper than those in shallower water. Even though the females may remain submerged while ovipositing (Corbet, 1980), females are prohibited from laying eggs on leaves that are too deep for them to reach. Further studies examining oviposition frequency and the depth of the floating vegetation from the surface are necessary to help rule out any negative consequences from such occurrences.

The correlation between survivorship of eggs and flow rate was explained by the deposition and growth of algae on the aquatic vegetation. Leaves in slower flow rates would accumulate more algae than vegetation with increased flow (Siva-Jothy et al. 1995). However, this assumption was found incorrect; our results showed more algae leaf cover in the faster flow rate groups (Table 1). There was also no relationship between the algae leaf cover and the amount of eggs (Figure 3); even though number of eggs and algae cover was significant between algae groups 1(0-20%) and 2 (40-60%), there was not a preference for oviposition based on algae cover because there was no statistical difference between algae group 1 and 3.

This study only examined physical parameters of site quality; however, oviposition is a result of both site quality and damselfly mating behavior (Gibbons and Pain, 1992). The quantitative data gathered in this study was not coupled with behavioral observations, which limits the conclusions that can be gathered by this study. Although oviposition preference for intermediate flow rate is present, whether this preference is female or male dependent is unknown. Whether the sites were colonized first by females or males is unknown. If females claim these new vegetative sites first, they avoid being harassed by males wanting to copulate; most females have enough stores of sperm from previous matings to lay their eggs without copulating with the resident male guarding oviposition access (Waage 1987). It is for these same reasons that Waage hypothesized that female damselflies prefer oviposition sites with other females; the second female may benefit from distracted males with their territorial behavior directed at the first female by avoiding harassment by other males.

With male occupation occurring first, female choice of mates may also influence oviposition preference. The biggest males with the most endurance to win elevated competitions between other males occupy the best territories (Forsyth and Montgomerie 1986). *C. haemorrhoidalis* male territorial disputes were most common in fast-flowing parts of the streams while slower streams were often undefended (Gibbons and Pain 1992). Male *C. haemorrhoidalis* and *C. s. xanthostoma* courted males by falling onto the surface of the water and floating with the current before regaining flight. Males that performed this strategy had a higher mating success. With this performance, males advertise the flow-rate quality of their territory and along with their fitness (Gibbons and Pain 1992). This same behavior has been observed in *Calopteryx maculata* (Johnson 1962). Males may prefer territories with intermediate flow rates as a result of the females' choice for such oviposition sites.

Studies of oviposition choice have only begun to be studied. While this study only examined flow rate and algae leaf coverage, site quality such as temperature, stream depth, types of vegetation chosen, along with the amount of sun and shade present have yet to be considered as oviposition variables. Studies analyzing damselfly egg growth and development and oviposition quality may further determine if intermediate flow rates are preferred to maximize egg viability. Further research is also necessary to determine where this flow-rate preference originated: whether female choice determines the sexually selection of males that occupy such territories or whether the fittest males occupy the territories with optimal flow rates that determine where females oviposit.

ACKNOWLEDGEMENTS

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TABLES

Average Algae Coverage within Flow Rate Group	
Group Number	Average Algae Coverage (%)
1 (0.0-0.2 m/s)	2.85
2 (0.2-0.4 m/s)	9.72*
3 (0.4-0.6 m/s)	5.98

Table 1. Percent leaves with algae coverage within each flow rate group.

Frequency of Oviposition within Flow Rate Group Categories	
Group Number	Frequency
1 (0.0-0.2 m/s)	0.13
2 (0.2-0.4 m/s)	0.33
3 (0.4-0.6 m/s)	0.12

Table 2. Frequency of leaves with oviposits within each flow group.

FIGURE LEGENDS

Figure 1. Artificial oviposition site leaf set up.

Figure 2. The average egg number in each flow rate group. Flow rate group 1 is 0.0-0.2 m/s, flow group 2 is 0.2-0.4 m/s, and flow rate group 3 is 0.4-0.6 m/s.

Figure 3. The average egg number in each alage group. Algae cover group 1 represents 0-20% coverage, group 2 represents 20-40% leaf cover and group 3 represents 40-60% leaf cover.

FIGURES

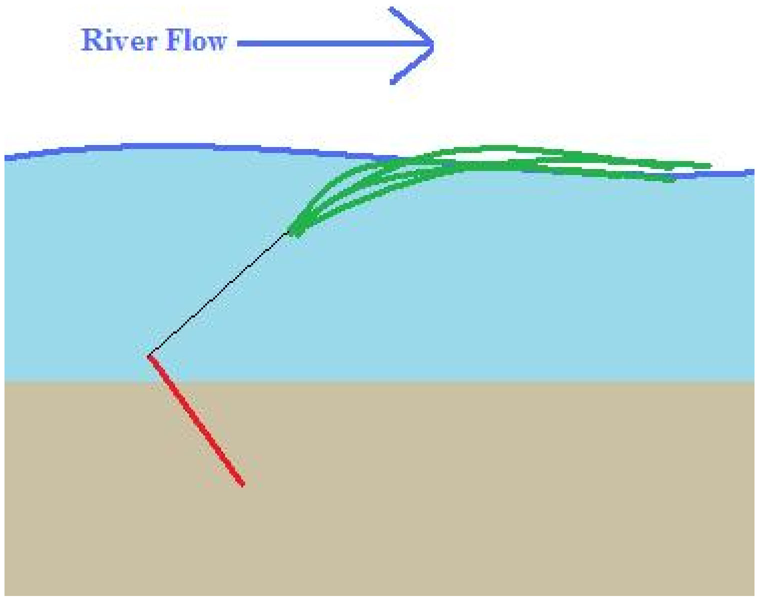

Figure 1.

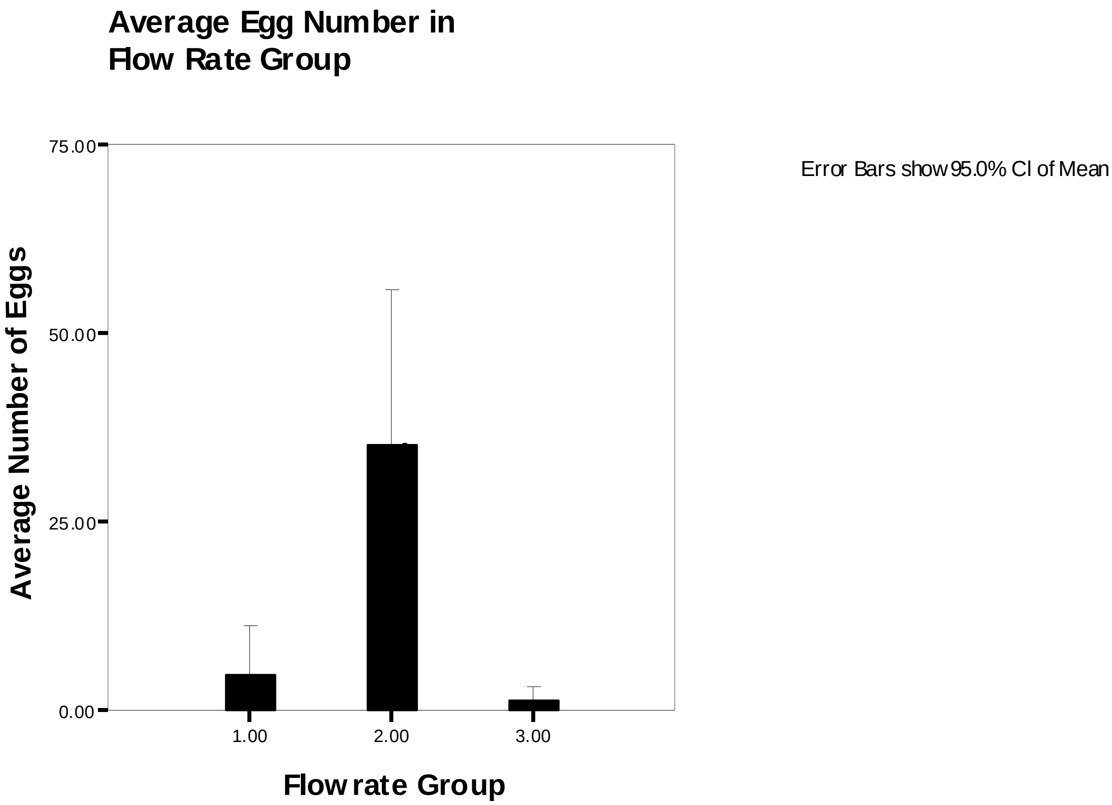
Average Egg Number in
Flow Rate Group
Error Bars show 95.0% CI of Mean
Average Number of Eggs
75.00
50.00
25.00
0.00
1.00
2.00
3.00
Flow rate Group

Figure 2.

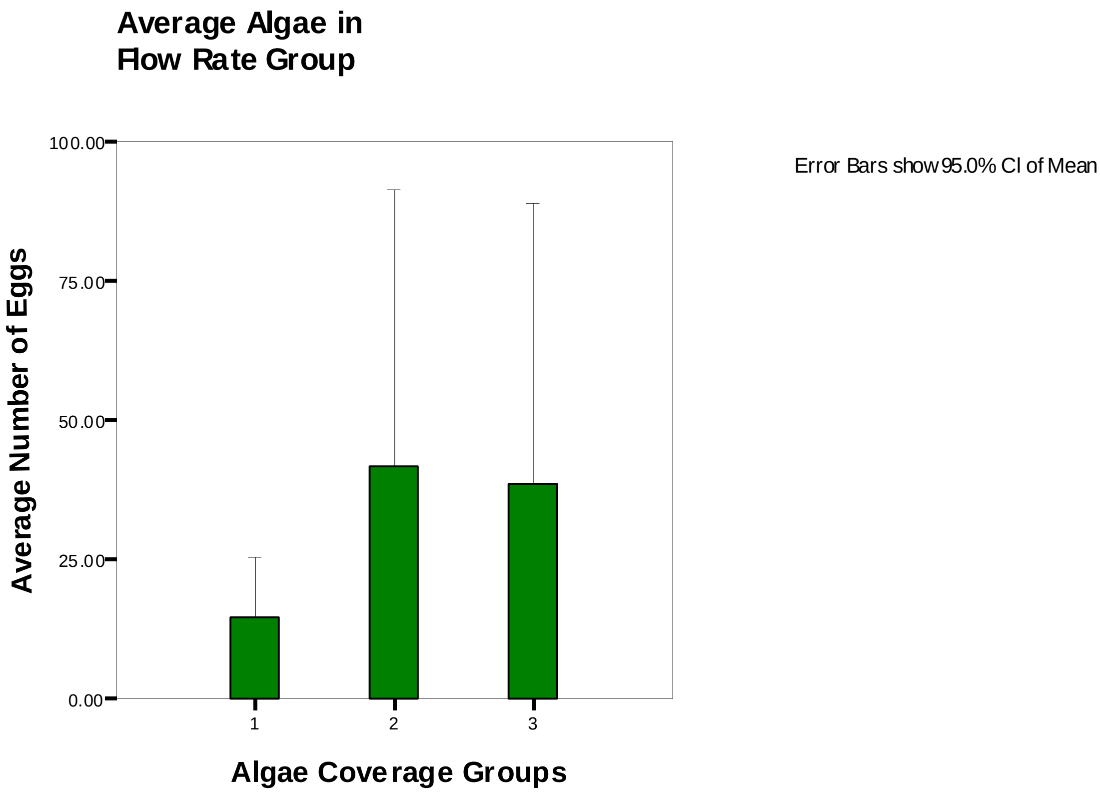
Average Algae in
Flow Rate Group
100.00
75.00
50.00
25.00
0.00
Average Number of Eggs
1
2
3
Algae Coverage Groups
Error Bars show 95.0% CI of Mean

Figure 3.